Master of Public Health Field Experience Report

LABORATORY GUIDE TO INTERPRETATION OF ENTERIC PATHOGENS

by

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submitted in partial fulfillment of the requirements for the degree

MASTER OF PUBLIC HEALTH

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Field Experience Site:
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Summary

This report details an experience with the North Carolina Division of Public Health Department of Health and Human Services (Communicable Disease Branch). The Communicable Disease branch works to unite local, state, and federal health agencies to monitor outbreaks, public health investigations, and health departments in the region. Real-time surveillance for the state is completed using the North Carolina Electronic Disease Surveillance System (NC EDSS), which requires state health officials to report various diseases for epidemiologic data and monitoring. Miscommunications over laboratory results and disease classification via the electronic-based reporting prompted the creation of a guide for laboratory interpretation of enteric pathogens for field use in North Carolina Health Departments. The guide was designed for quick reference in day-to-day use and in the hopes of providing resources for nurses across the state to better aid in disease classification and provide more reliable epidemiologic data. This guide serves as the capstone project.

In addition to work completed on enteric pathogens, the student generated an online survey linked to a database for a Zika Virus Registry in order to track infected mothers, their pregnancy progress, and infant health. This database consolidates information about Zika virus outbreaks across North Carolina and also generates reports to assist with tracking and categorizing cases and data points of interest.

The student assisted in updating templates used for survey outbreaks and disease classification for the North Carolina Communicable Disease Manual. The student also had the opportunity to participate in outbreak investigations regarding Naegleria fowleri, Escherichia coli O157, and Salmonella.

Subject Keywords: *Laboratory, Interpretation, Enteric, Pathogens, North Carolina*

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Chapter 1 - Field Experience Scope of Work

The Field Experience took place at the North Carolina Department of Health and Human Services on 225 North McDowell Street in Raleigh, NC 27603. The preceptor was Nicole Lee, MPH, a Foodborne Epidemiologist in the Communicable Disease Branch of the North Carolina Division of Public Health. North Carolina's health departments are managed by the local county, with the state overseeing results from all the individual local departments and aiding whenever necessary.

The primary focus of this Field Experience was to help create a guide for the laboratory interpretation of enteric pathogens. The guide is a practical resource to address miscommunicating results interpretations and to consolidate resources in order to minimize time spent verifying disease classification, testing methodology, control measures, etc. The guide include
s resources for: case definitions, control measures, and necessary reports for the most common reportable pathogen in North Carolina (to maintain brevity the number of pathogens addressed in the final document is limited to the most common). Background information for each disease is also provided, including symptoms, incubation period, duration of illness, and communicability.

The externship encompassed additional projects such as developing an online survey linked to the Zika Virus Database to help track and gather information regarding Zika virus outbreaks in the United States and assisting with disease outbreak investigations within the state. Disease outbreak investigations included developing, consolidating, and researching educational points for public statements regarding a Naegleria fowleri outbreak in North Carolina and interviewing individuals associated with a Salmonella outbreak within the state to help narrow down potential food sources and environmental origins.

Chapter 2 - Objectives and Activities

Learning Objectives:

1. Apply knowledge and experience from KSU Doctor of Veterinary Medicine and Master of Public Health programs to real-world situations and contribute successfully to the Communicable Disease Branch
2. Expand knowledge on communicable disease, state testing guidelines, and criteria for reporting various diseases with North Carolina.
3. Understand the protocols and actions taken by state departments of health in communicable disease outbreaks to better prepare for a career in public veterinary practice.
4. Understand the role of the Centers for Disease Control (CDC) and how federal and state guidelines contribute to disease recognition, tracking, containment, and public health awareness.
5. Demonstrate the value of professional development gained with the North Carolina Department of Health through field experience products and final reporting for the Master of Public Health degree.

Activities Performed

1. Designed and implemented a guide for North Carolina nurses to assess communicable diseases and better report testing results to improve accuracy of data compiled by the state.
    - Met with representatives of the North Carolina State Laboratory to discuss various laboratory testing, interpretations, and editing to final document.
    - Met with representatives of North Carolina Health Department (nurses and epidemiologists) to discuss difficulties with data entry, interpretation of laboratory results, and editing to final document.
2. Developed an outbreak investigation survey template (SurveyMonkey) that could be utilized as a base construct for future outbreak investigations within the state to minimize response time to future outbreaks.
3. Assisted with developing a plan to transfer outbreak investigation data in the National Outbreak Reporting System (NORS) from manual to direct input.
    - This project was ultimately abandoned after initial groundwork due to the current departmental structuring. Outbreak data is shared through the public health division at North Carolina (details on respiratory, sexually transmitted diseases, enteric pathogens, etc.). The enteric department had hoped to improve collection of outbreak investigation data by consolidating the systems to direct input; however, as this would only apply to enteric pathogens, it would potentially interfere with templates designed for multi-departmental use. This would create a system that would require double data entry, which would eliminate the entire purpose of switching to a more direct system.
4. Developed and transcribed a Zika Outbreak Survey to an online template to assist the CDC in collecting information regarding Zika exposure within North Carolina.
5. Attended the State Laboratory for Public Health to shadow processing and testing of rabies suspect submissions.

- Previous Rabies vaccination status and personal protective equipment were required for entry to the laboratory. There the student was able to partake in the testing process of rabies sampling and testing including practicing appropriate safety measures, brain extraction techniques from various species (ranging from bats to cows), preparing appropriate slide specimens from cerebellar and brain stem samples, slide staining, and result interpretation. The laboratory uses Direct Fluorescent Antibody (DGA) testing for all rabies submissions. Rabies submissions for the day are given unique barcodes and assessed for risk prior to handling. The positive and negative control slides are prepared from previous lab submissions and used to compare results to. If a result is positive, additional testing would be performed to identify the rabies type variant. All positive tissue samples are permanently stored and logged for the state and CDC records.

6. Participated in outbreak investigations including conference calls, preparing talking points for the public, and conducting interviews for the department.
    - Participated in conference calls that included discussion and planning with local health department staff, hospital staff, health care facility staff, and CDC representatives. Some cases involved additional discussions with city planning and water engineers. Examples of conference calls include:
        - *E. coli* O157 outbreak amongst campers at a primitive camping site
        - Multi-state *Salmonella spp.* outbreak
        - *N. fowleri* outbreak in North Carolina
    - Prepared talking point documents and researched educational points for brochures and public announcements regarding the *N. fowleri* outbreak.
    - Conducted interviews utilizing the National Hypothesis Generating Questionnaire on behalf of the CDC and North Carolina to help determine potential causes for a multi-state outbreak of *Salmonella spp.*
    - Participated in on-call support for rabies and other communicable disease with the state veterinarian and epidemiologists. Rabies prevention and control within the state requires assessing animal and human exposures, referring cases, and testing suspect animals for disease. Referral calls can receive direct guidance from state authorities on how to handle suspect cases, which contributes to accurate monitoring and response.
7. Updated Investigation Steps for communicable diseases (including Listeriosis, Salmonellosis, Trichinosis, and Vibriosis) on the North Carolina State Department website for public reference.

Products Developed

1. *Guide to the Laboratory Interpretation of Enteric Pathogens* – the capstone project to be described below in Chapter 3; involved integration across departments and with the state laboratory.

2. *Zika Registry Database* – the student assisted with creating a Microsoft Access database for the CDC Zika Registry forms necessary to follow Zika suspect,

probable, or confirmed maternal and infant cases. The database allowed for forms to be consolidated in one place and for the generation of reports based on various variables contained within each form (see Chapter 4). The student helped to develop the necessary variables, functions, and relationships to provide a working database that could track and categorize data as appropriate. The student also entered data from the various Zika cases reported across the state of North Carolina.

3. *N. fowleri* talking points and public outreach information (see Chapter 4).

4. *Outbreak Investigation Survey Template*

Chapter 3 - Laboratory Guide to the Interpretation of Enteric Pathogens

Introduction

Appropriate surveillance and reporting for communicable diseases is vital for public health epidemiology and appropriate responses to protect the public. Epidemiology reporting is key to a state's ability to detect outbreaks rapidly, with enhanced technology capacity (e.g. automated electronic laboratory-based reporting, cluster-detection software, geocoding, etc.) being a primary method to improve surveillance within states (Hadler and others 2015). North Carolina developed the North Carolina Electronic Disease Surveillance System (NC EDSS) as an incentive for health departments to move towards online surveillance of diseases within the state. NC EDSS is a part of the Public Health Information Network (PHIN) and it connects the Department of Public Health, 86 local health departments (LHDs), eight HIV/STD Regional Offices, and the Department of Environment Health and Natural Resources (DENR) ("NC EDSS", n.d.). The system allows better case reporting, outbreak management, automatic receipt of laboratory reports, and pre-set statistical reports that facilitate outbreak management.

The North Carolina Department of Public Health, Communicable Disease Branch, located in Raleigh, North Carolina utilizes the NC EDSS for reporting of communicable enteric diseases. Disease surveillance in the state involves active collection, analysis, and interpretation of disease cases that often begins with physicians, school administrators, child care operators, medical facilities, and restaurant operators reporting cases or suspected cases to their local health department (NC Health & Human Services, n.d). Local health departments in turn then report the results to the NC Division of Public Health for further analysis. The Communicable Disease Manual lists diseases that are reportable in North Carolina with case definitions established by the Council of State and Territorial Epidemiologists (NC Health & Human Services, n.d.).

Difficulties with electronic systems still exist, with one of the more pertinent issues within the state being incorrect data entry. This has caused diseases to be logged inappropriately, which leads to incorrect data or lost data points. Reporting errors during infectious disease outbreaks can have a substantial impact on result accuracy, especially if the errors are not accounted for statistically (White & Pagano, 2010). Further difficulties reported in other states include first line responders being unaware of which diseases need to be reported or which department is responsible for the reporting ("Mandatory Reporting", 1990).

The project designed to help rectify this situation was the creation of a "Laboratory Guide to the Interpretation of Enteric Pathogens." This guidebook was intended as a quick in-field reference for healthcare professionals responsible for logging initial confirmed disease cases to help with appropriate categorization for those first responders and reporters within the state. It also highlights which diseases should be reported immediately, the turnaround time for laboratory results, and additional resources should there be remaining questions.

Methods

Research was conducted via consolidating resources available from both the Centers for Disease Control and Prevention and the North Carolina Department of Public Health. This included general resources for each communicable disease from the CDC website (CDC "Diseases & Conditions", 2017) and state requirements for reporting from the North Carolina Division of Public Health Communicable Disease Manual (NC Department of Health & Human Services, 2017). In addition, to reduce mistakes, resources and examples were provided to assist health professionals initiating a report to accurately and efficiently search state forms for identifying disease classifications. These example documents were provided by the State Department.

Feedback & Recommendations

Initial instructions and recommendations were provided by the head nurses at the State Department of Public Health who described the general confusion and errors they noted from interactions with local health departments.

Feedback for the initial product was positive, but requests were made to shorten the original document (such as removing less common reportable pathogens such as *Vibrio spp*.). Examples of actual laboratory results were added in addition to brief reporting descriptions. This was to help provide examples of common reporting errors. For instance, some state laboratory results will have "bacterial culture" listed on their forms that report a polymerase chain reaction (PCR) test. This would lead to the PCR results incorrectly being logged as a culture result.

The rough draft of the original guidebook was then reviewed by the state nurses and also again by representatives at the State Laboratory of Public Health. Editing recommendations included agreement with shortening of the initial length and also additional information regarding the type of laboratory testing available for each pathogen. They also requested additional information regarding testing and turnaround times to help ease communication with local health departments. Lastly, they requested that tests not performed at the State Laboratory be removed from the guide to decrease confusion.

Final

The final guidebook begins with a brief introduction of basic testing methods and guides that have previously not been available to health officials. It also introduces the testing methods utilized by the State Laboratory and various turnaround time for tests within state laboratories and from the CDC. It then proceeds to provide examples of keywords used in State Laboratory results that health departments can use to identify the type of testing utilized for reporting. A generalized table is provided to summarize the most common North Carolina reportable enteric pathogens.

After the introduction, the guidebook is organized alphabetically within pathogen categories (i.e. "bacteria", "virus", and "parasite"). Each pathogen has a one to two page summary that describes the exposure, symptoms, testing methods, and communicability

of the disease. There is also a quick reference side panel for laboratory testing and additional references for that pathogen. See **Appendix 1** for the complete guidebook.

Chapter 4 - Additional Products

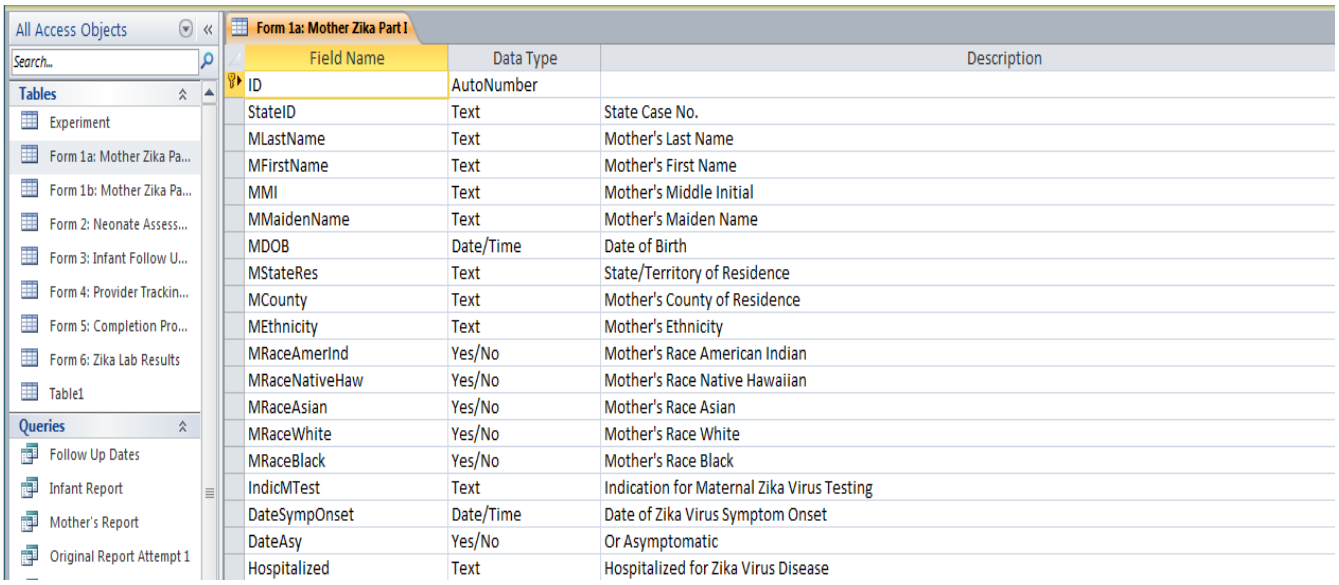
Product 2: Zika Registry Database

The student was asked to take CDC forms available to state departments for the reporting of Zika Virus infections and to duplicate the forms on an online database via Microsoft Access. These forms included a Mother's Health Assessment, Assessment at Delivery (Infant), Infant Health Follow Up Assessments, Provider Tracking Information, Completion Progress, and Zika Laboratory Results (Mother/Infant) (**see Appendix 2**).

These databases were then able to be compiled into three reports – one for mother's results and follow up information, one for infant's results and follow up information, and one to auto-generate follow-up dates so that infants could better be tracked and care-givers contacted during the appropriate times.

Every question on each form had to be reformatted and tied to the State ID for that case on the online database questionnaire (see Figure 1.1). This State ID had to remain constant across multiple forms so that when reports were generated, the same mother and child were linked across forms. These variables would then convert into digital version of the paper forms, allowing for input of the paper forms into the online database with ease.

