Evaluation of Biological Agent Clinical Sampling and Analysis

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November 2025 | IDA Product 3005891

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The Systems and Analyses Center conducted this work under contract HQ0034-24-D-0020, Project FN-6-5721, "FY25 Analysis for OTSG," for U.S. Army Office of the Surgeon General (OTSG) Executive Agent. The views, opinions, and findings should not be construed as representing the official position of the U.S. government or the sponsoring organization.

This paper was originally published in May 2021 as IDA Paper P-21576. It had limited distribution due to distribution restrictions for a small number of sources it cited. IDA researchers have presented the publicly-releasable results of P-21576 at multiple meetings of North Atlantic Treaty Organization (NATO) working groups and panels, and several Allies have requested the paper. As a result, IDA is republishing the paper with minor modifications so that it is publicly releasable. The conclusions and recommendations from the original paper are unclassified and are therefore unchanged.

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Executive Summary

Note: This work was originally completed and published in May 2021.[1]

Overview

The U.S. Department of Defense (DOD) should refine its procedures for employing emerging diagnostic devices for biological warfare (BW) diseases, such as Next Generation Diagnostic System (NGDS) Increments 1 and 2, to overcome potential performance issues that are not clearly identified/captured in system test procedures nor data. Critical gaps exist in the data that describes the performance of diagnostic tests employed by—or soon to be employed by—DOD, such as the NGDS 1 and 2. Specifically, data do not exist to characterize the probability that these systems will detect biological exposure events when analyzing specimens collected during the earliest stages of multiple BW-related diseases of concern or to understand how detectability of diseases varies during the course of disease. As a result, the DOD is unable to create evidence-based tactics, techniques, and procedures (TTPs) for the use of BW diagnostics.

To improve the likelihood of diagnostics *contributing* to situational awareness rather than *confounding* it, we make the following recommendations:

- Include requirements for sensitivity over the course of illness (particularly the early stages) in Capability Development Documents (CDDs) for diagnostic systems that are intended to be used for early diagnosis.
- Conduct experiments to characterize diagnostic sensitivity over the course of illness (particularly the early stages) for the systems of interest (e.g., NGDS 2) to inform analysis-based TTPs for use of those systems using the methods described in this paper.
  - Update initial/full operational capability numbers according to analysis-based TTPs and
  - Update doctrine accordingly.

[1] The Executive Summary has minor editorial changes from Kristen A. Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*, IDA P-21576 (Institute for Defense Analyses, May 2021), CONTROLLED UNCLASSIFIED INFORMATION. Only UNCLASSIFIED information is cited here.

- Ensure that diagnostic users and clinicians are educated on the performance of diagnostics at various stages of disease, especially for rarely diagnosed diseases, and whether diagnostics may need to be used again at set intervals.
- Incorporate rule-out testing for influenza/common diseases as a first step of forward sampling and analysis TTPs.

The U.S. Army Office of the Surgeon General (OTSG) tasked the Institute for Defense Analyses (IDA) to evaluate how various TTPs for the collection and analysis of clinical specimens influence the timing and accuracy of diagnostic test results. Analysis compared the use of diagnostics at lower roles of care (Role 1 or 2) and those at higher roles of care (Role 3 or outside-of-theater), which drove the included recommendations.

In the absence of effective environmental sampling, the military medical system will likely provide the first indication of a biological attack. Generating early diagnostic results, however, can be skewed by various clinical, operational, technological, and pathological factors. As shown in Figure ES-1, generating an accurate diagnostic result for a given disease requires the collection of the correct clinical specimen at a stage in the disease at which sufficient pathogen indicators are present in that specimen so that a given diagnostic tool can detect it. Furthermore, medical personnel require some form of motivation or exposure indicator (e.g., early signs/symptoms or intelligence) to collect the specimen and analyze it with a given test. We examined all four components in our analysis for this paper.

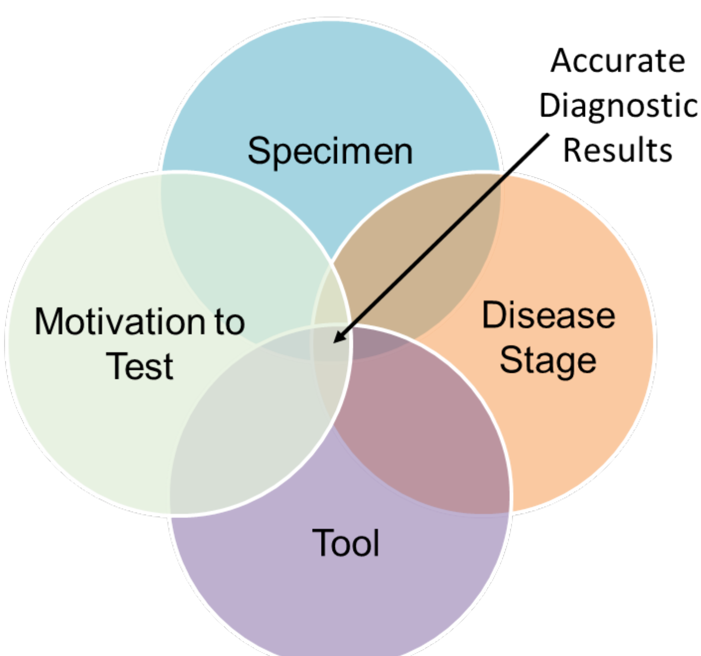

Figure ES-1. Requirements for an Accurate Diagnostic Result

Based on a scientific literature review, we characterized the timing during a given disease's progression that a diagnostic test can generate accurate results (see Figure ES-2). For multiple combinations of diseases and diagnostic technologies of interest, we were unable to find sufficient data to characterize the window of opportunity during the course of a disease when the diagnostic technology would generate accurate results (orange). Of the disease and diagnostic technology combinations of interest for which sufficient data were available (blue), high probability of detection at symptom onset was not common, which could limit the utility of far-forward diagnostics.

Disease	Specimen Type	Bacterial / Viral / Toxin Isolation	PCR (In-House)	PCR (Cartridge)	Antigen (ELISA / Mass Spec)	Antigen (Rapid)
Plague	Blood	Sufficient Test Results Data Found (Non-Human Primate Data)	Sufficient Test Results Data Found (Non-Human Primate Data)	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found
	Sputum	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found
Q Fever	Blood	Test Does not Exist	Sufficient Test Results Data Found (Non-Human Primate Data)	Insufficient Test Results Data Found	Test Does not Exist	Test Does not Exist
Tularemia	Blood	Sufficient Test Results Data Found (Non-Human Primate Data)	Sufficient Test Results Data Found (Non-Human Primate Data)	Sufficient Test Results Data Found (Non-Human Primate Data)	Insufficient Test Results Data Found	Test Does not Exist
	Sputum	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Test Does not Exist
Ebola	Blood	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Sufficient Test Results Data Found (Human Data)	Sufficient Test Results Data Found (Human Data)	Insufficient Test Results Data Found
VEE	Blood	Sufficient Test Results Data Found (Non-Human Primate Data)	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Test Does not Exist
Anthrax	Blood	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Insufficient Test Results Data Found
SEB	Blood	Test Does not Exist	Test Does not Exist	Test Does not Exist	Insufficient Test Results Data Found	Insufficient Test Results Data Found
Influenza	TNS	Sufficient Test Results Data Found (Human Data)	Sufficient Test Results Data Found (Human Data)	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Sufficient Test Results Data Found (Human Data)
	NPA	Sufficient Test Results Data Found (Human Data)	Sufficient Test Results Data Found (Human Data)	Insufficient Test Results Data Found	Insufficient Test Results Data Found	Sufficient Test Results Data Found (Human Data)

Sufficient Test Results Data Found
Insufficient Test Results Data Found
Test Does not Exist
Human Data
Non-Human Primate Data

Note: PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay; VEE = Venezuelan equine encephalitis; SEB = staphylococcal enterotoxin B.

Figure ES-2. Availability of Diagnostic Test Results

To further assess the disease and diagnostic technology combinations for which sufficient data exists, we developed a stochastic, individual-based model to simulate disease progression, patient movement, and clinical specimen collection and analysis following a biological exposure event. We then used this model to determine how changes in TTPs for specimen collection and analysis affect the timing and accuracy of diagnostic results. Due to the large gaps in the data that were available, especially for systems that the DOD currently employs or will soon employ, we could not make concrete conclusions regarding the fielding of or TTPs for specific diagnostic capabilities within the medical system or the use of those systems for shipped samples. However, we present broad conclusions regarding the design of various specimen collection and analysis TTPs in the context of disease and diagnostic test attributes in addition to the recommendations listed above.

Conclusions

- Critical gaps exist in the data that describe the performance of diagnostic tests employed by—or soon to be employed by—DOD. Specifically, data do not exist to characterize the probability of detection of these systems when analyzing specimens collected during the early stages of multiple diseases of concern.

- TTPs for use of diagnostics are not one-size-fits-all; rather, they must be designed specifically to suit the progression of disease and its detectability with a given diagnostics. However, the DOD currently cannot design a BW disease detection TTP to suit all diseases of interest, because it lacks information on how detectability of diseases varies during the course of each disease for its fielded BW-related diagnostics.
- Testing at lower roles of care has the most benefit when individuals first present with non-severe symptoms, assuming that the probability of detection is high enough that they can be detected using diagnostics at lower roles.

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1. Introduction

Note: This work was originally completed and published in May 2021.[1]

A. Background

In the case of covert employment, a biological attack may not be recognized until individuals present to the medical system.[2] If this is the case, a diagnostic system could provide the first indication of an attack and the identity of the agent. Earlier situational awareness facilitates the implementation of countermeasures in time to decrease casualties and fatalities. However, fielding diagnostics capabilities farther forward will not necessarily provide earlier situational awareness. This paper, written for the U.S. Army Office of the Surgeon General (OTSG), examines how quickly diagnostic results could be obtained given different diseases, technologies, and specimen collection procedures.

After realizing that an attack has occurred and determining which agent has been used in the attack, two types of responses can be implemented. The first are those responses that directly decrease casualties and fatalities from the attack itself. These responses can include post-exposure prophylaxis (PEP), antibiotic treatments, and restriction of movement (ROM) (in the case of contagious diseases). The second are those responses that provide greater situational awareness to inform medical and operational decisions and may indirectly decrease casualties. These responses can include intelligence gathering and preparing relevant countermeasures at nearby units in case of additional attacks or the spread of the disease from one unit to another (if contagious). Many responses can only be implemented after knowing that an attack with the given agent has occurred, and earlier responses could lead to fewer casualties or fatalities.[3] The desire to implement responses as quickly as possible has led to the suggestion that diagnostics should be performed as early and as far forward as possible so that the unit knows quickly whether an attack or outbreak has occurred.[4]

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[1] This chapter has minor editorial changes from Kristen A. Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*, IDA P-21576 (Institute for Defense Analyses, May 2021), CONTROLLED UNCLASSIFIED INFORMATION. Only UNCLASSIFIED information is cited here.

[2] Headquarters, Department of the Army (HQDA), *Army Biological Defense Strategy* (HQDA, 2021), 7.

[3] Julia K. Burr et al., *Emerging Infectious Diseases Study*, IDA Paper P-5302 (Institute for Defense Analyses, August 2016), vi, https://apps.dtic.mil/sti/citations/AD1165599.

[4] Ibid., ix.

Generating early diagnostic results can be confounded by various clinical, operational, technological, and pathological factors. As shown in Figure 1, generating an accurate diagnostic result for a given disease requires the collection of the correct clinical specimen at a stage in the disease at which there is a sufficient quantity of pathogen indicators in that specimen (often based on bacterial or viral load) such that a given diagnostic tool can detect it. Furthermore, medical personnel require some impetus to collect the specimen and analyze it with a given test. The correct specimen could be a blood, sputum, nasopharyngeal, or other specimen. Some diseases, such as Q fever, are diagnosed with blood specimens, while others, such as influenza, are diagnosed with nasopharyngeal specimens.[5] The correct test could be, for example, a polymerase chain reaction (PCR), bacterial, viral, or toxin isolation, or antigen test. Chapter 2 provides more information about those tests.

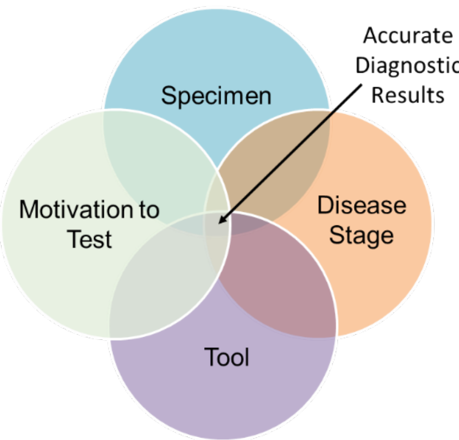

Figure 1. Requirements for an Accurate Diagnostic Result

To use the correct diagnostic tool and the correct specimen, a clinician must have a motivation to test. For example, if large numbers of individuals reported to a medical treatment facility (MTF), a clinician may use a diagnostic panel that tests for biological warfare (BW) agents since a large influx of patients could indicate a biological attack. Similarly, intelligence or knowledge of local disease outbreaks could influence which diagnostic panels a clinician may run for an individual with a non-specific presentation. Disease-specific signs and/or symptoms may also motivate a clinician to run a given diagnostic test.

Even if the correct test and specimen are obtained, diagnostics do not always produce positive results at all points in the disease progression, especially early. For many diseases,

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[5] United States Army Medical Research Institute of Infectious Diseases (USAMRIID), *Medical Management of Biological Casualties Handbook*, 9th ed. (USAMRIID, 2020), 221–224. https://usamriid.health.mil/assets/docs/training/USAMRIIDs_Blue_Book_9th_edition_PDF_format.pdf.

the pathogen indicators can be low at symptom onset and increase over time.[6] Therefore, the disease progression or stage is also an important component of achieving an accurate diagnostic result. This paper analyzes all four key components—specimen, tool, motivation to test, and disease stage—to determine the utility of different tactics, techniques, and procedures (TTPs) for the analysis of clinical specimens with diagnostic tests to inform situational awareness following a biological event and to inform the subsequent response process.

B. Objectives, Methodology, and Paper Organization

This paper has three main objectives:

- Perform a literature review that characterizes when during a given disease's progression it would be detectable using various diagnostic technologies.
- Use the results of the literature review and modeling to evaluate clinical sampling and analysis TTPs associated with each diagnostic in terms of the timing of when diagnostic results would be available to inform situational awareness under various sampling and analysis TTPs.
- Draw conclusions that may inform updates to diagnostic TTPs that enhance situational awareness and determine where there are gaps in the scientific literature that may preclude informing updates.

To fulfill the first objective, we performed a literature review that focused on those diagnostic tools and specimens available for diseases of interest. We also looked at available information that might relate changes in pathogen indicators for a disease of interest to the progression of symptoms over time. To fulfill the second objective, we modeled means by which the diagnostic results over time would affect how quickly a positive diagnosis could be obtained given different TTP options for collecting and analyzing clinical specimens with diagnostic tests. Not all diseases or disease-tool combinations included in our literature review had sufficient information to model them, but we modeled the combinations for which we had sufficient information. To fulfill the third objective, we synthesized our results for all diseases and drew conclusions across them.

Although this paper looks at the utility of diagnostic systems at lower roles of care or taking of samples at a lower role of care that can then be shipped to a higher role of care for analysis, we include in our analysis technologies that could be used at a lower role of care (e.g., cartridge-based PCR, rapid antigen tests) and technologies that could not (e.g.,

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[6] For example, see Frederick Koster et al., "Milestones in Progression of Primary Pneumonic Plague in Cynomolgus Macaques," *Infection and Immunity* 78, no. 7 (July 2010): 2946, doi:10.1128/IAI.01296-09.

bacterial/viral isolation, laboratory-based antigen tests). Within this paper, a lower role of care is assumed to be Role 1 or 2, and a higher role of care is assumed to be Role 3 or above (see Section 3.B). This assumption allows for a comparison of systems and allows us to determine whether developing or using technologies at a lower role of care provides earlier situational awareness than using technologies at a higher role of care. This paper does not analyze specific systems and does not address qualities of a specific system that would allow it to be used at a lower role of care (e.g., weight, size) beyond the general diagnostic technology type. In addition, it focuses on diagnosis for situational awareness, not for care of individual patients, and does not address environmental sampling/testing using diagnostic systems or testing of asymptomatic individuals.

The diseases of interest were those that are included on the Next Generation Diagnostic System (NGDS) Warrior Panel (except for Marburgvirus) and some additional diseases to provide a range of diseases (see Table 1). More information about the NGDS 1 (currently fielded) and NGDS 2 (in development) systems is included in Chapter 3. We did not include Marburgvirus in our analysis due to its similarly to Ebolavirus, although diagnostic systems for Marburgvirus may not necessarily have the same accuracy for infected individuals as those for Ebolavirus.

Table 1. Diseases Included in this Analysis

Disease	Pathogen Type	Early Symptom Presentation	Scope Note
Plague	Bacteria	Moderate, Non-Specific	NGDS 1 (Warrior Panel)
Q Fever	Bacteria	Moderate, Non-Specific	NGDS 1 (Warrior Panel)
Tularemia	Bacteria	Severe, Non-Specific	NGDS 1 (Warrior Panel)
Ebola	Virus	Moderate, Non-Specific	NGDS 1 (Warrior Panel)
VEE	Virus	Severe, Non-Specific	Included to broaden scope
Anthrax	Bacteria	Moderate, Non-Specific	NGDS 1 (Warrior Panel)
SEB	Toxin	Severe, Non-Specific	Included to broaden scope
Influenza	Virus	Moderate, Non-Specific	Included to broaden scope because tests are available at MTFs

Notes: Colors are for presentation only, not used elsewhere in this paper. The abbreviations used in this table are defined in Appendix E of this paper.

Sources:

Presence on Next Generation Diagnostic Systems (NGDS) Warrior Panel taken from COL David Hammer, “Building the CBR Diagnostic Family of Systems,” briefing presented at the Pennsylvania State University OTA Symposium January 15–16, 2019 (Joint Program Executive Office for Chemical, Biological, Radiological, and Nuclear Defense, January 15, 2019), 11, https://abrl.psu.edu/wp-content/uploads/2019/01/JPEO-PSU-OTA-Symposium_20190115_MCS-DX.pdf.

Early symptom severity is taken from North Atlantic Treaty Organization (NATO), *NATO Planning Guide for the Estimation of CBRN Casualties*, NATO Standard AMedP-7.5, Edition A Version 1 (NATO Standardization Office, October 2017), https://nso.nato.int/nso/nsdd/main/standards/ap-details/1788/EN.

As shown in Table 1, this paper looks at three viruses, four bacteria, and one toxin. All the resulting diseases start with a non-specific, sometimes severe, illness. This means that it is unlikely that any of these diseases could be initially diagnosed based on symptoms alone. Anthrax, plague, and Ebolavirus disease (EVD) develop more specific symptoms later on in the course of illness, and these symptoms may then be recognized by a clinician. EVD, plague, and influenza are all contagious diseases, while anthrax, Q fever, tularemia, VEE, and SEB are noncontagious. Most threats listed are considered BW threats; however, influenza is not. Influenza is a common disease that produces similar initial symptoms to many BW agents. Therefore, we included influenza in our analysis to compare its diagnosis with other diseases since ruling out influenza may be the first step that a clinician could take before determining that a disease may have occurred from a biological attack.

In this paper, Chapter 1 provides an introduction of and motivation for the analysis. Chapter 2 discusses how different diagnostic technologies work and what their requirements are. Chapter 3 provides an overview of current and potential future concepts of use for diagnostics within the Department of Defense (DOD) and an overview of our model. Chapters 4 through 11 discuss the results of the literature review and modeling (where appropriate) for each disease. Chapter 12 synthesizes the results for all diseases and provides broad conclusions and recommendations.

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2. Diagnostic Technologies

Note: This work was originally completed and published in May 2021. [7]

In this paper, we focus on the three major categories of diagnostic technologies used in evaluating acute infections: bacterial or viral isolation, PCR assays, or antigen tests. Many specimen types, including nasal swabs, blood, or sputum, can be used in these tests, although not all specimen types may be appropriate for every disease. Serology is not typically used to diagnose acute infections, and so it is mentioned only briefly. This chapter gives a broad overview of each technique, which helps inform our modeling and recommendations for diagnostic TTPs.

A. Bacterial/Viral Isolation

Bacterial or viral isolation has been the traditional method of diagnosis for decades. It is only within the past 20–30 years that antigen or PCR assays have become more prevalent. Bacterial and viral isolation are usually longer and more involved techniques than PCR or antigen tests but can still have utility, especially for diseases that do not have PCR or antigen tests available or for new strains of diseases. In addition, for many diseases bacterial or viral isolation is still the gold standard against which other tests, such as PCR, are measured.

Bacterial isolation generally requires adding sample to appropriate media and culturing the bacteria over the course of days.[8] Different bacteria may require different media or growing conditions, which can affect how long the process takes. Detecting the presence of bacteria was originally done by hand, but automated techniques have been developed, including mass spectrometry or fluorescence assays.[9]

Viral isolation generally requires growing the virus in cell culture or in live animals. Viral isolation is typically done in dedicated virology facilities rather than general

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7 This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

8 Pierre-Edouard Fourier et al., "Modern Clinical Microbiology: New Challenges and Solutions," *Nature Reviews Microbiology* 11, no. 8 (August 2013): 576, doi:10.1038/nrmicro3068.

9 Fourier et al., "Modern Clinical Microbiology"; J. W. Snyder, G. K. Munier, and C. L. Johnson, "Direct Comparison of the BD Phoenix System with the MicroScan WalkAway System for Identification and Antimicrobial Susceptibility Testing of *Enterobacteriaceae* and Nonfermentative Gram-Negative Organisms," *Journal of Clinical Microbiology* 46, no. 7 (July 2008): 2328, doi:10.1128/JCM.00075-08.

laboratories.[10] As with bacteria, different viruses can require different cell cultures to grow, and the isolation procedure can take different lengths of time. Some viruses can also be difficult—if not impossible—to grow on cell lines. Viruses are typically quantified or assessed by looking at the morphological changes of the cultured cells over time under a microscope.[11] A plaque assay, for example, stains and counts plaques formed within a cell culture infected with a virus.[12] Automated techniques are also being developed for detecting the presence of virus.[13]

Bacterial or viral isolation procedures can require high biocontainment facilities. These biocontainment requirements are designated by biosafety levels (BSLs) between one and four.[14] Table 2 gives the BSL requirements for culture or isolation of each disease-causing agent discussed in this paper. Biosafety level 1 (BSL-1), which is commonly used for organisms that do not cause disease in humans, uses standard laboratory procedures, with no additional safeguards. BSL-2, which is common for pathogens that cause disease in humans but do not usually transmit via inhalation, uses biosafety cabinets for aerosol or splash-generating procedures and additional personal protective equipment (PPE) as needed. BSL-3, which is common for pathogens that can cause disease in humans via inhalation, uses biosafety cabinets for all procedures and enhanced PPE and ventilation requirements, potentially with respiratory protection required. BSL-4, which is used for dangerous agents that can be life-threatening, have no treatment, and can cause disease via inhalation, uses airlocks, a positive-pressure suit, and many other barriers to ensure that the agent does not escape into the environment or infect the laboratory worker. There are few BSL-4 facilities in the world.

Due to the BSL requirements and difficult laboratory procedures, all bacterial or viral isolation for all the disease-causing agents discussed in this paper should be performed at a designated laboratory facility and cannot be moved to a lower role of care (see Section 3.A). Most of these designated laboratory facilities would be outside of the theater. BSL-4 capability is only available within the DOD at the U.S. Army Medical Research Institute

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[10] Gregory A. Storch, "Diagnostic Virology," *Clinical Infectious Diseases* 31, no. 3 (September 2000): 739, https://doi.org/10.1086/314015.

[11] Ibid., 740.

[12] Diana Juarez et al., "Assessment of Plaque Assay Methods for Alphaviruses," *Journal of Virological Methods* 187, no. 1 (January 2013): 186, https://doi.org/10.1016/j.jviromet.2012.09.026.

[13] Allyson L. Masci et al., "Integration of Fluorescence Detection and Image-Based Automated Counting Increases Speed, Sensitivity, and Robustness of Plaque Assays," *Molecular Therapy: Methods & Clinical Development* 14 (September 13, 2019): 270–274. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6717064/.

[14] Centers for Disease Control and Prevention, *Biosafety in Microbiological and Biomedical Laboratories*, 6th ed. (Centers for Disease Control and Prevention, June 2020), 68–69, https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf. The BSL descriptions in the rest of this paragraph are from this CDC source.

for Infectious Diseases (USAMRIID). BSL-3 capability within the DOD is only available at major medical centers, largely in the continental United States. The BSL level used for a diagnostic test would likely depend on what agents may be most likely or suspected.

Table 2. BSL Requirements for Disease-Causing Agents Discussed in this Paper

Bacteria/Virus/Toxin	BSL Requirement
Bacillus anthracis	BSL-3
Yersinia pestis	BSL-3
Coxiella burnetii	BSL-3
Francisella tularensis	BSL-3
Venezuelan Equine Encephalitis Virus	BSL-3
Ebolavirus	BSL-4
Influenza virus	BSL-2[a]
Staphylococcal Enterotoxin B	BSL-2

[a] BSL-2 procedures are used for seasonal influenzas. BSL-3 procedures or higher are used for historical influenzas (e.g., the 1918 pandemic strain) or animal influenza A viruses.

Source: Requirements taken from Centers for Disease Control and Prevention, *Biosafety in Microbiological and Biomedical Laboratories*, 6th ed. (Centers for Disease Control and Prevention, June 2020), https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.

B. PCR

PCR is the most common diagnostic method for many diseases in the modern age. PCR has fewer laboratory safety concerns and requires a shorter amount of time than bacterial or viral isolation. It is also more sensitive and specific than most antigen assays.[15] Many PCR tests inactivate the bacterium or virus early in the process so that the sample can be processed outside of BSL-2 or higher conditions. PCR amplifies a specific target sequence or set of target sequences from the deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) of bacteria or viruses. Bacteria carry their genetic code in DNA, while some viruses carry theirs in RNA and other viruses in DNA. Although there are several methods of PCR, most follow the same basic method.

PCR tests amplify a specific target sequence within the bacterial or viral genome by replicating it exponentially until the amount of that target sequence is large enough so that it can be detected by some method (e.g., fluorescent dye).[16] If RNA is amplified, as is the case for some viral genomes, then the RNA must first be transcribed into DNA using an

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[15] Caroline Chartrand et al., "Diagnostic Accuracy of Rapid Antigen Detection Tests for Respiratory Syncytial Virus Infection: Systematic Review and Meta-Analysis," *Journal of Clinical Microbiology* 53, no. 12 (December 2015): 3738, doi:10.1128/JCM.01816-15.

[16] Samuel Yang and Richard E. Rothman, "PCR-Based Diagnostics for Infectious Diseases: Uses, Limitations, and Future Applications in Acute-Care Settings," *The Lancet Infectious Diseases* 4, no. 6 (June 2004): 338, https://doi.org/10.1016/S1473-3099(04)01044-8.

enzyme called reverse transcriptase so that it can be used in PCR.[17] Therefore, PCR tests for viruses with RNA genomes are reverse transcriptase–polymerase chain reaction (RT-PCR) tests. To replicate the target sequence, the DNA strands are first separated from each other. Then, short nucleotide sequences called primers, which attach to the target sequence, are used.[18] A heat-stable DNA polymerase then attaches to the primers and replicates the DNA. The DNA is then denatured again, and the process restarts. In this way, the target sequence is amplified exponentially. The number of cycles required before the target sequence is detected via a fluorescent dye or other method is called the cycle threshold (Ct) value. The Ct value is sometimes used as a proxy for the concentration of the target sequence present in the original specimen.[19]

PCR assays can be developed in-house or can use cartridges or other commercially available platforms. PCR assays can also be developed for a single target sequence, or multiple genes. By being able to amplify multiple target sequences, some PCR assays, such as NGDS, can detect multiple species simultaneously. In addition, testing for multiple target sequences could enable better detection of different strains (since not all strains of a bacterium/virus may contain the target sequence) and differentiation between similar species. If a bacterium or virus does not contain the target sequence, even if it is the agent of interest, it may not be detected via PCR.

An in-house assay is one that is developed by the laboratory itself rather than as a commercially available kit. Therefore, in-house assays may require additional steps, such as DNA extraction or post-PCR processing. In-house assays are often more complex and require laboratory personnel to run, unlike some easier-to-use cartridge assays.[20] Some cartridge-based assays also can be performed outside of a laboratory, even at a point-of-care setting. Thus, some cartridge-based PCR systems could potentially be used at lower roles of care.[21] Even when the same target sequence is used, the sensitivity can be different by orders of magnitude across different PCR assays (whether in house or cartridge).[22] This difference could be due to the detection technology used or the purity of the sample used in PCR. Therefore, sensitivity cannot be generalized across different systems. In addition,

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[17] Ibid.

[18] Ibid.

[19] Ibid.

[20] Salvatore Petralia and Sabrina Conoci, “PCR Technologies for Point of Care Testing: Progress and Perspectives,” *ACS Sensors* 2, no. 7 (July 28, 2017): 876, https://pubs.acs.org/doi/pdf/10.1021/acssensors.7b00299.

[21] Ibid.

[22] Shuo Su et al., “Diagnostic Strategies for Ebola Virus Detection,” *The Lancet Infectious Diseases* 16, no. 3 (March 01, 2016): 295, https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(16)00049-9/fulltext.

test sensitivity can be dependent on the user's ability, since he or she may perform the assay incorrectly if they have limited experience.

PCR assays typically take between 1 and 3 hours to run. The time can be lengthened for some in-house assays that require a DNA extraction step beforehand or additional post-PCR processing/analysis of results. Many cartridge-based assays perform all three of these steps within the cartridge system.[23] The DNA extraction and post-PCR processing steps, when performed outside of a cartridge, are usually done in a laboratory by skilled technicians, so ensuring that a cartridge can go from sample to easily read output is key to making it an appropriate point-of-care diagnostic.[24] Since the PCR assay requires many cycles to amplify the gene sufficiently, decreasing the runtime for traditional PCR assays is difficult, although some technologies in development may allow for that in the future.[25]

Other methods of detecting DNA in clinical specimens, such as Loop-Mediated Isothermal Amplification (LAMP), are being researched, although most are still in the development stage.[26] In addition, next-generation sequencing techniques, which can sequence portions of the bacterial/viral genome, are in development for clinical diagnosis.[27] Next-generation sequencing can be especially important when identifying particular strains of a bacterium/virus or when investigating new strains or new traits (e.g., antibiotic resistance), although currently they can take days to weeks to achieve results.

C. Antigen Assays

Antigen assays, especially rapid point-of-care tests such as lateral flow assays, can be faster and easier to use than PCR tests or bacterial/viral isolation.[28] Rapid antigen tests, however, usually have a lower sensitivity than PCR tests or isolation.[29] Like PCR tests, most antigen tests inactivate the bacterium or virus early in the process, so the test can be

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[23] Petralia and Conoci, "PCR Technologies for Point of Care Testing," 877–879.

[24] Hanliang Zhu et al., "The Vision of Point-of-Care PCR Tests for the COVID-19 Pandemic and Beyond," *TrAC Trends in Analytical Chemistry* 130 (September 2020): 115984, https://doi.org/10.1016/j.trac.2020.115984.

[25] Ibid.

[26] Robin Augustine et al., "Loop-Mediated Isothermal Amplification (LAMP): A Rapid, Sensitive, Specific, and Cost-Effective Point-of-Care Test for Coronaviruses in the Context of COVID-19 Pandemic," *Biology* 9, no. 8 (August 2020): 182, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464797/.

[27] Wei Gu, Steve Miller, and Charles Y. Chiu, "Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection," *Annual Reviews of Pathology* 14 (January 24, 2019): 319, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6345613/.

[28] Chartrand et al., "Diagnostic Accuracy of Rapid Antigen Detection Tests," 3738.

[29] Jacqueline Dinnes et al., "Rapid, Point-of-Care Antigen and Molecular-Based Tests for Diagnosis of SARS-CoV-2 Infection" (Cochrane Library, 2020), 2, https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013705.pub2/full.

performed outside the laboratory setting. Antigen assays test for specific proteins, or antigens, produced by the virus or bacterium. They can be either laboratory-based tests, such as mass spectrometry or Enzyme-Linked Immunosorbent Assay (ELISA), or rapid point-of-care tests. Since antigen assays test for specific proteins, the proteins tested must be present in all bacterial/viral strains of interest. If the protein is not present in a given strain, the test will be negative even if the bacterium/virus is indeed present.

Laboratory tests typically require lengthy protocols and extensive equipment to perform but can often have very low limits of detection.[30] Two of the major types are mass spectrometry and ELISA. Mass spectrometry uses the mass-to-charge ratio to identify proteins.[31] Different types of mass spectrometry use different methods to charge the particles and to separate them by mass-to-charge ratio, but all use the same basic identification method. Tandem mass spectrometry (MS/MS) performs two rounds of mass spectrometry to further separate out proteins from each other.[32] Mass spectrometry can often be incredibly sensitive, sometimes down to the zeptomole (10^{-21} moles) range.[33] Mass spectrometry, however, uses large equipment and can take hours to run, so it usually must be performed in a laboratory.

ELISA uses antibodies to detect a given antigen or other protein/peptide using plates, antibodies, and reporter molecules.[34] Antigens are attached to the plate, and then antibodies that are attached to a reporter molecule are added in. When the appropriate antibody binds to the antigen, the reporter molecule is activated and will fluoresce or otherwise produce a signal that can be detected.[35] Additional antibodies may be required between the antigen and the reporter molecule in indirect or sandwich ELISA assays, but the same basic premise applies. Although ELISA assays are often less cumbersome than mass spectrometry assays, they still usually must be performed in a laboratory.

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[30] Renato C. Lins et al., "Zeptomole per Milliliter Detection and Quantification of Edema Factor in Plasma by LC-MS/MS Yields Insights into Toxemia and the Progression of Inhalation Anthrax," *Analytical and Bioanalytical Chemistry* 411, no. 12 (May 2019): 2493, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6988385/.

[31] "Protein Mass Spectrometry," Thermo Fisher Scientific, accessed January 27, 2021, https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-mass-spectrometry.html.

[32] Ibid.

[33] Lins et al., "Zeptomole per Milliliter Detection and Quantification," 2493.

[34] "What is an ELISA (Enzyme-Linked Immunosorbent Assay)?" Thermo Fisher Scientific, accessed January 27, 2021, https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html.

[35] Ibid.

In contrast to the laboratory-based approaches, rapid antigen tests typically can be done at point-of-care settings and do not require a laboratory capability.[36] In addition, many rapid antigen tests can be performed by individuals with minimal training. However, the tests performed by these individuals are less sensitive than laboratory-based antigen tests. Rapid antigen tests can take less than 30 minutes to run.[37] Many tests use test strips coated with antibodies to easily determine whether the antigen is present.[38] Some tests also use readers to further simplify the process of determining whether the antigen is present.[39] Although rapid antigen tests can be less sensitive than other tests, they can produce results quickly, which can be beneficial in some circumstances.

D. Serology/Antibody Tests

Serology or antibody tests determine whether an individual has antibodies against the disease of interest. It can take a week or more to develop antibodies against a given disease, making it difficult to use these tests against acute infections.[40] In addition, antibodies against a given illness can circulate in the body for years after the infection, making it difficult to determine whether the antibodies are from the current infection or a prior infection. Due to this delay and our focus on how quickly diagnostic results can be attained after exposure, we did not include serology or antibody tests in our analysis.

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[36] "Rapid Influenza Diagnostic Tests," Centers for Disease Control and Prevention (CDC), accessed January 28, 2021, last updated October 25, 2016, https://www.cdc.gov/flu/professionals/diagnosis/clinician_guidance_ridt.htm.

[37] Chartrand et al., "Diagnostic Accuracy of Rapid Antigen Detection Tests," 3738.

[38] Benjamin D. Grant et al., "SARS-CoV-2 Coronavirus Nucleocapsid Antigen-Detecting Half-Strip Lateral Flow Assay Toward the Development of Point of Care Tests Using Commercially Available Reagents," *Analytical Chemistry* 92, no. 16 (2020): 11305, https://pubs.acs.org/doi/10.1021/acs.analchem.0c01975.

[39] "Rapid Influenza Diagnostic Tests," Centers for Disease Control and Prevention (CDC).

[40] M. Jana Broadhurst, Tim J. G. Brooks, and Nira R. Pollock, "Diagnosis of Ebola Virus Disease: Past, Present, and Future," *Clinical Microbiology Reviews* 29, no. 4 (October 2016): 774, doi:10.1128/CMR.00003-16; Peter M. Schneeberger et al., "Real-Time PCR with Serum Samples Is Indispensable for Early Diagnosis of Acute Q Fever," *Clinical and Vaccine Immunology* 17, no. 2 (February 2010): 289, doi:10.1128/CVI.00454-09.

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3. Diagnostics Concepts of Use within the DOD and Model Overview

Note: This work was originally completed and published in May 2021. [41]

The quantitative portion of our analysis uses a stochastic, individual-based model that simulates the collection and analysis of clinical specimens from individuals infected during a BW attack. The model tracks the disease progression of each individual and their movement through the theater medical system. Specimens are collected and analyzed based on user-specified triggers and the total number of true positive and false negative diagnostic results are stochastically determined based on the performance of a given test when analyzing specimens collected at various stages of the disease. Figure 2 shows an overview of this process.

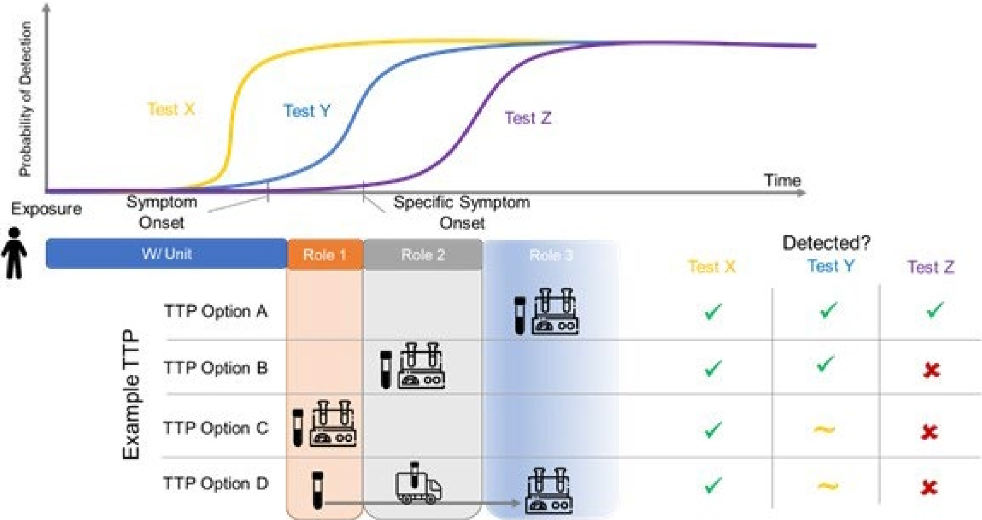

Figure 2. Schematic Depiction of the Interplay Between Diagnostic Probability of Detection Over the Course of Disease Progression and Sampling and Analysis TTP Utility

As shown in the top part of Figure 2, different tests for a given disease can have different test positivity rates along the course of disease. Our literature review provides that information for each disease-test combination that we could find. More information on the literature review is found in each of the disease-specific chapters (see Chapters 4 through

[41] This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

11). We then model different TTP options and determine whether a given test comes back positive or negative. Figure 2 shows four TTP options: a test taken at Role 3 (Option A), a test taken at Role 2 (Option B), a test taken at Role 1 (Option C), or a sample taken at Role 1 and then shipped to Role 3 (Option D). More information on the roles of care is provided in the next section. As shown, Test X has a high positivity rate along the whole symptom progression, so it provides positive results for all the illustrative TTP options. Test Y has a low positivity rate at the beginning of symptom progression, but that positivity rate increases as specific symptoms develop. Therefore, Test Y provides positive results for TTP options A and B, those taken later in the disease, but sometimes provides false negative results for TTP options C and D. Test Z has a low positivity rate until the very end of the symptom progression, so it only provides positive results for TTP option A. Although the results shown in Figure 2 are notional, the same logic applies for all the modeling used in this paper for specific disease-test-TTP combinations.

The model does not simulate the attack itself, but rather the user specifies a given number of individuals who were infected as a result of some attack. This approach allowed us to consider various adversary concepts of BW employment by parametrically varying the number of infected individuals. We modeled attacks that infected 1,000, 100, or 10 individuals. Human-to-human transmission of contagious diseases was not included in the analysis. In addition, the model does not account for the effect of BW defensive capabilities such as PEP and treatment. As a result, in a given simulation, all infected individuals become ill and progress through an untreated disease progression. The model uses discrete 0.1 day (2.4 hour) time steps; therefore, the timing of events are rounded accordingly.

The primary output of our model is the median time at which the first accurate positive diagnostic result is obtained.[42] In addition, our model tracks the number of casualties, severe casualties, and fatalities over time. Therefore, we can correlate the timing of the diagnostic results with the casualty metrics. The model enabled us to analyze how changing various components of the specimen collection and analysis process impacts when diagnostic results would be available following an attack. More information about the technical details of this model is found in Appendix E.

A. Roles of Care and Patient Movement

The military medical system is divided into four roles of care: Role 1 through Role 4.[43] In our paper, we assume that the attacks occur in a deployed force and that individuals

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[42] In the event that some trials do not produce any positive diagnostic results, the median is calculated from the set of trials in which at least one positive diagnostic result is generated.

[43] Joint Chiefs of Staff, *Joint Health Services,* Joint Publication 4-02 (Joint Chiefs of Staff, 11 December 2017, Incorporating Change 1, 28 September 2018), II-1, https://www.jcs.mil/Portals/36/Documents/Doctrine/pubs/jp4_02ch1.pdf.

progress through the roles of care accordingly, without skipping roles of care. Role 1 is often the first place that an individual would go to in the medical system when he or she became ill.[44] Role 1 provides unit-level medical care, including routine sick call. If an individual is sick enough to require more advanced care or is unable to immediately return to duty, he or she is evacuated to a higher role of care.[45] Role 2 provides advanced trauma management and further emergency medical care. Role 2 typically has limited holding capacity, so individuals requiring in-patient care and/or a recovery time longer than a few days (typically 3 days) would be moved to Role 3.[46] Role 3 is a theater hospital and has more capability to take care of inpatients. If an individual requires even longer medical care, he or she may be transferred to a Role 4 facility, which is an out-of-theater medical facility that can facilitate longer term care.[47] Since we focused our analysis on theater medical care, we did not include Role 4 in our analysis. Within this paper, we characterize Role 1 and 2 facilities as "forward" MTFs, with Role 1 being "farther forward."

Within our model, we assume that individuals move through roles of care based on the severity of their symptoms. We assume the medical system has unlimited capacity to transport and hold patients. We also only model one MTF facility at each role of care, even though for the larger attacks, individuals would likely report to multiple MTFs. Figure 3 shows the logic. The symptom severity for each disease was determined from Allied Medical Publication (AMedP)-7.5.[48] For those diseases that do not have symptom profiles characterized in AMedP-7.5 (EVD and influenza), we used the AMedP-7.5 methodology to create our own symptom profiles (explained further in Appendix E). As shown in Figure 3, within our model, an individual with mild or moderate initial symptoms reports to the Role 1 MTF for sick call and is treated as an outpatient. An individual who reports to the Role 1 MTF with severe or very severe symptoms is treated as an inpatient and is sent up to a Role 2 and then a Role 3 facility. This progression is consistent with AMedP-7.5 since "moderate" symptoms are defined as symptoms requiring outpatient care and "severe" symptoms are defined as symptoms requiring inpatient care.[49] "Mild" symptoms require only self-care, and "very severe" symptoms require intensive care.

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[44] Ibid.

[45] Ibid.

[46] Ibid.

[47] Ibid., II-3.

[48] North Atlantic Treaty Organization (NATO), *NATO Planning Guide for the Estimation of CBRN Casualties*, NATO Standard AMedP-7.5, Edition A Version 1 (NATO Standardization Office, October 2017), 1–10, https://nso.nato.int/nso/nsdd/main/standards/ap-details/1788/EN.

[49] Ibid.

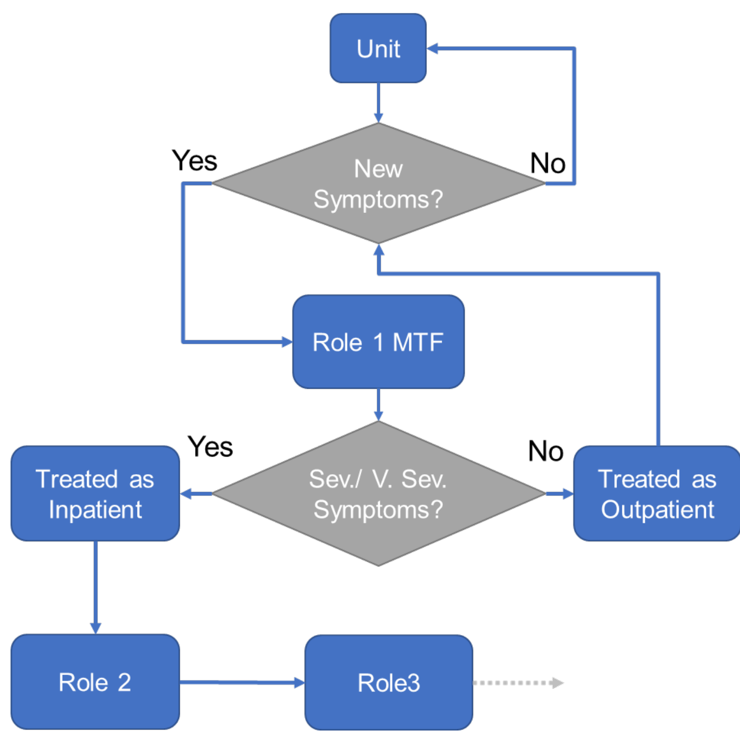

Figure 3. Model Logic for Patient Movement Through the Medical System

Within our model, we used distributions to determine the incubation period and durations of each symptom severity for each disease. The distributions were taken from AMedP-7.5,[50] with the exception of EVD and influenza. The distributions for those two diseases are explained further in Appendix E. In addition to the time that it takes to develop symptoms or more severe symptoms, the model builds in time spent at a given MTF and the time to transit from one role of care to another. Table 3 lists these parameters.

