CHALLENGE SET 2010

PARTICIPANT SUMMARY REPORT

January 2011

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

Specimen #1, Stool Culture: *E. coli* O157:H7 (STEC)

	Your Result	Intended Result
Identification	-No Specimen Shipped-	*E. coli* O157:H7
Referral of Isolate		Isolate should be referred to MDH-PHL
Sorbitol		Negative
Direct detection of STEC O157 and/or Shiga toxin		Positive for STEC O157 and/or Shiga toxin
Serotyping for STEC O157 or O157:H7		Positive for O157 or O157:H7

Goals and Objectives: The organism in Specimen 1 was a Shiga toxin-producing strain of *E. coli* O157:H7. Due to shipping costs this isolate was not included in the challenge set sent to North Dakota labs, however, a narrative has been included for your review. This organism was chosen with the goal of demonstrating the continuing need for diligent detection, reporting, and referral of this stool pathogen to your state PHL in the context of new CDC guidelines for the diagnosis of STEC infections by clinical laboratories. The objectives were to assess the ability of laboratories to detect, report, and refer this significant enteric pathogen; to illustrate the potential patient treatment and public health benefits of the new 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 (6). However, certain strains may cause significant intestinal and extraintestinal disease, including urinary tract infections, bacteremia, and meningitis. 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. Fresh produce can also become contaminated through runoff from cattle farms or contaminated irrigation sources. Agricultural workers or those with direct contact with animals or animal environments may also be at risk for occupational exposure to O157. The disease can also be easily spread from person to person in settings such as day care centers, schools, and long-term care facilities, due in part to the relatively low infectious dose of O157 (<200 CFU) (6). Over the past several years, there have been numerous outbreaks in Minnesota caused by *E. coli* O157. In 2009, a multistate O157 outbreak involving raw cookie dough affected 80 people in 31 states, eight of which were in Minnesota (2). More recently, MDH identified an outbreak associated with consumption of unpasteurized (raw) milk and dairy products, resulting in eight O157 infections, including one case of hemolytic uremic syndrome (HUS) (MDH, unpublished data)

About STEC and *E. coli* O157:H7: 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). In 2009, six culture-confirmed cases of O157 STEC infection and eight culture-confirmed cases of non-O157 STEC infection were reported to the NDDoH. The non-O157 isolates included (Five) O26:H11, (Two) O111: non-motile, and (One) 0121:H19 serotypes.

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* (6). 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). Antibiotics like ciprofloxacin and trimethoprim-sulfamethoxazole (SXT) that interfere with DNA synthesis can also trigger both phage replication and toxin production in O157 (4).

While STEC, including O157, are most often associated with diarrheal illness, 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). 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).

Public Health Surveillance: The most reliable mechanism for detecting bacterial foodborne disease outbreaks, including STEC, is the submission of isolates by clinical microbiology laboratories to ND-PHL 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 ND-PHL. Additional testing performed at ND-PHL 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 patterns are also 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 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 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.

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). **Table 1** summarizes the use of biosafety cabinets among the participating laboratories that performed stool cultures. Thirty-nine (60%) of these laboratories reported using a biosafety cabinet (BSC) only for setting up cultures from primary stool specimens, while 15 (23%) reported using a BSC for both primary set-up and manipulation of isolates from growing cultures. Eight laboratories (12%) reported that a BSC is not used for any part of the stool culture process.

Table 1 – Use of Class II biological safety cabinets for handling stool culture specimens and isolates

Response	Frequency	Count
BSC is used only when setting up primary specimen	60.0%	39
BSC is used only when manipulating isolates from growing culture	3.1%	2
BSC is used for both primary set up and when manipulating isolates	24.6%	16
BSC is not used at all when working with stool cultures	12.3%	8
	Total Responses	**65**

Direct Testing for Shiga Toxins: In 2009, CDC issued new recommendations for diagnosis of STEC infections by clinical laboratories. The NDDoH 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. The recommendations include screening 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 STEC using a test that detects the Stx genes or the toxins they encode (3). While O157 and other STEC can be directly detected in fecal specimens using a variety of commercially available methods, it is recommended that Shiga toxin testing be performed on growth from broth culture or primary isolation media, since this method is more sensitive and specific than direct testing of stool (3, 6). In accordance with the CDC recommendations, ND-PHL 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). Specimens or enrichment broths in which Shiga toxin or STEC is detected but from which O157 STEC cannot be cultured should be sent to ND-PHL for further testing. A copy of the CDC guidelines for diagnosis of STEC infections by clinical laboratories can be accessed through the CDC website at http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5812a1.htm

The standard practice for use of non-culture/rapid diagnostic methods for detection of STEC by participating laboratories is summarized in **Table 2**. Among the 65 laboratories that performed stool cultures, 29 (45%) reported using at least one non-culture method for direct detection of STEC; 26 of those laboratories correctly reported positive results for both Stx1 and Stx2, or O157 STEC. Twenty-five (38%) of the 65 participating laboratories reported using non-culture/rapid diagnostic methods in combination with stool culture for the detection of STEC as part of a routine stool culture, which is the practice recommended by ND-PHL.

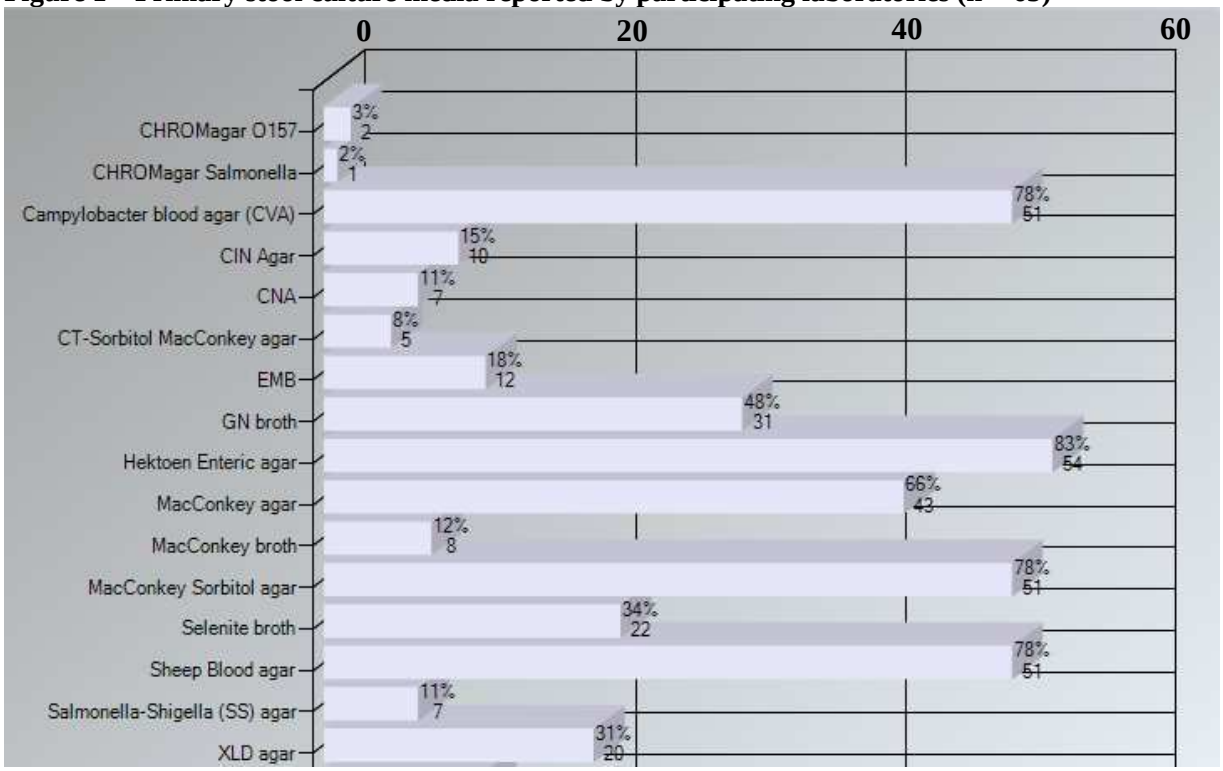
Table 2 – Standard practice for laboratories performing non-culture/rapid diagnostic methods for detection of STEC (participants could choose more than one response)

Response	Frequency	Count
Not applicable - non-culture/rapid diagnostic tests are not performed for STEC and/or Shiga toxin.	40.0%	26
Non-culture methods are performed in conjunction with routine stool culture set up.	38.5%	25
Non-culture methods are performed only by special request from the ordering physician.	15.4%	10
Non-culture methods are performed by our normal reference laboratory only.	10.8%	7
Stool culture confirmation is performed only if non-culture methods POSITIVE for STEC and/or Shiga toxin.	3.1%	2
Only non-culture methods are performed - stool culture confirmation is not performed.	1.5%	1
Other (specify):	1.5%	1
	Number of Labs Responding	**65**

Enrichment, Isolation, and Screening Methods: Culture for O157 and other STEC should be performed on patients with bloody diarrhea and/or HUS; however, since there are no culture-based methods for differentiating O157 from other STEC strains, screening for the presence of Shiga toxin is the best means of detecting STEC in fecal specimens. Some testing methods for the detection of Shiga toxin use a separate broth enrichment; however, if toxin testing is not part of the routine stool culture algorithm, the use of an additional broth enrichment has not been shown to significantly improve recovery of O157 from clinical specimens with low numbers of organisms (6).

O157 and other STEC are indistinguishable from commensal *E. coli* strains on MacConkey agar since virtually all STEC are able to ferment lactose. However, while roughly 80% of commensal *E. coli* can ferment sorbitol, a majority of O157 STEC cannot; 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). While sorbitol-fermenting O157 STEC have been isolated from patients in central Europe, they are still very rare in the U.S. (6). Primary culture and enrichment media used by participating laboratories for routine stool cultures are summarized in **Figure 1**. The most frequently reported primary media used by participating laboratories for stool cultures were Hektoen enteric agar, Campylobacter blood agar, MacConkey agar with sorbitol, sheep blood agar, and MacConkey agar.

Figure 1 – Primary stool culture media reported by participating laboratories (n = 65)



Biochemical Identification: STEC have biochemical reactions similar to other *E. coli*, with the exception of sorbitol fermentation (most STEC are negative). In addition, virtually all O157 STEC lack β-glucuronidase activity and will be negative by the MUG test. Commercial identification systems can readily identify isolates as suspected O157 STEC; however, most will recommend confirmation by serological methods (6).

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, serotypes of STEC other than H7 or O157 are also known to cause disease; therefore, negative serological tests for O157 do not necessarily exclude other 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 65 participating laboratories that performed stool cultures, 19 (29%) also performed serotyping on this isolate; all 19 laboratories that performed serotyping correctly reported the O157 or O157:H7 serotype.

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). 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, 13 of the 65

participating laboratories (20%) reported that AST would be routinely performed on this isolate in-house or by a reference laboratory.

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 the North Dakota Department of Health; Division of Disease Control. In addition to case reporting, culture isolates or positive toxin screening broths from all cases should be sent to ND-PHL for complete serotyping and molecular analysis by PFGE. Among participating laboratories, 58 (89%) reported that this isolate would be referred to MDH-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 #1, Tabulated Results

65/102 (64%) Laboratories performed stool cultures for pathogenic enteric bacteria

ORGANISM IDENTIFICATION

Identification: Intended answer = ***E. coli* O157:H7, with referral to MDH-PHL**
Acceptable Answers (58/65 = 89%): The following responses were considered acceptable when submitted individually with referral to MDH-PHL or in combination with a report of "No *Salmonella*, *Shigella*, or *Campylobacter* isolated" with referral to MDH-PHL.