Figure 1.1 Variable Examples



Field Name	Data Type	Description
ID	AutoNumber	
StateID	Text	State Case No.
MLastName	Text	Mother's Last Name
MFirstName	Text	Mother's First Name
MMI	Text	Mother's Middle Initial
MMaidenName	Text	Mother's Maiden Name
MDOB	Date/Time	Date of Birth
MStateRes	Text	State/Territory of Residence
MCounty	Text	Mother's County of Residence
MEthnicity	Text	Mother's Ethnicity
MRaceAmerInd	Yes/No	Mother's Race American Indian
MRaceNativeHaw	Yes/No	Mother's Race Native Hawaiian
MRaceAsian	Yes/No	Mother's Race Asian
MRaceWhite	Yes/No	Mother's Race White
MRaceBlack	Yes/No	Mother's Race Black
IndicMTest	Text	Indication for Maternal Zika Virus Testing
DateSympOnset	Date/Time	Date of Zika Virus Symptom Onset
DateAsy	Yes/No	Or Asymptomatic
Hospitalized	Text	Hospitalized for Zika Virus Disease

Once forms were generated via Access, the program could also be utilized to generate reports to analyze the data or to provide additional information on when retesting would be required. This was important for follow-up and tracking Zika outbreaks within the state, as mothers would need to be contacted after initial exposure for additional follow-up reports. The database could generate reports (see Figure 1.2) and future follow-up dates for certain ID numbers, which would ultimately improve tracking of the disease within

North Carolina. It was hoped that it could eventually generate automatic follow-up responses with various ID numbers to eliminate human error or forgetfulness associated with case tracking. The following example report shows how from the estimated delivery date (EDD), appropriate dates for follow up forms such as the Maternal Health History or acknowledgement by the department can be generated.

Figure 1.2 Example Report

Mother's Report

Mother's State ID:	Mother's Last Name	Mother's First Name	Final Results	EDD	Delivery Date	Acknowledgement Date	Maternal Health History (MHH)
[redacted]	[redacted]	[redacted]	Pending	4/27/2016	4/29/2016		5/12/2016
[redacted]	[redacted]	[redacted]	Pending	7/1/2016	6/10/2016		6/10/2016
[redacted]	[redacted]	[redacted]	Indeterminate	5/3/2016	5/2/2016		5/25/2016
[redacted]	[redacted]	[redacted]	Pending	7/7/2016	6/25/2016		6/24/2016
[redacted]	[redacted]	[redacted]	Pending	9/10/2016			5/26/2016
[redacted]	[redacted]	[redacted]	Pending	10/29/2016			7/7/2016
[redacted]			Pending				
[redacted]			Pending				
[redacted]			Pending				
[redacted]	[redacted]	[redacted]	Pending	7/11/2016			6/27/2016
[redacted]			Positive				

Total Records 11 | Acknowledgements Complete 0 | MHH Complete: 7

Product 3: *N. fowleri* Talking Points

The student had the opportunity to participate in a *Naegleria fowleri* outbreak (Primary Amoebic Meningoencephalitis or PAM). The case was unique in that it involved the death of an Ohio native who was exposed while visiting North Carolina. This led to a combined effort across states and with the CDC to identify the risk of *N. fowleri* infections associated with the exposure at a whitewater recreational facility in North Carolina.

This outbreak investigation was interesting in numerous ways. First, due to the multiple states involved and the rareness of the disease (only 111 cases have been reported in the U.S. from 1962-2008), communication had to be facilitated between multiple state departments and national facilities (Yoder and others, 2010). Initial talking points were adapted from publications utilized in Florida during a similar outbreak in 2014 (Weister, 2014). Initial investigation involved narrowing down the point of exposure of the patient, who had died from the infection after leaving North Carolina. At this time, the only freshwater exposure point had been at the National Whitewater Center in Charlotte, North Carolina.

N. fowleri is an ubiquitous free-living amoeba found in most warm freshwater in the United States that can cause an often-times fatal disease called primary amebic meningoencephalitis (PAM) (CDC “Parasites – N. fowleri”, 2017). Infection usually occurs when infected water is forced up the victim’s nostrils, allowing the amoeba to push past the cribriform plate and invade the brain. This presumed route of infection helped to limit the investigation for the exposure location to a site that involved warm freshwater and also potential for water being forced up nostrils.

Identification of a potential exposure location directed field investigators to the Center to take water samples for further testing. People from multiple state who were potentially exposed also had to be notified, and the center was temporarily closed during the investigation. The highly publicized nature of the case within North Carolina led to new legislation being rapidly proposed for the regulation of whitewater facilities within the state, which made prudent and accurate responses for the public even more important (Foster & Bruno, 2016). While the center did standard testing for coliforms in the water there were no state recommendations or protocols at the time for water sites to test for *N. fowleri* in water. The student was asked to prepare talking points and frequently asked questions (FAQs) that could be distributed throughout the North Carolina State Department and be utilized to answer news medias’ and the public’s concerns.

Further investigation at the associated facility found that the facility had unique features that made it prone to *Naegleria fowleri* growth, as all eleven water samples from the park were consistently positive for high amounts of the amoeba (Foster & Bruno, 2016). This made it a unique situation to which the original CDC control measures could not be applied, and consequently talking points and FAQs had to be adjusted accordingly (**see Appendix 3**). The Whitewater facility referenced a paper with its public response regarding water disinfection via ultraviolet light and how they followed such practices at their facility (Foster & Bruno, 2016; Sarkar & Gerba, 2012). This prompted research into how effective UV light penetration would be given their current water supply and algal growth. It was ultimately deemed after consultation, that the UV disinfection would be inadequate given the unique algal growth situation at the park that promoted high levels of the amoeba in its waters (especially when compared to the source from which the park drew its water). Even the initial paper suggested that factors such as organism concentration, disinfectant concentration, temperature, pH, and interfering substances could impact the efficacy of both chlorine and UV light as N. fowleri disinfectants (Sarkar & Gerba, 2012). Additional research has also shown that biofilms in field experiments may be more resistant to chlorine disinfection, allowing N. fowleri survival despite chlorine levels found to be effective in the laboratory (Miller and others, 2015).

The structure of the pools within the park promoted algal growth, which was believed to contribute to a biofilm and to create an environment suitable to excess *N. fowleri* growth. Ultimately, consultation with water engineers and other experts prompted the decision to temporarily close the whitewater rafting at the facility until a time where the engineering could be redone. In response, the facility drained the upper level pools to thoroughly clean algal growth to better improve efficacy of their filtration system.

Chapter 5 - Core Competencies

The courses required for the Masters of Public Health program at Kansas State University, and those courses that augmented my education from the Doctor of Veterinary Medicine program, provided competency for me while on this field experience in the following way:

Biostatistics

These courses gave me a better understanding of the analysis necessary for the information gathered. It helped me to have an appreciation of *why* it was vital that we had accurate reporting from local health departments. It also helped me understand the projects that other students were working on during my stay with the North Carolina Public Health Department, especially in regards to survey analysis of Salmonellosis cases in North Carolina. At the time of my field experience project, I had not completed all of my biostatistics courses; however, I still was able to apply what I had learned regarding study designs and quantitative analysis to both designing programs for data analysis and interpretation of research papers.

Environmental Health Sciences

This course was beneficial in assessing risk analysis and the level of appropriate exposure of various chemicals. In my field experience, it was useful for both understanding risk associated with various laboratory tests and also with recommendations made for water sanitation during the Whitewater Center *N. fowleri* outbreak. Sanitation methods had to be recommended that would be both efficacious yet safe for the employees and future guests of the park. The course helped to provide a broader insight into public health and how human and animal health are not always linked to diseases, but also to toxins and occupational hazards.

Epidemiology

Epidemiology was one of the most helpful core competencies for my field experience, as many of the projects I was involved with were essentially applied epidemiology. Drawing results from clusters of disease outbreaks such as identifying a source of infection or assessing which groups were most at risk relied on epidemiology knowledge. This was particularly apparent in all outbreak investigations – especially when localizing the cause of *Salmonella* and *E. coli* outbreaks.

Gathering data ethically and a manner that both helped the Health Department and the individuals involved took recognition of epidemiologic patterns and knowledge of how epidemiologic data can be utilized legally.

Identifying past epidemiologic patterns was also useful in helping to project future problems or incompetency within the state department or recurring outbreak patterns within the state.

Health Service Administration

This course greatly impacted my field experience as much of what I had learned from public communication needed to be applied for communication with the public. For instance, I had to simplify my original talking points provided during outbreaks to make

the information more accessible for all educational levels. This was a key discussion we had during MPH 720.

Recognizing the bureaucracy associated with public health systems and how local health department decisions are intertwined and affect the state department was also crucial for organizing my capstone project. It was also important to realize how healthcare employers do not necessarily communicate with the laboratory component of healthcare, which can greatly impact epidemiologic reporting and management on the state level.

Social and Behavioral Science

This course was beneficial in how it allowed us to develop talking points and answer concerns of the general public – making for an effective intervention before panic could affect the public (especially during the *N. fowleri* outbreak). It also provided excellent recommendations for addressing the public's concerns in an efficient and professional manner. This was important as we had to reformat our talking points with growing information regarding the unique factors of the Whitewater center that made our initial assumption regarding *N. fowleri* risk change. It was important to be honest and forthcoming with the public, and MPH 818 helped me build the necessary communication skills to apply these practices during my field experience with ease.

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Appendix 1 – Laboratory Guidebook to the Interpretation of Enterics

Laboratory Guide to Enteric Pathogens

Division of Public Health, Communicable Disease Branch
North Carolina Department of Health and Human Services

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7/8/2016

Overview

With advancements in laboratory techniques and the importance of laboratory results on disease classification, proper interpretation of laboratory results is increasingly important for determining public health action.

Information regarding testing and shipping requirements for the State Laboratory of Public Health (SLPH) can be found in *SCOPE – A Guide to Service* at: http://slph.ncpublichealth.com/doc/administration/SCOPE-Final-Rev-30116.pdf

The purpose of this document is to provide North Carolina health department employees with a practical guide to interpretation of reportable enteric pathogen laboratory results. Pathogen summaries will be provided along with discussion on common errors in regards to lab results and disease classification. This guide is by no means an all-inclusive discussion, but the author hopes that its brevity will provide a useful quick reference.

Laboratory Diagnosis

Sample Collecting

It is important to note that positive pathogen isolation does not necessarily guarantee that that pathogen is the underlying cause of disease. Clinical symptoms and laboratory test results should all be considered when making a diagnosis or outbreak categorization. A negative sample means that the pathogen was not present in that sample at that time. This could be for a variety of reasons:

- the patient is no longer shedding
- the sample was not collected properly or tested properly (human error)
- the patient did not have the pathogen being tested for (separate etiology)

Generally, pathogen recovery is more likely when samples are collected close to the onset date of disease.

Interpretation

What type of specimen?

- **Stool** – the *preferred* sample due to the fact it is non-invasive and can provide a wealth of information about many enteric pathogens (*Salmonella spp., E. coli, Shigella spp., Vibrio spp., Campylobacter spp.);* rectal swabs can also be utilized.
- **Blood** – typically spun down for serum samples for antibody-antigen testing; most often necessary for Hepatitis A or Typhoid Fever testing; can also detect bacteremic infections (*Salmonella spp*. or *E. coli* infections for instance)
- **Sterile site** – cerebrospinal fluid, synovial (joint) fluid, urine, and other immunoprivileged sites
  - *Urine* is a more common sterile specimen, but contamination during collection can make it non-ideal for culture and other tests
- **Miscellaneous** – vomitus, food sample, etc.

"Any clinical specimen" on disease investigation forms and diagnosis steps can refer to any of the above (excepting food samples).

Was the sample collected correctly?

It is vital samples are collected properly (e.g. amount of sample, amount of media, etc.) in order to obtain laboratory results. Poor sample taking can hamper a disease investigation by preventing laboratory diagnosis.

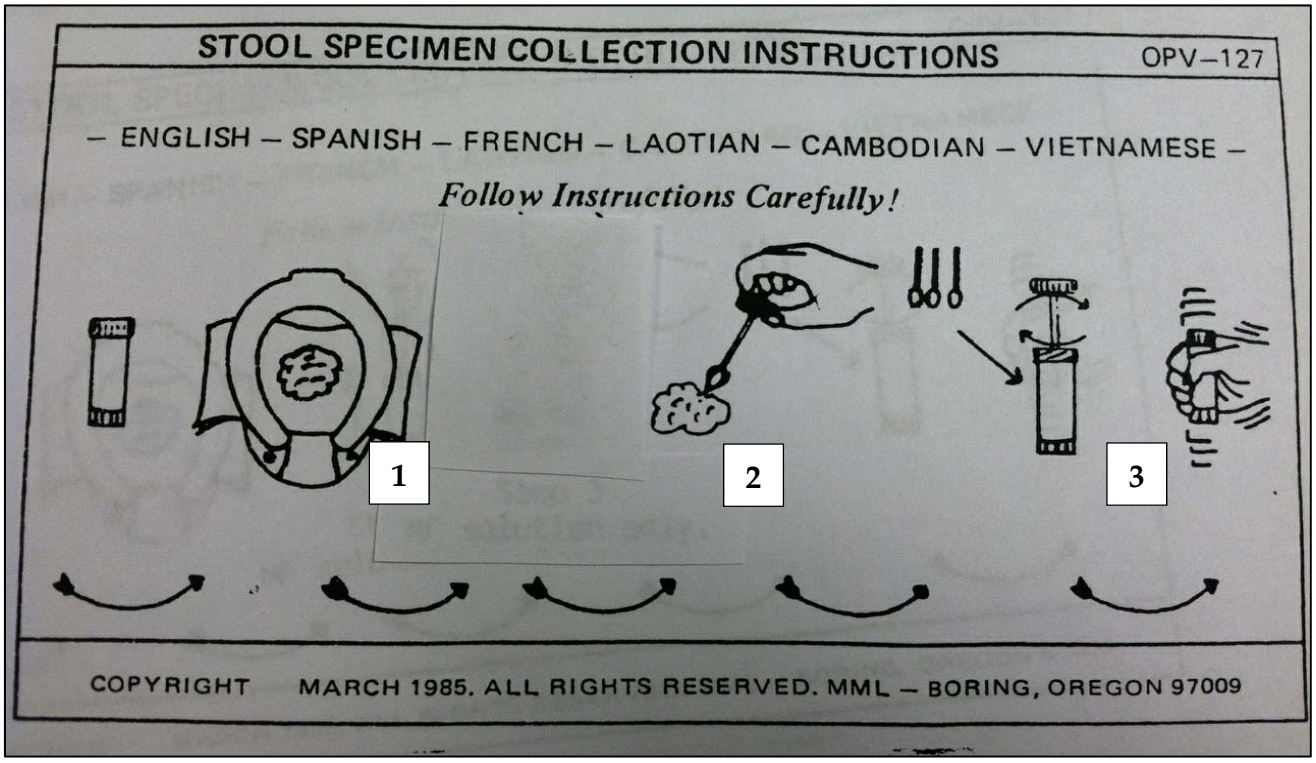
Stool Specimens in particular are prone to errors such as dumping of the Cary Blair preservative or wrong amounts of stool being added to the collection cup. Some Health Departments collect raw stool samples while others will allow the patient to collect samples for themselves, which can contribute to errors. The following document was prepared to assist in standardizing collection technique:

Stool Collection Procedures for Testing at

North Carolina State Lab for Public Health*

This is a vial of Cary Blair preservative that can be used to test stool for norovirus and enteric diseases (i.e. *Salmonella, Shigella, Campylobacter, Yersinia, E. coli*).

Vials that are used for testing of ova and parasites are yellow and have formalin preservative in them.



1. Put plastic wrap over the toilet to catch the stool OR collect the stool in a plastic container
2. Use the spork to add the stool to the vial of media BEING SURE NOT TO FILL ABOVE THE FILL LINE ON THE LABEL
3. Screw the top tightly and shake to ensure the media mixes with the stool

NOTE: Do not let water or urine touch the stool specimen.

It is important to have the proper ratio of stool to preservative in order to ensure that there is no overgrowth of normal flora in the stool and to keep any organisms that are in the stool viable during transport.

When stool is added to the vial of Cary Blair preservative, the total mixture should not go above the marked line on the label.

This picture is an example of what NOT to do because there is too much stool in this vial.

There is a spork connected to the cap of the Cary Blair vial that can be used to add stool to the vial – being sure to not overfill it above the red line on the label.

Because of the amount of preservative already in the vial, you don't have to add a lot of stool in order to make sure the mixture does not go above the red line on the vial's label.

Here are the inner and outer containers that can be used to ship stool specimens.

Put the vial in the inner container.

Place the inner container in the outer container AND put the requisition in this outer container as well so that it will not get soiled if the vial spills in the inner container.

NOTES:

- SLPH doesn't usually accept raw stool unless there is a special pre-approved circumstance. Raw stool will need to be kept cool since there is no preservative in it. The raw stool will need to be put into a stool cup and placed in a sealed plastic bag. The specimen should then be kept cool after collection and during transport with cold packs.