Table 3. Model Parameters for Time Spent at MTFs and Patient Movement

Parameter	Value
Movement from Unit to Role 1 plus time spent at Role 1 (Mild/Mod)	Constant- 2.4 hours[a]
Movement from Unit to Role 1 plus time spent at Role 1 plus movement to Role 2 (Sev./V. Sev.)	Uniform- 2–4 hours[b]
Time spent at Role 2 plus movement to Role 3	Uniform- 6–10 hours[b]

Note: (Mild/Mod) = patient has mild or moderate symptoms; (Sev./V. Sev.) = patient has severe or very severe symptoms

[a] Assumed to be a single time step as modeled.

[b] Based on subject-matter-expert judgment.

[50] Ibid., 5-18–5-95.

B. Diagnostic Systems and Roles of Care

Along with the capabilities for patient care, different roles of care have different capabilities for diagnostics. A Role 1 facility does not have laboratory capabilities, so diagnostic testing could be limited to rapid antigen test or a cartridge-based PCR test that requires no laboratory support, although these capabilities are not currently at Role 1.[51] A Role 2 facility only has limited laboratory capabilities, so it would also only be able to use a rapid antigen test, such as an influenza test, or a cartridge-based PCR test. A Role 3 facility could have more extensive laboratory capabilities and could sustain more complicated cartridge-based PCR tests or antigen tests, but it does not have the resources for complicated tests or procedures that require high BSL containment. DOD does maintain additional laboratory capabilities at overseas laboratory facilities and at USAMRIID, which can perform additional diagnostic methods, including bacterial or viral isolation procedures that may require BSL-3 or higher facilities.[52]

Currently, DOD uses the NGDS 1 system and its accompanying Warrior panel to identify BW agents in clinical specimens.[53] The Warrior panel tests for anthrax, tularemia, plague, Q fever, Ebola, and Marburg (see Table 1). NGDS 1 is a BioFire FilmArray 2.0 instrument, which is a cartridge-based PCR system.[54] An NGDS 2 system is currently in development for use as far forward as Role 1.[55] DOD has been working with the Cepheid GeneXpert system, which is a cartridge-based PCR system that is described as "man-portable."[56]

Within our model, we assume that each type of system could be employed at each role of care and that each system takes a certain amount of time to run. Although the time between different PCR or antigen systems can vary by disease and by technology, we aimed to keep them consistent. The time and resource requirements for PCR are based on a cartridge-based system, although some of the PCR data used within the model are from in-house assays, which may require more resources or time. We assume, however, that the

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[51] Joint Chiefs of Staff, *Joint Health Services,* Joint Publication 4-02, II-2.

[52] Burr et al., *Emerging Infectious Diseases Study*, 45.

[53] COL David Hammer, "Building the CBR Diagnostic Family of Systems" (briefing presented at the Pennsylvania State University OTA Symposium January 15–16, 2019, Joint Program Executive Office for Chemical, Biological, Radiological, and Nuclear Defense, 15 January 2019), 10, https://abrl.psu.edu/wp-content/uploads/2019/01/JPEO-PSU-OTA-Symposium_20190115_MCS-DX.pdf.

[54] Ibid.

[55] Joint Requirements Office for Chemical, Biological, Radiological, and Nuclear Defense, "NGDS 2 Biological CDD Update," briefing, July 2025, 5.

[56] Joint Program Executive Office for Chemical, Biological, Radiological, and Nuclear Defense, "JPM CBRN Medical COVID-19 Diagnostic Capability: Cepheid GeneXpert," accessed 23 September, 2025, https://www.jpeocbrnd.osd.mil/Portals/90/jpm-cbrnd-medical-cepheid-slick-sheet.pdf.

general performance of cartridge-based systems when analyzing specimens collected at various stages of a given disease is the same as that of an in-house system. We provide additional discussion on the limitations of this assumption and the details of which PCR assay is included for each disease in each of the applicable disease chapters.

In addition, the time to isolate bacteria or virus can vary by days depending on the disease (e.g., 2 to 3 days for plague and up to 10 days for Ebolavirus Disease).[57] Transport time to an out-of-theater laboratory or a Role 4 could vary based on the specific theater or other operational factors. In most instances, our modeling showed that the bacterial/viral isolation results came days after the results from other tests, so the exact amount of time that it takes to run these tests or ship the samples out of theater was largely inconsequential for our analysis. Therefore, we used 4 days as a median value for transporting and running the isolation assay, although it could take much longer or much shorter depending on the disease and situation/theater. Table 4 includes the values used within our model for all assays.

Table 4. Technology by Role of Care and Time to Produce Results, as Modeled

Technology	Roles of Care as Modeled	Time to Run Assay
PCR	Role 1, 2, or 3	2 hours
Antigen (rapid)	Role 1, 2, or 3	30 minutes
Antigen (laboratory)	Role 3	4 hours
Bacterial/viral isolation	Out-of-theater lab or Role 4	4 days[a]

[a] Includes time to ship the specimen to the laboratory.

1. Specimen Shipment

In most situations, a diagnostic test at a given MTF will likely be used only to test individuals currently being cared for at that MTF. In some situations, however, a specimen may be collected at one MTF and then shipped to another for analysis. Current doctrine states that for chemical, biological, radiological, or nuclear (CBRN) exposures, where time is of the essence, “expedited shipment and processing of environmental samples and clinical specimens within the AOR [area of responsibility] and, if needed, outside of it, is crucial to effective treatment and medical recovery of force personnel. JFS [Joint Force Surgeon] in cooperation with the component command surgeons should therefore maintain a unified tracking system within their OAs [operational areas] that allows medical personnel to quickly identify the closest in-theater laboratory with appropriate analytical

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[57] USAMRIID, *Medical Management of Biological Casualties Handbook*, 54, 102.

capabilities for routing specimens and samples."[58] Shipment of samples may be especially helpful for diseases such as Q fever, which does not produce severe symptoms requiring hospitalization but may need to be diagnosed with a system only located at the Role 3 or higher.

Because of this guidance, we included specimen shipment within our model. Specimen shipment was modeled by assuming that a specimen was collected at a role of care and then, at a specified frequency, shipped to a higher role of care for analysis. We assumed that the timing of the first specimen transport was determined from a uniform distribution from 0 to 12 hours after the attack and then all specimen transports afterwards were at 12-hour intervals from the first specimen transport time. We analyzed a scenario that involved shipping specimens collected at a Role 1 MTF for analysis at a Role 3 MTF and an alternative scenario that entailed shipping specimens collected in the theater medical system for analysis at an out-of-theater laboratory or Role 4 facility. Shipment time is assumed to be a uniform distribution of 5–8 hours from a Role 1 to a Role 3. For shipment outside of theater, the shipment time is accounted for in the time it takes to run the diagnostic test (see Table 4).

C. Triggers for Specimen Collection

A diagnostic system is only beneficial if a clinician has a trigger to use that system. Individuals entering the MTF—even a larger-than-normal number of individuals—with moderate, non-specific symptoms may not necessarily trigger the use of diagnostics for BW agents. We included influenza in our analysis along with BW agents since clinicians would likely first rule out influenza before progressing to using diagnostics for BW agents.

Within our model, we included three different triggers for specimen collection and use of a diagnostic modality. The first trigger was the onset of moderate, non-specific symptoms. This trigger would be the least likely to occur in most settings, although the use of diagnostics at the first sign of symptoms could be more likely if local intelligence suggested that a biological attack was likely. The second trigger was the onset of disease-specific symptoms. The disease-specific symptoms could include more severe symptoms requiring inpatient care and symptoms that could provide an indication of the disease (e.g., coughing up blood and rapid onset of severe symptoms in the case of plague). The third trigger was the occurrence of 10 or more individuals with the same symptoms, which could provide an indication that a BW attack had occurred. As noted in Section 3.B, unless specimens are shipped, they may not be taken until an individual has reported to the MTF where the diagnostic system is located. For example, if a diagnostic test is located at a

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[58] Joint Chiefs of Staff, *Joint Health Services,* Joint Publication 4-02, V-17.

Role 3 MTF, then specimen collection and subsequent analysis with that test may not occur until the patient has arrived at that facility unless the specimen is shipped beforehand.

Within our model, we did not address combining multiple TTP options. However, our modeling results can still illustrate the effect of employing multiple types of tests or testing at multiple locations. For example, consider a TTP option in which individuals who receive a negative test at a forward role of care are tested again at a higher role of care. In this case, the timing of the first positive diagnostic result can be determined by comparing the timing of the first positive result from the TTP option involving only far-forward testing to the timing of the first positive result from the TTP option involving only testing at a higher role of care. The timing of the first positive result from the combined TTP option would be the earlier of the times of the first positive results from the two constituent TTP options.

If two tests were done in sequence at a given facility (e.g., a rapid antigen influenza test before a PCR test for BW agents at a Role 1 MTF), then the timing of positive results from the second test would need to be delayed by the amount of time that it takes to run the first test.

In the model, specimens are collected and analyzed from all infected individuals who meet the specimen collection trigger. For example, if the specimen collection trigger is the onset of moderate, non-specific symptoms at a Role 1 MTF, then the model will simulate the collection of a specimen from every individual when he or she reports for sick call. The model, however, does not include post-mortem testing, so individuals who die before being tested are never tested. Furthermore, the model simulates the analysis of these specimens without regard to any specimen analysis capacity at a given location. While this limitation should not impact the timing of the first diagnostic result following an attack, it will impact the timing of subsequent diagnostic results that, in reality, may require queuing before analysis.

D. Summary of Data Availability

For most BW agents, blood and sometimes sputum would be the most commonly used specimen types. Respiratory diseases, such as influenza, may require throat or nasal swabs instead. Other specimen types, such as urine, are used less frequently for these diseases. As discussed in later chapters, we could not locate scientific literature that described test probabilities of detection over the course of symptom progression (which is relative to quantities of pathogen indicator in a specimen) for many combinations of diagnostic system, disease, and specimen type. The lack of this data meant that we could not quantify how the probability of detection of a given diagnostic would change depending on when during the course of disease a specimen was collected. The lack of sufficient data constrained what we were able to quantitatively analyze and ultimately the extent of the conclusions we could draw. Figure 4 shows the combinations of disease, specimen type,

and diagnostic technologies for which we could obtain sufficient data to model (blue) and those combinations for which we could not obtain the requisite data (orange).

Disease	Specimen Type	Bacterial / Viral / Toxin Isolation	PCR (In-House)	PCR (Cartridge)	Antigen (ELISA / Mass Spec)	Antigen (Rapid)
Plague	Blood					
	Sputum					
Q Fever	Blood					
Tularemia	Blood					
	Sputum					
Ebola	Blood					
VEE	Blood					
Anthrax	Blood					
SEB	Blood					
Influenza	TNS					
	NPA					

Sufficient Test Results Data Found

Insufficient Test Results Data Found

Test Does not Exist

Human Data

Non-Human Primate Data

Note: The abbreviations used in this figure are defined in Appendix E of this paper.

Note: This work was originally completed and published in May 2021.

Figure 4. Availability of Diagnostic Test Results

In Figure 4, combinations shown in gray are those which do not have tests available (e.g., we could not find any rapid antigen tests available for Venezuelan Equine Encephalitis Virus (VEEV) disease). A further discussion of the conclusions based on this figure is provided in Chapter 12. As also shown in Figure 4, much of the information we found was on non-human primates (NHPs), and we often had to generalize from a single test (often in-house) to cartridge-based tests, which may not use the same gene/antigen targets or which may have different probabilities of detection. Therefore, the results of our analysis may not be able to be used in decisions related to basis of issue plans of or procedures for specific DOD systems. The results, however, do show trends for what basis of issue may be more or less useful depending on the probability of detection over symptom progression and disease characteristics. In addition, the model described in this paper can be used to inform basis of issue plans and procedures for specific DOD systems if data for those specific systems become available at a later point.

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4. Plague

Note: This work was originally completed and published in May 2021. [59]

A. Plague Symptom Progression

Pneumonic plague can occur after an individual inhales *Yersinia pestis*. It is a fast-moving, two-stage disease.[60] The first stage is characterized by moderate, non-specific symptoms and lasts approximately 1 day. The second stage, also lasting approximately 1 day, is characterized by a bloody cough and progressive pneumonia, leading to death in 100% of people if untreated.[61]

We assume that individuals first arrive at a Role 1 MTF with moderate, non-specific symptoms and later progress to a Role 3 or higher MTF when the second, more severe stage of disease begins. This progression is consistent with the symptom profile given in AMedP-7.5.[62]

B. Plague Diagnostic Types

Plague can be diagnosed via antigen test, PCR, or bacterial isolation using blood or deep respiratory secretion/sputum specimens.[63] Deep respiratory secretions or sputum can be difficult to obtain early in the disease since individuals are coughing less and sometimes the specimens can be too viscous to easily handle and test, especially with rapid tests.[64] Although sputum specimens have been used in pneumonic plague outbreaks previously,[65]

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[59] This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

[60] Jacob L. Kool, “Risk of Person-to-Person Transmission of Pneumonic Plague,” *Clinical Infectious Diseases* 40, no. 8 (April 15, 2005): 1167, https://doi.org/10.1086/428617.

[61] Ibid.

[62] NATO, *AMedP-7.5*, 5-57–5-58.

[63] Christian E. Demeure et al., “*Yersinia pestis* and Plague: An Updated View on Evolution, Virulence Determinants, Immune Subversion, Vaccination, and Diagnostics,” *Genes & Immunity* 20 (2019): 363–365, https://www.nature.com/articles/s41435-019-0065-0.

[64] Ibid., 365.

[65] Rindra Randremanana et al., “Epidemiological Characteristics of an Urban Plague Epidemic in Madagascar, August–November, 2017: An Outbreak Report,” *The Lancet Infectious Diseases* 19, no. 5 (May 01, 2019): 539, https://doi.org/10.1016/S1473-3099(18)30730-8.

we could not find sufficient information on the identification of plague in sputum over the disease progression, so we could not include sputum specimens in our modeling.

Bacterial isolation is the gold standard for plague diagnosis, but it can take up to 4 days for the bacteria to be cultured, plus transport time.[66] In addition, *Y. pestis* must be handled in contained conditions, so the bacterial culture must be done at a laboratory under BSL-3 conditions rather than at a Role 3 or lower facility.

PCR tests for plague can amplify one or more genes of interest from *Y. pestis*. Tests for multiple genes are often used because single gene tests typically cannot differentiate between *Y. pestis* and *Yersinia pseudotuberculosis*, a closely related bacterium that can also cause human disease.[67] *Y. pestis* is also included on the Warrior panel for the NGDS, and blood or sputum specimens can be used to diagnose plague on the system.[68]

Rapid and laboratory antigen tests have been developed to diagnose plague. Most antigen tests detect the F1 antigen, although tests for other antigens have also been developed.[69] Rapid antigen tests, some of which are commercially available, are typically dipstick tests that test for the F1 antigen.[70] They can have higher false positive or false negative rates than other diagnostic methods but are easier to use.[71] Laboratory antigen tests have also been developed but require more extensive equipment.[72] Few of those tests have been commercially developed.

World Health Organization (WHO) guidance states that if plague is suspected, the patient should have diagnostic specimens taken but should receive antibiotics before

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[66] World Health Organization, *Interregional Meeting on Prevention and Control of Plague*, Antananarivo, Madagascar, 1–11 April 2006, WHO/HSE/EPR/2008.3 (World Health Organization, 2008), 5, https://www.who.int/csr/resources/publications/WHO_HSE_EPR_2008_3w.pdf.

[67] Alvin Stewart et al., “A Quadruplex Real-Time PCR Assay for the Detection of *Yersinia pestis* and Its Plasmids,” *Journal of Medical Microbiology* 57, Pt. 3 (March 2008): 324, doi:10.1099/jmm.0.47485-0.

[68] Brian Taylor, *Evaluation of the NGDS Warrior Panel Using Synthetic Material on the FilmArray*, HT-15-1099 (Battelle Memorial Institute, May 2016), 1.

[69] Yehuda Flashner et al., “The Search for Early Markers of Plague: Evidence for Accumulation of Soluble *Yersinia pestis* LcrV in Bubonic and Pneumonic Mouse Models of Disease,” *FEMS Immunology & Medical Microbiology* 59, no. 2 (July 1, 2010): 197, https://doi.org/10.1111/j.1574-695X.2010.00687.x.

[70] Minoarisoa Rajerison et al., “Performance of Plague Rapid Diagnostic Test Compared to Bacteriology: A Retrospective Analysis of the Data Collected in Madagascar,” *BMC Infectious Diseases* 20, article number 90 (2020): 6 pp, https://doi.org/10.1186/s12879-020-4812-7.

[71] H. Tomaso et al., “Comparison of Hand-Held Test Kits, Immunofluorescence Microscopy, Enzyme-Linked Immunosorbent Assay, and Flow Cytometric Analysis for Rapid Presumptive Identification of *Yersinia pestis*,” *Journal of Clinical Microbiology* 45, no. 10 (October 2007): 3405, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2045319/.

[72] Jim Aiken, *Next Generation Diagnostic System-Bio: Scientific Technical Information (STI) Relevance and Gap Analysis* (Battelle Memorial Institute, August 2015), 29.

definitive diagnostic results can be obtained.[73] This regimen is followed because pneumonic plague is a fast-moving disease, and antibiotics should be given as soon as possible to prevent death. This WHO guidance also means that treatment could be provided before the diagnostic result is finalized, especially for a patient whose symptoms begin after a plague outbreak is known or suspected.

C. Probability of Detection Over the Course of the Disease

Two papers described the diagnostic accuracy over time and tied them to symptom progression. One paper looked at the results of bacterial isolation tests over time in African Green monkeys[74] and the other looked at the results of bacterial isolation tests and PCR over time in cynomolgus monkeys.[75] Both papers looked only at whole blood specimens. Previous analysis has found that African Green monkeys are extremely sensitive to *Y. pestis* and may not be a good model animal.[76] Due to that finding and the fact that the two papers had similar results (but Koster et al. included two forms of diagnosis), we used the data in Koster et al.

Koster et al. exposed 12 monkeys to *Y. pestis* via aerosol and evaluated their symptom progression over time.[77] Three monkeys were sacrificed every 24 hours and quantitative bacterial cultures of whole blood and different organs were performed. All monkeys developed symptoms 2 to 3 days after infection.[78] The authors also used an in-house PCR test amplifying a single gene, which gave positive results slightly earlier in the symptom progression than bacterial isolation did. Both tests detected infection only after symptom onset.[79] Given the available data, we characterized the probability of detection for the combinations of technology, specimen, and stage of disease, as shown in Table 5. To translate the results in monkeys to the human progression of disease, we used logistic regression techniques as described in Appendix E.

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[73] World Health Organization, *Interregional Meeting on Prevention and Control of Plague*, 48.

[74] R. Colby Layton et al., "Primary Pneumonic Plague in the African Green Monkey as a Model for Treatment Efficacy Evaluation," *Journal of Medical Primatology* 40, no. 1 (February 2011): 6–17, doi:10.1111/j.1600-0684.2010.00443.x.

[75] Koster et al., "Milestones in Progression of Primary Pneumonic Plague," 2946–2955.

[76] North Atlantic Treaty Organization (NATO), *Technical Reference Manual NATO Planning Guide for the Estimation of CBRN Casualties*, NATO Standards-Related Document AMedP-7.5-1, Edition A, Version 1 (NATO Standardization Office, January 2018), 23-2, https://nso.nato.int/nso/nsdd/main/standards/srd-details/91/EN.

[77] Koster et al., "Milestones in Progression of Primary Pneumonic Plague," 2946.

[78] Ibid.

[79] Ibid., 2948.

Table 5. Plague Diagnostic Probabilities of Detection Included in Modeling Analysis

Technology (Specimen)	Stage of Disease	Probability of Detection
In-house PCR (blood)	Symptom Onset	5%
	Onset of Stage 2	100%
Bacterial isolation (blood)	Symptom Onset	5%
	Onset of Stage 2	40%

D. Modeling Analysis

As described in Table 5, we were able to locate sufficient data to model two plague diagnostics technologies: an in-house PCR and bacterial isolation. Our quantitative analysis of these technologies focused primarily on the timing of positive results from a PCR test under various clinical sampling TTPs. The actual performance of currently or potentially fielded PCR technologies may differ from that of the in-house PCR test for which we could obtain the requisite data for our model. Accordingly, the quantitative results presented here should be interpreted as illustrating general trends in how various TTP options affect the timing of diagnostic results and not as precise predictive forecasts of the performance of any specific PCR capability.

The modeling analysis of plague specifically focused on how the following TTP components impact the timing of diagnostic results following an attack:

- The location of PCR technology in the theater medical system,
- The stage of the disease at which a specimen would be taken,
- Delaying the collection of clinical specimens until multiple individuals report to the medical system following an attack,
- Shipping specimens taken at a lower role of care for testing at a higher role of care, and
- Use of bacterial isolation at a reachback laboratory.

In addition to determining how different TTP components impact the timing of diagnostic results following the attack, we also looked at what information would be available from syndromic indicators by the time that diagnostic results can be obtained.

Table 6 lists the specific TTP options we modeled for plague and the corresponding median time of the first positive diagnostic result. We simulated attacks that infect either 10, 100, or 1,000 individuals. Figure 5 shows a representative time progression of plague cases by severity level following an attack as well as the median time of the first positive diagnostic result for the TTP options shown in Table 6.

Table 6. Median Time (Days) of First True Positive Diagnostic Result Following a Plague Attack for Various Sampling and Analysis TTP Options

Option	Technology (Specimen)	Test Location	Specimen Collection Trigger	Median Time of First True Positive Result (Days)		
				10 Infections	100 Infections	1,000 Infections
A	In-house PCR (blood)	Role 3 MTF	Patient arrival at Role 3 MTF	3.8	3.0	2.7
B	In-house PCR (blood)	Role 2 MTF	Patient arrival at Role 2 MTF	3.4	2.7	2.3
C	In-house PCR (blood)	Role 1 MTF	Patient arrival at Role 1 MTF with severe symptoms	3.3	2.6	2.2
D	In-house PCR (blood)	Role 1 MTF	Patient arrival at Role 1 MTF with any symptoms	3.6	2.7	1.7
E	In-house PCR (blood)	Role 1 MTF	Patient arrival at Role 1 MTF with any symptoms; start sampling on the tenth case	7.2	3.0	1.9
F	In-house PCR (blood)	Role 3 MTF	Patient arrival at Role 1 MTF with any symptoms; specimens shipped from Role 1 MTF to Role 3 MTF every 12 hours	4.2	3.1	2.2
G	Bacterial isolation (blood)	Laboratory	Patient arrival at Role 3 MTF	8.3	7.2	6.7

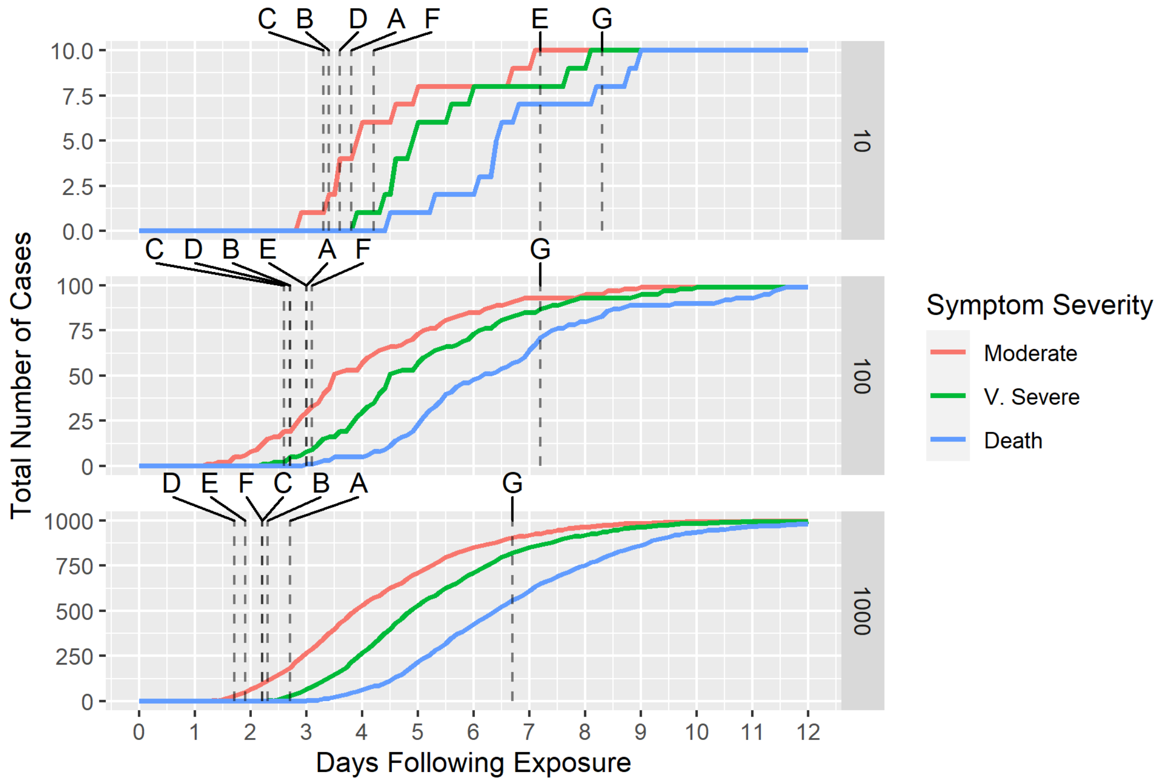

Figure 5. Representative Total Numbers of Plague Cases by Severity Level over Time for Attacks That Infect 10, 100, or 1,000 Individuals and Median Time of First Positive Diagnostic Result for TTP Options Listed in Table 6 (Vertical Dashed Lines)

1. Location of the PCR Technology in the Theater Medical System

We compared TTP options A, B, and C to analyze how the location of a PCR test within the medical system impacts the timing of the first positive results. For all three of these TTP options, specimens are obtained from individuals with severe symptoms when they reach the MTF at which the PCR test is located. As shown in Table 6, our modeling demonstrates that running a PCR test on severe plague patients at a Role 1 MTF will generate positive results approximately half a day sooner than testing patients when they arrive at a Role 3 MTF. The difference in the timing of the first positive result between TTP option A and either B or C is directly proportional to the time it takes to treat and move the patient though the roles of medical system. The specific results shown in Table 6 reflect our choice in modeling parameters to describe patient movement. If theater patient movement is faster or slower than what we modeled, then the timing of diagnostic results would shift accordingly.

2. Disease Stage at Which a Specimen Is Collected

We compared TTP options C and D to illustrate the impact of collecting specimens from individuals at symptom onset as compared to waiting for the onset of the more severe and specific symptoms associated with the second stage of plague. Even though the onset

of stage 1 symptoms occurs 1 day before the onset of the severe stage two symptoms, the timing of the first positive diagnostic result is not hastened by the same amount of time. As shown in Table 6, for the attack that caused 1,000 infections, collecting the specimen from individuals at symptom onset—as compared to the onset of more severe symptoms—hastened the time of the first positive diagnostic result from a PCR at the Role 1 by 0.5 days. However, for attacks that caused 10 or 100 infections, testing specimens taken from individuals at symptom onset delays the median time of the first positive diagnostic result. In other words, even though specimens are being taken *earlier*, the median time of the first positive result is occurring *later*. This counter-intuitive result is due to the low PCR probability of detection for specimens taken at symptom onset.

As shown in Table 5, the probability of detection of the in-house PCR for which we could obtain sufficient data to model is much lower when analyzing a blood specimen taken at symptom onset (5%) as compared to the same specimen taken later in the disease progression (100%). Accordingly, analyzing specimens taken at symptom onset can generate multiple false negative results before the first true positive result. In the case of the specific PCR technology that we modeled, the 5% probability of detection results in an average of 19 false negatives for every 1 true positive. The delay in obtaining the first positive result caused by these false negatives depends on the number of times the test is run. The high rate of individuals reporting to the medical system following a large attack can provide the opportunity to test large numbers of individuals, thus increasing the chances of getting a positive result even with a low probability of detection. However, if testing for plague is halted after repeated negative results or testing is delayed because the Role 1 MTF only has the capability to analyze a limited number of specimens, a positive result may be delayed until patients are retested at another location.

The specific performance of the in-house PCR test for which we could obtain sufficient data for inclusion in our model may or may not represent the performance of other PCR tests. That being said, the counterintuitive phenomenon of earlier specimen collection resulting in delayed diagnostic results could occur with any test that has a sufficiently low probability of detection during the early stages of the disease. Therefore, knowledge of a test's probability of detection at various stages of a disease is critical in developing clinical sampling and analysis TTPs and interpreting the results generated from such TTPs.

3. Delaying the Collection of Clinical Specimens until Multiple Cases Report

Given the generic non-specific early symptoms of plague, it is possible that medical personnel would not test for plague during the first stage of the disease even if a test was available. We compared TTP option E to other options to analyze the timing of positive diagnostic results associated with delaying collecting specimens until 10 individuals infected during the attack report to the medical system. As shown in Table 6, this TTP

option resulted in a median time of the first positive results of 7.2, 3.0, and 1.9 days for attacks that cause 10, 100, or 1,000 infections, respectively. When compared to testing the first patient who reports to the medical system (TTP option D), delaying collecting specimens until the tenth case delays the median time of the first positive result from a PCR at a Role 1 by 0.2, 0.3, and 3.6 days for the attacks that caused 1,000, 100, and 10 infections, respectively.

For attacks that generate substantial numbers of initial infections, large numbers of individuals will likely develop symptoms within a short time of each other. Therefore, the delay due to waiting for multiple individuals to report to the medical system is small. However, if the attack only infects a smaller number of individuals, then the timing of symptom onset for the first few individuals is more likely to be spread out over a longer period of time. As a result, the delay due to waiting for multiple individuals to report to the medical system can become longer than if the attack infected a larger number of individuals. In such a case, the first positive diagnostic result may come from tests at a higher role of care or the onset of severe, disease-specific symptoms instead of a PCR capability at a Role 1 MTF.

4. Shipping Specimens Taken at Lower Roles of Care for Testing at Higher Roles of Care

To analyze the impact of specimen shipping, we simulated the collection of specimens at symptom onset at a Role 1 MTF that were analyzed with a PCR test at a Role 3 MTF (TTP option F in Table 6) and compared it to other options. When compared to testing specimens taken at symptom onset with a collocated test (TTP option D), shipping those same specimens delays the timing of the first positive result. The extent to which the results are delayed depends on how frequently specimens are shipped and the transportation time. Given our choice of model parameters, collecting specimens at symptom onset at a Role 1 MTF and shipping them to a Role 3 MTF—as compared to analyzing the specimens with a collocated test—delays the median time of the first positive result by approximately half a day. If the frequency with which specimens are shipped or the time it takes to transport those specimens is different than what we modeled, then the delay will be shifted accordingly.

For the simulated attack that infects 1,000 individuals, collecting specimens at symptom onset and shipping them for analysis at a Role 3 MTF (TTP option F) provides diagnostic results sooner than analyzing a specimen collected from the patient when they arrive at the Role 3 (TTP option A). However, for the simulated attacks that only infected 10 or 100 individuals, waiting for the arrival of the patient at the Role 3 (TTP option A) is the faster option. Again, this counterintuitive result that delaying the collection of a specimen can, in some circumstances, result in diagnostic results sooner is attributed to the low probability of detection at symptom onset for the PCR test under consideration.

Even though the specimens are being analyzed at a higher role of care, the bacterial load of the specimens still reflect that of the individuals at the time the specimen was collected. In other words, if a diagnostic test has a low probability of detection when analyzing specimens collected at symptom onset, it will exhibit that same performance regardless of where the test is physically located.

Shipping specimens from lower to higher roles of care eliminates the need to field, staff, maintain, and operate diagnostic systems at forward MTFs, although it would likely have additional packing and documentation requirements to ship samples appropriately. Instead, shipping specimens requires the logistical capability to package and transport specimens. Dedicated and routine transportation assets would likely reduce the time that it takes to move specimens through the theater but would require converting existing assets or fielding additional capabilities. Using ad-hoc or opportunistic transportation methods to move specimens through the theater would reduce the need for specialized assets but could potentially introduce significant delays if such opportunities are not frequent or predictable.

5. Bacterial Isolation at Reachback Laboratory

The final TTP option that we modeled for plague involves the use of bacterial isolation at a reachback laboratory (TTP option G in Table 6). Our modeling results indicate that these diagnostic results would not be available until about a week after the attack (see Table 6). As shown in Figure 5, nearly all infected individuals will have developed symptoms by the time bacterial isolation results are available. Even if substantially delayed relative to in-theater tests and therefore not the *only* test one would use, testing at a reachback laboratory—whether bacterial isolation or some other test such as genetic sequencing—can serve as gold-standard confirmatory test and provide more detailed analyses (e.g., identifying disease strain).

6. Syndromic Indicators

In addition to the results of diagnostic technologies, awareness of an attack can also come from the observation of unusual numbers of individuals reporting to the medical system. Table 7 shows representative timing of when certain numbers of total individuals with a given symptom severity would occur following the attack. For example, with 10 initial infections, 3 individuals develop very severe symptoms by 4.1 days after the attack. As shown in Table 7, the occurrence of at least 10 cases with symptoms and individuals with very severe symptoms occurs before or about the same time as the first positive diagnostic result for attacks that caused 100 or 1,000 infections. The first individual develops very severe symptoms by 3.2, 2.5, or 1.9 days after the attack for attacks that caused 10, 100, or 1,000 initial infections, respectively (see Table 7). In contrast the fastest diagnostic result across all TTP options produces the first positive by 3.3, 2.6, or 1.7 days after the attack for attacks that caused 10, 100, or 1,000 initial

infections, respectively (see Table 6). Thus, the onset of disease-specific, very severe symptoms, such as coughing up blood, may provide an earlier indication to clinicians that the causative agent is plague than using an actual diagnostic system, given the assumptions within our model.

Table 7. Representative Time (Days) When Various Numbers of Total Cases by Severity Level Occur

		Time of Total Number of Cases by Severity Level								
		10 Infections			100 Infections			1,000 Infections		
	Total Number of Cases	1	3	5	1	5	10	1	10	50
Symptom Severity	Moderate	2.2	3.1	4.4	1.5	2.2	2.6	0.9	1.5	2.0
	Very Severe	3.2	4.1	5.4	2.5	3.2	3.6	1.9	2.5	3.0
	Death	5.1	5.5	6.7	3.6	4.2	4.4	2.3	3.3	4.1

While the generic early symptoms of plague may not lead medical personnel to specifically suspect plague, the sudden appearance of numerous individuals with the same symptoms suggests the occurrence of a biological event. The rapid progression from moderate disease to the disease's very severe life-threatening symptoms is fairly unique to plague. When taken together with the large number of individuals with similar symptoms, the first few cases of very severe disease and potentially death suggest a large-scale plague exposure event. Even though these syndromic indicators may lack the confidence that diagnostic tests provide, they may be the first indication that an attack occurred and can trigger the initial decision and response processes.

E. Implications for Response Implementation

One of the principal responses to a plague attack is the administration of PEP. In general, PEP is effective at preventing casualties when administered to individuals before symptom onset.[80] Therefore, we can look at the number of individuals in the infected population who have not yet developed symptoms as a metric to generally assess the timeliness of the diagnostic results produced by a given TTP option. As shown in Figure 5, all the assessed TTP options involving PCR can provide a positive result before the onset of symptoms in the majority of the infected population when 100 or 1,000 individuals are initially infected. However, a significant portion of casualties occur within a few days of the first diagnostic results. Therefore, if multiple days are required for the response decision process or to administer the countermeasure, then the portion of infected individuals who receive PEP before developing symptoms may be significantly reduced.

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[80] NATO, *AMedP-7.5*, 5-58.

To illustrate this point, consider the use of a PCR test at a Role 3 MTF (TTP option A) with 1,000 infected individuals. Our modeling shows that at the time of the first positive diagnostic result (day 2.7), 171 of the 1,000 infected individuals have developed symptoms. If PEP was immediately administered at this time, 829 of the 1,000 infected individuals would receive PEP before the onset of their symptoms. However, if the administration of PEP is delayed by 1, 3, or 5 days, then the number of individuals who could receive PEP before the onset of their symptoms drops to 550, 176, or 50, respectively.

Although not accounted for in our model, plague is a contagious disease and responding to a plague attack will likely include efforts to limit transmission. Previous Institute for Defense Analyses (IDA) analyses found that the administration of PEP is a highly effective method of preventing secondary plague casualties.[81] In addition to PEP, other response measures such as ROM can also be implemented. Previous IDA analysis found that a contagious disease outbreak resulting from a BW attack can spread to multiple locations within a theater in a few days.[82] Therefore, the sampling and analysis TTP options that we modeled may not provide sufficient early warning to implement an ROM protocol that contains the outbreak to one location. Additional discussion of the timeliness and effectiveness of various contagious disease response measures can be found in IDA paper P-10877.[83]

F. Evaluation

Plague produces early moderate non-specific symptoms that rapidly progress to life-threatening very severe pneumonia and death in untreated individuals. Diagnosing plague during the early stage of the disease would likely require additional indicators beyond symptom presentation. Such indicators could include the observation of numerous individuals with similar symptom presentation or a specimen collection and analysis TTP that involves analyzing specimen collected from individuals with moderate symptoms on a test that can detect plague. Otherwise, accurate diagnosis of individuals would likely be delayed until the onset of the very severe symptoms that occur 1 day after initial symptom onset.

In general, the various PCR-based TTP options that we modeled resulted in similar timing of the first positive diagnostic result, except when there were only 10 initial infections. This similar timing of first positive results is driven by two factors. First, the PCR test that we modeled has very low test probability of detection when analyzing

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81 Burr et al., *Emerging Infectious Diseases Study*, vi.

82 Ibid., 92.

83 Julia K. Burr et al., *Controlling the Spread of Contagious Disease in an Operational Environment*, IDA Paper P-10877 (Institute for Defense Analyses, February 2020), https://apps.dtic.mil/sti/citations/AD1116631.

specimens collected at symptom onset. Second, plague has a very rapid progression from its early moderate symptoms to very severe symptoms. Our modeling suggests early far-forward sampling TTPs provide limited early warning compared to more conventional sampling and analysis TTPs that involve testing at higher roles of medical care. The rapid progression of illness may mean that small periods of time could influence how many people could be saved by PEP/treatment, but those effects may be eclipsed by the time that it takes to implement mitigation procedures after receiving the diagnostic result. Sampling and analysis TTP components that mitigate the operational burden of early testing (i.e., only sampling after observing numerous cases or shipping specimens for analysis in a centralized location in the theater) can delay the timing of diagnostic results.

The exact modeling results presented here are driven by the performance of the in-house PCR that we simulated. A PCR test with a higher probability of detection when analyzing specimens collected at symptom onset may improve the timing of diagnostic results from TTPs that involve testing at symptom onset, although we do not have information on diagnostic systems available to the DOD to determine whether they have a higher probability of detection than the one modeled. Any potential improvements in diagnostic result timeliness from such TTPs will be limited by the rapid progression of plague. Waiting for the onset of severe symptoms before collecting a specimen for analysis will only delay diagnostic results by about a day. Therefore, TTP options that only test individuals with severe symptoms may result in minimal delays, especially if DOD diagnostic systems have a low positivity rate at symptom onset, although making specific recommendations at this time on the best TTP options for plague is impossible without more information.

5. Q Fever

Note: This work was originally completed and published in May 2021. [84]

A. Q Fever Symptom Progression

Q fever is a disease caused by the bacterium *Coxiella burnetii*. Normally, the disease is zoonotic, transmitted by infected sheep or goats, but *C. burnetii* is also listed as a Category B bioterror agent.[85] Q fever produces moderate non-specific symptoms, which makes it difficult to diagnose or to distinguish from more common diseases.[86] In most individuals, the disease resolves on its own within 1–3 weeks, but, in a few cases, especially in those with previous heart conditions, chronic Q fever can occur.[87]

Within our model, we assume that Q fever only produces non-specific, moderate symptoms and does not have a second stage of specific symptoms. This progression is consistent with the symptom profile given in AMedP-7.5.[88] Therefore, we assume that individuals only arrive at a Role 1 MTF and never progress to higher roles of care. Specimens from the individual, however, could be shipped to a higher role of care or a specialized diagnostics laboratory in theater or in the continental United States.

B. Q Fever Diagnostic Types

Since Q fever only produces moderate, generic symptoms, epidemiological factors are usually required to know to test for the disease. Epidemiological factors from naturally occurring Q fever include contact with livestock or living near areas known to have Q fever outbreaks among livestock. The two major types of tests used to diagnose Q fever are PCR tests and serology.[89] Both tests use whole blood or, occasionally, serum specimens.

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84 This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

85 Marcus Panning et al., "High Throughput Detection of *Coxiella burnetii* by Real-Time PCR with Internal Control System and Automated DNA Preparation," *BMC Microbiology* 8 (19 May 2008): 1, https://doi.org/10.1186/1471-2180-8-77.

86 Alicia Anderson et al., "Diagnosis and Management of Q Fever – United States, 2013: Recommendations from CDC and the Q Fever Working Group," *Morbidity and Mortality Weekly Report (MMWR)* 62 (RR-03) (29 March 2013): 1, https://pubmed.ncbi.nlm.nih.gov/23535757/.

87 Ibid., 2.

88 NATO, *AMedP-7.5*, 5–63.

89 Anderson et al., "Diagnosis and Management of Q Fever- United States, 2013," 9.

Therefore, only whole blood specimens are modeled in our paper. Serology tests detect antibodies, which typically do not appear until a week or more after the onset of symptoms. Serology tests also cannot differentiate between an acute infection or a past infection. Therefore, serology tests are not recommended for determining acute Q fever infection and are not modeled within our paper (see Section 2.D).[90]

PCR tests can detect bacterial DNA earlier in the disease progression than serology tests. Several PCR tests have been developed using different gene targets, although only a few of these tests are available commercially.[91] Q fever is included on the Warrior Panel for NGDS 1 (see Table 1). No antigen tests have been developed for Q fever, and bacterial isolation is not recommended because the bacteria are highly infectious and the culturing process for *C. burnetii* is difficult and time consuming.[92]

C. Probability of Detection Over the Course of the Disease

We located three papers that discuss the positive and negative PCR results over time compared to symptom progression. Two of these papers looked at human exposures, while the third used NHP exposures. One difficulty with using the two papers on human exposures was that most of the specimens were taken from individuals late in the disease progression, and therefore the results focused more on serology results than on PCR results. In addition, many people were given antibiotics before their specimen was taken, which could lead to a negative PCR result even with an active Q fever infection. Turra et al. tested 27 individuals with Q fever for serology and PCR.[93] Schneeberger et al. tested the sera of 65 patients with acute Q fever for serology and PCR.[94] Neither study was relevant to our efforts because most results focused on the later portion of the disease.

One study using cynomolgus monkeys was selected to form the basis of our Q fever model.[95] In this study, 10 monkeys were exposed to *C. burnetii* via aerosol, and their symptom progression was evaluated over time along with regular blood specimens. All the monkeys developed symptoms 6 days after infection. PCR tests were performed with an in-house assay using one of two genes: *IS1111* or *com1*. All the monkeys tested for *IS1111*

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90 Ibid.

91 Ibid., 10.

92 Ibid., 11.

93 M. Turra et al., "Diagnosis of Acute Q Fever by PCR on Sera during a Recent Outbreak in Rural South Australia," *Annals of the New York Academy of Science* 1078, no. 1 (October 2006): 566–569, https://doi.org/10.1196/annals.1374.112.

94 Schneeberger et al., "Real-Time PCR with Serum Samples," 286–290.

95 Gerald B. Howe et al., "Real-Time PCR for the Early Detection and Quantification of *Coxiella burnetii* as an Alternative to the Murine Bioassay," *Molecular and Cellular Probes* 23, nos. 3–4 (June–August 2009): 127–131, https://doi.org/10.1016/j.mcp.2009.01.004.

had positive results for the first 8 days of disease, while those tested for *com1* had fewer positive results, especially early in the disease progression. Given the available data, we characterized the probability of detection for the combinations of test, specimen, and stage of disease, as shown in Table 8. Since Q fever only has one disease stage, we only listed the probability of detection on the first day of symptoms rather than throughout the disease progression. Additional technical details describing how we obtained these specific values can be found in Appendix E.