- Possible *Escherichia coli* O157
- Possible *Escherichia coli* O157:H7
- *Escherichia coli* O157
- *Escherichia coli* O157:H7
- Shiga toxin-producing *Escherichia coli* (STEC)
- *Escherichia coli*, sorbitol negative

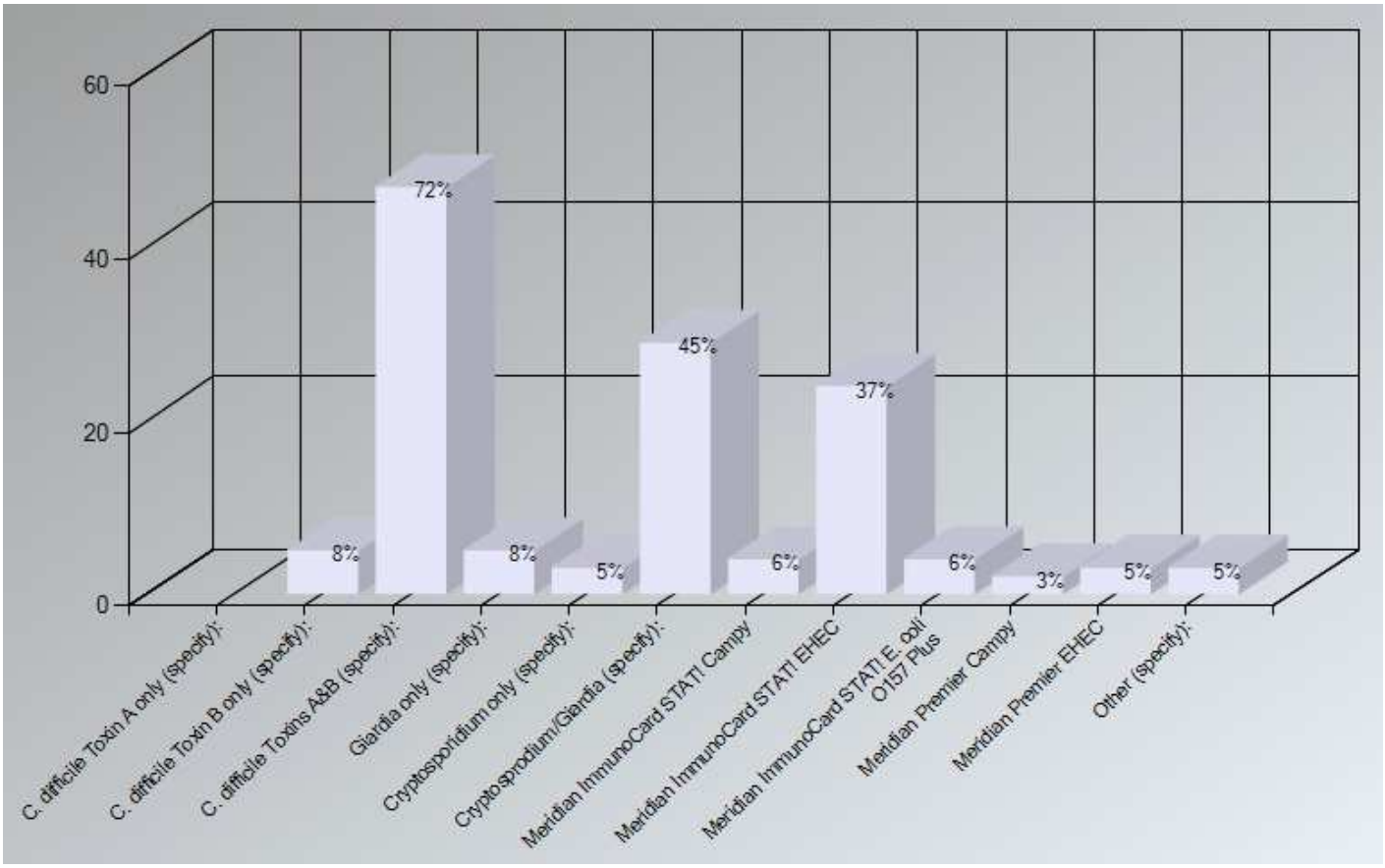
Unacceptable Answers (7/65 = 11%): The following responses, and any responses that did not indicate referral to MDH-PHL, were considered unacceptable.

- *Escherichia coli* (without serotype or sorbitol reaction)
- No *Salmonella*, *Shigella*, or *Campylobacter* isolated (when not reported in combination with any other identification).

USE OF NON-CULTURE METHODS

Figure 2 – Use of non-culture/rapid diagnostic methods for the detection of enteric pathogens (participants could choose more than one response)

Figure 2 summarizes data from laboratories reporting the use of non-culture/rapid diagnostic methods for the diagnosis of a variety of enteric pathogens, including *C. difficile*, *Giardia*, *Cryptosporidium*, *Campylobacter*, and EHEC (STEC). The use of these methods is monitored by MDH due to their potential effect on isolate submission and disease surveillance activities.



REFERENCES

1. The Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – 10 States, 2009. MMWR Morb Mortal Wkly Rep. 2010;59(14):418-422.
2. The Centers for Disease Control and Prevention [Internet]. Atlanta (GA): Multistate outbreak of *E. coli* O157:H7 infections linked to eating raw refrigerated, prepackaged cookie dough [modified 2009 Aug 7, accessed 2010 June 17]. Available from: http://www.cdc.gov/ecoli/2009/0807.html
3. Gould LH, Bopp C, Strockbine N, Atkinson R, Baselski V, Body B, Carey R, Crandall C, Hurd S, Kaplan R, Neill M, Shea S, Somsel P, Tobin-D'Angelo M, Griffin PM, Gerner-Smidt P. Recommendations for diagnosis of shiga toxin-producing *Escherichia coli* infections by clinical laboratories. MMWR Morb Mortal Wkly Rep. 2009;58(RR12):1-14.
4. McGannon CM, Fuller CA, Weiss AA. Different classes of antibiotics differentially influence Shiga toxin production. Antimicrob Agents Chemother. 2010 Jun 28 (e-publication ahead of print).
5. Minnesota Department of Health. Annual summary of communicable diseases reported to the Minnesota Department of Health, 2009. Disease Control Newsletter. 2010;38(1):1-32.
6. Nataro JP, Bopp CA, Fields PI, Kaper JB, and Strockbine NA. *Escherichia*, *Shigella*, and *Salmonella*. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, and Landry ML, editors. Manual of Clinical Microbiology. 9th ed. Vol 1. Washington, DC: ASM Press; 2007. p. 670-87.
7. The National Institutes of Health (US) and Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, D.C.: U.S. Government Printing Office; 2007.
8. U.S. Department of Transportation, Pipeline and Hazardous Materials Safety Administration. Transporting Infectious Substances Safely. Washington, D.C.: U.S. Government Printing Office; 2006.
9. U.S. Senate [Internet]. Washington, D.C.: Daily Digest – Tuesday, November 30, 2010 [modified 2010 Nov 30, accessed 2010 Dec 1]. Available from: http://thomas.loc.gov/cgi-bin/query/B?r111:@FIELD(FLD003+d)+@FIELD(DDATE+20101130)

Specimen #2, Body Fluid Culture: *Streptococcus* Group A (*S. pyogenes*)

Submitter: 2209	**Your Result**	**Intended Result**
Identification	Streptococcus, Group A (S. pyogenes)	*Streptococcus* Group A (*S. pyogenes*)
Referral of Isolate		Isolate should be referred to ND-PHL

Goals and Objectives: The organism in Specimen 2 was beta-hemolytic *Streptococcus* Group A (*S. pyogenes*). The goal of this challenge was to illustrate the potential for this organism, which is typically associated with acute exudative pharyngitis, to cause potentially fatal invasive disease. The objectives were to describe an unusual presentation of streptococcal disease, demonstrate the significant infection prevention challenges that can sometimes be associated with Group A *Streptococcus*, and assess the ability of North Dakota sentinel site laboratories to detect, report, and refer invasive isolates of Group A *Streptococcus* (GAS) to ND-PHL.

About the genus *Streptococcus*: Members of the genus *Streptococcus* are catalase-negative, facultatively anaerobic gram-positive cocci that tend to grow in chains in liquid media. Streptococci ferment glucose and several other carbohydrates to lactic acid (11). Various species express characteristic cell wall proteins, carbohydrates, and fatty acids that can be used for identification. One of the most useful characteristics of streptococcal isolates is hemolysis on sheep blood agar, since it can guide both empiric treatment of streptococcal infections and diagnostic testing (3).

About beta-hemolytic streptococci and GAS: Beta-hemolytic streptococci have historically been characterized by their unique carbohydrate surface antigens using the Lancefield system (11). Some species may belong to more than one Lancefield group, depending on the surface antigens expressed (3). *S. pyogenes* is the only significant human pathogen of the Lancefield Group A streptococci (GAS). Beta-hemolytic members of the various Lancefield groups represent a spectrum of human disease, ranging from asymptomatic carriage to acute focal infections and potentially life-threatening invasive disease (1, 10). GAS itself can cause a wide variety of infections, but it is the most common bacterial cause of acute pharyngitis (or “strep throat”), accounting for 15-30% of pediatric cases, and 5-10% of adult cases. Other infections caused by GAS include impetigo, cellulitis, and necrotizing fasciitis. Toxins produced by GAS can also cause scarlet fever, which is characterized by a diffuse, erythematous rash. In rare cases, scarlet fever may progress to streptococcal toxic shock syndrome (STSS) resulting from the massive cytokine response to the superantigen toxins (11). An estimated 11,500 cases of these invasive GAS infections occur each year in the U.S. according to the Centers for Disease Control and Prevention (CDC) (10). Respiratory droplets or direct contact with localized soft tissue infections are the most common routes of transmission.

One of the primary virulence factors of GAS is the surface M protein, which mimics human cell surface markers and allows the organism to evade phagocytosis. Cross-reactivity with the M protein can result in sequelae that include glomerulonephritis, acute rheumatic fever, and heart valve damage following pharyngitis or impetigo. More than 120 M protein subtypes of GAS have been identified, with M1 and M3 accounting for about half of invasive GAS disease (1, 10). Infection with one particular M subtype imparts immunity to subsequent infections of the same subtype, which is the basis for a 26-valent vaccine that has successfully completed phase 2 trials in adults (1, 9, 10). It is estimated that this proposed vaccine could prevent 49-63% of pediatric invasive GAS infections and 43-50% of invasive GAS infections in persons age 65 years and older. However, there is concern about potential cross-reactivity between the M types included in the vaccine and human tissue proteins that could trigger sequelae like acute rheumatic fever (10).

Reservoirs of invasive GAS isolates: Children with GAS pharyngitis have been shown to be a significant reservoir for M types with increased invasive potential (5). Post-operative and post-partum

outbreaks of GAS infection have also been linked to asymptomatic carriage in health-care workers (1). The patient history provided for this specimen was based on an actual case that was part of a nosocomial outbreak of invasive GAS infections involving three surgical patients in a California hospital in 1996. Two of the three patients died from GAS septicemia, shock, and cardiopulmonary arrest; the only healthcare worker who had contact with all three patients was the surgeon, who began taking a course of penicillin before adequate surveillance cultures could be collected. Although subsequent surveillance cultures were negative for GAS, the surgeon was restricted from patient care until a 10-day course of rifampin and penicillin was completed; no additional cases were reported following the course of treatment (1).

Nosocomial outbreaks of invasive GAS infection emphasize the importance of adherence to infection prevention strategies in healthcare settings, as well as prompt reporting of invasive GAS infections in order to identify any potential outbreaks. Good communication between infection prevention specialists and the microbiology laboratory can have a significant impact on the prevention, detection, and elimination of outbreaks associated with healthcare workers. Aggressive mitigation strategies, including surveillance cultures from healthcare workers with direct patient contact, can also be beneficial in identifying and treating potential sources of nosocomial infection.

Public Health Surveillance: In 2009 only 18 cases of invasive GAS were reported to the North Dakota Department of Health; Division of Disease Control with no associated reports of death. Clusters of invasive GAS infection are investigated by the North Dakota Department of Health; Division of Disease Control with further testing and banking of isolates performed by ND-PHL when submitted by clinical laboratories.

Emerging antimicrobial resistance in GAS and other beta-hemolytic streptococci: Penicillin is the drug of choice for treating most non-invasive GAS infections, as all strains identified are universally susceptible (11). Treatment of invasive infections, including toxic shock syndrome, often includes the addition of clindamycin as means of targeting protein synthesis to limit toxin production (6). Until recently, macrolides like erythromycin were historically used in patients allergic to penicillin since resistance in beta-hemolytic streptococci was uncommon in the U.S. However, the same mobile genetic elements responsible for the emergence of macrolide resistance (M phenotype) and inducible and constitutive clindamycin resistance (MLS_i and MLS_c phenotypes, respectively) in staphylococci and Group B *Streptococcus* have begun to emerge in GAS. Studies of pharyngeal GAS isolates have indicated that macrolide resistance patterns have increased in recent years and periodically exceed 10% of clinical isolates in some facilities (4). Among macrolide-resistant isolates, 27-43% may also have inducible resistance to clindamycin in some settings (4, 8). The M, MLS_i, and MLS_c phenotypes can be differentiated by the double-disk diffusion test (D-test) using erythromycin and clindamycin disks, as well as by PCR for specific resistance genes.

In February 2010, MDH-PHL identified four invasive GAS isolates submitted between September and December 2009 that exhibited the same unusual multidrug resistance pattern. All four of the isolates were resistant to erythromycin, clindamycin, and tetracycline, and had elevated MIC values for telithromycin. An investigation by the MDH IDEPC division determined that three of the cases were young men who have sex with men (MSM) and residents of the Twin Cities metropolitan area. Two of the three case patients were also methamphetamine users. Analysis by PFGE showed that two of the isolates had indistinguishable PFGE patterns, while a third differed by one band. The fourth isolate had a distinct PFGE pattern, and was not considered to be part of the cluster. Although laboratories, Infection Preventionists, and Infectious Disease doctors statewide were notified of the cluster and asked to look for additional cases, no additional cases were identified through enhanced surveillance (MDH, unpublished data).