- Requisition, collection and shipment details can be found under the "Microbiology" tab at SLPH's website: http://slph.ncpublichealth.com/

*Special thanks to Nicole Lee, Foodborne Epidemiologist, Division of Public Health, Communicable Disease Branch NC Department of Health and Human Services for preparing these procedures.

Interpretation Continued:

What is the turnaround time?

Once submitted, it generally takes between 7-10 work days ("turnaround time") for Enteric reference identifications and clinical cultures.

In North Carolina, public health utilizes a courier system that ships samples to Mocksville, NC to minimize costs. This means it can take additional days **before** samples arrive to the state laboratory (up to three days if over the weekend). Testing can take a few additional days once samples arrive, which accounts for the wide range in turnaround times. Pathogens do not always "read the textbook" on growth or other laboratory tests and it can be difficult to predict when exactly results will be available.

Check the SCOPE document for a more accurate estimation of turnaround time for each specific test or pathogen for the NC State Laboratory.

Keep in mind that results submitted directly to the CDC can take weeks to months for turnaround. Truly negative cultures require ~40 work days and susceptibility testing can take ~21 days.

What type of test?

Recognizing the differences between tests and how that affects a result's interpretation is vital for proper disease classification. More time intensive and accurate tests are preferred as "gold standards" for disease identification, but frequently faster methods are necessary for quick turnaround time for patient management.

Culture Techniques: Specialized media is inoculated with specimen samples. Media plates are streaked via sterile technique in order to produce isolated bacterial colonies after growth. These colonies can then be stained or further tested to determine the nature of the pathogen (e.g. antimicrobial susceptibility). Enteric microorganisms are typically grown on blood agars or MacConkey agars (which have high salts that usually kill non-enteric bacteria). Anaerobic culture is rarely performed and usually not cost-effective or informative for Enterobactericeae.

Culture is the "gold standard" for bacterial enteric identification and is usually required in a case report for "confirmed" status.

Polymerase Chain Reaction (PCR): PCR is a technique utilized to amplify DNA fragments to a level of detection that can be utilized in diagnosis of diseases. Heat stable polymerases (Taq) are utilized to allow a single piece of DNA to be used as a template for replication, and thermal cycling is used to separate and

reform DNA repeatedly until an adequate amount is produced. PCR testing tends to have a **faster turn-around time** than culture, and is thus preferred in a clinical setting because it allows the patient to be managed earlier in presentation.

PCR identifies DNA. This DNA could be from a viable (alive) organism or a nonviable (dead) organism. It does not guarantee active infection or disease status.

PCR positives are most often classified as "suspect" cases.

No matter the case classification, control measures must be applied!

Enzyme-linked immunosorbent assay (ELISA): an enzyme immunoassay (EIA) that measures the presence of a molecule or pathogen through the use of antibody and color change. Generally used to detect viral pathogens or distinguish STEC toxins. Sera are the preferred sample.

Immunofluorescent Assay (IFA): tests that bind antibodies to a fluorochrome, which produces fluorescence under ultraviolet light. Immunofluorescence can be detected by **direct (DFAs)** or **indirect** methods, with pathogen-specific antibodies binding to antigens located in tissue or cell samples. Generally used to detect viral pathogens with sera the preferred sample.

Pulsed-field gel Electrophoresis (PFGE): a test that separates DNA molecules by applying an electric field to a gel matrix. The size of the molecules dictates how far they move across the gel. It is generally used for genotyping (determining specific genetics), and the CDC recommends its use for identifying *Eschericia coli O157:H7 (STEC), Salmonella serotypes, Shigella sonnei,* and *Shigella flexneri.* Note that the pathogen first must be isolated by culture **before** PFGE can be performed:

See the following for information on how to create gels and run PFGEs if interested: https://www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf

Antibody vs. Antigen

An **antibody** is a protein created by the body's immune system in response to a pathogen.

An **antigen** is a protein or part of a pathogen.

Misleading Laboratory Labeling

Laboratories will often mislabel results as a "culture". This is generally due to the data inputting software not allowing for selection of more specialized tests such as an EIA or PCR.

Sometimes tests will not be labeled at all!

PCR and culture are not the same. One should always try to confirm sample source and laboratory test type.

The following pages will contain a few example laboratory results and suggestions on how to identify the type of test utilized. Keep in mind that there is not a uniform results page utilized by laboratories, so there will be much variation in actual practice.

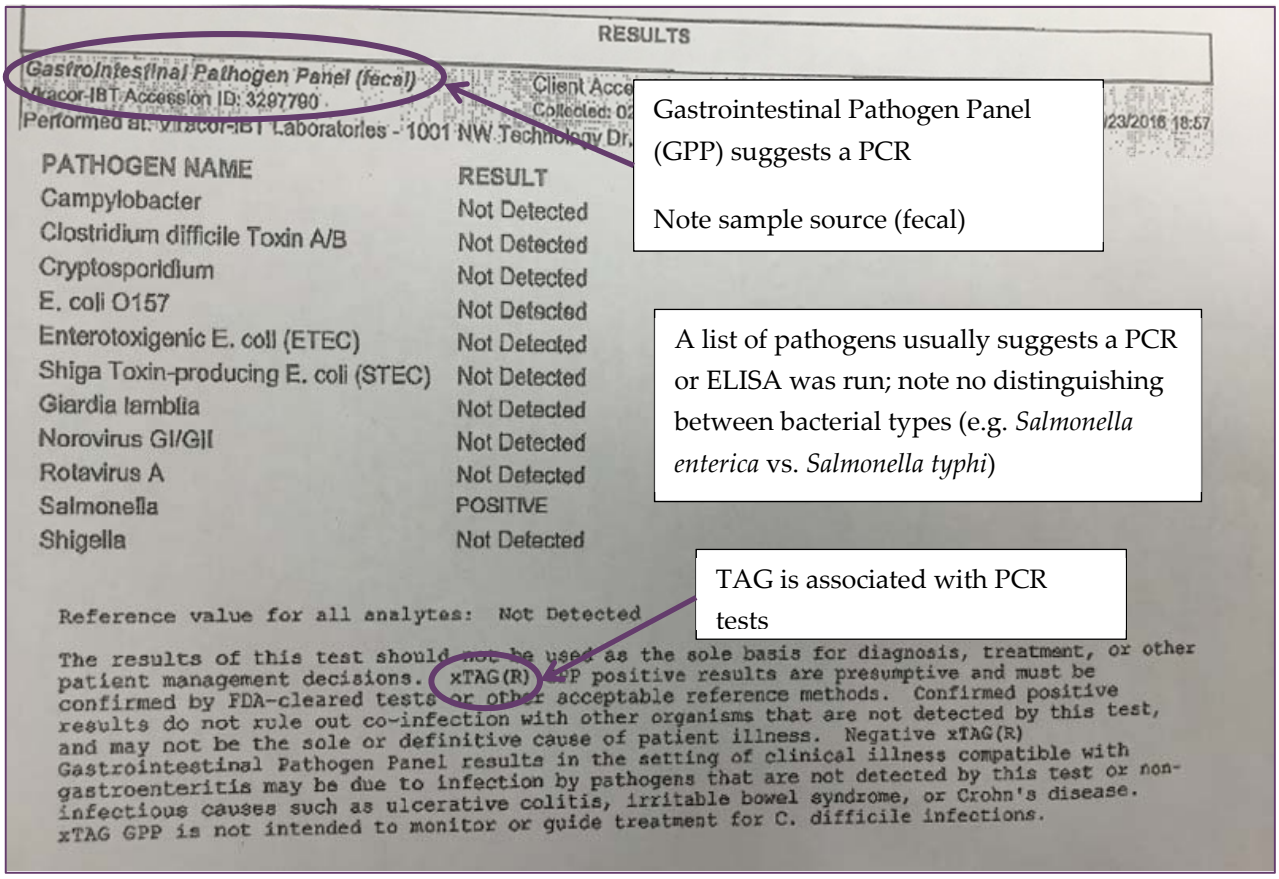
Name of Test	Tips on What to Look For	Important Points
Culture (Isolation of pathogen)	✓ Distinguishes between species (e.g. *Campylobacter jejuni* vs. *Campylobacter fetus*) indicates a culture and additional biochemical tests occurred ✓ Mentions growth or isolation ✓ Mentions susceptibility testing	• Specific pathogen found • Think "growth" • Generally will use stool sample
Polymerase Chain Reaction (PCR)	✓ "GI Panel" ✓ Multiple pathogens being tested for at once indicates PCR ✓ TAG or taq mentioned ✓ Organism can be dead or alive; not indication of active infection	• Broad pathogen panel • Think "DNA" • Stool Sample
ELISA	✓ Discussion of antibodies (Ab) or antigen (Ag) ✓ Sera dilution ✓ Usually for viral pathogens ✓ "MAC", "IgM", "IgG", "NS1", or "PRNT"	• Broad pathogen panel • Serum Sample • Type of EIA • Color change
IFA	✓ Direct (DFAs) vs. indirect fluorescence ✓ Sera dilution ✓ Discussion of antibodies (Ab) or antigen (Ag) ✓ Usually for viral pathogens or parasitic infections (such as Cryptosporidiosis)	• DFAs most common • Serum sample

References

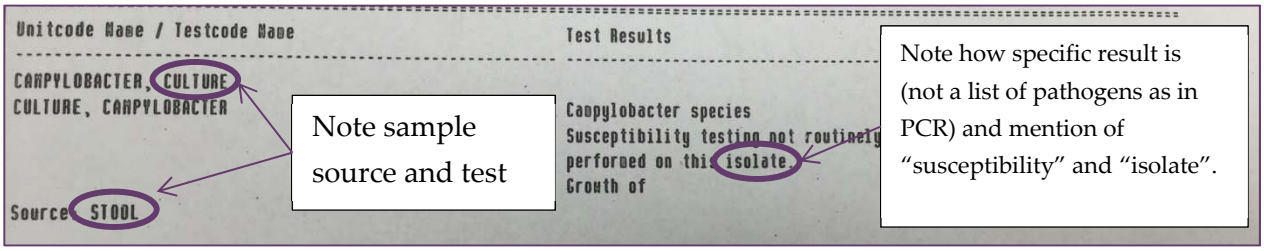
Nietfeld JC. (2013). Veterinary Microbiology. 3rd edn (eds McVey SD, Kennedy M, and Chengappa MM), Wiley-Blackwell Press, Ames, IA 50014, pp.167-174

North Carolina. (2015). SCOPE – A Guide to Services. North Carolina State Laboratory of Public Health, Division of Public Health, Department of Health and Human Services.

Laboratory Diagnosis

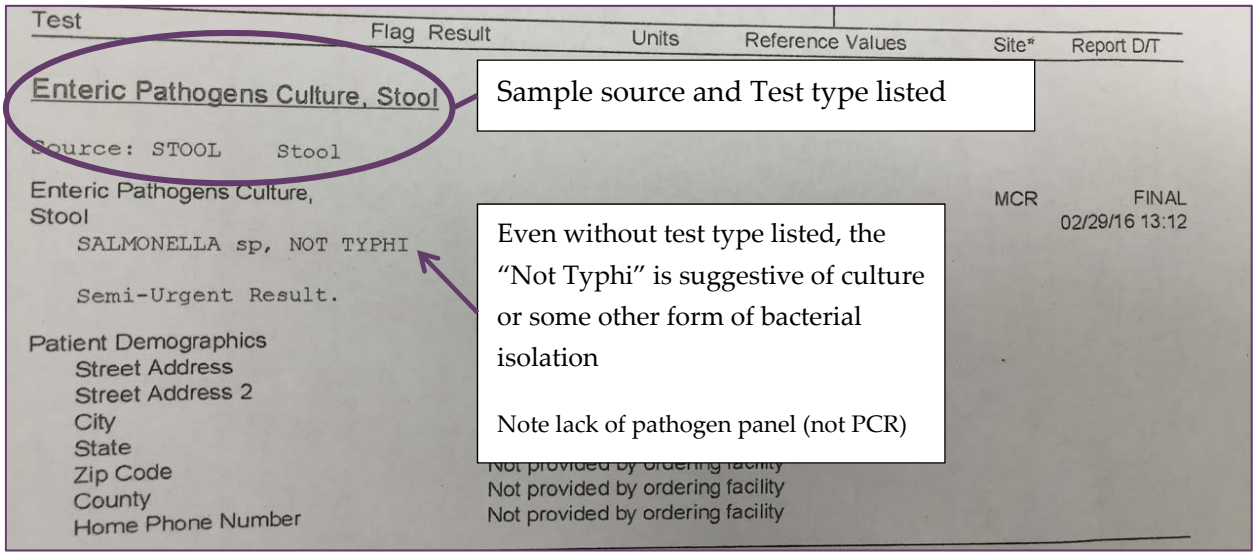


Polymerase Chain Reaction (PCR) Results



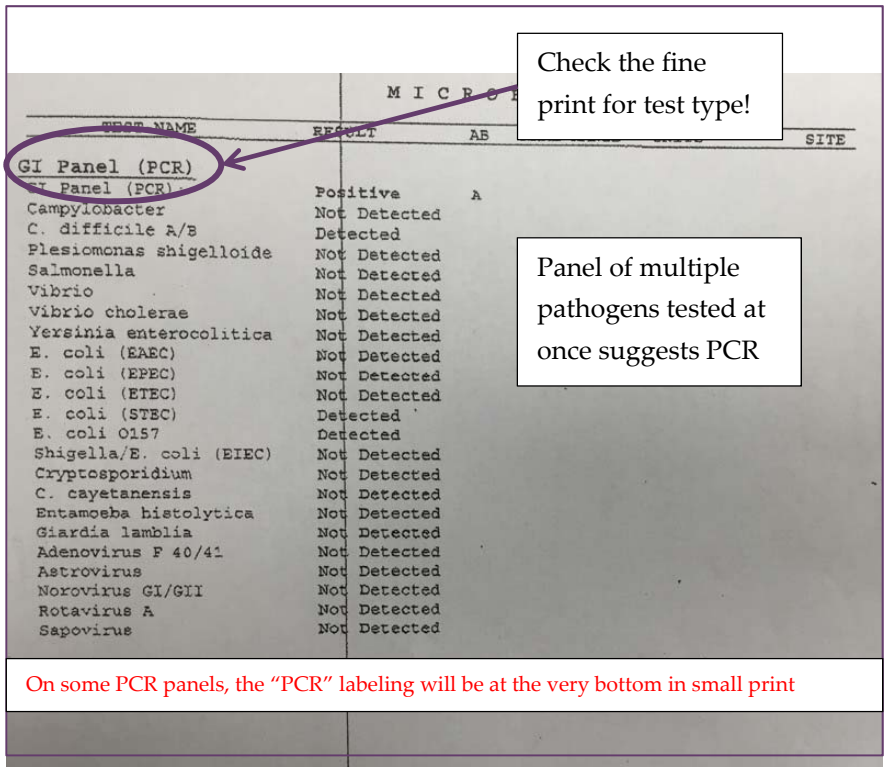
***Campylobacter* Culture (Isolation) Result**

Laboratory results may often say "culture" without being a culture/isolation test or not be labeled at all– make sure to double check so results are interpreted correctly!



***Salmonella sp.* Culture (Isolation) Results**

When species or subspecies are distinguished from one another it most often is a culture.



***E. coli* and *C. difficile* Positive PCR Panel**

This PCR does distinguish between various types of E. coli – the presence of other pathogens that were tested for simultaneously, however, should still discourage labeling these results as a culture.

Which Disease First?

Prioritizing Disease Investigations

All pathogens listed are important and need to be investigated and reported. Most generally need to be reported within 24 hours.

With that in mind, the following is a **subjective suggestion** of which should be prioritized in a practical setting based on

Most enteric pathogens in this guide must be reported within 24 hours. ***Botulism must be reported immediately to the CDC.***

lowest infectious dose[1, 2], lethality, and highest prevalence according to NC surveillance data (see following page):

Immediately Report: Botulism (Clostridium botulinum), Cholera◊, and Hepatitis A*

High Priority: *Salmonella spp.*, *Shigella sp.*, Shiga-toxin producing *E. coli* (STEC/EHEC/VTEC), *Listeria monocytogenes*◊, all *Vibrio*◊ (excluding Cholera)

Intermediate priority: Cryptosporidium, Norovirus (multiple cases), and Typhoid Fever◊/Paratyphoid Fever◊

Low Priority: *Campylobacter spp.*, *Clostridium perfringens*, Cyclosporiasis◊, Trichinosis◊, and other foodborne diseases (i.e. Staphylococcal illness, ciguatera, mushroom toxins, scombroid poisoning, etc.)

