



# **CHALLENGE SET 2011**

## **PARTICIPANT SUMMARY REPORT**

March 2012

**NORTH DAKOTA DEPT. OF HEALTH**  
Submitter #: 2209



**NORTH DAKOTA DEPARTMENT OF HEALTH**  
**DIVISION OF LABORATORY SERVICES-**  
**MICROBIOLOGY**

A collaboration to address:  
Bioterrorism  
Quality Assurance  
Foodborne Diseases  
Antimicrobial Resistance  
Emerging Infectious Diseases

**2011 NDLRN/MLS CHALLENGE SET RESULTS SUMMARY**

<b>Submitter Number: 2209</b>	<b>Facility Name: NORTH DAKOTA DEPT. OF HEALTH</b>	<b>Email Address: hrsease@nd.gov</b>
<b>Specimen 1 – Blood</b>		
<b>Your Response</b>	<b>Intended Response</b>	
Organism identification	Bordetella holmesii	<i>Bordetella holmesii</i> ; <i>Staphylococcus</i> coagulase negative
Referral	Our lab would not refer this isolate to any other facility for further testing.	Isolate should be referred to ND-PHL if <i>B. pertussis</i> or <i>Francisella tularensis</i> cannot be ruled out
Gram stain	Gram - negative coccobacilli	Gram-negative bacilli or coccobacilli
Oxidase	Negative	Negative
Urea	Negative	Negative
Nitrate	Not performed	Negative
<b>Specimen 2 – Wound</b>		
<b>Your Response</b>	<b>Intended Response</b>	
Organism identification	Vibrio vulnificus	<i>Vibrio vulnificus</i>
Referral	Our lab would not refer this isolate to any other facility for further testing.	Isolate should be referred to ND-PHL
Gram stain	Gram - negative bacilli	Gram-negative bacilli
Oxidase	Not performed	Positive
Motility	Positive	Positive
<b>Specimen 3 – Stool</b>		
<b>Your Response</b>	<b>Intended Response</b>	
Organism identification	Escherichia coli	No <i>Salmonella</i> , <i>Shigella</i> , <i>Campylobacter</i> , <i>E. coli</i> O157 isolated.
Referral	Our lab would not refer this isolate to any other facility for further testing.	Isolate should be referred to ND-PHL if STEC cannot be ruled out
Direct detection of STEC	Negative	Negative
Serotyping for O157 or O157:H7	Not performed	Negative
<b>Specimen 4 – Abscess</b>		
<b>Your Response</b>	<b>Intended Response</b>	
Organism identification	Burkholderia thailandensis	Possible <i>Burkholderia pseudomallei</i>
Referral	Our lab would not refer this isolate to any other facility for further testing.	Isolate should be referred to ND-PHL if <i>B. mallei</i> or <i>B. pseudomallei</i> cannot be ruled out
Gram stain	Gram - negative bacilli	Gram-negative bacilli
Arginine dihydrolase	Positive	Positive
Catalase	Positive	Positive
Motility	Positive	Positive
Oxidase	Positive	Positive

## **Specimen #1, Blood Culture: *Bordetella holmesii***

**Goals and Objectives:** The organisms in Specimen 1 were *Bordetella holmesii* and a strain of coagulase-negative *Staphylococcus* to simulate a potential contaminant. *B. holmesii* was chosen due to its role as a newly recognized pathogen, primarily in immunocompromised patients, and to reinforce the importance of identifying or referring a slow-growing, Gram-negative organism from a blood source for identification. The objectives were to assess the ability of NDLRN laboratories to identify this unusual pathogen; determine the blood culture systems currently in use throughout the state; and to illustrate key differences between *B. holmesii* and other similar organisms. During the course of analyzing the responses received from this specimen, it was noted that several laboratories were unable to rule out *Francisella tularensis* or *Yersinia pestis* based on classical biochemical testing. Reports of possible *F. tularensis* or *Y. pestis* were an unexpected result, since the intent of choosing *B. holmesii* was not to simulate a potential bioterrorism agent, but to highlight a newly recognized pathogen in a uniquely susceptible patient population. However, the results also illustrate the importance of diligent use of the LRN Sentinel guidelines when working with unusual blood culture isolates, and the importance of education and training on how to recognize, rule out, or refer potential bioterrorism agents to the North Dakota public health lab (NDDoH; Division of Laboratory Services).

**About *Bordetella*:** The genus *Bordetella*, in the family *Alcaligenaceae*, is comprised of eight species, most of which cause illness in humans and/or warm blooded animals. Most species are fastidious, with *B. pertussis* being the most sensitive to toxic substances and metabolites found in common culture media (7). *B. pertussis* is the most well-known member of this genus and is responsible for the respiratory disease whooping cough. *B. parapertussis*, and occasionally *B. holmesii* have been isolated in patients with pertussis-like symptoms who test negative for *B. pertussis*.

**About *Bordetella holmesii*:** Previously known as CDC nonoxidizer group 2 (NO-2), *B. holmesii* mainly causes illness such as bacteremia and endocarditis in patients with underlying conditions; however it has also been isolated from respiratory sources (5). The organism is biochemically inert, making it difficult to identify in the clinical laboratory setting. Molecular methods such as PCR can also be problematic for identification due to the genetic similarity among *B. pertussis*, *B. bronchiseptica*, and *B. holmesii*. Most PCR methods use a genetic element known as IS481 as the amplification target. This element is present in high copy numbers in *B. pertussis*, but is also found in *B. holmesii* and *B. bronchiseptica*, which can lead to false positives in *B. pertussis* assays (4, 5). Recently developed multiplex PCR methods are able to differentiate *Bordetella* species by using amplification targets unique to each species (4). Since 2007 only one isolate of *B. holmesii* has been referred to the North Dakota public health lab for identification. Since 2000, MDH has only identified seven isolates of *B. holmesii*; one isolate was incorrectly identified and submitted as a *B. pertussis* isolate and six were from blood cultures that were referred to MDH for identification. It should be noted that one of the blood culture isolates was referred from a laboratory that was unable to rule out *F. tularensis* and *Y. pestis*.

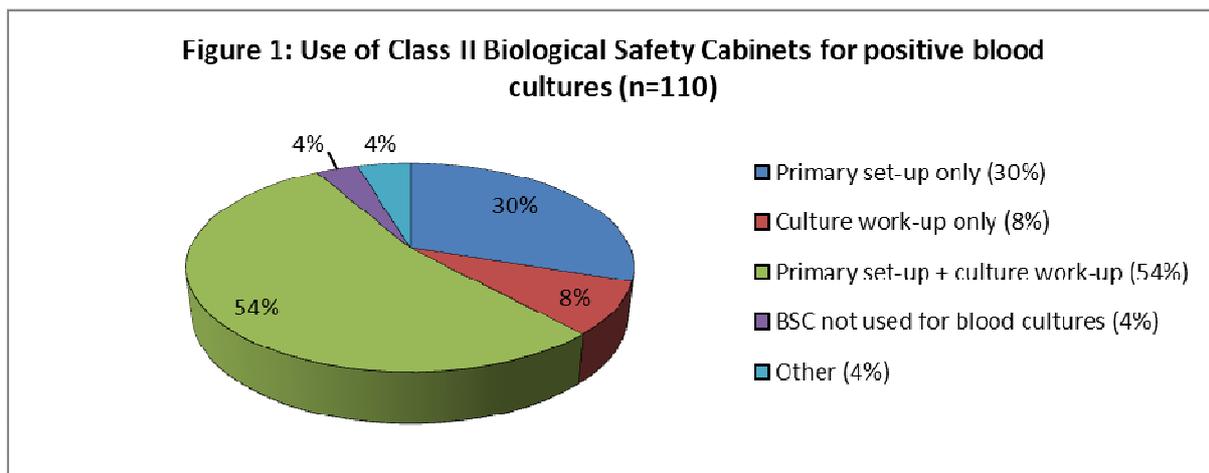
**Disease and Risk Factors:** *Bordetella holmesii* was first characterized in blood cultures from septicemic patients with underlying health conditions (6). Since so few cases have been reported in the literature, little is known about the disease presentation and risk factors for *B. holmesii*, although it has been primarily isolated in patients who are functionally or anatomically asplenic, in addition to patients with underlying sickle cell disease (5). While respiratory symptoms have been reported in patients with *B. holmesii* infection, the organism is almost exclusively associated with septicemia in patients with associated risk factors. Respiratory symptoms associated with *B. holmesii* infection are described as “pertussis-like,” although they are usually milder than those of whooping cough (7). Symptoms in otherwise healthy individuals may be mild and self-limiting. Since *B. holmesii* infection is not reportable

to NDDoH or CDC, the cases that have been documented may not represent the full clinical spectrum of disease or the rate of incidence in the population (5).

### Laboratory Identification of *Bordetella holmesii*

**Collection and Transport:** Specimens should be collected as quickly as possible after the onset of symptoms and before any antibiotic treatment has begun (7). *B. holmesii* may be isolated from blood or respiratory specimens, including nasopharyngeal swabs submitted for *B. pertussis* testing (1). It should be noted that recovery of *B. holmesii* from nasopharyngeal swabs may be inhibited by transport medium containing cephalexin to suppress growth of normal nasopharyngeal flora (7). Blood cultures do not require any specialized collection procedures since most commercial blood culture systems contain substances specifically designed to absorb inhibitory compounds and promote growth of most bacteria.

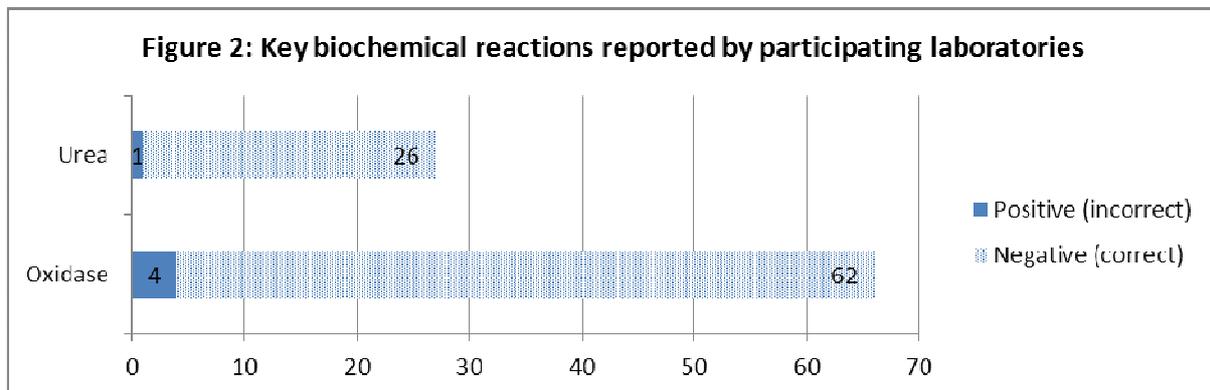
**Laboratory Safety:** Clinical specimens and cultures suspected or known to contain *Bordetella* species should be handled under BSL2 conditions. Any procedure that may produce infectious aerosols (such as vortexing bacterial suspensions, or manipulating blood culture bottles) should be performed inside a Class II biosafety cabinet (2). As mentioned previously, some laboratories may not be able to differentiate *B. holmesii* from the potential bioterrorism agents *Francisella tularensis* or *Yersinia pestis*. Cultures suspected of containing potential bioterrorism agents, or for which these agents cannot be ruled out by LRN Sentinel laboratory methods, represent a significant risk of laboratory exposure and should be handled only in a biosafety cabinet under BSL3 conditions and practices. Sampling of positive blood culture bottles and other routine characterization procedures such as preparing Gram stains, preparing bacterial suspensions, and performing subcultures and biochemical tests, especially catalase testing, can generate dangerous aerosols. Therefore, all manipulation of positive blood cultures should be performed in a BSC (2). **Figure 1** summarizes how participating laboratories reported using a BSC for handling positive blood cultures. It should be noted that additional responses classified as “other” included: using the BSC for primary set up and when there was a high index of suspicion for a highly transmissible agent; using the BSC for culture work-up based on colony morphology and Gram stain appearance; using the BSC for plating samples from liquid media; or using the BSC based on patient history. One laboratory did not provide a response.