Laboratory Identification of GAS

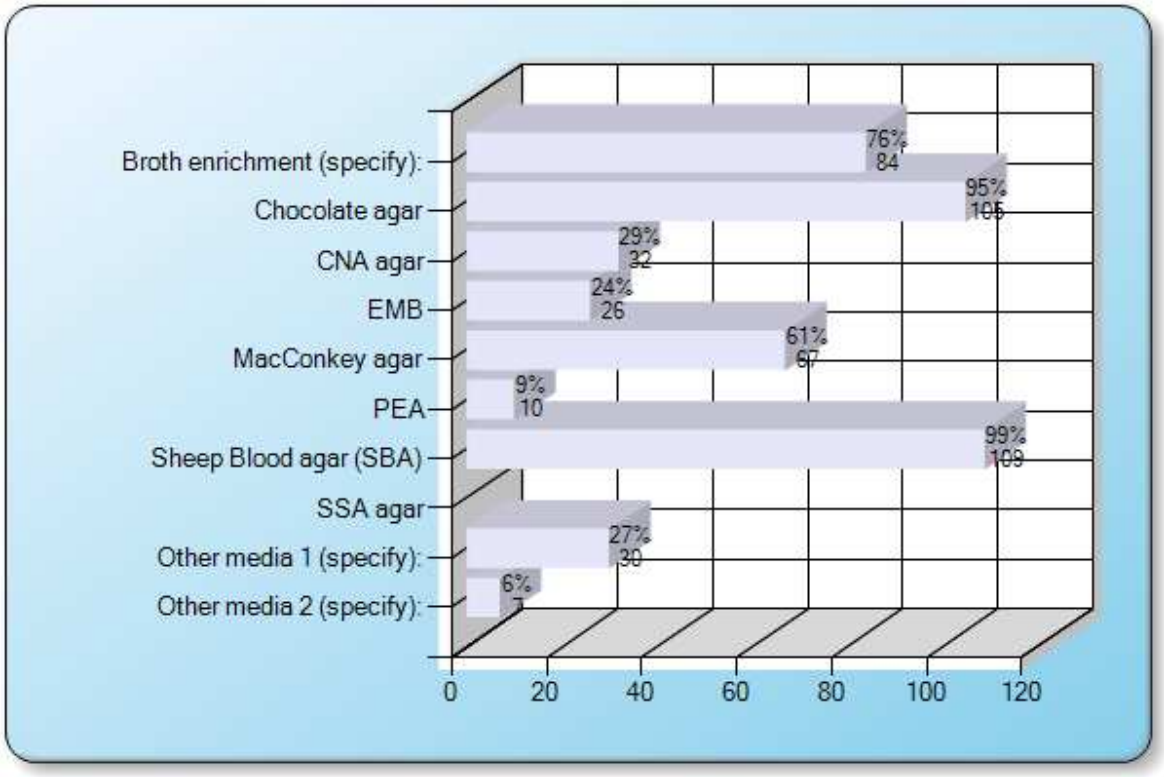
Collection and Transport: GAS can be isolated from virtually any clinical specimen, including throat and wound swabs, aspirates, respiratory specimens, body fluids, blood, and tissues. While moist transport medium is preferred for swab specimens, dry swabs are also acceptable (11).

Laboratory Safety: Clinical specimens and cultures suspected or known to contain GAS can be handled safely under BSL2 conditions. However, certain procedures that may produce infectious aerosols (such as sampling from blood culture bottles or handling large volumes of other body fluids) should be performed inside a Class II biological safety cabinet (9). Among participating laboratories, 46% (51/110) reported using a BSC for setting up cultures from primary pleural fluid specimens, while 39% (43/110) reported using a BSC for handling primary specimens and manipulating isolates from pleural fluid cultures. Thirteen laboratories (12%) reported that a BSC is not used for any part of the pleural fluid culture process.

Rapid Detection Methods: A wide variety of rapid methods have been developed for the direct detection of the Group A carbohydrate antigen in pharyngeal swabs, facilitating prompt initiation of antibiotic treatment for the prevention of sequelae and reduced risk of transmission. The sensitivity and specificity of rapid tests vary greatly among methods and manufacturers; therefore, throat culture remains the gold standard for detection of GAS, and culture backup is recommended by national advisory committees for the confirmation of negative results from these rapid tests. However, more recently developed molecular methods, particularly real-time PCR, have been shown to be more sensitive than throat culture, making culture backup unnecessary for negative PCR results (11).

Enrichment, Isolation, and Screening Methods: Primary culture and enrichment media used by participating laboratories for routine pleural fluid cultures are summarized in **Figure 1**. The most commonly used primary media reported by participating laboratories were sheep blood agar (109/110, 99%), chocolate agar (105/110, 95%), various broth enrichments (84/110, 76%), and MacConkey agar (67/110, 61%). Among the media reported in the “Other” category, a significant number of laboratories (23/110, 21%) also reported using various anaerobic or blood culture media for setting up primary cultures from pleural fluid specimens. Initial processing of sterile body fluids submitted for culture should include a concentrated Gram stain and inoculation of broth enrichment medium or blood culture bottles for recovery of low numbers of organisms (12). On sheep blood agar, GAS will appear as strongly beta-hemolytic, moist, glistening, translucent grayish white colonies >0.5mm in diameter after 24 hours of incubation.

Figure 1 – Primary media used for inoculation of pleural fluid specimens by participating laboratories (n = 110)



Biochemical Identification: GAS is catalase negative, PYR positive, and will give a negative CAMP reaction (11). GAS can be reliably identified by a variety of commercially available biochemical methods, as well as a variety of serological Lancefield grouping methods. Out of the 110 participating laboratories, 44 (40%) used an automated or commercial biochemical identification system to identify this isolate, while 41 laboratories (37%) reported using a rapid EIA or latex agglutination method in combination with classical methods for identification.

Antimicrobial Susceptibility Testing (AST): For patients not allergic to penicillin, AST of GAS isolates for the beta-lactam antibiotics (penicillins and cephalosporins) is not typically necessary due to the universal susceptibility of GAS to these drugs. The Clinical and Laboratory Standards Institute (CLSI) recommends that all GAS isolates that demonstrate elevated penicillin MIC values should be sent to a reference laboratory for confirmation (2). As mentioned previously, the M, MLS_c, and MLS_i phenotypes can be differentiated by use of the D-test or nucleic acid detection of the specific resistance genes. Among the 104 laboratories that correctly identified this isolate as GAS, 44% (48/104) reported that AST would not be routinely performed or would only be performed under special circumstances (e.g. patient allergy, special request by the physician, etc).

Reportable Disease Rule: Rapid and accurate diagnosis of invasive GAS infections by clinical laboratories is crucial for prompt initiation of treatment regimens to prevent potentially fatal outcomes. In North Dakota, all cases of invasive GAS infection (i.e. GAS isolated from normally sterile body sites), as well as cases of streptococcal toxic shock syndrome, must be reported to North Dakota Department of Health; Division of Disease Control in order to identify potential clusters of invasive disease or unusual clinical presentations of GAS infection. In addition to case reporting, culture isolates from all reportable cases should be sent to ND-PHL. Among participating laboratories, 73% (80/110) correctly reported that this isolate would be referred to their state-PHL. For more information about

the ND Communicable Disease Reporting Rule, please visit:
http://www.health.state.nd.us/Disease/Documents/ReportableConditions.pdf

Specimen #2, Tabulated Results

110/119 (92%) Laboratories performed pleural fluid cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = ***Streptococcus* Group A (*S. pyogenes*), with referral to a state-PHL**

Acceptable Answers (80/110 = 73%): The following responses were considered acceptable in combination with referral to a state-PHL.
79/110 (72%) *Streptococcus* Group A (*S. pyogenes*)
1/110 (1%) *Streptococcus*, beta-hemolytic

Unacceptable Answers (30/110 = 27%): The following responses, when submitted individually, or any responses that did not indicate referral to a state-PHL, were considered unacceptable.
25/110 (23%) *Streptococcus*, Group A (*S. pyogenes*), without referral to a state-PHL
2/110 (2%) *Streptococcus*, beta-hemolytic, without referral to a state-PHL
1/110 (<1%) Gram-positive cocci, NOS, without referral to a state-PHL
1/110 (<1%) *Micrococcus* species
1/110 (<1%) *Streptococcus*, beta-hemolytic, untypeable, without referral to a state-PHL

REFERENCES

1. The Centers for Disease Control and Prevention. Nosocomial Group A streptococcal infections associated with asymptomatic health-care workers – Maryland and California, 1997. MMWR Morb Mortal Wkly Rep. 1999;48(08):163-166.
2. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S20.Wayne, PA: CLSI; 2010.
3. Facklam R. What happened to the streptococci: overview of taxonomic and nomenclature changes. 10biol Rev. 2002;15(5):612-630.
4. Green MD, Beall B, Marcon MJ, Allen CH, Bradley JS, Dashefsky B, Gilsdorf JR, Schutze GE, Smith C, Walter EB, Martin JM, Edwards KM, Barbadora KA, Wald ER. Multicentre surveillance of the prevalence and molecular epidemiology of macrolide resistance among pharyngeal isolates of Group A streptococci in the USA. J Antimicrob Chemother. 2006;57:1240-1243.
5. Haukness HA, Tanz RR, Thomson RB Jr, Pierry DK, Kaplan EL, Beall B, Johnson D, Hoe NP, Musser JM, Shulman ST. The heterogeneity of endemic community pediatric Group A streptococcal pharyngeal isolates and their relationship to invasive isolates. J Infect Dis. 2001;185:915-20.
6. Lappin E, Ferguson AJ. Gram-positive toxic shock syndromes. Lancet Inf Dis. 2009;9(6):281-290.
7. Minnesota Department of Health. Annual summary of communicable diseases reported to the Minnesota Department of Health, 2009. Disease Control Newsletter. 2010;38(1):1-32.
8. Myers AL, Jackson MA, Selverangan R, Goering RV, Harrison C. Genetic commonality of macrolide-resistant Group A beta hemolytic streptococcus pharyngeal strains. Ann 10biol Antimicrob. 2009;8:33-39.
9. The National Institutes of Health (US) and Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, D.C.: U.S. Government Printing Office; 2007.
10. O'Loughlin RE, Roberson A, Cieslak PR, Lynfield R, Gershman K, Craig A, Albanese BA, Farley MM, Barrett NL, Spina NL, Beall B, Harrison LH, Reingold A, Van Beneden C, Active Bacterial Core Surveillance Team. The epidemiology of invasive Group A streptococcal infection and potential vaccine implications: United States, 2000-2004. Clin Infect Dis. 2007;45:853-862.
11. Spellerberg B, Brandt C. *Streptococcus*. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, and Landry ML, editors. Manual of Clinical Microbiology. 9th ed. Vol 1. Washington, DC: ASM Press; 2007. p. 412-429.
12. York MK, Church DL. Paratechnical processing of specimens for aerobic bacteriology. In: Lynne S. Garcia, editor. Clinical Microbiology Procedures Handbook. 3rd ed. Vol 1. Washington, DC: ASM Press; 2007. p. 3.3.1.1.-3.3.1.11.

Specimen #3, Urine Culture: *Klebsiella pneumoniae*, carbapenemase producer

Submitter #: 2209	Your Result	Intended Result
Identification	*Klebsiella pneumoniae*, carbapenemase producer	*Klebsiella pneumoniae*, carbapenemase producer
Referral of Isolate		Would refer isolate to ND-PHL
Susceptibilities		
Ertapenem	Resistant	Resistant
Imipenem	Resistant	Intermediate or Resistant
Meropenem	Resistant	Intermediate or Resistant
Cefotaxime	Not performed	Resistant
Ceftazidime	Not performed	Resistant
Ceftriaxone	Not performed	Resistant
Modified Hodge Test	Positive	Positive

Goals and Objectives: The isolate in this specimen was a carbapenemase-producing *Klebsiella pneumoniae* with resistance mediated by the bla_{KPC} gene. This same organism was sent in Challenge Set Seven (2009). The goal in resending this organism was to reinforce the need for clinical laboratories and their healthcare teams to maintain vigilant detection, reporting, and infection prevention strategies in order to prevent carbapenem resistant *Enterobacteriaceae* (CRE) from acquiring a stronghold in North Dakota, as they have in other parts of the United States. The objectives were to re-assess the ability of North Dakota laboratories to recognize carbapenem resistance and perform additional phenotypic testing; evaluate changes in antimicrobial susceptibility testing practices among North Dakota sentinel site laboratories between Challenge Sets Seven and Eight; and to provide information to assist laboratories in the detection of CRE and carbapenemases.