*Due to possible exposure from food handling and need for post-exposure prophylaxis in a short window of time.

◊ These pathogens require additional supplemental forms that have to be completed for the CDC

Contacts

For any suspected or confirmed cases of **Botulism, Cholera, or Hepatitis A**, immediately contact the Epidemiologist on Call at **(919) 733 - 3419.**

Additional Report Forms for CDC

Listeriosis – http://www.cdc.gov/listeria/surveillance.html

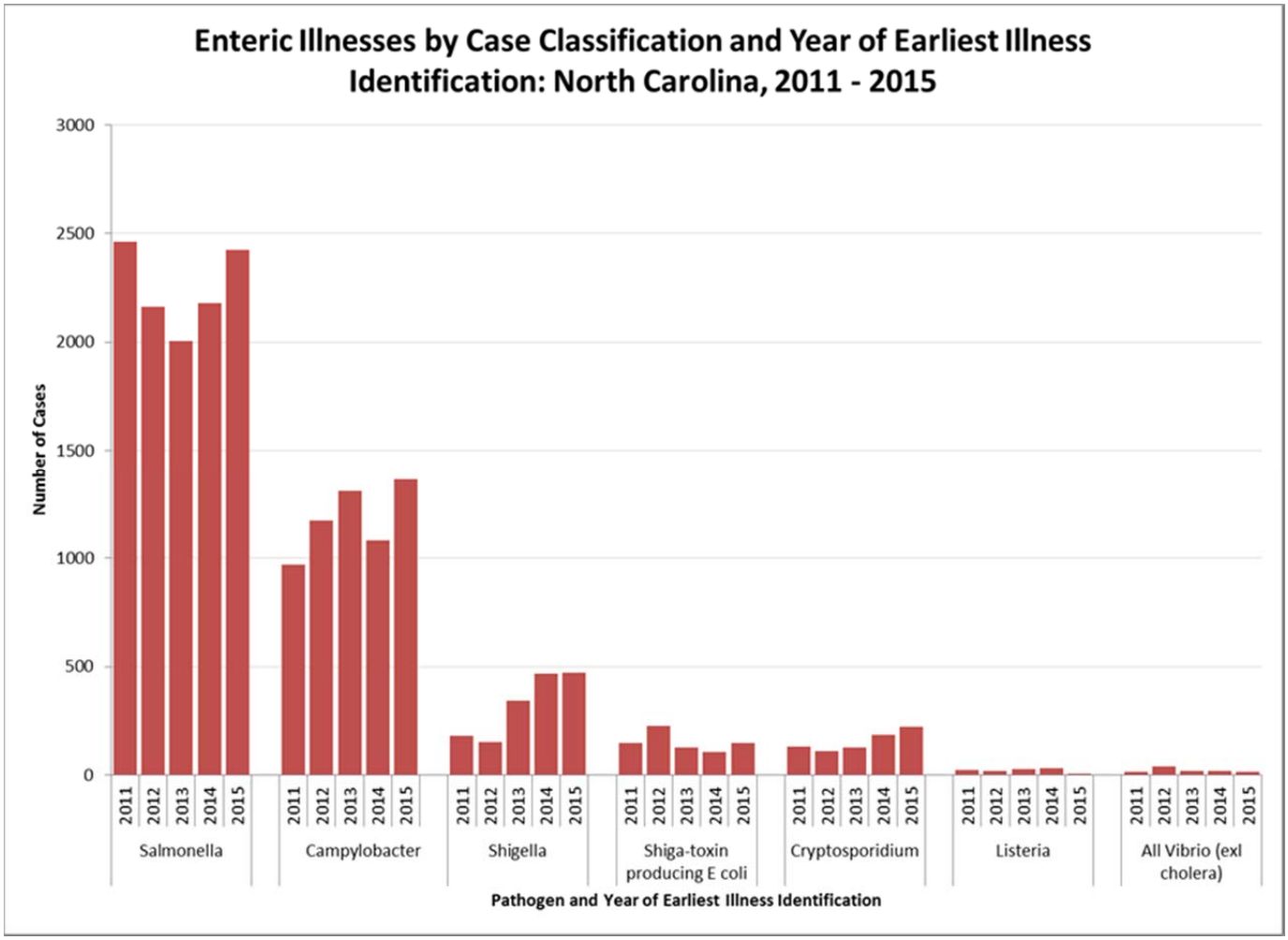
Typhoid/Paratyphoid Fever – http://www.cdc.gov/nationalsurveillance/PDFs/typhi-surveillance-form.pdf

Vibrio spp. (including Cholera) – http://www.cdc.gov/vibrio/surveillance.html

Trichinosis – http://www.cdc.gov/parasites/trichinellosis/health_professionals/index.html

Cyclosporiasis – http://www.cdc.gov/parasites/cyclosporiasis/surveillance.html

Please complete and fax to "Enterics" at **(919) 733 - 0490** OR scan the completed form and attach to NCEDSS event.



References

1. Centers for Disease Control and Prevention. *Section VIII - Agent Summary Statements: Bacterial Agents.* Centers for Disease Control and Prevention Publications. Web.

2. Y. Hara-Kudo and K. Takatori (2011). Contamination level and ingestion dose of foodborne pathogens associated with infections. Epidemiology and Infection, 139, pp 1505-1510. doi:10.1017/S095026881000292X.

DIATHERIX | LABORATORY REPORT

Diatherix Laboratories, Inc.
601 Genome Way / Suite 2100 / Huntsville, AL 35806
Phone: 866.979.4242 / Fax: 256.327.0984 / CLIA ID: 01D1085737

PATIENT:

ID:

Gender: Age: DOB: Ethnicity:

ORDERING PHYSICIAN:

Name:

Phone:

SPECIMEN:

Source: Type:

Specimen ID:

Accession ID:

Collected:

Received:

Reported:

CLIENT:

Name:

Code:

Address:

Gastrointestinal Panel:

Adenovirus 40, 41
Not reportable
Norovirus
Not reportable as a single case
Rotavirus
Not reportable

Enterohemorrhagic E. coli (EHEC)
Shiga-like toxin gene (stx1)
Shiga-like toxin gene (stx2)
Reportable

Enteropathogenic E. coli (EPEC)
Not reportable – has virulence markers but does not produce shiga toxin

Enterotoxigenic E. coli (ETEC)
Not reportable

Enteroinvasive E. coli (EIEC)/Shigella
Reportable- Shigella

(EIEC) Salmonella enterica
Reportable

Campylobacter jejuni
Reportable

Vibrio parahaemolyticus
Not reportable

Clostridium difficile (Toxin B gene)
Not reportable

Cryptosporidium parvum
Reportable

Giardia lamblia
Not reportable

COMMENTS

Testing performed by TEM-PCR™ (Target Enriched Multiplex Polymerase Chain Reaction)

Patent US 7,851,148 B2

Linking Diagnostics to Therapeutics ™

Interpreting GI Pathogen Panels

Bacteria	
Campylobacter (jejuni, coli and upsaliensis)	*Reportable*
Clostridium difficile (toxin A/B)	Not Reportable
Plesiomonas shigelloides	Not Reportable
Salmonella	*Reportable*
Yersinia enterocolitica	Not Reportable
Vibrio (parahaemolyticus, vulnificus and cholerae)	*Reportable*
Vibrio cholerae	*Reportable*
Diarrheagenic E. coli/Shigella	
Enteroaggregative *E. coli* (EAEC)	Not Reportable
Enteropathogenic *E. coli* (EPEC)	Not Reportable
Enterotoxigenic *E. coli* (ETEC) *lt/st*	Not Reportable
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	*Reportable*
E. coli O157	*Reportable*
Shigella/Enteroinvasive *E. coli* (EIEC)	*Reportable (this is **Shigella**)*
Parasites	
Cryptosporidium	*Reportable*
Cyclospora cayetanensis	*Reportable*
Entamoeba histolytica	Not Reportable
Giardia lamblia	Not Reportable
Viruses	
Adenovirus F 40/41	Not Reportable
Astrovirus	Not Reportable
Norovirus GI/GII	Not Reportable
Rotavirus A	Not Reportable
Sapovirus (I, II, IV and V)	Not Reportable

Note: GI Pathogen Panels are polymerase chain reaction (PCR) tests and should be marked as such in the lab package of NCEDSS. These tests are not cultures.

For more information related to *Escherichia coli* types, please click on the link below:

http://www.cdc.gov/ecoli/general/index.html#what-are-shiga-toxin

Created 7/12/2016 NC DPH Enterics Team

Summary of Reportable Enteric Pathogens in North Carolina

Organism	Common Name of Illness	Onset Time After Ingestion	Signs & Symptoms	Duration	Food Sources	Diagnostic Test
Campylobacter jejuni	Campylobacteriosis	2-5 days	(Bloody) diarrhea, cramps, fever, vomiting	2-10 days	Raw and undercooked poultry, raw milk, contaminated water	Culture CIDTs (PCR, IFA, & EIA)
Ciguatera toxin	Ciguatera poisoning (marine toxin)	Minutes to 30 hours	Nausea, vomiting, diarrhea, cramps, excessive sweating, headache, muscle aches, weakness, itching, dizziness, burning ("pins and needles"), reversal of temperature sensation in mouth, nightmares, or hallucinations	1-4 weeks	Contaminated tropical reef fish (barracuda, grouper ,sea bass, snapper, mullet)	Generally not applicable
Clostridium botulinum	Botulism	12-72 hours	Vomiting, diarrhea, blurred vision, double vision, difficulty swallowing, muscle weakness; respiratory failure and death	Variable	Improperly canned foods (home canned vegetables, fermented fish, baked potatoes in aluminum foil)	PCR, ELISA, MS, mouse bioassay
Clostridium perfringens	Perfringens food poisoning	8-16 hours	Intense abdominal cramps, watery diarrhea	Usually 24 hours	Meats, poultry, gravy, dried or precooked foods, time and/or temperature-abused foods	Culture, PCR,
Cryptosporidium	Intestinal cryptosporidiosis	2-10 days	(Water) diarrhea), stomach cramps, upset stomach, slight fever	3 weeks; May be remitting and relapsing over weeks to months	Uncooked food or food contaminated by an ill handler after cooking; contaminated drinking water; contact with infected animal	Biopsy, oocysts in stool, staining, microscopy, PCR, DFA, rapid card, EIA

Summary of Reportable Enteric Pathogens in North Carolina

Organism	Common Name of Illness	Onset Time After Ingestion	Signs & Symptoms	Duration	Food Sources	Diagnostic Test
Cyclospora cayetanensis	Cyclosporiasis	1-14 days; usually 1 week	(Watery) diarrhea, loss of appetite, substantial loss of weight, stomach cramps, nausea, vomiting, fatigue	May be remitting and relapsing over weeks to months	Various types of fresh produce (imported berries, lettuce, basil)	Oocysts in stool, microscopy, PCR, DFA, EIA
E. coli Shiga – toxin producing (EHEC/STEC/VTEC)	STEC infection	1-3 days	Watery diarrhea, abdominal cramps, some vomiting	3-7 or more days	Water or food contaminated with human feces	Culture, PCR, EIA, serology
E. coli O157:H7	O157 STEC infection	1-8 days	Severe (often bloody) diarrhea, abdominal pain and vomiting. Usually, little or no fever is present. More common in children 4 years or younger. Can lead to kidney failure	5-10 days	Undercooked beef (especially hamburger), unpasteurized milk and juice, raw fruits and vegetables (e.g. sprouts), and contaminated water	Culture, PCR, EIA, serology
Hepatitis A	Hepatitis	28 days average (15-50 days)	Diarrhea, dark urine, jaundice, and flu-like symptoms, i.e., fever, headache, nausea, and abdominal pain	Variable, 2 weeks-3 months	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters	PCR, antibody detection

Summary of Reportable Enteric Pathogens in North Carolina

Organism	Common Name of Illness	Onset Time After Ingestion	Signs & Symptoms	Duration	Food Sources	Diagnostic Test
Listeria monocytogenes	Listeriosis	9-48 hours for gastrointestinal symptoms; 2-6 weeks for invasive disease	Fever, muscle aches, nausea or diarrhea. Pregnant women may have mild flu-like illness, and infection can lead to premature delivery or stillbirth. Elderly or immunocompromised patients may develop bacteremia or meningitis	Variable	Unpasteurized milk, soft cheeses made with unpasteurized milk, ready-to-eat deli meats	Culture
Mushroom Poisoning	Mushroom poisoning (**varies on mushroom consumed)** *Amanita smithiana* Amatoxin	1-3 hours 1-2 hours	Acute gastroenteritis; nausea, vomiting, abdominal cramping, diarrhea; hallucinations Acute gastroenteritis; renal failure within 24 hours Liver toxicity; rhabdomyolysis	4-24 hours	Mushroom consumption; oftentimes mistake a toxic mushroom for an edible variety	Generally not applicable; some tests exist for *Amanita* spp., but they are difficult to come by and expensive.
Noroviruses	Viral gastroenteritis, winter diarrhea, food poisoning	12-48 hours	Nausea, vomiting (>50%), abdominal cramping, diarrhea, fever, headache. Diarrhea more in adults; vomiting more in children	12-60 hours	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler; shellfish from contaminated waters	PCR, EIA

Summary of Reportable Enteric Pathogens in North Carolina

Organism	Common Name of Illness	Onset Time After Ingestion	Signs & Symptoms	Duration	Food Sources	Diagnostic Test
Salmonella sp.	Salmonellosis	6-48 hours	Diarrhea, fever (commonly), abdominal cramps, vomiting	4-7 days	Eggs, poultry, meat, unpasteurized milk or juice, cheese, contaminated fruits and vegetables	Culture, PCR
Salmonella Typhi	Typhoid Fever	1-2 weeks	Sustained fever, weakness, abdominal pains, headache, loss of appetite, rash (with rose colored spots), constipation	4-6 weeks	Fecal-oral, contaminated food or water; usually from traveling	Culture, PCR, serology
Scombroid or histamine fish poisoning	Scrombrotoxic fish poisoning	2 minutes-2 hours	Rash, diarrhea, flushing, sweating, abdominal pain, headache, vomiting, burning or swelling of mouth, and metallic taste	Usually resolves within a few hours	Bacterially spoiled fish (tuna, mackerel, bonito)	Generally not applicable
Shigella	Shigellosis or Bacillary dysentery	4-7 days	Abdominal cramps, fever, tenesmus, and diarrhea. Stools may have blood and mucus	24-48 hours	Raw produce, contaminated drinking water, uncooked foods and cooked foods that are not reheated after contact with an infected food handler	Culture, PCR
Staphylococcus aureus	Staphylococcal food poisoning	1-6 hours	Sudden onset of severe nausea and vomiting. Abdominal cramps. Diarrhea and fever may be present.	24-48 hours	Unrefrigerated or improperly refrigerated meats, potato and egg salads, cream pastries	Culture, PCR

Summary of Reportable Enteric Pathogens in North Carolina

Organism	Common Name of Illness	Onset Time After Ingestion	Signs & Symptoms	Duration	Food Sources	Diagnostic Test
Trichinella spp.	Trichinellosis (Trichinosis)	1-2 days More severe 2 weeks	Nausea, diarrhea, vomiting, abdominal pain, muscle pain, fever, swelling of face, weakness, headache, chills, rash, cough, constipation	8 weeks; Most symptoms resolve within a few months	Raw or undercooked infected meat (pig or bear meat usually)	Muscle biopsy, PCR, antibody test, DFA, EIA
Vibrio cholera	Cholera	2-3 days	Profuse watery diarrhea, vomiting, leg cramps, rapid loss of body fluids leading to dehydration, shock, and death	1-10 days	Fecal-contaminated water or food sources; poor sanitation; rarely undercooked or raw shellfish	Culture, PCR, rapid test
Vibrio parahaemolyticus	V. parahaemolyticus infection	4-96 hours	Watery (occasionally bloody) diarrhea, abdominal cramps, nausea, vomiting, and fever	2-5 days	Undercooked or raw seafood, such as shellfish	Culture, PCR
Vibrio vulnificus	V. vulnificus infection	1-7 days	Vomiting, diarrhea, abdominal pain, blood borne infection. Fever, bleeding within the skin, ulcers requiring surgical removal. Can be fatal to persons with liver disease or weakened immune systems	2-8 days	Undercooked or raw seafood such as shellfish (especially oysters) *Infection usually associated with wound exposure to brackish water	Culture, PCR

Hemolytic Uremic Syndrome (HUS) is also reportable within 24 hours

*Chart was adapted from the FDA's "Foodborne Illness-Causing Organisms in the U.S." (www.fda.gov), 10A NCAC 41.A0101 "Reportable Diseases and Conditions", and the NC Public Health Communicable Disease Manual; special thanks to Vanessa Greene, RN; Foodborne/TATP Nurse Consultant with the Division of Public Health, Communicable Disease Branch, NC Department of Health and Human Services

15 June 2016

Cholera

Cholera O1 or O139

Vibrio cholerae

Cholera is a disease caused by gram-negative, non-spore forming bacterial rods.