**Enrichment, Isolation, and Screening Methods:** *B. holmesii*, unlike *B. pertussis*, will grow on most commercially available media, so specialized enrichment or isolation media are not routinely necessary. However, growth may be slow, especially on differential media such as MacConkey agar (6). Colonies on sheep blood agar are typically small, round, and convex with a brown or green pigment after 48 hours, although pigmentation is more apparent on Mueller-Hinton agar (3, 6). Colonies on MacConkey agar will

be clear or colorless. *B. holmesii* will appear as a small, Gram-negative coccobacillus by Gram stain. Among the participating laboratories that performed a Gram stain, 81/109 (74%) reported a correct result of either Gram-negative bacilli or Gram-negative coccobacilli.

**Biochemical Identification:** *Bordetella* species in general are small, Gram-negative coccobacilli; all are catalase positive, non-fermenting obligate aerobes, with the exception of the recently-characterized *B. petrii* (7). *B. holmesii* can be differentiated from *B. pertussis* by its lack of oxidase activity, from *B. parapertussis* by its inability to hydrolyze urea, and from *B. bronchiseptica* by its lack of motility (1, 6). *B. holmesii* can further differentiated from *Acinetobacter* species by its pigment production on Mueller-Hinton agar (3). Key biochemical reactions reported by participating laboratories are summarized in **Figure 2**.



*B. holmesii* is a challenging organism to identify in the laboratory due to its lack of biochemical activity. In fact, only one laboratory was able to correctly identify this isolate as *B. holmesii* using PCR, and only one other laboratory was able to obtain a genus level identification. Commercial identification systems may be unable to give a definitive identification for *B. holmesii* isolates; this may lead to misidentification of the organism as a *Pseudomonas* species or *Acinetobacter* species (3). Isolates submitted to the North Dakota public health lab (NDDoH-Division of Laboratory Services) for identification are characterized by a lack of biochemical activity, growth on sheep blood agar, and pigment production on Mueller-Hinton agar. Among the 110 participating laboratories, 69 (63%) reported using at least one commercial identification system.

The Gram stain morphology, colony morphology, and biochemical reactions of *B. holmesii* could be mistaken for those of *Francisella tularensis*, a potential bioterrorism agent. Among participating laboratories, 14% (15/110) reported an identification of possible *F. tularensis*. While the goal of this challenge was to compare and contrast *B. holmesii* and other *Bordetella* species, results obtained from participating laboratories demonstrated that the disease presentation and biochemical reactions of this organism would be similar to those expected in a case of tularemia. However, *F. tularensis* could be ruled out in this case by Gram stain morphology, pigment production, and growth on sheep blood agar. *F. tularensis* appears as an extremely tiny Gram-negative coccobacillus, much smaller than cells of *B. holmesii*; however, since both of these organisms are so infrequently isolated, laboratory staff may be unfamiliar with the differences in microscopic morphology. *F. tularensis* does not produce a pigment on culture media, and most strains will not grow on sheep blood agar due the organism's requirement for cysteine.

This profile is also similar to *Yersinia pestis*, another potential bioterrorism agent. *Y. pestis* is not fastidious, and grows well on most commercial media. Its Gram stain appearance may be similar to that of *B. holmesii*, although *Y. pestis* may exhibit bipolar staining. The colony morphology of *Y. pestis* is also distinct, as older colonies have a characteristic "fried egg" appearance, with irregular edges surrounding a

distinct raised center. For more information about the algorithms for ruling out or referring potential bioterrorism agents, please refer to the NDLRN Bench Guide for Bioterrorism Agents provided to your laboratory. Guidelines for LRN Advanced Sentinel Laboratories may also be found on the ASM website; <http://www.asm.org/index.php/policy/sentinel-level-clinical-microbiology-laboratory-guidelines.html>

**Antimicrobial Susceptibility Testing:** Interpretive criteria for antimicrobial susceptibility testing have not been established for *Bordetella holmesii*. However, studies have shown that the minimum inhibitory concentration (MIC) values for cephalosporins and other  $\beta$ -lactam antibiotics are higher than the established breakpoints for these drugs in other Gram-negative bacilli (5). The same studies have shown relatively low MIC values for carbapenems, fluorquinolones, and trimethoprim-sulfamethoxazole (5). Since *B. holmesii* can be difficult to identify biochemically, susceptibility testing may be performed and results reported based on erroneous identification results. Among participating laboratories, 35/110 (32%) reported that antimicrobial susceptibility testing would be performed in house, even without a confirmed species-level identification. An additional 34 laboratories (31%) reported that the isolate would be sent to a reference laboratory for susceptibility testing.

**Reportable Disease Rule:** *Bordetella holmesii* is not reportable to the NDDoH or to CDC, and the NDDoH does not currently perform active surveillance for *B. holmesii*; however, any unusual, slow-growing isolate, particularly those from blood cultures, which cannot be readily identified by the methods used in your laboratory, should be sent to the North Dakota public health lab (NDDoH; Division of Laboratory Services) or another reference laboratory for identification. In North Dakota, all suspected cases of *Francisella tularensis* or *Yersinia pestis* infection must be reported immediately by telephone to the NDDoH; Division of Disease Control at 1.800.472.2180 or 701-328-2378. In addition to telephone notification, culture isolates from all cases must be sent to the NDDoH; Division of Laboratory Services for confirmatory testing. Among the 110 participating laboratories, 33 (30%) reported that they would refer this isolate to the state PHL for further testing, while 53 (48%) reported that they would refer the isolate to a reference laboratory other than the state PHL. The laboratories that would refer the isolate to the state-PHL included all but one of the laboratories that reported an identification of possible *F. tularensis*. Thirteen laboratories correctly followed the LRN Advanced Sentinel protocols and notified the state-PHL by telephone that they were unable to rule out *F. tularensis* or *Y. pestis*. For more information regarding the North Dakota Communicable Disease Reporting Rule please visit: <http://www.health.state.nd.us/Disease/Documents/ReportableConditions.pdf>

## **Specimen #1, Tabulated Results**

110/119 (92%) Laboratories perform blood cultures

### **ORGANISM IDENTIFICATION**

**Intended response (2/110, 2%): *Bordetella holmesii***

**Acceptable responses (55/110, 50%):**

- Gram-negative bacilli, oxidase negative with referral to state-PHL or another reference laboratory (22/110, 20%)
- Gram-negative coccobacilli, NOS with referral to state-PHL or another reference laboratory (13/110, 12%)
- Gram-negative bacilli, NOS with referral to state-PHL or another reference laboratory (12/110, 11% )
- Non-fermenting Gram-negative bacilli with referral to state-PHL or another reference laboratory (5/110, 5%)
- Gram-negative bacilli, refer for ID (3/110, 3%)

**Unacceptable responses (53/110, 48%):**

- Possible *Francisella* species with referral to state-PHL (14/110, 13%)
- Coagulase-negative *Staphylococcus*, without a Gram-negative organism reported (10/110, 9%)
- *Acinetobacter lwoffii* (5/110, 5%)
- *Staphylococcus epidermidis*, without a Gram-negative organism reported (4/110, 4%)
- Possible *Yersinia pestis* with referral to state-PHL (2/110, 2%)
- Gram-negative bacilli, oxidase positive (2/110, 2%)
- *Oligella urealytica* (2/110, 2%)
- *Aggregatibacter (Haemophilus) aphrophilus* (1/110, <1%)
- *Bacillus* species (1/110, <1%)
- *Bordetella bronchiseptica* (1/110, <1%)
- *Chromobacterium violaceum* (1/110, <1%)
- *Escherichia coli* (1/110, <1%)
- Gram-positive bacilli, NOS (1/110, <1%)
- Gram-positive cocci, NOS (1/110, <1%)
- No growth (1/110, <1%)
- Non-fermenting Gram-negative bacilli without referral to state-PHL or another reference laboratory (1/110, <1%)
- *Oligella urethralis* (1/110, <1%)
- Possible *Francisella* species, without referral to state-PHL (1/110, <1%)
- Possible *Haemophilus* species (1/110, <1%)
- *Staphylococcus aureus* (1/110, <1%)
- *Staphylococcus* species, without a Gram-negative organism reported (1/110, <1%)

## REFERENCES

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2. The National Institutes of Health (US) and Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories. 5<sup>th</sup> ed. Washington, D.C.: U.S. Government Printing Office; 2007.
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4. Reischl U, Lehn N, Sanden GN, and Loeffelholz MJ. Real-time PCR assay targeting IS481 of *Bordetella pertussis* and molecular basis for detecting *Bordetella holmesii*. *J Clin Microbiol.* 2001;39(5):1963-1966.
5. Shepard CW *et al.* *Bordetella holmesii* bacteremia: a newly recognized clinical entity among asplenic patients. *Clin Infect Dis.* 2004;38:799-804.
6. Weyant RS *et al.* *Bordetella holmesii* sp. nov., a new Gram-negative species associated with septicemia. *J Clin Microbiol.* 1995;33(1):1-7.
7. Wirsing von Konig CH, Riffelmann M, and Coenye T. *Bordetella* and Related Genera. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, and Warnock DW, editors. *Manual of Clinical Microbiology.* 10<sup>th</sup> ed. Vol 1. Washington, DC: ASM Press; 2011. p. 739-750.

## **Specimen #2, Wound Culture: *Vibrio vulnificus***

**Goals and Objectives:** The organism in Specimen 2 was *Vibrio vulnificus*. This organism was chosen with the goal of raising awareness of this uncommonly isolated organism and illustrating the potential for illness in susceptible patient populations. The objectives were to assess the ability of NDLRN laboratories to identify this unusual pathogen and to determine the current standard of practice among NDLRN laboratories for referral of this isolate to the North Dakota public health lab (NDDoH; Division of Laboratory Services) in accordance with the Communicable Disease Reporting Rule.

**About *Vibrio*:** This genus consists of several well-described human pathogens, including the causative agents of cholera (toxigenic *Vibrio cholerae*) and other potentially fatal human infections. The halophilic *Vibrio* species typically require sodium concentrations between 0.029 and 4.1% for growth (5). *Vibrios* are most commonly isolated in aquatic environments, with their distribution depending on water temperature, salt concentration, and the availability of other nutrients. Non-halophilic species (e.g. *V. cholerae* and *V. mimicus*) may be found in freshwater rivers and lakes, as well as marine environments. *Vibrio* concentrations typically peak in the summer months (5).