***Enterobacteriaceae* Family:** Members of the *Enterobacteriaceae* family are gram-negative bacilli that do not form spores; most are oxidase negative, grow well on MacConkey agar, ferment various sugars to produce acid and gas, and reduce nitrate to nitrite. *Enterobacteriaceae* comprise a significant proportion of the intestinal normal flora of humans. However, several species are significant human pathogens and cause serious nosocomial infections including 70% of urinary tract infections (UTI), 50% of bloodstream infections, and a significant percentage of intestinal infections (6).

***Klebsiella pneumoniae*:** *Klebsiella* species are among the four most common causes of healthcare-associated and community-associated UTIs (1). Certain strains of *K. pneumoniae* can be associated with asymptomatic colonization of the intestinal, urinary, and respiratory tracts and can cause potentially fatal pneumonia, septicemia, and meningitis (6). Most infections caused by *K. pneumoniae* are healthcare-associated or occur in patients that have other underlying conditions leaving them vulnerable to disease. *K. pneumoniae* is second only to *E. coli* in causing bacteremia secondary to a UTI (5).

Laboratory identification: Microscopically, *K. pneumoniae* organisms appear as medium-sized gram-negative bacilli. Due to polysaccharide capsule production, colonies appear mucoid on solid agar media. *K. pneumoniae* ferments most sugars and is non-motile, indole and oxidase negative, and lysine, Voges-Proskauer and malonate positive (6). In this challenge, nearly all participants (97%) correctly identified this organism, which is readily identified by commercial methods.

***Klebsiella* and Antibiotics:** *Klebsiella* is inherently resistant to carbenicillin and ampicillin (6). Some strains also produce extended spectrum ß-lactamases (ESBL) which can cause resistance to the third generation cephalosporins and aztreonam. More recently, strains have been identified that produce

carbapenemases, which cause resistance by hydrolyzing carbapenems (ertapenem, imipenem, meropenem, and doripenem) as well as penicillins, cephalosporins, and aztreonam (10).

Carbapenemases and Other Resistance Mechanisms: Carbapenemases are a sub-class of ß -lactamase enzymes that are classified by their specific resistance mechanisms (e.g., KPC, SME, etc.). Organisms producing the *Klebsiella pneumoniae* carbapenemase (KPC) are the most common type of carbapenem resistant *Enterobacteriaceae* (CRE) in the United States. The bla_{KPC} gene (*bla* stands for ß-lactamase) is plasmid-encoded, is specific for production of the KPC enzyme, and causes resistance to the carbapenem group of antibiotics in *Enterobacteriaceae* (2, 9). KPC is most commonly found in *K. pneumoniae*, but can also be found in several other *Enterobacteriaceae* (such as *K. oxytoca*, *E. coli*, *C. freundii*, *S. marcesens, and E. cloacae*) (8, 9). KPC represents an emerging bacterial resistance mechanism and has been reported in 35 states to date, but since there is no requirement to report KPC to CDC, there may well be other states that have seen this organism. KPC is currently most prevalent in the northeastern part of the U.S., particularly in New York, New Jersey and Maryland (2, 8). Other carbapenem resistance mechanisms, such as the New Delhi metallo-beta-lactamase (NDM) and the Verona Integron-encoded metallo-beta-lactamase (VIM), have recently been identified in the U.S. and raise additional concerns about the identification and control of CRE (2).

Importance of Detecting Carbapenem Resistant *Enterobacteriaceae* (CRE): Detection and notification of CRE is important for both therapeutic and infection prevention and control reasons. The carbapenems used in a hospital setting are ertapenem, imipenem, meropenem and less frequently doripenem. These antibiotics have broad-spectrum activity and are often used empirically for potentially life-threatening infections such as sepsis. They are also used for treating infections caused by gram-negative bacilli that are multi-drug resistant (i.e. *Pseudomonas aeruginosa*, *Acinetobacter* spp.) and for ESBL-producing *Enterobacteriaceae*. Therefore, if carbapenem-resistant organisms are present in a healthcare setting there are significant limitations to the treatment options for the most critically ill patients. Studies regarding patients with a *Klebsiella pneumoniae* carbapenemase (KPC) show poor clinical outcomes, increased hospital expenditures, and increased mortality (9). In addition, KPC enzymes are typically encoded on plasmids that can easily transfer the resistance mechanism from one organism to another and from one patient to another. Collectively, these features demonstrate the significance of rapid laboratory detection and reporting of CRE to clinicians and infection preventionists so that infection prevention and control measures can be implemented. Any CRE, regardless of resistance mechanism, is clinically significant and should trigger implementation of infection prevention and control measures, including implementation of contact precautions (2, 8). The earlier CRE is identified and reported, the sooner the patient can be placed on appropriate treatment and the more likely that spread throughout the healthcare facility will be prevented. If a CRE is identified, your laboratory may be requested to test for additional antibiotics such as tigecycline or polymyxins (9).

A Healthcare Team Approach in Response to CRE: A team approach, which includes laboratorians, clinicians, infection preventionists, and pharmacists, is necessary to effectively identify and control CRE. This approach will not only help to prevent transmission, but will reinforce better antibiotic stewardship and overall prevention of healthcare associated infections (2). For infection control purposes, it is important that all members of the team have access to the carbapenem antibiotic results that are tested, even if the laboratory may normally suppress the reporting of the carbapenem antibiotics on the patient's report. Additionally, it is not necessary to know the mechanism of resistance (i.e. KPC) to trigger therapy or infection control measures. Among the 113 laboratories that correctly identified the organism in this challenge, 91 (81%) indicated that infection control, the nursing station, the physician, or some combination of the three, would be notified with the results. Four laboratories (4%) indicated that they would only notify the Public Health Laboratory and 8 laboratories (7%) would take no further action. For specific infection prevention and control guidelines please refer to the North Dakota Department of Health (NDDoH) Division of Disease Control website under "Current Conditions": http://www.ndhealth.gov/disease/.

Laboratory Challenges in the Detection of CRE and Carbapenemases

There are several challenges in detecting CRE and carbapenemase production; one is inconsistent adoption of new CLSI interpretive criteria for carbapenems; second is that not all screening carbapenem antibiotics are equally sensitive and specific; thirdly phenotypic tests are challenging to perform and interpret; and fourth, certain species (e.g. *Proteus*) can have elevated imipenem MICs due to mechanisms other than KPC.

Challenge 1 – CLSI Breakpoints: A major challenge for consistency in carbapenemase detection and reporting is recognizing that a carbapenemase-producing organism may not demonstrate resistance to carbapenems using the old January 2010 CLSI breakpoints and common antimicrobial susceptibility testing (AST) methods. In June 2010, CLSI released the "Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement (June 2010 Update) – M100-S20-U". The new guidelines set lower carbapenem breakpoints – see **Table 1**. CLSI states that if laboratories adopt the new breakpoints, initial screening and confirmatory testing can be abandoned for patient therapy purposes; however, confirmatory testing may be useful for epidemiological purposes (4). The new breakpoints are not without controversy and may be debated for some time.

In this challenge, laboratories were asked if they had adopted the new June 2010 CLSI carbapenem breakpoints. Of the 113 laboratories correctly identifying this organism, seven (6%) have adopted the new breakpoints, 26 (23%) have not adopted, 61 (54%) have not adopted, but plan to when their automation manufacturer has implemented the change, and 19 (17%) did not indicate if they had adopted. Most laboratories are choosing to wait until manufacturers of their commercial systems are cleared through FDA to modify the reportable ranges in their products. However, laboratories that would like to implement the new breakpoints sooner can perform in-house validations of methods using the new breakpoints and start reporting immediately. The danger in using the old (higher) January 2010 CLSI breakpoints is that a carbapenemase-producing isolate may test and be reported as susceptible to a carbapenem, increasing the potential for therapeutic failure (8). If your laboratory is considering adopting and reporting AST results using the new June 2010 CLSI interpretations for carbapenems, or making any other changes to reporting or testing practices, it is very important that they are implemented in consultation with your infectious disease practitioners, pharmacy, and the infection control committee (4).

In addition to the challenges of adopting the new breakpoints, some laboratories are using AST panels that do not test for all of the carbapenems and that may not have MIC dilutions low enough to detect potential carbapenemase-producers. Some KPC organisms may demonstrate elevated meropenem and imipenem MICs of 2 to 4 μg/ml (considered susceptible by old breakpoints). One study involving several automated AST methods showed that a meropenem or ertapenem MIC value of ≥ 1 μg/ml was a sensitive indicator of a KPC (8). However, many laboratories are using commercial AST panels and cards that do not have dilutions low enough to detect an MIC of 1 μg/ml. In some cases, the lowest MIC value is ≤4 μg/ml. **NDPHL strongly encourages all laboratories to contact technical representatives of their commercial systems to upgrade susceptibility cards and panels to newer versions that have lower MIC limits for the carbapenems and include at least two carbapenem antibiotics.**

Table 1: Carbapenem breakpoints CLSI M100 S20 and M100-S20-U (June 2010 Update) (3, 4)

	CLSI M100-S20 (Jan. 2010)			CLSI M100-S20 (June 2010)		
	Susceptible	**Intermediate**	**Resistant**	**Susceptible**	**Intermediate**	**Resistant**
Ertapenem	≤ 2	4	≥ 8	≤ 0.25	0.5	≥ 1
Imipenem	≤4	8	≥16	≤ 1	2	≥ 4
Meropenem	≤4	8	≥16	≤ 1	2	≥ 4

Challenge 2 – Inequality of Screening Antibiotics: Another difficulty in determining KPC production is that KPCs do not hydrolyze all carbapenems equally; therefore, it is important that laboratories attempt to use the most sensitive screening method and antibiotic, which several experts suggest to be ertapenem (9). However, one study determined ertapenem to be overly sensitive and that the best screening scheme was to examine AST reports for imipenem and meropenem MICs that were ≥ 2 µg/ml (Intermediate by new breakpoints) for either antibiotic; these organisms would then be subjected to a modified Hodge test (MHT) confirmation using meropenem. This approach decreased false positive results that can be caused by using ertapenem MICs to screen (7). Laboratories must determine the best approach based on which antibiotics and methods are available for the most effective screening. Please refer to the NDPHL website for lab protocols for detecting KPC organisms and performing the MHT.
www.ndhealth.gov/microlab

Challenge 3 – Phenotypic Testing: An additional challenge to the detection of KPC is that the phenotypic testing methods available for confirming carbapenemases (e.g. MHT) can also produce positive or equivocal results for organisms that display other mechanisms of resistance (e.g. metallo-beta-lactamases). While there are PCR methods for the detection of the bla_{KPC} gene, these tests are mainly used in reference lab settings. Some organisms may also have more than one mechanism of resistance, which can further complicate the interpretation of phenotypic confirmatory tests (9). However, since KPC is the most common carbapenemase and the other mechanisms of resistance are rare, an organism with a positive modified Hodge test has a high probability of being a KPC.

Challenge 4 – *Proteus* spp.: To further complicate matters, CLSI points out that "Imipenem MICs for *Proteus* spp., *Providencia* spp., and *Morganella morganii* tend to be higher (i.e. MICs in the new intermediate or resistant range) than meropenem or doripenem MICs. These isolates may have elevated MICs by mechanisms other than production of carbapenemases."(4)

Laboratory Screening and Confirmation of Carbapenemase-producing *Enterobacteriaceae*

Following are examples of methods that laboratories can employ to assist in the detection of carbapenem-producing organisms.

Disk diffusion testing: A simple method to screen for carbapenemases is to perform disk diffusion with ertapenem (10 µg) or meropenem (10 µg) disks. Using imipenem disks is not recommended because it has been shown in several studies to be a less sensitive indicator of KPC production. New zone sizes of 16-21 mm for ertapenem and 14-21 mm for meropenem are considered screen positive, and laboratories should proceed to the modified Hodge Test (MHT) for confirmation (4, 8).

Manually review AST reports: It is suggested that laboratories perform daily reviews of all *Enterobacteriaceae* AST reports for elevated carbapenem MIC values and positive screen results in order to detect possible carbapenemases. There is debate among experts as to whether detection of carbapenemases should be based on the combination of AST results for at least one resistant third generation cephalosporin and elevated MIC's to the carbapenems or whether elevated carbapenem MICs alone are indicative (3, 4, 8). In addition, since KPCs are inhibited by clavulanic acid they may also be detected in ESBL screens; therefore, it might also be prudent to check all *K. pneumoniae* that test resistant to the extended-spectrum cephalosporins (ceftazidime, ceftriaxone, and cefotaxime) (8, 10). It is important that laboratories consider all of these methods, and develop internal protocols with their healthcare team to ensure detection of carbapenem-producing organisms.