Cholera survives for days in water and in a wide variety of food and drinks for 1-14 days; 1-35 days in ice; 1-7 days on fomites at room temperature.

Ingestion of fecal contaminated water or food source; poor sanitation; rarely undercooked shellfish

Incubation Time: 2-3 days

Duration of Illness: 1-10 days

Exposure: Fecal-oral through ingestion of contaminated food and water; rarely consumption of undercooked or raw seafood; 2-3 days incubation time in the host once exposed.

Symptoms: Profuse watery diarrhea, vomiting, leg cramps, rapid loss of body fluids leading to dehydration, shock, and death. Symptoms can last from 1-10 days.

Testing: Culture is the preferred diagnostic; Crystal VC® dipstick rapid test (high false positives).

Communicability: Patients may shed bacteria before clinical onset of illness and for up to two weeks after illness has resolved; carrier states can exist.

Note that only toxigenic strains (O1 or O139) are reportable

Laboratory Information

Reportable: within 24 hours

Sample: Stool or vomitus

Test Usually Run: Culture, PCR, rapid test

Turn Around Time: 7-10 work days (state); 8 weeks (CDC)

Culture is the Gold Standard

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/CHOLERA_CD.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/CHOLERA_LHD_STEPS.pdf

Additional Forms:
http://www.cdc.gov/vibrio/surveillance.html

References

Centers for Disease Control and Prevention (CDC) (2014). *Cholera*. Centers for Disease Control and Prevention. Web.

North Carolina Division of Public Health Communicable Disease Branch (2013). *2012 North Carolina Division of Public Health Communicable Disease Manual.* North Carolina Department of Health and Human Services. Web.

North Carolina. (2015). *SCOPE – A Guide to Services.* North Carolina State Laboratory of Public Health, Division of Public Health, Department of Health and Human Services.

Public Health Agency of Canada. *Cholera.* Public Health Agency of Canada Pathogen Safety Data Sheets and Risk Assessment. Web.

Which E. coli is Which?

Different E. coli

E. coli is a common bacteria found in the environment, foods, and intestines of normal humans. Pathogenic strains are distinguished from each other based on the pathogenesis of the type of diarrhea they cause and the type of toxins they produce (which consequently leads to excessive acronym use).

Enterotoxigenic *E. coli* (ETEC) – the cause of traveler's diarrhea, this type of *E. coli* produces **enterotoxins** (heat stable and labile), and has fimbriae that allow for intestinal attachment.

Diffusely adherent *E. coli* (DAEC) – forms a diffuse attachment on enterocytes and contributes to increased permeability of cells leading to fluid and electrolyte loss.

So What about STEC?

STEC refers to "shiga-toxin producing *E. coli*". STEC can also be called "Verocytotoxin-producing *E. coli* (VTEC)" or "enterohemorrhagic *E. coli* (EHEC)".

STEC = VTEC = EHEC

***ETEC*, *EPEC*, *EAEC*, *EIEC*, and *DAEC* types of E.coli, while pathogenic, are generally not as dangerous as *STEC* and consequently not reportable**

Enteropathogenic *E. coli* (EPEC) – common cause of diarrheal outbreaks in children; attaches and effaces intestinal wall; mechanisms not completely understood.

Enteroaggregative *E. coli* (EAEC) – attaches to the surfaces of intestinal cells and essentially forms a biofilm on the surface; leads to sloughing of cells.

Enteroinvasive *E. coli* (EIEC) – causes a syndrome very similar to Shigellosis; bacteria actually bind and enter intestinal cell, damaging the intestinal wall via cell destruction.

STEC is reportable. It is estimated that 5-10% of those diagnosed with STEC infections have the capacity to develop **hemolytic uremic syndrome (HUS)**, another reportable condition.

STEC serotypes of concern include O157:H7 and O104:H4. These are arguably the most researched types of EHEC. O and H refer to different types of proteins found on the bacteria that are unique to that type of bacteria and consequently used to identify it.

STEC bacteria produce "Shiga-like toxin" (also called verotoxin). It is very similar, but **different** from the toxin produced by *Shigella dysenteriae.*

E. coli Infection, Shiga Toxin Producing (STEC/VTEC/EHEC)

E. coli Infection

Escherichia coli O157:H7

E. coli infections are caused by Gram-negative non-spore forming rods. Reportable E.coli infections include those that produce Shiga toxin (isolates O157:H7, O26, O45, O103, O111, and O121).

Can survive 1.5 hours in food and water; 16 months on dry surfaces.

Ingestion of undercooked beef (viz. hamburger), unpasteurized milk and juice, raw fruits and vegetables (viz. sprouts)

Incubation Time: 1-8 days

Duration of Illness: 5-10 days

Exposure: Consumption of undercooked beef (usually hamburger), contaminated water, unpasteurized milk and juice, or raw fruits and vegetables (such as sprouts); direct contact with animals; 1-8 day incubation time.

Symptoms: Often bloody diarrhea and abdominal cramps; rarely Hemolytic Uremic Syndrome (HUS); symptoms generally last 5-10 days.

Testing:

- O157:H7 must be cultured; *E.coli* cultures that are isolated can then be tested (EIA or PCR) to confirm Shiga-toxin production
- Elevated antibody titers via serology
- Identification of Shiga toxin (EIA)
- Presence of Shiga toxin gene (PCR)

Communicability: Person-to-person transfer possible, but uncommon.

Laboratory Information

Reportable: within 24 hours

Sample: Stool

Test Usually Run: Culture, PCR, EIA, serology

PFGE

Turn Around Time: 7-10 work days (state); 8 weeks (CDC)

Culture is the Gold Standard

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/ECOLI_INFECTION-SHIGA_TOXIN_PRODUCING_CD_2016.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/E_COLI_LHD_STEPS.pdf

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Public Health Agency of Canada. *Escherichia coli.* Public Health Agency of Canada Pathogen Safety Data Sheets and Risk Assessment. Web.

Salmonellosis

Salmonellosis

Salmonella spp.

A disease caused by Gram-negative rod shaped bacteria. They can survive for days (or months depending on serotype) in the environment and food.

Exposure: Consumption of contaminated foods, contact with infected feces, animals, or people. Milk, egg, and poultry products pose the most risk. 6-48 hour incubation period

Symptoms: Four clinical manifestations: gastroenteritis, bacteremia, enteric fever, and asymptomatic. Usual symptoms include diarrhea, fever (commonly), abdominal cramps, and vomiting. Asymptomatic or extraintestinal infections may occur. Symptoms usually resolve in 4-7 days.

Consumption of contaminated foods such as eggs, poultry, meat, unpasteurized milk, juice, or cheese; contact with pet reptiles

Incubation Time: 6-48 hours

Duration of Illness: 4-7 days

Testing: Culture is the preferred diagnostic; PCR; PFGE

Communicability: Humans spread disease while they shed it in their feces (1-2 weeks); certain carriers can spread the disease for years; zoonotic.

Laboratory Information

Reportable: within 24 hours

Sample: Stool

Test Usually Run: Culture or PCR

Turn Around Time: 4-7 business days (state); 8 weeks (CDC)

Culture is the Gold Standard

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/SALMONELLOSIS_CD.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/SALMONELLA_LHD_STEPS.pdf

Additional Forms:
http://www.cdc.gov/nationalsurveillance/PDFs/typhi-surveillance-form.pdf

References

Centers for Disease Control and Prevention (CDC) (2014). *Salmonella*. Centers for Disease Control and Prevention. Web.

North Carolina Division of Public Health Communicable Disease Branch (2013). *2012 North Carolina Division of Public Health Communicable Disease Manual.* North Carolina Department of Health and Human Services. Web.

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Public Health Agency of Canada. *Salmonella.* Public Health Agency of Canada Pathogen Safety Data Sheets and Risk Assessment. Web.

(2013). Veterinary Microbiology. 3rd edn (eds McVey SD, Kennedy M, and Chengappa MM), Wiley-Blackwell Press, Ames, IA 50014,

Typhoid Fever

Typhoid Fever

Salmonella Typhi

Typhoid fever is caused by *Salmonella Typhi*, a gram-negative rod bacteria.

Survivability outside of host depends on serotype (weeks to months).

Exposure: Fecal-oral through ingestion of contaminated food and water; usually from travelling; 1-2 week incubation time in the host once exposed.

Symptoms: Sustained fever, weakness, abdominal pains, headache, loss of appetite, rash (with rose colored spots), nonproductive cough, bradycardia, anorexia, diarrhea, and constipation. Usually resolves within 4-6 weeks.

Testing: Culture is the preferred diagnostic with serotyping performed; PCR; serum testing; Widal test

In a Widal test, blood is added to various chemicals to see if it agglutinates (indicating the presence of Typhoid antibodies to the O or H antigens of *Salmonella typhi*).

Communicability: Can be spread human-to-human; carrier states exist and can infect others.

Fecal-oral exposure to contaminated food or water; poor hygiene

Incubation Time: 1-2 weeks

Duration of Illness: 4-6 weeks

Laboratory Information

Reportable: within 24 hours; Carrier state within 7 days

Sample: Stool, Serum

Test Usually Run: Culture or PCR; serology (serum)

Turn Around Time: 4-7 business days (state); months from CDC

Culture is the Gold Standard

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/TYPHOID_FEVER_ACUTE_CD.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/TYPHOID_ACUTE_LHD_STEPS.pdf

Additional Forms:
http://www.cdc.gov/nationalsurveillance/PDFs/typhi-surveillance-form.pdf

References

Centers for Disease Control and Prevention (CDC) (2014). *Typhoid*. Centers for Disease Control and Prevention. Web.

North Carolina Division of Public Health Communicable Disease Branch (2013). *2012 North Carolina Division of Public Health Communicable Disease Manual.* North Carolina Department of Health and Human Services. Web.

North Carolina. (2015). *SCOPE – A Guide to Services.* North Carolina State Laboratory of Public Health, Division of Public Health, Department of Health and Human Services.

Shigellosis

Shigellosis ("Bacillary dysentery")

Shigella spp.

Shigellosis is a disease caused by Gram-negative rod shaped bacteria.

It can survive for months on dry surfaces, days on vegetables, over three hours on fingers, and for 2-28 days on metal utensils. Flies can also carry the organism.

Exposure: Fecal-oral; poor hand hygiene; raw produce; contaminated drinking water and (un)cooked foods that are not reheated after contact with an infected food handler such as tossed salads, chicken, and shellfish. Incubation time is 4-7 days post consumption.

Symptoms: fever, (bloody, mucoid) diarrhea, abdominal pain, nausea, vomiting, tenesmus. A small amount of people can develop blood poisoning, Reiter's syndrome, chronic arthritis, seizures, and hemolytic uremic syndrome (HUS). Symptoms usually resolve after two days.

Testing: Culture and PCR; Culture is the gold standard. PFGE for serotyping.

Communicability: People tend to be infectious while symptomatic, and for up to 4 weeks after symptoms resolve. Asymptomatic carriers can shed for months.

Exposure to raw produce, contaminated drinking water, uncooked foods; poor hand hygiene

Incubation Time: 4-7 days

Duration of Illness: 24-48 hours

Laboratory Information

Reportable: within 24 hours

Sample: Stool

Test Usually Run: Culture or PCR; PFGE for serotypes post-culturing

Turn Around Time: 4-7 business days (state); 8 weeks (CDC)

Culture is the Gold Standard

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/SHIGELLOSIS_CD.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/SHIGELLOSIS_LHD_STEPS.pdf

Let's Talk About Toxins

Shigella spp. produce Shiga Toxin (Stx). The toxin is a virulence factor that causes vascular lesions that lead to hemorrhagic colitis and hemolytic uremic syndrome.

Keep in mind that toxins are different from spores.

Toxins are produced by actively reproducing cells and help to facilitate bacterial spread through the host.

Endospores are dormant non-reproductive structures produced by certain bacteria in order to survive inhospitable conditions (pH, temperature extremes, etc.). Spores can germinate into actively reproducing bacteria in ideal conditions.

References

Centers for Disease Control and Prevention (CDC) (2014). *Shigella*. Centers for Disease Control and Prevention. Web.

Moxley R. (2013). Veterinary Microbiology. 3rd edn (eds McVey SD, Kennedy M, and Chengappa MM), Wiley-Blackwell Press, Ames, IA 50014, pp.95-100

North Carolina Division of Public Health Communicable Disease Branch (2013). *2012 North Carolina Division of Public Health Communicable Disease Manual.* North Carolina Department of Health and Human Services. Web.

North Carolina. (2015). *SCOPE – A Guide to Services.* North Carolina State Laboratory of Public Health, Division of Public Health, Department of Health and Human Services.

Public Health Agency of Canada. *Shigella.* Public Health Agency of Canada Pathogen Safety Data Sheets and Risk Assessment. Web.

Vibrio spp.

Vibriosis

Vibriosis is caused by *Vibrio spp.*, which are Gram-negative rod shaped bacteria.

More infections occur between May and October when ocean water temperatures are warmer.

Exposure: consumption of undercooked or raw seafood such as shellfish; exposure of wounds to salt water; incubation time is 24 hours

Symptoms: Vomiting, (watery) diarrhea, abdominal pain, nausea, fever, and chills. Symptoms usually resolve within 3 days.

Testing: Culture is the preferred diagnostic.

Communicability: No evidence of person to person spread.

Consumption of undercooked or raw seafood such as shellfish (especially oysters) or wounds exposed to brackish or salt water

Incubation Time: 24 hours

Duration of Illness: 3 days

Laboratory Information

Reportable: within 24 hours

Sample: Stool

Test Usually Run: Culture or PCR

Turn Around Time: 2-7 business days; 8 weeks CDC

Culture is the Gold Standard

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/VIBRIO_INFECTION_OTHER_CD.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/VIBRIO_OTHER_LHD_STEPS.pdf

Additional Forms:
http://www.cdc.gov/vibrio/surveillance.html

References

Centers for Disease Control and Prevention (CDC) (2014). *Vibrio spp*. Centers for Disease Control and Prevention. Web.

North Carolina Division of Public Health Communicable Disease Branch (2013). *2012 North Carolina Division of Public Health Communicable Disease Manual.* North Carolina Department of Health and Human Services. Web.

North Carolina. (2015). *SCOPE – A Guide to Services.* North Carolina State Laboratory of Public Health, Division of Public Health, Department of Health and Human Services.

Listeriosis

Listeriosis

Listeria monocytogenes

Listeriosis is a zoonotic disease caused by anaerobic, Gram-positive coccobacillus.

***L. monocytogenes* can grow at low temperatures (even in refrigerators).** It is found normally in nature.

Exposure: Ingestion of unpasteurized milk, unpasteurized soft cheeses, ready to eat deli meats; in rare cases transplacental transmission. It incubates for 9-48 hours.

Symptoms: Fever, muscle aches, nausea, diarrhea; flu-like symptoms in pregnant women in addition to premature birth, stillbirth, and abortion. Meningitis can also occur. Listeriosis has a high mortality rate.

Testing: Culture is the preferred diagnostic; usefulness of fluorescent antibody testing or PCR is not established; atypical testing with NC state lab

Consumption of unpasteurized milk, unpasteurized soft cheeses, and read-to-eat deli meats

Incubation Time: 9-48 hours

Duration of Illness: Variable

Communicability: Can be spread from mother to child during pregnancy and childbirth; zoonotic

Laboratory Information

Reportable: within 24 hours

Sample: Blood or spinal fluid

Test Usually Run: Culture

Turn Around Time: 7-10 business days (state); 8 weeks (CDC)

Culture is the Gold Standard

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/LISTERIOSIS_CD.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/LISTERIOSIS_LHD_STEPS.pdf

Additional Forms:
http://www.cdc.gov/listeria/surveillance.html

References

Centers for Disease Control and Prevention (CDC) (2014). *Listeriosis*. Centers for Disease Control and Prevention. Web.