Table 8. Q Fever Diagnostic Probabilities of Detection Included in Modeling Analysis

Technology (Specimen)	Stage of Disease	Probability of Detection
In-house PCR, *IS111* gene (blood)	Symptom Onset	100%
In-house PCR, *com1* gene (blood)	Symptom Onset	50%

D. Modeling Analysis

As described in Section 5.B, most Q fever patients only experience a moderate disease and therefore would not require transportation to roles of medical care beyond a Role 1 MTF. As a result, our modeling analysis focuses on the timing of positive diagnostic results generated from PCR tests or specimens taken at a Role 1 MTF. The specific performance of currently or potentially fielded PCR technologies may differ from the performance of the in-house PCR tests for which we could obtain the requisite data needed for our model. Accordingly, the quantitative results presented in this chapter should be interpreted as illustrating the general trends in how various TTP options affect the timing of diagnostic results and not as precise predictive forecasts of the performance of any specific PCR capability.

The modeling analysis of Q fever specifically focused on how the following TTP components impact the timing of diagnostic results following an attack:

- Delaying the collection of clinical specimens until multiple individuals report to the medical system following an attack,
- Shipping specimens taken at a lower role of care for testing at a higher role of care, and
- The probability of detection of a PCR test at a Role 1 MTF.

In addition to determining how different TTP components impact the timing of diagnostic results following the attack, we also looked at what information would be available from syndromic indicators by the time that diagnostic results can be obtained.

Table 9 lists the specific TTP options that we modeled for Q fever and the corresponding median time of the first positive diagnostic result. We simulated attacks that infected either 10, 100, or 1,000 individuals. Figure 6 shows a representative time progression of Q fever casualties following an attack as well as the median time of the first positive diagnostic result for the TTP options shown in Table 9.

Table 9. Median Time (Days) of First True Positive Diagnostic Result Following a Q Fever Attack for Various Sampling and Analysis TTP Options and the Time (Days)

Option	Technology (Specimen)	Test Location	Specimen Collection Trigger	Median Time of First True Positive Result (Days)		
				10 Infections	100 Infections	1,000 Infections
A	In-house PCR, *IS111* gene (blood)	Role 1 MTF	Patient arrival at Role 1 MTF	7.4	6.8	6.4
B	In-house PCR, *com1* gene (blood)	Role 1 MTF	Patient arrival at Role 1 MTF	7.6	7.0	6.6
C	In-house PCR, *IS111* gene (blood)	Role 1 MTF	Patient arrival at Role 1 MTF; start sampling on tenth case	9.2	7.5	6.9
D	In-house PCR, *com1* gene (blood)	Role 1 MTF	Patient arrival at Role 1 MTF; start sampling on tenth case	9.2	7.5	6.9
E	In-house PCR, *IS111* gene (blood)	Role 3 MTF	Patient arrival at Role 1 MTF with any symptoms; specimens shipped from Role 1 MTF to Role 3 MTF every 12 hours	7.7	7.2	6.8
F	In-house PCR, *com1* gene (blood)	Role 3 MTF	Patient arrival at Role 1 MTF with any symptoms; specimens shipped from Role 1 MTF to Role 3 MTF every 12 hours	8	7.3	6.9

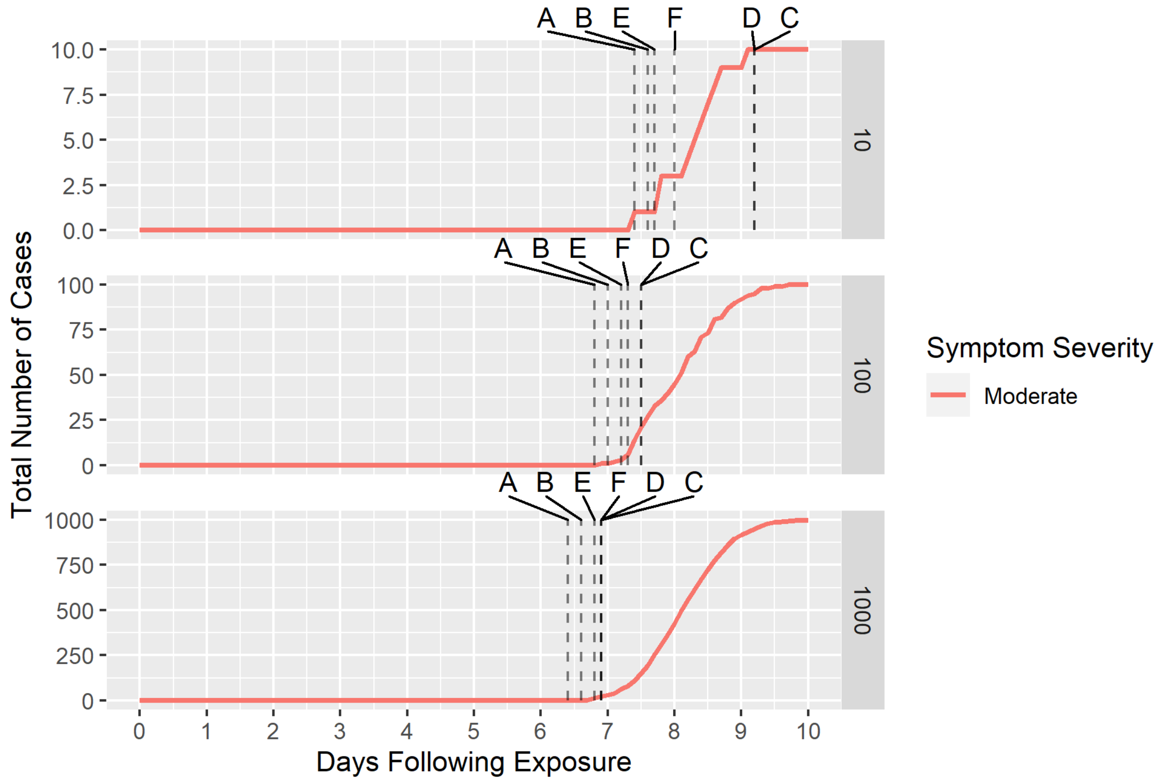

Figure 6. Representative Total Numbers of Q Fever Cases by Severity Level over Time for Attacks That Infect 10, 100, or 1000 Individuals and Median Time of First Positive Diagnostic Result for TTP Options Listed in Table 9 (Vertical Dashed Lines)

1. Delaying the Collection of Clinical Specimens until Multiple Cases Report

Since Q fever produces only a moderate, generic symptoms and does not progress to more severe symptoms, it is likely that clinicians would assume that the disease was influenza without negative influenza tests or other indicators of a biological attack. This assumption may delay testing for Q fever. We compared options C and D to options A and B to analyze the timing of positive diagnostic results associated with the collection of specimens after 10 individuals infected during the attack report to the medical system. Option C tests for the *IS111* gene, which has a 100% positive rate at symptom onset, and option D tests for the *com1* gene, which has a 50% positive rate at symptom onset. As shown in Table 9, waiting to run a diagnostic test until 10 individuals have arrived at the MTF with moderate symptoms leads to a delay in the positive diagnostic result of 1.6–1.8, 0.5–0.7, or 0.3–0.5 days for an attack infecting 10, 100, or 1,000 individuals, respectively.

As would be expected, the delay in the positive diagnosis time due to waiting for 10 individuals to arrive at the MTF is less for 1,000 initial infections than for 10 or 100 initial infections. This difference is because so many individuals report to the MTF at once with 1,000 initial infections that waiting for 10 individuals to report does not require much time. In the case of 10 initial infections, however, waiting until 10 individuals report to the MTF

means that everyone who had been initially infected has reported by that point. The difference between testing for the two genes—*IS111* and *com1*—was negligible when testing on the tenth individual due to the large number of individuals reporting to the MTF by the time that the tenth individual arrives in the 100 or 1,000 initial infection scenarios (see Figure 6).

2. Shipping Specimens Taken at Lower Roles of Care for Testing at Higher Roles of Care

Q fever produces only moderate symptoms, which means that an individual would be unlikely to require inpatient care or need to be transported beyond a Role 1 MTF. Therefore, as modeled, those individuals sick with Q fever would never be tested using a system at Role 2 or 3 unless specimens are shipped from a Role 1 MTF. We compared options E and F to options A and B to test how shipping samples could impact the timing of diagnostic results.

In options E and F, specimens are taken at the Role 1 MTF but are then shipped to a Role 3 MTF every 12 hours. As modeled, this protocol leads to a slower diagnostic time of 0.3–0.4 days across all numbers of initial infections and across both gene targets. The difference, however, may be worth avoiding the costs of putting diagnostic systems at every Role 1 MTF. As discussed in Section 4.D.4, however, shipping specimens would require packaging and logistics to ensure that specimens are shipped regularly between MTFs.

3. Probability of Detection of PCR Test at Role 1 MTF

We compared TTP options A and B to analyze how the probability of detection of a PCR test impacts the timing of positive diagnostic results. For both of these TTP options, specimens are collected from all individuals at symptom onset when they report to the Role 1 MTF where the test is located. For all three attack sizes that we considered, using the less sensitive PCR test that amplifies the *com1* gene only delays the median time of the first positive diagnostic result by 0.2 days. Even though the PCR test that amplifies the *com1* gene is half as sensitive at symptom onset (50% as compared to 100%), a sufficient flow of cases reports to the Role 1 MTF, generally minimizing the delays associated with the test producing false negative results.

Even though a less sensitive PCR test is able to provide positive diagnostic results in about the same time as the more sensitive test, it will also generate false negative results. For the in-house PCR test that amplifies the *com1* gene, a probability of detection of 50% means it will generate an average of one false negative test for every true positive. As discussed in the Chapter 4, these false negative results could confound the situational awareness provided by the diagnostic—especially if the probability of detection of the test is unknown or otherwise not accounted for when interpreting the results. In addition, the

higher false negative rate could lead to improper diagnosis of those individuals who test negative, which could interfere with their treatment unless the treatment is provided to all individuals in the affected unit as a whole rather than based on diagnostic result. The use of diagnostics for testing of individuals is outside the scope of this paper, however, since it is largely focused on diagnostic results at the population level for situational awareness.

4. Syndromic Indicators

Except in the case of 10 initial infections, most diagnostic results are produced well before most individuals become symptomatic (see Figure 6). In addition, Q fever only produces moderate, non-specific symptoms, so it may be mistaken for influenza or another common disease. Even cases of large numbers of individuals with moderate, non-specific symptoms in a deployed setting may be assumed to be a normal influenza outbreak, especially during the winter months. The number of individuals would likely be even more than 10, although we could not find a specific number and it would likely depend upon the number of individuals for which a given Role 1 is responsible, the time of year, and the endemic diseases in the area (see Section 3.C). It is possible, however, that if no specimens can be taken at a Role 1 (whether tested at the Role 1 or shipped to a higher role of care), the outbreak may only be detected by observing a large number of individuals all reporting moderate, non-specific symptoms. Table 10 shows the timing when a certain number of total cases would occur following an attack.

Table 10. Time (Days) When Various Numbers of Total Cases by Severity Level Occur

		Time of Total Number of Cases by Severity Level								
		10 Infections			100 Infections			1,000 Infections		
	Total Number of Cases	1	3	5	1	5	10	1	10	50
Symptom Severity	Moderate	7.5	7.6	7.7	6.9	7.2	7.4	6.5	6.8	7.2

E. Implications for Response Implementation

Q fever can be treated with antibiotics, and PEP can be effective at preventing Q fever.[96] Therefore, a timely diagnostic result may allow for the administration of PEP before many individuals become ill. Q fever is not a contagious disease, so no ROM would need to be implemented to contain it. To see how different diagnostic TTP options may affect the number of casualties who could be saved by PEP, we looked at the number of casualties that would occur by the time each TTP option may produce a result. As shown in Figure 6, all the TTP options can produce results before the majority of individuals

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[96] NATO, *Technical Reference Manual*, 24-11–24-14.

become symptomatic in the 100 and 1,000 initial infections cases. However, casualty numbers increase relatively soon after diagnostic results are obtained. Because of this phenomenon, if PEP cannot be administered for multiple days after the diagnostic result, then the portion of infected individuals who receive PEP before developing symptoms may be significantly reduced.

To illustrate this point, consider the use of a PCR test at a Role 1 MTF using the *IS111* gene (TTP option A) with 1,000 infected individuals. The first positive result occurs on day 6.4. At that time, one infected individual has developed symptoms. If PEP was immediately administered at that time, 999 of the 1,000 infected individuals would receive PEP before symptom onset. If the administration of PEP is delayed by 1, 3, or 5 days, however, then the individuals who could receive PEP before symptom onset drops to 914, 44, or 0, respectively.

Treatment with antibiotics for Q fever is recommended even if test results come back negative, as long as epidemiological factors and symptom progression are consistent with those of the disease.[97] However, Q fever, due to its moderate, non-specific illness, would likely only be suspected after a positive diagnostic result or other indications (e.g., environmental sampling or local intelligence). If treatment is given to an individual without a diagnostic result for that specific individual, this protocol could decrease the necessity of running diagnostic specimens in additional people after the first positive diagnostic result.

F. Evaluation

Q fever only produces moderate, non-specific symptoms. Thus, to reliably diagnose Q fever, a physician would either need additional indicators to suggest a biological attack (e.g., a large number of individuals all becoming ill at once) or TTPs would need to be in place such that he or she would test anyone with moderate, non-specific symptoms for Q fever regardless of the presence of other indicators. In addition, since individuals with Q fever do not progress to a more severe form of disease and would likely not progress beyond a Role 1 MTF, specimens for Q fever would need to be taken at the Role 1 MTF and either tested there or shipped to a higher role of care.

Shipping specimens from a Role 1 MTF or waiting until 10 individuals develop symptoms delayed diagnostic results by 0.2–0.7 days in all cases, except when there were only 10 initial infections (see Table 9). Even with the delay, the diagnostic result, in most cases, was obtained before the majority of infected individuals become symptomatic (see Figure 6). As noted previously, however, the exact modeling results presented here are driven by the performance of the in-house PCR test. Another PCR test, such as a cartridge-based test or one that tests for other genes, may have a higher or lower probability of

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[97] USAMRIID, *Medical Management of Biological Casualties Handbook*, 59.

detection as shown by the difference in the probability of detection for the *IS111* gene and the *com1* gene (see Table 8). Using a different test could affect the results of the modeling, although between tests for the two genes, similar trends were observed (see Table 9).

6. Tularemia

Note: This work was originally completed and published in May 2021. [98]

A. Tularemia Symptom Progression

Pneumonic tularemia is a disease that can be caused by inhaling the bacterium *Francisella tularensis.* Pneumonic tularemia has two stages. The first stage is characterized by the sudden onset of severe but non-specific symptoms.[99] The second stage can either be similar symptoms with an eventual recovery or a worsening condition that leads to death.[100]

We assume that individuals first arrive at a Role 1 with non-specific, severe symptoms and progress quickly to a Role 3 or higher MTF due to the need for care. This progression is consistent with the symptom profile given in AMedP-7.5.[101]

B. Tularemia Diagnostic Types

Tularemia can be diagnosed by PCR, bacterial isolation, or antigen test, using either blood or sputum specimens. We could find little information on the concentration of *F. tularensis* in sputum specimens, although the NGDS can detect *F. tularensis* in spiked synthetic sputum specimens.[102] One study of accidental laboratory exposures found that *F. tularensis* bacteria could be cultured from sputum specimens, but many specimens were taken after antibiotics had been started.[103] We could also find little information on antigen test results compared to symptom progression, although some antigen tests against *F. tularensis* have been developed.[104]

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[98] This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

[99] Samuel Saslaw et al., "Tularemia Vaccine Study. II. Respiratory Challenge," *Archives of Internal Medicine* 107 (May 1961): 705–706, doi:10.1001/archinte.1961.03620050068007.

[100] NATO, *Technical Reference Manual*, 25-11.

[101] NATO, *AMedP-7.5*, 5-67.

[102] Taylor, *Evaluation of the NGDS Warrior Panel*, 12.

[103] Lt. Col. Edwin L. Overholt et al., "An Analysis of Forty-Two Cases of Laboratory-Acquired Tularemia: Treatment with Broad Spectrum Antibiotics," *The American Journal of Medicine* 30, no. 5 (May 1961): 790, https://doi.org/10.1016/0002-9343(61)90214-5.

[104] Roland Grunow et al., "Detection of *Francisella tularensis* in Biological Specimens Using a Capture Enzyme-Linked Immunosorbent Assay, an Immunochromatographic Handheld Assay, and a PCR,"

Bacterial isolation has been used for diagnosis of tularemia for decades, but it can take considerable resources and time. Culturing *F. tularensis* can take 2–4 days due to its slow growth on media.[105] In addition, bacterial isolation must be performed under BSL-3 conditions, so it must be performed at a laboratory rather than an MTF.

PCR assays have been developed to test for tularemia, including in-house assays and the GeneXpert system.[106] PCR assays can have higher sensitivities than bacterial isolation for blood specimens and can be performed much more quickly.[107] In addition, cartridge-based PCR systems can be used outside of a laboratory setting, potentially at a Role 3 or lower MTF.

C. Probability of Detection Over the Course of the Disease

We found three studies that include information about the percent positive PCR or bacterial isolation results for tularemia in NHP models. All the studies used whole blood specimens. None looked at sputum. One study only included bacterial isolation and used rhesus monkeys.[108] The other two studies included bacterial isolation and PCR and used cynomolgus monkeys.[109] We used the two studies that included bacterial isolation and PCR since we wanted to include information about both types of assays.

Guina et al. exposed 33 cynomolgus monkeys to *F. tularensis* via aerosol and evaluated their symptom progression over time.[110] Most developed symptoms within 2–3 days. Bacteria were detected via isolation by 4–6 days after infection in most animals and via PCR by 3–5 days after infection. Guina et al. used an in-house PCR assay. We used their data for our bacterial isolation and in-house PCR models.

Clinical and Diagnostic Laboratory Immunology 7, no. 1 (January 2000): 86, doi:10.1128/cdli.7.1.86-90.2000.

[105] Matthew J. Hepburn and Andrew J. H. Simpson, "Tularemia: Current Diagnosis and Treatment Options," *Expert Review of Anti-Infective Therapy* 6, no. 2 (April 2008): 233, https://doi.org/10.1586/14787210.6.2.231.

[106] Padmapriya P. Banada et al., "Sensitive Detection of *Francisella tularensis* Directly from Whole Blood by Use of the GeneXpert System," *Journal of Clinical Microbiology* 55, no. 1 (January 2017): 291, https://jcm.asm.org/content/55/1/291.

[107] Ibid., 295.

[108] Robert L. Schricker et al., "Pathogenesis of Monkeys Aerogenically Exposed to *Francisella tularensis* 425," *Infection and Immunity* 5, no. 5 (May 1972): 734, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC422433/.

[109] Banada et al., "Sensitive Detection of *Francisella* tularensis," 291; Tina Guina et al., "The Cynomolgus Macaque Natural History Model of Pneumonic Tularemia for Predicting Clinical Efficacy Under the Animal Rule," *Frontiers in Cellular and Infection Microbiology* 8, article 99 (April 2018): 18pp., doi:10.3389/fcimb.2018.00099.

[110] Guina et al., "The Cynomolgus Macaque Natural History Model."

We also included information on the GeneXpert assay from Banada et al. since that is a cartridge-based assay and a similar GeneXpert system might be used for NGDS 2.[111] Banada et al. exposed 29 cynomolgus monkeys to *F. tularensis* via aerosol and evaluated their symptom progression over time. The results for their in-house PCR assay and bacterial isolation were similar to those of Guina et al., and the GeneXpert assay was slightly more sensitive early in the disease progression than the in-house PCR assay.[112] Guina et al., however, included information for each animal, including symptom progression, so we used their results within our model for the bacterial isolation and in-house PCR assays and Banada et al.'s results for the GeneXpert assay only. We characterized the probability of detection for the combinations of technology, specimen, and stage of disease, as shown in Table 11. Additional technical details describing how we obtained these specific values can be found in Appendix E.

Table 11. Tularemia Diagnostic Probabilities of Detection Included in Modeling Analysis

Technology (Specimen)	Stage of Disease	Probability of Detection
In-house PCR (blood)	Symptom Onset	30%
	Onset of Stage 2	100%
GeneXpert assay (blood)	Symptom Onset	30%
	Onset of Stage 2	100%
Bacterial isolation (blood)	Symptom Onset	10%
	Onset of Stage 2	90%

D. Modeling

As shown in Table 11, we located sufficient data to model three tularemia diagnostics: an in-house PCR, the GeneXpert assay, and bacterial isolation. However, we modeled the GeneXpert assay and the in-house PCR as having identical probabilities of detection at symptom onset and at the onset of stage 2 of the disease. As discussed in Section 3.B, we modeled all tests using PCR technology with a 2-hour run time. Therefore, the characterization of the in-house PCR and the GeneXpert assay are identical in the model, so we present their results together. In addition, we modeled the incubation period for tularemia from AMedP-7.5. A further discussion of how the incubation period was determined is found in Appendix A.

Our quantitative analysis of these tests focused primarily on the timing of positive results from a test using PCR technology under various clinical sampling TTPs. The actual

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[111] Banada et al., "Sensitive Detection of *Francisella* tularensis," 291.

[112] Ibid., 295.

performance of currently or potentially fielded tests using PCR technology may differ from that of the in-house PCR test for which we have sufficient data to model. The performance of the GeneXpert may differ from what we model due to the different GeneXpert system that would be used for NGDS 2, although the differences would likely be smaller. Accordingly, the quantitative results presented here should be interpreted as illustrating general trends in how various TTP options affect the timing of diagnostic results and not precise predictive forecasts of the performance of any specific diagnostic capability.

The modeling analysis of tularemia specifically focused on how the following TTP components impact the timing of diagnostic results following an attack:

- The location of PCR technology tests in the theater medical system,
- Delaying the collection of clinical specimens until multiple individuals report to the medical system following an attack,
- Shipping specimens collected from patients at a lower role of care for analysis at a higher role of care, and
- Use of bacterial isolation at a reachback laboratory.

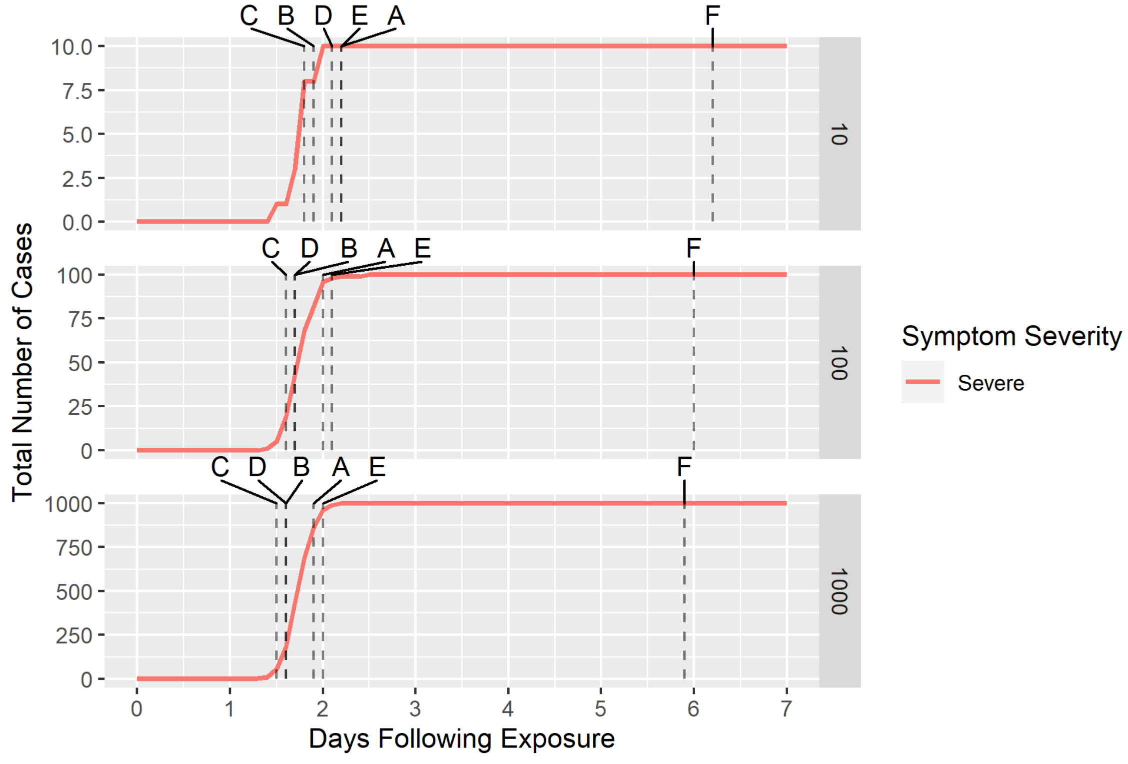
In addition to determining how different TTP components impact the timing of diagnostic results following the attack, we also looked at what information would be available from syndromic indicators by the time that diagnostic results can be obtained.

As previously mentioned, tularemia's disease progression begins with severe symptoms. Therefore, we did not analyze the difference between collecting specimens at symptom onset as compared to the onset of severe symptoms as was done for other diseases.

Table 12 lists the specific TTP options that we modeled for tularemia and the corresponding median time of the first positive diagnostic result. In addition, Figure 7 shows a representative time progression of total tularemia cases by severity level following an attack and the median time of the first positive diagnostic result for the TTP options shown in Table 12. These results assume that no treatment is given during the course of illness. Treatment is discussed further in Subsection 6.E.

Table 12. Median Time (Days) of First True Positive Diagnostic Result Following a Tularemia Attack for Various Sampling and Analysis TTP Options

Option	Technology (Specimen)	Test Location	Specimen Collection Trigger	Median Time of First True Positive Result (Days)		
				10 Infections	100 Infections	1,000 Infections
A	In-house PCR or GeneXpert (blood)	Role 3 MTF	Patient arrival at Role 3 MTF	2.2	2.0	1.9
B	In-house PCR or GeneXpert (blood)	Role 2 MTF	Patient arrival at Role 2 MTF	1.9	1.7	1.6
C	In-house PCR or GeneXpert (blood)	Role 1 MTF	Patient arrival at Role 1 MTF with severe symptoms	1.8	1.6	1.5
D	In-house PCR or GeneXpert (blood)	Role 1 MTF	Patient arrival at Role 1 MTF with severe symptoms; start sampling on tenth case	2.1	1.7	1.6
E	In-house PCR or GeneXpert (blood)	Role 3 MTF	Patient arrival at Role 1 MTF with severe symptoms; specimens shipped from Role 1 MTF to Role 3 MTF every 12 hours	2.2	2.1	2.0
F	Bacterial isolation (blood)	Laboratory	Patient arrival at Role 3 MTF	6.2	6.0	5.9



Note: The onset of very severe symptoms and the occurrence of deaths in the infected population occurs beyond the range of this figure. This figure should not lead to the conclusion that the attacks do not result in fatalities—the case fatality rate for tularemia is 75%.

Figure 7. Representative Total Numbers of Tularemia Cases by Severity Level over Time for Attacks That Infect 10, 100, or 1,000 Individuals and Median Time of First Positive Diagnostic Result for TTP Options Listed in Table 12 (Vertical Dashed Lines)

1. Location of PCR Technology in the Theater Medical System

We compared TTP options A, B, and C to analyze how the location of a PCR technology test in the theater medical system impacts the timing of the first positive diagnostic result from that system. For all three of these TTP options, specimens are collected from individuals when they arrive at the MTF at which the diagnostic is located. As was the case in the plague analysis, placing the diagnostic test at lower roles of care improves the timing of the first diagnostic result by the amount of time it takes to move the patient through the medical system. Given our choice of patient transportation modeling parameters, locating a PCR test at a Role 2 (TTP option B) or Role 1 MTF (TTP option C) as compared to a Role 3 MTF (TTP option A) reduces the time of the first positive result by 0.3 and 0.4 days, respectively. Again, if theater patient movement is faster or slower than what we modeled, then the timing the first diagnostic result would shift accordingly.

2. Delaying the Collection of Clinical Specimens Until Multiple Cases Report

We compared TTP option D to the other options to analyze the timing of positive diagnostic results associated with delaying specimen collection until 10 individuals reported to the medical system. As shown in Figure 7, all infected individuals develop symptoms within a very short period of time. Therefore, for the simulated attacks that infected 100 or 1,000 individuals, waiting for 10 individuals to develop symptoms (TTP option D) only delays the collection of specimens by 0.1 days compared to collecting specimens from everyone who reports to a Role 1 MTF (TTP option C). For the simulated attack that infects 10 individuals, this delay is 0.3 days. Even when the collection of specimens is delayed until 10 cases have reported to the medical system (TTP option D), the timing of the first positive result from a PCR test at a Role 1 MTF occurs before the result from the same test placed at a Role 3 MTF (TTP option A). These results use the incubation period distribution determined in Appendix A, which takes dose effects on incubation period into account.

3. Shipping Specimens Taken at Lower Roles of Care for Testing at Higher Roles of Care

To analyze the impact of specimen shipping, we simulated the collection of specimens at a Role 1 MTF that were analyzed with a PCR test at a Role 3 MTF (TTP option E). When compared to analyzing the specimen at the Role 1 MTF (TTP option C), shipping specimens delays the time of the first positive result by the amount of time it takes for the specimen to reach the Role 3 MTF. For our choice of modeling parameters, this corresponds to a delay of 0.5 days. If the actual time it takes specimens to reach a Role 3 MTF is faster or slower, then the delay will shorter or longer accordingly.

The initial symptoms of tularemia are severe, and therefore, we modeled patients being transported up through the roles of medical care as fast as possible upon symptom onset. Collecting specimens at symptom onset at a Role 1 MTF and shipping them to a Role 3 MTF (TTP option E) generates positive diagnostic results around the same time as waiting for the patient to arrive at the Role 3 MTF and testing him or her there (TTP option A). The timing of when both these TTP options generate positive results is tied to who can be moved through the theater quicker: the specimens or the patients.

4. Bacterial Isolation at Reachback Laboratory

The final TTP option that we modeled for tularemia involves the use of bacterial isolation at a reachback laboratory (TTP option F), which we compared to the other options. Our modeling results indicate that the median time of the first positive result from the laboratory test would be around day 6. As shown in Figure 7, all infected individuals will have developed symptoms by the time the results are available from bacterial isolation. Even if substantially delayed relative to in-theater tests, testing at a reachback laboratory—

whether bacterial isolation or some other test such as genetic sequencing—can serve as gold-standard confirmatory test and provide more detailed analyses (e.g., identifying the pathogen strain).

5. Syndromic Indicators

As shown in Figure 7 and Table 13, all the individuals infected during the attack can develop symptoms within a very short window of time. These large numbers of individuals reporting to the medical system with the severe symptoms of early tularemia will likely serve as strong indication that a biological event has occurred. Even though the early symptoms of tularemia are severe, they are also generic. Therefore, medical personnel may suspect a biological event, but the early symptoms alone may not strongly suggest tularemia. As shown in Table 13, the onset of worsening symptoms and death occurs 10 and 16 days following the onset of initial symptoms. The occurrence of 5, 10, and 50 cases with severe symptoms for attacks that caused 10, 100, and 1,000 infections occurs about the same time as the first positive diagnostic results from a PCR at a Role 1 MTF (TTP option C).

Table 13. Representative Time (Days) When Various Numbers of Total Cases by Severity Level Occur

		Time of Total Number of Cases by Severity Level								
		10 Infections			100 Infections			1,000 Infections		
	Total Number of Cases	1	3	5	1	5	10	1	10	50
Symptom Severity	Severe	1.6	1.7	1.7	1.5	1.5	1.6	1.3	1.5	1.6
	Very Severe	10.6	10.7	10.8	10.5	10.6	10.6	10.3	10.5	10.0
	Death	16.6	16.7	16.8	16.5	16.6	16.6	16.3	16.5	16.6

E. Implications for Response Implementation

Tularemia can be treated with antibiotics, and PEP can be effective at preventing illness.[113] Therefore, a timely response may allow for the administration of PEP before too many casualties occur. To characterize the ways in which different TTP options may affect the number of casualties that could be prevented with PEP, we looked at the number of casualties that would occur by the time each TTP option may produce its first positive result.

As shown in Figure 7, given the very narrow window in time in which the casualties occur given the dose-dependent incubation period modeled (Appendix A), the number of

[113] NATO, *Technical Reference Manual* , 25-13–25-15.

individuals who have not yet developed symptoms at the time of the first diagnostic result varies substantially based on which TTP option is employed. For example, consider the attack that caused 1,000 infections. Collecting specimens and analyzing them with a PCR test at a Role 1 MTF (TTP option C) can produce a positive result when only 56 of the 1,000 infected individuals have developed symptoms. In comparison, if specimen collection and analysis is delayed until the patient arrives at a Role 3 MTF (TTP option A), then 896 of the 1,000 infected individuals will have developed symptoms by the time of the first positive diagnostic result.

Even though certain TTP options are able to provide positive results before the point at which most people develop symptoms, any delays in the response decision or PEP administration processes may cause PEP to be administered too late to prevent significant casualties, given the incubation period in our model. This situation would occur because even if PEP could be administered at the moment of the first positive diagnostic result, within our model, all pre-symptomatic infected individuals are within a day of becoming casualties due to the high doses that individuals receive and the dose-dependent incubation period. Even though AMedP-7.5 specifies that PEP can completely prevent infected individuals from becoming casualties if administered at any point before symptom onset, it is likely that the efficacy of PEP in preventing casualties can be significantly reduced in people who are just about to develop their symptoms.

F. Evaluation

According to AMedP-7.5, tularemia produces severe yet non-specific symptoms early in the disease. The sudden and simultaneous appearance of severe disease in numerous previously healthy individuals would likely serve as an indicator of a biological event. However, given the non-specific nature of these symptoms, diagnostic results would likely be required to confirm the identity of the disease. The presence of early severe symptoms may facilitate diagnosis by allowing medical personnel to rule out common influenza-like illnesses. Accordingly, TTPs that only involve the collection of specimens from individuals with severe symptoms would be adequate to detect tularemia at symptom onset.

In general, all the specimen collection and analysis TTPs that we modeled generated positive results approximately 1.5–2 days following an attack (see Table 12). The exception to this scenario is the use of bacterial isolation at a reachback laboratory. Those results would be available approximately 6 days following an attack. Our modeling of attacks that infected 100 or 1,000 individuals showed that collecting specimens from individuals at a Role 3 MTF or shipping specimens for analysis at a Role 3 MTF results in obtaining the first diagnostic result after most infected individuals develop symptoms. Even if specimen collection and analysis were conducted at Role 1 MTFs, delays in the response decision and implementation process and the potentially low effectiveness of PEP administered immediately before symptom onset due to the short, dose-dependent

incubation period may make it difficult to prevent infected individuals from becoming casualties.

7. Ebolavirus Disease (EVD)

Note: This work was originally completed and published in May 2021. [114]

A. EVD Symptom Progression

EVD is caused by *Ebolavirus*. In this chapter, we focus on *Zaire ebolavirus*, although there are other Ebolaviruses, such as *Sudan ebolavirus*, which can also produce disease in humans. *Zaire ebolavirus* typically produces a two-stage disease. In the first stage, the individual has non-specific moderate symptoms such as high fever, malaise, fatigue, and body aches.[115] The first stage typically lasts 3 to 5 days. The second stage of disease is characterized by severe gastrointestinal symptoms, which either resolve or lead to death.[116] The second stage lasts a week or more, with a prolonged convalescence in those who survive. We assume that individuals first arrive at a Role 1 with moderate, non-specific symptoms and later progress to a Role 3 or higher MTF when the second, more severe stage of disease begins. Further information on the disease progression parameters is included in Appendix A.

B. EVD Diagnostic Types

The 2014–2015 West Africa outbreak led to the development of additional diagnostic platforms for EVD.[117] EVD can be diagnosed via viral isolation, PCR, or antigen test. Viral isolation is seldom performed now that PCR and antigen tests are available since it requires a BSL-4 facility.[118] All these tests usually use blood or serum specimens. Throat, saliva, or urine specimens infrequently are positive for Ebolavirus.[119]

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[114] This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

[115] Daniel S. Chertow et al., “Ebola Virus Disease in West Africa- Clinical Manifestations and Management,” *The New England Journal of Medicine* 371 (November 27, 2014): 2054, doi:10.1056/NEJMp1413084.

[116] Ibid.

[117] Broadhurst, Brooks, and Pollock, “Diagnosis of Ebola Virus Disease,” 773.

[118] Ibid., 774.

[119] Tomasz A. Leski et al., “Use of the FilmArray System for Detection of *Zaire ebolavirus* in a Small Hospital in Bo, Sierra Leone,” *Journal of Clinical Microbiology* 53, no. 7 (July 2015): 2369, doi:10.1128/JCM.00527-15.

Both cartridge-based and in-house PCR assays have been developed for *Zaire ebolavirus*. Some, such as the GeneXpert system, have very low limits of detection.[120] PCR assays are usually more sensitive than rapid or laboratory-based antigen tests.[121] There can be up to 100-fold variation in how sensitive different PCR assays are, however, even between cartridge-based assays. The NGDS Biothreat–Ebola (BT-E) system was found to be much less sensitive than the GeneXpert system.[122] In addition, many cartridge-based PCR assays inactivate the virus within the sample during the test, so they are easier to use outside of a BSL-4 laboratory.[123] In-house assays can take 4–6 hours to run, while cartridge-based assays can take 90 minutes or less to run.[124]

Rapid and laboratory-based antigen tests have been developed for *Zaire ebolavirus.* Rapid antigen tests typically can be performed at a point-of-care setting but also have high limits of detection, which means that they may not be able to detect individuals early in their disease progression.[125] Antigen levels, like other pathogen indicators for EVD, tend to increase over the disease progression until the individual recovers.[126] Antigen levels remain high if the individual does not recover. Laboratory-based tests can have higher sensitivities but typically take longer to run and require specialized equipment and well-trained technicians.[127]

C. Probability of Detection Over the Course of the Disease

The viral load for *Ebolavirus* typically increases over the progression of symptoms until death, or stays the same or slightly increases until the individual begins to recover.[128] Unlike the other diseases included in the paper, the results for EVD diagnosis over symptom progression were taken from combining limit-of-detection data along with viral

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[120] Benjamin A. Pinsky et al., "Analytical Performance Characteristics of the Cepheid GeneXpert Ebola Assay for the Detection of Ebola Virus," *PLoS One* 10, no. 12 (2015): e0142216, https://doi.org/10.1371/journal.pone.0142216.

[121] Broadhurst, Brooks, and Pollock, "Diagnosis of Ebola Virus Disease," 777.

[122] Pinsky et al., "Analytical Performance Characteristics of the Cepheid GeneXpert Ebola Assay," e0142216.

[123] Broadhurst, Brooks, and Pollock, "Diagnosis of Ebola Virus Disease," 785.

[124] Ibid., 782.

[125] Su et al., "Diagnostic Strategies for Ebola Virus Detection," 295.

[126] T. G. Ksiazek et al., "Clinical Virology of Ebola Hemorrhagic Fever (EHF): Virus, Virus Antigen, and IgG and IgM Antibody Findings among EHF Patients in Kikwit, Democratic Republic of the Congo, 1995," Supplement, *Journal of Infectious Diseases* 179, suppl. 1 (February 1999): S180, https://doi.org/10.1086/514321.

[127] Ibid.

[128] Oumur Faye et al., "Use of Viremia to Evaluate the Baseline Case Fatality Ratio of Ebola Virus Disease and Inform Treatment Studies: A Retrospective Cohort Study," *PLoS Medicine* 12, no. 12 (December 1, 2015): e1001908, https://doi.org/10.1371/journal.pmed.1001908.

load over time for the results of PCR-based cartridge tests. All the papers looked at humans rather than NHPs. We used the results for the GeneXpert system, which has a low limit of detection (73 copies/mL).[129] This number is well below the copies/mL typically observed in individuals infected with Ebolavirus during any time after symptom onset until recovery.[130] It is true also for the RealStar cartridge-based system.[131] Therefore, we assumed for our model that the cartridge-based PCR system would produce positive results 100% of the time for individuals with EVD, although, in a real-world situation, the positivity rate may be lower. Although the limits of detection are low for these systems, there is no evidence that they may be used for screening pre-symptomatic individuals, and data from *Sudan ebolavirus* using an in-house PCR assay could not detect Ebolavirus in any samples taken before symptom onset.[132]

Less information is available about antigen tests, although we were able to find some studies dealing with ELISA-based antigen tests. Although multiple rapid antigen tests are available for EVD, we could not find information on their ability to detect antigen in samples from symptomatic people over the course of their disease. Some information is available regarding laboratory-based antigen tests, such as ELISA, but both papers tested few individuals within 3 days of symptom onset. Rowe et al. found that all serum specimens from individuals tested 3–6 days after symptom onset were positive via ELISA, but they only tested 1 individual between 0 and 3 days after symptom onset, and his or her result was negative.[133] Ksiazek et al. found that almost all the serum specimens from 5–10 days after symptom onset were positive for antigen via ELISA, although an exact value could not be determined.[134] They did not include information about percent positive tests taken earlier than 5 days after symptom onset. As modeled, individuals progress to the second, more severe stage of disease a median of 4.3 days after the onset of symptoms (see Appendix A). Therefore, we assume that individuals who are tested at the onset of severe symptoms have a 100% chance of being positively diagnosed via ELISA assay of serum. We do not, however, have sufficient information to determine the positivity rate at

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[129] Pinsky et al., "Analytical Performance Characteristics of the Cepheid GeneXpert Ebola Assay," e0142216.

[130] Faye et al., "Use of Viremia to Evaluate the Baseline Case Fatality Ratio," e1001908.

[131] Pinsky et al., "Analytical Performance Characteristics of the Cepheid GeneXpert Ebola Assay," e0142216.

[132] Jonathan S. Towner et al., "Rapid Diagnosis of Ebola Hemorrhagic Fever by Reverse Transcription–PCR in an Outbreak Setting and Assessment of Patient Viral Load as a Predictor of Outcome," *Journal of Virology* 78, no.8 (April 2004): 4341, doi:10.1128/jvi.78.8.4330-4341.2004.

[133] Alexander K. Rowe et al., "Clinical, Virologic, and Immunologic Follow-Up of Convalescent Ebola Hemorrhagic Fever Patients and Their Household Contacts, Kikwit, Democratic Republic of the Congo," Supplement, *Journal of Infectious Diseases* 179, suppl. 1 (1999): S30, https://doi.org/10.1086/514318.

[134] Ksiazek et al., "Clinical Virology of Ebola Hemorrhagic Fever (EHF)," S179.

symptom onset for antigen assays within our model. Table 14 shows the final test sensitivity results for PCR and antigen tests we used in our modeling.

Table 14. EVD Diagnostic Probabilities of Detection Included in Modeling Analysis

Technology (Specimen)	Stage of Disease	Test Sensitivity
PCR cartridge (blood)	Symptom Onset	100%
	Onset of Stage 2	100%
Antigen ELISA (blood)	Symptom Onset	NA*
	Onset of Stage 2	100%

* We were unable to obtain sufficient data to characterize the test sensitivity of the ELISA antigen test on specimens collected at symptom onset. Therefore, we only modeled its use on specimens collected at the onset of the second stage of the disease.

D. Modeling

As described in Table 14, we located sufficient data to include two EVD diagnostics in our modeling analysis: PCR cartridge and ELISA antigen assay. Our quantitative analysis focused primarily on the timing of positive diagnostic results from a PCR cartridge under various specimen collection and analysis TTPs. Specifically, we focused on how the following TTP components impact the timing of diagnostic results following an attack.

- The location of PCR cartridge test in the theater medical system,
- The stage of the disease at which a specimen is collected,
- Delaying the collection of clinical specimens until multiple individuals report to the medical system following an attack,
- Shipping specimens taken at a lower role of care for testing at a higher role of care, and
- Use of ELISA antigen assay at a Role 3 MTF.

In addition to determining how different TTP components impact the timing of diagnostic results following the attack, we also looked at what information would be available from syndromic indicators by the time that diagnostic results can be obtained.

Table 15 lists the specific TTP options we included in our modeling analysis and the corresponding median time of the first positive diagnostic result following an attack. Figure 8 shows a representative time progression of EVD cases by severity level following an attack and the median time of the first positive diagnostic result for the TTP options shown in Table 15.