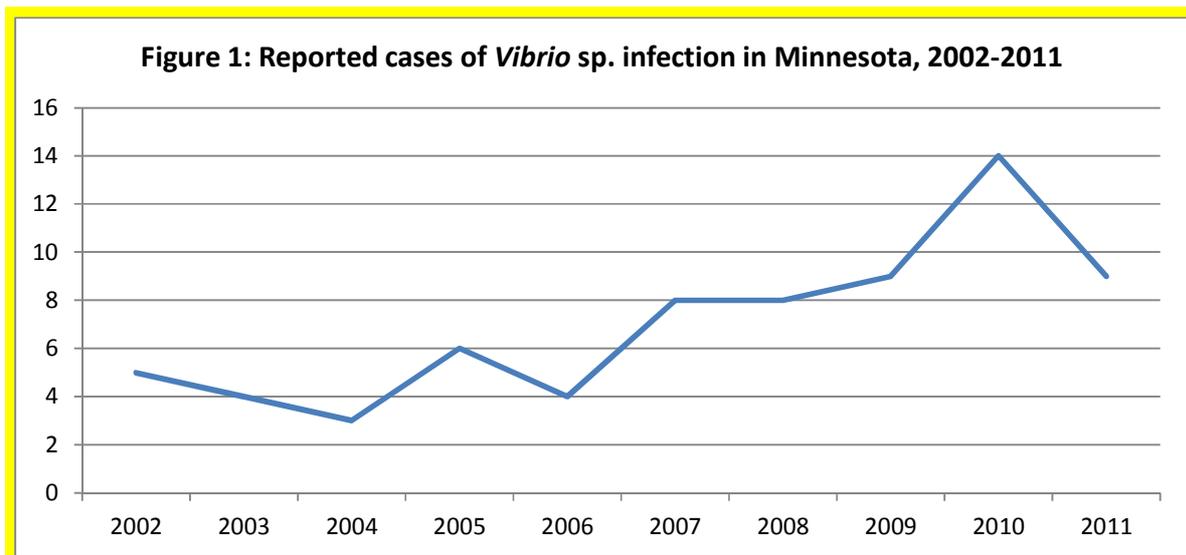
**About *Vibrio vulnificus*:** This species is typically found in warm coastal marine environments with water temperatures between 9 and 31°C and elevated salt concentrations (4). *V. vulnificus* is halophilic and expresses an array of cytotoxins, including a hemolysin, metalloprotease, and other virulence factors that are responsible for the significant tissue damage and hemodynamic disruptions associated with *V. vulnificus* infections. There are three biotypes of *V. vulnificus*, each occupying a specific environmental niche: Biotype 1 is found worldwide in salt or brackish waters, while biotypes 2 and 3 are primarily associated with commercial seafood farming in the Far East and Israel, respectively (4). Biotype 1 is associated with a wide spectrum of human disease, and is the most frequently isolated (4). Although *Vibrio* deaths are rare in the U.S., *V. vulnificus* accounts for greater than 90% of the *Vibrio*-associated deaths in the U.S. each year (5).

**Disease and Risk Factors:** In the U.S., *V. vulnificus* is primarily found in seafood from the Gulf Coast region, particularly oysters. Since the organism is ubiquitous in this environment, the presence of *V. vulnificus* is not considered an indicator of poor water quality. Symptoms appear within 1-7 days of exposure, and usually manifest as one of three clinical syndromes: gastroenteritis, primary sepsis, or wound infection (1). Gastroenteritis occurs after consuming raw or undercooked seafood contaminated with *V. vulnificus*, particularly raw oysters. Symptoms may be mild to moderate, or even self-limited, and include nausea, vomiting, diarrhea, fever, and chills. *V. vulnificus* gastroenteritis is rarely fatal, although ingestion is also believed to be the route of entry for primary sepsis, which is characterized by disseminated illness without a primary focus of infection. Symptoms of primary sepsis are usually preceded by gastrointestinal symptoms, and include abrupt onset of fever and chills followed by the appearance of cutaneous lesions on the trunk or lower limbs, including hemorrhagic bullae, necrotizing fasciitis or vasculitis. Sepsis is usually accompanied by thrombocytopenia and disseminated intravascular coagulopathy. Mortality from *V. vulnificus* primary sepsis may exceed 50% even with aggressive treatment. Wound infections are associated with traumatic injuries (e.g. cuts and scrapes from wading or swimming in contaminated water, handling contaminated shellfish, etc.) or exposure of preexisting wounds to contaminated salt water. Symptoms are similar to sepsis, although the cutaneous lesions are limited to the site of infection or affected limbs. Wound infections may be mild to moderate or more severe with hemorrhagic bullae, cellulitis, and tissue necrosis requiring aggressive surgical debridement or amputation (4).

According to data from the CDC, greater than 95% of all *V. vulnificus* cases report eating raw oysters within 7 days of symptom onset (5). While *V. vulnificus* is found in all Gulf Coast seafood during the

summer months, not all individuals who consume seafood will become ill. Risk factors for infection include liver disease (e.g. cirrhosis, hepatitis), diabetes, immune suppression or deficiency, and iron metabolism disorders. Patients without underlying health conditions may still be susceptible to *V. vulnificus* infection, although their symptoms are typically much less severe and result in dramatically lower mortality (4). Increased incidence of *Vibrio* infections has also been observed following large-scale weather disasters, as was seen after Hurricane Katrina made landfall on the Gulf Coast in 2005 (2).

**Public Health Surveillance:** *Vibrio* illness is infrequent in North Dakota, with only six cases reported to the NDDoH; Division of Disease Control since 2007. *Vibrio* illness is also infrequent in Minnesota with only 70 cases reported to MDH since 2002 (MDH, unpublished data). **Figure 1** shows the incidence of *Vibrio* illness during that time, including two cases of *Vibrio vulnificus* infection. In collaboration with CDC, MDH-PHL serves as a national sentinel site for the FoodNet program, which conducts active surveillance for key foodborne pathogens, including *Vibrio* species. In 2010, FoodNet sites representing health departments in seven states and three metropolitan counties reported 193 cases of foodborne *Vibrio* infection, representing a 115% increase in overall *Vibrio* infections compared to the period from 1996-1998; 25 of these isolates (13%) were *V. vulnificus* (3). Of the 193 cases of *Vibrio* infections in 2010, 45 were hospitalized and 6 died (3).



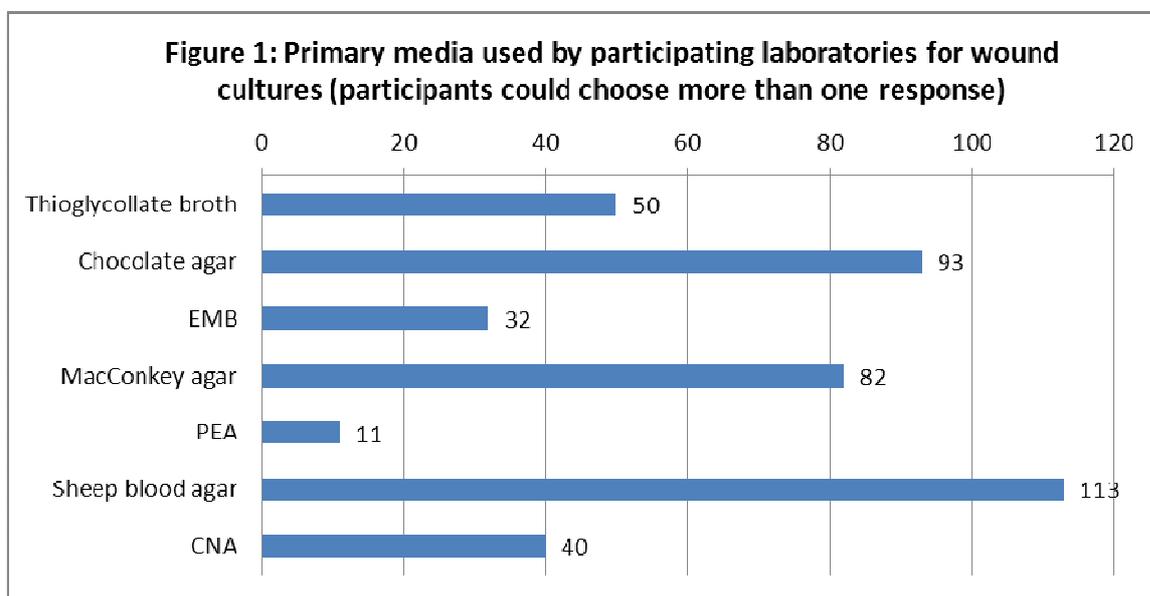
### Laboratory Identification of *Vibrio vulnificus*

**Collection and Transport:** Specimens should be collected as quickly as possible after the onset of symptoms and before any antibiotic treatment has begun (5). The ideal specimen from gastroenteritis infections is freshly passed stool collected in a sterile container, although rectal swabs with visible fecal staining or vomitus are also acceptable from patients with acute illness. If specimens cannot be processed immediately (i.e. within 2-4 hours of collection), then they should be collected in an approved transport medium such as Cary-Blair, Stuart's, or Amies medium (5). Buffered glycerol is not acceptable for the collection of stool samples for *Vibrio* species. Wound or blood cultures do not require any specialized collection procedures since *V. vulnificus* is typically found in pure culture from these sites and most commercial media contain enough salt to support growth.

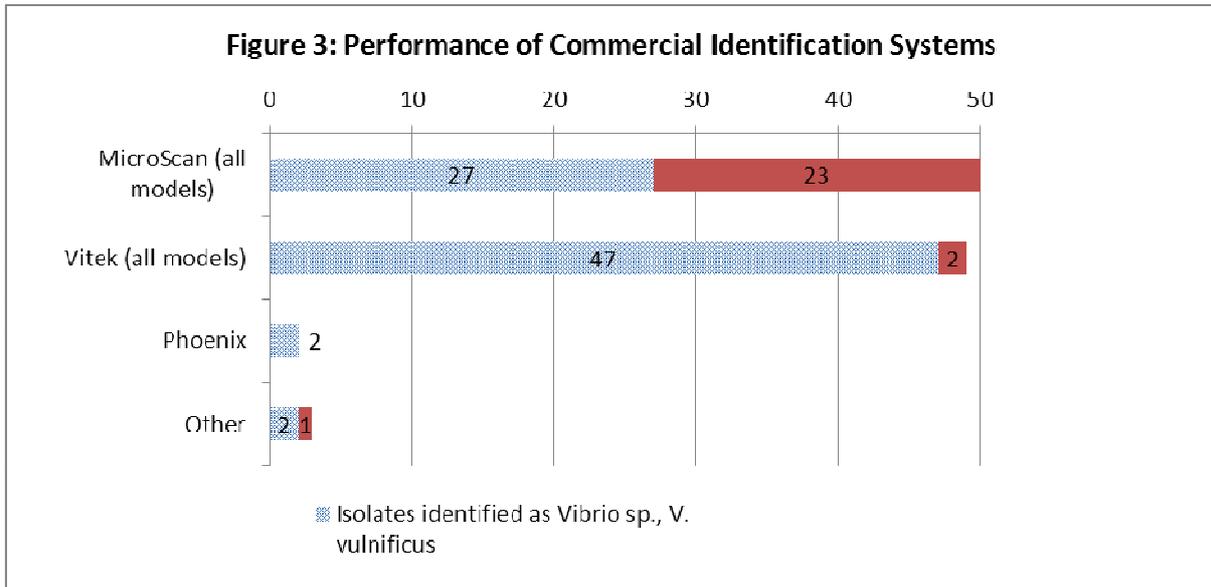
**Laboratory Safety:** Like all enteric pathogens, clinical specimens and cultures suspected or known to contain *Vibrio vulnificus* should be handled under BSL2 conditions. Any procedure that may produce

infectious aerosols (such as vortexing bacterial suspensions, or manipulating blood culture bottles) should be performed inside a Class II biosafety cabinet (6).