Narayanan S. (2013). Veterinary Microbiology. 3rd edn (eds McVey SD, Kennedy M, and Chengappa MM), Wiley-Blackwell Press, Ames, IA 50014, pp.223-227

North Carolina Division of Public Health Communicable Disease Branch (2013). *2012 North Carolina Division of Public Health Communicable Disease Manual.* North Carolina Department of Health and Human Services. Web.

North Carolina. (2015). *SCOPE – A Guide to Services.* North Carolina State Laboratory of Public Health, Division of Public Health, Department of Health and Human Services.

Public Health Agency of Canada. *Listeria.* Public Health Agency of Canada Pathogen Safety Data Sheets and Risk Assessment. Web.

Vibrio vulnificus

Vibriosis

Vibriosis is caused by *Vibrio vulnificus* and other *Vibrio spp.*, which are Gram-negative rod shaped bacteria.

More infections occur between May and October when ocean water temperatures are warmer.

Exposure: consumption of undercooked or raw seafood such as shellfish; incubation time is 1-7 hours.

Symptoms: Vomiting, diarrhea, abdominal pain, blood borne infection, fever, bleeding within the skin, ulcers requiring surgical removal. Can be fatal to immunocompromised individuals or individuals with compromised liver function. Symptoms usually resolve in 2-8 days.

Testing: Culture is the preferred diagnostic

Communicability: No evidence of person-to-person spread.

Consumption of undercooked or raw seafood such as shellfish (especially oysters)

Incubation Time: 1-7 hours

Duration of Illness: 2-8 days

Laboratory Information

Reportable: within 24 hours

Sample: Stool

Test Usually Run: Culture or PCR

Turn Around Time: 7-10 business days (state); 8 weeks (CDC)

Culture is the Gold Standard

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/VIBRIO_VULNIFICUS_INFECTION_CD.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/VIBRIO_VULNIFICUS_LHD_STEPS.pdf

Additional Forms:
http://www.cdc.gov/vibrio/surveillance.html

References

Centers for Disease Control and Prevention (CDC) (2014). *Vibrio vulnificus*. Centers for Disease Control and Prevention. Web.

North Carolina Division of Public Health Communicable Disease Branch (2013). *2012 North Carolina Division of Public Health Communicable Disease Manual.* North Carolina Department of Health and Human Services. Web.

North Carolina. (2015). *SCOPE – A Guide to Services.* North Carolina State Laboratory of Public Health, Division of Public Health, Department of Health and Human Services.

Cryptosporidiosis

Cryptosporidiosis

Cryptosporidium parvum

Cryptosporidium parvum is a zoonotic intracellular parasite with a complicated life cycle.

It can survive for 6 months at 20°C in the environment.

Exposure: Fecal-oral; direct contact with infected humans or animals, contaminated water, and aerosols. Incubation time is 2-10 days.

Symptoms: Diarrhea (longer than 72 hours), abdominal cramping, vomiting, anorexia, myalgia, nausea, malaise, fatigue. Symptoms usually last up to 3 weeks, but can be remitting and relapsing in immunocompromised individuals.

Testing: Cryptosporidium can be tested for in stool, biopsy, and tissue samples via direct fluorescent antibody (DFA), polymerase chain reaction (PCR), enzyme; rapid card; immunoassay (EIA), or light microscopy.

Communicability: Highly contagious from human to human and zoonotic; oocysts can be shed for up to 50 days post cessation of diarrhea.

Exposure via fecal-oral route or from direct contact with infected humans or animals

Incubation Time: 2-10 days

Duration of Illness: 3 weeks; may be remitting and relapsing over weeks to months in immunocompromised individuals

Laboratory Information

Reportable: within 24 hours

Sample: Muscle biopsy, stool

Test Usually Run: DFA is gold standard; PCR; rapid card; EIA

Turn Around Time: 2-7 business days

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/CRYPTOSPORIDIOSIS_CD.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/CRYPTOSPORIDIOSIS_LHD_STEPS.pdf

References

Bowman DD. (2013). *Georgis' Parasitology for Veterinarians*. 10th Elsevier, Amsterdam, Netherlands. Print.

Centers for Disease Control and Prevention (CDC) (2014). *Cryptosporidium*. Centers for Disease Control and Prevention. Web.

North Carolina Division of Public Health Communicable Disease Branch (2013). *2012 North Carolina Division of Public Health Communicable Disease Manual.* North Carolina Department of Health and Human Services. Web.

North Carolina. (2015). *SCOPE – A Guide to Services.* North Carolina State Laboratory of Public Health, Division of Public Health, Department of Health and Human Services.

Public Health Agency of Canada. *Cryptosporidium.* Public Health Agency of Canada Pathogen Safety Data Sheets and Risk Assessment. Web.

Cyclosporiasis

Cyclosporiasis

Cyclospora cayetanensis

Cyclospora spp. are coccidian parasites with complicated life cycles. They can survive in soil and in water for 2 months at 4°C and 7 days at 37°C

Exposure: Consumption of untreated water or contaminated food, or contact with contaminated soil. The oocysts require 7-13 days to mature outside the host.

Symptoms: (Watery) diarrhea, appetite loss, substantial weight loss, stomach cramps, nausea, vomiting, fatigue, low grade fever. Opportunistic infection among AIDS patients.

Testing: Cryptosporidium can be tested for in stool, biopsy, and tissue samples via direct fluorescent antibody (DFA), polymerase chain reaction (PCR), enzyme immunoassay (EIA), or light microscopy.

Communicability: No person-to-person transmission reported.

Exposure via consumption of contaminated food or water, or contact with contaminated soil

Maturation Time Outside Host: 7-13 days

Duration of Illness: Variable; may be remitting and relapsing over weeks to months in immunocompromised individuals

Laboratory Information

Reportable: within 24 hours

Sample: Stool

Test Usually Run: **PCR**, DFA, EIA

Turn Around Time: 21 business days

Investigation Resources

Case Definition:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/casedefs/CYCLOSPORIASIS_CD.pdf

Investigation Steps:
http://epi.publichealth.nc.gov/cd/lhds/manuals/cd/invest/CYCLOSPORIASIS_LHD_STEPS.pdf

Additional Forms:
http://www.cdc.gov/parasites/cyclosporiasis/surveillance.html

References

Bowman DD. (2013). *Georgis' Parasitology for Veterinarians*. 10th Elsevier, Amsterdam, Netherlands. Print.

Centers for Disease Control and Prevention (CDC) (2014). *Cyclosporiasis*. Centers for Disease Control and Prevention. Web.

North Carolina Division of Public Health Communicable Disease Branch (2013). *2012 North Carolina Division of Public Health Communicable Disease Manual.* North Carolina Department of Health and Human Services. Web.

North Carolina. (2015). *SCOPE – A Guide to Services.* North Carolina State Laboratory of Public Health, Division of Public Health, Department of Health and Human Services.

Public Health Agency of Canada. *Cyclospora spp.* Public Health Agency of Canada Pathogen Safety Data Sheets and Risk Assessment. Web.

Main Points to Remember

- Control measures should be applied to all confirmed, probable, and suspect cases!
- PCR detects DNA – from both alive and potentially dead organisms!
- Culture tends to be the gold standard!

E. coli Infections: **STEC = EHEC = VTEC**

Lab results that read "culture" may actually be PCR or EIA results. Double check!

Think twice before entering "serology" for enteric pathogens!

E.coli O157 = STEC infection

Specify O1 or O139 for cholera

Lab turnaround time can take weeks. Be patient with your laboratories! Call the foodborne team if expedited results are needed

Clinical specimens include blood, stool, urine, synovial joint fluid, vomitus, etc.

Do not overfill fecal sample cups!

Appendix 2 – Zika Virus Database Original Documents

Infant's State/Territory ID _____ Registry ID _____
Mother's State/Territory ID _____

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Pregnancy and Zika Virus Surveillance—Infant Follow-Up Form

These data are considered confidential and will be stored in a secure database at the Centers for Disease Control and Prevention

Please return completed form via SAMS or secure FTP—request access from ZIKApregnancy@cdc.gov
The form can also be sent by encrypted email to this address or by secure fax to 404-718-1013 or 404-718-2200

Infant follow up: ☐ 2 months ☐ 6 months ☐ 12 months

IFU.1. State/Territory reporting _____ **IFU.2.** Date of infant examination _____/_____/____

IFU.3. Infant's State/Territory ID _____	**IFU.4.** Mother's State/Territory ID _____	**IFU.5.** DOB: ____/_____/_____	**IFU.6.** Sex: ☐ Male ☐ Female ☐ Ambiguous/undetermined

IFU.7. Infant Death: ☐ No ☐ Yes, date _____/_____/____ or Age at death _____ ☐ Unknown

IFU.8. Weight:	**IFU.9.** Length:	**IFU.10.** Head circumference:
_____grams **or** ____ lbs_____ oz	_____ cm **or** _____ in	_____ cm **or** _____ in

IFU.11. Infant findings for corrected age at examination: (*For infants born preterm, please account for corrected age: chronological age minus weeks born before 40 weeks' gestation)*

Check all that apply

☐ Microcephaly (head circumference <3%ile)
☐ Excessive and redundant scalp skin
☐ Arthrogryposis (congenital joint contractures)
☐ Congenital Talipes Equinovarus (clubfoot)
☐ Hypertonia/Spasticity ☐ Hyperreflexia ☐ Irritability ☐ Tremors
☐ Splenomegaly ☐ Hepatomegaly ☐ Skin rash ☐ Microphthalmia
☐ Absent red reflex ☐ Swallowing/feeding difficulties ☐ Other

IFU.12. *Please list other abnormal findings:*

IFU.13. Development assessment for corrected age at examination: (*For infants born preterm, please account for corrected age: chronological age minus weeks born before 40 weeks' gestation)*
☐ Normal ☐ Abnormal ☐ Unknown

IFU.14. If developmental delay, in what area? *Please check all that apply*
☐ Gross motor ☐ Fine motor ☐ Cognitive, linguistic and communication ☐ Socio-Emotional

Special Studies Since Last Follow-up

IFU.15. Imaging study: ☐ Cranial ultrasound ☐ MRI ☐ CT ☐ Other _____
☐ Not Performed ☐ Unknown

IFU.16. Date: _____/_____/_____

IFU.17. Findings: *check all that apply*
☐ Microcephaly ☐ Cerebral (brain) atrophy ☐ Cerebellar atrophy ☐ Intracranial calcification
☐ Ventricular enlargement ☐ Lissencephaly ☐ Pachygyria ☐ Hydranencephaly
☐ Porencephaly ☐ Abnormality of corpus callosum ☐ Other abnormalities

IFU.18. *Please describe below*

Infant's State/Territory ID _____ Registry ID _____
Mother's State/Territory ID _____

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IFU.19. Imaging study: ☐ Cranial ultrasound ☐ MRI ☐ CT ☐ Other _____
☐ Not Performed ☐ Unknown
IFU.20. Date: _____/_____/_____

IFU.21. Findings: *check all that apply*
☐ Microcephaly ☐ Cerebral (brain) atrophy ☐ Cerebellar atrophy ☐ Intracranial calcification
☐ Ventricular enlargement ☐ Lissencephaly ☐ Pachygyria ☐ Hydranencephaly
☐ Porencephaly ☐ Abnormality of corpus callosum ☐ Other abnormalities
IFU.22. (*please describe below*)

IFU.23. Hearing screening or re-screening: ☐ Not performed ☐ Performed ☐ Unknown
IFU.24. *If performed:* Date: ____/____/____ **IFU.25.** ☐ Pass ☐ Fail or referred,
IFU.26. *Please describe*

IFU.27. Audiological evaluation: ☐ Not performed ☐ Performed ☐ Unknown
IFU.28. *If performed:* Date: ____/____/____ **IFU.29.** ☐ Normal ☐ Abnormal,
IFU.30. *Please describe*

IFU.31. Retinal exam (with dilation): ☐ Not Performed ☐ Performed ☐ Unknown
IFU.32. *If performed:* Date: _____/_____/_____
IFU.33. Findings: *Check all that apply:*
☐ Microphthalmia ☐ Chorioretinitis ☐ Macular pallor ☐ Other retinal abnormalities
IFU.34. *Please describe*

IFU.35. Other abnormal tests/results/diagnosis (include dates): ☐ No ☐ Yes
IFU.36. Date: _____/_____/_____
IFU.37. *Please describe*

Health Department Information

IFU.38. Name of person completing form: _____
IFU.39. Phone: _____ IFU.40. Email: _____
IFU.41. Date of form completion_____/_____/____

Internal use only

Date entered ____/_____/_____ ***Data Entry POC Initials:*** _____	***Data Entry Notes*:**

Registry ID _____ **State/Territory ID** _____

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Exp. 08/31/2016

Pregnancy and Zika Virus Surveillance—Maternal Health History Form

These data are considered confidential and will be stored in a secure database at the Centers for Disease Control and Prevention.

Please return completed form via SAMS or secure FTP—request access from ZIKApregnancy@cdc.gov
The form can also be sent by encrypted email to this address or by secure fax to 404-718-1013 or 404-718-2200

MHH.1. State/Territory ID: _____	**MHH.2. Maternal Age at Diagnosis:** _____	**MHH.3. State/Territory reporting:** _____ **MHH.4. County reporting:** _____

MHH.5. Ethnicity: ☐ Hispanic or Latino ☐ Not Hispanic or Latino

MHH.6. Race *(check all that apply)*:
☐ American Indian or Alaskan Native ☐ Asian ☐ Black or African-American
☐ Native Hawaiian or other Pacific Islander ☐ White

MHH.7. Indication for maternal Zika virus testing: ☐ Exposure history only, no known fetal abnormalities
☐ Exposure history and fetal abnormalities

MHH.8. Date of Zika virus symptom onset: _____/_____/_____ **OR** **MHH.9.** ☐ Asymptomatic

MHH.10. If symptomatic, gestational age at onset: _____(weeks, days)

MHH.11. If gestational age or date not known, trimester of symptom onset _____ (1^{st}, 2^{nd}, 3^{rd})

MHH.12. Symptoms of mother's Zika virus disease: *(check all that apply)*

☐ Fever*(if measured)* _____°F **or** _____°C ☐ Arthralgia ☐ Conjunctivitis ☐ Rash

☐ Other clinical presentation_____

MHH.13. If rash, check all that apply ☐ Maculopapular ☐ Petechial ☐ Purpuric ☐ Pruritic

Describe rash distribution_____

MHH.14. Hospitalized for Zika virus disease ☐ No ☐ Yes ☐ Unknown

MHH.15. Maternal Death ☐ No ☐ Yes ☐ Unknown **If yes, describe**_____
MHH.16. If yes, date of death _____/_____/_____ _____

History of Exposure

MHH.17. What was the suspected mode of Zika virus transmission?
☐ Human-mosquito-human (vector) ☐ Sexual ☐ Other, please specify_____ ☐ Unknown

MHH.18. Did the woman spend time in any areas outside the US states or US territories where there was active Zika virus transmission during the periconceptional period or during pregnancy?
(http://www.cdc.gov/zika/geo/active-countries.html)

☐ No ☐ Yes ☐ Unknown (*If 'no' or 'unknown', skip to question 26)*

MHH.19. *If yes*, please characterize the type of travel:
☐ Incoming travel (one way travel to US states from an area with active Zika virus transmission)
☐ Incoming travel (one way travel to US territories from an area with active Zika virus transmission)
☐ Outgoing and incoming travel (roundtrip from US states to an area with active Zika virus transmission)
☐ Outgoing and incoming travel (roundtrip from US territories to an area with active Zika virus transmission)

If incoming or outgoing travel, please list location and dates of travel:

MHH.20. Country of exposure (1) _____	**MHH.21**. Start Date ____/_____/_____ End Date ____/_____/_____ ☐ Start date is same as LMP
MHH.22. Country of exposure (2)	**MHH.23**. Start Date ____/_____/_____ End Date ____/_____/_____

Registry ID _____ **State/Territory ID** _____

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Pregnancy and Zika Virus Surveillance—Maternal Health History Form

These data are considered confidential and will be stored in a secure database at the Centers for Disease Control and Prevention.