Table 15. Median Time (Days) of First True Positive Diagnostic Result Following an EVD Attack for Various Sampling and Analysis TTP Options

Option	Technology (Specimen)	Test Location	Specimen Collection Trigger	Median Time of First True Positive Result (Days)		
				10 Infections	100 Infections	1,000 Infections
A	PCR cartridge (blood)	Role 3 MTF	Patient arrival at Role 3 MTF	4.8	2.3	1.3
B	ELISA Antigen (blood)	Role 3 MTF	Patient arrival at Role 3 MTF	4.9	2.4	1.4
C	PCR cartridge (blood)	Role 2 MTF	Patient arrival at Role 2 MTF	4.4	1.9	1.0
D	PCR cartridge (blood)	Role 1 MTF	Patient arrival at Role 1 MTF with severe symptoms	4.2	1.8	0.8
E	PCR cartridge (blood)	Role 1 MTF	Patient arrival at Role 1 MTF with any symptoms	1.8	0.5	0.2
F	PCR cartridge (blood)	Role 1 MTF	Patient arrival at Role 1 MTF with any symptoms; start sampling on tenth case	24.2	2.2	0.5
G	PCR cartridge (blood)	Role 3 MTF	Patient arrival at Role 1 MTF with any symptoms; specimens shipped from Role 1 MTF to Role 3 MTF every 12 hours	2.2	0.9	0.7

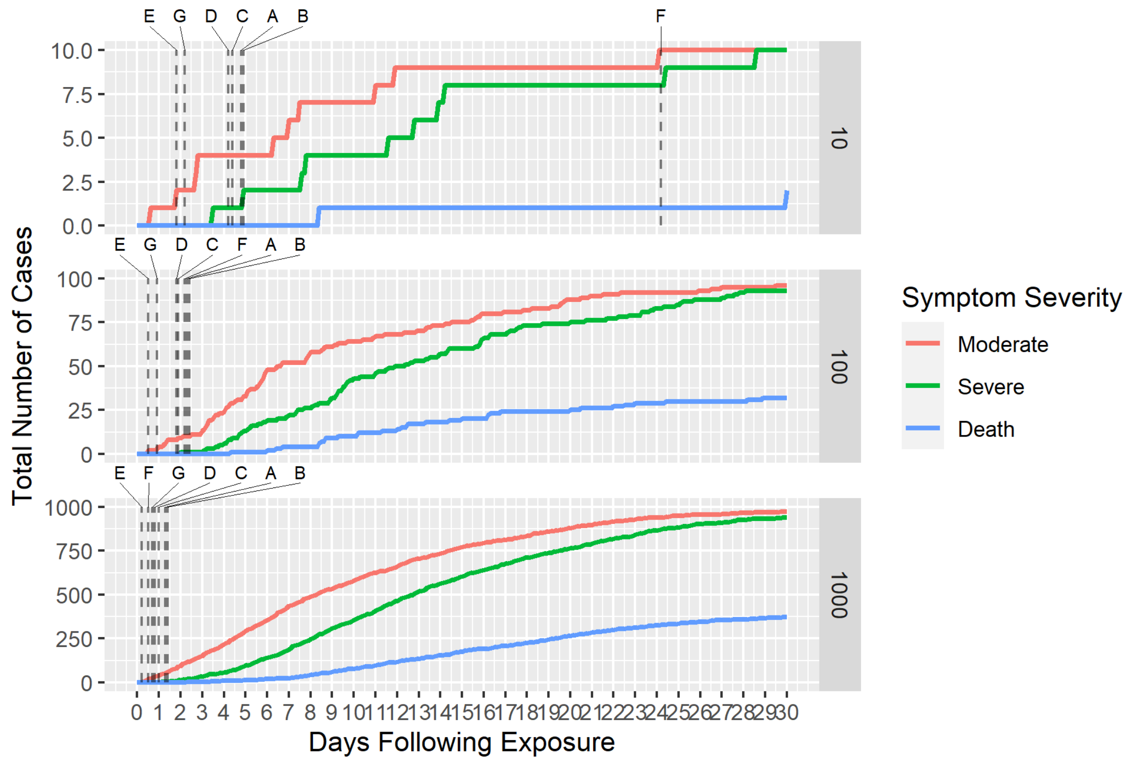

Figure 8. Representative Total Numbers of EVD Cases by Severity Level over Time for Attacks That Infect 10, 100, or 1,000 Individuals and Median Time of First Positive Diagnostic Result for TTP Options Listed in Table 15 (Vertical Dashed Lines)

The distributions that we used to model the durations of the incubation period and stage 1 of EVD have large variances relative to their mean. Because we used these distributions directly as they are reported in the literature and did not truncate or otherwise manipulate them, our model features some individuals who develop symptoms and subsequently enter the second stage of EVD within 1 day of the attack. We acknowledge that this timing is likely artificially fast. That being said, we are not confident enough in our understanding of what the minimum incubation period or minimum length of the first stage of illness would be following an inhalational exposure to Ebolavirus to truncate the distributions reported in the scientific literature. As a result of this limitation, the timing of the first diagnostic results shown in Table 15 may also be artificially fast. However, we believe the relative timing of the results from the various TTP options to each other does reflect the relative impact of the various TTP components under consideration.

1. Location of PCR Cartridge Test in Theater Medical System

We compared TTP options A, C, and D to analyze how the location of a PCR cartridge test within the theater medical system impacts the timing of the first diagnostic result from that test. For all three of these TTP options, specimens are collected from individuals with severe symptoms when they arrive at the MTF where the test is located. Placing a

diagnostic test at lower roles of care improves the timing of the first diagnostic result by the time it takes to move the patient through the roles of the medical system. Given our choice of patient transportation modeling parameters, locating a PCR cartridge test at a Role 1 MTF (TTP option D) as compared to a Role 3 MTF (TTP option A) reduces the time of the first positive result by approximately 0.5 days. If theater patient movement is faster or slower than what we modeled, then the timing of the first positive diagnostic result from these TTP options would shift accordingly.

2. Disease Stage at which Specimen is Collected

We compared TTP options D and E to other options to analyze the impact of collecting specimens from individuals at symptom onset as compared to waiting for the onset of the more severe symptoms associated with the second stage of EVD. Our modeling indicates that analyzing specimens collected at symptom onset (TTP option D) provides positive diagnostic results 0.6 days, 1.3 days, and 2.4 days sooner than collecting specimens at the onset of stage 2 symptoms (TTP option E). As shown in Table 14, we modeled the PCR cartridge test as having the same sensitivity when analyzing specimens collected at symptom onset as those collected at the onset of the second stage of the disease. Therefore, delaying specimen collection until the onset of the second stage of the disease only changes the timing of when specimens are collected, not the likelihood that those specimens will generate positive results.

Given EVD's initial moderate, non-specific presentation, analyzing specimens collected at symptom onset (TTP option D) would either require the collection and analysis of specimens from all individuals with moderate, non-specific symptoms, only collecting specimens from individuals after ruling out more common influenza-like illness, or some other indication that an EVD attack has occurred.

3. Delaying the Collection of Clinical Specimens Until Multiple Cases Report

Given EVD's moderate, non-specific early symptoms, medical personnel would likely assume that stage 1 EVD patients were infected with some other influenza-like illness. Accordingly, medical personnel may not run a PCR test for EVD until more common infections could be ruled out or until there was other evidence of a biological attack. We compared TTP option F to other options to analyze how delaying specimen collection until the tenth case reports to the medical system delays the timing of diagnostic results. When compared to testing every symptomatic case at a Role 1 MTF (TTP option E), waiting for the tenth case before initiating testing delays the timing of positive results by 0.3, 1.7, and 22.4 days for attacks causing 1,000, 100, and 10 infections, respectively. As shown in Figure 8, infected individuals develop symptoms over a wide period of time (i.e., the incubation period of EVD has large variance). Therefore, attacks infecting smaller numbers of individuals can result in weeks between when the first and

tenth case report to the medical system. In a real-world scenario, however, one of the first nine cases would likely be retested at a higher role of care after progressing to more severe symptoms. We did not address combining multiple TTP options within our model, although if an individual was retested later during symptom progression or tested after severe symptoms develop or if 10 individuals report to an MTF, then the timing of positive results for the combination would follow the lower of the two TTP options.

4. Shipping Specimens Taken at Lower Roles of Care for Testing at Higher Roles of Care

Given EVD's early moderate symptoms, before the first positive diagnostic result for EVD, infected individuals would likely not be transported to higher roles of care until the onset of the second stage of the disease. Therefore, if a PCR test was only available at a Role 3 MTF, then collecting specimens at a Role 1 MTF and shipping them to the Role 3 MTF could provide diagnostic results earlier than if the test was only run when the patient arrived at the Role 3 MTF.

We compared TTP option F to other options to analyze how shipping specimens collected at lower roles of care for analysis at higher roles of care impacts the timing of diagnostic results. When compared to collecting and analyzing specimens at a Role 3 MTF (TTP option A), collecting specimens at a Role 1 MTF and shipping them to a Role 3 MTF (TTP option G) provides diagnostic results 0.6, 1.4, and 2.6 days sooner for 1,000, 100, and 10 initial infections, respectively. Our modeling shows that for attacks infecting 1,000 individuals, shipping specimens collected at symptom onset (TTP option G) generates positive diagnostic results around the same time that would happen if the test was located at the Role 1 MTF and specimens were collected at the onset of stage 2 (TTP option D). For the attacks that caused fewer infections, specimen shipping can result in positive diagnostic results approximately 1 day or more sooner than collecting and analyzing specimens at stage 2 onset at a Role 1 MTF (TTP option D).

5. ELISA Antigen Test at a Role 3 MTF

In addition to PCR tests, we also modeled the use of an ELISA antigen test at a Role 3 MTF. As shown in Table 14, we modeled the antigen test with the same sensitivity as the PCR test. However, as shown in Table 4, we modeled laboratory antigen tests as requiring 4 hours to run instead of the 2 hours to run a PCR test. Therefore, the only difference between analyzing specimens with a PCR test at a Role 3 MTF (TTP option A) instead of an ELISA antigen test (TTP option B) is the delay associated with the antigen assay's slightly longer run time. As a result, our modeling indicates that results from an ELISA antigen test at a Role 3 MTF would be available around the same time as results from a PCR test. Most of the benefit from using antigen rather than PCR tests for EVD comes from rapid antigen, for which we could not find sufficient data to model. A rapid antigen

test could be used at a lower role of care but has lower sensitivity than laboratory-based tests. Therefore, we could not determine the utility of it based on the laboratory-based test results.

6. Syndromic Indicators

As shown in Figure 8, individuals infected during an EVD attack will develop symptoms over a relative long period of time as compared to the other diseases we analyzed. Accordingly, the diagnostic results generated by the various TTP options we modeled occur before symptom onset in most of the infected individuals and in nearly all the infected individuals for the two larger sized attacks. Our modeling results in Table 16, suggest that when diagnostic results are first available from tests at a Role 3 MTF, 50, 10, or 3 individuals would have symptoms following an attack that infects 1,000, 100, or 10 individuals. Multiple individuals have onset of severe symptoms or die after the first diagnostic results from the various TTP options that we modeled.

Table 16. Representative Time (Days) When Various Numbers of Total Cases by Severity Level Occur

		Time of Total Number of Cases by Severity Level								
		10 Infections			100 Infections			1,000 Infections		
	Total Number of Cases	1	3	5	1	5	10	1	10	50
Symptom Severity	Moderate	0.6	2.7	6.3	0.5	1.2	2.1	0.3	0.4	1.2
	Very Severe	3.5	7.6	11.6	2.0	3.8	4.7	0.7	1.8	3.7
	Death	8.4	30.0	NA*	4.3	8.4	9.3	2.1	4.7	8.4

* The case fatality rate of EVD is 40%. The representative model trial from which these data are obtained did not result in five fatalities.

E. Implications for Response Implementation

Currently, no approved PEP is available for EVD, and therefore all individuals infected during the attack will become casualties.[135] Treatment consists of intensive supportive care and administration of a recently approved monoclonal antibody.[136] Positive diagnostic results could trigger the administration of the monoclonal antibody treatment or the process of making sufficient doses of it available.

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[135] USAMRIID, *Medical Management of Biological Casualties Handbook*, 91.

[136] "FDA Approves Treatment for Ebola Virus," U.S. Food and Drug Administration (FDA), accessed March 9, 2021, last updated December 21, 2020, https://www.fda.gov/drugs/drug-safety-and-availability/fda-approves-treatment-ebola-virus.

EVD is a contagious disease. Although transmission of the disease is not accounted for in our modeling analysis, additional casualties beyond those infected during the attack may occur. EVD is primarily transmitted by direct contact with bodily fluids, so avoiding direct physical contact with ill individuals and using PPE when personnel are required to interact with an ill individual can greatly reduce spread of disease.[137] Therefore, we do not anticipate that a significant spread of EVD would occur through a military population. That being said, ROM can reduce further casualties in the exposed population and limit the spread of the disease to other populations. Previous IDA analysis found that a contagious disease outbreak resulting from a BW attack can spread to multiple locations within a theater in a few days.[138] The ability of ROMs to reduce or stop the spread of a disease in a population depends on when the restrictions are implemented and the extent to which individuals in the population move and interact with each other and with other populations. Additional analyses would be required to understand how the timing of diagnostic results affect the ability of restrictions of movement to mitigate the operational impact of an EVD attack.

F. Evaluation

EVD produces early moderate, non-specific symptoms that progresses to more severe gastrointestinal symptoms and potentially death. Given the larger variance in the duration of the incubation period, individuals infected during an attack may report to the medical system over a longer period of time than other diseases we analyzed. That being said, as shown in Figure 8 and Table 16, hundreds of individuals may develop symptoms within a few days of the attack, which would likely serve as an indication that a biological event is occurring. Given the generic nature of these early symptoms, medical personnel may need to rule out more common infections before considering EVD. However, the onset of the characteristic hemorrhagic symptoms of the second stage of the disease could allow medical personnel to rule out common influenza-like illnesses.

Generating diagnostic results during the early stages of EVD would require a specimen collection TTP that involves testing everyone with influenza-like illness unless other indications beyond the patients' symptoms suggest EVD. Our modeling suggests that delaying specimen collection and analysis until individuals enter the second stage of disease delays the timing of the first positive diagnostic result by approximately 1, 2, or 3 days for attacks that infect 1,000, 100, or 10 individuals. However, even with these delays, most infected individuals will have not yet developed symptoms by the time the first

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[137] William A. Fischer II, David J. Weber, and David A. Wohl, "Personal Protective Equipment: Protecting Health Care Providers in an Ebola Outbreak," *Clinical Therapeutics* 37, no. 11 (1 November 2015): 2403, https://doi.org/10.1016/j.clinthera.2015.07.007.

[138] Burr et al., *Emerging Infectious Diseases Study*, 87.

positive diagnostic result is available. The delay in situational awareness, however, could delay the implementation of ROM and other responses, which may be of great concern due to the high lethality and contagious nature of EVD.

Due to the lack of PEP, all infected individuals will become casualties. Therefore, the results of diagnostic tests can do little to prevent the initial casualties resulting from the attack. However, early diagnostic results can inform the implementation of ROM protocols that can reduce the spread of the disease in the exposed population and in other populations in the theater and ready MTFs for the influx of EVD patients, who typically require high levels of containment and PPE for medical personnel.

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8. Venezuelan Equine Encephalitis Virus (VEEV) Disease

Note: This work was originally completed and published in May 2021. [139]

A. VEEV Disease Symptom Progression

VEEV disease is caused by the Venezuelan Equine Encephalitis (VEE) virus. Although it is called "Venezuelan Equine Encephalitis," encephalitis symptoms rarely occur in adults with this disease.[140] Symptoms, instead, start out severe and non-specific, and improve over time.[141] We assume that individuals first arrive at a Role 1 with non-specific, severe symptoms and progress quickly to a Role 3 or higher MTF due to the need for care. This progression is consistent with the symptom profile given in AMedP-7.5.[142]

B. VEEV Disease Diagnostic Types

VEEV disease is most commonly diagnosed via viral isolation, typically using plaque neutralization assays of blood specimens. These assays can require days to complete and involve growing VEEV in cell culture to determine the viral load.[143] Therefore, the assays must be done in a laboratory rather than at an MTF.

PCR and antigen tests have been developed, although they are less commonly used than viral isolation. Many PCR tests can only detect some VEEV subtypes, generally the subtypes that most commonly cause human disease.[144] Some tests have been developed

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[139] This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

[140] Michele A. Zacks and Slobodan Paessler, "Encephalitic Alphaviruses," *Veterinary Microbiology* 140, nos. 3–4 (27 January 2010): 284, https://doi.org/10.1016/j.vetmic.2009.08.023.

[141] NATO, *AMedP-7.5*, 5-78.

[142] Ibid.

[143] Douglas S. Reed et al., "Genetically Engineered, Live, Attenuated Vaccines Protect Nonhuman Primates Against Aerosol Challenge with a Virulent IE Strain of Venezuelan Equine Encephalitis Virus," *Vaccine* 23, no. 24 (May 2005): 3141, https://doi.org/10.1016/j.vaccine.2004.12.023.

[144] Bettina Linssen et al., "Development of Reverse Transcription-PCR Assays Specific for Detection of Equine Encephalitis Viruses," *Journal of Clinical Microbiology* 38, no. 4 (April 2000): 1527, doi:10.1128/JCM.38.4.1527-1535.2000.

that do detect all VEEV subtypes, but none are commercially available.[145] Laboratory-based antigen tests have also been developed, although none are commercially available and most require complicated laboratory techniques.[146] We found no information on the results of PCR or antigen tests compared to symptom progression, so were unable to include those results in our model.

C. Probability of Detection Over the Course of the Disease

Since viral isolation is the most common assay for diagnosing VEEV disease, we were only able to find information on the results of that assay compared to symptom progression. Five papers looked at viral isolation tests of blood specimens and at symptom progression results over time in NHPs.[147] All these papers found similar results, and all five used cynomolgus monkeys as the test subjects. All studies found that symptoms began approximately 1 day after infection and that viremia was observable in the first 3 days of symptoms. Symptoms persisted for approximately 5 to 7 days in all studies, so VEEV was not able to be diagnosed late in the disease. Since all the studies had similar results, we combined them for our modeling.

Table 17 gives the sensitivity results included in our analysis.

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[145] María Belén Pisano et al., "Specific Detection of All Members of the Venezuelan Equine Encephalitis Complex: Development of a RT-Nested PCR," *Journal of Virological Methods* 186, nos. 1–2 (December 2012): 203, https://doi.org/10.1016/j.jviromet.2012.05.009.

[146] Wei-Gang Hu et al., "Development of Immunofiltration Assay by Light Addressable Potentiometric Sensor with Genetically Biotinylated Recombinant Antibody for Rapid Identification of Venezuelan Equine Encephalitis Virus," *Journal of Immunological Methods* 289, nos. 1–2 (June 2004): 27, https://doi.org/10.1016/j.jim.2004.03.007.

[147] Douglas S. Reed et al., "Aerosol Infection of Cynomolgus Macaques with Enzootic Strains of Venezuelan Equine Encephalitis Viruses," *Journal of Infectious Diseases* 189, no. 6 (March 15, 2004): 1013–1017, https://doi.org/10.1086/382281; Reed et al., "Genetically Engineered, Live, Attenuated Vaccines Protect Nonhuman Primates," 3139–3147; Lesley C. Dupuy et al., "Immunogenicity and Protective Efficacy of a DNA Vaccine Against Venezuelan Equine Encephalitis Virus Aerosol Challenge in Nonhuman Primates," *Vaccine* 28, no. 46 (28 October 2010): 7345–7350, https://doi.org/10.1016/j.vaccine.2010.09.005; Lesley C. Dupuy et al., "A DNA Vaccine for Venezuelan Equine Encephalitis Virus Delivered by Intramuscular Electroporation Elicits High Levels of Neutralizing Antibodies in Multiple Animal Models and Provides Protective Immunity to Mice and Nonhuman Primates," *Clinical and Vaccine Immunology* 18, no. 5 (May 2011): 707–716, doi:10.1128/CVI.00030-11; Shannan L. Rossi et al., "IRES-Containing VEEV Vaccine Protects Cynomolgus Macaques from IE Venezuelan Equine Encephalitis Virus Aerosol Challenge," *PLoS Neglected Tropical Diseases* 9, no. 5 (May 2015): e0003797, doi:10.1371/journal.pntd.0003797.

Table 17. VEEV Disease Diagnostic Probabilities of Detection Included in Modeling Analysis

Technology (Specimen)	Stage of Disease	Test Sensitivity
Viral isolation (blood)	Symptom Onset	100%
Viral isolation (throat or nasal swab)	Symptom Onset	100%
Viral isolation (serum)	Symptom Onset	100%

D. Modeling

As described in Table 17, we were able to locate sufficient data to model viral isolation using a blood, throat/nasal swab, or serum specimen. Within our model, all three of these sample types have the same sensitivity and the same model assumptions. Therefore, we can look at the results for all three simultaneously. Small differences in the results for each of these specimen types are due to the stochastic nature of the model. Since only data for viral isolation procedures were available, only a few of the TTP options could be analyzed for VEEV disease. In addition, VEEV disease begins with severe symptoms, so testing at a later stage of disease is not applicable for this disease. The modeling analysis of VEEV disease specifically focused on how the following TTP components impact the timing of diagnostic results following an attack:

- Delaying the collection of clinical specimens until multiple individuals report to the medical system following an attack, and
- Use of viral isolation at a reachback laboratory.

In addition to determining how different TTP components impact the timing of diagnostic results following the attack, we also looked at what information would be available from syndromic indicators by the time that diagnostic results can be obtained.

Table 18 lists the specific TTP options we modeled for VEEV disease and the corresponding median time of the first positive diagnostic result. Figure 9 shows a representative time progression of VEEV disease cases by severity level following an attack and the median time of the first positive diagnostic result for the TTP options shown in Table 18.

Table 18. Median Time (Days) of First True Positive Diagnostic Result Following a VEEV Attack for Various Sampling and Analysis TTP Options

Option	Technology (Specimen)	Test Location	Specimen Collection Trigger	Median Time of First True Positive Result (Days)		
				10 Infections	100 Infections	1,000 Infections
A	Viral isolation (blood)	Laboratory	Patient arrival at Role 3 MTF	4.9	4.5	4.4
B	Viral isolation (throat or nasal swab)	Laboratory	Patient arrival at Role 3 MTF	4.9	4.5	4.4
C	Viral isolation (serum)	Laboratory	Patient arrival at Role 3 MTF	4.8	4.5	4.4
D	Viral isolation (blood)	Laboratory	Patient arrival at Role 3 MTF with any symptoms; start sampling on tenth case	8.6	4.9	4.5

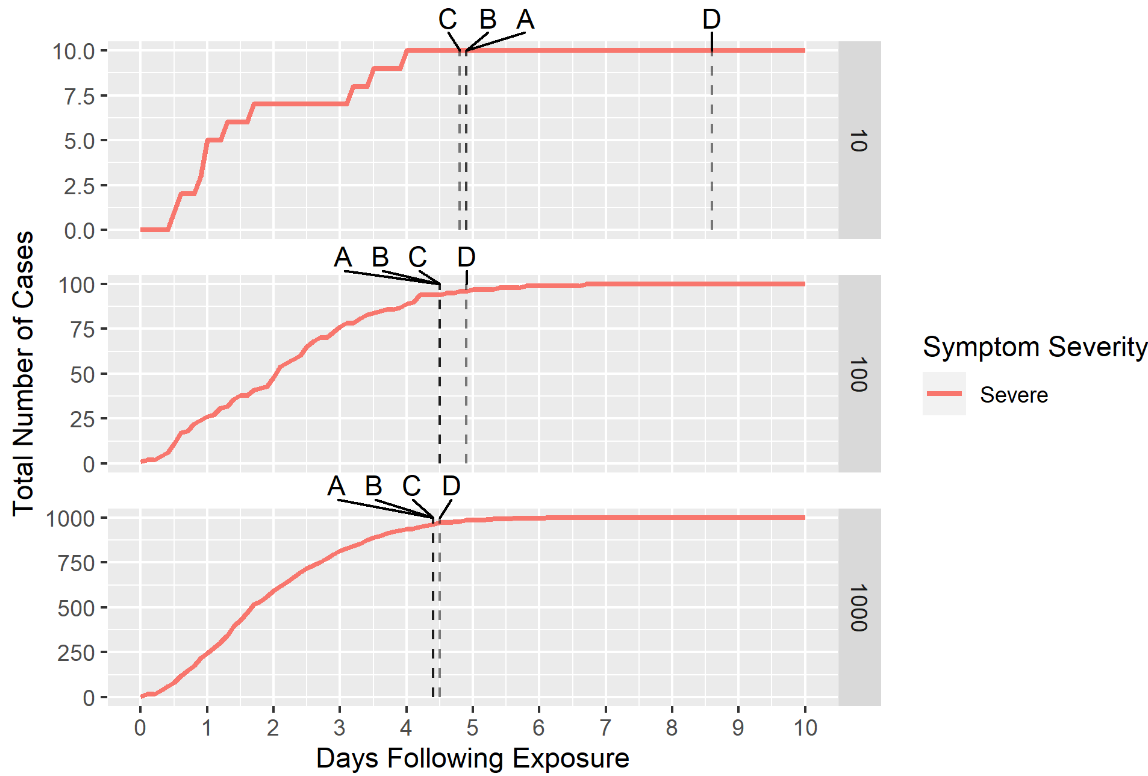

Figure 9. Representative Total Numbers of VEEV Disease Cases by Severity Level over Time for Attacks That Infect 10, 100, or 1,000 Individuals and Median Time of First Positive Diagnostic Result for TTP Options Listed in Table 18 (Vertical Dashed Lines)

1. Delaying the Collection of Clinical Specimens until Multiple Cases Report

Since VEEV produces non-specific severe symptoms of disease, it is possible that medical personnel would not take a specimen to test for VEEV upon the first patient's arrival at the Role 3 MTF, especially given the time and laboratory equipment required for VEEV isolation. We compared option D to option A to analyze the timing of positive diagnostic results associated with collecting specimens until 10 individuals infected during the attack report to the medical system. As shown in Table 18, delaying sampling until 10 individuals become ill delays the result by 0.1 days for 1,000 initial infections, 0.4 days for 100 initial infections, and 3.7 days for 10 initial infections.

Since VEEV disease typically has a short incubation period, individuals become symptomatic quickly after the attack, especially in situations with large numbers of initial infections. Therefore, the delay in a diagnostic result is minimal when large numbers of people are initially infected. In addition, since the test takes days to run after the specimen is collected, the difference is minimal compared to the distribution of cases (see Figure 9). When only a few individuals are infected, the delay can be much greater because the test is only performed on the last individual infected.

2. Viral Isolation at Reachback Laboratory

All the TTP options included in our model for VEEV disease use viral isolation at a reachback laboratory. Therefore, diagnostic results are not available for at least 4.5 days after the attack, even in the case of 1,000 initial infections (see Table 18). Previous studies found that the test was 100% sensitive for blood, serum, or throat/nasal swab specimens (see Table 17). Therefore, the results are the same for all specimen types. The timing of results is similar across 10, 100, and 1000 initial infections: 4.5–4.9 days because of the typically short incubation period for VEEV disease.

3. Syndromic Indicators

Since the results from viral isolation take so long to produce, virtually all individuals have become ill by that time (see Figure 9). Especially for the larger attacks, the sheer number of ill individuals presenting to the medical system should be a clue that a biological attack has occurred. However, since the severe symptoms produced by VEEV disease are non-specific, clinicians may not have an indication that VEEV is the source of disease without diagnostic results or additional intelligence information. Individuals will recover over time, and VEEV disease is rarely fatal. As noted in the Section 8.E, no treatments or PEP are available for VEEV disease. Table 19 shows the timing when a certain number of total individuals with a given symptom severity would occur following the attack. Since VEEV disease begins with severe symptoms and is generally not fatal, only values for severe symptoms are shown in Table 19.

Table 19. Time (Days) When Various Numbers of Total Cases by Severity Level Occur

		Time of Total Number of Cases by Severity Level								
		10 Infections			100 Infections			1,000 Infections		
	Total Number of Cases	1	3	5	1	5	10	1	10	50
Symptom Severity	Moderate	0.4	0.5	0.8	0.2	0.5	0.7	0.1	0.2	0.3

E. Implications for Response Implementation

No specific treatment or PEP is available for VEEV disease. Test results, however, could inform supportive care and would show that the disease at hand is non-contagious and viral, therefore allowing for the relaxation of ROM measures or antibiotics use if those measures had been put into effect at the onset of the outbreak. Due to the lack of a specific treatment or PEP and the non-contagious nature of VEE, the timely knowledge of VEE as the causative agent would be less important than for other diseases that are contagious or that have specific PEP or treatment.

F. Evaluation

Overall, the various TTP options that we explored in the modeling analysis for viral isolation assays have virtually identical model results. All the TTP options used viral isolation procedures that must be performed at a reachback laboratory, which means that the positive diagnostics results are typically produced 4.5–4.9 days after the attack. VEEV disease has a short incubation period, so delaying the collection of specimens until after 10 individuals became ill did not delay results by much in the case of 100 or 1,000 initial infections. In addition, the short incubation period means that most individuals become ill before the diagnostic results are available. No PEP or treatment is available for VEEV disease, although diagnostic results may be able to inform supportive care or additional theater responses, such as halting ROM, since VEEV disease is not contagious. Supportive care or additional theater responses, however, may not need to be as quickly implemented as PEP or ROM for other diseases, which could limit the utility of a faster diagnostic technology, such as PCR.

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9. Anthrax

Note: This work was originally completed and published in May 2021. [148]

A. Anthrax Symptom Progression

Anthrax is a disease caused by the bacterium *Bacillus anthracis*. Inhalational anthrax occurs after inhalation of anthrax spores and produces a two-stage illness.[149] The first, the prodromal stage, produces moderate, non-specific symptoms that are difficult to distinguish from other respiratory diseases. The prodromal stage lasts an average of 4 days.[150] After the prodromal stage, the individual passes to the fulminant stage, which has more severe and specific symptoms, including respiratory distress, a widened mediastinum, and cardiovascular collapse.[151] The fulminant stage progresses rapidly and typically results in death within 1 day unless treatment is provided early.[152]

We assume that individuals first arrive at a Role 1 MTF with moderate, non-specific symptoms and later progress to a Role 3 or higher MTF when the second, more severe stage of disease begins. This progression is consistent with the symptom profile given in AMedP-7.5.[153]

B. Anthrax Diagnostic Types

Anthrax can be diagnosed via bacterial isolation, PCR, or antigen testing, typically using blood specimens. Because anthrax produces moderate, non-specific symptoms at the beginning of the disease, it is often difficult to diagnose by symptoms alone early in the disease.[154] Bacterial isolation was used to diagnose some of those who were infected with

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[148] This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

[149] Jon-Erik C. Holty et al., "Systematic Review: A Century of Inhalational Anthrax Cases from 1900 to 2005," *Annals of Internal Medicine* 144, no. 4 (February 21, 2006): 273, doi:10.7326/0003-4819-144-4-200602210-00009.

[150] Ibid.

[151] John A. Jernigan et al., "Bioterrorism-Related Inhalational Anthrax: The First 10 Cases Reported in the United States," *Emerging Infectious Diseases* 7, no. 6 (November-December 2001): 933–944, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2631903/.

[152] Holty et al., "Systematic Review: A Century of Inhalational Anthrax Cases," 273.

[153] NATO, *AMedP-7.5*, 5-21.

[154] Nathaniel Hupert et al., "Accuracy of Screening for Inhalational Anthrax after a Bioterrorist Attack," *Annals of Internal Medicine* 139, no 5 (Pt. 1) (September 2, 2003): 337,

anthrax during the 2001 attacks.[155] PCR tests for anthrax in blood or serum have been developed as laboratory protocols, and some tests have been marketed commercially. Anthrax is included on the NGDS 1 Warrior panel.[156]

Most antigen tests for anthrax detect the lethal factor (LF), protective antigen, or edema factor (EF) in blood or serum. Most of the published methods for detecting these antigens use immunoassays or mass spectrometry.[157] Both of these technologies are labor intensive, require large machines, and are unlikely to be employed at a Role 3 or lower facility. It is theoretically possible, however, that lateral flow assays or other point-of-care antigen assays could be developed to detect anthrax antigens. However, mass spectrometry and other laboratory-based testing can have much lower limits of detection than other methods, so the different limit of detection would need to be investigated if point-of-care antigen tests for anthrax are developed.[158]

C. Diagnostic Results Over Time

Multiple papers described PCR, antigen test, or bacterial isolation results over time. Unfortunately, however, these results could not be correlated to symptom progression, so anthrax could not be included within our model. Although pathological changes can occur in NHPs due to anthrax, most subjects do not have outward signs or symptoms.[159] Occasionally, some monkeys will have transient fevers, but those fevers do not last the duration of disease and fever is not present in all monkeys. Therefore, we could not determine how the test results over time would be related to symptom progression and can only discuss anthrax diagnostics qualitatively rather than quantitatively.

In those papers that provided antigen test or PCR results over time, most antigen tests provided results as early in the disease progression as PCR results and sometimes earlier. These antigen tests, however, as noted in Section 9.B, used very sensitive technologies that would be unlikely to be used in a Role 3 or lower facility. Some antigens, such as LF, have

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https://journals.lww.com/infectdis/fulltext/2004/03000/accuracy_of_screening_for_inhalation_anthrax_after.52.aspx.

[155] Centers for Disease Control and Prevention, "Update: Investigation of Anthrax Associated with Intentional Exposure and Interim Public Health Guidelines, October 2001," *Morbidity and Mortality Weekly Report (MMWR)* 50, no. 41 (October 19, 2001): 889, https://www.cdc.gov/mmwr/PDF/wk/mm5041.pdf.

[156] Taylor, *Evaluation of the NGDS Warrior Panel*, 1.

[157] Lins et al., "Zeptomole per Millilieter Detection and Quantification," 2493–2509.

[158] Ibid.

[159] C. C. Berdjis et al., "Pathogenesis of Respiratory Anthrax in *Macaca mulatta*," *British Journal of Experimental Pathology* 43, no. 5 (October 1962): 516, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2095137/; Cynthia A. Rossi et al., "Identification of a Surrogate Marker for Infection in the African Green Monkey Model of Inhalation Anthrax," *Infection and Immunity* 76, no. 12 (December 2008): 5792–5796, doi:10.1128/IAI.00520-08

triphasic profiles, where their values peak at two different times between infection and death, with lower values in between.[160] Boyer et al. found that antigen mass spectrometry results were positive in whole blood from 24–48 hours after infection until death and that PCR results were also generally positive after 48 hours.[161] Bacteremia, as detected by bacterial isolation, was positive at 48 hours, often negative at 72 hours, and then positive again at 96 hours until death, mirroring the LF levels. EF showed the same triphasic profile as LF in serum but at lower levels.[162] Rossi et al. found more variation in the PCR and electrochemiluminescence assay results over time in whole blood from African Green Monkeys that were given low or high doses of anthrax but similarly found that antigen results were positive at the same times after infection as PCR results.[163] Depending on the sensitivity of a given assay, this triphasic nature may lead to false negative results during the symptom progression or may be inconsequential if the assay is sensitive enough. Since we could not find information on symptom progression in primates, we cannot relate the triphasic profile to symptom onset.

D. Modeling

As discussed in Section 9.C, we were not able to directly model how quickly anthrax diagnosis could occur relative to symptom progression. If further information is available in the future, this analysis could be performed.

E. Evaluation

Since symptoms were not always present or detectable in the animals used to determine bacterial isolation, antigen, and PCR results over time, we could not determine how likely, relative to symptom onset, it would be to receive a positive test result for anthrax. Further animal studies may be required for that information.

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[160] Anne E. Boyer et al., "Kinetics of Lethal Factor and Poly-D-Glutamic Acid Antigenemia during Inhalation Anthrax in Rhesus Macaques," *Infection and Immunity* 77, no. 8 (August 2009): 3435, doi:10.1128/IAI.00346-09.

[161] Ibid., 3434.

[162] Lins et al., "Zeptomole per Millilieter Detection and Quantification," 2501.

[163] Rossi et al., "Identification of a Surrogate Marker for Infection," 5794–5796.

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10. Staphylococcal Enterotoxin B (SEB)

Note: This work was originally completed and published in May 2021. [164]

A. SEB Symptom Progression

SEB is one of many serotypes in the family of staphylococcal enterotoxins (SEs). These toxins are produced by the *Staphylococcus aureus* bacterium and are part of a large family of similar toxins known as pyrogenic toxin superantigens (PTSAgs).[165] The SE toxins are most commonly encountered in the context of food poisoning when food contaminated with preformed toxin is ingested. When ingested via food products, the effects frequently manifest as nausea, vomiting, and diarrhea, and symptoms such as fever and hypotension are rarely observed in this route of intoxication.[166]

Limited clinical data are available on the effects of SEB due to aerosol exposure; however, laboratory accidents have happened in which the toxin became airborne and exposures occurred, and some individuals were intentionally exposed during the Project Whitecoat trials.[167] Sidell described the clinical course in nine patients who were exposed to airborne SEB.[168] Symptom onset was rapid, within 3–4 hours, and persisted for 3–4 days

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[164] This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

[165] Martin M. Dinges, Paul M. Orwin, and Patrick M. Schlievert, "Exotoxins of *Staphylococcus aureus*," *Clinical Microbiology Reviews* 13, no. 1 (January 2000): 16, doi:10.1128/cmr.13.1.16-34.2000.

[166] Ibid; Kamal U. Saikh, Robert G. Ulrich, and Teresa Krakauer, "Staphylococcal Enterotoxin B and Related Toxins Produced by *Staphylococcus aureus* and *Streptococcus pyogenes*," in *Textbook of Military Medicine: Medical Aspects of Biological Warfare*, ed. Joel Bozue, Christopher K. Cote, and Pamela J. Glass (Office of the Surgeon General, Borden Institute, U.S. Army Medical Department Center and School, Health Readiness Center of Excellence, 2018), 403–414, https://fas.org/irp/threat/cbw/medical.pdf; Teresa Krakauer and Bradley G. Stiles, "The Staphylococcal Enterotoxin (SE) Family: SEB and Siblings," *Virulence* 4, no. 8 (November 15, 2013): 759, doi:10.4161/viru.23905.

[167] COL Phillip R. Pittman et al., "An Assessment of Health Status among Medical Research Volunteers Who Served in the Project Whitecoat Program at Fort Detrick, Maryland," *Military Medicine* 170, no. 3 (March 2005): 185, doi:10.7205/milmed.170.3.183.

[168] Robert G. Ulrich et al., "Staphylococcal enterotoxin B and related Pyrogenic Toxins" in *Textbook of Military Medicine: Medical Aspects of Biological Warfare*, ed. Brigadier General Russ Zajtchuk and Ronald F. Bellamy (Borden Institute, Walter Reed Army Medical Center, 1997), 626–628, https://www.researchgate.net/publication/237283636_STAPHYLOCOCCAL_ENTEROTOXIN_B_AND_RELATED_PYROGENIC_TOXINS.

with fever up to 106°F.[169] Respiratory symptoms consisting of a non-productive cough were present, 89% of those exposed experienced headaches, and most patients experienced some form of gastrointestinal distress.[170] The clinical findings reported by Sidell are similar to those detailed from exposures documented by the United States Army between 1963 and 1964, with fever and cough being the predominant symptoms of exposure.[171]

B. SEB Diagnostic Types

Examination of clinical specimens to determine whether a patient has been exposed to SEs can involve looking for the toxin directly or examining changes in the patient's physiology. SEs are stable molecules in a variety of environments, so, in theory, they should be able to be detected from clinical specimens. However, these toxins bind strongly and quickly to their targets on immune cells, resulting in very low levels of free toxin that can be detected in clinical specimens.[172] The low levels of free toxin in a patient renders direct detection possible only with very sensitive laboratory-based antigen assays, such as an ELISA, to detect the low levels of free toxin. A variety of ELISA methods have been developed with various sensitivities; however, these particular assays are not commercially available and would need to be used by personnel with expertise in performing this type of work.

PCR assays are also available that test for the toxin gene; however, in the context of diagnosis following aerosol exposure to the toxin, these assays would be of limited use because the genetic material needed for these assays may not be present after the toxin is purified and prepared for use on the battlefield. Even if some nucleic acid material remains following purification, the toxin gene would need to be the genetic material remaining in order for a diagnostic based on PCR to provide a result that is specific for SEB rather than for *Staphylococcus aureus* in general. In addition, the DNA could degrade after the toxin has entered the body, and, since there is no bacteria or virus to replicate the DNA, there would not be enough DNA to be detected.

Exposure to SEB frequently results in robust seroconversion such that antibody detection can be performed by ELISA 1 or 2 weeks after initial exposure. Unfortunately, an approach to diagnosis that relies solely on antibody detection will likely lead to numerous false positive results because of the high prevalence of antibodies to

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[169] Ibid., 626.

[170] Ibid., 627.

[171] Janice M. Rusnak et al., "Laboratory Exposures to Staphylococcal Enterotoxin B," *Emerging Infectious Diseases* 10, no. 9 (September 2004): 1544, doi:10.3201/eid1009.040250.

[172] E. Cook et al., "Measurement of Staphylococcal Enterotoxin B in Serum and Culture Supernatant with a Capture Enzyme-Linked Immunosorbent Assay," *Clinical and Vaccine Immunology* 14, no. 9 (September 2007): 1094, doi:10.1128/CVI.00183-07.

staphylococcal superantigens such as the SEs. The potential for high false positive rates is supported by research into the role of various SEs in different disease states, which have demonstrated that nearly all healthy study subjects have antibodies to SEB.[173] In addition, the delay in time for antibody response makes it outside of the scope of this paper (see Section 2.D).

Very little data that describe the kinetics of SEB in human patients have been published; however, one study in mice examined the pharmacokinetics of injected SEB.[174] The results of this study indicated that SEB concentrations became detectable after 5 minutes, peaking after 30 minutes and falling below the femtomolar detection limits of the assay after 24 hours.[175] The kinetics exhibited by SEB in this model suggest that toxin detection is not likely to be easily performed in field conditions since the toxin will no longer be present in the blood shortly after symptoms occur. Vabulas et al. were able to detect SEB in mouse urine up to 48 hours after toxin challenge,[176] and this finding suggests that urine specimens may be useful for field detection although no assays currently exist that can use this specimen. No data are available for diagnosis following aerosol exposures to SEB.

Clinical assays that focus on antibody detection will not be able to provide actionable results in time to be useful because of the lag period between exposure to SEB, the antigen, and the immune systems production of antibody in response. The other complicating factor for antibody-based assays is the high percentage of people who already have antibodies to various SEs such as SEB.[177]

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[173] D. E. Campbell, A. S. Kemp, "Production of Antibodies to Staphylococcal Superantigens in Atopic Dermatitis," *Archives of Disease in Childhood* 79, no. 5 (November 1998): 400–404, doi:10.1136/adc.79.5.400; Ross D. LeClaire and Sina Bavari, "Human Antibodies to Bacterial Superantigens and Their Ability to Inhibit T-Cell Activation and Lethality," *Antimicrobial Agents and Chemotherapy* 45, no. 2 (February 2001): 460–463, doi:10.1128/AAC.45.2.460-463.2001; Elke Schröeder et al., "Prevalence of Serum Antibodies to Toxic-Shock-Syndrome-Toxin-1 and to Staphylococcal Enterotoxins A, B and C in West-Germany," *Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene. Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitolog*y 270, nos. 1–2 (November 1988): 110–114, https://doi.org/10.1016/S0176-6724(88)80146-9.

[174] Ramunas Vabulas et al., "Rapid Clearance of the Bacterial Superantigen Staphylococcal Entertoxin B In Vivo," *Infection and Immunity* 64, no. 11 (November 1996): 4567–4573, doi:10.1128/IAI.64.11.4567-4573.1996.

[175] Ibid. 4570.

[176] Ibid.

[177] Campbell and Kemp, "Production of Antibodies to Staphylococcal Superantigens," 400–404; LeClaire and Bavari, "Human Antibodies to Bacterial Superantigens," 460–463; Schröeder et al., "Prevalence of Serum Antibodies to Toxic-Shock-Syndrome-Toxin-1," 110–114.

C. Modeling

We were unable to develop an appropriate model because the data necessary to make a model were not available.

D. Evaluation

Since only mouse data were available using injection rather than aerosol, we could not determine how likely, relative to symptom onset, a positive test result for SEB following aerosol exposure would be available. Further animal studies may be required for that information. In addition, the technologies currently available for SEB would not be able to be deployed at a lower role of care. Also, no PEP or treatment is available for SEB, and, since it is a toxin, it is non-contagious. Therefore, the utility of a quick diagnostic would likely be more limited than for other diseases.

11. Influenza

Note: This work was originally completed and published in May 2021. [178]

A. Influenza Symptom Progression

Although influenza is not a BW agent, it is a common disease that can influence readiness during larger outbreaks.[179] During the winter influenza season, thousands of military personnel become ill with influenza each week.[180] In addition, many diseases described in this paper produce moderate, non-specific symptoms at symptom onset, before progressing to more severe symptoms. Therefore, medical personnel may assume that the disease is influenza until after influenza is ruled out. Thus, we included influenza in our analysis of diagnostics so we could see how the diagnosis of influenza may differ from that of other diseases.

Influenza symptoms typically include fever, cough, fatigue, and other non-specific symptoms.[181] In most cases, symptoms resolve within 1 week. Therefore, in most instances, individuals would not be hospitalized, and we assume within our model that individuals only arrive at a Role 1 MTF and then leave the medical system. Specimens from the individual, however, could be shipped to a higher role of care or could be shipped to a specialized diagnostics laboratory in theater or in the continental United States. Further discussion of the disease parameters used for influenza is found in Appendix A.

B. Influenza Diagnostic Types

Influenza can be diagnosed via viral isolation, antigen tests (whether rapid or laboratory based), or PCR. Unlike the other agents described in this paper, influenza virus is usually not detectable in blood,[182] so nasal or throat swabs or washes must be used

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[178] This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

[179] HQDA, *Army Biological Defense Strategy*, 4.

[180] Jose L. Sanchez and Michael J. Cooper, "Influenza in the US Military: An Overview," *Journal of Infectious Diseases and Treatment* 2, no. 1 (2016): 1, https://infectious-diseases-and-treatment.imedpub.com/influenza-in-the-us-military-an-overview.php?aid=8781.

[181] Sanchez and Cooper, "Influenza in the US Military," 1.

[182] Susan L. Stramer et al., "Sensitive Detection Assays for Influenza RNA Do Not Reveal Viremia in US Blood Donors," *The Journal of Infectious Diseases* 205, no 6 (March 15, 2012): 886, doi:10.1093/infdis/jir863.

instead. Therefore, medical personnel would not be able to use a single specimen to test for influenza and the other agents discussed in this paper simultaneously.