**Enrichment, Isolation, and Screening Methods:** *Vibrio vulnificus* will grow on most commercially available media, so specialized enrichment or isolation media are not routinely necessary. However, thiosulfate-citrate-bile salts-sucrose (TCBS) agar can be a useful screening medium for *Vibrio* species in stool cultures due to the selective properties of the bile salts and thiosulfate, and the differential properties of sucrose. *V. vulnificus* will most often produce green colonies on TCBS since most strains do not ferment sucrose (5). Unlike other *Vibrio* species that are lactose-negative, *V. vulnificus* ferments lactose and will produce pink colonies on MacConkey agar (5). On non-selective media, *Vibrio* species in pure culture may produce a variety of colony morphologies, giving the appearance of a mixed culture (5). The primary media used for wound cultures by participating laboratories are summarized in **Figure 2**.



**Biochemical Identification:** Vibrios are Gram-negative straight, curved, or comma-shaped rods that are catalase and oxidase positive (5). *Vibrio* species are motile, with some species demonstrating swarming activity on solid media. *Vibrio* species also require various concentrations of sodium ions for growth, which, in addition to the oxidase reaction, can be helpful in differentiating them from *Enterobacteriaceae*. *V. vulnificus* are biochemically similar to other *Vibrio* species, with fermentation of glucose (without gas production) and positive motility. *V. vulnificus* will also have a negative Voges-Proskauer reaction and a positive lysine decarboxylase reaction, although media for both tests should be supplemented with NaCl if *Vibrio* is suspected (5). Among the 69 laboratories that performed oxidase testing, 96% (66/69) correctly reported a positive result for this isolate. Only 6% (7/115) of participating laboratories reported performing motility testing on this isolate; five of these laboratories correctly reported positive results. This organism can also be identified by commercial identification systems, although there may be difficulty with some systems due to the organism's requirement for salt (5). **Figure 3** summarizes the identifications reported by participating laboratories compared to the commercial identification systems used. It should be noted that the organism identification reported by a given laboratory did not necessarily correspond to the identification obtained by the commercial identification system.



**Antimicrobial Susceptibility Testing:** *Vibrio vulnificus* is susceptible to several classes of antimicrobials, and several drugs have shown to be reliably effective in treatment of *V. vulnificus* infections. Antimicrobial therapy alone has been shown to be minimally effective in cases of severe soft tissue infection, where surgical intervention is considered a requirement for treatment (4). The recommended treatment regimen is a combination of ceftazidime and doxycycline for 7-14 days (4). Overall, 73 of the 115 participating laboratories (63%) reported that antimicrobial susceptibility testing (AST) would be performed on this isolate. Among those laboratories that identified the isolate as *Vibrio* species or *V. vulnificus*, 65% (53/82) reported that AST would be performed.

**Reportable Disease Rule:** Rapid and accurate diagnosis by clinical laboratories is crucial for the detection of outbreaks, timely public health interventions, and detection of *Vibrio* species. In North Dakota, all suspected or confirmed cases of *Vibrio* infection, regardless of species or source, must be reported to NDDoH; Division of Disease Control immediately by telephone due to the significant clinical and epidemiologic impact of *Vibrio* disease. In addition to case reporting, culture isolates from all cases must be sent to the ND state public health lab (NDDoH; Division of Laboratory Services) for complete characterization and serotyping. Among the 82 participating laboratories that identified this isolate as *V. vulnificus* or *Vibrio* species, 61 (74%) reported that they would refer the isolate to their state-PHL. For more information regarding the North Dakota Communicable Disease Reporting Rule, please visit: <http://www.health.state.nd.us/Disease/Documents/ReportableConditions.pdf>

## **Specimen #2, Tabulated Results**

115/118 (98%) Laboratories performed wound cultures

### **ORGANISM IDENTIFICATION**

#### **Identification:**

**Intended response (43/115, 37%):** *Vibrio vulnificus* with referral to state-PHL

**Acceptable responses (37/115, 32%):**

- *Vibrio* species with referral to state-PHL (18/115, 16%)
- Gram-negative bacilli, oxidase positive with referral to state-PHL or another reference laboratory (11/115, 10%)
- Gram-negative bacilli, NOS with referral to state-PHL or another reference laboratory (7/115, 6%)
- Gram-negative coccobacilli, NOS with referral to state-PHL or another reference laboratory (1/115, <1%)

**Unacceptable Answers (35/115 = 30%):**

- *Vibrio vulnificus* without referral to state-PHL (15/115, 13%)
- *Vibrio* species without referral to state-PHL (6/115, 5%)
- *Vibrio parahaemolyticus* (2/115, 2%)
- Possible *Burkholderia pseudomallei* (2/115, 2%)
- *Aeromonas* species (1/115, <1%)
- Gram-positive bacilli, NOS (1/115, <1%)
- *Pasteurella multocida* (1/115, <1%)
- *Pasteurella* species (1/115, <1%)
- *Plesiomonas* species (1/115, <1%)
- Possible *Brucella* species (1/115, <1%)
- *Pseudomonas* species (1/115, <1%)
- *Staphylococcus epidermidis*; *Moraxella* species (1/115, <1%)
- *Yersinia pseudotuberculosis* (1/115, <1%)
- *Yersinia* species (1/115, <1%)

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**Specimen #3, Stool Culture: *E. coli* sorbitol negative (No *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* O157 isolated)**

**Goals and Objectives:** The organism in Specimen 3 was a sorbitol-negative strain of *E. coli* that is also negative for Shiga toxin production. This organism was chosen with the goal of demonstrating the continuing need for diligent detection, reporting, and referral of potential Shiga toxin-producing *E. coli* (STEC) isolates to the North Dakota public health lab (NDDoH; Division of Laboratory Services) in the context of CDC guidelines for the diagnosis of STEC infections by clinical laboratories. The objectives were to assess the ability of NDLRN laboratories to differentiate STEC from *E. coli* isolates that do not produce Shiga toxin; to illustrate the potential benefits to patient management and public health benefits of implementing the CDC STEC screening guidelines; and to determine typing and toxin screening or detection methods currently in use throughout the state.

**About *Escherichia coli*:** *E. coli* is a member of the family *Enterobacteriaceae* and a ubiquitous component of the normal intestinal flora in healthy humans (7). However, certain strains may cause significant intestinal and extraintestinal disease, including urinary tract infections, bacteremia, and meningitis. Although *E. coli* is phenotypically and genetically similar to *Shigella*, the two genera are classified separately due to the clinically significant differences in their disease presentations (6). Much like similar organisms, such as *Salmonella* and *Shigella*, *E. coli* can be serotyped based on the somatic (O) and flagellar (H) antigens. Serotypes associated with intestinal disease can be further categorized based on the specific syndromes they cause: Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive *E. coli* (EIEC).

**Disease and Risk Factors:** *E. coli* O157 (or simply O157) is a colonizer of the intestinal tract of cattle and other ruminants and is often associated with foodborne diarrheal illness caused by consumption of undercooked ground beef that has been contaminated with intestinal contents during processing. Due to the large scale of modern beef production in the U.S., outbreaks associated with ground beef are often widespread and highly publicized. STEC infection can progress to hemolytic uremic syndrome (HUS), which can result in thrombocytopenia, hemolytic anemia, and kidney failure. HUS is typically associated with infections in children, while a similar syndrome (thrombotic thrombocytopenic purpura, or TTP) is more typical in adults. Approximately 8% of O157 cases will develop HUS or TTP, and although other STEC serotypes can cause HUS, more than 80% of HUS cases in North America are associated with O157 infection (3, 6).

In addition to outbreaks in ground beef, there have been numerous outbreaks caused by *E. coli* O157 involving food products such as unpasteurized (raw) milk, fresh produce, and hazelnuts (5). A large outbreak of enteroaggregative hemorrhagic *E. coli* (EAHEC) in Europe in 2011 was traced to sprouts grown in Germany and France from a single lot of fenugreek seeds that originated in Egypt (1). Fresh produce can become contaminated through runoff from cattle farms, contaminated irrigation sources, or wild animals. In addition to being at increased risk of occupational exposure, agricultural workers or those with direct contact with animals or animal environments may also serve as potential reservoirs of O157. The disease can also be easily spread from person to person in settings such as day care centers, due in part to the relatively low infectious dose of O157 (<200 CFU) (7).

**About STEC and *E. coli* O157:H7:** Members of the STEC group express one or both of the Shiga toxins Stx1 and Stx2, which are virtually identical to the toxins produced by *Shigella dysenteriae*. In STEC, the Stx toxins consist of two subunits; the B subunit binds host cell surface receptors, while the enzymatically active A subunit disrupts protein synthesis by cleaving ribosomal RNA. Genes encoding the Stx toxins are embedded into the *E. coli* genome through a lysogenic bacteriophage that expresses the toxins in

response to stress signals. Lysis of bacterial cells during a lytic phage cycle releases the toxins and new phage particles that can infect other non-toxigenic *E. coli* cells in the normal intestinal flora and result in greater toxin production (4). STEC virulence is determined by toxin production; strains producing only Stx2 are more likely to be associated with HUS than those that express both toxins or only Stx1 (3, 4).

More than 150 *E. coli* serotypes, including O157:H7, make up the Shiga toxin-producing *E. coli* (STEC) group. In North America and Europe, the most commonly isolated forms of STEC are the O157:H7 and O157:non-motile (NM) serotypes, although more than 150 other STEC serotypes have been identified. In the United States, O157 is the most commonly isolated STEC and accounts for an estimated 73,000 infections each year, while other non-O157 strains, including O111:non-motile and O26:H11, are more commonly isolated in other countries (3, 6). A large O104:H7 STEC outbreak occurred in Europe in 2011, resulting in 852 cases of HUS and 32 deaths; six cases (four of HUS and two of diarrheal illness) occurred in U.S. residents who had traveled to Europe during the outbreak (1). From September 2010 through December 2011, 21 cases of STEC infection were reported to the NDDoH; of these cases, nine were confirmed as O157. The predominant serotypes among the remaining 12 non-O157 cases included O121:H19, O111:nonmotile, O103:H2, O145:nonmotile and O157:nonmotile. In 2010, 257 cases of STEC infection were reported to MDH; of these cases, 140 (54%) were confirmed as O157 by culture. The predominant serotypes among the remaining 117 cases included O26, O103, and O111, accounting for 61% of the non-O157 serotypes isolated. Culture confirmation could not be performed on 12 of those isolates, so O157 could not be ruled out (5).

**Public Health Surveillance:** In North Dakota, outbreaks of foodborne illnesses are detected through two primary mechanisms. North Dakota residents can call the NDDoH; Division of Disease Control at 701.328.2378 or 1.800.472.2180 to report suspected cases of foodborne illness. However, the most reliable mechanism for detecting bacterial foodborne disease outbreaks, including STEC, is the submission of isolates by clinical microbiology laboratories to the North Dakota public health lab for additional characterization as part of the North Dakota Disease Reporting Rule. All reported cases of suspected or confirmed STEC infections, including O157, are investigated by the NDDoH; Division of Disease Control in conjunction with further testing of isolates submitted to the North Dakota public health lab. Additional testing performed at the North Dakota public health lab may include serotyping for somatic O and flagellar H antigens, and pulsed field gel electrophoresis (PFGE), a process in which the bacterial DNA is fragmented and separated in an agarose gel to generate specific patterns or “fingerprints.” These patterns are compared to those of other North Dakota STEC isolates to track disease, detect outbreaks, and prevent additional cases.