_____	☐ Start date is same as LMP
MHH.24. Country of exposure (3) _____	**MHH.25**. Start Date ____/_____/_____ End Date ____/_____/_____ ☐ Start date is same as LMP

MHH.26. **Was the Zika virus exposure within the 50 states, DC, or territories?** ☐ No ☐ Yes ☐ Unknown

***If yes,* separately list each state or territory where Zika virus exposure occurred, and dates of possible exposure:**

MHH.27. State or territory 1 _____	**MHH.28**. Start Date _____/_____/_____ End Date _____/_____/_____ ☐ Start date is same as LMP ☐ Still at location
MHH.29. State or territory 2 _____	**MHH.30**. Start Date _____/_____/_____ End Date _____/_____/_____ ☐ Start date is same as LMP ☐ Still at location
MHH.31. State or territory 3 _____	**MHH.32**. Start Date _____/_____/_____ End Date _____/_____/_____ ☐ Start date is same as LMP ☐ Still at location

MHH.33. If suspected mode of transmission is sexual, was the pregnant woman's sexual partner(s):
☐ Male ☐ Female *Please check all that apply*

MHH.34. Did any sexual partner(s) have an illness that included fever, rash, joint pain, or pink eye during or within 2 weeks of spending any time in an area with active Zika virus transmission?
☐ No ☐ Yes ☐ Unknown

MHH.35. If yes, was there unprotected sexual contact while partner(s) had this illness?
☐ No ☐ Yes ☐ Unknown

MHH.36. Did partner have a test that demonstrated laboratory evidence of Zika virus infection? ☐ No ☐ Yes ☐ Unknown

Maternal Health History *(Underlying maternal illness)*

MHH.37. Diabetes ☐ No ☐ Yes ☐ Unknown
MHH.38. Maternal Phenylketonuria (PKU) ☐ No ☐ Yes ☐ Unknown
MHH.39. Hypothyroidism ☐ No ☐ Yes ☐ Unknown
MHH.40. High Blood Pressure or Hypertension ☐ No ☐ Yes ☐ Unknown
MHH.41. **Other underlying illness(es):** ☐ No ☐ Yes ☐ Unknown
MHH.42. If yes, specify: _____

Pregnancy Information

MHH.43. Last menstrual period (LMP): _____/_____/_____	**MHH.44. Estimated delivery date (EDD):** _____/_____/_____

MHH.45. Estimated delivery date based on (*check all that apply*):
☐ LMP ☐ U/S (1st trimester) ☐ U/S (2nd trimester) ☐ U/S (3rd trimester)
☐ Other, specify _____

OB History: **MHH.46.** # pregnancies (including current pregnancy) _____ **MHH.47.** # living children _____
MHH.48. # miscarriages _____ **MHH.49.** # elective terminations _____

MHH.50. Prior fetus/infant with microcephaly: ☐ No ☐ Yes ☐ Unknown
MHH.51. If yes, cause genetic?: ☐ No ☐ Yes ☐ Unknown

MHH.52. Gestation: ☐ Single ☐ Twins ☐ Triplets+

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Substance use during this pregnancy:	**MHH.53.** Alcohol use: ☐ No ☐ Yes ☐ Unknown **MHH.54**. Cocaine use: ☐ No ☐ Yes ☐ Unknown **MHH.55.** Smoking: ☐ No ☐ Yes ☐ Unknown	
Complications during current pregnancy		
MHH.56.	Toxoplasmosis infection:	☐ No ☐ Yes ☐ Unknown
MHH.57.	Cytomegalovirus infection:	☐ No ☐ Yes ☐ Unknown
MHH.58.	Herpes Simplex infection:	☐ No ☐ Yes ☐ Unknown
MHH.59.	Rubella infection:	☐ No ☐ Yes ☐ Unknown
MHH.60.	Syphilis infection:	☐ No ☐ Yes ☐ Unknown
MHH.61.	Fetal genetic abnormality:	☐ No ☐ Yes, **MHH.62.** Diagnosis _____ ☐ Unknown
MHH.63.	Gestational diabetes:	☐ No ☐ Yes ☐ Unknown
MHH.64.	Pregnancy-related hypertension:	☐ No ☐ Yes ☐ Unknown
MHH.65.	Intrauterine death of a twin:	☐ No ☐ Yes ☐ Unknown
MHH.66.	Other: ☐ No ☐ Yes ☐ Unknown **MHH.67.** If yes, please specify _____	

MHH.68. Medications during pregnancy: ☐ No ☐ Yes ☐ Unknown

MHH.69. If yes, specify (*please specify type and see guide for further instructions):*

Pregnancy Losses: *Please also complete pertinent sections of neonatal assessment form*

MHH.70. Did this pregnancy end in miscarriage (<20 weeks of gestation)?
☐ No ☐ Yes ☐ Unknown **MHH.71.** Date: _____/_____/_____ *or* gestational age_____ *weeks*
MHH.72. Please describe any abnormalities noted _____

MHH.73. Did this pregnancy end in stillbirth (intrauterine fetal demise) (≥20 weeks of gestation)?
☐ No ☐ Yes ☐ Unknown **MHH.74.** Date: _____/_____/_____ *or* gestational age_____ *weeks*
MHH.75. Please describe any abnormalities noted_____

MHH.76. Was this pregnancy terminated?
☐ No ☐ Yes ☐ Unknown **MHH.77.** Date: _____/_____/_____ *or* gestational age _____ *weeks*
MHH.78. Please describe any abnormalities noted _____

Maternal Prenatal Imaging and Diagnostics

MHH.79. Date(s) of ultrasound(s): ___/___/___ ☐ **MHH.80**. *Check if date approximated* **MHH.81**. *If date not known,* Gestational age	**MHH.82. Overall fetal ultrasound results:** ☐ Normal ☐ Abnormal			
	MHH.83. ☐ Reported by patient/healthcare provider **MHH.84.** ☐ Ultrasound report			
	MHH.85. Head Circumference (HC) _____cm **MHH.86.** ☐ Normal ☐ Abnormal (*by physician report*)			
	MHH.87. Biparietal diameter (BPD) _____cm **MHH.88.** Femur length (FL) _____cm **MHH.89.** Abdominal circumference (AC) _____cm			
	MHH.90. ☐ Symmetrical intrauterine growth restriction (IUGR) (<5% EFW) ☐ Asymmetrical IUGR (HC<FL or HC <AC)			
	MHH.91. Microcephaly	☐ No ☐ Yes	**MHH.92.** Intracranial calcifications	☐ No ☐ Yes
	MHH.93. Encephalocele	☐ No ☐ Yes	**MHH.94.** Ventriculomegaly	☐ No ☐ Yes
	MHH.95. Cerebral atrophy	☐ No ☐ Yes	**MHH.96.** Ocular anomalies	☐ No ☐ Yes

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_____ *(weeks, days)*	**MHH.97.** Cerebellar abnormalities	☐ No ☐ Yes	**MHH.98.** Corpus callosum abnormalities	☐ No ☐ Yes
	MHH.99. Arthrogryposis	☐ No ☐ Yes	**MHH.100.** Lissencephaly	☐ No ☐ Yes
	MHH.101. Pachygyria	☐ No ☐ Yes	**MHH.102.**Hydranencephaly	☐ No ☐ Yes
	MHH.103. Porencephaly	☐ No ☐ Yes	**MHH.104.**Hydrops	☐ No ☐ Yes
	MHH.105. Ascites	☐ No ☐ Yes		
	MHH.106. Other	☐ No ☐ Yes If yes, describe:		
MHH.107. Description of abnormal ultrasound findings:				
MHH.108. Date(s) of Ultrasound(s): ____/____/____ ☐ **MHH.109.** *check if date approximated*	**MHH.111. Overall fetal ultrasound results:** ☐ Normal ☐ Abnormal			
	MHH.112. ☐ Reported by patient/healthcare provider *or* **MHH.113.** ☐ Ultrasound report			
	MHH.114. Head Circumference (HC) _____cm **MHH.115.** ☐ Normal ☐ Abnormal (*by physician report*)			
	MHH.116. Biparietal diameter (BPD) _____cm **MHH.117.** Femur length (FL) _____cm **MHH.118.** Abdominal circumference (AC) _____cm			
	MHH.119. ☐ Symmetrical IUGR (<5% EFW) ☐ Asymmetrical IUGR (HC<FL or HC <AC)			
	MHH.120. Microcephaly	☐ No ☐ Yes	**MHH.121.** Intracranial calcifications	☐ No ☐ Yes
	MHH.122. Encephalocele	☐ No ☐ Yes	**MHH.123.** Ventriculomegaly	☐ No ☐ Yes
	MHH.124. Cerebral atrophy	☐ No ☐ Yes	**MHH.125.** Ocular anomalies	☐ No ☐ Yes
MHH.110. *if date not known,* gestational age _____ *(weeks, days)*	**MHH.126.** Cerebellar abnormalities	☐ No ☐ Yes	**MHH.127.** Corpus callosum abnormalities	☐ No ☐ Yes
	MHH.128. Arthrogryposis	☐ No ☐ Yes	**MHH.129.** Lissencephaly	☐ No ☐ Yes
	MHH.130. Pachygyria	☐ No ☐ Yes	**MHH.131.** Hydranencephaly	☐ No ☐ Yes
	MHH.132. Porencephaly	☐ No ☐ Yes	**MHH.133.** Hydrops	☐ No ☐ Yes
	MHH.134. Ascites	☐ No ☐ Yes		
	MHH.135. Other	☐ No ☐ Yes If yes, describe:		
MHH.136. Description of abnormal ultrasound findings:				
MHH.137. Date(s) of Ultrasound(s): ____/____/____ ☐ **MHH.138.** *check if date approximated* **MHH.139.***if date not*	**MHH.140. Overall fetal ultrasound results:** ☐ Normal ☐ Abnormal			
	MHH.141. ☐ Reported by patient/healthcare provider **MHH.142.** ☐ Ultrasound report			
	MHH.143. Head Circumference (HC)_____cm **MHH.144.** ☐ Normal ☐ Abnormal (*by physician report*)			
	MHH.145. Biparietal diameter (BPD) _____cm **MHH.146.** Femur length (FL) _____cm **MHH.147.**Abdominal circumference (AC) _____cm			
	MHH.148. ☐ Symmetrical IUGR (<5% EFW) ☐ Asymmetrical IUGR (HC<FL or HC <AC)			
	MHH.149. Microcephaly	☐ No ☐ Yes	**MHH.150.** Intracranial calcifications	☐ No ☐ Yes
	MHH.151. Encephalocele	☐ No ☐ Yes	**MHH.152.** Ventriculomegaly	☐ No ☐ Yes

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known, gestational age _____ *(weeks, days)*	**MHH.153.** Cerebral atrophy	☐ No ☐ Yes	**MHH.154.** Ocular anomalies	☐ No ☐ Yes
	MHH.155. Cerebellar abnormalities	☐ No ☐ Yes	**MHH.156.** Corpus callosum abnormalities	☐ No ☐ Yes
	MHH.157. Arthrogryposis	☐ No ☐ Yes	**MHH.158.** Lissencephaly	☐ No ☐ Yes
	MHH.159. Pachygyria	☐ No ☐ Yes	**MHH.160.** Hydranencephaly	☐ No ☐ Yes
	MHH.161. Porencephaly	☐ No ☐ Yes	**MHH.162.** Hydrops	☐ No ☐ Yes
	MHH.163. Ascites	☐ No ☐ Yes		
	MHH.164. Other	☐ No ☐ Yes If yes, describe:		

MHH.165. Description of abnormal ultrasound findings:

****For additional ultrasounds or MRIs, please request a supplementary imaging form****

MHH.166. Fetal MRI performed: ☐ No ☐ Yes (If yes, please answer questions below)

MHH.167. Date(s) of MRI(s): ___/___/___ ☐ **MHH.168.** *check if date is approximated*	**MHH.170. Overall fetal MRI results:** ☐ Normal ☐ Abnormal Describe:_____
	MHH.171. ☐ Reported by patient/healthcare provider **MHH.172.** ☐ MRI report
	MHH.173. Head Circumference (HC) ___cm **MHH.174.** ☐ Normal ☐ Abnormal (*by physician report*)
	MHH.175. Biparietal diameter (BPD) _____cm **MHH.176.** Femur Length (FL) _____cm **MHH.177.** Abdominal circumference (AC) _____cm
	MHH.178. ☐ Symmetrical IUGR (<5% EFW) ☐ Asymmetrical IUGR (HC<FL or HC<AC)

MHH.169. *if date not known,* gestational age _____ *(weeks, days)*	**MHH.179.** Encephalocele	☐ No ☐ Yes	**MHH.180.** Intracranial calcifications	☐ No ☐ Yes
	MHH.181. Ventriculomegaly	☐ No ☐ Yes	**MHH.182.** Cerebral atrophy	☐ No ☐ Yes
	MHH.183. Ocular anomalies	☐ No ☐ Yes	**MHH.184.** Cerebellar abnormalities	☐ No ☐ Yes
	MHH.185. Arthrogryposis	☐ No ☐ Yes	**MHH.186.** Corpus callosum abnormalities	☐ No ☐ Yes
	MHH.187. Lissencephaly	☐ No ☐ Yes	**MHH.188**. Pachygyria	☐ No ☐ Yes
	MHH.189. Hydranencephaly	☐ No ☐ Yes	**MHH.190.** Porencephaly	☐ No ☐ Yes
	MHH.191. Hydrops	☐ No ☐ Yes	**MHH.192.** Ascites	☐ No ☐ Yes
	MHH.193. Other	☐ No ☐ Yes	**MHH. 194.** If yes, describe:	

MHH.195. Description of abnormal MRI findings:

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MHH.196. Amniocentesis performed: ☐ No ☐ Yes **MHH.197**. I*f yes,* date performed: _____/_____/_____

MHH.198. If date unknown, gestational age at time of amniocentesis _____(weeks, days)

MHH.199. Amniotic fluid Zika virus testing: ☐ Not performed ☐ Yes

MHH. 200. If yes, test results: ☐ Negative for Zika ☐ PCR+ Zika

MHH.201. Non-Zika infection detected: ☐ No ☐ Yes

MHH. 202. If yes, what infection(s) detected_____

MHH.203. Genetic abnormality detected: ☐ No ☐ Yes

MHH.204. If yes, please describe: _____

For reporting additional lab results, please use lab form

Health Department Information

MHH.205. Name of person completing form: _____

MHH.206. Phone: _____ **MHH.207. Email:** _____

MHH.208. Date form completed ____/____/____

Internal use only

Date entered____/_____/_____ ***Data Entry POC Initials:*** _____	***Data Entry Notes*:**

Pregnancy and Zika Virus Surveillance—Neonate Assessment Form

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Please return completed form via SAMS or secure FTP—request access from ZIKApregnancy@cdc.gov
The form can also be sent by encrypted email to this address or by secure fax to 404-718-1013 or 404-718-2200
Contact Pregnancy & Birth Defects Task Force phone number: 770-488-7100

NAD.1. Infant's State/Territory ID _____	**NAD.2. Mother's State/Territory ID** _____	**NAD.3. DOB:** _____/_____/_____ ☐Live birth ☐Stillbirth	**NAD.4. Sex:** ☐ Male ☐ Female ☐ Ambiguous/undetermined
NAD.5. Gestational age at delivery: _____ weeks _____ days	**NAD.6. Based on**: (*check all that apply*) ☐ LMP ___/___/___ ☐ U/S (1st trimester) ☐ U/S (2nd trimester) ☐ U/S (3rd trimester) ☐ Other_____		
NAD.7. State/Territory reporting: _____	**NAD.8. County reporting:** _____		
NAD.9. Delivery type: ☐ Vaginal ☐ Caesarean section **NAD.10.** Delivery complication: ☐ No ☐ Yes **NAD.11.** If yes, please describe: _____	**NAD.12. Arterial Cord blood pH** (*if performed*): _____ **NAD.13. Venous Cord blood pH** (*if performed*): _____		
NAD.14. Placental exam (based on path report): ☐ No ☐ Yes **NAD.15.** If yes, ☐ Normal ☐ Abruption ☐ Inflammation ☐ Other abnormality (*please describe*)			
NAD.16. Apgar score: 1 min _____ / 5 min _____	**NAD.17. Infant temp** (*if abnormal*): _____ °F **or** _____ °C		