Viral isolation is one of the gold standards for diagnosis but can take days to complete. Newer techniques allow for results within 24–48 hours of the start of the test, but this amount of time is still much longer than that required for PCR or antigen tests.[183] In addition, viral isolation for influenza must be performed in a BSL-2 laboratory.

PCR tests are also considered a gold standard for influenza diagnosis. PCR tests can be laboratory-based tests or rapid tests. Rapid tests typically use cartridges or other methods so that the test can be done easily at an MTF and can often take approximately 1 hour.[184] Rapid PCR tests are usually more accurate than rapid antigen tests for influenza, although they can be less accurate than laboratory-based PCR tests.[185]

Antigen tests can be either laboratory-based or rapid tests, as for PCR. Rapid antigen tests are commonly used at point-of-care facilities and generate results within 30 minutes.[186] Newer antigen test technologies tend to be more accurate than older ones, although both are often less accurate than PCR tests.[187] However, because antigen tests are easy to use and produce rapid results, they are still useful diagnostic tests at MTF facilities.

C. Probability of Detection Over the Course of the Disease

We located four papers that included information about the results of influenza diagnostics relative to symptom progression. All these papers examined humans rather than NHPs. For our model, we chose information from Cheng et al.[188] and Lee et al.[189] Tsang

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[183] Sai Vikram Vemula et al., "Current Approaches for Diagnosis of Influenza Virus Infections in Humans," *Viruses* 8, no. 4 (April 12, 2016): 96, doi:10.3390/v8040096.

[184] Laura M. Vos et al., "Rapid Molecular Tests for Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses: A Systematic Review of Diagnostic Accuracy and Clinical Impact Studies," *Clinical Infectious Diseases* 69, no. 7 (September 13, 2019): 1243, doi:10.1093/cid/ciz056.

[185] Joanna Merckx et al., "Diagnostic Accuracy of Novel and Traditional Rapid Tests for Influenza Infection Compared with Reverse Transcriptase Polymerase Chain Reaction: A Systematic Review and Meta-Analysis," *Annals of Internal Medicine* 167, no. 6 (September 19, 2017): 399–400, https://pubmed.ncbi.nlm.nih.gov/28869986/.

[186] Vemula et al., "Current Approaches for Diagnosis, 96.

[187] Merckx et al., "Diagnostic Accuracy of Novel and Traditional Rapid Tests," 401–402.

[188] Peter K. C. Cheng et al., "Performance of Laboratory Diagnostics for the Detection of Influenza A(H1N1)v Virus as Correlated with the Time After Symptom Onset and Viral Load," *Journal of Clinical Virology* 47, no. 2 (February 2010): 182–185, https://doi.org/10.1016/j.jcv.2009.11.022.

[189] Chang-Seop Lee, Ju-Hyung Lee, and Cheon-Hyeon Kim, "Time-Dependent Sensitivity of a Rapid Antigen Test in Patients with 2009 H1N1 Influenza," *Journal of Clinical Microbiology* 49, no. 4 (April 2011): 1702, doi:10.1128/JCM.02247-10.

et al. looked at the transmission of influenza through households.[190] Therefore, they followed up with household members who were known to be sick with influenza and took diagnostic specimens each day. Since they were looking at household transmission, most of the individuals whom they tested were children (68% were under 18).[191] Children tend to have higher viral loads of influenza, so the results may not be comparable.[192] In addition, they only used an in-house PCR assay instead of using multiple techniques. Therefore, we did not include that information in our model. Carrat et al. summarized information from human challenge studies of influenza, which included information on viral isolation levels over time. We did not include information from that paper either, since no PCR or antigen test information was included and most of the challenge studies used intranasal infection, which may produce a milder disease than through respiratory droplets or other natural means.[193]

For our modeling, we largely took parameters from Cheng et al.[194] In this paper, the scientists tested 587 nasopharyngeal aspirate or throat and nasal swab specimens from different influenza-positive individuals using a rapid antigen test, an in-house PCR assay, or viral isolation. They also noted how many days after symptom onset the specimen was taken, which enabled us to correlate the diagnostic results with symptom progression.[195] They did not include specimens taken after antiviral treatment was given. This paper used the ESPLINE rapid antigen test, which is more commonly used in Asian countries. Cheng et al. did not include information on rapid antigen test results for throat or nasal swabs. Therefore, we used information from Lee et al. for that information.[196] Lee et al. used a different rapid antigen test, the SD Bioline test, also more commonly used in Asian countries. We could not find a paper that described the results of antigen tests more commonly used in the United States over the course of disease. Lee et al. similarly used previous nasopharyngeal swab specimens to determine how well the rapid antigen test or PCR could diagnose individuals compared to symptom progression.[197] Table 20 shows the final test probability of detection values we used in our modeling.

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[190] Tim K. Tsang et al., "Individual Correlates of Infectivity in Influenza A Virus Infections in Households," *PLoS One* 11, no. 5 (May 6, 2016): e0154418, doi:10.1371/journal.pone.0154418.

[191] Ibid.

[192] Fabrice Carrat et al., "Time Lines of Infection and Disease in Human Influenza: A Review of Volunteer Challenge Studies," *American Journal of Epidemiology* 167, no. 7 (April 1, 2008): 776, doi:10.1093/aje/kwm375.

[193] Ibid., 781.

[194] Cheng et al., "Performance of Laboratory Diagnostics," 182–185.

[195] Ibid., 183.

[196] Lee, Lee, and Kim, "Time-Dependent Sensitivity of a Rapid Antigen Test," 1702.

[197] Ibid.

Table 20. Influenza Diagnostic Probability of Detection Values Included in Modeling Analysis

Technology (Specimen)	Stage of Disease	Probability of Detection
In-house PCR (TNS)	Symptom Onset	90%
Rapid antigen (TNS)	Symptom Onset	77%
Viral isolation (TNS)	Symptom Onset	94%
In-house PCR (NPA)	Symptom Onset	100%
Rapid antigen (NPA)	Symptom Onset	36%
Viral isolation (NPA)	Symptom Onset	100%

Note: The abbreviations used in this table are defined in Appendix E of this paper.

D. Modeling

Our modeling of influenza used the same analytic approach that we used for the other diseases of interest despite the differences between an influenza outbreak and a BW attack. This approach was used to facilitate comparisons between the timing of diagnostic results for influenza to those of the other diseases of interest and for completeness. As was done for other diseases we modeled all the diagnostic test-specimen combinations for which we could obtain sufficient data—even if some of these combinations are unlikely to be used in a deployed medical environment. In addition, we simulated the simultaneous infection of either 10, 100, or 1,000 individuals and did not model the spread of the disease beyond these initially infected individuals. In most natural influenza outbreaks, 1,000 individuals would not be infected on a single day for a population, unlike what may occur from an intentional attack with anthrax, plague, or another disease assessed in this paper. A large influenza outbreak would likely derive from contagion rather than from a single event. Therefore, larger numbers of initial infections on a single day are unrealistic for influenza but are included for comparability with other diseases.

As described in Table 20, we were able to locate sufficient data to model three influenza diagnostics technologies (an in-house PCR, a rapid antigen test, and viral isolation) and two specimen types (nasopharyngeal aspirate and throat and nasal swabs). Although we include results for all these combinations in our summary modeling table (Table 21), not all these combinations would be typically employed in routine influenza screening at an MTF. Viral isolation, for example, is a complicated process that can require a BSL-2 or higher laboratory facility (Section 2.A). Nasopharyngeal aspirate specimens are also much more difficult to obtain than throat or nasal swabs since they require a suction

device.[198] Therefore, a typical MTF would likely use a throat or nasal swab specimen rather than a nasopharyngeal aspirate specimen. The model results for these diagnostic tests or specimen types are included in Table 21 for completeness but are discussed minimally in this section.

Our quantitative analysis of these technologies focuses primarily on the timing of positive diagnostic results under various clinical sampling TTPs. The focus of the analysis for influenza is on the timing of influenza results and how that timing may differ from other diseases. This paper, as a whole, looks at clinical sampling for biological agents, and influenza is included because it would be logical for medical personnel to test for it before other diseases are considered. Therefore, we focus this chapter on TTPs for influenza that may be most likely to be used before testing for a BW agent. The modeling analysis of influenza, therefore, focuses primarily on how the following TTP components impact the timing of diagnostic results following exposure:

- The type of point of care test used (rapid antigen vs. PCR),
- Delaying the collection of clinical specimens until multiple individuals report to the medical system following an attack, and
- Use of viral isolation at a reachback laboratory or use of nasopharyngeal aspirates.

In addition to determining how different TTP components impact the timing of diagnostic results following the attack, we also looked at what information would be available from syndromic indicators by the time that diagnostic results can be obtained.

We simulated simultaneous infections of either 10, 100, or 1,000 individuals. In addition to positive diagnostic results, the presence of numerous individuals with similar symptoms reporting to the medical system can also inform situation awareness. Figure 10 shows a representative time progression of influenza cases by severity level following an attack and the median time of the first positive diagnostic result for the TTP options shown in Table 21.

[198] Terho Heikkinen et al., "Nasal Swab versus Nasopharyngeal Aspirate for Isolation of Respiratory Viruses," *Journal of Clinical Microbiology* 40, no. 11 (November 2002): 4337, doi:10.1128/jcm.40.11.4337-4339.2002.

Table 21. Median Time (Days) of First True Positive Diagnostic Result Following a Given Number of Initial Influenza Infections for Various Sampling and Analysis TTP Options

Option	Technology (Specimen)	Test Location	Specimen Collection Trigger	Median Time of First True Positive Result (Days)		
				10 Infections	100 Infections	1,000 Infections
A	In-house PCR (TNS)	Role 1 MTF	Patient arrival at Role 1 MTF	0.9	0.6	0.5
B	Rapid Antigen (TNS)	Role 1 MTF	Patient arrival at Role 1 MTF	0.8	0.5	0.4
C	In-house PCR (TNS)	Role 1 MTF	Patient arrival at Role 1 MTF; start sampling on tenth case	2.7	0.9	0.6
D	Rapid Antigen (TNS)	Role 1 MTF	Patient arrival at Role 1 MTF; start sampling on tenth case	2.6	0.8	0.5
E	In-house PCR (TNS)	Role 3 MTF	Patient arrival at Role 1 MTF with any symptoms; specimens shipped from Role 1 MTF to Role 3 MTF every 12 hours	1.3	1.1	0.9
F	Rapid Antigen (TNS)	Role 3 MTF	Patient arrival at Role 1 MTF with any symptoms; specimens shipped from Role 1 MTF to Role 3 MTF every 12 hours	1.3	1.0	0.8
G	In-house PCR (NPA)	Role 1 MTF	Patient arrival at Role 1 MTF	0.8	0.6	0.5
H	Rapid Antigen (NPA)	Role 1 MTF	Patient arrival at Role 1 MTF	1.0	0.6	0.4
I	In-house PCR (NPA)	Role 1 MTF	Patient arrival at Role 1 MTF; start sampling on tenth case	2.7	0.9	0.6
J	Rapid Antigen (NPA)	Role 1 MTF	Patient arrival at Role 1 MTF; start sampling on tenth case	2.5	0.8	0.5
K	In-house PCR (NPA)	Role 3 MTF	Patient arrival at Role 1 MTF with any symptoms; specimens shipped from Role 1 MTF to Role 3 MTF every 12 hours	1.3	1.1	0.9

Option	Technology (Specimen)	Test Location	Specimen Collection Trigger	Median Time of First True Positive Result (Days)		
				10 Infections	100 Infections	1,000 Infections
L	Rapid Antigen (NPA)	Role 3 MTF	Patient arrival at Role 1 MTF with any symptoms; specimens shipped from Role 1 MTF to Role 3 MTF every 12 hours	1.4	1.1	0.9
M	Viral isolation (TNS)	Laboratory	Patient arrival at Role 1 MTF	4.8	4.5	4.4
N	Viral isolation (NPA)	Laboratory	Patient arrival at Role 1 MTF	4.7	4.5	4.4

Note: The abbreviations used in this table are defined in Appendix E of this paper.

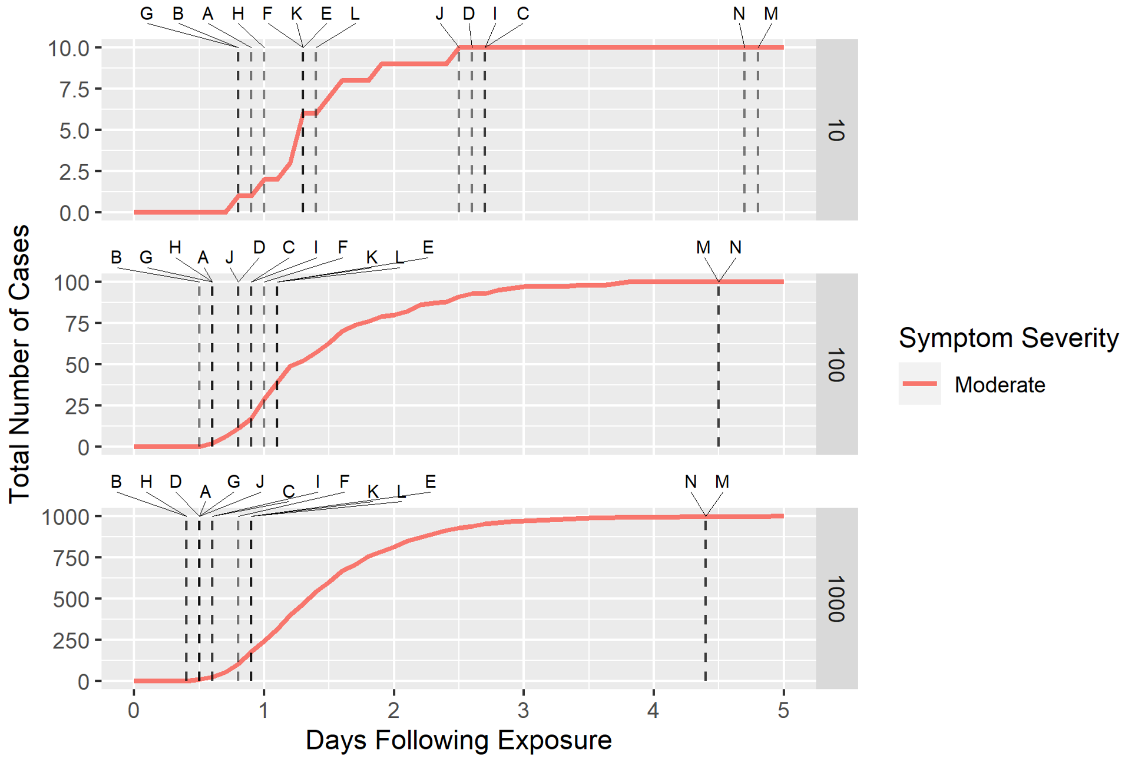

Figure 10. Representative Total Numbers of Influenza Cases by Severity Level over Time for 10, 100, or 1,000 Individuals Initially Infected and Median Time of First Positive Diagnostic Result for TTP Options Listed in Table 21 (Vertical Dashed Lines)

1. Type of Test Used (Rapid Antigen vs. PCR)

We compared TTP options A and B to look at how the type of test influences how quickly positive diagnostic results can be obtained (see Table 21). PCR tests are typically more sensitive than rapid antigen tests for influenza (see Table 20). Rapid antigen tests, however, are typically easier to employ at a lower role of care, often less expensive, and produce results more quickly.[199] The difference in timing of positive diagnostic results between rapid antigen tests and PCR in our modeling was only 0.1 days (see Table 21), suggesting that the choice of test had minimal effect on the timing of results. The incubation period for influenza is short, and most individuals report to the medical system within the first two days of exposure (see Figure 10).

Positive results for influenza tests given to anyone with influenza symptoms, as modeled, came back within a day of infection for any of the numbers of initial infections modeled. Thus, testing individuals for influenza, especially when multiple individuals report to the medical system with moderate, non-specific symptoms, could quickly determine that individuals did have influenza. Looking at time after multiple individuals

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[199] Vemula et al., "Current Approaches for Diagnosis," 96.

report to the medical system rather than time relative to infection, positive test results usually come back, as modeled, within 0.1 days, for either an antigen or a PCR test (see Table 20). This modeling supports the idea that taking influenza tests first is an important step in the diagnosis of potential BW agents, since BW panels could be used after multiple individuals test negative for influenza. Rapid influenza tests are suitable for use at farther forward MTFs, although they may not be available at Role 1. Therefore, additional rapid influenza tests at Role 1 may be required to screen individuals for influenza more quickly. In addition, PCR or antigen influenza tests are usually 90% or more specific, which makes it unlikely that multiple individuals would receive positive influenza results without having influenza (false positives).[200]

2. Delaying the Collection of Clinical Specimens until Multiple Cases Report

It is possible that a clinician would simply assume that individuals had influenza and not bother administering a test until multiple cases with moderate, non-specific symptoms reported to the medical system. This situation may especially occur if testing supplies are limited or unavailable at a given MTF. We compared TTP options C and D to options A and B to analyze the timing of positive diagnostic results associated with delaying collecting specimens until 10 infected individuals report to the medical system. As shown in Table 21, these TTP options resulted in a median time of the first positive results of 2.6–2.7 and 0.8–0.9 days for 10 or 100 initial infections, respectively. As with options A and B, the difference between the rapid antigen test and the PCR test results timing was only 0.1 days. When compared to testing the first patient who reports to the medical system (TTP options A and B), delaying collecting specimens until the tenth case delays the median time of the first positive result by 1.8 and 0.3 days for 10 or 100 initial infections, respectively.

As noted in the previous subsection, obtaining a quick result for influenza can help clinicians rapidly determine whether an individual is sick with a common disease or something more serious, such as a BW agent. Therefore, waiting to collect specimens for influenza until after multiple individuals have reported may unnecessarily delay diagnosis to determine whether individuals have influenza or a have been exposed to a BW agent.

We modeled TTP Options E and F to analyze the delay in diagnostic results associated with collecting specimens at a Role 1 MTF and shipping them to higher MTF for testing. As shown in Table 21, shipping specimens results in a delay of 0.4–0.5 days across both test types and all initial infection categories. This delay meant that positive diagnostic results were available on days 1.3 and 1.0–1.1 for 10 and 100 initial infections,

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[200] Michael L. Jackson and Kenneth J. Rothman, "Effects of Imperfect Test Sensitivity and Specificity on Observational Studies of Influenza Vaccine Effectiveness," *Vaccine* 33, no. 11 (March 2015): 1314, https://doi.org/10.1016/j.vaccine.2015.01.069.

respectively. The difference was a maximum of 0.1 days between PCR and rapid antigen test results, as seen in the other options. As noted in the previous subsection, a quick diagnostic result for influenza may allow clinicians to confirm or rule out influenza and provide treatment accordingly. The 0.4- to 0.5-day delay in diagnostic results for shipping specimens would delay the results. Since influenza tests, especially rapid antigen tests, are easy to use and can be deployed at lower roles of care, our model results support the idea that influenza diagnostics at Role 1 or 2 MTFs would be useful for TTPs to screen for potential BW exposures.

3. Viral Isolation at Reachback Laboratory or Use of Nasopharyngeal Aspirates

Some options included in our model would be unlikely to be used in a deployed setting. These options include using nasopharyngeal aspirate specimens at Role 1 or 2 and testing for influenza using viral isolation at a reachback laboratory. These options are included here for completeness, although they are less relevant for our conclusions.

Nasopharyngeal aspirate specimens are taken using a suction system that is cumbersome to use compared to throat or nasal swabs, especially at a lower role of care.[201] As shown in Table 21, the results for nasopharyngeal aspirate specimens are similar to those for throat or nasal swabs. Therefore, according to our model and its assumptions, nasopharyngeal aspirate specimens may not confer benefits beyond throat and nasal swabs.

Viral isolation at a reachback laboratory can take days to complete since it requires complicated laboratory procedures and must be done at a BSL-2 or higher facility (see Section 2.A). Therefore, as modeled, positive viral isolation results do not come until 4.4–4.8 days after initial infection (see Table 21), which is well after results from any other TTP option that we modeled and would be available and after almost every individual has become symptomatic (see Figure 10). Therefore, viral isolation would not be useful for routine screening of suspected influenza patients. Viral isolation and other laboratory procedures, however, can be useful if disease dynamics are sufficiently abnormal (e.g., more severe or more contagious) and the virus needs to be typed more accurately or sequenced to determine whether a strain is novel.

4. Syndromic Indicators

In instances where influenza tests are unavailable or scarce, clinicians may assume that the cause of disease is influenza without testing. Table 22 shows representative timing of when certain numbers of total individuals with symptoms would occur following a given number of initial infections. Since influenza produces non-specific symptoms, the numbers of ill individuals would likely not provide any more situational awareness than the typical

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[201] Heikkinen et al., "Nasal Swab versus Nasopharyngeal Aspirate," 4337.

assumption that the moderate, non-specific symptoms were caused by influenza. In addition, as noted earlier, in an influenza outbreak, large numbers of individuals infected on a given day would be unlikely. Still, Table 22 shows that given the short incubation period of influenza, case numbers can quickly increase given a certain number of initial infections.

Table 22. Representative Time (Days) When Various Numbers of Total Cases by Severity Level Occur

		Time of Total Number of Cases by Severity Level								
		10 Infections			100 Infections			1,000 Infections		
	Total Number of Cases	1	3	5	1	5	10	1	10	50
Symptom Severity	Moderate	0.8	1.2	1.3	0.6	0.7	0.8	0.5	0.5	0.7

5. Implications for Response Implementation

Although no PEP is available for influenza, antiviral treatments are available. Antiviral treatments for influenza such as Tamiflu can decrease symptom duration by approximately 1 day and should be given early in the disease progression.[202] Delaying antiviral treatment, therefore, can decrease its utility; however, diagnostic results can be provided quickly, and Tamiflu could be sent to a Role 1 within hours, though supplies may be limited.

In addition, influenza is a contagious disease. This attribute is not accounted for in the model and would impact the actual distribution of arrival times to the medical system for symptomatic individuals, especially given the short incubation period for influenza. Previous IDA analyses found that influenza outbreaks can spread quickly, reaching 20% of the force within a week after a single initial infection.[203] In most influenza outbreaks, ROM is not required. In a large influenza outbreak, however, ROM and other measures (e.g., isolation) may be implemented to prevent further spread of disease, although the short incubation time makes these actions difficult to use successfully to contain influenza outbreaks completely.[204] Additional discussion of the timeliness and effectiveness of various contagious disease response measures for influenza can be found in IDA Paper P-10877.[205]

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[202] Joanna Dobson et al., “Oseltamivir Treatment for Influenza in Adults: A Meta-Analysis of Randomised Controlled Trials,” *Lancet* 385, no. 9979 (May 2, 2015): 1736, doi:10.1016/S0140-6736(14)62449-1.

[203] Burr et al., *Emerging Infectious Diseases Study*, 74.

[204] Ibid., vi.

[205] Burr et al., *Controlling the Spread of Contagious Disease*.

E. Evaluation

As noted in the previous section, timely diagnosis of influenza could allow clinicians to quickly determine whether ill individuals reporting to an MTF are the result of a natural influenza outbreak or another cause, such as a BW attack. Influenza has a short incubation period, which means that individuals become ill and report to the MTF shortly after infection (see Table 22). Testing individuals when they report to the Role 1 MTF results in a positive diagnosis within 1 day of initial infection, even with low numbers of initial infections (see Table 21). The results did not change significantly for a rapid antigen test vs. a PCR test. Other TTP options, such as shipping specimens and waiting until 10 individuals reported to the MTF generally delayed results by 0.3 days or more. Although this amount of time is a relatively short period, it may make it more difficult to use if the purpose of testing for influenza is to screen for whether an individual has influenza or another, more serious disease. Thus, it may be best to prioritize influenza tests at lower roles of care and then use tests for BW agents only after influenza tests have come back negative (or if other intelligence is available that suggests a BW attack has occurred). This approach would add the time it takes to run the influenza test (15 minutes for a rapid antigen test or 1 hour for a PCR) to time to achieve a positive result for each TTP option for other diseases.

It is worth reiterating that the exact modeling results presented here are driven by the performance of the in-house PCR and the rapid antigen test that we simulated. Most PCR tests for influenza have high probability of detection, and most rapid antigen tests have medium probability of detection when analyzing specimens collected at symptom onset,[206] although the exact numbers could be different between different systems. The in-house assay and the rapid antigen test are not the exact tests that would be used at an MTF. The difference in timing of results, however, was small between the more sensitive PCR test and the less sensitive rapid antigen test, likely due to the short incubation period and quick arrival of infected individuals to the MTF.

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[206] Jackson and Rothman, "Effects of Imperfect Test Sensitivity and Specificity," 1314.

12. Conclusions and Recommendations

Note: This work was originally completed and published in May 2021. [207]

This paper evaluates how various TTPs for the collection and analysis of clinical specimens influence the timing and accuracy of diagnostic test results for BW diseases and influenza. The analysis included a scientific literature review, the results of which we used to characterize the times during a given disease's progression that a given diagnostic test can generate accurate results. The literature review results were fed into a model to determine how quickly different TTP options could produce results, and then overall conclusions were determined based on the literature review and the model results.

A. Data Limitations

One of the major findings of the analysis was the limited data available in the scientific literature comparing diagnostic probability of detection to symptom progression. Figure 11 depicts the data gaps that we found in our literature review. Figure 11 is the same as Figure 4 but is repeated to further explain the conclusions that we can draw from this figure. Within the figure, the disease-specimen-test combinations for which we could find sufficient data are shown in blue and those for which we could not find sufficient data are shown in orange. Those combinations for which diagnostics do not exist (e.g., we could find no rapid antigen tests for Q fever) are shown in gray.

As shown in Figure 11, we found insufficient data on cartridge-based PCR assays or antigen assays (either laboratory or rapid) to enable us to characterize the probability of detection over the course of disease progression for most of the diseases that we examined. We could not find sufficient data on tests run with sputum specimens. Most of the sufficient data that we could find were on limited numbers of primates or humans, which means that the data that were available were often of low confidence. These difficulties limited our ability to draw conclusions across diseases and specimen types. Additional experiments would need to be conducted to fill in these gaps.

[207] This chapter has minor editorial changes from Bishop et al., *Evaluation of Biological Agent Clinical Sampling and Analysis*.

Disease	Specimen Type	Bacterial / Viral / Toxin Isolation	PCR (In-House)	PCR (Cartridge)	Antigen (ELISA / Mass Spec)	Antigen (Rapid)
Plague	Blood	Sufficient (Non-Human Primate Data)	Sufficient (Non-Human Primate Data)	Insufficient	Insufficient	Insufficient
	Sputum	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient
Q Fever	Blood	Test Does not Exist	Sufficient (Non-Human Primate Data)	Insufficient	Test Does not Exist	Test Does not Exist
Tularemia	Blood	Sufficient (Non-Human Primate Data)	Sufficient (Non-Human Primate Data)	Sufficient (Non-Human Primate Data)	Insufficient	Test Does not Exist
	Sputum	Insufficient	Insufficient	Insufficient	Insufficient	Test Does not Exist
Ebola	Blood	Insufficient	Insufficient	Sufficient (Human Data)	Sufficient (Human Data)	Insufficient
VEE	Blood	Sufficient (Non-Human Primate Data)	Insufficient	Insufficient	Insufficient	Test Does not Exist
Anthrax	Blood	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient
SEB	Blood	Test Does not Exist	Test Does not Exist	Test Does not Exist	Insufficient	Insufficient
Influenza	TNS	Sufficient (Human Data)	Sufficient (Human Data)	Insufficient	Insufficient	Sufficient (Human Data)
	NPA	Sufficient (Human Data)	Sufficient (Human Data)	Insufficient	Insufficient	Sufficient (Human Data)

Sufficient Test Results Data Found
Insufficient Test Results Data Found
Test Does not Exist
Human Data
Non-Human Primate Data

Note: The abbreviations used in this table are defined in Appendix E of this paper.
Note: This work was originally completed and published in May 2021.

Figure 11. Availability of Data Describing Diagnostic Probability of Detection over the Course of Disease Progression

A rapid antigen test or a cartridge-based PCR test would be the most likely test used at a lower role of care (see Chapter 2). Since we could not find sufficient information on cartridge-based PCR tests for many diseases, our models often extrapolated from in-house PCR tests. As noted in Chapter 2, in-house PCR tests can be more complicated and take longer to perform than a cartridge-based test. In addition, in-house assays or different cartridge-based assays may test for different genes or have higher or lower limits of detection than the cartridge-based assays (e.g., NGDS 1 or NGDS 2) that the military uses. Therefore, the data from in-house assays cannot be directly used to inform decisions on how to best use specific instruments such as NGDS 1 or NGDS 2.

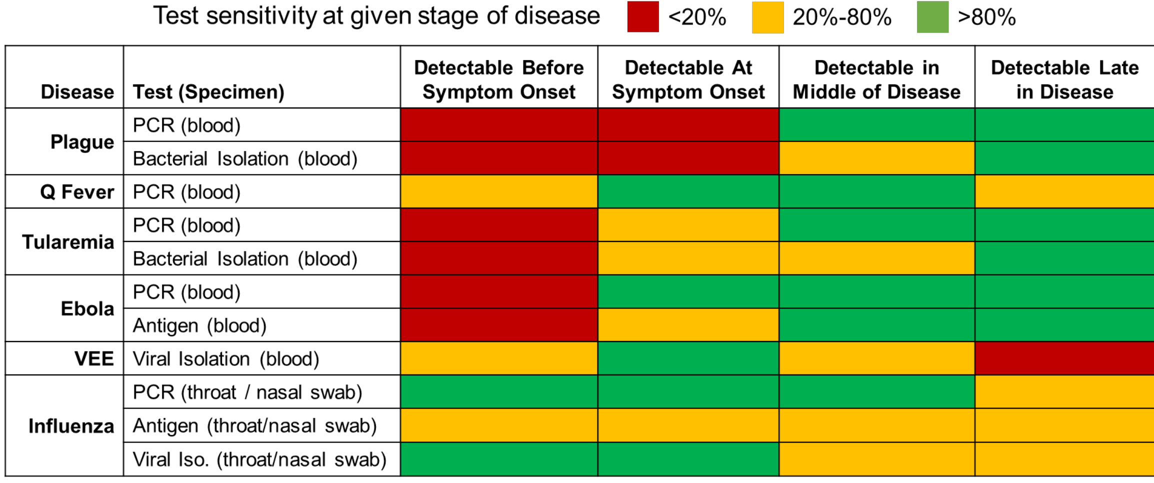
This paper thus makes general conclusions about types of tests and cannot make conclusions about or recommend TTPs for specific systems. To properly inform decisions about NGDS systems, especially those that may be employed farther forward, such as NGDS 2, information about the probability of detection throughout symptom progression is necessary. Where possible, this requirement should be included within Capability Development Documents (CDDs) to best inform the basis of issue plans of future diagnostics systems. In addition, that information should be provided to clinicians and users so that they can best determine the utility of the diagnostic system in a given situation.

- ***Conclusion.*** *Critical gaps exist in the data that describe the performance of diagnostic tests employed by—or soon to be employed by—DOD. Specifically, data do not exist to characterize the probability of detection of these systems when analyzing specimens collected during the early stages of multiple diseases of concern.*

- ***Recommendation.*** *Include requirements for sensitivity over the course of illness (particularly the early stages) in CDDs for diagnostic systems that are intended to be used for early diagnosis.*
- ***Recommendation.*** *Conduct experiments to characterize diagnostic sensitivity over the course of illness (particularly the early stages) for the systems of interest (e.g., NGDS 2) to inform analysis-based TTPs for use of those systems using the methods described in this paper.*
  - *Update initial/full operational capability numbers according to analysis-based TTPs.*
  - *Update doctrine, including ATP 4-02.84,*[208] *accordingly.*

B. Literature Review and Model Results

When sufficient data were available (the blue boxes in Figure 11), we determined the likelihood of a positive test result during the disease progression. These results, taken directly from the literature review, are shown in Figure 12.



Note: This work was originally completed and published in May 2021.

Figure 12. Probability of Detection at a Given Stage of Disease

As shown in Figure 12, some diseases, such as influenza, tend to produce higher pathogen indicators at the beginning of (or even before) symptom onset, while others, such as EVD or plague, tend to only produce higher pathogen indicators late in the disease. This

[208] Headquarters, Department of the Army (HQDA), *Multi-Service Tactics, Techniques, and Procedures for Treatment of Biological Warfare Agent Casualties*, ATP 4-02.84/MCRP 3-40A.3/NTRP 4-02.23/ AFMAN 44-156_IP (HQDA, November 2019), https://armypubs.army.mil/ProductMaps/PubForm/Details.aspx?PUB_ID=1008190.

information, along with other disease attributes, can influence what TTP options would be more or less useful to obtaining positive diagnostic results quickly. The results of this paper could also help inform the specimen collection guidance as described in ATP 4-02.84 Table A-2.[209] We could not give specific recommendations for this chart due to the lack of data from our literature review, but some of our literature review indicates that parts of the table should be updated:

- Adding PCR or antigen tests for EVD rather than only recommending viral culture, and
- Making the clinical sampling guidance clearer about whether it is timed based on the time from exposure or the time from symptom onset (which would be dependent on incubation period).

Thus, the guidance in ATP 4-02.84 should be reexamined with current methods to determine whether it should be altered. Additional information from conducting experiments on currently or soon-to-be fielded diagnostic technologies within DOD could help inform new updates to ATP 4-02.84.

Although our model results could not directly inform the basis of issue plans for specific diagnostic systems within the medical system, they do give some indications for what sorts of disease attributes would make concepts such as specimen shipment or waiting to test until the onset of severe symptoms more or less valuable. Conclusions related to the attributes are discussed Subsections 12.B.1–12.B.3. The main conclusion for all three is as follows:

- ***Conclusion.*** *TTPs for use of diagnostics are not one-size-fits-all, but rather they must be designed specifically to suit the progression of disease and its detectability with given diagnostics. However, DOD currently cannot design a BW disease detection TTP to suit all diseases of interest, because it lacks information on how detectability of diseases varies during the course of each disease for its fielded BW-related diagnostics.*

1. Pathogen Indicator Levels at Symptom Onset

Of the disease-specimen-test combinations that we could model, the likelihood of a given combination yielding a positive diagnosis at symptom onset ranged from 5% to 100% (see Figure 12). In most situations, the difference between moderate or higher (> 40%) positivity rates did not greatly impact the timing of a first diagnostic result, especially when the numbers of initial infections are large. This lack of impact occurs because enough individuals report to the medical system over time such that a false negative does not delay

[209] Ibid., A-5–A-6.

the results significantly. This phenomenon may not be true for especially low positivity rates at symptom onset, however.

We can use the results from our plague model to analyze how a low pathogen indicator level at symptom onset can influence the utility of different diagnostic options. As modeled, the positivity rate at symptom onset for plague was especially low (5%). This low rate means that if specimens were taken at symptom onset, almost all individuals tested would have false negative results and only a few true positives would be detected. Our modeling results suggest that given a sufficiently large flow of patients into the medical system and a sufficient testing capacity, the delay caused by these false negative results could be relatively small.

However, clinicians may cease running the test before generating the first true positive result for two reasons. First, without knowing the probability of detection at different points of disease progression, a user may incorrectly conclude that false negative results are true negative results. Therefore, the presence of a single or relatively few true positive results could be incorrectly dismissed as false positives. Second, testing capacity at a facility could become exhausted before the first true positive result occurring. In either case, situational awareness and subsequent response could be confounded until testing can be done at higher roles of care. This delay, in turn, can impede the implementation of disease-specific countermeasures.

That being said, these problems could potentially be mitigated if the low probability of detection of the test in the early stage of disease was known. For example, if clinicians were aware of the low probability of detection of the diagnostic that they were using, then they may be less likely to misclassify a false negative as a true negative or a true positive as a false positive. Furthermore, if a test with low probability of detection at symptom onset is intended to be used at a lower role of care, then a sufficient quantity of those tests should be available along with the capacity to run them to enable the large number of tests that is likely needed to produce a positive result.

We did not include false positive results within our analysis, which is a limitation of this paper. Even a system with a very low rate of false positives may cause frequent false positives if TTPs are used that often or always tests individuals with non-specific symptoms. False positive information could undermine confidence in the diagnostic information used by decision makers. This situation may be especially true when false negatives are common for a disease early in symptom onset since a true positive result along with many false negative results may appear to be another false positive.

The numerous data gaps that we identified in our literature review suggest that military clinicians may be put in the position of using diagnostic tests for which they have little if any knowledge of its performance. This lack of knowledge could result in the misinterpretation of diagnostic results, which, in turn, could delay the administration of

time-critical response measures that can prevent casualties and fatalities. Furthermore, by not knowing the probability of detection of a given test at symptom onset, the utility of employing that test to lower roles of care cannot be known and could result in the unnecessary fielding of diagnostic capabilities to a given role of care with limited utility at that role of care.

- ***Recommendation.*** *Ensure that diagnostic users and clinicians are educated on the performance of diagnostics at various stages of disease, especially for rarely diagnosed diseases, and whether diagnostics may need to be used again at set intervals.*

2. Initial and Maximum Symptom Severity

We modeled some of the diseases as beginning with a moderate, non-specific illness (EVD, plague, Q fever, influenza), while we modeled others as beginning with severe, non-specific symptoms (tularemia, VEEV disease) (see Table 1). Although within the model, diseases cause one or the other symptom severity only, in reality, not all individuals with a given disease may have the same initial symptom severity. As modeled, once an individual develops severe symptoms, he or she moves through the roles of care until he or she is an inpatient at a Role 3 facility (since AMedP-7.5 defines severe symptoms as those that require inpatient care).[210] Thus, as modeled, for individuals who initially present with severe symptoms of tularemia or VEEV disease, the time between taking a specimen at Role 1 at symptom onset and taking a specimen once the patient has been moved to Role 3 is based on only the time that it takes the patient to move from a Role 1 to a Role 3 (see Table 12 and Table 18).

This progression is different from a disease that first produces moderate, non-specific symptoms. If a disease, such as plague or EVD, first presents with moderate, non-specific symptoms and then later progresses to more severe symptoms, then, as modeled, an individual may first present to a Role 1 facility for sick call and then leave the medical system until he or she becomes ill enough to require in-person care (see Figure 3). For a disease such as EVD, where the time between the onset of moderate, non-specific symptoms and more severe symptoms can be long, this situation could lead to a delay in diagnostic results of days, especially when there are few initial infections (see Table 15).

Some diseases, such as influenza and Q fever, usually do not progress beyond moderate severity. In that case, individuals would typically not be moved beyond a lower role of care and would instead recover outside the medical system. A specimen would need to be taken at the lower role of care and either tested there or shipped to a higher role of care and tested at that facility. If regular specimen shipment procedures were in place, tests

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[210] NATO, *AMedP-7.5*, 1–10.

can be kept at higher roles of care with specimens shipped to them, which could decrease the cost compared to maintaining many systems at lower roles of care. Otherwise, a test at a lower role of care would be required in addition to any tests at higher roles of care.

3. Speed of Disease Progression

As noted in the previous subsection, collecting specimens at the onset of more severe symptoms later in the disease rather than at symptom onset can result in a significant delay if the time between symptom onset and the onset of severe symptoms is long. For some diseases, such as plague, the onset of severe symptoms is soon after symptom onset, which negates some of the benefit of taking specimens at symptom onset since the timing of taking diagnostic specimens is not very different between the two. Due to the fast progression of illness, however, short delays in achieving a diagnostic result may lead to many more individuals becoming ill or developing severe disease during that time frame, depending on the incubation period.

In addition, some of the diseases modeled have highly variable incubation periods (e.g., EVD), while others have less variable incubation periods (e.g., tularemia). For diseases that have less variable incubation periods, many people can arrive at an MTF within a short window of time and could potentially be tested. This situation could mean that a less sensitive test can still give positive results in some people, although there will also be many false negative results. Accordingly, diagnostic results can be obtained more quickly for such diseases than for those with highly variable incubation periods, where only a few individuals report to an MTF on a given day. The number of initial infections also influences such calculus since a large number of infected individuals will result in a correspondingly large number of individuals who develop symptoms on a given day, even in the case of diseases with highly variable incubation periods.

C. Broader Conclusions

Along with the conclusions that we could draw about specific diseases or disease attributes in earlier chapters, we were also able to glean some broader conclusions about the benefits of testing for different diseases earlier rather than later. Although testing at a lower role of care can often lead to earlier diagnostic results, the cost—fiscal and other—could be quite high for maintaining a diagnostic system at these lower roles. In addition, the benefit from testing at a lower role generally only comes from testing individuals early in their disease progression when they may only have moderate, non-specific symptoms. When individuals have more severe symptoms, they should be quickly moved through the roles of care, thus reducing the benefit of testing at a lower role of care (see Subsection 12.B.2). TTPs, however, must incorporate results from all relevant diseases since the user of a diagnostic system would not know which disease a patient has until after the diagnostic has been run.

- ***Conclusion.*** *Testing at lower roles of care has the most benefit when individuals first present with non-severe symptoms, assuming that the pathogen indicator levels are high enough that they can be detected using diagnostics at lower roles of care.*

Testing at the onset of moderate, non-specific symptoms may produce the fastest diagnostic result for some of the diseases modeled but also would require significant resources and time. In the majority of cases, the moderate, non-specific symptoms result from influenza or other common diseases rather than from a BW attack. In addition, different specimens are required for influenza than for most biological agents (throat or nasal swab vs. blood), so tests could not be run for both simultaneously using the same specimen. Therefore, it may be a better use of resources to test individuals for influenza and/or other more common diseases first. As noted in Chapter 11, influenza tests can be easy to use in point-of-care settings and typically have high true positive rates early in the disease progression. Influenza tests also typically have low false positive rates, so it is unlikely that multiple individuals exposed to a BW agent would test positive for influenza without also being exposed to influenza. Thus, only testing for BW agents after many influenza tests come back negative could result in less time and resources required for diagnostics (especially for more costly BW panel systems).

- ***Recommendation.*** *Incorporate rule-out testing for influenza/common diseases as a first step of forward sampling and analysis TTPs.*

Overall, this paper examined means by which different sampling and analysis TTPs could influence the timing of diagnostic results for different diseases. Unfortunately, significant gaps exist in the scientific literature related to the rate of positive results for different diagnostic systems, especially those used by the military, or in development for such use. Moreover, few reports link such results to symptom progression. This reality limited the direct conclusions on which we could draw specific TTP options. This paper, however, describes the gaps that are present in the literature and also draws some general conclusions based on disease and diagnostic system attributes. Future diagnostic systems, such as new technologies developed as part of the coronavirus-19 (COVID-19) response, could allow for additional capabilities at lower roles of care, although additional research would be required to determine how that may influence diagnostics within the DOD in the mid-term.

D. Recommendations

From this paper, we provide the following recommendations for the DOD and restate them here:

- Include requirements for sensitivity over the course of illness (particularly the early stages) in CDDs for diagnostic systems that are intended to be used for early diagnosis.
- Conduct experiments to characterize diagnostic sensitivity over the course of illness (particularly the early stages) for the systems of interest (e.g., NGDS 2) to inform analysis-based TTPs for use of those systems using the methods described in this paper.
  - Update initial/full operational capability numbers according to analysis-based TTPs.
  - Update doctrine, including ATP 4-02.84,[211] accordingly.
- Ensure that diagnostic users and clinicians are educated on the performance of diagnostics at various stages of disease, especially for rarely diagnosed diseases, and whether diagnostics may need to be used again at set intervals.
- Incorporate rule-out testing for influenza/common diseases as a first step of forward sampling and analysis TTPs.

[211] HQDA, *Multi-Service Tactics, Techniques, and Procedures for Treatment of Biological Warfare Agent Casualties*.

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Appendix A.
Additional Technical Modeling Information

Note: This work was originally completed and published in May 2021.

Derivation of Disease Progression Models

As discussed in Section 3.A, we used Allied Medical Publication (AMedP)-7.5[212] as our source for the disease progression and severity models. However, AMedP-7.5 does not contain models for Ebolavirus disease (EVD) or influenza. Therefore, we developed our own models for those two diseases. The following two sections—Ebola Virus Disease Progression Model and Influenza Disease Progression Model—describe how we derived these models.

Ebola Virus Disease Progression Model

As discussed in Section 7.A, *Zaire ebolavirus* produces a two-stage disease. In the first stage, the individual has non-specific moderate symptoms such as high fever, malaise, fatigue, and body aches.[213] The first stage typically lasts 3 to 5 days. The second stage of disease is characterized by severe gastrointestinal symptoms, which either resolve or lead to death.[214] The second stage lasts a week or more, with a prolonged convalescence in those who survive. We used a case fatality rate of 40%.[215]

We used epidemiological data captured during the 2014–2015 West Africa EVD epidemic to characterize the distributions for the duration of the incubation period, stage 1 and stage 2 of the disease. Agua-Agum et al. fit gamma distributions to data observed in Guinea, Liberia, and Sierra Leone during the first year of the 2014 epidemic.[216] We used

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[212] North Atlantic Treaty Organization (NATO), *NATO Planning Guide for the Estimation of CBRN Casualties*, NATO Standard AMedP-7.5, Edition A Version 1 (NATO Standardization Office, October 2017), 1–10, https://nso.nato.int/nso/nsdd/main/standards/ap-details/1788/EN.

[213] Daniel S. Chertow et al., "Ebola Virus Disease in West Africa- Clinical Manifestations and Management," *The New England Journal of Medicine* 371 (November 27, 2014): 2054, doi:10.1056/NEJMp1413084.

[214] Ibid.