Modified Hodge Test (MHT): The modified Hodge or "cloverleaf" test is a phenotypic confirmation method for the detection of carbapenemase production, although it does not identify bla_{KPC} as the specific mechanism. CLSI recommends using either ertapenem or meropenem disks when performing MHT. The new June 2010 CLSI document, states that if the MHT is positive, and your laboratory is using old January 2010 CLSI breakpoints, then the carbapenems should be reported as "resistant" (this is a change

from the old recommendation to report just the MIC without interpretation); if using the new June 2010 breakpoints, report MIC and interpretations as tested (3, 4). If your laboratory has isolated an *Enterobacteriaceae* isolate that is MHT positive, please send an isolate to ND-PHL and notify the NDDoH Division of Disease Control. ND-PHL continues to encourage laboratories to implement the MHT for the confirmation of carbapenemase production. The number of laboratories performing MHT has increased from 23 last year to 33 this year, with 32 laboratories (97%) correctly indicating a positive MHT result – nearly the same percentage as last year.

For additional information on the MHT, refer to the June CLSI M100-S20-U document for the entire protocol for confirmation of carbapenemases (4). CDC's protocol for the MHT was included in last year's challenge set packet, and can also be found on the ND-PHL website at: www.ndhealth.gov/microlab

PCR for bla_{KPC}: Only one laboratory in this challenge indicated having the ability to perform PCR for bla_{KPC}. For the purposes of implementing infection prevention and control measures it is not necessary to know the mechanism of resistance (i.e. bla_{KPC}). However, some clinicians or infectious disease specialists may request this information. Knowing if an organism is positive for bla_{KPC} may be important in an epidemiologic investigation (3, 4). MDH-PHL performs PCR for the bla_{KPC} gene to confirm the resistance mechanism for appropriate isolates, as defined on page 6. When appropriate the ND-PHL will coordinate with the MDH-PHL to perform PCR for the bla_{KPC} gene.

A note about ESBLs: The isolate in this challenge set was also tested for ESBL production in both the MDH-PHL and CDC laboratories. The results obtained demonstrate the incredible challenge phenotypic tests present. In the MDH-PHL the isolate screened positive for ESBL, but the CLSI disk confirmation test results were not clearly interpretable. The CDC-AST laboratory had similar results and they explained that "the bla_{KPC} enzyme can also mimic a positive ESBL double disk test as the bla_{KPC} enzyme can be inhibited by clavulanate by varying degrees." This isolate was not tested by a PCR method for any other resistance mechanisms except the bla_{KPC}; however, since this isolate was multi-drug resistant, the determination of ESBL production would not be necessary for clinical treatment. It is important to note that the January 2010 CLSI M100-S20 standards document significantly revised the breakpoints for some of the cephalosporins and aztreonam when testing *Enterobacteriaceae* (see **Table 2)**. The breakpoints have been lowered, which may result in increased reporting of resistance and a decreased need to perform phenotypic (double disk) testing for ESBL determination. This too is controversial and laboratories should include members of the healthcare team if deciding to implement the new breakpoints. In this challenge, 27 laboratories (24%) indicated adoption of these new breakpoints.

Table 2: ESBL revised breakpoints CLSI M100 S19 and S20 (4)

	CLSI M100-S19 (2009)			**CLSI M100-S20 (Jan. and June 2010)**		
	Susceptible	**Intermediate**	**Resistant**	**Susceptible**	**Intermediate**	**Resistant**
Cefazolin	≤ 8	16	≥ 32	≤ 1	2	≥ 4
Cefotaxime	≤8	16-32	≥64	≤ 1	2	≥ 4
Ceftizoxime	≤8	16-32	≥64	≤ 1	2	≥ 4
Ceftriaxone	≤8	16-32	≥64	≤ 1	2	≥ 4
Ceftazidime	≤8	16	≥32	≤ 4	8	≥ 16
Aztreonam	≤8	16	≥32	≤ 4	8	≥ 16

North Dakota Epidemiologic Surveillance of CRE: Laboratorians and the rest of the healthcare team play an integral role in assisting the NDDoH in its goal of keeping carbapenemase-producing organisms, especially KPCs, from establishing a stronghold in North Dakota. Per the ND Communicable Disease Reporting Rule, all cases in which organisms show reduced susceptibility to carbapenem should be reported to the NDDoH Division of Disease Control and an isolate should be submitted to the ND-PHL.

NDDoH Division of Disease Control is available for consultation regarding patient management, including surveillance and infection prevention and control measures.

When Should Laboratories Submit Isolates?
NDDoH encourages all laboratories to submit possible carbapenemase-producing isolates for epidemiological purposes. The following isolate submission guidelines were developed based on the best knowledge currently available. Please submit all *Enterobacteriaceae* isolates that are:

- Resistant to at least one carbapenem (June 2010 CLSI breakpoints): ≥ 1 µg/ml for ertapenem or ≥ 4 µg/ml for meropenem or imipenem; AND
- Resistant to at least one 3rd generation cephalosporin (by Jan. 2010 CLSI breakpoints)

Some isolates of *Morganella*, *Serratia*, *Providencia*, *Citrobacter*, *Enterobacter*, *Hafnia*, *Proteus*, and *Yersinia* may have different resistance mechanisms that may result in elevated carbapenem MICs. Therefore, submit these isolates ONLY if they are:

- Resistant to at least two carbapenem antibiotics (June 2010 CLSI breakpoints); AND
- Resistant to at least one 3rd generation cephalosporin.

Note: Based on the data from this challenge set, some laboratories may not have the capability to implement all of these rules for submission of isolates, but each laboratory must employ the most effective methods that they have available to detect CRE. It should be a goal of all laboratories to have the most current and effective AST panel of carbapenem antibiotics available for testing so that CRE can be readily detected and controlled.

What to Include when Submitting an Isolate:

- A copy of a printed report from the commercial AST method (please include any antibiotics that may normally be suppressed on the patient report)
- Results of the MHT (it is understood that some laboratories do not perform MHT however NDDoH encourages laboratories to institute the MHT or have access to a reference laboratory that can perform confirmatory testing)
- Results from any other supplemental AST methods.

Specimen #1, Tabulated Results

116/116 (100%) = Laboratories performed urine cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = *Klebsiella pneumoniae*, carbapenemase producer
Acceptable Answers (113/116 = 97%): An identification of *Klebsiella pneumoniae*, submitted either individually or in combination with any of the following responses, was considered acceptable.

83/116 (73%)	*Klebsiella pneumoniae*
8/116 (7%)	*Klebsiella pneumoniae*; possible carbapenemase producer
7/116 (6%)	*Klebsiella pneumoniae*; carbapenemase producer
4/116 (3%)	*Klebsiella pneumoniae*; possible carbapenemase producer; possible extended spectrum ß-lactamase (ESBL) producer
4/116 (3%)	*Klebsiella pneumoniae*; possible carbapenemase producer; ESBL producer
2/116 (2%)	*Klebsiella pneumoniae*; carbapenemase producer; ESBL producer
2/116 (2%)	*Klebsiella pneumoniae*; possible ESBL producer
2/116 (2%)	*Klebsiella pneumoniae*; ESBL producer
1/116 (<1%)	*Klebsiella pneumoniae*; carbapenemase producer; possible ESBL producer

Unacceptable Answers (3/116 = 3%)

1/116 (<1%)	*Klebsiella oxytoca*

1/116 (<1%) *Lactobacillus* sp.
1/116 (<1%) *Raoultella ornithinolytica*

Challenge Set Organism Characteristics: The isolate in this challenge was a KPC producer and laboratories should have detected resistance to ertapenem and non-susceptible results to imipenem, and meropenem (based on old Jan. 2010 CLSI breakpoints). In addition, the 3rd generation cephalosporins (ceftriaxone, cefotaxime, ceftazidime, and ceftizoxime) should have been resistant. Of the 113 laboratories that correctly identified this organism as *K. pneumoniae* and reported AST results, 87 (77%) reported results for at least one carbapenem, this is nearly identical to last year's challenge set; 95 (84%) laboratories reported results for at least one 3rd generation cephalosporin. Furthermore, 77 (68%) of laboratories correctly reported at least one carbapenem as being non-susceptible; and 90 (80%) correctly reported at least one 3rd generation cephalosporin as resistant. Reported MIC results for the carbapenems varied remarkably, ranging from 2-32 µg/ml. This variation may be partly due to differences in AST panel configurations, AST methods used, as well as variations in inoculum density due to capsule production by *K. pneumoniae* (9). See **Table 3** for carbapenem results and **Table 4** for 3rd generation cephalosporin results reported by participating laboratories.

Table 3: AST results from 87 laboratories reporting carbapenems

Antibiotic	Ertapenem 2009 (n=36)	Ertapenem 2010 (n=40)	Imipenem 2009 (n=76)	Imipenem 2010 (n=79)	Meropenem 2009 (n=22)	Meropenem 2010 (n=20)
Report = non-susceptible (I or R)	34 (94%)	40 (100%)	60 (78%)	67 (85%)	17 (77%)	17 (85%)
Report = susceptible	1 (3%)	0	14 (19%)	10 (13%)	2 (9%)	0
No interpretation provided	1 (3%)	0	3 (4%)	2 (2%)	3 (14%)	3 (15%)

Note: some labs reported more than one carbapenem

Table 4: AST results from 95 laboratories reporting 3rd generation cephalosporins

Antibiotic	Cefotaxime 2009 (n = 35)	Cefotaxime 2010 (n = 35)	Ceftazidime 2009 (n=65)	Ceftazidime 2010 (n=61)	Ceftriaxone 2009 (n=88)	Ceftriaxone 2010 (n=82)
Report = resistant	24 (69%)	31 (89%)	57 (88%)	57 (93%)	69 (78%)	76 (93%)
Report = intermediate	0	0	0	0	0	3 (4%)
Report = susceptible	2 (6%)	1 (3%)	0	0	8 (9%)	0
No interpretation provided	9 (25%)	3 (9%)	8 (12%)	4 (7%)	11 (13%)	3 (4%)

Note: some labs reported more than one 3rd generation cephalosporin; one lab reported Ceftizoxime – resistant.

REFERENCES

1. Abbott SL. *Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas*, and Other *Enterobacteriaceae*. In: Murray, PR, Baron EJ, Jorgensen JH, Pfaller MA, and Landry ML, editors. Manual of Clinical Microbiology, 9th ed. Vol 1. Washington, DC: ASM Press, 2007. p. 698-715.
2. Centers for Disease Control and Prevention, 2010 [Internet]. Atlanta (GA): Public Health update of Carbapenem-Resistant Enterobacteriaceae (CRE) producing metallo-beta-lactamases (NDM,VIM, IMP) in the U.S. reported to CDC [modified 2010 Dec 13, accessed 2011 Jan 6]. Available from: http://www.cdc.gov/HAI/organisms/cre.html
3. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S20.Wayne, PA: CLSI; 2010.
4. Clinical and Laboratory Standards Institute.. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement (June 2010 Update). CLSI document M100-S20-U. Wayne, PA: CLSI; June 2010.
5. Donnenberg MS. *Enterobacteriaceae*. In: Mandell GL, Bennett JE, and Dolin R, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 7th ed. Vol 2. Philadelphia: Churchill Livingstone Elsevier, 2010. p.2826-2827.
6. Farmer III JJ, Boatwright KD, and Janda JM. *Enterobacteriaceae*: Introduction and Identification. In: Murray, PR, Baron EJ, Jorgensen JH, Pfaller MA, and Landry ML, editors. Manual of Clinical Microbiology, 9th ed. Vol 1. Washington, DC: ASM Press, 2007. p. 649-669.
7. Lonsway, DR, Wong BK, Anderson KF, and Patel JB. Evaluation of Testing Schemes for the Detection of KPC-Producing *Enterobacteriaceae*. 110th ASM General Meeting. May 2010. San Diego, CA.
8. Patel, JB, Rasheed JK, and Kitchel B. Carbapenemases in *Enterobacteriaceae*: Activity, Epidemiology, and Laboratory Detection, Clin Microbiol Newsletter. 2009; 31(8):55-62.
9. Schuetz AN. *Klebsiella pneumoniae* Carbapenemases (KPCs): When to suspect and how to detect them. ASCP Lab Q. Clinical Laboratory 2009; 17:125-131.
10. Sundin DR. Hidden Beta-Lactamases in the *Enterobacteriaceae* – Dropping the Extra Disks for Detection, Part II. Clin Microbiol Newsletter. 2009; 31(7);47-52.

Specimen #4, Blood Culture: *Oligella ureolytica*

Submitter: 2209	Your Result	Intended Result
Identification	*Oligella ureolytica*	*Oligella ureolytica*
Referral of Isolate	NA	Isolate should be referred to ND-PHL if *Brucella* spp. cannot be ruled-out
Gram stain	Small Gram-negative coccobacilli	Tiny or small Gram-negative coccobacilli or bacilli/rods
Catalase	Positive	Positive
Nitrate	Not Performed	Positive
Oxidase	Positive	Positive
Urea	Positive	Positive

Goals and Objectives: The organism in specimen 4 was *Oligella ureolytica* and was the same organism that was sent out in Challenge Set one in 2002 and Challenge Set Five in 2007. As in past Challenge Sets, this isolate was chosen as a surrogate for *Brucella* species; therefore, this report will focus on describing *Brucella* rather than *Oligella ureolytica*. The goal of this challenge was to emphasize the role of *Brucella* as one of the most common laboratory acquired infections (LAI), as well as a potential agent of bioterrorism. The objectives were to illustrate the importance of proper biosafety practices in the clinical laboratory and to assess the ability of LRN Advanced Sentinel Laboratories to rule-out and refer potential *Brucella* isolates to ND-PHL.