Physical Examination

NAD.18. Birth head circumference: _____☐ cm _____☐ in **NAD.19.** ☐ molding present **NAD.20.** Physican report: ☐ Normal ☐ Abnormal	**NAD.21. Birth weight:** _____☐ grams _____☐ lbs/oz	**NAD.22. Birth length:** _____ ☐ cm _____ ☐ in
NAD.23. *Repeat* head circumference: _____☐ cm _____☐ in ☐ <24hrs ☐ 24–35hrs ☐ 36–48hrs ☐ >48hrs **NAD.24.** Physican report: ☐ Normal ☐ Abnormal	**NAD.25. Admitted to Neonatal Intensive Care Unit:** ☐ No ☐ Yes *If yes,* reason: _____ **NAD.26. Neonatal death:** ☐ No ☐ Yes Date __/__/____ *or* age at death_____ days	
NAD.27. Microcephaly (head circumference <3%ile): ☐ No ☐ Yes	**NAD.28. Seizures:** ☐ No ☐ Yes	
NAD.29. Neurologic exam*:* (*check all that apply*) ☐ Not performed ☐ Unknown ☐ Normal ☐ Hypertonia/Spasticity ☐ Hyperreflexia ☐ Irritability ☐ Tremors ☐ Other neurologic abnormalities **NAD.30.** (*please describe below*)		
NAD.31. Splenomegaly *by physical exam*: ☐ No ☐ Yes ☐ Unknown **NAD.32.** (*please describe*)	**NAD.33. Hepatomegaly** *by physical exam*: ☐ No ☐ Yes ☐ Unknown **NAD.34.** (*please describe*)	**NAD.35. Skin rash** *by physical exam:* ☐ No ☐ Yes ☐ Unknown **NAD.36.** (*please describe*)

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NAD.37. Other abnormalities identified: *please check all that apply*

☐ Microphthalmia ☐ Absent red reflex ☐ Excessive and redundant scalp skin
☐ Arthrogryposis (congenital joint contractures) ☐ Congenital Talipes Equinovarus (clubfoot)
☐ Other abnormalities **NAD.38.** (*please describe below*)

Neonate Imaging and Diagnostics

NAD.39. Hearing screening : *(date:____/_____/_____)*

NAD.40. ☐ Pass ☐ Fail or referred ☐ Not performed **NAD.41.** *(please describe below)*

NAD.42. Retinal exam (with dilation): ☐ Not Performed ☐ Performed ☐ Unknown

NAD.43. *If performed: (date: _____/_____/_____)*

NAD.44. *please check all that apply:*

☐ Microphthalmia ☐ Chorioretinitis ☐ Macular pallor ☐ Other retinal abnormalities

NAD.45. (*please describe below)*

NAD.46. Imaging study: ☐ Cranial ultrasound ☐ MRI ☐ CT ☐ Not Performed

NAD.47. *(date: _____/_____/_____)*

NAD.48. Findings: *check all that apply*

☐ Encephalocele ☐ Microcephaly ☐ Cerebral (brain) atrophy ☐ Cerebellar abnormalities
☐ Intracranial calcification ☐ Ventricular enlargement ☐ Lissencephaly ☐ Pachygyria
☐ Hydranencephaly ☐ Porencephaly ☐ Abnormality of corpus callosum
☐ Other abnormalities **NAD.49.** (*please describe below*)

NAD.50. Imaging study: ☐ Cranial ultrasound ☐ MRI ☐ CT ☐ Not Performed

NAD.51. *(date: _____/_____/_____)*

NAD.52. Findings: *check all that apply*

☐ Encephalocele ☐ Microcephaly ☐ Cerebral (brain) atrophy ☐ Cerebellar abnormalities
☐ Intracranial calcification ☐ Ventricular enlargement ☐ Lissencephaly ☐ Pachygyria
☐ Hydranencephaly ☐ Porencephaly ☐ Abnormality of corpus callosum
☐ Other abnormalities **NAD.53.** (*please describe below*)

Infant's State/Territory ID_____ Mother's State/Territory ID _____

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NAD.54. Imaging study: ☐ Cranial ultrasound ☐ MRI ☐ CT **NAD.55.** *(date: _____/_____/_____)*
☐ Not Performed

NAD.56. Findings: *check all that apply*
☐ Encephalocele ☐ Microcephaly ☐ Cerebral (brain) atrophy ☐ Cerebellar abnormalities
☐ Intracranial calcification ☐ Ventricular enlargement ☐ Lissencephaly ☐ Pachygyria
☐ Hydranencephaly ☐ Porencephaly ☐ Abnormality of corpus callosum
☐ Other abnormalities **NAD.57.** (*please describe below*)

NAD.58. Was a lumbar puncture performed: ☐ Yes ☐ No ☐ Unknown **NAD.59.** *(date: _____/_____/_____)*

NAD.60.	Toxoplasmosis infection:	☐ Negative ☐ Positive ☐ Unknown
NAD.61.	Cytomegalovirus infection:	☐ Negative ☐ Positive ☐ Unknown
NAD.62.	Herpes Simplex infection:	☐ Negative ☐ Positive ☐ Unknown
NAD.63.	Rubella infection:	☐ Negative ☐ Positive ☐ Unknown
NAD.64.	Syphillis infection:	☐ Negative ☐ Positive ☐ Unknown

NAD.65. Other tests/results/diagnosis (*include dates*):

Health Department Information

NAD.66. Name of person completing form: _____

NAD.67. Phone: _____

NAD.68. Email: _____ **NAD.69. Date of form completion**_____/_____/____

FOR INTERNAL CDC USE ONLY
Mother ID: **State/territory ID:**

Appendix 3 – N. Fowleri Talking Points with Edits

Primary Amebic Meningoencephalitis (PAM) caused by *Naegleria fowleri*

Talking Points

- *Naegleria fowleri* is a commonly and naturally found amoeba in warm freshwater environments world wide
    - Lakes, rivers, hot springs, poorly maintained or under chlorinated swimming pools, and soil
    - Infections are most common during the summer months of July, August, and September

- In rare cases it can travel up the nose and into the brain, causing primary amebic meningoencephalitis (PAM), which is often fatal
    - Usually from underwater submersion or other water-related activities that force water up the nose (e.g., swimming underwater, diving)
    - People do not get *Naegleria* infections by drinking contaminated water

- It very rarely causes infections; less than 10 cases have been reported annually for the past 53 years in the United States

- No approved treatment available
    - Miltefosine may help and is available from the CDC.

- Symptoms begin 1 to 9 days after swimming underwater or nasal exposure to warm freshwater
    - *Symptoms:* headache, fever, nausea, vomiting, stiff neck; in later disease stages – confusion, lack of attention to surroundings or people, loss of balance and bodily control, seizures, hallucinations, and death
    - Death usually occurs within 5 days after symptoms appear
- According to the Centers for Disease Control and Prevention (CDC), the only known way to prevent Naegleria infections is to refrain from water-related activities. However, some measures that might reduce risk by limiting the chance of contaminated water going up the nose include:

    - Avoid water-related activities in warm freshwater during periods of high water temperature and low water levels.

    - Hold the nose shut or use nose clips when taking part in water-related activities in bodies of warm freshwater.

- Avoid digging in or stirring up the sediment while taking part in water-related activities in shallow, warm freshwater areas.
- If you are irrigating, flushing, or rinsing your sinuses (for example, by using a neti pot), use water that has been:
  - distilled;
  - sterilized;
  - previously boiled for 1 minute (at elevations above 6,500 feet, boil for 3 minutes) and left to cool;
  - or filtered, using a filter with an absolute pore size of 1 micron or smaller.
  - Rinse the irrigation device after each use with water that has been distilled, sterilized, filtered, or previously boiled and leave the device open to air dry completely.

For additional information: http://www.cdc.gov/parasites/naegleria/

Frequently Asked Questions

What is Naegleria?

Naegleria fowleri is a microscopic amoeba found in warm bodies of water worldwide. These freshwater bodies of water include lakes, rivers, and hot springs. It can also be found in soil, industrial water waste, and underchlorinated swimming pools. *N. fowleri* cannot live in salt water. It very rarely infects humans.

What temperatures put water at risk for Naegleria?

Naegleria fowleri enjoys warm temperatures. It grows best at high temperatures up to 115°F (46°C) and is less likely to be found in the water as temperatures decline below 77°F (25°C). Waters with total chlorine levels above 0.5mg/L also have decreased *N. fowleri* risk. Infection is most common during the summer months of July, August, and September.

How common are Naegleria infections?

Naegleria fowleri infections are extremely rare. There have been less than 10 cases reported annually for the past 53 years.

From 2006 to 2015, 37 infections were reported in the U.S. Of those cases, 33 people were infected by exposure to recreational water, three people were infected after performing nasal irrigation using tap water and one person was infected by tap water used on a backyard slip-n-slide.

How does *Naegleria* cause disease?

Naegleria fowleri causes disease in humans when it enters the body through the nose. This occurs when water is forced up the nose usually during warm freshwater activities such as swimming or diving. The amoeba travels from the nose to the brain, where it feeds on brain tissue and can cause primary amebic meningoencephalitis (PAM). The infection cannot be spread from person-to-person. One cannot be infected from drinking water.

What are the symptoms of *Naegleria fowleri* infection?

Symptoms generally start 1 to 9 days after swimming or nasal exposure to the amoeba in warm water. Initial signs include headache, fever, nausea, and stiff neck. Later signs include confusion, lack of attention to surroundings, loss of balance, loss of bodily control, seizures, hallucinations, and death. The disease progresses rapidly and usually results in death within 5 days once symptoms begin. Unfortunately, infection is almost always fatal.

How is Naegleria treated?

Treatment is aimed at reducing brain swelling and other supportive care. There are no approved drugs although several are being tested in laboratories. There have been reported survivals after treatment with miltefosine. A clear treatment plan is still unclear.

Where are people most at risk?

People are most at risk in the southern United States during summer months if they swim or dive in freshwater sites (such as lakes, rivers, or hot springs). Infections do occur worldwide. Bodies of water are most at risk when temperatures are hot and water levels are low.

How can people protect themselves?

According to the Centers for Disease Control and Prevention (CDC), the only known way to prevent *Naegleria* infections is to refrain from water-related activities. However, some measures that might reduce risk by limiting the chance of contaminated water going up the nose include:

- Avoid water-related activities in warm freshwater during periods of high water temperature and low water levels.

- Hold the nose shut or use nose clips when taking part in water-related activities in bodies of warm freshwater.
- Avoid digging in or stirring up the sediment while taking part in water-related activities in shallow, warm freshwater areas.
- If you are irrigating, flushing, or rinsing your sinuses (e.g. by using a neti pot), use water that has been:
    - distilled;
    - sterilized;
    - previously boiled for 1 minute (at elevations above 6,500 feet, boil for 3 minutes) and left to cool;
    - or filtered, using a filter with an absolute pore size of 1 micron or smaller.
    - Rinse the irrigation device after each use with water that has been distilled, sterilized, filtered, or previously boiled and leave the device open to air dry completely.

For additional information: http://www.cdc.gov/parasites/naegleria/

North Carolina FAQ

How common is *Naegleria fowleri* in North Carolina?

There have only been four cases of primary amebic meningoencephalitis (PAM) reported to the CDC from North Carolina. Two cases were in 1991, one case in 1998, and one case in 2003. This is a fairly low percentage of the total number of cases (37) reported across the nation to the CDC in the past ten years. *N. fowleri* infections are extremely rare.

Where does the US National Whitewater Center water come from?

The US National Whitewater Center draws its water from permitted well water supply and from the Charlotte-Mecklenburg water supply.

Is it safe to go in the water at US National Whitewater Center?

~~The risk associated with *Naegleria fowleri* at US National Whitewater Center is presumed equal to the risk of swimming in *any* freshwater body of water in the United States or abroad. *N. fowleri* is an extremely rare infection, but there is always a risk due to how commonly it is found in nature. The only way to truly limit infection is to abstain from all water activities in warm, freshwater bodies of water (including lakes, hot springs, and rivers). See the aforementioned safety measures to help reduce risk.~~

The risk associated with *Naegleria fowleri* at the US National Whitewater Center is still being determined by the CDC and state health departments. At this time it is presumed equal to or greater than the risk of swimming in any freshwater body of water in the United States and individuals should take appropriate precautions. The only way to truly prevent infection is to abstain from all water activities in warm, freshwater bodies of water.

~~What should you do if you were exposed (water up the nose) at US National Whitewater Center?~~

~~First and foremost remain calm as the likelihood of contracting an infection is very low. Cases almost always occur in isolation; more than one person rarely gets sick at the same place and time. The risk associated with *Naegleria fowleri* at US National Whitewater Center is presumed equal to the risk of swimming in *any* freshwater body of water in the United States or abroad. If you experience symptoms such as headache, fever, nausea, and stiff neck within 9 days of water exposure, then seek medical aid from your doctor. Be sure to include your concerns about *Naegleria fowleri* and your involvement in water activities. If it has been more than nine days since your visit to the National Whitewater Center and you are free of the symptoms of Naegleria, you are unlikely to be infected with *N. fowleri*.~~

Is the case associated with the US National Whitewater Center being investigated?

Yes. The Mecklenburg County Health Department is collaborating with the NC Division of Public Health, the Ohio Department of Health and the Centers for Disease Control and Prevention to investigate the case.

I participated in the rafting at the WWC at the same time as teen who died, what should I do?

If you experience symptoms such as headache, fever, nausea, and stiff neck within 9 days of water exposure, then seek medical aid from your doctor. Be sure to include your concerns about *Naegleria fowleri* and your involvement in water activities. If it has been more than nine days since your visit to the National Whitewater Center and you are free of the symptoms of Naegleria, you are unlikely to be infected with *N. fowleri*.

I went underwater (at WWC, or lake, or river) and now I feel sick, what should I do?

If you experience symptoms such as headache, fever, nausea, and stiff neck within 9 days of water exposure, then seek medical aid from your doctor. Be sure to include your concerns about *Naegleria fowleri* and your involvement in water activities. If it has been more than nine days since your visit to the National Whitewater Center and you are free of the symptoms of Naegleria, you are unlikely to be infected with *N. fowleri*.

I went underwater (at WWC, or lake or river) and am well, should I see a doctor?

No.

I went underwater, am well but would like to be tested? How can I get tested?

Testing for *Naegleria fowleri* involves a lumbar puncture to obtain spinal fluid. It is not recommended that people without symptoms get tested.

Can I take a vaccine or medicine to prevent this infection?

No.

~~**Should the WWC close?**~~

~~No. The risk associated with *Naegleria fowleri* at US National Whitewater Center is presumed equal to the risk of swimming in *any* freshwater body of water in the United States or abroad. *N. fowleri* is an extremely rare infection, but there is always a risk due to how commonly it is found in nature. The only way to truly limit infection is to abstain from all water activities in warm, freshwater bodies of water (including lakes, hot springs, and rivers).~~

Is the water at the WWC rafting venue as safe as a public pool?

The water at the WWC is not considered a pool and therefore does not have to be inspected and cleaned in the same manner as a pool.

What is the risk of exposure underwater at WWC rafting, lake or river?

The risk is very low, but not zero. *Naegleria fowleri* can be found in natural bodies of water, excluding saltwater. Infection causing illness from *Naegleria fowleri* is very rare. There have only been 4 cases reported from NC to CDC in the past 25 years. There have been 37 cases in the past 10 years nationally.

The risk of exposure underwater at WWC is still undetermined, but is presumed to be equal or greater than risk found at natural bodies of freshwater.

Possible Future questions:

~~**Since the test result were negative on the water samples taken at the WWC, does that mean I am safe to participate in rafting at the WWC?**~~

~~Negative results mean that the organism was not found in the sample taken. The risk associated with Naegleria fowleri at US National Whitewater Center is presumed equal to the risk of swimming in any freshwater body of water in the United States or abroad. N. fowleri is an extremely rare infection, but there is always a risk due to how commonly it is found in nature.~~

~~**Since the test results were positive on the water samples taken at the WWC, can I safely participate in rafting at the WWC?**~~

~~*Naegleria fowleri* is a commonly found amoeba in warm bodies of freshwater word wide. The US National Whitewater Center is presumed equal to the risk of swimming in any freshwater body of water in the United States or abroad.~~

I am immunosuppressed (or pregnant), is it more risky for me to participate in rafting at the WWC or go under at river or lake?

Probably not. Primary amebic meningoencephalitis (PAM), the illness caused by *Naegleria fowleri*, typically affects young, healthy people.

For additional information: http://www.cdc.gov/parasites/naegleria/