The North Dakota public health lab also collaborates with the Centers for Disease Control and Prevention (CDC) to perform national STEC surveillance and investigation of multi-state potential outbreaks. Clinical laboratories are the foundation of these enhanced surveillance programs through submission of culture isolates, clinical specimens, and survey data on laboratory practices. PFGE patterns from North Dakota STEC cases are uploaded into the national PFGE database known as PulseNet, which is used by CDC and other public health agencies to compare DNA similarities and identify potential multistate and national outbreaks

### **Laboratory Identification of O157 STEC**

**Collection and Transport:** Specimens should be collected as quickly as possible after the onset of symptoms and before any antibiotic treatment has begun (3). The ideal specimen is freshly passed stool collected in a sterile container, although rectal swabs with visible fecal staining are also acceptable if no other specimen is available. If specimens collected for STEC testing cannot be processed immediately (i.e. within 1-2 hours of collection), then they should be refrigerated or frozen at -70°C in an approved transport medium such as Cary-Blair, Stuart’s, or Amies medium (6). However, specific collection and

transport conditions may depend on the organism suspected since some stool pathogens may not survive freezing.

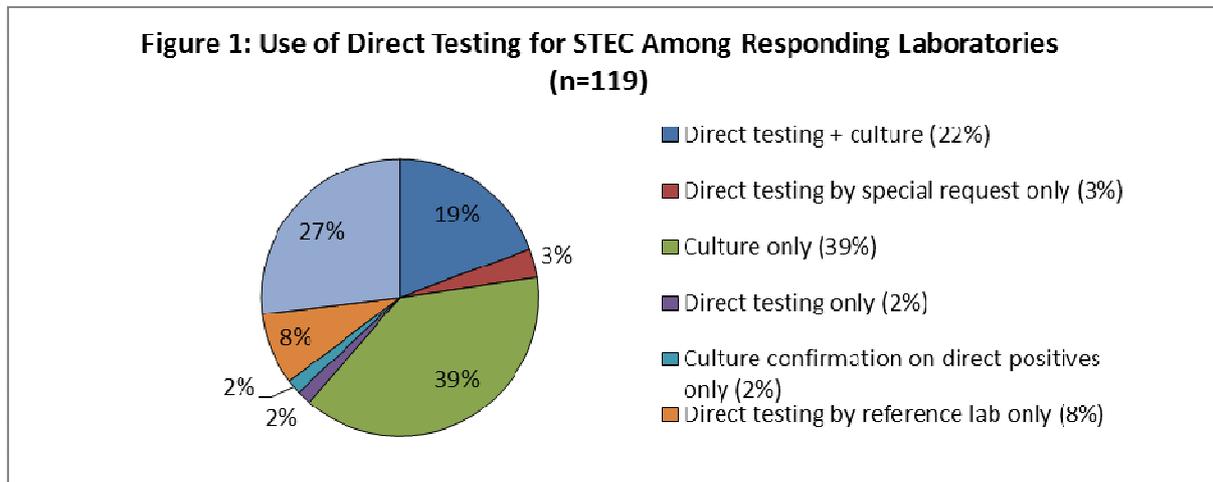
Cultures of toxigenic strains of *E. coli*, including O157, have been classified as Infectious Substances, Category A UN 2814 by the U.S. Department of Transportation under 49 CFR part 173 (8). Shippers are ultimately responsible for using their best professional judgment when considering the correct classification of any isolate submitted for shipment by commercial carrier (e.g. FedEx or UPS). In addition, anyone who packages and ships hazardous materials, including Category A infectious substances, must receive documented training on the U.S. Department of Transportation regulations. The NDDoH public health laboratory provides a free on line packaging and shipping training course. For more information and directions on how to access the course contact Jan Trythall at: [jtrythal@nd.gov](mailto:jtrythal@nd.gov) or call 701-328-6295.

**Laboratory Safety:** Like all enteric pathogens, clinical specimens and cultures suspected or known to contain O157 and other STEC should be handled under BSL2 conditions. Any procedure that may produce infectious aerosols (such as vortexing bacterial suspensions, or manipulating enrichment broths) should be performed inside a Class II biosafety cabinet (7).

**Direct Testing for Shiga Toxins:** In 2009, CDC issued recommendations for detection of STEC infections by clinical laboratories. These guidelines were distributed with the results of the 2010 Challenge Set and can be accessed through the CDC website at <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5812a1.htm>. NDDoH strongly supports the implementation of these guidelines as a means of enhancing the detection of STEC and improving the clinical outcome of patients with STEC infections, particularly among those with O157 infections. The recommendations include testing stool specimens from all patients with acute community-acquired diarrhea for O157 STEC using selective and differential culture media, as well as simultaneous screening for non-O157 STEC using a test that directly detects the Stx genes or the toxins they encode, either in fecal specimens or on growth from broth enrichment or primary isolation media (3).

In accordance with the CDC recommendations, NDDoH public health lab recommends using a combination of both stool culture and toxin screening methods to ensure that all STEC serotypes, including non-O157 serotypes, can be detected. A testing strategy that combines toxin detection and culture also allows early diagnosis, prompt initiation of appropriate therapy, improved patient outcomes, and timely public health responses to potential outbreaks or emerging STEC strains (3). The standard of practice for the use of direct STEC testing among responding laboratories is summarized in **Figure 1**. Among the 78 laboratories that performed stool cultures, 31 (40%) reported using at least one method for direct detection of STEC in stool specimens. Of those 31 laboratories, 23 (74%) reported using the direct method in combination with stool culture for the detection of STEC as part of a routine stool culture, which is the practice recommended by NDDoH public health lab. Two laboratories reported using direct methods alone for the detection of STEC without culture; one uses PCR, while the other does not routinely culture for *E. coli* O157 from stool specimens and uses a commercial kit alone.

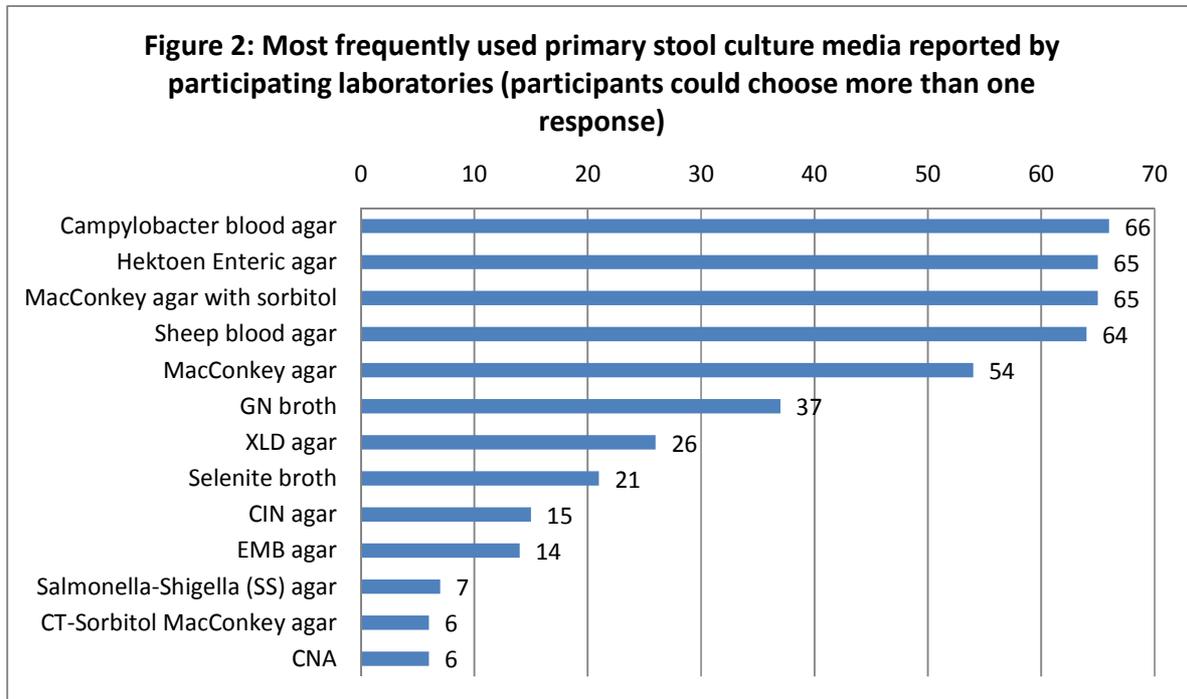
**Enrichment, Isolation, and Screening Methods:** Culture for O157 and other STEC should be performed on all patients with diarrhea, especially those with bloody diarrhea and/or HUS. However, since there are few practical culture-based methods for detecting STEC other than O157, direct screening for the presence of Shiga toxin in combination with routine stool culture is the best means of detecting non-O157 STEC that may go undetected if culture is used alone. While direct testing methods may require the use of an initial broth enrichment, the use of an additional broth enrichment for routine culture has not been shown to significantly improve recovery of O157 from clinical specimens (6).



O157 and other STEC are indistinguishable from commensal *E. coli* strains on MacConkey agar since virtually all STEC are able to ferment lactose. Roughly 80% of commensal *E. coli* can also ferment sorbitol in addition to lactose, while the majority of O157 STEC cannot. While sorbitol-fermenting O157 STEC have been isolated from patients in central Europe, they are still very rare in the U.S. (6). Therefore, primary media for isolation of O157 STEC should include MacConkey agar with sorbitol (SMAC). More selective media include a variety of chromogenic agars and SMAC supplemented with cefixime and tellurite (CT-SMAC). It should be noted, however, that most non-O157 STEC serotypes are sorbitol positive and will not be detected if SMAC or CT-SMAC is the only method of STEC screening from stool cultures (2). The most frequently reported primary media used by the 78 participating laboratories for stool cultures are summarized in **Figure 2**.

**Biochemical Identification:** In the U.S., virtually all O157 STEC lack  $\beta$ -glucuronidase activity and will be negative by the MUG test. Commercial identification systems can readily identify isolates as suspected O157 STEC based on a combination of  $\beta$ -glucuronidase and sorbitol reactions; however, most will recommend confirmation by serological methods (6). Of the 78 participating laboratories, 56 (72%) reported using a commercial or automated system for identification of this isolate. Vitek users accounted for 28 (50%) of these laboratories, while 24 laboratories (43%) reported using MicroScan platforms. Two laboratories (4%) reported using API panels for identification, one laboratory (2%) reported using the Phoenix system, and one laboratory (2%) reported using the Trek Sensititre system. There were distinct reporting differences among the systems used for identification. The one Phoenix user, and 11 of the 24 MicroScan users (46%), identified this isolate as a possible O157. In contrast, only one of the 28 Vitek users (4%) reported possible O157.

**Serotyping:** Most STEC antisera and latex agglutination reagents commercially available to clinical laboratories are limited to the detection of the O157 or O157:H7 serotype, since this is the serotype most commonly associated with severe human disease. However, negative serological tests for O157 do not necessarily exclude other non-O157 STEC. Screening by latex agglutination or O157-specific antiserum should be performed on colorless (non-fermenting) colonies on SMAC. Since other organisms can cross-react with O157 antiserum and latex agglutination reagents, biochemical identification is necessary to confirm presumptive O157 STEC isolates. In order to detect possible non-O157 STEC, any suspicious colonies on SMAC that are negative by latex agglutination or O157-specific antisera should also be screened for production of Shiga toxins (6). Among the 78 participating laboratories that performed stool cultures, 22 (28%) also performed serotyping on this isolate; five laboratories correctly reported negative serotyping results. None of the remaining 17 laboratories reported their serotyping results, although two of these laboratories went on to report possible O157 or O157:H7.