About *Oligella ureolytica*: *O. ureolytica* is an oxidase-positive, indole-negative, asaccharolytic, nonfermenting gram-negative coccobacillus. It is most often isolated from urinary tract infections and would be an unusual organism to find in a blood culture. A variety of commercial identification systems can readily identify *O. ureolytica* (7). Among the 112 participating laboratories, 14 (13%) correctly identified this organism as *Oligella ureolytica*.

Clinical Significance of *Brucella* species: *Brucella* species are facultative intracellular pathogens that cause brucellosis, a zoonosis usually transmitted to humans by contact with infected animals or consumption of contaminated animal products. Brucellosis can be acquired through abraded skin, inhalation of infectious aerosols, or consumption of undercooked meat or unpasteurized milk or cheese from infected animals (4, 7). The acute phase of the disease typically lasts up to eight weeks and includes non-specific “flu-like” illness with fever, sweats, chills, headaches, muscle pain, and back pain. The organism can also affect the spleen and liver, the reproductive and nervous systems, and can cause osteomyelitis and meningitis (4, 7). Hallmarks of chronic brucellosis can mimic miliary tuberculosis and include focal suppurative lesions of the liver, spleen and bones, arthritis, depression, and periodic relapsing or “undulant” fever (7, 10). Because brucellae can cause deep-seated infections and long-term sequelae, relapse and chronic disease are common. Although brucellosis is associated with significant morbidity, the mortality rate is <2% if left untreated (4, 7).

Incidence and Risk Factors: Approximately 100 cases of brucellosis are reported in the United States each year, the majority of which are in immigrants or travelers returning from endemic areas including southeastern Europe, the Mediterranean basin, the Middle East, Asia, Africa, the Caribbean, and Central and South America (7). *B. melitensis* is the most commonly isolated species in the U.S. and is typically associated with consumption of unpasteurized milk or soft cheeses. Veterinarians, livestock farmers, and slaughterhouse workers may be exposed to *B. abortus* (cattle) and *B. suis* (pigs) either by direct contact with infected animals or through infectious aerosols. *B. canis* is usually isolated from kennel-bred dogs, and although capable of causing a similar disease, is less virulent in humans than other species of *Brucella* (7).

While laboratory-acquired brucellosis represents only 2% of the reported brucellosis cases in the U.S., it is still one of the most commonly reported causes of laboratory-acquired infections (3, 7, 10).

Laboratorians have a case incidence rate of 641 per 100,000 while the rate of incidence in the general population is 0.08 per 100,000; this represents a greater than 8,000-fold increase in the relative risk of brucellosis among laboratory workers as compared to the general population (2). Laboratorians may be at increased risk for exposure to brucellae due to the diagnostic challenges presented by the non-specific nature of both acute and chronic symptoms. These challenges may be especially difficult in developed countries where incidence of zoonotic brucellosis is low. Clinicians with a low index of suspicion may not consider brucellosis to be a likely diagnosis or fail to communicate their suspicion to the clinical microbiology laboratory prior to submission of specimens for testing. In addition, laboratorians may be less familiar with the phenotypic characteristics of brucellae, which may result in inadvertent handling of cultures without adequate biosafety precautions (1, 12). Through its Division of Disease Control, the North Dakota Department of Health (NDDoH) investigates all cases of brucellosis reported by clinical laboratories in order to determine the need for prophylaxis of laboratory staff who have potentially been exposed.

Treatment: Since brucellae are intracellular organisms, long-term combination antibiotic therapy is required for successful treatment of brucellosis. The World Health Organization (WHO) currently recommends a six week course of doxycycline in combination with rifampin (11). Post-exposure prophylaxis consisting of a combination of oral doxycycline plus rifampin for 3 weeks is often indicated for laboratory workers with occupational exposure to *Brucella* (9). In cases of documented exposure to *Brucella*, laboratorians may be monitored for symptoms of brucellosis or seroconversion for up to three months (9).

***Brucella* and Bioterrorism:** *B. suis* was the first biological agent to be weaponized by the United States during its offensive biological warfare program in 1954 (10). Since brucellae have a low infective dose (10-100 organisms) and are easily aerosolized, they have the potential to be effective bioterrorism agents (10). LRN Advanced Sentinel Laboratories are responsible for ruling-out and referring any suspected brucellae to ND-PHL, which serves as the LRN Reference Laboratory for North Dakota (1). In addition, CDC has classified *B. abortus*, *B. melitensis*, and *B. suis* as Select Agents. The reference laboratory (laboratory that confirms the identification of the select agent – ND-PHL) must complete APHIS/CDC Form 4 Section 1 within seven calendar days after identification for all entities in possession of the specimen or isolate at the time of the identification. Furthermore, within seven calendar days after identification of a select agent or toxin, the identified select agent or toxin must be transferred to a select agent registered laboratory (ND-PHL) or destroyed on-site by a recognized sterilization or inactivation process.(5). More information about the Select Agent Program may be found at www.selectagent.gov.

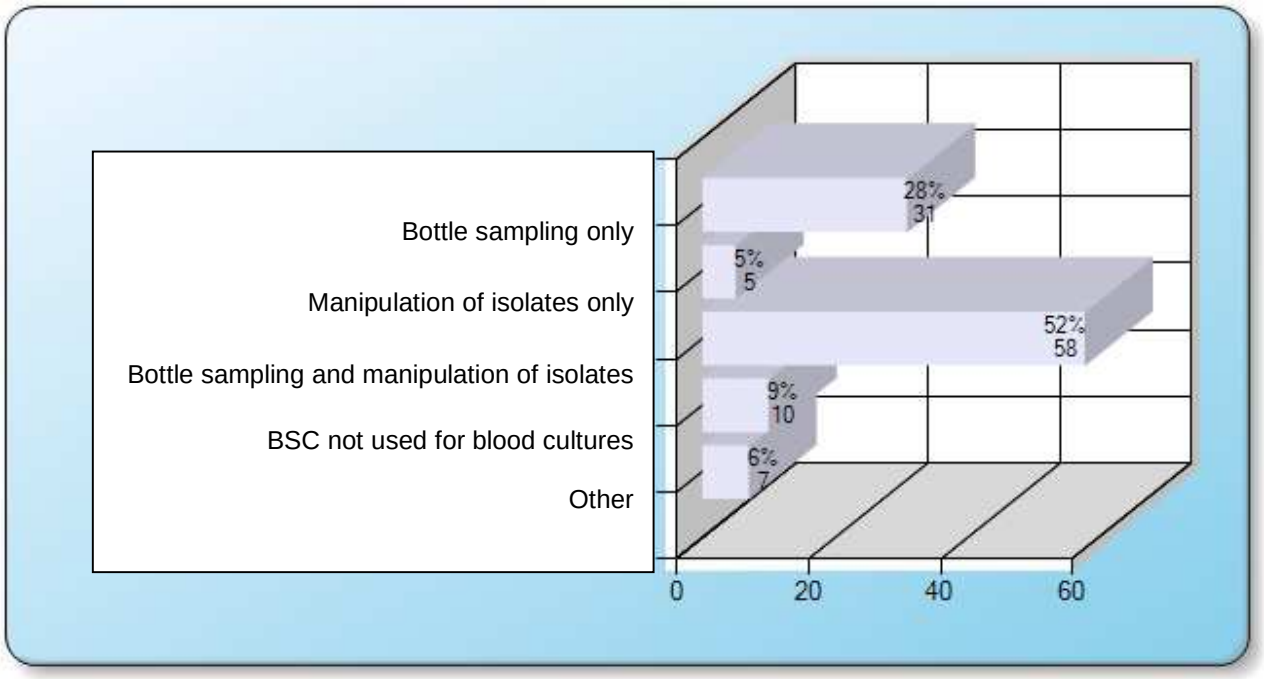
Laboratory Identification of *Brucella* species

Collection and Transport: Blood and bone marrow are the specimens of choice for the isolation of brucellae (7, 10). Other important specimens include tissue biopsies, abscess aspirates, and body fluids. Clinical specimens submitted for testing can be packaged and shipped as Biological Substance, Category B UN3373; however, confirmed cultures of *B. abortus*, *B. melitensis*, and *B. suis* are classified as Infectious Substance, Category A UN2814 for the purposes of packaging and shipping by the U.S. Department of Transportation (6). Training on packaging and shipping regulations is available online through the ND-PHL. Please call 701-328-6295 and request access to the on-line course. Additional information on packaging and shipping may be found on the ND-PHL website at www.ndhealth.gov/microlab.

Laboratory Safety: Due to the dramatically increased risk of brucellosis among laboratorians, it is critically important that clinical specimens and cultures potentially containing *Brucella* be handled appropriately. 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 and procedures that may generate aerosols (e.g. grinding

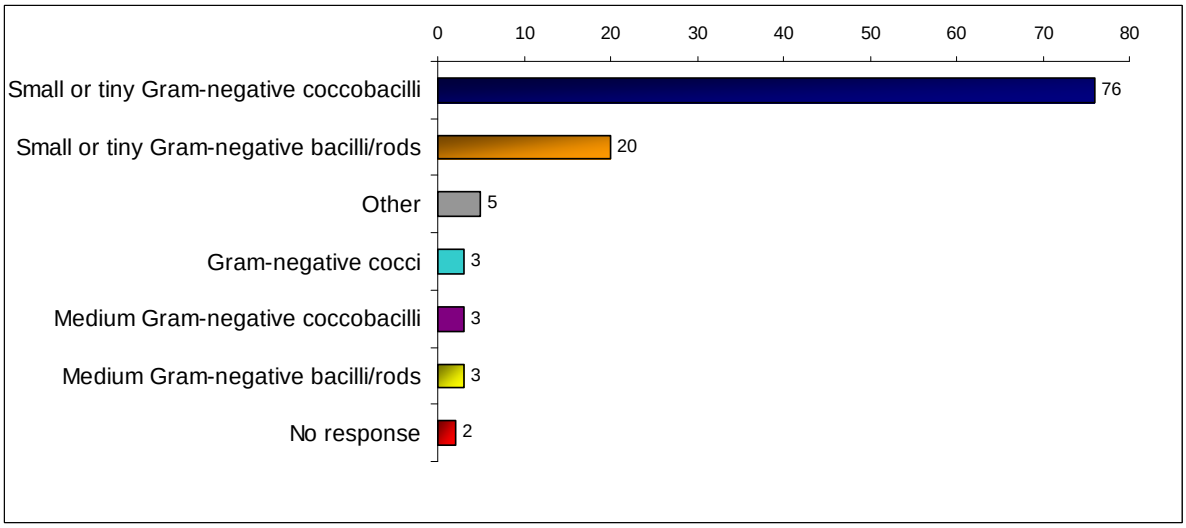
tissues, aliquoting body fluids, etc) (8, 10). BSL3 practices, containment equipment, and facilities are also recommended for manipulation of any cultures suspected or known to contain *Brucella* species (8). The potential for clinically relevant exposure to *Brucella* increases exponentially after specimens have been incubated in solid and liquid culture media, which can generate very large amounts of viable *Brucella* organisms. While *Brucella* can be recovered from a variety of specimens, it is most commonly isolated from blood and bone marrow cultures (13). 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, especially for those cultures with extended times to positivity. **Figure 1** summarizes how participating laboratories reported using a BSC for handling positive blood cultures.

Figure 1 - Use of Class II biological safety cabinets (BSC) for handling positive blood cultures (n=111). Note: One laboratory did not indicate how the BSC was used.



Microscopic Morphology: Brucellae are very small (0.5 to 0.7 by 1.5 µm), faintly staining, gram-negative coccobacilli whose microscopic morphology has been described as “fine sand” (1, 7, 10). These characteristics make *Brucella* especially difficult to visualize in smears from positive blood culture bottles due to background staining of red blood cells and other debris. Despite these difficulties, Gram stain is one of the key tests for those laboratories with limited microbiology resources, who may rely solely on macroscopic and microscopic characteristics of this organism to rule-out or refer potential isolates to ND-PHL (ND LRN Reference Laboratory). The Gram stain results reported by participating laboratories are summarized in **Figure 2**.