[215] François Lamontagne et al., "Evidence-Based Guidelines for Supportive Care of Patients with Ebola Virus Disease," *Lancet* 391, no. 10121 (February 17, 2018): 700, doi:10.1016/S0140-6736(17)31795-6.

[216] Junerlyn Agua-Agum et al., "West Africa Ebola Epidemic After One Year—Slowing but Not Yet Under Control," *New England Journal of Medicine* 372, no. 6 (February 5, 2015): 584–587, https://pubmed.ncbi.nlm.nih.gov/25539446/.

the incubation period that was fit to all the observed incubation period data. The result is a gamma distribution with a mean of 10.3 days and a standard deviation of 8.2 days.

Agua-Agum et al. did not fit distributions for the duration of the initial moderate symptoms of the disease. Instead, they fit distributions to the duration of the time from symptom onset to hospital admission. Given the severe symptoms of the second stage of EVD, we assume that hospitalization and the onset of severe symptoms would occur at the same time. However, we know that the medical infrastructure of in West Africa may have resulted in delays between the onset of severe symptoms and hospitalization, especially early in the outbreak before treatment was better streamlined. As a result, the distributions fit to the symptom onset to hospitalization data may overestimate the duration of the first stage of the disease. Agua-Agum et al. report distributions fit to data observed during different time periods within the first year of the epidemic. We selected the distribution corresponding to the latest reported period of time (1 October to 25 November 2014), with the assumption that as the epidemic response progressed, the delays from severe symptom onset to hospitalization decreased. The resulting distribution that we used for the duration of the first stage of the disease is a gamma distribution with a mean of 4.3 days and a standard deviation of 3.7 days.

As previously mentioned, the duration of the second stage of the disease is shorter in those who die as a result of their infection as compared to those who recover. For fatalities, we used the time from hospitalization to death reported by Agua-Agum et al. to represent the duration of the second stage of the disease. To be consistent with our choice of distribution for the first stage of the disease, we used the distribution fit to the data observed in the latest reported period of time. The resulting distribution for the second stage of the disease for fatalities is a gamma distribution with a mean of 3.8 days and a standard deviation of 3.3 days. For survivors, we used the time from hospitalization to discharge reported by Agua-Agum et al. to represent the duration of the second stage of the disease. Again, we used the distribution fit to the data observed in the latest reported period of time. The resulting distribution for the duration of the second stage of the disease in survivors is a gamma distribution with a mean of 10.6 days and a standard deviation of 6.2 days. Table A-1 summarizes the EVD disease progression model.

Table A-1. EVD Disease Progression Model

Disease Stage	Symptom Severity	Distribution	Mean (Days)	Standard Deviation (Days)
Incubation Period		Gamma	10.3	8.2
Stage 1	Moderate	Gamma	4.3	3.7
Stage 2 (Fatalities)	Severe	Gamma	3.8	3.3
Stage 2 (Survivors)	Severe	Gamma	10.6	6.2

Influenza Disease Progression Model

As discussed in Section 11.A, we modeled influenza as a single-stage disease with moderate symptoms. We used the incubation period distribution reported by Lessler et al. for influenza A as the incubation period in our influenza model.[217] The distribution for the incubation period is a lognormal distribution with a mean of 1.52 days and a standard deviation of 0.66 days. The duration of the first—and only—stage of the disease was modeled as a uniform distribution with a minimum of three days and a maximum of 7 days.[218] Table A-2 summarizes the influenza disease progression model.

Table A-2. Influenza Disease Progression Model

Disease Stage	Symptom Severity	Distribution
Incubation Period		Lognormal (mean = 1.52 days, standard deviation = 0.66 days)
Stage 1	Moderate	Uniform (minimum = 3 days, maximum = 7 days)

Accounting for the Dose-Dependent Incubation Periods of Q Fever and Tularemia

The disease progression models for Q fever and tularemia in AMedP-7.5 each use a dose-dependent incubation period. As discussed in the beginning of Chapter 3, our model did not simulate the attack itself; rather, the user specifies a given number of individuals who were infected as a result of the attack. Because the model does not simulate the attack itself, the doses received by the infected individuals were not characterized. Therefore, we simulated representative attacks that provided representative dose profiles in an exposed population.

We used the Hazard Prediction and Assessment Capability (HPAC) to simulate the transport and dispersion of representative Q fever and tularemia attacks. These attacks were based on those conducted in a previous Institute for Defense Analyses (IDA) analysis that developed realistic and representative biological warfare (BW) attacks on U.S. military targets.[219] The attacks each consist of a single backpack point sprayer that released a dry preparation of either *Coxiella burnetii* or *Francisella tularensis* approximately 1.5 km

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[217] Justin Lessler et al., "Incubation Periods of Acute Respiratory Viral Infections: A Systematic Review," *Lancet* 9, no. 5 (May 2009): 291–300, doi:10.1016/S1473-3099(09)70069-6.

[218] "Clinical Signs and Symptoms of Influenza," Centers for Disease Control and Prevention (CDC), last updated August 31, 2020, https://www.cdc.gov/flu/professionals/acip/clinical.htm.

[219] Sean Oxford, Doug Schultz, and Scott Weinrich, "Five Example Biological Warfare Attacks for the Medical Planners' Toolkit (MPTk) and the Joint Medical Planning Tool (JMPT)," memorandum (Institute for Defense Analyses, 2 October 2020).

upwind of the Marshalling Area of an airport of debarkation (APOD). The modeling parameters were taken directly from the previous IDA analysis, with changes made to account for the use of *C. burnetii* and *F. tularensis* instead of the originally used *Yersinia pestis*. Table A-3 shows the modeling parameters.

Table A-3. Transport and Dispersion Modeling Parameters

Parameter	Information
Target	APOD
PAR	3,321
Biological weapon	2.275 kg of *C. burnetii* or *F. tularensis* in a stationary sprayer
Release description	Singe stationary point source just north of the APOD's Marshalling Area
Release height	1.5 m
Release time	1300
Release duration	12 min
Release rate	0.0032 kg/s
Agent particle size distribution	Mass median diameter: 5 μm Geometric standard deviation: 1.5 μm
Day/night environmental decay rate	3.37×10^{-4}/3.37×10^{-4} 1/s (*C. burnetii*)* 1.22×10^{-3}/4.22×10^{-4} 1/s (*F. tularensis*)*
Particle density	1,000 kg/m^3
Dry mass fraction	100%
Active fraction	60%
Terrain	"Cultivated" land cover; no terrain
Meteorology	Wind direction: 0 degrees Wind speed: 2.439 kph Temperature: -0.2°C Humidity: 70.3% Pasquill-Gifford-Turner stability class: 5
CFU/mg agent	5.0×10^5 (*C. burnetii*)* 5.0×10^5 (*F. tularensis*)*
Personnel breathing rate	15 liters/minute

* HPAC default value

Note: The abbreviations used in this table are defined in Appendix E of this paper.

The result of the transport and dispersion modeling was the dose received by each entity location in the PAR. We used the dose-dependent incubation periods from AMedP-7.5 to determine the corresponding duration of incubation periods for each entity location. We then created a distribution of incubation periods for the population by weighting the incubation period of each entity location by the corresponding dose-dependent probability of infection. The result was an empirical Q fever and a tularemia incubation period distribution that corresponded to the representative attack. For the final

step we used R's fit.dist function to fit various parametric distributions to the empirical distributions. We used the Akaike information criterion (AIC) of each fit to determine which distribution to select. For Q fever, the fitted Weibull, lognormal, and gamma distributions had AICs of 2687, 2710, and 2682. Figure A-1 shows a histogram of the data and the three fitted distributions for Q fever. For tularemia, the AIC for the fit Weibull, lognormal, and gamma distributions had AICs of -956, -1422, and -1412. Figure A-2 shows a histogram of the data and the three fit distributions for tularemia.

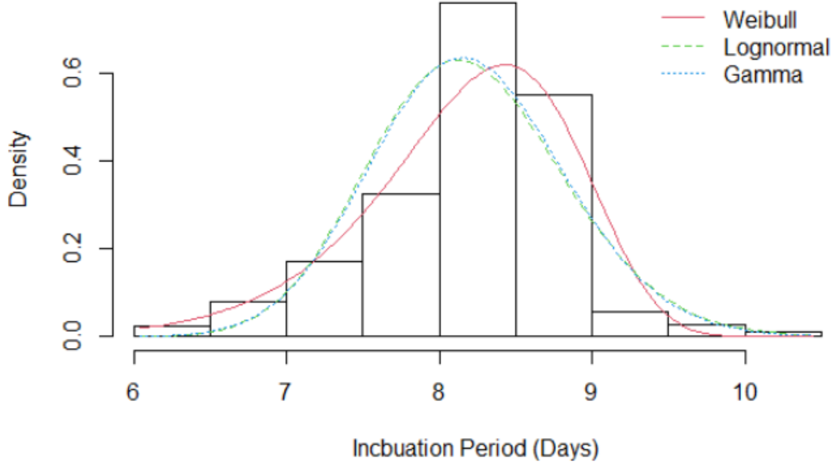

Figure A-1. Fit Distributions and Incubation Period Data for Q Fever

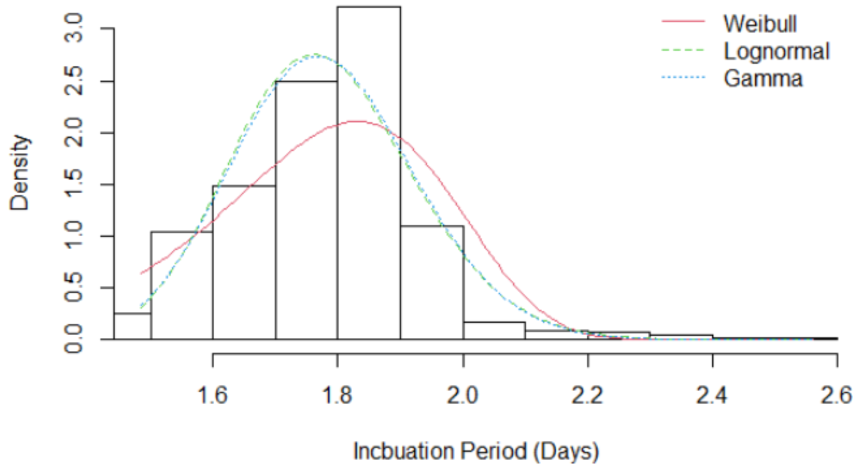

Figure A-2. Fit Distributions and Incubation Period Data for Tularemia

Table A-4 summarizes the incubation period distributions that we generated with this methodology. A Q fever incubation period of 8.2 days is relatively short but is consistent

with what is expected following exposure to a large inhalational dose.[220] The incubation period for tularemia is usually 3 to 6 days but can vary from 1 to 21 days.[221] The incubation period distribution that we obtained is on the lower end of these ranges, but, as was the case for Q fever, a shorter incubation period is expected for larger inhalational doses.[222]

Table A-4. Representative Incubation Period Distributions for Q Fever and Tularemia

Disease	Distribution	Mean	Standard Deviation
Q Fever	Gamma	8.20 days	0.63 days
Tularemia	Lognormal	1.78 days	0.15 days

Technical Details for Determining Diagnostic Results Over Time

The following sections—Plague, Q Fever, and Tularemia—provide additional technical explanation on how we converted the diagnostic test data that we found in the scientific literature to the specific probability of detection values that we used in our model.

Plague

As discussed in Section **Error! Reference source not found.**, we used Koster et. al. as the source of data to inform our choice of probability of detection values.[223] The experiment continuously monitored the internal body temperature of the non-human primate (NHP) subjects. Three subjects were sacrificed every 24 hours post exposure, and their blood was analyzed with an in-house PCR test and with bacterial isolation. Three subjects were not sacrificed and died as a result of their infection. Table A-5 shows pertinent data from the experiment. Symptom onset (defined by us as the onset of fever) occurred 55–60 hours post exposure in the 6 subjects that were not sacrificed before symptom onset.

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[220] North Atlantic Treaty Organization (NATO), *Technical Reference Manual NATO Planning Guide for the Estimation of CBRN Casualties*, NATO Standards-Related Document AMedP-7.5-1, Edition A, Version 1 (NATO Standardization Office, January 2018), 24-5—24-7, https://nso.nato.int/nso/nsdd/main/standards/srd-details/91/EN.

[221] United States Army Medical Research Institute of Infectious Diseases (USAMRIID), *Medical Management of Biological Casualties Handbook*, 9th ed. (USAMRIID, 2020), 70, https://usamriid.health.mil/assets/docs/training/USAMRIIDs_Blue_Book_9th_edition_PDF_format.pdf.

[222] Ibid.

[223] Frederick Koster et al., "Milestones in Progression of Primary Pneumonic Plague in Cynomolgus Macaques," *Infection and Immunity* 78, no. 7 (July 2010): 2946–2955, doi:10.1128/IAI.01296-09.

Table A-5. Number of Positive Diagnostic Tests over Time in Cynomolgus Monkeys

Hours After Exposure	In-House PCR	Bacterial Isolation
24	0 of 3	0 of 3
48	0 of 3	0 of 3
72	3 of 3	1 of 3
Death (70, 92, 94 hours)	3 of 3	3 of 3

We encountered several challenges in using the data from Koster et. al. First, although we could use fever as a surrogate for the onset of symptoms, the study did not report the timing of symptoms that we could use as a surrogate for the onset of the second stage of plague. Therefore, we scaled the timing of the data to account for the differences in disease duration between the NHP subjects and humans and looked at the probability of detection at the scaled time corresponding to the onset of stage 2 symptoms in humans.

The mode of the time from exposure to symptom onset in the NHPs was 57.5 hours. In comparison, our plague disease progression model uses an average incubation period of 4.3 days (103.2 hours)—approximately twice as long. The average time from symptom onset to death in the NHP was 27.8 hours. In comparison, the corresponding time in our plague model is 2.5 days (60 hours)—again, approximately twice as long. Therefore, we scaled the times reported in Koster et. al. by a factor of two.

The second challenge with using the data was the infrequency in which the data is reported. To overcome this issue, we used a method of interpolating between the reported values. We fit a logistic regression to the scaled data to provide interpolated estimates of the test probability of detection. Figure A-3 shows the results of the logistic regressions and the scaled time of symptom onset and corresponding time of stage 2 onset.

The points in Figure A-3 represent the data from Koster et al. A value of 1.00 indicates that the sample was positive, and a value of 0.00 indicates that the sample was negative. The horizontal location of the points represents the time after symptom-onset that the sample was collected after being scaled to account for the longer duration of disease in humans as compared to the NHP subjects. The first vertical line represents the scaled median time of symptom onset in Koster et al. The duration of the first stage of plague is 1 day. Therefore, we looked at the predicted probability of detection 1 day after the scaled symptom onset time, which is shown as the second vertical line.

Our final step was to use the curves shown in Figure A-3 to inform our estimates of the tests' probability of detection at symptom onset and at the onset of stage 2. Directly using the values predicted by the regression at the scaled time of symptom onset resulted in an estimated probability of detection of 2.2% and 5.5% for the in-house PCR and bacterial isolation, respectively. Unfortunately, the data reported by Koster et al. show the in-house PCR test performing better than bacteria isolation at the first sample after

symptom onset (72 hours post exposure). This contradictory result is likely due to the very small sample sizes used in the experiment. Given that the data suggest the performance of the in-house PCR should be no worse than that of bacterial isolation, we used a test probability of detection at symptom onset of 5% for both diagnostic tests. No such inconsistencies occurred when using the results of the logistic regression to inform the probability of detection at the onset of stage 2. Therefore, we used the predicted values of 100% and 40% as the probabilities of detection of the in-house PCR test and bacterial isolation at the onset of stage 2, respectively.

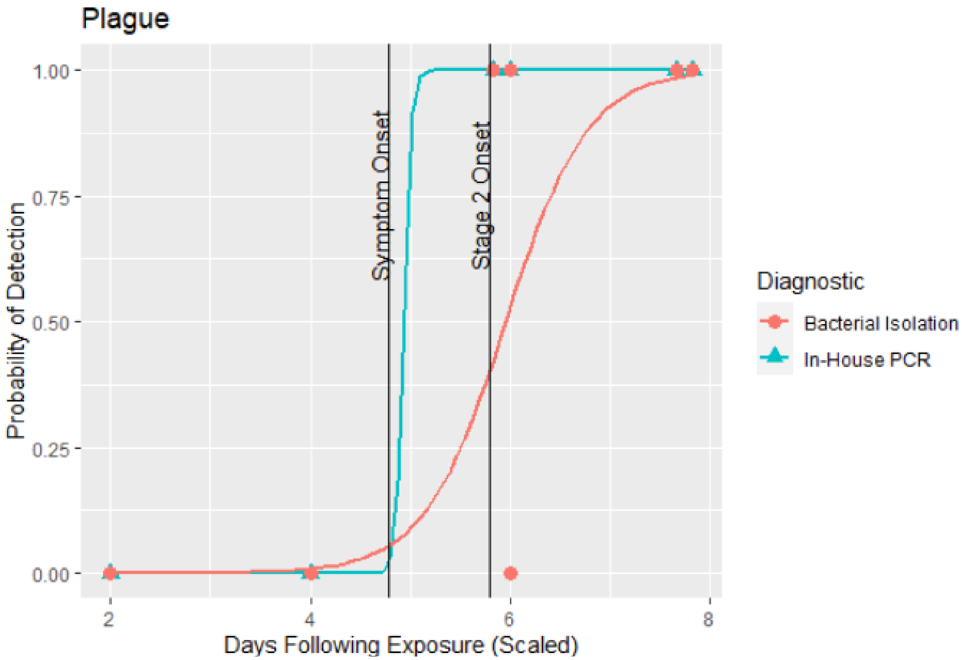

Figure A-3. Logistic Regression of Scaled Data and the Scaled Time of Symptom and the Corresponding Time of Stage 2 Onset

Q Fever

As discussed in Section 5.C, we used the data from Howe et al. to inform our choice of probability of detection values for an in-house PCR test.[224] Table A-6 summarizes the pertinent data from Howe et al. As shown in the table, all NHP subjects developed symptoms at the same experimental time point—6 days post exposure. Therefore, we simply used the percentage of positive results at that time as our values for probability of detection at symptom onset.

[224] Gerald B. Howe et al., "Real-Time PCR for the Early Detection and Quantification of *Coxiella burnetii* as an Alternative to the Murine Bioassay," *Molecular and Cellular Probes* 23, nos. 3–4 (June–August 2009): 127–131, https://doi.org/10.1016/j.mcp.2009.01.004.

Table A-6. Number of NHP Subjects with Clinical Signs and Number of Positive In-House PCR Assay Results over Time

Day Following Exposure	Clinical Signs of Illness	In-House PCR, *IS11* Gene	In-House PCR, *com1* Gene
0	0 out of 10	0 out of 10	0 out of 10
2	0 out of 10	0 out of 10	0 out of 10
4	0 out of 10	4 out of 10	0 out of 10
6	10 out of 10	10 out of 10	5 out of 10

Tularemia

As discussed in Section 6.C, we used the data from Guina et al. to inform our choice of probability of detection values for the in-house PCR assay and bacterial isolation.[225] Guina et al. tracked the internal temperature of each NHP subject and analyzed daily blood samples with the in-house PCR assay and bacterial isolation. They reported the time of fever onset and the time of the first positive diagnostic result for each diagnostic test for each subject. From these data, we calculated the time relative to symptom onset for the first positive diagnostic result for each test subject. As is discussed subsequently, we only considered the data from subjects that died as a result of their infection. Table A-7 summarizes the results

As was the case for the plague data discussed previously, the progression of tularemia in the NHP subjects occurred much faster than what would be expected in humans. Therefore, we scaled the tularemia data as we did for the plague data. The average time from symptoms onset to death in the Guina et al. study was 167 hours. Our tularemia disease progression model uses an average time from symptom onset to death of 15 days (360 hours), which is approximately twice that which was observed in the NHP subjects. Therefore, we used a scaling factor of two to adjust the data to account for the differences in disease progression between the NHP subjects and humans.

[225] Tina Guina et al., "The Cynomolgus Macaque Natural History Model of Pneumonic Tularemia for Predicting Clinical Efficacy Under the Animal Rule," *Frontiers in Cellular and Infection Microbiology* 8, article 99 (April 2018): 18pp., doi:10.3389/fcimb.2018.00099.

Table A-7. Time Relative to Symptom Onset of First Positive Diagnostic Result from Guina et al.

	Bacterial Isolation		In-House PCR	
Day of First Positive Result Relative to Symptom Onset	Number of Subjects	Percent of Total Subjects Tested	Number of Subjects	Percent of Total Subjects Tested
-1	1	3%	0	0%
0	1	3%	3	30%
1	10	28%	6	60%
2	13	36%	1	10%
3	6	17%	0	0%
4	1	3%	0	0%
5	1	3%	0	0%
Never Detected	3	8%	0	0%
Total	36	100%	10	100%

In an approach that was similar to the one that we used for the plague data, we fit logistic regressions to the data shown in Table 18. The results of these regressions are shown in Figure A-4. The predicted values for probability of detection at symptom onset are 13.1% and 27.2% for bacterial isolation and in-house PCR, respectively. We rounded these values to a single significant figure for use in our model (i.e., 10% and 30%). The corresponding predictions at onset of stage 2 are 95.6% and 100.0% for bacterial isolation and in-house PCR, respectively. We also rounded these values to a single significant figure for use in our analysis; however, we opted to round the probability of detection of bacterial isolation down from 95.6% to 90% instead of rounding up to 100% to reflect the fact that bacterial isolation never detected the disease in some subjects (see Table A-7).

As discussed in Section 6.C, we used the data from Banada et al. to inform our choice of probability of detection values for the GeneXpert assay.[226] Unfortunately, Banada et al. did not report the time of symptom onset for each subject. Instead, they reported the percentage of febrile subjects and the number of positive GeneXpert assays at each day post exposure. Table A-8 shows the pertinent data from Banada et al.

[226] Padmapriya P. Banada et al., "Sensitive Detection of *Francisella tularensis* Directly from Whole Blood by Use of the GeneXpert System," *Journal of Clinical Microbiology* 55, no. 1 (January 2017): 291, https://jcm.asm.org/content/55/1/291.

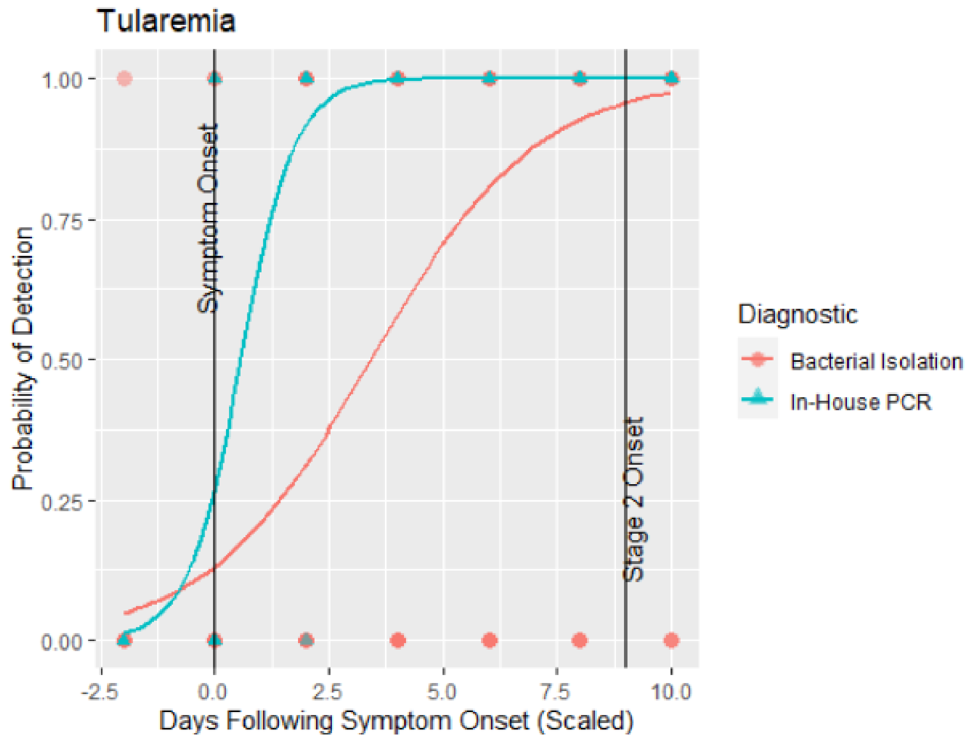

Figure A-4. Logistic Regression of Scaled Data and the Time of Symptom Onset and the Corresponding Time of Stage 2 Onset: In-House PCR and Bacterial Isolation

Table A-8. Percentage of Febrile Subjects and Number of Positive GeneXpert Assay Results over Time

Day Post Exposure	Percentage of Febrile Subjects	Number of Positive Results
1	57%	4 of 19
2	88%	3 of 18
3	100%	3 of 5
4	100%	5 of 6
5	100%	7 of 9
6	100%	10 of 10
7	100%	1 of 1

The experiments conducted by Banada et al. and Guina et al. used the same NHP species for subjects (cynomolgus macaques), the same strain of tularemia (Schu S4), and similar exposure doses (up to 2,182 CFU). Therefore, we used the same scaling factor of 2 to account for the difference in disease duration between the NHP subjects and humans. We fit a logistic regression to the scaled GeneXpert assay results (see Figure A-5).

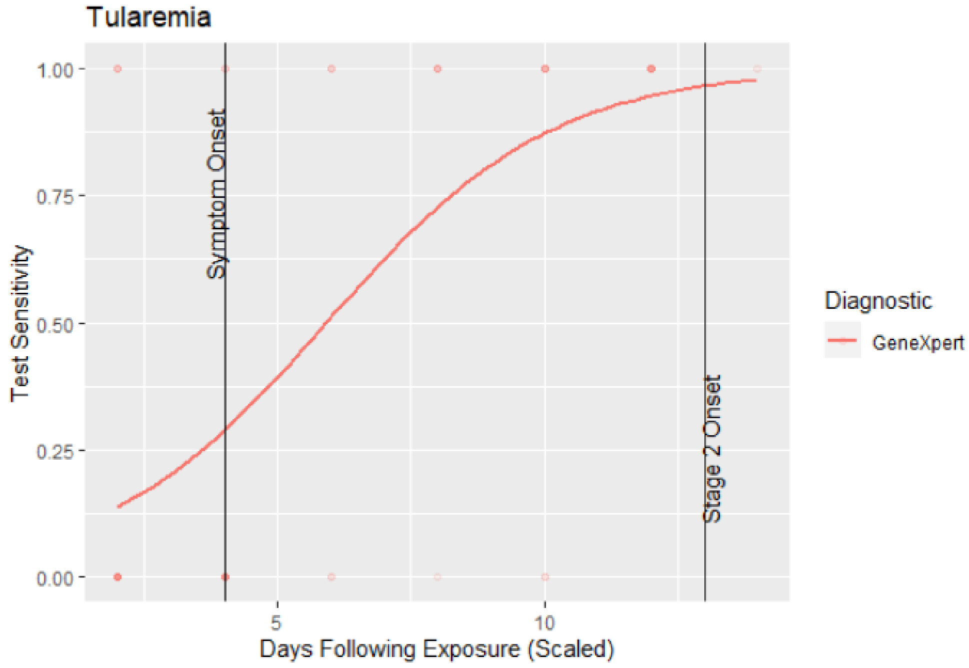

Figure A-5. Logistic Regression of Scaled Data and the Time of Symptom Onset and the Corresponding Time of Stage 2 Onset: GeneXpert PCR

The time of symptom onset shown in Figure A-5 was calculated using the exposure doses reported in Banada et al. and the dose-dependent incubation period described in AMedP-7.5. The time of stage 2 onset was simply 9 days after symptom onset, as described by our tularemia disease progression model. Based on the regression in Figure A-5, the probability of detection of the GeneXpert assay is 28.9% and 96.6% at symptom onset and stage 2 onset, respectively. As was done for the in-house PCR and bacterial isolation values, we rounded the probabilities of detection to a single significant figure for use in our model (i.e., 30% and 100%).

Additional Technical Discussion of Model

As discussed in Chapter 3, we developed a stochastic individual-based model that simulates the collection and analysis of clinical specimens from individuals infected during a BW attack. The model tracks the disease progression of each infected individual and their movement through the theater medical system. The following information supplements the discussion of the model presented in Chapter 3 by providing additional technical details. The model was implemented in code written in Python 3.8.3 and requires pandas 1.0.5 and numpy 1.18.5. The python code is presented in Appendix B. The minimum hardware requirements are estimated to be 16 GB of Random Access Memory (RAM). We post-processed and visualized the model results in R 4.0.3 with the plotting package ggplot.

Initializing the Population

The model does not simulate the attack; rather, the user specifies a given number of infected individuals. The first step of each simulation is to initialize the infected individuals. The durations of each individual's latent period, stage1, and stage 2 of disease and the result of their infection (i.e., death or recovery) are determined via random draws from the associated probability distributions for a given disease. The disease durations for each individual are combined to generate a timeline that characterizes that individual's disease progression. A symptom severity level timeline is then created for each individual based on the severity levels associated with each stage of the disease and the individual's disease progression timeline.

The final step in initializing each individual is determining his or her movement through the medical system. As discussed in Section 3.B, an individual's movement through the medical system is governed by his or her symptom severity. Therefore, the location of the individual in the medical system over time is determined from his or her symptom severity timeline using the logic flow shown in Figure 3 and the movement parameters shown in Table 3. The result is a patient location timeline for each individual.

Simulate Specimen Collection and Analysis

After the population has been initialized, the next step is to simulate the specimen collection and analysis process. For each individual, the model determines the result of the diagnostic test (i.e., positive or negative) and the timing of that result. The result of the diagnostic test is stochastically determined from the probability of detection of the test when analyzing a given specimen collected at a given stage of disease. The timing of the diagnostic result is determined from the timing of when the specimen is collected, plus the time it takes to ship the sample (if applicable), and the time to run the test itself. Again, as discussed in Section 3.C, the model does not account for any delays in the specimen collection and analysis process due to testing capacity at a given location. We assume that the specimen can immediately start to be analyzed as soon as it reaches the location of the diagnostic test, without any queuing delays.

As discussed in Section 3.C, we modeled three triggers for specimen collection. The first trigger was the onset of generic, non-specific symptoms. When this trigger is used, the model simulates the collection of a specimen from each individual when he or she first reports to a Role 1 MTF. The result of the diagnostic test is determined using the corresponding probability of detection at symptom onset.

The second trigger for specimen collection is the onset of severe symptoms. For diseases with mild or moderate symptoms in the first stage, this trigger corresponds to the onset of the seconds stage of the disease. For diseases that have severe symptoms in the first stage, this trigger corresponds to the onset of the first stage of the disease. In general, when this trigger is used, the timing of specimen collection from an individual is the time

at which that individual enters the location where the test is located. The exception is the use of specimen shipping, discussed in the next section. The result of the diagnostic test is determined using the corresponding probability of detection at the onset of the applicable stage of the disease.

The third trigger for specimen collection is the occurrence of 10 individuals with symptoms. In this case, the time at which the tenth individual develops his or her symptoms is determined and only the specimen that would be collected at or subsequent to that time are simulated. The remainder of the specimen collection and analysis process occurs in the same way as discussed previously.

Specimen Shipping

In general, in our model, specimens are not collected from individuals until they arrive at the MTF where the diagnostic test is located. The exception is when specimen shipping is used. Our model can simulate the shipping of specimens collected at a Role 1 MTF for analysis with a test located at a Role 3 MTF. Two parameters are used to characterize specimen shipping: (1) the frequency with which specimens are shipped and (2) the time it takes to transport them. The frequency with which specimens are shipped is a user-specified parameter. For example, if the shipping frequency is 12 hours, then once every 12 hours, all the specimens that have been collected over the previous 12 hours are shipped to the Role 3 MTF. The timing of the first shipment relative to the attack is randomly determined with equal probability. A stochastically determined transportation time is determined for each shipment. A uniform distribution with a minimum of 5 hours and a maximum of 8 hours is used to model the transportation times.

The shipping of specimens for analysis at either an out-of-theater laboratory or a Role 4 MTF is handled differently in our model. As discussed in Section 3.B, the time required to transport specimens to these out of theater locations is accounted for in the time it takes to run the diagnostic test (see Table 4).

Outputs

The principal unprocessed outputs from each trial of the model are lists of the following:

- The times of each positive diagnostic result,
- The times of each negative diagnostic result, and
- The times of the onset of symptoms by severity level for each infected person.

We applied summary statistics on the results across the trials for a given set of inputs to generate the final results presented in this paper.

Appendix B.
Model Code and Input Data

Note: This work was originally completed and published in May 2021.

The model is run through execution of `Main.py`, which calls various support functions defined in `Support_Functions.py`. Global model inputs are specified in the following csv files: `P_Detect.csv`, `Diagnostics_Run_Times.csv`, `Diseases.csv`, `MTF.csv`, and `Sample_Shipment_Times.csv`. The parameters used for a given simulation are specified for each disease in their own input file: `Plague.csv`, `Tularemia.csv`, `Flu.csv`, `Ebola.csv`, `VEE.csv`, and `QFever.csv`. Data post-processing and visualization were performed using a separate R code. All code and csv files are included below.

Main.py

```
#Main.py
#By: Robert Cubeta, 4/6/2021
#This Code is UNCLASSIFIED

import pandas as pd
import numpy as np
import time
from Support_Functions import *

run_csv = ‘Ebola.csv’  # File specifying a batch of runs

dt = 0.1  # Simulation timestep

# Read model parameters
df_disease, df_mtf_delay_times, df_p_detect, df_ship_times, df_run_times, df_runs = Read_In
puts(run_csv)

assert (len(np.unique(df_runs[‘N_Diags_Report’]))==1) and (len(np.unique(df_runs[‘N_Cases_R
eport’]))==1), \
    ‘Runs will try to report different numbers of diagnostic results or cases. Make sure N_
Diags_Report and N_Cases_Report columns are constant’
```

```
#  Initialize lists to be updated with results
results_lists = []
time_line_results_lists = []

#  Iterate over each run in the run csv
for index, row in df_runs.iterrows():
    disease, diagnostic, sample, cases_start_sampling, SL_trigger_sample, test_loc, direct_
to_R2, ship_samples, \
    ship_samples_freq, n_pos_diags_to_report, n_symptoms_to_report,  trials, N = row

    print('run ', index, ' out of ', df_runs.shape[0]-1)

    # Parse input parameters into dictionaries
    SL_dict = Create_Severity_Levels_Dict(disease, df_disease)  # Dict. of severity levels
by disease stage for survivors/fatalities
    p_detects = [df_p_detect.loc[(disease, diagnostic, sample)]['Symptom Onset'],
                 df_p_detect.loc[(disease, diagnostic, sample)]['Stage2 Onset']]  # List of
diagnostic test sensitivity at symptom onset and stage 2 onset
    mtf_delays = Create_MTF_Delay_Dict(df_mtf_delay_times)  #Dict. of  min and max times a
person spends at each MTF + time to move to next
    ship_times = Create_Ship_Times_Dict(df_ship_times)  # Nested dict. of min and max times
it takes to ship a sample from one MTF to another
    run_time = df_run_times.loc[(disease, diagnostic, sample)]['Run Time (Days)']  # Time (
days) to run the diagnostic test used in the given run
    run_time = int(round(run_time/dt, 0))
    cfr = df_disease['CFR'][disease]['Surv']  # Case fatality rate of disease

# Iterate over each trial
    for trial in range(trials):
        # Perform random draws
        sampled_dis_durations = Determine_Durations(N, disease, df_disease)  # Dictionary w
ith keys of disease stage and values of lists of the durations for each person

        # Initialize population
        pop = [Individual(disease, dt, n, sampled_dis_durations, SL_dict, p_detects, mtf_de
lays, cfr, direct_to_R2)
               for n in range(N)]  # List of the objects that represent the individuals in
the population
```

```
        # Determine the time each true positive (diags_times) and false negative (fn_times)
        diags_times, fn_times = Determine_Time_of_Diagnoses(pop,test_loc,cases_start_sampli
ng,SL_trigger_sample,ship_times,dt,run_time,ship_samples,ship_samples_freq)

        ###
        # Parse and format model results for reporting
        ###

        # Convert times from timesteps to days and sort
        diags_days = [round(time * dt,1) if time is not None else None for time in diags_ti
mes]
        diags_days_sorted = sorted(diags_days, key=lambda x: (x is None, x))  # Sorts and p
uts all Nones at end.
        diags_days_to_report = [diags_days_sorted[n-1] if len(diags_days_sorted)>=n else No
ne
                                for n in n_pos_diags_to_report]  # List of the day on which
the user specified number of positive results occur
        fn_days = [round(time*dt,1) for time in sorted(fn_times)]

        # Determine the time when a user specified number of people w/a given symptom sever
ity occur
        symptom_days_to_report = {}
        cases_at_diags_to_report= {} #total number of cases at each SL on the day that a gi
ven number of diagnostic results occur
        symptom_time_lines ={}
        #Determine the time when each person develops symptoms of each SL. None if SL never
experienced
        for sl in [1,2,3,4,'Death']:
            symptom_days = [round(person.symptom_times[sl] * dt,1)
                    if person.symptom_times[sl] is not None
                    else None
                    for person in pop]  # Days at which each person develops symptoms of a
given severity level
            symptom_days_sorted = sorted(symptom_days, key=lambda x: (x is None, x))
            symptom_days_to_report[sl] = [symptom_days_sorted[n-1] if len(symptom_days_sort
ed)>=n else None
                                          for n in n_symptoms_to_report]
            symptom_days_sorted_noNone = [x for x in symptom_days_sorted if x is not None]

            # Determine the number of people who develop symptoms of a given severity level
```

```
each timestep
            if len(symptom_days_sorted_noNone) > 0:
                symptom_time_lines[sl] = np.histogram(symptom_days_sorted_noNone, bins=np.a
range(-0.05,max(symptom_days_sorted_noNone)+0.15,0.1))[0].astype(int).tolist()
            else:
                symptom_time_lines[sl] = []

            assert np.sum(symptom_time_lines[sl]) == len(symptom_days_sorted_noNone), "Hist
ogram dropping case data"

            # Determine the number of cases at the time of the user-specified number positi
ve results of interest
            if len(symptom_days_sorted_noNone) == 0:
                cases_at_diags_to_report[sl] = [None for diag_day_to_report in diags_days_t
o_report]
            else:
                cases_at_diags_to_report[sl] = [len([day for day in symptom_days_sorted_noN
one if day <= diag_day_to_report])
                                                if diag_day_to_report is not None
                                                else None
                                                for diag_day_to_report in diags_days_to_rep
ort]

        results_list = [disease, diagnostic, sample, cases_start_sampling, SL_trigger_sampl
e, test_loc, direct_to_R2, ship_samples, ship_samples_freq, N, trial]
        results_list += diags_days_to_report

        # Determine the number of positive and negative results per timestep
        tp_days = [day for day in diags_days_sorted if day is not None]  # Time of true pos
itive results
        if len(tp_days) > 0:
            tp_time_line = np.histogram(tp_days, bins=np.arange(-0.05, max(tp_days) + 0.15,
0.1))[0].astype(int).tolist()  # Offset bins to avoid confusion of which edge of the bin is
inclusive
        else:
            tp_time_line = []
        if len(fn_days) > 0:
            fn_time_line = np.histogram(fn_days, bins=np.arange(-0.05,max(fn_days)+0.15,0.1
))[0].astype(int).tolist()
        else:
```

```
            fn_time_line = []

        time_line_results_lists.append([disease, diagnostic, sample, cases_start_sampling,
SL_trigger_sample, test_loc, direct_to_R2, ship_samples, ship_samples_freq, N, trial,'TP']
+
                                       tp_time_line)
        time_line_results_lists.append([disease, diagnostic, sample, cases_start_sampling,
SL_trigger_sample, test_loc, direct_to_R2, ship_samples, ship_samples_freq, N, trial,'FN']
+
                                      fn_time_line)
        for sl in [1,2,3,4,'Death']:
            if type(sl) != str:
                lab = 'SL'+str(sl)
            else:
                lab = sl
            time_line_results_lists.append([disease, diagnostic, sample, cases_start_sampli
ng, SL_trigger_sample, test_loc, direct_to_R2, ship_samples, ship_samples_freq, N, trial,la
b] +
                                       symptom_time_lines[sl])

        for x in range(len(diags_days_to_report)):
            for sl in [1,2,3,4,'Death']:
                results_list += [cases_at_diags_to_report[sl][x]]

        for sl in [1,2,3,4,'Death']:
            results_list += symptom_days_to_report[sl]
        results_lists.append(results_list)


cols = ['Disease', 'Diagnostic', 'Sample', 'Cases_To_Start_Sampling', 'SL_Trigger_To_Collec
t_Sample', 'Test_Location',
        'Report_Direct_to_R2', 'Ship_Samples', 'Ship_Samples_Freq', 'N_Infected', 'Trial']
cols += ['Day_of_%s_Pos_Res' %(n) for n in n_pos_diags_to_report]

for n in n_pos_diags_to_report:
   cols += ['Cases_with_SL%s_at_Day_of_%s_Pos_Res' %(sl,n) for sl in [1,2,3,4,'Death']]

for sl in [1,2,3,4,'Death']:
    cols += ['Day_of_%s_Cases_SL%s' %(n,sl) for n in n_symptoms_to_report]
```

```
df_results = pd.DataFrame(results_lists, columns=cols)
time_stamp = time.strftime("%Y%m%d-%H%M%S")


time_line_cols = ['Disease', 'Diagnostic', 'Sample', 'Cases_To_Start_Sampling', 'SL_Trigger
_To_Collect_Sample', 'Test_Location',
        'Report_Direct_to_R2', 'Ship_Samples', 'Ship_Samples_Freq', 'N_Infected', 'Trial','
Result']
max_t = max([len(res) for res in time_line_results_lists])-len(time_line_cols)
time_line_cols += [round(x*dt,1) for x in range(max_t)]
df_time_line_results = pd.DataFrame(time_line_results_lists, columns=time_line_cols)
df_time_line_results.to_csv('Outputs//Time_Line_Results//%s_%s.csv'%(run_csv[:-4],time_stam
p))
```