**Antimicrobial Susceptibility Testing:** Prompt treatment of suspected or confirmed O157 STEC infections with parenteral volume expansion can reduce the risk of kidney damage due to HUS in uncomplicated cases; however, antibiotic treatment can increase the risk of HUS (3). Antibiotics like ciprofloxacin and trimethoprim-sulfamethoxazole (SXT) that disrupt DNA synthesis can trigger both phage replication and toxin production in O157. Treatment with antimicrobials that interfere with DNA synthesis can preferentially trigger the replication of the bacteriophage encoding Stx2 (the toxin most associated with HUS), thus potentially leading to a dramatic increase in toxin production when such drugs are used for treatment (4). Therefore, routine antimicrobial susceptibility testing (AST) of this isolate would not be indicated. However, 14 of the 78 participating laboratories (18%) reported that AST would be routinely performed on this isolate, regardless of reported identification.

**Reportable Disease Rule:** Rapid and accurate diagnosis by clinical laboratories is crucial for the detection of outbreaks, timely public health interventions, and detection of emerging non-O157 STEC strains. In North Dakota, all suspected or confirmed cases of STEC infection, regardless of serotype, must be reported to NDDoH; Division of Disease Control within seven days of identification due to the significant clinical and epidemiologic impact of STEC disease. Whenever there is clinical suspicion of HUS the NDDoH; Division of Disease Control should be notified immediately by telephone: 1.800.472.2180. In addition to case reporting, culture isolates or positive toxin screening broths from all cases must be sent to the North Dakota public health lab (NDDoH; Division of Laboratory Services) for complete serotyping and molecular analysis by PFGE. Specimens or enrichment broths in which Shiga toxin or STEC is detected but from which O157 STEC cannot be cultured should be sent to the North Dakota public health lab (NDDoH; Division of Laboratory Services) for further testing. Isolates that are negative for Shiga toxin production by direct methods (as was the case with this organism) are not required to be referred to the North Dakota public health lab. However, if there is clinical suspicion of HUS and all testing for STEC is negative, then the specimen should be referred to North Dakota public health lab for additional testing using methods not routinely available to clinical

microbiology laboratories. Among the 78 participating laboratories, 21 (27%) reported that this isolate would be referred to the appropriate state public health lab. Of these 21 laboratories, nine (43%) reported negative results for direct Shiga toxin testing. For more information regarding the North Dakota Communicable Disease Reporting, please visit:  
<http://www.health.state.nd.us/Disease/Documents/ReportableConditions.pdf>

### **Specimen #3, Tabulated Results**

78/119 (66%) Laboratories performed stool cultures for pathogenic enteric bacteria

#### **ORGANISM IDENTIFICATION**

##### **Identification:**

**Intended response (20/78, 26%):** No *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* O157 isolated

**Acceptable responses (31/78, 40%):**

- *Escherichia coli*, sorbitol negative with referral to the appropriate state public health lab or another reference laboratory, if direct testing for STEC was positive or not performed (9/78, 12%)\*
- Possible *Escherichia coli* O157:H7 with referral to the appropriate state public health lab, if direct testing for STEC was not performed (6/78, 8%)
- No enteric pathogens (6/78, 8%)
- Normal flora, no pathogens (4/78, 5%)
- No *Salmonella*, *Shigella*, *Campylobacter* isolated, if *E. coli* O157 is not part of the routine stool culture (2/78, 3%)
- Possible *Escherichia coli* O157 with referral to the appropriate state public health lab, if direct testing for STEC was not performed (2/78, 3%)
- Gram-negative bacilli, NOS with referral to the appropriate state public health lab or another reference laboratory, if direct testing for STEC was not performed (2/78, 3%)

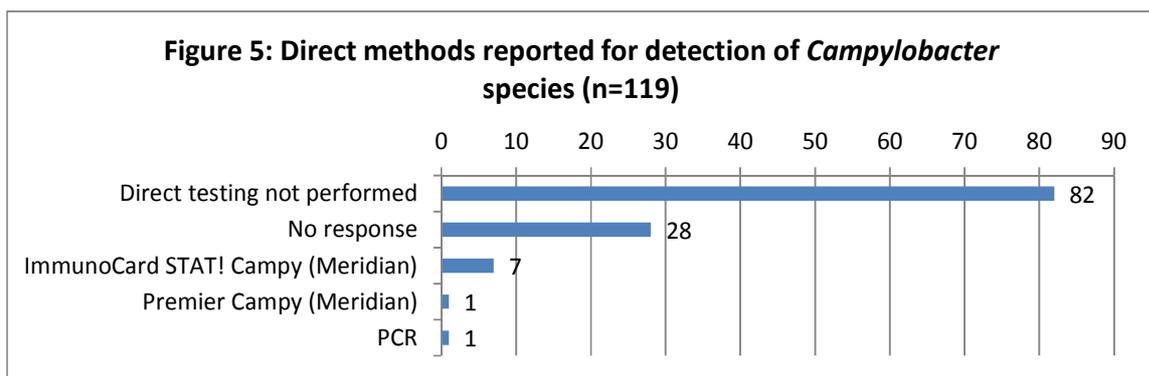
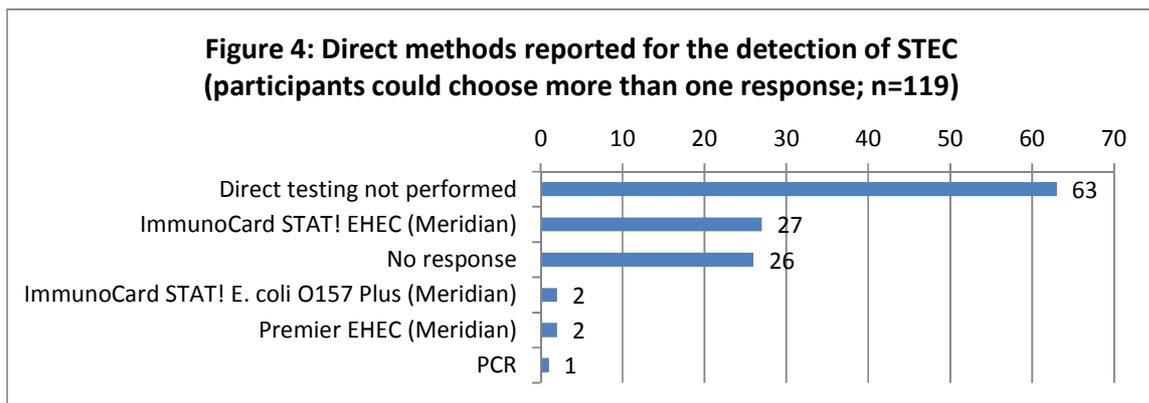
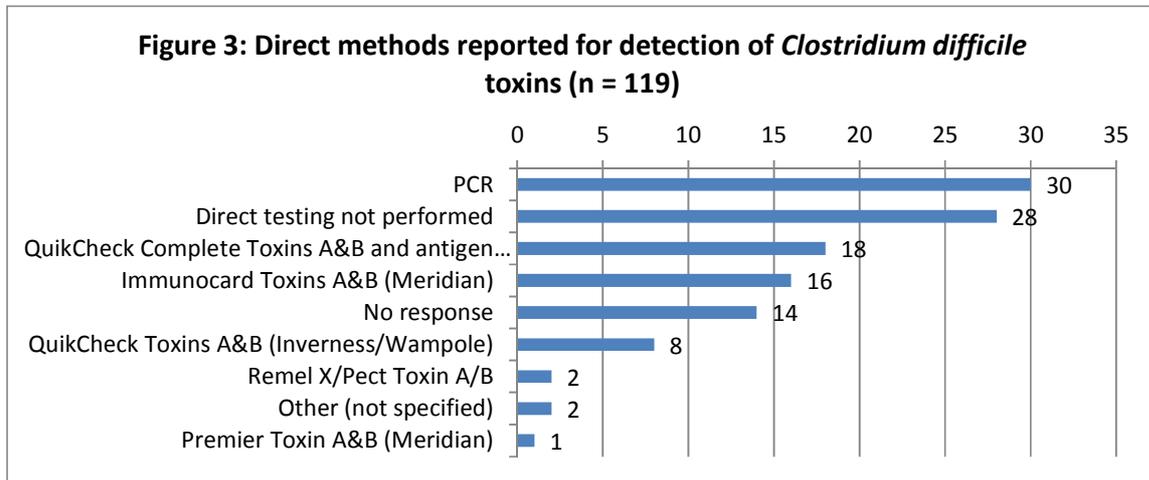
\* It is not necessary to refer isolates that are negative for toxin production to the North Dakota public health lab unless there is clinical suspicion of STEC infection or HUS.

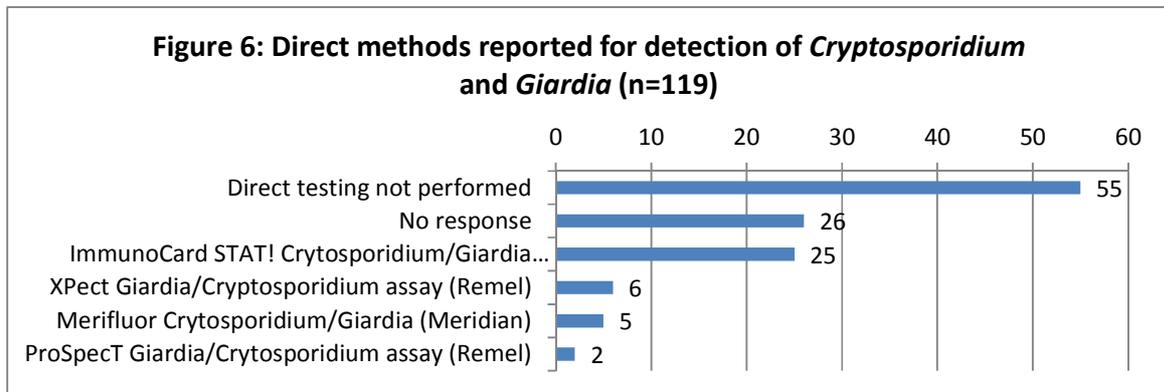
**Unacceptable Answers (27/78 = 35%):**

- *Escherichia coli*, without serotype or sorbitol reaction (16/78, 21%)
- *Escherichia coli*, sorbitol negative without referral to the appropriate state public health lab or another reference laboratory (3/78, 4%)
- Possible *Escherichia coli* O157 without referral to the appropriate state public health lab, or if results were not reported for direct testing for STEC (3/78, 4%)
- Possible *Escherichia coli* O157:H7 if direct testing for STEC was negative, or if results were not entered (2/78, 3%)
- *Vibrio vulnificus* (2/78, 3%)\*\*
- No *Salmonella*, *Shigella*, *Campylobacter* isolated, if *E. coli* O157 is included in the routine stool culture (1/78, 1%)

\*\*Reports of *Vibrio vulnificus* were confirmed with both reporting laboratories, and were most likely the result of inadvertent duplication of Challenge Set Specimen #2 sent from MDH-PHL or duplicate set-ups in the receiving laboratory.

**Figures 3-6:** These figures summarize the specific testing methods used by participating laboratories for the direct detection of certain enteric pathogens from stool samples. This information is utilized by the department of health to monitor trends in the use of these methods and their potential effects on the detection and reporting of certain enteric pathogens.