Figure 2 – Reported Gram stain results (n=112)
Intended result: Gram-negative coccobacilli or bacilli/rods



Isolation Methods: While brucellae are slow-growing organisms, they are not nutritionally fastidious and will grow on a variety of laboratory media, including sheep blood and chocolate agars. Although growth on MacConkey is variable, Thayer-Martin agar will also support the growth of most *Brucella* strains and is a good choice if a selective agar is needed. Plates should be taped shut and incubated in 5-10% CO_2 for seven days before discarding as no growth. Primary cultures of brucellae will show very little or no visible growth on chocolate or blood agar at 24 hours. After 48 hours or more, colonies are tiny, raised, white to cream, glistening, and nonhemolytic. Growth in commercial blood culture systems may be delayed until 3 to 4 days after inoculation due to slow growth of the organism, although most modern automated blood culture systems are able to detect growth of brucellae within the routine incubation period (7, 10). For those facilities that use biphasic or manual broth blood culture techniques, current guidelines recommend incubation for 21 days with blind subculturing every 7 days followed by terminal subculture (1).

Biochemical Identification: The biochemical differences between *Brucella* species and several similar organisms are summarized **Table 1**. *Brucella* may be confused with *Haemophilus* because of its Gram stain morphology and similar biochemical profile; however, *Brucella* species do not require Factors X and V. The *Brucella* LRN Advanced Sentinel Laboratory algorithm for presumptive identification or rule out of *Brucella* species includes catalase, oxidase, urea and nitrate (the complete algorithm can be found at the end of this document). All *Brucella* species are positive for catalase, oxidase, and urea, with *B. suis* strains typically turning urea positive within 5 minutes (1).

Table 1 - Common biochemical reactions for *Brucella* and similar organisms.

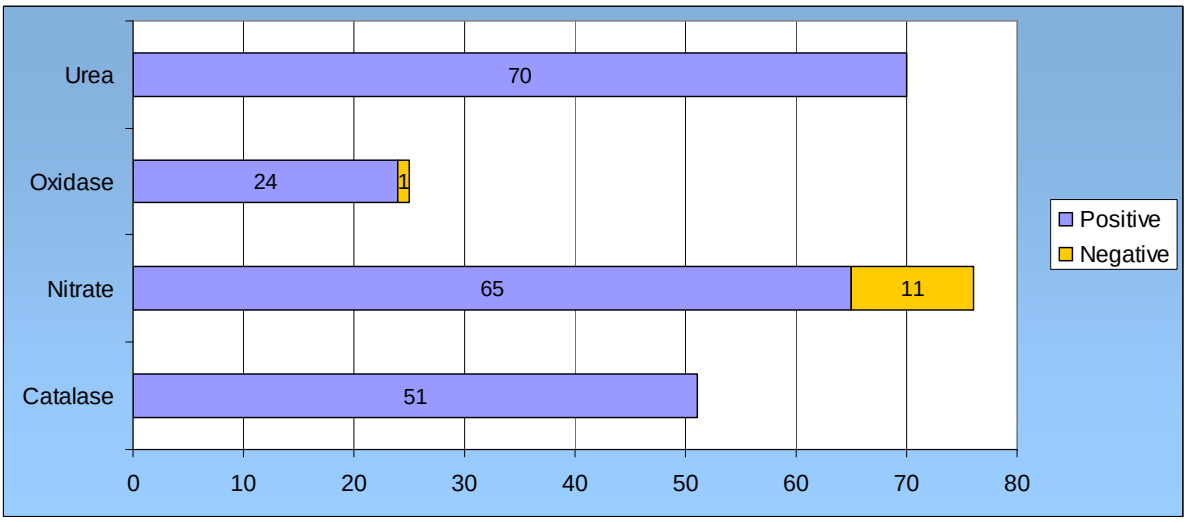
	***Brucella* spp.**	***Oligella ureolytica***	***Francisella tularensis***	***Haemophilus influenzae***	***Psychrobacter phenlpyruvicus****	***Bordetella bronchiseptica***
Urea	+	+	-	+/-	+	+
Oxidase	+	+	-	v	+	+
Nitrate	+	+	-	+	+/-	+
Motility	-	+	-	-	-	+

[* Plump gram negative coccobacilli]

It is important to note that commercial identification systems should not be used to attempt identification of *Brucella* species. The Vitek 2 platform is able to identify *B. melitensis* only, and most other systems currently do not include *Brucella* species in the database of identified organisms, resulting in a higher probability of misidentification (7). In addition, preparation of bacterial suspensions used in

some automated identification systems can generate potentially dangerous aerosols that may put laboratorians at risk of exposure. Any slow-growing organism with colony and microscopic morphologies consistent with *Brucella* that are positive for catalase, oxidase, and urea can be presumptively identified as *Brucella* species and should be submitted to ND-PHL (LRN Reference Laboratory) for confirmation (1, 7, 10). **Figure 3** summarizes the results obtained for key biochemical reactions by participating laboratories. It should be noted that 54% of participating laboratories reported acceptable responses for this organism in 2006, while 70% gave acceptable responses in 2010. This represents a significant improvement in the ability of ND sentinel site laboratories to recognize the characteristics of *Brucella* and refer potential isolates to ND-PHL.

Figure 3 – Results reported by participating laboratories for key biochemical reactions (n=112)



Reportable Disease Rule: In North Dakota, all cases of suspected or confirmed brucellosis must be reported to NDDoH; Division of Disease Control immediately by telephone due to the significant public health threat that *Brucella* represents. If your laboratory is unable to rule out *Brucella* species in any specimen as outlined in the guidelines for LRN Advanced Sentinel Laboratories, **the isolate must be referred to the LRN Reference Laboratory (ND-PHL) and <u>not</u> to your regular reference laboratory**. ND-PHL has rapid and classic LRN methods that can confirm a presumptive identification of *Brucella* and identify the species. Since regular commercial laboratories do not have these LRN assays, referral to a commercial reference laboratory may delay confirmation and species identification and put additional laboratory personnel at risk for exposure. Among participating laboratories, 74 (66%) reported that this isolate would be referred to ND-PHL, or that they would contact ND-PHL for further instructions. For more information about the North Dakota Communicable Disease Reporting Rule, please visit: http://www.health.state.nd.us/Disease/Documents/ReportableConditions.pdf

Specimen #4, Tabulated Results

112/119 (94%) Laboratories performed routine blood cultures

ORGANISM IDENTIFICATION

Identification: Intended result = ***Oligella ureolytica***

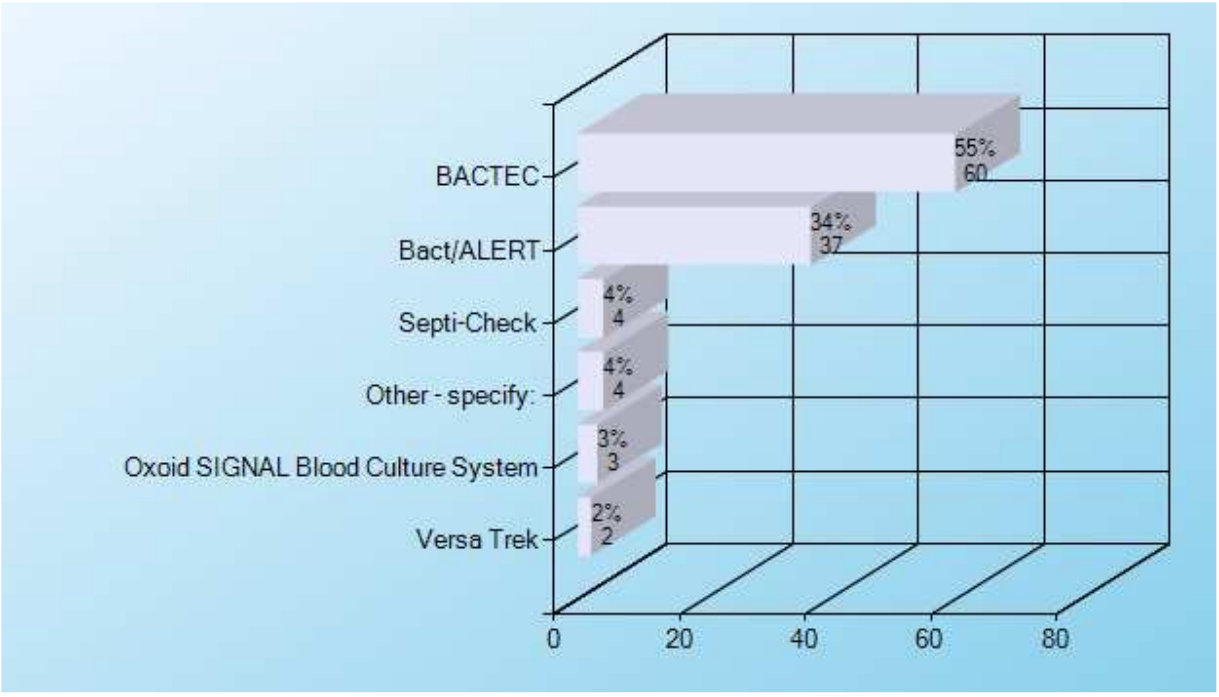
Acceptable Answers (78/112 = 70%):

48/112 (43%) Possible *Brucella* species, would refer to state-PHL
14/112 (13%) *Oligella ureolytica*
12/112 (11%) Gram-negative coccobacilli, NOS; would refer to state-PHL
2/112 (2%) Oxidase-positive gram-negative coccobacilli; would refer to state-PHL
1/112 (<1%) Gram-negative bacilli, NOS; would refer to state-PHL
1/112 (<1%) *Oligella* species

Unacceptable Answers (34/112 = 30%): The following responses, when submitted individually, and any responses that did not indicate referral to ND-PHL, were considered unacceptable. **Note:** An identification of *Brucella* species was considered unacceptable since the methods available to most clinical laboratories in North Dakota only allow presumptive identification of these organisms. Any suspected isolate of *Brucella* species should be submitted to the LRN Reference Laboratory (ND-PHL) for confirmatory testing.

8/112 (7%)	Gram negative coccobacilli, NOS; would not refer to appropriate state-PHL
4/112 (4%)	No identification reported
3/112 (3%)	Possible *Haemophilus* species
3/112 (3%)	*Brucella* species
1/112 (<1%)	Possible *Brucella* species; would not refer to state-PHL
1/112 (<1%)	*Bordetella bronchiseptica*
1/112 (<1%)	*Burkholderia cepacia*
1/112 (<1%)	Possible *Francisella* species
1/112 (<1%)	Gram-negative bacilli, NOS; would not refer to state-PHL
1/112 (<1%)	Gram-positive bacilli, NOS
1/112 (<1%)	Gram-positive cocci, NOS
1/112 (<1%)	*Haemophilus influenzae*
1/112 (<1%)	*Haemophilus* species
1/112 (<1%)	*Klebsiella pneumoniae*/Gram-negative bacilli, NOS
1/112 (<1%)	*Klebsiella pneumoniae*/Gram-positive cocci, NOS
1/112 (<1%)	*Micrococcus* species
1/112 (<1%)	*Oligella urethralis*
1/112 (<1%)	*Pantoea* species
1/112 (<1%)	*Pasteurella* species
1/112 (<1%)	*Staphylococcus aureus*

Figure 4 – Blood culture systems used by participating laboratories (n=110). Two laboratories that submitted identification for this isolate did not indicate which blood culture method was used.