Support Functions Code

```
#Main.py
#By: Robert Cubeta, 4/6/2021
#This Code is UNCLASSIFIED

import numpy as np
import pandas as pd
import ast

def Read_Inputs(run_csv):
    """
    Returns various dataframes containing the model input parameters for the runs specified
in run_csv.
    """
    df_diseases = pd.read_csv('Inputs//Diseases.csv', index_col=[0, 1])
    for disease in set([i[0] for i in df_diseases.index.values]):
        assert df_diseases['CFR'][disease]['Surv'] == df_diseases['CFR'][disease]['Fat'], \
            'CFR rates do not match for %s' % disease

    df_mtf_delay_times = pd.read_csv('Inputs//MTF.csv', index_col=0)
    df_p_detect = pd.read_csv('Inputs//P_Detect.csv', index_col=[0, 1, 2])
    df_ship_times = pd.read_csv('Inputs//Sample_Shipment_Times.csv', index_col=0)
    df_run_times = pd.read_csv('Inputs//Diagnostics_Run_Times.csv', index_col=[0, 1, 2])

    df_runs = pd.read_csv('Inputs//Run_CSVs//%s' % run_csv)
    df_runs['N_Diags_Report'] = df_runs['N_Diags_Report'].apply(lambda x: list(ast.literal_
```

```
eval(x)))
    df_runs['N_Cases_Report'] = df_runs['N_Cases_Report'].apply(lambda x: list(ast.literal_
eval(x)))
    df_runs['Cases_To_Start_Sampling'] = df_runs['Cases_To_Start_Sampling'].apply(lambda x:
int(x))
    df_runs['SL_Trigger_To_Collect_Sample'] = df_runs['SL_Trigger_To_Collect_Sample'].apply
(lambda x: int(x))
    df_runs['Infected'] = df_runs['Infected'].apply(lambda x: int(x))
    df_runs['Trials'] = df_runs['Trials'].apply(lambda x: int(x))

    return ([df_diseases, df_mtf_delay_times, df_p_detect, df_ship_times, df_run_times, df_
runs])

def Create_Severity_Levels_Dict(disease, df_disease):
    """"""
    Returns a dictionary with keys survivor or fatality and the values are another dictiona
ry where the keys are the
    stages of the disease and the values are the severity levels of that stage.
    """""
    SL_dict = {}
    for outcome in ['Surv', 'Fat']:
        SL_dict[outcome] = {'inc': 0,
                            'stage_1': df_disease['Stage_1_SL'][disease][outcome],
                            'stage_2': df_disease['Stage_2_SL'][disease][outcome],
                            'stage_3': df_disease['Stage_3_SL'][disease][outcome]}
    return(SL_dict)

def Create_MTF_Delay_Dict(df_mtf_delay_times):
    """""
    R1_out: time spent at Role 1 MTF as outpatient
    R1_in: time spent at Role 1 MTF + time to get to Role 2 MTF
    R_2: time spent at Role 2 MTF + time to get to Role 3 MTF
    Returns dictionary with the min ('low') and max ('high') times for the uniform distribu
tion that characterizes these dwell times
    """""
    r1_out_delay_low = df_mtf_delay_times.loc['R1_Out']['Low']
    r1_out_delay_high = df_mtf_delay_times.loc['R1_Out']['High']
    r1_in_delay_low = df_mtf_delay_times.loc['R1_In']['Low']
    r1_in_delay_high = df_mtf_delay_times.loc['R1_In']['High']
    r2_delay_low = df_mtf_delay_times.loc['R2']['Low']
```

```
    r2_delay_high = df_mtf_delay_times.loc['R2']['High']

    mtf_delays = {'R1_Out': [r1_out_delay_low, r1_out_delay_high],
                  'R1_In': [r1_in_delay_low, r1_in_delay_high],
                  'R2': [r2_delay_low, r2_delay_high]}
    return(mtf_delays)

def Create_Ship_Times_Dict(df_ship_times):
    """
    Returns dictionary with the min (low) and max (high) times it takes to ship a sample fr
om one MTF to another
    """
    r1_r2_low = df_ship_times.loc['R1-R2']['Low']
    r1_r2_high = df_ship_times.loc['R1-R2']['High']
    r1_r3_low = df_ship_times.loc['R1-R3']['Low']
    r1_r3_high = df_ship_times.loc['R1-R3']['High']
    r1_lab_low = df_ship_times.loc['R1-Lab']['Low']
    r1_lab_high = df_ship_times.loc['R1-Lab']['High']
    r1_dict = {'R2':[r1_r2_low,r1_r2_high], 'R3': [r1_r3_low,r1_r3_high], 'Lab': [r1_lab_lo
w,r1_lab_high]}

    r2_r3_low = df_ship_times.loc['R2-R3']['Low']
    r2_r3_high = df_ship_times.loc['R2-R3']['High']
    r2_dict = {'R3': [r2_r3_low, r2_r3_high]}

    shipment_times = {'R1': r1_dict, 'R2': r2_dict}
    return(shipment_times)

def Determine_Durations(N, disease, df_disease):
    """
    Sample disease duration distributions
    Returns a dictionary with keys of the disease stages and values of a list of durations-
-one for each person in pop
    """
    outcomes = ['Surv','Fat']
    stages_dict = {}
    for outcome in outcomes:
        incubations = Draw_From_Dist(df_disease['Incubation_Dist'][disease][outcome],
                                     df_disease['Incubation_Param1'][disease][outcome],
                                     df_disease['Incubation_Param2'][disease][outcome],
```

```
                                            N)
        stage_1s = Draw_From_Dist(df_disease['Stage_1_Dist'][disease][outcome],
                                  df_disease['Stage_1_Param1'][disease][outcome],
                                  df_disease['Stage_1_Param2'][disease][outcome],
                                  N)
        stage_2s = Draw_From_Dist(df_disease['Stage_2_Dist'][disease][outcome],
                                  df_disease['Stage_2_Param1'][disease][outcome],
                                  df_disease['Stage_2_Param2'][disease][outcome],
                                  N)
        stage_3s = Draw_From_Dist(df_disease['Stage_3_Dist'][disease][outcome],
                                  df_disease['Stage_3_Param1'][disease][outcome],
                                  df_disease['Stage_3_Param2'][disease][outcome],
                                  N)
        stages_dict[outcome] = {'incubation':incubations, 'stage_1s':stage_1s, 'stage_2s':s
tage_2s,'stage_3s':stage_3s}
    return(stages_dict)

def Draw_From_Dist(distribution, param_1, param_2, n):
    """
    Samples from a specified distribution
    Returns a list of the values
    """
    assert distribution in ['lognormal', 'gamma', 'uniform', 'none', 'constant', 'weibull',
'discrete_80_20'],  \
        '%s not defined in Draw_From_Dist()' %distribution

    if distribution == 'none':
        times = [0]*n
    else:
        if distribution == 'lognormal':
            mean = param_1
            std = param_2
            mu = np.log(mean**2/np.sqrt(std**2+mean**2))
            sigma = np.sqrt(np.log(1+std**2/mean**2))
            times = np.random.lognormal(mu,sigma,n)

        elif distribution == 'gamma':
            mean = param_1
            std = param_2
            shape = mean**2/std**2
```

```
            scale = std**2/mean
            times = np.random.gamma(shape,scale,n)

        elif distribution == 'uniform':
            min = param_1
            max = param_2
            times = np.random.uniform(min,max,n)

        elif distribution == 'constant':
            constant = param_1
            times = [constant]*n

        elif distribution == 'weibull':
            mean = param_1
            std = param_2
            assert mean in [1.94] and std in [1.24], 'Weibull parameters not included.'
            if mean == 1.94 and std == 1.24:
                shape = 1.60
                scale = 2.16
            times = scale * np.random.weibull(shape,n)

        elif distribution == 'discrete_80_20':
            day_80 = param_1
            day_20 = param_2
            times = [param_1 if i < 0.8 else param_2 for i in np.random.uniform(0,1,n)]

    if n == 1:
        times = times[0]
    return(times)

class Individual:
    """
    Object representing each individual in the population
    """
    #All times in number of timesteps
    def __init__(self, disease, dt, n, sampled_dis_durations, SL_dict, p_detects, mtf_delay
s, cfr, direct_to_R2):
        self.disease = disease
        self.cfr = cfr
        self.disease_stages = Determine_Disease_Stages(self,n,sampled_dis_durations,dt)  #
```

```
List where each element is the disease stage the person is in at that time step
        self.SL_dict = SL_dict[self.outcome]  # Dictionary with keys disease stage, values
severity level for person's outcome (fatality vs survivor)
        self.severity_levels = Determine_Severity_Levels(self)  # List where each element i
s the severity level of the person at each time step
        self.locations = Determine_Locations(self,mtf_delays,dt,direct_to_R2)  # List of pe
rson's location at each timestep
        self.p_detects_symptom_onset, self.p_detects_stage2_onset = p_detects  # Diagnostic
test sensitivity at symptom onset & stage 2 onset
        self.p_detect = Determine_P_Detect(self)  # List of probability of detection if dia
gnostic was run on specimens collected at that time
        self.symptom_times = Determine_Symptom_Times(self)  # Dictionary with keys for seve
rity level and values for the timestep the person reached that severity level

def Determine_Disease_Stages(person,n,sampled_dis_durations,dt):
    """
    Returns a list where each element is the disease stage the person is in at that time st
ep
    """
    if np.random.uniform(0,1) < person.cfr:
        person.outcome = 'Fat'
    else:
        person.outcome = 'Surv'

    person.incubation = sampled_dis_durations[person.outcome]['incubation'][n]
    person.stage_1 = sampled_dis_durations[person.outcome]['stage_1s'][n]
    person.stage_2 = sampled_dis_durations[person.outcome]['stage_2s'][n]
    person.stage_3 = sampled_dis_durations[person.outcome]['stage_3s'][n]

    # Convert from days to number of timesteps
    person.incubation, person.stage_1, person.stage_2, person.stage_3 = [int(round(x/dt, 0)
) for
                                                                   x in [person.incubation,
                                                                        person.stage_1,
                                                                        person.stage_2,
                                                                        person.stage_3]]
    person.total_illness = person.incubation + person.stage_1 + person.stage_2 + person.sta
ge_3

    disease_stages = ['inc'] * person.incubation + \
```

```
                              ['stage_1'] * person.stage_1 + \
                              ['stage_2'] * person.stage_2 + \
                              ['stage_3'] * person.stage_3
    return(disease_stages)

def Determine_Severity_Levels(person):
    """
    Returns a list where each element is the severity level of the person at each time step
    """
    person.stage_1_SL = person.SL_dict['stage_1']
    person.stage_2_SL = person.SL_dict['stage_2']
    person.stage_3_SL = person.SL_dict['stage_3']

    severity_levels = [person.SL_dict[t] for t in person.disease_stages]
    return (severity_levels)

def Determine_Locations(person, mtf_delays, dt,direct_to_R2):
    """
    Returns list specifying person's location at every timestep
    """
    # Random draws to determine time at each MTF /transport time
    delay_r1_out_days = np.random.uniform(mtf_delays['R1_Out'][0],mtf_delays['R1_Out'][1])
    delay_r1_in_days = np.random.uniform(mtf_delays['R1_In'][0], mtf_delays['R1_In'][1])
    delay_r2_days = np.random.uniform(mtf_delays['R2'][0], mtf_delays['R2'][1])

    # Convert from days to timesteps
    delay_r1_out = int(round(delay_r1_out_days/dt, 0))
    delay_r1_in = int(round(delay_r1_in_days/dt, 0))
    delay_r2 = int(round(delay_r2_days/dt, 0))

    # Person is in their unit while incubating the disease
    locations = ['Unit'] * person.incubation

    # If first symptoms are severity level 3 (severe) or worse, they go to Role 1 MTF as an
inpatient and then are transported to higher roles of care
    if person.stage_1_SL > 2:
        #  direct_to_R2 is  deprecated feature in which person could report directly to a R
ole 2 MTF instead of a Role 1 MTF
        if not direct_to_R2:
            locations += ['R1'] * delay_r1_in
```

```
            locations += ['R2'] * delay_r2

    #  If person's stage 1 severity level is <= 2 (mild or moderate) and their stage 2 seve
rity level is >2,
        # they first report to the Role 1 MTF as an outpatient, go back to their unit until
onset of stage 2,
        # then they return to Role 1 MTF as in inpatient before being moved to higher roles
of care
    elif person.stage_2_SL > 2:
        # Again, "direct to Role 2" is a deprecated feature we did not use in the analysis
        if not direct_to_R2:
            locations += ['R1'] * delay_r1_out + \
                         ['Unit'] * max((person.stage_1 - delay_r1_out),0) + \
                         ['R1'] * delay_r1_in + \
                         ['R2'] * delay_r2
        else:
            locations += ['R2'] * delay_r1_out + \
                         ['Unit'] * max((person.stage_1 - delay_r1_out), 0) + \
                         ['R2'] * delay_r2
    # If person's severity level is <= 2 for first two stages of disease, they first report
to the Role 1 MTF as an outpatient,
        #then go back to their unit until the onset of stage 3 symptoms
        # at which point they return to the Role 1 MTF for inpatient treatment before being
moved to higher roles of care
    elif person.stage_3_SL > 2:
        if not direct_to_R2:
            locations += ['R1'] * delay_r1_out + \
                         ['Unit'] * max((person.stage_1 + person.stage_2 - delay_r1_out),0)
+ \
                         ['R1'] * delay_r1_in + \
                         ['R2'] * delay_r2
        else:
            locations += ['R2'] * delay_r1_out + \
                         ['Unit'] * max((person.stage_1 + person.stage_2 - delay_r1_out), 0
) + \
                         ['R2'] * delay_r2

    # If person's severity level is always <= 2, they first report to the Role 1 MTF as an
outpatient,
        # Then return to their unit for the duration of their disease
```

```
    else:
        if not direct_to_R2:
            locations += [‘R1’] * delay_r1_out + \
                         [‘Unit’] * max((person.stage_1 + person.stage_2 + person.stage_3 -
delay_r1_out),0)
        else:
            locations += [‘R2’] * delay_r1_out + \
                         [‘Unit’] * max((person.stage_1 + person.stage_2 + person.stage_3 -
delay_r1_out), 0)

    # Truncate locations list so it is same length as their disease duration
    if len(locations) > person.total_illness:
        locations = locations[:person.total_illness]
    # Pad the right of their location list with Role 3 to reflect them spending the remaini
ng duration of their disease there
    else:
        time_in_R3 = person.total_illness - len(locations)
        locations += [‘R3’] * time_in_R3

    return (locations)

def Determine_P_Detect(person):
    “““
    Returns list of diagnostic test sensitivity if specimen was collected at that time step
    Note, this is step function with values equal to the sensitivity at symptom onset for t
he duration of stage 1, and
    equal to the sensitivity at onset of stage 2 for duration of stage 2
    “““
    p_detect = [0.] * person.incubation
    p_detect += [person.p_detects_symptom_onset] * person.stage_1
    p_detect += [person.p_detects_stage2_onset] * (person.total_illness - person.stage_1 -
person.incubation)

    return(p_detect)

def Determine_Symptom_Times(person):
    “““
    Returns dictionary with key for each severity level and values for the time (in timeste
ps) the person reached that stage of disease
    “““
```

```
    symptom_times = {}
    sls = set(person.severity_levels)
    for sl in [1,2,3,4]:
        if sl in sls:
            onset_time = person.severity_levels.index(sl)
        else:
            onset_time = None
        symptom_times[sl] = onset_time

    if person.outcome == 'Fat':
        symptom_times['Death'] = person.total_illness
    else:
        symptom_times['Death'] = None

    return(symptom_times)

def Determine_Time_of_Diagnoses(pop, test_loc, cases_start_sampling, SL_trigger_sample, shi
p_times, dt,run_time,ship_samples,ship_samples_freq):
    """
    Returns the time of each true positive and each false negative
    """
    symptom_onsets_dt = sorted([person.incubation for person in pop])

    # If sampling does not start until after x cases, determine when that is
    if cases_start_sampling == 0:
        dt_start_sampling = 0
    else:
        dt_start_sampling = symptom_onsets_dt[cases_start_sampling-1]

    # Account for the shipping of samples if applicable
    if ship_samples:
        max_time = max(person.total_illness for person in pop) * dt
        #Sample shipping occurs at fixed times (e.g., every 12 hours), so first determine w
hen first samples are shipped after the attack
        if ship_samples_freq > dt:
            first_ship = np.random.uniform(0,ship_samples_freq)
            ship_schedule = np.arange(first_ship, max_time + 3*ship_samples_freq, ship_samp
les_freq) #  Arbitrary pad of 3*shipping freq on end
            ship_schedule = [int(round(t/dt,0)) for t in ship_schedule]
        else:
```

```
            ship_schedule = list(range(0,int(round(max_time/dt,0))))

        # Determine a random sample transport time for each ship schedule time for each pos
sible ship route.
        # That way all samples shipped at one time will take the same amount of time to tra
nsport
        random_ship_times = {'R1':{},'R2':{},'R3':{}}
        for sample_loc_option, val in ship_times.items():
            for test_loc_option, times in val.items():
                random_ship_times[sample_loc_option][test_loc_option] = np.random.uniform(s
hip_times[sample_loc_option][test_loc_option][0],
                                                                                  ship_times[samp
le_loc_option][test_loc_option][1],
                                                                                  len(ship_schedu
le))

    result_times = []  # True positive result times
    fn_times = []  # False negative result times
    # Determine when the diagnostic result is available for each person
    for person in pop:
        # Some people never get a test run b/c testing starts after they develop symptoms
        if person.incubation < dt_start_sampling:
            result_time = None
        # The following ways in which a person could not be tested don't occur in any of ou
r runs, but are included to make the model robust
        elif max(person.severity_levels) < SL_trigger_sample:
            result_time = None
        elif (not ship_samples) and (test_loc != 'Lab') and (test_loc not in person.locatio
ns):
            result_time = None
        elif (not ship_samples) and (test_loc == 'Lab') and ('R3' not in person.locations):
            result_time = None

        # Determine time the sample is taken
        else:
            if ship_samples:
                sample_time = next(x for x, val in enumerate(person.severity_levels) if
                                   val >= SL_trigger_sample)  # First time step with SL >=
threshold
            elif not ship_samples and test_loc == "Lab":
```

```
            sample_time = next(x for x, val in enumerate(zip(person.severity_levels, pe
rson.locations)) if
                                            val[0] >= SL_trigger_sample and val[1] == 'R3')  # First
time step with SL>=thresh & person at R3
        else:
            sample_time = next(x for x, val in enumerate(zip(person.severity_levels,per
son.locations)) if
                                            val[0] >= SL_trigger_sample and val[1] == test_loc)  # F
irst time step with SL >= threshold & person in same MTF as test

        sample_loc = person.locations[sample_time]  # Location of person when sample is
collected
        sample_bioav = person.p_detect[sample_time]  # Test sensitivity of diagnostic f
or sample collected at that time

        # Account for time it takes to ship sample
        if ship_samples:
            #Determine when sample will be shipped
            next_shipment_time = [t for t in ship_schedule if t >= sample_time][0]
            next_shipment_delay = next_shipment_time - sample_time

            next_shipment_index = ship_schedule.index(next_shipment_time)
            ship_time = random_ship_times[sample_loc][test_loc][next_shipment_index]
            ship_time = int(round(ship_time/dt, 0))
        else:
            next_shipment_delay = 0
            ship_time = 0

        if not ship_samples and test_loc != "Lab":
            assert ship_time == 0, "trying to ship sample even though its prohibited"

        result_time = sample_time + next_shipment_delay + ship_time + run_time  # Time
diagnostic result available
        result = np.random.uniform(0,1) <= sample_bioav  # Boolean result of diagnostic
test

        if result == False:
            fn_times.append(result_time)
            result_time = None
```

```
        result_times.append(result_time)
    return(result_times, fn_times)
```

Visualization R Code

```
library(tidyverse)
library(ggrepel)
library(hash)

diseases <- c('Plague', 'QFever', 'Tularemia', 'VEE', 'Flu', 'Ebola')
disease <- diseases[6]

f.hash <- hash()
f.hash[['Plague']] <- 'parsed_Plague_20210203-172853'
f.hash[['QFever']] <- 'parsed_QFever_20210203-181306'
f.hash[['Tularemia']] <- 'parsed_Tularemia_20210203-180857'
f.hash[['VEE']] <- 'parsed_VEE_Lab_20210212-163119'
f.hash[['Flu']] <- 'parsed_Flu_20210203-175519'
f.hash[['Ebola']] <- 'parsed_Ebola_Combined_1500_Trials'

ttp.hash <- hash()
ttp.hash[['Plague']] <- 43
ttp.hash[['QFever']] <- 19
ttp.hash[['VEE']] <- 4
ttp.hash[['Tularemia']] <- 25
ttp.hash[['Flu']] <- 19
ttp.hash[['Ebola']]<- 31

x.hash <- hash()
x.hash[['Plague']] <- 12
x.hash[['QFever']] <- 10
x.hash[['Tularemia']] <- 7
x.hash[['VEE']] <- 10
x.hash[['Flu']] <- 5
x.hash[['Ebola']] <- 30

plague.labs.df <-  data.frame('TTP'=c('A','B','C','D','E','F','G'),
                              '10'=c(3.8,3.4,3.3,3.6,7.2,4.2,8.3),
                              '100'=c(3.0,2.7,2.6,2.7,3.0,3.1,7.2),
                              '1000'=c(2.7,2.3,2.2,1.7,1.9,2.2,6.7))
```

```
Qfever.labs.df <- data.frame('TTP' = c('A','B','C','D','E','F'),
                              '10' = c(7.4, 7.6, 9.2, 9.2, 7.7, 8),
                              '100' = c(6.8, 7.0, 7.5, 7.5, 7.2, 7.3),
                              '1000' = c(6.4, 6.6, 6.9, 6.9, 6.8, 6.9))
VEE.labs.df <- data.frame('TTP' = c('A','B','C','D'),
                           '10' = c(4.9, 4.9, 4.8, 8.6),
                           '100' = c(4.5, 4.5, 4.5, 4.9),
                           '1000' = c(4.4, 4.4, 4.4, 4.5))
Tularemia.labs.df <- data.frame('TTP' = c('A','B','C','D','E','F'),
                                 '10' = c(2.2, 1.9, 1.8, 2.1, 2.2, 6.2),
                                 '100' = c(2.0, 1.7, 1.6, 1.7, 2.1, 6.0),
                                 '1000' = c(1.9, 1.6, 1.5, 1.6, 2.0, 5.9))
Flu.labs.df <- data.frame('TTP' = c('A','B','C','D','E','F','G','H','I','J','K','L','M','N')
,
                           '10' = c(0.9, 0.8, 2.7, 2.6, 1.3, 1.3, 0.8, 1.0, 2.7, 2.5, 1.3, 1.
4, 4.8, 4.7),
                           '100' = c(0.6, 0.5, 0.9, 0.8, 1.1, 1.0, 0.6, 0.6, 0.9, 0.8, 1.1, 1
.1, 4.5, 4.5),
                           '1000' = c(0.5, 0.4, 0.6, 0.5, 0.9, 0.8, 0.5, 0.4, 0.6, 0.5, 0.9,
0.9, 4.4, 4.4))
Ebola.labs.df <- data.frame('TTP' = c('A','B','C','D','E','F','G'),
                             '10' = c(4.8, 4.9, 4.4, 4.2, 1.8, 24.2, 2.2),
                             '100' = c(2.3, 2.4, 1.9, 1.8, 0.5, 2.2, 0.9),
                             '1000' = c(1.3, 1.4, 1.0, 0.8, 0.2, 0.5, 0.7))
df <- read.csv(sprintf('Outputs//Time_Line_Results//Parsed/%s.csv',f.hash[[disease]]),row.na
mes = 1)

if(disease=='Plague'){
  df.labs<-plague.labs.df
  sevs <- c('Moderate', 'V. Severe', 'Death')
}
if(disease=='QFever'){
  df.labs<-Qfever.labs.df
  sevs <- c('Moderate')
}
if(disease=='VEE'){
  df.labs<-VEE.labs.df
  sevs <- c('Severe')
}
if(disease=='Tularemia'){
```

```
  df.labs<-Tularemia.labs.df
  sevs <- c('Severe')
}
if(disease=='Flu'){
  df.labs<-Flu.labs.df
  sevs <- c('Moderate')
}
if(disease=='Ebola'){
  df.labs <- Ebola.labs.df
  sevs <- c('Moderate', 'Severe', 'Death')
}

df2 <- df %>%
  filter(TTP %in% unique(df$TTP)[ttp.hash[[disease]]:(ttp.hash[[disease]]+2)], percentile==0
.5) %>%
  select(-c(2:11))

df3 <- df2 %>%
  gather(key='Day',value='Cases',-c(TTP,N_Infected,Day_First_Diag,percentile,Result))

df3$Day <- as.numeric(gsub('X','',df3$Day))

df4 <- df3 %>%
  group_by(TTP,Result) %>%
  replace_na(list(Cases=0)) %>%
  mutate("Total_Cases"=cumsum(Cases))

df4$Result <- recode_factor(factor(df4$Result), 'SL1'='Mild','SL2'='Moderate','SL3'='Severe'
,
                            'SL4'='V. Severe','Death'='Death','TP'='True Positives','FN'='Fa
lse Negatives')
df4 <- filter(df4, !Result %in% c('True Positives','False Negatives'))
df4 <- filter(df4, Result %in% sevs)


df.TTP.labs <- df.labs %>%
  gather(key='N_Infected',value='t',-TTP)

df.TTP.labs$N_Infected <- as.numeric(gsub('X','',df.TTP.labs$N_Infected))
df.TTP.labs <- mutate(df.TTP.labs, "y.pos"=N_Infected*1)
```

```
ggplot() +
  geom_line(data=df4, aes(x=Day,y=Total_Cases,color=Result),size=1) +
  geom_segment(data=df.TTP.labs, aes(x=t, xend=t, y=0, yend=y.pos),size=0.5,linetype='dashed
',alpha=0.5) +
  facet_grid(rows=vars(N_Infected), scales = "free_y") +
  scale_x_continuous(limits=c(0,x.hash[[disease]]), breaks=c(seq(0,x.hash[[disease]],1))) +
  xlab('Days Following Exposure') +
  ylab('Total Number of Cases') +
  scale_color_discrete(name="Symptom Severity") +
  theme(panel.spacing = unit(1., "lines")) +
  geom_text_repel(data=filter(df.TTP.labs,N_Infected==1000), aes(x=t,y=y.pos,label=TTP), yli
m = c(1110, 1250), segment.size = 0.2, size=2.5, min.segment.length = 0) +
  geom_text_repel(data=filter(df.TTP.labs,N_Infected==100), aes(x=t,y=y.pos,label=TTP), ylim
= c(111, 125), segment.size = 0.2, size=2.5, min.segment.length = 0) +
  geom_text_repel(data=filter(df.TTP.labs,N_Infected==10), aes(x=t,y=y.pos,label=TTP), ylim
= c(11.1, 12.5), segment.size = 0.2, size=2.5, min.segment.length = 0) +
  coord_cartesian(clip = "off") +
  labs(title = '')

ggsave(sprintf('.//Plots//%s.png',disease))
```

Input Data

P_Detect.csv

Probability of detection at symptom onset and Stage 2 onset for each disease, diagnostic test, and specimen (sample).

```
Disease,Diagnostic,Sample,Symptom Onset,Stage2 Onset

QFever,PCR_IS111,Blood,1,

QFever,PCR_com1,Blood,0.5,

Plague,Culture,Blood,0.05,0.4

Plague,PCR,Blood,0.05,1

Tularemia,Culture,Blood,0.1,0.9

Tularemia,PCR,Blood,0.3,1

Tularemia,Panel,Blood,0.3,1

Flu,PCR,NasoPhary_Asp,1,
```

```
Flu,Antigen,NasoPhary_Asp,0.36,

Flu,Isolation,NasoPhary_Asp,1,

Flu,PCR,Throat_Nasal_Swab,0.9,

Flu,Antigen,Throat_Nasal_Swab,0.768,

Flu,Isolation,Throat_Nasal_Swab,0.94,

VEE,Isolation,Blood,1,

VEE,Isolation,Throat_Swab,1,

VEE,Plaque_Assay,Serum,1,

Ebola,PCR,Blood,1,1

Ebola,Antigen,Blood,,1
```

Diagnostics_Run_Times.csv

The time it takes to run each diagnostic (in days).

```
Disease,Diagnostic,Sample,Run Time (Days)

QFever,PCR_IS111,Blood,0.083333333

QFever,PCR_com1,Blood,0.083333333

Plague,Culture,Blood,4

Plague,PCR,Blood,0.083333333

Tularemia,Culture,Blood,4

Tularemia,PCR,Blood,0.083333333

Tularemia,Panel,Blood,0.0625

Flu,PCR,NasoPhary_Asp,0.083333333

Flu,Antigen,NasoPhary_Asp,0.041666667

Flu,Isolation,NasoPhary_Asp,4

Flu,PCR,Throat_Nasal_Swab,0.083333333

Flu,Antigen,Throat_Nasal_Swab,0.041666667

Flu,Isolation,Throat_Nasal_Swab,4

VEE,Isolation,Blood,4

VEE,Isolation,Throat_Swab,4

VEE,Plaque_Assay,Serum,4
```

```
Ebola,PCR,Blood,0.083333333

Ebola,Antigen,Blood,0.166666667
```

Diseases.csv

The parameters to characterize the distributions for the duration of each stage of each disease. Two parameters are available to specify each stage of the disease. The parameters are specified according to Table **B-1**.

Table B-1**. Distribution Parameters Specified in Diseases.csv**

Distribution	Parameter 1	Parameter 2
lognormal	Mean (days)	STD (days)
uniform	Min (days)	Max (days)
gamma	Mean (days)	STD (days)
constant	value(days)	-1
weibull	Mean(days	STD (days))
discrete_80_20	duration 80% of the time	duration 20% of the time

Note: The abbreviations used in this table are defined in Appendix E of this paper.

```
Disease,Outcome,CFR,Incubation_Dist,Incubation_Param1,Incubation_Par
am2,Stage_1_Dist,Stage_1_Param1,Stage_1_Param2,Stage_1_SL,Stage_2_Di
st,Stage_2_Param1,Stage_2_Param2,Stage_2_SL,Stage_3_Dist,Stage_3_Par
am1,Stage_3_Param2,Stage_3_SL

Ebola,Surv,0.4,gamma,10.3,8.2,gamma,4.3,3.7,2,gamma,10.6,6.2,3,none,
-1,-1,-1

Ebola,Fat,0.4,gamma,10.3,8.2,gamma,4.3,3.7,2,gamma,3.8,3.3,3,none,-
1,-1,-1

Plague,Surv,1,lognormal,4.3,1.8,constant,1,-
1,2,lognormal,1.5,1.2,4,none,-1,-1,-1

Plague,Fat,1,lognormal,4.3,1.8,constant,1,-
1,2,lognormal,1.5,1.2,4,none,-1,-1,-1

QFever,Surv,0,gamma,8.196359534,0.630945152,lognormal,12.1,6.66,2,no
ne,-1,-1,-1,none,-1,-1,-1

QFever,Fat,0,gamma,8.196359534,0.630945152,lognormal,12.1,6.66,2,non
e,-1,-1,-1,none,-1,-1,-1

Tularemia,Surv,0.75,lognormal,1.778758419,0.146133667,constant,12,-
1,3,constant,28,-1,3,constant,84,-1,2
```

```
Tularemia,Fat,0.75,lognormal,1.778758419,0.146133667,constant,9,-
1,3,constant,6,-1,4,none,-1,-1,-1

Flu,Surv,0,lognormal,1.52,0.66,uniform,3,7,2,none,-1,-1,-1,none,-1,-
1,-1

Flu,Fat,0,lognormal,1.52,0.66,uniform,3,7,2,none,-1,-1,-1,none,-1,-
1,-1

VEE,Surv,0,weibull,1.94,1.24,discrete_80_20,2,3,3,lognormal,3.47,2.8
,2,lognormal,4.84,3.81,1

VEE,Fat,0,weibull,1.94,1.24,discrete_80_20,2,3,3,lognormal,3.47,2.8,
2,lognormal,4.84,3.81,1
```

MTF.csv

The minimum (Low) and maximum (High) time (in days) for the following:

- R1_Out: time at a Role 1 MTF as an outpatient
- R1_In: time at a role 1 MTF as an inpatient + time to get to Role 2 MTF
- R2: time at a Role 2 MTF + time to get to Role 3 MTF

```
Parameter,Low,High

R1_Out,0.1,0.1

R1_In,0.083333333,0.166666667

R2,0.25,0.416666667
```

Sample_Shipment_Times.csv

The minimum (Low) and maximum (High) time in days for the following:

- R1-R2: ship a sample from a Role 1 to a Role 2 (not used in analysis)
- R1-R3: ship a sample from a Role 1 to a Role 3.
- R2-R3: ship a sample from a Role 2 to a Role 3 (not used in analysis).
- R1-Lab: ship a sample from a Role 1 to a an out of theater lab (accounted for in the run time of the out of theater diagnostic).

```
Route,Low,High

R1-R2,0.0625,0.104166667

R1-R3,0.208333333,0.333333333

R2-R3,0.145833333,0.229166667
```

```
R1-Lab,0,0
```

Disease-Specific Run Files

Batches of runs for each disease; columns are as follows:

- Disease.
- Diagnostic: diagnostic test.
- Sample: clinical specimen.
- Cases_To_Start_Sampling: number of cases that must occur before the collection/analysis of samples.
- SL_Trigger_To_Collect_Sample: symptom severity level that triggers specimen collection.
- Test_Location: location of diagnostic test.
- Direct_to_R2: does patient report directly to a Role 2 at symptom onset instead of a Role 1 MTF (deprecated, not used in analysis)?
- Ship_Samples: are samples shipped?
- Ship_Samples_Freq: frequency in days that samples are shipped.
- N_Diags_Report: the number of positive diagnostics results for which the time of the specified number of diagnostic results are reported (deprecated, not used in analysis).
- N_Cases_Report: the number of cases with any symptoms for which the time of the specified number of cases are reported (deprecated, not used in analysis).
- Trials: number of trials run.
- Infected: number of infected individuals.

Plague.csv

```
Disease,Diagnostic,Sample,Cases_To_Start_Sampling,SL_Trigger_To_Coll
ect_Sample,Test_Location,Direct_to_R2,Ship_Samples,Ship_Samples_Freq
,N_Diags_Report,N_Cases_Report,Trials,Infected

Plague,PCR,Blood,0,4,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100
0

Plague,PCR,Blood,0,4,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100
0

Plague,PCR,Blood,0,4,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100
0
```

```
Plague,PCR,Blood,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100
0

Plague,PCR,Blood,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100
0

Plague,PCR,Blood,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10
00

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,100
0

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,1
000

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,
1000

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500
,1000

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,100
0

Plague,PCR,Blood,0,4,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100

Plague,PCR,Blood,0,4,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100

Plague,PCR,Blood,0,4,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100

Plague,PCR,Blood,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100

Plague,PCR,Blood,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100

Plague,PCR,Blood,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10
0

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,100

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,1
00

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,
100

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500
,100

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,100

Plague,PCR,Blood,0,4,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Plague,PCR,Blood,0,4,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10
```

Plague,PCR,Blood,0,4,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Plague,PCR,Blood,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Plague,PCR,Blood,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Plague,PCR,Blood,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,10

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,1
0

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,
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Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500
,10

Plague,PCR,Blood,0,4,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,10

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0

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,1
000

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,
1000

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500
,1000

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,100
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Plague,PCR,Blood,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,100

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,1
00

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,
100

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500
,100

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,100

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,10

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,1
0

```
Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,10

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500,10

Plague,PCR,Blood,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,10

Plague,Culture,Blood,0,4,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,1000

Plague,Culture,Blood,0,4,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,100

Plague,Culture,Blood,0,4,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10
```

Tularemia.csv

```
Disease,Diagnostic,Sample,Cases_To_Start_Sampling,SL_Trigger_To_Collect_Sample,Test_Location,Direct_to_R2,Ship_Samples,Ship_Samples_Freq,N_Diags_Report,N_Cases_Report,Trials,Infected

Tularemia,PCR,Blood,0,3,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,1000

Tularemia,PCR,Blood,0,3,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,1000

Tularemia,PCR,Blood,0,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,1000

Tularemia,PCR,Blood,5,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,1000

Tularemia,PCR,Blood,10,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,1000

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,1000

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,1000

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,1000

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500,1000
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Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,
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Tularemia,Panel,Blood,0,3,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,1000

Tularemia,Panel,Blood,0,3,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,1000

Tularemia,Panel,Blood,0,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,1000

Tularemia,Panel,Blood,5,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,1000

Tularemia,Panel,Blood,10,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
00,1000

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,50
0,1000

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,
500,1000

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”
,500,1000

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]
”,500,1000

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,50
0,1000

Tularemia,PCR,Blood,0,3,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,
100

Tularemia,PCR,Blood,0,3,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,
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Tularemia,PCR,Blood,0,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,
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Tularemia,PCR,Blood,5,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,
100

Tularemia,PCR,Blood,10,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500
,100

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,
100
```

```
Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,50
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Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,5
00,100

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,
500,100

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,
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Tularemia,Panel,Blood,0,3,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
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Tularemia,Panel,Blood,0,3,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,100

Tularemia,Panel,Blood,0,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,100

Tularemia,Panel,Blood,5,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,100

Tularemia,Panel,Blood,10,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
00,100

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,50
0,100

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,
500,100

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”
,500,100

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]
”,500,100

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,50
0,100

Tularemia,PCR,Blood,0,3,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,
10

Tularemia,PCR,Blood,0,3,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,
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Tularemia,PCR,Blood,0,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,
10
```

Tularemia,PCR,Blood,5,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,PCR,Blood,10,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,PCR,Blood,0,3,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,0,3,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,0,3,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,0,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,5,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,10,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Panel,Blood,0,3,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,10

Tularemia,Culture,Blood,0,3,Lab,FALSE,FALSE,,"[1,5,10]","[1,10,100]",500,1000

Tularemia,Culture,Blood,0,3,Lab,FALSE,FALSE,,"[1,5,10]","[1,10,100]",500,100

Tularemia,Culture,Blood,0,3,Lab,FALSE,FALSE,,"[1,5,10]","[1,10,100]",500,10

Flu.csv

Disease,Diagnostic,Sample,Cases_To_Start_Sampling,SL_Trigger_To_Collect_Sample,Test_Location,Direct_to_R2,Ship_Samples,Ship_Samples_Freq,N_Diags_Report,N_Cases_Report,Trials,Infected

Flu,PCR,NasoPhary_Asp,0,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",500,1000

Flu,PCR,NasoPhary_Asp,5,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",500,1000

Flu,PCR,NasoPhary_Asp,10,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",500,1000

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,1,"[1,5,10]","[1,10,100]",500,1000

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.5,"[1,5,10]","[1,10,100]",500,1000

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.25,"[1,5,10]","[1,10,100]",500,1000

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.125,"[1,5,10]","[1,10,100]",500,1000

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0,"[1,5,10]","[1,10,100]",500,1000

Flu,Antigen,NasoPhary_Asp,0,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",500,1000

Flu,Antigen,NasoPhary_Asp,5,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",500,1000

Flu,Antigen,NasoPhary_Asp,10,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",500,1000

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,1,"[1,5,10]","[1,10,100]",500,1000

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Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,10
0]”,500,1000

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,1
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Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,
100]”,500,1000

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]
”,500,1000

Flu,PCR,Throat_Nasal_Swab,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]
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”,500,1000

Flu,PCR,Throat_Nasal_Swab,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100
]”,500,1000

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]
”,500,1000

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,10
0]”,500,1000

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,1
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Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,
100]”,500,1000

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]
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Flu,Antigen,Throat_Nasal_Swab,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,
100]”,500,1000

Flu,Antigen,Throat_Nasal_Swab,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10
,100]”,500,1000

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,
100]”,500,1000

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,1
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```
Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,
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Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1
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Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,
100]”,500,1000

Flu,PCR,NasoPhary_Asp,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,100

Flu,PCR,NasoPhary_Asp,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
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Flu,PCR,NasoPhary_Asp,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
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Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,50
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Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,
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Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”
,500,100

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]
”,500,100

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,50
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Flu,Antigen,NasoPhary_Asp,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]
”,500,100

Flu,Antigen,NasoPhary_Asp,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]
”,500,100

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]”,500,100

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]
”,500,100

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,10
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Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,1
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Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0,"[1,5,10]","[1,10,100]
",500,100

Flu,PCR,Throat_Nasal_Swab,0,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]
",500,100

Flu,PCR,Throat_Nasal_Swab,5,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]
",500,100

Flu,PCR,Throat_Nasal_Swab,10,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100
]",500,100

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,1,"[1,5,10]","[1,10,100]
",500,100

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.5,"[1,5,10]","[1,10,10
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Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.25,"[1,5,10]","[1,10,1
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Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.125,"[1,5,10]","[1,10,
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Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0,"[1,5,10]","[1,10,100]
",500,100

Flu,Antigen,Throat_Nasal_Swab,0,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,
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Flu,Antigen,Throat_Nasal_Swab,5,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,
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Flu,Antigen,Throat_Nasal_Swab,10,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10
,100]",500,100

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,1,"[1,5,10]","[1,10,
100]",500,100

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.5,"[1,5,10]","[1,1
0,100]",500,100

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.25,"[1,5,10]","[1,
10,100]",500,100

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.125,"[1,5,10]","[1
,10,100]",500,100
```

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,
100]”,500,100

Flu,PCR,NasoPhary_Asp,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,10

Flu,PCR,NasoPhary_Asp,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,10

Flu,PCR,NasoPhary_Asp,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
00,10

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,50
0,10

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,
500,10

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”
,500,10

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]
”,500,10

Flu,PCR,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,50
0,10

Flu,Antigen,NasoPhary_Asp,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]
”,500,10

Flu,Antigen,NasoPhary_Asp,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]
”,500,10

Flu,Antigen,NasoPhary_Asp,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100
]”,500,10

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]
”,500,10

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,10
0]”,500,10

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,1
00]”,500,10

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,
100]”,500,10

Flu,Antigen,NasoPhary_Asp,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]
”,500,10

```
Flu,PCR,Throat_Nasal_Swab,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Flu,PCR,Throat_Nasal_Swab,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Flu,PCR,Throat_Nasal_Swab,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,10

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,10

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,10

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500,10

Flu,PCR,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,10

Flu,Antigen,Throat_Nasal_Swab,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Flu,Antigen,Throat_Nasal_Swab,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Flu,Antigen,Throat_Nasal_Swab,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500,10

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,500,10

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,500,10

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,500,10

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,500,10

Flu,Antigen,Throat_Nasal_Swab,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,10

Flu,Isolation,Throat_Nasal_Swab,0,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,500,1000
```

```
Flu,Isolation,Throat_Nasal_Swab,5,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,
10,100]”,500,1000

Flu,Isolation,Throat_Nasal_Swab,10,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1
,10,100]”,500,1000

Flu,Isolation,NasoPhary_Asp,0,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,1
00]”,500,1000

Flu,Isolation,NasoPhary_Asp,5,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,1
00]”,500,1000

Flu,Isolation,NasoPhary_Asp,10,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,
100]”,500,1000

Flu,Isolation,Throat_Nasal_Swab,0,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,
10,100]”,500,100

Flu,Isolation,Throat_Nasal_Swab,5,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,
10,100]”,500,100

Flu,Isolation,Throat_Nasal_Swab,10,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1
,10,100]”,500,100

Flu,Isolation,NasoPhary_Asp,0,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,1
00]”,500,100

Flu,Isolation,NasoPhary_Asp,5,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,1
00]”,500,100

Flu,Isolation,NasoPhary_Asp,10,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,
100]”,500,100

Flu,Isolation,Throat_Nasal_Swab,0,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,
10,100]”,500,10

Flu,Isolation,Throat_Nasal_Swab,5,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,
10,100]”,500,10

Flu,Isolation,Throat_Nasal_Swab,10,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1
,10,100]”,500,10

Flu,Isolation,NasoPhary_Asp,0,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,1
00]”,500,10

Flu,Isolation,NasoPhary_Asp,5,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,1
00]”,500,10

Flu,Isolation,NasoPhary_Asp,10,2,Lab,FALSE,TRUE,0,”[1,5,10]”,”[1,10,
100]”,500,10
```

Ebola.csv

```
Disease,Diagnostic,Sample,Cases_To_Start_Sampling,SL_Trigger_To_Coll
ect_Sample,Test_Location,Direct_to_R2,Ship_Samples,Ship_Samples_Freq
,N_Diags_Report,N_Cases_Report,Trials,Infected

Ebola,PCR,Blood,0,3,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100
0

Ebola,PCR,Blood,0,3,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100
0

Ebola,PCR,Blood,0,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100
0

Ebola,PCR,Blood,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100
0

Ebola,PCR,Blood,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100
0

Ebola,PCR,Blood,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,10
00

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,1000,100
0

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,1000,1
000

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”,1000,
1000

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]”,1000
,1000

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,1000,100
0

Ebola,Antigen,Blood,0,3,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000
,1000

Ebola,PCR,Blood,0,3,R3,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100

Ebola,PCR,Blood,0,3,R2,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100

Ebola,PCR,Blood,0,3,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100

Ebola,PCR,Blood,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100

Ebola,PCR,Blood,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,1000,100
```

```
Ebola,PCR,Blood,10,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",1000,10
0

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,1,"[1,5,10]","[1,10,100]",1000,100

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0.5,"[1,5,10]","[1,10,100]",1000,1
00

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0.25,"[1,5,10]","[1,10,100]",1000,
100

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0.125,"[1,5,10]","[1,10,100]",1000
,100

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0,"[1,5,10]","[1,10,100]",1000,100

Ebola,Antigen,Blood,0,3,R3,FALSE,FALSE,,"[1,5,10]","[1,10,100]",1000
,100

Ebola,PCR,Blood,0,3,R3,FALSE,FALSE,,"[1,5,10]","[1,10,100]",1000,10

Ebola,PCR,Blood,0,3,R2,FALSE,FALSE,,"[1,5,10]","[1,10,100]",1000,10

Ebola,PCR,Blood,0,3,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",1000,10

Ebola,PCR,Blood,0,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",1000,10

Ebola,PCR,Blood,5,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",1000,10

Ebola,PCR,Blood,10,2,R1,FALSE,FALSE,,"[1,5,10]","[1,10,100]",1000,10

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,1,"[1,5,10]","[1,10,100]",1000,10

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0.5,"[1,5,10]","[1,10,100]",1000,1
0

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0.25,"[1,5,10]","[1,10,100]",1000,
10

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0.125,"[1,5,10]","[1,10,100]",1000
,10

Ebola,PCR,Blood,0,2,R3,FALSE,TRUE,0,"[1,5,10]","[1,10,100]",1000,10

Ebola,Antigen,Blood,0,3,R3,FALSE,FALSE,,"[1,5,10]","[1,10,100]",1000
,10
```

VEE.csv

```
Disease,Diagnostic,Sample,Cases_To_Start_Sampling,SL_Trigger_To_Coll
ect_Sample,Test_Location,Direct_to_R2,Ship_Samples,Ship_Samples_Freq
,N_Diags_Report,N_Cases_Report,Trials,Infected
```

```
VEE,Isolation,Blood,0,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500
,1000

VEE,Isolation,Blood,5,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500
,1000

VEE,Isolation,Blood,10,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,1000

VEE,Isolation,Throat_Swab,0,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100
]”,500,1000

VEE,Isolation,Throat_Swab,5,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100
]”,500,1000

VEE,Isolation,Throat_Swab,10,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,10
0]”,500,1000

VEE,Plaque_Assay,Serum,0,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,
500,1000

VEE,Plaque_Assay,Serum,5,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,
500,1000

VEE,Plaque_Assay,Serum,10,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”
,500,1000

VEE,Isolation,Blood,0,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500
,100

VEE,Isolation,Blood,5,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500
,100

VEE,Isolation,Blood,10,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,100

VEE,Isolation,Throat_Swab,0,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100
]”,500,100

VEE,Isolation,Throat_Swab,5,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100
]”,500,100

VEE,Isolation,Throat_Swab,10,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,10
0]”,500,100

VEE,Plaque_Assay,Serum,0,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,
500,100

VEE,Plaque_Assay,Serum,5,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,
500,100
```

```
VEE,Plaque_Assay,Serum,10,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”
,500,100

VEE,Isolation,Blood,0,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500
,10

VEE,Isolation,Blood,5,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,500
,10

VEE,Isolation,Blood,10,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,10

VEE,Isolation,Throat_Swab,0,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100
]”,500,10

VEE,Isolation,Throat_Swab,5,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100
]”,500,10

VEE,Isolation,Throat_Swab,10,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,10
0]”,500,10

VEE,Plaque_Assay,Serum,0,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,
500,10

VEE,Plaque_Assay,Serum,5,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,
500,10