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### **Specimen #4, Lung Abscess Aspirate: *Burkholderia thailandensis***

**Goals and Objectives:** The organism in Specimen 4 was *Burkholderia thailandensis*, which is a surrogate for the Category B bioterrorism agent *Burkholderia pseudomallei*. The goal was to emphasize the significance of *B. pseudomallei* as a very rarely isolated organism and potential bioterrorism agent that causes potentially fatal illness. The objective was to assess the ability of NDLRN laboratories to utilize the LRN Sentinel guidelines to rule-out and refer potential *Burkholderia mallei* and *B. pseudomallei* isolates to the North Dakota public health laboratory (NDDoH; Division of Laboratory Services).

**Clinical Significance of *Burkholderia* species:** Among the numerous *Burkholderia* species, only the *B. cepacia* complex, *B. mallei*, and *B. pseudomallei* are considered to be potential human pathogens. Members of the *B. cepacia* complex are significant pathogens in cystic fibrosis patients and cause chronic respiratory tract infection or acute pulmonary deterioration and sepsis (6). *B. mallei* and *B. pseudomallei* can infect animals as well as humans, and are the causative agents of glanders and melioidosis, respectively.

Glanders normally affects equine animals such as horses, mules, and donkeys. Although glanders has been eradicated from most of the world, persistent foci of epizootic infections still exist in the Middle East, Asia, Africa, and South America (7). Infection is caused by inhalation, cutaneous inoculation from contact with open wounds or mucous membranes, or ingestion of contaminated food or water. In cases of human infection, glanders may present as a cutaneous or systemic disease that may be characterized by pneumonia and sepsis. Infections may be acute or chronic, with symptoms depending on the route of infection. Involvement of the lymphatic system is more common in glanders than in melioidosis, with lymphadenopathy and suppurative abscesses in some cases. The case fatality rate for glanders in humans is near 95% if untreated (1).

Humans and animals become infected with *B. pseudomallei* by percutaneous inoculation or inhalation, and person-to-person spread is very uncommon. While the majority of *B. pseudomallei* infections remain asymptomatic, melioidosis may present with diverse symptoms including localized skin ulcers or abscesses, chronic pneumonia mimicking tuberculosis, or fulminant septic shock. Half of all melioidosis cases in endemic areas present as pneumonia with accompanying fatal septicemia, or mimicking other community acquired pneumonias or tuberculosis (6, 7). Although most melioidosis cases are acute due to recent infection, cases of latent infection with subsequent reactivation are well documented, with some cases reactivating several decades after exposure. This phenomenon, which has been called the "Vietnamese time bomb," has been documented in veterans of the Vietnam War. The fatality rate for melioidosis varies from 15-75%, depending on risk factors, mode of infection, and available treatments. Inhalation cases and those with bacteremia are associated with higher mortality (6). A recently-discovered *B. pseudomallei* toxin, BPSL1549, which is similar to the *E. coli* cytotoxic necrotizing factor 1, inhibits DNA helicase and protein synthesis activities in host cells. Toxin expression is variable among the *B. pseudomallei* strains that have been studied, which may partially account for the range of reported clinical syndromes and fatality rates (4).

**Incidence and Risk Factors:** Members of the genus *Burkholderia* are typically found as environmental organisms in soil, water, and on plants, including fruits and vegetables (6). *B. pseudomallei* is found in the soil of rice-growing regions of southeast Asia and northern Australia, with infections occurring in a seasonal pattern that coincides with the monsoon wet season. *B. thailandensis* is a non-pathogenic close relative of *B. pseudomallei* that is readily isolated from soil samples in the southern and central regions of Thailand. Physiologic risk factors associated with increased mortality from melioidosis include diabetes, alcohol abuse, chronic renal disease, and chronic lung disease (6). The only case of human *B. mallei* infection in the U.S. in the last 50 years was a recent laboratory-acquired infection in Maryland (1, 6).

Fewer than five cases of human *B. pseudomallei* infection are reported to the CDC each year; two laboratory-acquired cases were recently reported in Florida (2, 9).

**Treatment:** Treatment of *B. mallei* and *B. pseudomallei* infections requires intravenous ceftazidime or carbapenem therapy for 10 days followed by oral eradication therapy using trimethoprim-sulfamethoxazole with or without the addition of doxycycline for 20-24 weeks. Relapse occurs in up to 10% of cases, even following the full treatment regimen (6).

**Burkholderia and Bioterrorism:** Several countries have developed *B. mallei* and *B. pseudomallei* as potential biological weapons, which has led to their characterization by CDC as Category B threat agents (8). *B. mallei* was used by the German army in World War I as a biological weapon against horses and pack animals that were used extensively in the war effort (3). Since both *B. mallei* and *B. pseudomallei* are not found naturally in the U.S., isolation of either of these organisms from a patient without documented travel history to an endemic area should raise suspicion of a possible bioterrorism threat and should be reported immediately to MDH.

### Laboratory Identification of *Burkholderia mallei* and *Burkholderia pseudomallei*

**Collection and Transport:** *Burkholderia* species may be recovered from a wide range of clinical specimens, including urine, blood, sputum, BAL, wound swabs, or abscess aspirates. Clinical specimens submitted for testing can be packaged and shipped as Biological Substance, Category B (UN3373); however, suspected or confirmed cultures of *B. mallei* and *B. pseudomallei* are classified as Infectious Substance, Category A (UN2814) for the purposes of packaging and shipping by the U.S. Department of Transportation (10). The NDDoH public health laboratory provides a free on line packaging and shipping training course. For more information and directions on how to access the course contact Jan Trythall at: [jtrythal@nd.gov](mailto:jtrythal@nd.gov) or call 701-328-6295.

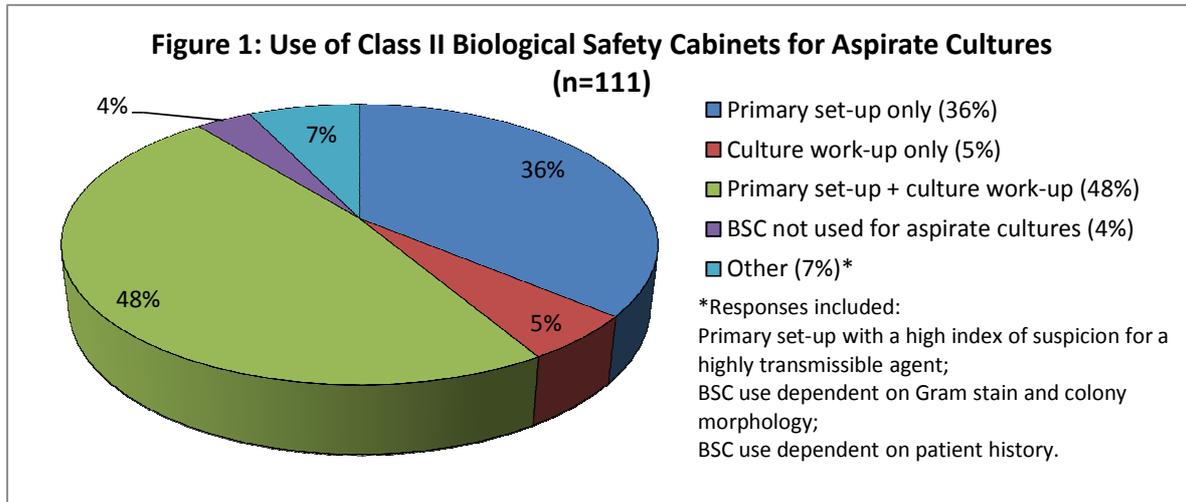
Both *B. pseudomallei* and *B. mallei* are Select Agents per 42 CFR part 1003, "Possession, Use and Transfer of Select Agents and Toxins; Final Rule." This rule states that isolates must be referred to a Select Agent registered laboratory (i.e. NDDoH; Division of Laboratory Services) or destroyed within 7 days of identification (5).

**Laboratory Safety:** Most clinical specimens can be safely handled using BSL-2 practices, containment equipment, and facilities, which should include the use of a Class II biological safety cabinet (BSC) for specimens with a high index of suspicion (8). BSL3 practices, containment equipment, and facilities are recommended for any procedures that may generate infectious aerosols (e.g. pipetting, vortexing, or centrifuging) and any manipulation of cultures suspected or known to contain *B. mallei* or *B. pseudomallei* (8). Sampling of positive blood culture bottles and other routine characterization procedures such as preparing Gram stains, preparing bacterial suspensions, and performing subcultures and biochemical tests, especially catalase testing, can generate dangerous aerosols. Therefore, all manipulation of positive blood cultures should be performed in a BSC (8). **Figure 1** summarizes how participating laboratories reported using a BSC for this specimen.

**Microscopic morphology:** *B. mallei* and *B. pseudomallei* appear as small, Gram-negative bacilli and are not easily differentiated from other similar organisms on the basis of Gram stain alone. However, *B. pseudomallei* may exhibit bipolar staining (7).

**Isolation Methods:** *Burkholderia* will grow on a variety of commercially prepared culture media, including sheep blood agar and chocolate agar. Certain strains of *B. mallei* may or may not grow on MacConkey agar, while *B. pseudomallei* will typically grow well on MacConkey agar in 24 hours. Colonies of *B. pseudomallei* will initially be smooth but may become dry and wrinkled with extended incubation (48-72 hours). Neither organism is hemolytic on sheep blood agar. Both *B. mallei* and *B.*

*pseudomallei* grow readily in broth-based blood culture systems within the standard incubation period, so specialized techniques and extended incubation conditions are not necessary (7).

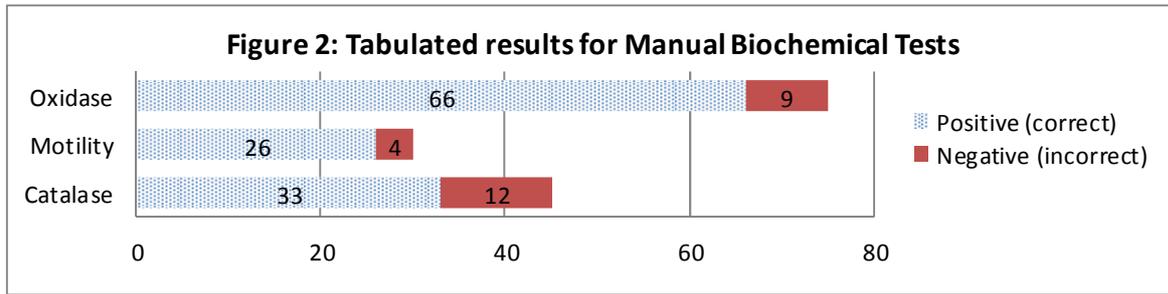


**Biochemical Identification:** As seen in **Table 1**, *B. thailandensis* has a biochemical profile nearly identical to that of *B. pseudomallei*, with the exception of L-arabinose assimilation (*B. thailandensis* will be positive). The Laboratory Response Network (LRN) algorithm for ruling out *B. mallei* and *B. pseudomallei* includes Gram stain morphology, indole, catalase, motility, and oxidase testing. Laboratories may also use polymixin B or colistin disk testing as part of the algorithm (both species will be resistant to either antimicrobial agent). The results of these manual biochemical tests are summarized in **Figure 2**. If your laboratory is unable to rule out *B. mallei* or *B. pseudomallei* using the methods outlined in the NDLRN Bench Guide for Bioterrorism Agents, then the isolate must be referred to North Dakota public health lab (NDDoH; Division of Laboratory Services) for confirmatory testing. The Guidelines for LRN Advanced Sentinel Laboratories can also be found on the ASM website at [www.asm.org](http://www.asm.org)