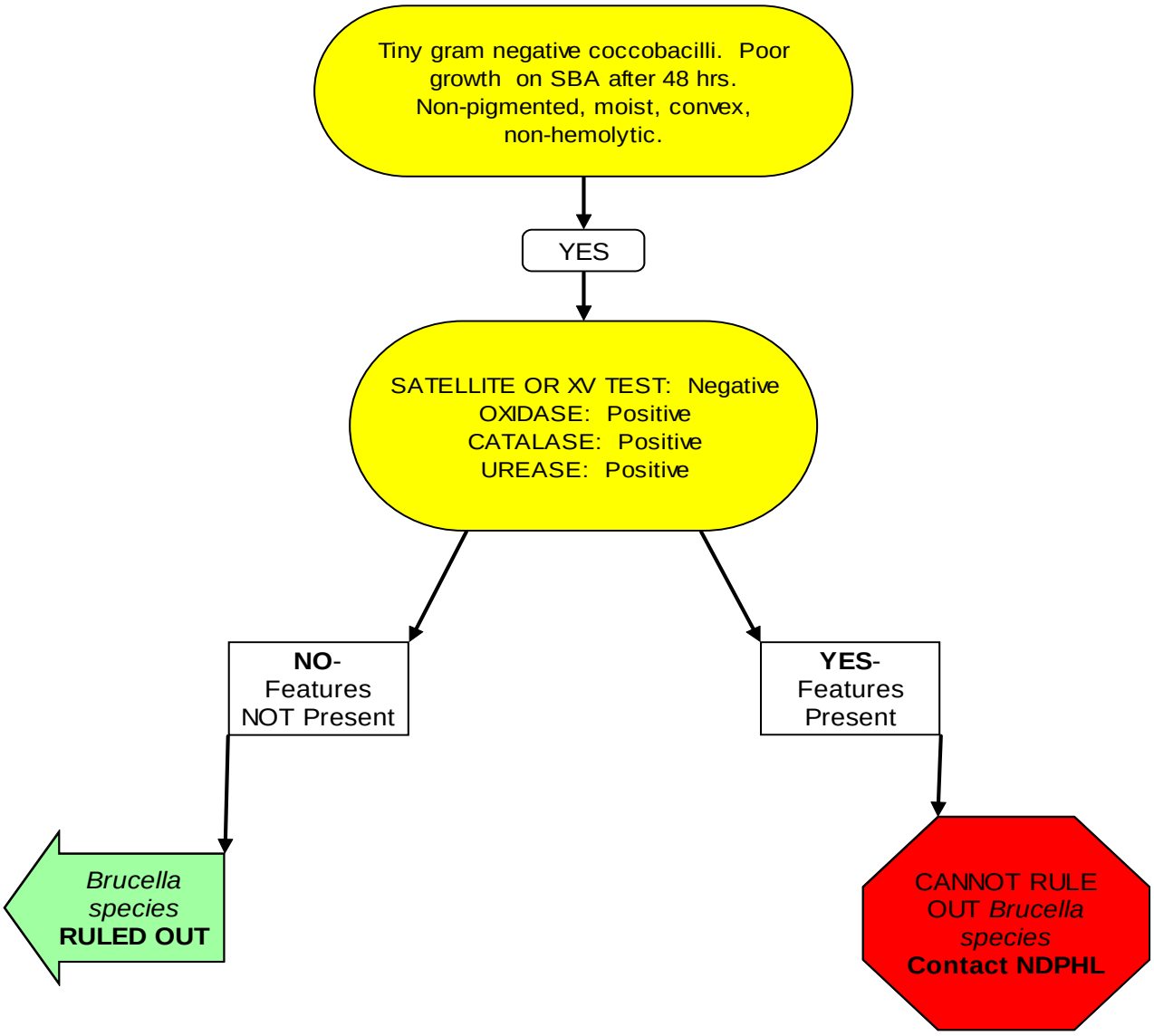


REFERENCES

1. American Society for Microbiology [Internet]. Washington, DC: Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism, *Brucella* spp. [modified 2004 Oct, accessed 2010 June 3]. Available from: http://www.asm.org/images/pdf/Brucella101504.pdf
2. Baron EJ, Miller JM. Bacterial and fungal infections among diagnostic laboratory workers: evaluating the risks. Diagn Microbiol Infect Dis. 2008;60(3):241-246.
3. Centers for Disease Control and Prevention. Laboratory-acquired brucellosis --- Indiana and Minnesota, 2006. MMWR Morb Mortal Wkly Rep. 2008;57(02):39-42.
4. Centers for Disease Control and Prevention (US) [Internet]. Atlanta, GA: Brucellosis (*Brucella melitensis*, *abortus*, *suis*, and *canis*) [modified 2005 Oct, accessed 2010 June 3]. Available from: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/brucellosis_t.htm
5. Department of Health and Human Services (US). Possession, Use and Transfer of Select Agents and Toxins, Final Rule 42 CFR part 72 and 73. Federal Register. 2005; 70(52):13294-13325.
6. Department of Transportation (US). 49 CFR Parts 171, 172, 173, and 175 Hazardous Materials: Infectious Substances; Harmonization with the United Nations Recommendations; Final Rule. Federal Register. 2006;71(106):32243-32263.
7. Lindquist D, Chu MC, and Probert WS. *Francisella* and *Brucella*. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, and Landry ML, editors. Manual of Clinical Microbiology. 9th ed. Vol 1. Washington, DC: ASM Press; 2007. p. 815-834.
8. The National Institutes of Health (US) and Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, D.C.: U.S. Government Printing Office; 2007.
9. Robichaud S, Liman M, Behr M, Rubin E. Prevention of laboratory-acquired brucellosis. Clin Infect Dis. 2004;38:e119-22.
10. Robinson-Dunn B, Shah S, Jacobson R, Luper D. Brucellosis—*Brucella* spp. In: Garcia LS, editor. Clinical Microbiology Procedures Handbook. 3rd ed. Vol 3. Washington, DC: ASM Press; 2007. p. 16.6.1-16.6.5.
11. World Health Organization, Food and Agriculture Organization of the United Nations, World Organisation for Animal Health. Treatment of brucellosis in humans. In: Brucellosis in humans and animals. Geneva, Switzerland: WHO Press; 2006. p. 36-41.
12. Yagupsky P, Baron EJ. Laboratory exposures to brucellae and implications for bioterrorism. Emer Infect Dis. 2005;11(8):1180-1185.
13. Young EJ. *Brucella* Species. In: Mandell GL, Bennett JE, and Dolin R, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 7th ed. Vol 2. Philadelphia: Churchill Livingstone Elsevier; 2010. p. 2921-2925.

NDLRN Bench Guide for Bioterrorism Agents

RULE-OUT/REFERRAL FOR BRUCELLA SPECIES



North Dakota Dept. of Health, Division of Microbiology 24/7 Emergency Contact Information
Monday-Friday 8 a.m. to 5 p.m. (701.328.6272)
After hours and weekends call the on call Microbiologist at: 701.400.2772 or
State Radio at (701.328.9921 or 800.472.2121)

NDLRN/MLS: Challenge Set 2010
Competency Exam

Specimen #1 – *E. coli* O157:H7

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 North Dakota 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. All of the following are performed at ND-PHL **(except)** to aid in epidemiologic investigations of O157 outbreaks? (choose one):
   a. PFGE patterns are uploaded into the National PulseNet database
   b. Pulsed-field gel electrophoresis (PFGE)
   c. Serotyping for somatic O and flagellar H antigens
   d. High-pressure liquid chromatography (HPLC)

4. **True** **False** A combination of stool culture and toxin screening methods 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 #2 – *Streptococcus* Group A (*S. pyogenes*)

1. The 26-valent vaccine for Group A *Streptococcus* (GAS) currently in clinical trials is based on immunity to the _____.
   a. Group A Lancefield carbohydrate antigen
   b. 16s ribosomal RNA sequence
   c. Catalase enzyme
   d. Surface M protein

2. Which of the following diseases is NOT typically associated with GAS?
   a. Gas gangrene
   b. Acute exudative pharyngitis
   c. Necrotizing fasciitis
   d. Acute rheumatic fever

3. The emerging M, MLS_i, and MLS_c antimicrobial resistance phenotypes can be detected and differentiated in GAS using which test?
   a. Cefoxitin disk diffusion
   b. Double-disk diffusion (D-test)
   c. Lancefield grouping
   d. β-lactamase (Cefinase) disk

4. According to the North Dakota Communicable Disease Reporting Rule laboratories must :
   a. Report invasive GAS infections to the NDDoH; Division of Disease Control
   b. Report all cases of streptococcal toxic shock syndrome
   c. Send an isolate to the ND-PHL for all reportable cases of GAS
   d. All of the above

5. **True** **False** Nosocomial outbreaks of fatal GAS infections have been linked to transient and asymptomatic carriage by healthcare workers.

Specimen #3 – *Klebsiella pneumoniae* – carbapenemase producer

1. **True** **False** Many infections caused by *K. pneumoniae* are healthcare-associated.

2. KPCs represent an emerging bacterial resistance mechanism and are currently more prevalent in which region of the United States?
   a. Southwest
   b. Pacific Northwest
   c. Midwest
   d. Northeast
   e. 'c' and 'd' above

3. Which of the following is not a carbapenem antibiotic?
   a. Imipenem
   b. Aztreonam
   c. Meropenem
   d. Ertapenem
   e. None of the above

4. Which antibiotic is not recommended for use in screening for KPC producers?
   a. Imipenem
   b. Meropenem
   c. Ertapenem
   d. None of the above

5. **True** **False** Discovering a carbapenem resistant *Klebsiella pneumoniae* is an urgent situation that requires immediate notification of the clinician and infection prevention staff.

6. Which of the following is a phenotypic confirmatory test for carbapenemase-producing *Enterobacteriaceae*?
   a. D-test
   b. Modified Hodge test
   c. Cefoxitin disk test
   d. None of the above

Specimen #4 – *Oligella ureolytica* (analog for *Brucella* species)

1. Which of the following statements most accurately describes the Gram stain morphology of *Brucella* species? (choose one)
   a. Curved, faintly-staining Gram-negative bacilli
   b. Tiny Gram-negative coccobacilli
   c. Plump Gram-negative bacilli with bipolar staining
   d. Large, boxy Gram-positive bacilli

2. Which of the following LRN Sentinel Lab test results can be used to rule out *Brucella*? (choose one)
   a. Requirement for X & V factors
   b. Oxidase positive
   c. Catalase positive
   d. Urea positive

3. Which *Brucella* species is most commonly isolated in the U.S.? (choose one)
   a. *B. abortus*
   b. *B. canis*
   c. *B. melitensis*
   d. *B. suis*

4. **True** **False** Laboratories that cannot rule out *Brucella* should send isolates to their routine reference laboratory for identification before contacting ND-PHL.

5. **True** **False** *Brucella* is one of the most common causes of laboratory-acquired infections.

NDLRN/MLS: Challenge Set 2010
Competency Exam
ANSWER SHEET (correct answers in bold)

Specimen #1 – *E. coli* O157:H7

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 North Dakota 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. All of the following are performed at ND-PHL **(except)** to aid in epidemiologic investigations of O157 outbreaks? (choose one):
   a. PFGE patterns are uploaded into the National PulseNet datatbase
   b. Pulsed-field gel electrophoresis (PFGE)
   c. Serotyping for somatic O and flagellar H antigens
   d. High-pressure liquid chromatography (HPLC)

4. **True** False A combination of stool culture and toxin screening methods 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 #2 – *Streptococcus* Group A (*S. pyogenes*)

1. The 26-valent vaccine for Group A *Streptococcus* (GAS) currently in clinical trials is based on immunity to the _____.
   a. Group A Lancefield carbohydrate antigen
   b. 16s ribosomal RNA sequence
   c. Catalase enzyme
   d. Surface M protein

2. Which of the following diseases is NOT typically associated with GAS?
   a. Gas gangrene
   b. Acute exudative pharyngitis
   c. Necrotizing fasciitis
   d. Acute rheumatic fever

3. The emerging M, MLS_i, and MLS_c antimicrobial resistance phenotypes can be detected and differentiated in GAS using which test?
   a. Cefoxitin disk diffusion
   b. Double-disk diffusion (D-test)
   c. Lancefield grouping
   d. β-lactamase (Cefinase) disk

4. According to the North Dakota Communicable Disease Reporting Rule laboratories must:
   a. Report invasive GAS infections to the NDDoH; Division of Disease Control
   b. Report all cases of streptococcal toxic shock syndrome
   c. Send an isolate to the ND-PHL for all reportable cases of GAS
   d. All of the above

5. **True** False Nosocomial outbreaks of fatal GAS infections have been linked to transient and asymptomatic carriage by healthcare workers.

Specimen #3 – *Klebsiella pneumoniae* – carbapenemase producer

1. **True** False Many infections caused by *K. pneumoniae* are healthcare-associated.

2. KPCs represent an emerging bacterial resistance mechanism and are currently more prevalent in which region of the United States?
   a. Southwest
   b. Pacific Northwest
   c. Midwest
   d. **Northeast**
   e. 'c' and 'd' above

3. Which of the following is **not** a carbapenem antibiotic?
   a. Imipenem
   b. **Aztreonam**
   c. Meropenem
   d. Ertapenem
   e. None of the above

4. Which antibiotic is **not** recommended for use in screening for KPC producers?
   a. **Imipenem**
   b. Meropenem
   c. Ertapenem
   d. None of the above

5. **True** False Discovering a carbapenem resistant *Klebsiella pneumoniae* is an urgent situation that requires immediate notification of the clinician and infection prevention staff.

6. Which of the following is a phenotypic confirmatory test for carbapenemase-producing *Enterobacteriaceae*?
   a. D-test
   b. **Modified Hodge test**
   c. Cefoxitin disk test
   d. None of the above

Specimen #4 – *Oligella ureolytica* (analog for *Brucella* species)

1. Which of the following statements most accurately describes the Gram stain morphology of *Brucella* species? (choose one)
   a. Curved, faintly-staining Gram-negative bacilli
   b. Tiny Gram-negative coccobacilli
   c. Plump Gram-negative bacilli with bipolar staining
   d. Large, boxy Gram-positive bacilli

2. Which of the following LRN Sentinel Lab test results can be used to rule out *Brucella*? (choose one)
   a. Requirement for X & V factors
   b. Oxidase positive
   c. Catalase positive
   d. Urea positive

3. Which *Brucella* species is most commonly isolated in the U.S.? (choose one)
   a. *B. abortus*
   b. *B. canis*
   c. *B. melitensis*
   d. *B. suis*

4. True **False** Laboratories that cannot rule out *Brucella* should send isolates to their routine reference laboratory for identification before contacting ND-PHL.

5. **True** False *Brucella* is one of the most common causes of laboratory-acquired infections.