VEE,Plaque_Assay,Serum,10,3,Lab,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”
,500,10
```

QFever.csv

```
Disease,Diagnostic,Sample,Cases_To_Start_Sampling,SL_Trigger_To_Coll
ect_Sample,Test_Location,Direct_to_R2,Ship_Samples,Ship_Samples_Freq
,N_Diags_Report,N_Cases_Report,Trials,Infected

QFever,PCR_IS111,Blood,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
00,1000

QFever,PCR_com1,Blood,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,1000

QFever,PCR_IS111,Blood,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
00,1000

QFever,PCR_com1,Blood,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,1000

QFever,PCR_IS111,Blood,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,
500,1000
```

QFever,PCR_com1,Blood,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
00,1000

QFever,PCR_IS111,Blood,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,5
00,1000

QFever,PCR_com1,Blood,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,50
0,1000

QFever,PCR_IS111,Blood,5,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,5
00,1000

QFever,PCR_com1,Blood,5,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,50
0,1000

QFever,PCR_IS111,Blood,10,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,
500,1000

QFever,PCR_com1,Blood,10,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,5
00,1000

QFever,PCR_IS111,Blood,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”
,500,1000

QFever,PCR_com1,Blood,0,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,
500,1000

QFever,PCR_IS111,Blood,5,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”
,500,1000

QFever,PCR_com1,Blood,5,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”,
500,1000

QFever,PCR_IS111,Blood,10,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]
”,500,1000

QFever,PCR_com1,Blood,10,2,R3,FALSE,TRUE,0.5,”[1,5,10]”,”[1,10,100]”
,500,1000

QFever,PCR_IS111,Blood,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]
”,500,1000

QFever,PCR_com1,Blood,0,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”
,500,1000

QFever,PCR_IS111,Blood,5,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]
”,500,1000

QFever,PCR_com1,Blood,5,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]”
,500,1000

```
QFever,PCR_IS111,Blood,10,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100
]”,500,1000

QFever,PCR_com1,Blood,10,2,R3,FALSE,TRUE,0.25,”[1,5,10]”,”[1,10,100]
”,500,1000

QFever,PCR_IS111,Blood,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100
]”,500,1000

QFever,PCR_com1,Blood,0,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]
”,500,1000

QFever,PCR_IS111,Blood,5,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100
]”,500,1000

QFever,PCR_com1,Blood,5,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100]
”,500,1000

QFever,PCR_IS111,Blood,10,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,10
0]”,500,1000

QFever,PCR_com1,Blood,10,2,R3,FALSE,TRUE,0.125,”[1,5,10]”,”[1,10,100
]”,500,1000

QFever,PCR_IS111,Blood,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,5
00,1000

QFever,PCR_com1,Blood,0,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,50
0,1000

QFever,PCR_IS111,Blood,5,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,5
00,1000

QFever,PCR_com1,Blood,5,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,50
0,1000

QFever,PCR_IS111,Blood,10,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,
500,1000

QFever,PCR_com1,Blood,10,2,R3,FALSE,TRUE,0,”[1,5,10]”,”[1,10,100]”,5
00,1000

QFever,PCR_IS111,Blood,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
00,100

QFever,PCR_com1,Blood,0,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,100

QFever,PCR_IS111,Blood,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
00,100
```

QFever,PCR_com1,Blood,5,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,50
0,100

QFever,PCR_IS111,Blood,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,
500,100

QFever,PCR_com1,Blood,10,2,R1,FALSE,FALSE,,”[1,5,10]”,”[1,10,100]”,5
00,100

QFever,PCR_IS111,Blood,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,5
00,100

QFever,PCR_com1,Blood,0,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,50
0,100

QFever,PCR_IS111,Blood,5,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,5
00,100

QFever,PCR_com1,Blood,5,2,R3,FALSE,TRUE,1,”[1,5,10]”,”[1,10,100]”,50
0,100

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References

Agua-Agum, Junerlyn, Archchun Ariyarajah, Bruce Aylward, Isobel M. Blake, Richard Brennan, Annie Cori, Christl A. Donnelly, et al. "West Africa Ebola Epidemic After One Year—Slowing but Not Yet Under Control." *New England Journal of Medicine* 372, no. 6 (February 5, 2015): 584–587. https://pubmed.ncbi.nlm.nih.gov/25539446/.

Aiken, Jim. *Next Generation Diagnostic System-Bio: Scientific Technical Information (STI) Relevance and Gap Analysis*. Battelle Memorial Institute, August 2015.

Anderson, Alicia, Henk Bijlmer, Pierre-Edouard Fournier, Stephen Graves, Joshua Hartzell, Gilbert J. Kersh, Gijs Limonard, et al. "Diagnosis and Management of Q Fever – United States, 2013: Recommendations from CDC and the Q Fever Working Group." *Morbidity and Mortality Weekly Report (MMWR)* 62 (RR-03) (29 March 2013): 1–30. https://pubmed.ncbi.nlm.nih.gov/23535757/.

Augustine, Robin, Anwarul Hasan, Survathi Das, Rashid Ahmed, Yasuyoshi Mori, Tsugunori Notomi, Bhavesh D. Kevadiya, and Avnesh S. Thakor. "Loop-Mediated Isothermal Amplification (LAMP): A Rapid, Sensitive, Specific, and Cost-Effective Point-of-Care Test for Coronaviruses in the Context of COVID-19 Pandemic." *Biology* 9, no. 8 (August 2020): 182. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464797/.

Banada, Padmapriya P., Srinidhi Deshpande, Soumitesh Chakravorty, Riccardo Russo, James Occi, Gabriel Meister, Kelly J. Jones, et al. "Sensitive Detection of *Francisella tularensis* Directly from Whole Blood by Use of the GeneXpert System." *Journal of Clinical Microbiology* 55, no. 1 (January 2017): 291–301. https://jcm.asm.org/content/55/1/291.

Berdjis, C.C., C. A. Gleiser, H. A. Hartman, R. W. Kuehne, and W. S. Gochenour. "Pathogenesis of Respiratory Anthrax in *Macaca mulatta*." *British Journal of Experimental Pathology* 43, no. 5 (October 1962): 515–524. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2095137/.

Bishop, Kristen A., Robert L. Cubeta, Jon M. Davis, and Lucas A. LaViolet. *Evaluation of Biological Agent Clinical Sampling and Analysis*. IDA P-21576. Institute for Defense Analyses, May 2021. CONTROLLED UNCLASSIFIED INFORMATION. Only UNCLASSIFIED information cited here.

Boyer, Anne E., Conrad P. Quinn, Alex R. Hoffmaster, Thomas R. Kozel, Elke Saile, Chung K. Marston, Ann Percival, et al. "Kinetics of Lethal Factor and Poly-D-Glutamic Acid Antigenemia during Inhalation Anthrax in Rhesus Macaques."

Infection and Immunity 77, no. 8 (August 2009): 3432–3441. doi:10.1128/IAI.00346-09.

Broadhurst, M. Jana, Tim J. G. Brooks, and Nira R. Pollock. "Diagnosis of Ebola Virus Disease: Past, Present, and Future." *Clinical Microbiology Reviews* 29, no. 4 (October 2016): 773–793. doi:10.1128/CMR.00003-16.

Burr, Julia K., Robert L. Cubeta, Jeffrey H. Grotte, Lucas A. LaViolet, Katherine M. Sixt, and Minica A. Smith. *Emerging Infectious Diseases Study*. IDA Paper P-5302. Institute for Defense Analyses, August 2016. https://apps.dtic.mil/sti/citations/AD1165599.

Burr, Julia K., Robert L. Cubeta, Lucas A. LaViolet, and Sean M. Oxford. *Controlling the Spread of Contagious Disease in an Operational Environment*. IDA Paper P-10877. Institute for Defense Analyses, February 2020. https://apps.dtic.mil/sti/citations/AD1116631.

Campbell, D. E., and A. S. Kemp. " Production of Antibodies to Staphylococcal Superantigens in Atopic Dermatitis." *Archives of Disease in Childhood* 79, no. 5 (November 1998): 400–404. doi:10.1136/adc.79.5.400.

Carrat, Fabrice, Elisabeta Vergu, Neil M. Ferguson, Magali Lemaitre, Simon Cauchemez, Steve Leach, and Alain-Jacques Valleron. "Time Lines of Infection and Disease in Human Influenza: A Review of Volunteer Challenge Studies." *American Journal of Epidemiology* 167, no. 7 (April 1, 2008): 775–785. doi:10.1093/aje/kwm375.

Centers for Disease Control and Prevention (CDC). "Clinical Signs and Symptoms of Influenza." Last updated August 31, 2020. https://www.cdc.gov/flu/professionals/acip/clinical.htm.

Centers for Disease Control and Prevention (CDC). "Rapid Influenza Diagnostic Tests." Accessed January 28, 2021. Last updated October 25, 2016. https://www.cdc.gov/flu/professionals/diagnosis/clinician_guidance_ridt.htm.

Centers for Disease Control and Prevention (CDC). "Update: Investigation of Anthrax Associated with Intentional Exposure and Interim Public Health Guidelines, October 2001." *Morbidity and Mortality Weekly Report (MMWR)* 50, no. 41 (October 19, 2001): 889–908. https://www.cdc.gov/mmwr/PDF/wk/mm5041.pdf.

Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical Laboratories*. 6th ed. Centers for Disease Control and Prevention, June 2020. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.

Chartrand, Caroline, Nicolas Tremblay, Christian Renaud, and Jesse Papenburg. "Diagnostic Accuracy of Rapid Antigen Detection Tests for Respiratory Syncytial Virus Infection: Systematic Review and Meta-Analysis." *Journal of Clinical Microbiology* 53, no. 12 (December 2015): 3738–3749. doi:10.1128/JCM.01816-15.

Cheng, Peter K. C., Kitty K. Y. Wong, Gannon C. Mak, Ann H. Wong, Anita Y. Y. Ng, Salina Y. K. Chow, et al. "Performance of Laboratory Diagnostics for the Detection of Influenza A(H1N1)v Virus as Correlated with the Time After Symptom Onset and

Viral Load." *Journal of Clinical Virology* 47, no. 2 (February 2010): 182–185. https://doi.org/10.1016/j.jcv.2009.11.022.

Chertow, Daniel S., Christian Kleine, Jeffrey K. Edwards, Roberto Scaini, Ruggero Giuliani, and Armand Sprecherand. "Ebola Virus Disease in West Africa- Clinical Manifestations and Management." *The New England Journal of Medicine* 371 (November 27, 2014): 2054–2057. doi:10.1056/NEJMp1413084.

Cook, E., X. Wang, N. Robiou, and B. C. Fries. "Measurement of Staphylococcal Enterotoxin B in Serum and Culture Supernatant with a Capture Enzyme-Linked Immunosorbent Assay." *Clinical and Vaccine Immunology* 14, no. 9 (September 2007): 1094–1101, doi:10.1128/CVI.00183-07.

Demeure, Christian E., Olivier Dussurget, Guillem Mas Fiol, Anne-Sophie Le Guern, Cyril Savin, and Javier Pizzaro-Cerdá. "*Yersinia pestis* and Plague: An Updated View on Evolution, Virulence Determinants, Immune Subversion, Vaccination, and Diagnostics." *Genes & Immunity* 20 (2019): 357–370. https://www.nature.com/articles/s41435-019-0065-0.

Dinges, Martin M., Paul M. Orwin, and Patrick M. Schlievert. "Exotoxins of *Staphylococcus aureus*." *Clinical Microbiology Reviews* 13, no. 1 (January 2000): 16–34. doi:10.1128/cmr.13.1.16-34.2000.

Dinnes, Jacqueline, Jonathan J. Deeks, Sarah Berhane, Melissa Taylor, Ada Adriano, Clare Davenport, Sabine Dittrich, et al. "Rapid, Point-of-Care Antigen and Molecular-Based Tests for Diagnosis of SARS-CoV-2 Infection." Cochrane Library, 2020. https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013705.pub2/full.

Dobson, Joanna, Richard J. Whitley, Stuart Pocock, and Arnold S. Monto. "Oseltamivir Treatment for Influenza in Adults: A Meta-Analysis of Randomised Controlled Trials." *Lancet* 385, no. 9979 (May 2, 2015): 1736. doi:10.1016/S0140-6736(14)62449-1.

Dupuy, Lesley C., Michelle J. Richards, Barry Ellefsen, Lillian Chau, Alain Luxembourg, Drew Hannaman, Brian D. Livingston, and Connie S. Schmaljohn. "A DNA Vaccine for Venezuelan Equine Encephalitis Virus Delivered by Intramuscular Electroporation Elicits High Levels of Neutralizing Antibodies in Multiple Animal Models and Provides Protective Immunity to Mice and Nonhuman Primates." *Clinical and Vaccine Immunology* 18, no. 5 (May 2011): 707–716. doi:10.1128/CVI.00030-11.

Dupuy, Lesley C., Michelle J. Richards, Douglas S. Reed, and Connie S. Schmaljohn. "Immunogenicity and Protective Efficacy of a DNA Vaccine Against Venezuelan Equine Encephalitis Virus Aerosol Challenge in Nonhuman Primates." *Vaccine* 28, no. 46 (28 October 2010): 7345–7350. https://doi.org/10.1016/j.vaccine.2010.09.005.

Faye, Oumur, Alessio Andronico, Ousmane Faye, Henrik Salje, Pierre-Yves Boëlle, N'Faly Magassouba, Elhadj Ibrahima Bah, et al. "Use of Viremia to Evaluate the Baseline Case Fatality Ratio of Ebola Virus Disease and Inform Treatment Studies: A Retrospective Cohort Study." *PLoS Medicine* 12, no. 12 (December 1, 2015): e1001908. https://doi.org/10.1371/journal.pmed.1001908.

Fischer, William A., II, David J. Weber, and David A. Wohl. “Personal Protective Equipment: Protecting Health Care Providers in an Ebola Outbreak.” *Clinical Therapeutics* 37, no. 11 (1 November 2015): 2402–2410. https://doi.org/10.1016/j.clinthera.2015.07.007.

Flashner, Yehuda, Morly Fisher, Avital Tidhar, Adva Mechaly, David Gur, Gideon Halperin, Eran Zahavy, et al. “The Search for Early Markers of Plague: Evidence for Accumulation of Soluble *Yersinia pestis* LcrV in Bubonic and Pneumonic Mouse Models of Disease.” *FEMS Immunology & Medical Microbiology* 59, no. 2 (July 1, 2010): 197–206. https://doi.org/10.1111/j.1574-695X.2010.00687.x.

Fourier, Pierre-Edouard, Michel Drancourt. Philippe Colson, Jean-Marc Rolain, Bernard La Scola, and Didier Raoult. “Modern Clinical Microbiology: New Challenges and Solutions.” *Nature Reviews Microbiology* 11, no. 8 (August 2013): 574–585. doi:10.1038/nrmicro3068.

Grant, Benjamin D., Caitlin E. Anderson, John R. Williford, Luis F. Alonzo, Veronika A. Glukjova, David. S. Boyle, Bernhard H. Weigl, and Kevin P. Nichols. “SARS-CoV-2 Coronavirus Nucleocapsid Antigen-Detecting Half-Strip Lateral Flow Assay Toward the Development of Point of Care Tests Using Commercially Available Reagents.” *Analytical Chemistry* 92, no. 16 (2020): 11305–11309. https://pubs.acs.org/doi/10.1021/acs.analchem.0c01975.

Grunow, Roland, Wolf Splettstoesser, Sahra McDonald, Christopher Otterbein, Tom O’Brien, Cecilia Morgan, Jenifer Aldrich, et al. “Detection of *Francisella tularensis* in Biological Specimens Using a Capture Enzyme-Linked Immunosorbent Assay, an Immunochromatographic Handheld Assay, and a PCR.” *Clinical and Diagnostic Laboratory Immunology* 7, no. 1 (January 2000): 86–90. doi:10.1128/cdli.7.1.86-90.2000.

Gu, Wei, Steve Miller, and Charles Y. Chiu. “Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection.” *Annual Reviews of Pathology* 14 (January 24, 2019): 319–338. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6345613/.

Guina, Tina, Lynda Lanning, Kristian S. Omland, Mark S. Williams, Larry A. Wolfraim, Stephen P. Heyse, Christopher R. Houchens, et al. “The Cynomolgus Macaque Natural History Model of Pneumonic Tularemia for Predicting Clinical Efficacy Under the Animal Rule.” *Frontiers in Cellular and Infection Microbiology* 8, article 99 (April 2018): 18 pp. doi:10.3389/fcimb.2018.00099.

Hammer, COL David. “Building the CBR Diagnostic Family of Systems.” Briefing presented at the Pennsylvania State University OTA Symposium January 15–16, 2019. Joint Program Executive Office for Chemical, Biological, Radiological, and Nuclear Defense, 15 January 2019. https://abrl.psu.edu/wp-content/uploads/2019/01/JPEO-PSU-OTA-Symposium_20190115_MCS-DX.pdf.

Headquarters, Department of the Army (HQDA). *Multi-Service Tactics, Techniques, and Procedures for Treatment of Biological Warfare Agent Casualties*. ATP 4-02.84/MCRP 3-40A.3/NTRP 4-02.23/AFMAN 44-156_IP. HQDA,

November 2019. https://armypubs.army.mil/ProductMaps/PubForm/Details.aspx?PUB_ID=1008190.

Heikkinen, Terho, Jane Marttila, Aimo A. Salmi, and Olli Ruuskanen. "Nasal Swab versus Nasopharyngeal Aspirate for Isolation of Respiratory Viruses." *Journal of Clinical Microbiology* 40, no. 11 (November 2002): 4337–4339. doi:10.1128/jcm.40.11.4337-4339.2002.

Hepburn, Matthew J., and Andrew J. H. Simpson. "Tularemia: Current Diagnosis and Treatment Options." *Expert Review of Anti-Infective Therapy* 6, no. 2 (April 2008): 231–240. https://doi.org/10.1586/14787210.6.2.231.

Holty, Jon-Erik C., Dena M. Bravata, Hau Liu, Richard A. Olshen, Katrhryn M. McDonald, and Douglas K. Owens. "Systematic Review: A Century of Inhalational Anthrax Cases from 1900 to 2005." *Annals of Internal Medicine* 144, no. 4 (February 21, 2006): 270–280. doi:10.7326/0003-4819-144-4-200602210-00009.

Howe, Gerald B., Bonnie M. Loveless, David Norwood, Philip Craw, David Waag, Marilyn England, John R. Lowe, et al. "Real-Time PCR for the Early Detection and Quantification of *Coxiella burnetii* as an Alternative to the Murine Bioassay." *Molecular and Cellular Probes* 23, nos. 3–4 (June–August 2009): 127–131. https://doi.org/10.1016/j.mcp.2009.01.004.

Hu, Wei-Gang, H. Gail Thompson, Azhar Z. Alvi, Les P. Nagata, Mavanur R. Suresh, and R. Elaine Fulton. "Development of Immunofiltration Assay by Light Addressable Potentiometric Sensor with Genetically Biotinylated Recombinant Antibody for Rapid Identification of Venezuelan Equine Encephalitis Virus." *Journal of Immunological Methods* 289, nos. 1–2 (June 2004): 27–35. https://doi.org/10.1016/j.jim.2004.03.007.

Hupert, Nathaniel, Gonzalo M. L. Bearman, Alvin I. Mushlin, and Mark A. Callahan. "Accuracy of Screening for Inhalational Anthrax after a Bioterrorist Attack." *Annals of Internal Medicine* 139, no 5 (Pt. 1) (September 2, 2003): 337–345. https://journals.lww.com/infectdis/fulltext/2004/03000/accuracy_of_screening_for_inhalation_anthrax_after.52.aspx.

Jackson, Michael L., and Kenneth J. Rothman. "Effects of Imperfect Test Sensitivity and Specificity on Observational Studies of Influenza Vaccine Effectiveness." *Vaccine* 33, no. 11 (March 2015): 1313–1316. https://doi.org/10.1016/j.vaccine.2015.01.069.

Jernigan, John A., David S. Stephens, David A. Ashford, Carlos Omenaca, Martin S. Topiel, Mark Galbraith, Michael Tapper, et al. "Bioterrorism-Related Inhalational Anthrax: The First 10 Cases Reported in the United States." *Emerging Infectious Diseases* 7, no. 6 (November-December 2001): 933–944. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2631903/.

Joint Chiefs of Staff. *Joint Health Services.* Joint Publication 4-02. Joint Chiefs of Staff, 11 December 2017, Incorporating Change 1, 28 September 2018. https://www.jcs.mil/Portals/36/Documents/Doctrine/pubs/jp4_02ch1.pdf.

Joint Program Executive Office for Chemical, Biological, Radiological, and Nuclear Defense. "JPM CBRN Medical COVID-19 Diagnostic Capability: Cepheid GeneXpert." Accessed 23 September, 2025. https://www.jpeocbrnd.osd.mil/Portals/90/jpm-cbrnd-medical-cepheid-slick-sheet.pdf.

Joint Requirements Office for Chemical, Biological, Radiological, and Nuclear Defense. "NGDS 2 Biological CDD Update." Briefing. July 2025.

Juarez, Diana, Kanya C. Long, Patricia Aguilar, Tadeusz J. Kochel, and Eric S. Halsey. "Assessment of Plaque Assay Methods for Alphaviruses." *Journal of Virological Methods* 187, no. 1 (January 2013): 185–189. https://doi.org/10.1016/j.jviromet.2012.09.026.

Kool, Jacob L. "Risk of Person-to-Person Transmission of Pneumonic Plague." *Clinical Infectious Diseases* 40, no. 8 (April 15, 2005): 1166–1172. https://doi.org/10.1086/428617.

Koster, Frederick, David S. Perlin, Steven Park, Trevor Brasel, Andrew Gigliotti, Edward Barr, Leslie Myers, et al. "Milestones in Progression of Primary Pneumonic Plague in Cynomolgus Macaques." *Infection and Immunity* 78, no. 7 (July 2010): 2946–2955. doi:10.1128/IAI.01296-09.

Krakauer, Teresa, and Bradley G. Stiles. "The Staphylococcal Enterotoxin (SE) Family: SEB and Siblings." *Virulence* 4, no. 8 (November 15, 2013): 759–773. doi:10.4161/viru.23905.

Ksiazek, T. G., P. E. Rollin, A. J. Williams, D. S. Bressler, M. L. Martin, R. Swanepoel, F. J. Burt, et al. "Clinical Virology of Ebola Hemorrhagic Fever (EHF): Virus, Virus Antigen, and IgG and IgM Antibody Findings among EHF Patients in Kikwit, Democratic Republic of the Congo, 1995." Supplement, *Journal of Infectious Diseases* 179, suppl. 1 (February 1999): S11–187. https://doi.org/10.1086/514321.

Lamontagne, François, Robert A. Fowler, Neill K. Adhikari, Srinivas Murthy, David M. Brett-Major, Michael Jacobs, Timothy M Uyeki, et al. "Evidence-Based Guidelines for Supportive Care of Patients with Ebola Virus Disease." *Lancet* 391, no. 10121 (February 17, 2018): 700–708. doi:10.1016/S0140-6736(17)31795-6.

Layton, R. Colby, Trevor Brasel, Andrew Gigliotti, Edward Barr, Steven Storch, Leslie Myers, Charles Hobbs, and Frederick Koster. "Primary Pneumonic Plague in the African Green Monkey as a Model for Treatment Efficacy Evaluation." *Journal of Medical Primatology* 40, no. 1 (February 2011): 6–17. doi:10.1111/j.1600-0684.2010.00443.x.

LeClaire, Ross D., and Sina Bavari. "Human Antibodies to Bacterial Superantigens and Their Ability to Inhibit T-Cell Activation and Lethality." *Antimicrobial Agents and Chemotherapy* 45, no. 2 (February 2001): 460–463. doi:10.1128/AAC.45.2.460-463.2001.

Lee, Chang-Seop, Ju-Hyung Lee, and Cheon-Hyeon Kim. "Time-Dependent Sensitivity of a Rapid Antigen Test in Patients with 2009 H1N1 Influenza." *Journal of Clinical Microbiology* 49, no. 4 (April 2011): 1702. doi:10.1128/JCM.02247-10.

Leski, Tomasz A., Rashid Ansumana, Chris R. Taitt, Joseph M. Lamin, Umaru Bangura, Joseph Lahai, George Mbayo, et al. "Use of the FilmArray System for Detection of *Zaire ebolavirus* in a Small Hospital in Bo, Sierra Leone." *Journal of Clinical Microbiology* 53, no. 7 (July 2015): 2368–2370. doi:10.1128/JCM.00527-15.

Lessler, Justin, Nicholas G. Reich, Ron Brookmeyer, Trish M. Perl, Kenrad E. Nelson, and Derek A. T. Cummings. "Incubation Periods of Acute Respiratory Viral Infections: A Systematic Review." *Lancet* 9, no. 5 (May 2009): 291–300. doi:10.1016/S1473-3099(09)70069-6.

Lins, Renato C., Anne E. Boyer, Zsuzsanna Kuklenyik, Adrian R. Woolfitt, Jason Goldstein, Alex R. Hoffmaster, Maribel Gallegos-Candela, et al. "Zeptomole per Milliliter Detection and Quantification of Edema Factor in Plasma by LC-MS/MS Yields Insights into Toxemia and the Progression of Inhalation Anthrax." *Analytical and Bioanalytical Chemistry* 411, no. 12 (May 2019): 2493–2509. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6988385/.

Linssen, Bettina, Richard M. Kinney, Patricia Aguilar, Kevin L. Russell, Douglas M. Watts, Oskar-Rüger Kaaden, and Martin Pfeffer. "Development of Reverse Transcription-PCR Assays Specific for Detection of Equine Encephalitis Viruses." *Journal of Clinical Microbiology* 38, no. 4 (April 2000): 1527, doi:10.1128/JCM.38.4.1527-1535.2000.

Masci, Allyson, Emily B. Menesale, Wei-Chiang Chen, Carl Co, Xiaohui Lu, and Svetlana Bergelson. "Integration of Fluorescence Detection and Image-Based Automated Counting Increases Speed, Sensitivity, and Robustness of Plaque Assays." *Molecular Therapy: Methods & Clinical Development* 14 (September 13, 2019): 270–274. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6717064/.

Merckx, Joanna, Rehab Wali, Ian Schiller, Chelsea Caya, Genevieve C. Gore, Caroline Chartrand, Nandini Dendukuri, and Jesse Papenburg. "Diagnostic Accuracy of Novel and Traditional Rapid Tests for Influenza Infection Compared with Reverse Transcriptase Polymerase Chain Reaction: A Systematic Review and Meta-Analysis." *Annals of Internal Medicine* 167, no. 6 (September 19, 2017): 394–409. https://pubmed.ncbi.nlm.nih.gov/28869986/.

North Atlantic Treaty Organization (NATO). *NATO Planning Guide for the Estimation of CBRN Casualties*. NATO Standard AMedP-7.5. Edition A Version 1. NATO Standardization Office, October 2017. https://nso.nato.int/nso/nsdd/main/standards/ap-details/1788/EN.

North Atlantic Treaty Organization (NATO). *Technical Reference Manual NATO Planning Guide for the Estimation of CBRN Casualties*. NATO Standards-Related Document AMedP-7.5-1. Edition A, Version 1. NATO Standardization Office, January 2018. https://nso.nato.int/nso/nsdd/main/standards/srd-details/91/EN.

Overholt, Lt. Col. Edwin L., Col. W. D. Tigertt, Paul J. Kadull, and Cmdr. Martha K. Ward. "An Analysis of Forty-Two Cases of Laboratory-Acquired Tularemia: Treatment with Broad Spectrum Antibiotics." *The American Journal of Medicine* 30, no. 5 (May 1961): 785–806. https://doi.org/10.1016/0002-9343(61)90214-5.

Oxford, Sean, Doug Schultz, and Scott Weinrich, "Five Example Biological Warfare Attacks for the Medical Planners' Toolkit (MPTk) and the Joint Medical Planning Tool (JMPT)." Memorandum. Institute for Defense Analyses, 2 October 2020.

Panning, Marcus, Jochen Kilwinski, Susanne Greiner-Fischer, Martin Peters, Stafanie Kramme, Dimitrios Frangoulidis, Hermann Meyer, et al. "High Throughput Detection of *Coxiella burnetii* by Real-Time PCR with Internal Control System and Automated DNA Preparation." *BMC Microbiology* 8 (19 May 2008): 1–8. https://doi.org/10.1186/1471-2180-8-77.

Petralia, Salvatore, and Sabrina Conoci. "PCR Technologies for Point of Care Testing: Progress and Perspectives." *ACS Sensors* 2, no. 7 (July 28, 2017): 876–891. https://pubs.acs.org/doi/pdf/10.1021/acssensors.7b00299.

Pinsky, Benjamin A., Malaya K. Sahoo, Johanna Sandlund, Marika Kleman, Medha Kulkarni, Per Grufman, Malin Nygren, et al. "Analytical Performance Characteristics of the Cepheid GeneXpert Ebola Assay for the Detection of Ebola Virus." *PLoS One* 10, no. 12 (2015): e0142216. https://doi.org/10.1371/journal.pone.0142216.

Pisano, María Belén, María Paz Sánchez Seco, Viviana Elizabeth Ré, Adrián A. Farías, Marta Silva Contigiani, and Antonio Tenorio. "Specific Detection of All Members of the Venezuelan Equine Encephalitis Complex: Development of a RT-Nested PCR." *Journal of Virological Methods* 186, nos. 1–2 (December 2012): 203–206. https://doi.org/10.1016/j.jviromet.2012.05.009.

Pittman, COL Phillip R., Sarah L. Norris, Kevin M. Coonan, and Kelly T. McKee, Jr. "An Assessment of Health Status among Medical Research Volunteers Who Served in the Project Whitecoat Program at Fort Detrick, Maryland." *Military Medicine* 170, no. 3 (March 2005): 183–187. doi:10.7205/milmed.170.3.183.

Rajerison, Minoarisoa, Marie Melocco, Voahangy Andrianaivoarimanana, Soloandry Rahajandraibe, Feno Rakotoarimanana, Andre Spiegel, Matherisoa Ratsitorahina, and Laurence Baril. "Performance of Plague Rapid Diagnostic Test Compared to Bacteriology: A Retrospective Analysis of the Data Collected in Madagascar." *BMC Infectious Diseases* 20, article number 90 (2020): 6 pp. https://doi.org/10.1186/s12879-020-4812-7.

Randremanana, Rindra, Voahangy Andrianaivoarimanana, Birgit Nikolay, Beza Ramasindrazana, Juliette Paireau, Quirine Astrid ten Bosch, Jean Marius Rakotondramanga, et al. "Epidemiological Characteristics of an Urban Plague Epidemic in Madagascar, August–November, 2017: An Outbreak Report." *The Lancet Infectious Diseases* 19, no. 5 (May 01, 2019): 537–545. https://doi.org/10.1016/S1473-3099(18)30730-8.

Reed, Douglas S., Cathleen M. Lind, Lawrence J. Sullivan, William D. Pratt, and Michael D. Parker. "Aerosol Infection of Cynomolgus Macaques with Enzootic Strains of Venezuelan Equine Encephalitis Viruses." *Journal of Infectious Diseases* 189, no. 6 (March 15, 2004): 1013–1017, https://doi.org/10.1086/382281.

Reed, Douglas S., Cathleen M. Lind, Matthew G. Lackemeyer, Lawrence J. Sullivan, William D. Pratt, and Michael D. Parker. "Genetically Engineered, Live, Attenuated Vaccines Protect Nonhuman Primates Against Aerosol Challenge with a Virulent IE Strain of Venezuelan Equine Encephalitis Virus." *Vaccine* 23, no. 24 (May 2005): 3139–3147. https://doi.org/10.1016/j.vaccine.2004.12.023.

Rossi, Cynthia A., Melanie Ulrich, Sarah Norris, Douglas S. Reed, Louise M. Pitt, and Elizabeth K. Leffel. "Identification of a Surrogate Marker for Infection in the African Green Monkey Model of Inhalation Anthrax," *Infection and Immunity* 76, no. 12 (December 2008): 5790–5801. doi:10.1128/IAI.00520-08.

Rossi, Shannan L., Kasi E. Russell-Lodrigue, Stephanie Z. Killeen, Eryu Wang, Grace Leal, Nicholas A. Bergren, Heather Vinet-Oliphant, et al. "IRES-Containing VEEV Vaccine Protects Cynomolgus Macaques from IE Venezuelan Equine Encephalitis Virus Aerosol Challenge." *PLoS Neglected Tropical Diseases* 9, no. 5 (May 2015): e0003797. doi:10.1371/journal.pntd.0003797.

Rowe, Alexander K., Jeanne Bertolli, Ali S. Khan, Rose Mukunu, J. J. Muyembe-Tamfum, David Bressler, A. J. Williams, et al. "Clinical, Virologic, and Immunologic Follow-Up of Convalescent Ebola Hemorrhagic Fever Patients and Their Household Contacts, Kikwit, Democratic Republic of the Congo." Supplement, *Journal of Infectious Diseases* 179, suppl. 1 (1999): S28–S35. https://doi.org/10.1086/514318.

Rusnak, Janice M., Mark Kortepeter, Robert Ulrich, Mark Poli, and Ellen Boudreau. "Laboratory Exposures to Staphylococcal Enterotoxin B." *Emerging Infectious Diseases* 10, no. 9 (September 2004): 1544–1549. doi:10.3201/eid1009.040250.

Saikh, Kamal U., Robert G. Ulrich, and Teresa Krakauer. "Staphylococcal Enterotoxin B and Related Toxins Produced by *Staphylococcus aureus* and *Streptococcus pyogenes*." In *Textbook of Military Medicine: Medical Aspects of Biological Warfare*, edited by Joel Bozue, Christopher K. Cote, and Pamela J. Glass, 403–414. Office of the Surgeon General, Borden Institute, U.S. Army Medical Department Center and School, Health Readiness Center of Excellence, 2018. https://fas.org/irp/threat/cbw/medical.pdf.

Sanchez, Jose L., and Michael J. Cooper. "Influenza in the US Military: An Overview." *Journal of Infectious Diseases and Treatment* 2, no. 1 (2016): 1–7. https://infectious-diseases-and-treatment.imedpub.com/influenza-in-the-us-military-an-overview.php?aid=8781.

Saslaw, Samuel, Henry T. Eigelsbach, John A. Prior, Henry E. Wilson, and Sally Carhart. "Tularemia Vaccine Study. II. Respiratory Challenge." *Archives of Internal Medicine* 107 (May 1961): 702–714. doi:10.1001/archinte.1961.03620050068007.

Schneeberger, Peter M., Mirjam H. A. Hermans, Erik J. van Hannen, Jeroen J. A. Schellekens, Alexander C. A. P. Leenders, and Peter C. Wever. "Real-Time PCR with Serum Samples Is Indispensable for Early Diagnosis of Acute Q Fever." *Clinical and Vaccine Immunology* 17, no. 2 (February 2010): 286–290. doi:10.1128/CVI.00454-09.

Schricker, Robert L., Henry T. Eigelsbach, John Q. Mitten, and William C. Hall. "Pathogenesis of Monkeys Aerogenically Exposed to *Francisella tularensis* 425." *Infection and Immunity* 5, no. 5 (May 1972): 734–744. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC422433/.

Schröeder, Elke, G. Kuntsmann, H. Hasbach, and G. Pulverer. "Prevalence of Serum Antibodies to Toxic-Shock-Syndrome-Toxin-1 and to Staphylococcal Enterotoxins A, B and C in West-Germany." *Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene. Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitolog*y 270, nos. 1–2 (November 1988): 110–114. https://doi.org/10.1016/S0176-6724(88)80146-9.

Snyder, J. W., G. K. Munier, and C. L. Johnson. "Direct Comparison of the BD Phoenix System with the MicroScan WalkAway System for Identification and Antimicrobial Susceptibility Testing of *Enterobacteriaceae* and Nonfermentative Gram-Negative Organisms." *Journal of Clinical Microbiology* 46, no. 7 (July 2008): 2327–2333. doi:10.1128/JCM.00075-08.

Stewart, Alvin, Benjamin Satterfield, Marissa Cohen, Kim O'Neill, and Richard Robison, "A Quadruplex Real-Time PCR Assay for the Detection of *Yersinia pestis* and Its Plasmids." *Journal of Medical Microbiology* 57, issue 3 (March 2008): 324–331. doi:10.1099/jmm.0.47485-0.

Storch, Gregory A. "Diagnostic Virology." *Clinical Infectious Diseases* 31, no. 3 (September 2000): 739–751. https://doi.org/10.1086/314015.

Stramer, Susan L., Cynthia Collins, Thomas Nugent, Megan Fuschino, John W. Heitman, Jacqueline Law, et al. "Sensitive Detection Assays for Influenza RNA Do Not Reveal Viremia in US Blood Donors." *The Journal of Infectious Diseases* 205, no 6 (March 15, 2012): 886–894. doi:10.1093/infdis/jir863.

Su, Shuo, Gary Wong, Xiangguo Qui, Gary Kobinger, Yuhai Bi, and Jiyong Zhou. "Diagnostic Strategies for Ebola Virus Detection." *The Lancet Infectious Diseases* 16, no. 3 (March 01, 2016): 294–295. https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(16)00049-9/fulltext.

Taylor, Brian. *Evaluation of the NGDS Warrior Panel Using Synthetic Material on the FilmArray*. HT-15-1099. Battelle Memorial Institute, May 2016.

Thermo Fisher Scientific. "Protein Mass Spectrometry." Accessed January 27, 2021. https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-mass-spectrometry.html.

Thermo Fisher Scientific. "What is an ELISA (Enzyme-Linked Immunosorbent Assay)?" Accessed January 27, 2021, https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html.

Tomaso, H., P. Thullier, E. Seibild, V. Guglielmo, A. Buckendahl, L. Rahalison, H. Neubauer, et al. "Comparison of Hand-Held Test Kits, Immunofluorescence Microscopy, Enzyme-Linked Immunosorbent Assay, and Flow Cytometric Analysis

for Rapid Presumptive Identification of *Yersinia pestis*.” *Journal of Clinical Microbiology* 45, no. 10 (October 2007): 3404–3407. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2045319/.

Towner, Jonathan S., Pierre E. Rollin, Daniel G. Bausch, Anthony Sanchez, Sharon M. Crary, Martin Vincent, William F. Lee, et al. “Rapid Diagnosis of Ebola Hemorrhagic Fever by Reverse Transcription–PCR in an Outbreak Setting and Assessment of Patient Viral Load as a Predictor of Outcome.” *Journal of Virology* 78, no.8 (April 2004): 4330–4341. doi:10.1128/jvi.78.8.4330-4341.2004.

Tsang, Tim K., Vicky J. Fang, Kwok-Hung-Chan, Dennis K. M. Ip, Gabriel M. Leung, J. S. Malik Peiris, Benjamin J. Cowling, and Simon Cauchemez. “Individual Correlates of Infectivity in Influenza A Virus Infections in Households.” *PLoS One* 11, no. 5 (May 6, 2016): e0154418. doi:10.1371/journal.pone.0154418.

Turra, M., G. Chang, D. Whybrow, G. Higgins, and M. Qiao. “Diagnosis of Acute Q Fever by PCR on Sera during a Recent Outbreak in Rural South Australia.” *Annals of the New York Academy of Science* 1078, no. 1 (October 2006): 566–569. https://doi.org/10.1196/annals.1374.112.

U.S. Food and Drug Administration (FDA). “FDA Approves Treatment for Ebola Virus.” Accessed March 9, 2021. Last updated December 21, 2020. https://www.fda.gov/drugs/drug-safety-and-availability/fda-approves-treatment-ebola-virus.

United States Army Medical Research Institute of Infectious Diseases (USAMRIID). *Medical Management of Biological Casualties Handbook*. 9th ed. USAMRIID, 2020. https://usamriid.health.mil/assets/docs/training/USAMRIIDs_Blue_Book_9th_edition_PDF_format.pdf.

Ulrich, Robert G., Sheldon Sidell, Thomas J. Taylor, Catherine L. Wilhelmsen, and David R. Franz. “Staphylococcal enterotoxin B and related Pyrogenic Toxins.” In *Textbook of Military Medicine: Medical Aspects of Biological Warfare*, edited by Brigadier General Russ Zajtchuk and Ronald F. Bellamy, 621–630. Borden Institute, Walter Reed Army Medical Center, 1997. https://www.researchgate.net/publication/237283636_STAPHYLOCOCCAL_ENTEROTOXIN_B_AND_RELATED_PYROGENIC_TOXINS.

Headquarters, Department of the Army (HQDA). *Army Biological Defense Strategy*. HQDA, 2021.

Vabulas, Ramunas, Renate Bittlingmaier, Klaus Heeg, Herman Wagner, and Thomas Miethke. “Rapid Clearance of the Bacterial Superantigen Staphylococcal Entertoxin B In Vivo.” *Infection and Immunity* 64, no. 11 (November 1996): 4567–4573. doi:10.1128/IAI.64.11.4567-4573.1996.

Vemula, Sai Vikram, Joangqin Zhao, Jikun Liu, Xue Wang, Santanu Biswas, and Indira Hewlett. “Current Approaches for Diagnosis of Influenza Virus Infections in Humans.” *Viruses* 8, no. 4 (April 12, 2016): 96. doi:10.3390/v8040096.

Vos, Laura M., Andrea H. L. Bruning, Johannes R. Reitsma, Rob Schuurman, Annelies Riezebos-Brilman, Andy I. M. Hoepelman, and Jan Jelrik Oosterheert. "Rapid Molecular Tests for Influenza, Respiratory Syncytial Virus, and Other Respiratory Viruses: A Systematic Review of Diagnostic Accuracy and Clinical Impact Studies." *Clinical Infectious Diseases* 69, no. 7 (September 13, 2019): 1243–1253. doi:10.1093/cid/ciz056.

World Health Organization. *Interregional Meeting on Prevention and Control of Plague*. Antananarivo, Madagascar, 1–11 April 2006. WHO/HSE/EPR/2008.3. World Health Organization, 2008. https://www.who.int/csr/resources/publications/WHO_HSE_EPR_2008_3w.pdf.

Yang, Samuel, and Richard E. Rothman. "PCR-Based Diagnostics for Infectious Diseases: Uses, Limitations, and Future Applications in Acute-Care Settings." *The Lancet Infectious Diseases* 4, no. 6 (June 2004): 337–348. https://doi.org/10.1016/S1473-3099(04)01044-8.

Zacks, Michele A., and Slobodan Paessler. "Encephalitic Alphaviruses." *Veterinary Microbiology* 140, nos. 3–4 (27 January 2010): 281–286. https://doi.org/10.1016/j.vetmic.2009.08.023.

Zhu, Hanliang, Haoquing Zhang, Sheng Ni, Marie Korabečná, Levent Yobas, and Paul Neuzil. "The Vision of Point-of-Care PCR Tests for the COVID-19 Pandemic and Beyond." *TrAC Trends in Analytical Chemistry* 130 (September2020): 115984. https://doi.org/10.1016/j.trac.2020.115984.

Appendix E.
Abbreviations

AFMAN	Air Force Manual
AIC	Akaike information criterion
AMedP	Allied Medical Publication
AoA	Analysis of Alternatives
AOR	area of responsibility
APOD	airport of debarkation
ATP	Army Techniques Publication
BSL	biosafety level
BT-E	Biothreat–Ebola
BW	biological warfare
CBRN	chemical, biological, radiological, or nuclear
CDD	Capability Development Document
CFU	colony forming unit
COVID-19	coronavirus-19
Ct	cycle threshold
DNA	deoxyribonucleic acid
DOD	Department of Defense
EF	edema factor
ELISA	Enzyme-Linked Immunosorbent Assay
EPR	Emergencies Preparedness, Response
EVD	Ebolavirus disease
FDA	Food and Drug Administration
HF	hemorrhagic fever
HPAC	Hazard Prediction and Assessment Capability
HQDA	Headquarters, Department of the Army
HSE	Health Security and Environment
IDA	Institute for Defense Analyses
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IP	Integrated Policy
JFS	Joint Force Surgeon
JMPT	Joint Medical Planning Tool
LAMP	Loop-Mediated Isothermal Amplification
LcrV	V-antigen protein

LF	lethal factor
MCRP	Marine Corps Reference Publication
Mod	Moderate
MPDS	Man Portable Diagnostic System
MS/MS	tandem mass spectrometry
MPTk	Medical Planners' Toolkit
MTF	medical treatment facility
OTSG	U.S. Army Office of the Surgeon General
NATO	North Atlantic Treaty Organization
NGDS	Next Generation Diagnostic System
NHP	non-human primate
NPA	nasopharyngeal aspirate
NSO	NATO Standardization Office
NTRP	Navy Tactical Reference Publication
OA	operational area
PCR	polymerase chain reaction
PEP	post-exposure prophylaxis
PPE	personal protective equipment
PAR	population at risk
PTSAg	pyrogenic toxin superantigen
RAM	Random Access Memory
RNA	ribonucleic acid
ROM	restriction of movement
RT-PCR	reverse transcriptase–polymerase chain reaction
SE	staphylococcal enterotoxin
SEB	Staphylococcal enterotoxin B
Sev.	Severe
STD	standard deviation
TAP	tularemia, anthrax, and plague
TNS	throat/nasal swab
TTPs	tactics, techniques, and procedures
TTX	tabletop exercise
USAMRIID	U.S. Army Medical Research Institute for Infectious Diseases
V Sev	Very Severe
VEE	Venezuelan Equine Encephalitis
VEEV	Venezuelan Equine Encephalitis Virus
WHO	World Health Organization

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