**Table 1: Common Biochemical Reactions for Differentiation of *Burkholderia* species (7).**

Biochemical test	<i>B. mallei</i>	<i>B. pseudomallei</i>	<i>B. thailandensis</i>
Gram stain*	Gram-negative coccobacilli	Gram-negative rod	Gram-negative rod
Growth on MAC*	Variable	Yes	Yes
Catalase*	Positive	Positive	Positive
Spot indole*	Negative	Negative	Negative
Motility*	Non-motile	Motile	Motile
Oxidase*	Variable	Positive	Positive
Colistin/polymixin B*	Resistant/variable	Resistant/resistant	Resistant/resistant
Arginine dihydrolase	Positive	Positive	Positive
Gas from Nitrate	No gas	Gas	Gas
Growth @ 42°C	No	Yes	Yes
TSI	K/K	Variable/K	Variable/K
L-arabinose assimilation	NA	Negative	Positive

\*LRN Sentinel test



**Table 2** summarizes the commercial platforms used and the corresponding results reported. Of the 111 participating laboratories, 88% (98/111) reported using at least one commercial system for identification of this isolate. It is important to note that only one sentinel laboratory was able to correctly identify the organism as *B. thailandensis* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Identifications of possible or confirmed *B. pseudomallei* were reported by 52% (25/48) of MicroScan users and 65% (28/43) of Vitek users. Although the goal of any clinical microbiology laboratory is the accurate and timely diagnosis of infectious diseases for the purposes of effective patient treatment, it is also important to consider the potential public health impact of certain agents. While some laboratories may have the capability of presumptively or definitively identifying *B. mallei* or *B. pseudomallei*, these organisms must still be referred to the nearest LRN Reference laboratory (i.e. North Dakota public health lab) for additional confirmatory testing and to ensure that a public health investigation can begin promptly. It should be noted in this case that while *B. thailandensis* is biochemically similar to *B. pseudomallei*, the organisms could not be differentiated using the LRN Sentinel algorithm or the commercial platforms currently in use (see Table 2). Therefore, it is important to consider identifications of *B. mallei* or *B. pseudomallei* as presumptive until confirmatory testing can be performed by an LRN Reference laboratory, especially since these organisms have the potential to be used as biological weapons (7).

**Table 2: Reported Identification for Laboratories using Automated or Commercial Systems (n=98).**

Reported Identification	MicroScan (all models)	Vitek (all models)	Phoenix	Other
Burkholderia sp	3	8	1	--
Burkholderia cepacia	4	4	1	1
Possible Burkholderia mallei	--	1	--	--
Burkholderia pseudomallei	8	17	--	--
Possible Burkholderia pseudomallei	17	11	--	1
Burkholderia thailandensis	--	--	--	2
Chromobacter violaceum	2	--	--	--
Gram-negative bacilli, NOS	4	--	--	--
Gram-negative bacilli, oxidase negative	2	--	--	1
Gram-negative bacilli, oxidase positive	6	2	--	--
Gram-positive bacilli, NOS	1	--	--	--
Pseudomonas fluorescens/putida	--	--	--	1
Vibrio species	1	--	--	--
<b>Total</b>	<b>48</b>	<b>43</b>	<b>2</b>	<b>5</b>

\*Results reflect the identification reported, not necessarily the identification obtained by the commercial system used.

\*\*Other systems included API 20NE, BBL OXI/FERM Tube II, and MALDI-TOF.

**Reportable Disease Rule:** In North Dakota, all cases of suspected or confirmed glanders or melioidosis must be reported to the NDDoH; Division of Disease Control immediately by telephone due to the significant public health threat that these infections potentially represent. If your laboratory is unable to rule out *B. mallei* or *B. pseudomallei* in any specimen as outlined in the guidelines for LRN Advanced Sentinel Laboratories, **the isolate must be referred to the LRN Reference Laboratory (North Dakota public health lab) and not to your regular reference laboratory.** The NDDoH public health lab has rapid and conventional LRN methods for confirming *B. pseudomallei* and *B. mallei* that regular commercial laboratories may not have. Referral to a commercial reference laboratory may delay confirmation and put additional laboratory staff at risk for exposure. Among the 111 participating laboratories, 74 (67%) reported that this isolate would be referred to the North Dakota public health lab or that they would contact the ND public health lab for further instructions, regardless of the reported identification. For more information regarding the North Dakota Communicable Disease Reporting Rule, please visit: <http://www.health.state.nd.us/Disease/Documents/ReportableConditions.pdf>

## **Specimen #4, Tabulated Results**

111/119 (93%) Laboratories perform aspirate cultures

### **ORGANISM IDENTIFICATION**

**Intended response (37/111, 33%):** *Burkholderia thailandensis*, or possible *Burkholderia pseudomallei* with referral to the appropriate state-PHL

#### **Acceptable responses (42/111, 38%)**

- *Burkholderia pseudomallei* with referral to the appropriate state-PHL (19/111, 17%)\*
- *Burkholderia* species with referral to the appropriate state-PHL (9/111, 8%)
- Gram-negative bacilli, oxidase positive, with referral to the appropriate state-PHL or other reference laboratory (8/111, 7%)
- Gram-negative bacilli, NOS, with referral to the appropriate state-PHL or other reference laboratory (5/111, 5%)
- *Burkholderia* species with referral to another reference laboratory (1/111, 1%)

\*Although this identification is technically incorrect, it was considered acceptable in combination with referral to the appropriate state-PHL since the commercial systems and LRN protocols used were unable to differentiate *Burkholderia thailandensis* from *B. pseudomallei*. Any such identification from a commercial system should be reported as “possible” until confirmed by the appropriate state-PHL.

#### **Unacceptable responses (32/111, 29%)**

- *Burkholderia cepacia* (10/111, 9%)
- *Burkholderia pseudomallei* without referral to the appropriate state-PHL (6/111, 5%)
- Possible *Burkholderia mallei* with referral to the appropriate state-PHL (3/111, 3%)\*\*
- Gram-negative bacilli, oxidase negative (3/111, 3%)
- *Burkholderia* species without referral to the appropriate state-PHL or other reference laboratory (2/111, 2%)
- *Chromobacter violaceum* (2/111, 2%)
- Possible *Bacillus anthracis* (1/111, 1%)
- Gram-negative bacilli, NOS, without referral to the appropriate state-PHL or other reference laboratory (1/111, 1%)
- Gram-positive bacilli, NOS (1/111, 1%)
- Gram-positive spore-forming bacillus, NOS (1/111, 1%)
- *Pseudomonas fluorescens/putida* (1/111, 1%)
- *Vibrio* species (1/111, 1%)

\*\*Although these laboratories would correctly refer the isolate to MDH-PHL, *B. mallei* could be ruled out by motility (*B. mallei* is negative).

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## NDLRN/MLS: Challenge Set 2011 Self-Study Quiz

### **Specimen #1** – *Bordetella holmesii*

- \_\_\_\_\_ is the genetic element common to *Bordetella pertussis*, *B. parapertussis*, and *B. holmesii* that is used as the target for some molecular assays, leading to misidentification of *B. holmesii* in some samples.
  - PBP2a
  - IS481
  - tcdD*
  - mecA*
- Which of the following biochemical reactions is characteristic of *Bordetella holmesii*?
  - Catalase positive
  - Arginine positive
  - Oxidase positive
  - Indole positive
- Bordetella holmesii* infections typically occur in patients with which underlying health condition:
  - IV drug use
  - Cystic fibrosis
  - Functional or anatomical asplenic
  - Phenylketonuria
- True False** *Bordetella holmesii* requires specialized culture media such as Regan-Lowe for growth.
- True False** *Bordetella holmesii* has biochemical and morphological similarities to the potential bioterrorism agents *Yersinia pestis* and *Francisella tularensis*.

### **Specimen #2** – *Vibrio vulnificus*

- All of the following syndromes may be associated with *Vibrio vulnificus* infection, EXCEPT:
  - Pneumonia
  - Gastroenteritis
  - Severe septicemia
  - Sever wound infection
- Consumption of which food item accounts for greater than 95% of all *V. vulnificus* infections in the U.S.?
  - Raw or undercooked shell eggs
  - Contaminated ground beef
  - Raw oysters from the Gulf Coast
  - Contaminated fresh fruit and vegetables
- Which of the following biochemical reactions is typical of *V. vulnificus*?
  - Oxidase negative
  - Lactose positive
  - VP positive
  - Lysine decarboxylase negative

4. Which culture medium can be most useful in screening for *V. vulnificus*?
  - a. CT-SMAC
  - b. Mueller-Hinton supplemented with X & V factors
  - c. Hektoen Enteric agar
  - d. TCBS
5. **True False** Since cases of *V. vulnificus* infection are typically not life-threatening, they are not reportable to NDDoH under the Disease Reporting Rule.

### **Specimen #3 – *Escherichia coli*, sorbitol negative**

1. All of the following statements regarding *E. coli* O157:H7 are true, **except** (choose one):
  - a. O157 is the most common STEC serotype in the U.S. and accounts for an estimated 73,000 STEC infections each year.
  - b. Antibiotics that target DNA replication can trigger toxin production in O157.
  - c. O157 can be differentiated from other *E. coli* serotypes by its lactose reaction on MacConkey agar.
  - d. O157 is a reportable disease under the NDDoH Communicable Disease Reporting Rule.
2. Which of the following is characteristic of *E. coli* O157:H7?
  - a. Sorbitol negative
  - b. Sorbitol positive
  - c. Oxidase positive
  - d. MUG positive
3. Which of the following tests is performed at NDDoH; Division of Laboratory Services to aid in epidemiologic investigations of O157 outbreaks? (choose one):
  - a. Voges-Proskauer test (VP)
  - b. Pulsed-field gel electrophoresis (PFGE)
  - c. High-pressure liquid chromatography (HPLC)
  - d. DNA hybridization probes
4. **True False** Stool culture combined with a toxin screening method is more sensitive than either method alone for the detection of STEC infections (including O157).
5. **True False** O157 is the only *E. coli* serotype capable of causing hemolytic uremic syndrome (HUS).

### **Specimen #4 – *Burkholderia thailandensis* (surrogate for *B. pseudomallei*)**

1. *Burkholderia pseudomallei* is endemic in which part of the world?
  - a. Northern Australia and Southeast Asia
  - b. Southwest United States
  - c. Ohio and Mississippi River valleys
  - d. Pacific Northwest
2. Which of the following LRN Sentinel Lab test results can be used to rule out *B. pseudomallei*? (choose one)
  - a. Arginine positive
  - b. Motility positive
  - c. Catalase positive
  - d. Indole positive

3. *B. pseudomallei* illness is known by what nickname?
  - a. Undulating fever
  - b. Rabbit fever
  - c. Vietnam timebomb
  - d. Woolsorter's disease
  
4. **True** **False** Laboratories that cannot rule out *B. pseudomallei* should send isolates to their routine reference laboratory for identification before contacting the NDDoH; Division of Laboratory Services.
  
5. **True** **False** It is not necessary to confirm an identification of *B. pseudomallei* from a commercial identification system before issuing a final report to the treatment provider.



## NDLRN/MLS: Challenge Set 2011 Self-Study Quiz

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  - IS481**
  - tcdD*
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5. **True** **False** It is not necessary to confirm an identification of *B. pseudomallei* from a commercial identification system before issuing a final report to the treatment provider.