

Specimen #1, Urine Culture: *Klebsiella pneumoniae*; carbapenemase producer

Submitter #:		
	Your Result	Intended Result
Identification		<i>Klebsiella pneumoniae</i>
Referral of Isolate		Would refer isolate to ND-PHL
Susceptibilities		
Ertapenem		Resistant
Imipenem		Intermediate or Resistant
Meropenem		Intermediate or Resistant
Cefotaxime		Resistant
Ceftazidime		Resistant
Ceftriaxone		Resistant
Modified Hodge Test		Positive

Intended Goal: The isolate in this specimen was a carbapenemase-producing *Klebsiella pneumoniae* with resistance mediated by the *bla_{KPC}*. It was chosen to emphasize the urgent infection control and prevention situation that these organisms present when identified. It was also chosen to illustrate the challenges laboratorians face with the ever-changing antimicrobial susceptibility testing (AST) breakpoints and guidelines.

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.(4)

The Genus *Klebsiella*: *Klebsiella* is one of two *Enterobacteriaceae* that are normally encapsulated (the other is *Raoultella*), and therefore appears mucoid when grown on agar plates. *Klebsiella* species are among the four most common causes of healthcare-associated and community-associated UTIs. Patient carriage of *Klebsiella* is highest in the stool and less frequent in the nasopharynx; however, hospitalization and antibiotic usage can increase fecal carriage rates dramatically.(1) *K. pneumoniae*, *K. oxytoca*, and *K. granulomatis* are the three *Klebsiella* species associated with human disease.(3)

***Klebsiella pneumoniae*:** Certain strains of *K. pneumoniae* can cause an array of human infections ranging from asymptomatic colonization of the intestinal, urinary, and respiratory tracts to potentially fatal pneumonia, septicemia, and meningitis.(4) Most infections caused by *K. pneumoniae* are healthcare-associated or occur in patients that have other underlying conditions that leave them vulnerable to disease. *K. pneumoniae* is second only to *E. coli* in causing bacteremia secondary to a UTI.(3)

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.(4) In this challenge, nearly all participants correctly identified this organism (116/118 – 98%), which is readily identified by automated methods.

Antimicrobial Resistance Mechanisms: *Klebsiella* is inherently resistant to carbenicillin and ampicillin.(4) Some strains also produce extended spectrum β -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.(9) Because of the significant treatment and infection control challenge these organisms present, it is important that laboratories have a process for identifying carbapenem resistant *Enterobacteriaceae* (CRE).

The Infection Control “Emergency” Surrounding Carbapenemase-producing Organisms: The carbapenems most frequently used in a clinical setting are ertapenem, imipenem and meropenem. 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. In addition, carbapenemases 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 why carbapenemase-producing *Enterobacteriaceae* can cause an infection control “emergency” in a health care setting and why it is important for laboratorians to quickly recognize and accurately report these organisms to clinicians so that infection prevention and control measures can be implemented. It is important that clinical laboratories have protocols in place for prompt notification of infection prevention staff of all *Enterobacteriaceae* isolates that are non-susceptible to carbapenems or test positive for a carbapenemase.(7) Not all carbapenem resistance in *Enterobacteriaceae* is mediated by a carbapenemase. However, regardless of mechanism, any CRE is clinically significant and should be regarded as an infection control issue. (6) See “Infection Prevention measures following identification of a KPC” later in the document.

Among the 116 laboratories that correctly identified the organism in this challenge, 84 (72%) indicated that infection control, the nursing station, the physician, or some combination of the three, would be notified with the results. Seven laboratories indicated that they would only notify their PHL and 25 laboratories would take no further action.

About Carbapenemases: Carbapenemases are a sub-class of β -lactamase enzymes that are classified by their specific resistance mechanisms. Due to the complexity and variety of β -lactamase resistance mechanisms (including AmpC and others), this document will concentrate on the *Klebsiella pneumoniae* carbapenemase (KPC). KPC is a serine β -lactamase that is found in several different genera of *Enterobacteriaceae* (such as *K. oxytoca*, *E. coli*, *C. freundii*, *S. marcescens*, and *E. cloacae*), but most commonly in *K. pneumoniae*.(6,8) KPC represents an emerging bacterial resistance mechanism and is currently more prevalent in the northeastern part of the U.S. (New York, New Jersey and Maryland), although it is being seen with more frequency in other parts of the country.(6)

Laboratory Challenge in the Detection of Carbapenemases: There are several challenges in detecting KPC production; one is recognizing that a KPC-producing organism may not demonstrate resistance to carbapenems using the current CLSI breakpoints when employing common antimicrobial susceptibility testing (AST) methods in clinical laboratories. Even if a KPC-producing isolate tests susceptible under current CLSI breakpoints, there is potential for therapeutic failure when using a carbapenem.(6) A second 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 is suggested to be ertapenem.(8) An additional challenge is that the phenotypic laboratory methods available for confirming KPC (e.g. modified Hodge test) can also produce positive or equivocal results for organisms that display other mechanisms of resistance. While there are PCR methods for the detection of the *bla* gene

(*bla* stands for β -lactamase), which are specific for production of the KPC enzymes, these tests are mainly used in reference lab settings. (8)

Screening Methods for Suspected Carbapenemases: The Clinical and Laboratory Standards Institute (CLSI) M100-S20 states: **“Enterobacteriaceae that are resistant to one or multiple agents in cephalosporin subclass III (Cefoperazone, Cefotaxime, Ceftazidime, Ceftizoxime, Ceftriaxone) and that demonstrate elevated MICs or reduced disk zone diameters to carbapenems may produce a carbapenemase despite the fact that the MICs or zone diameters may fall within the current susceptible range.”**(2) Therefore, it is suggested that laboratories review, daily, all *Enterobacteriaceae* AST reports for elevated carbapenem MIC values in order to detect possible KPCs. If there are susceptible but elevated MICs to any of the carbapenems, further testing should be considered using one of the methods described below. 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 flag positive for ESBL.(9)

Disk diffusion testing: CLSI recommends screening for carbapenemases by performing disk diffusion with ertapenem (10 μ g) or meropenem (10 μ g) disks. Using imipenem disks has been shown in several studies to be a less sensitive indicator of KPC production; furthermore, ertapenem does not display inoculum-effect like imipenem and meropenem.(2,6) Zone sizes of 19-21 mm for ertapenem and 16-21 mm for meropenem are considered screen positive, even though these are in the current “susceptible” interpretive categories; labs should proceed to the modified Hodge Test (MHT) for confirmation. CLSI also recommends broth microdilution as an alternative screening method; see the CLSI M100-S20 for further details.

Minimum Inhibitory Concentration (MIC) testing: When KPC isolates test susceptible to imipenem or meropenem, they often demonstrate elevated MICs of 2 to 4 μ g/mL. One study involving several automated AST methods showed that observation of MIC values \geq 1 μ g/mL for meropenem or imipenem were sensitive indicators for the presence of a KPC enzyme.(6) However, detecting these slightly elevated MIC values is a challenge since a large portion of the automated susceptibility panels and cards that laboratories are currently using do not have dilutions low enough to detect an MIC of 1 μ g/mL. In some cases, the lowest MIC value is \leq 4 μ g/mL. ND-PHL encourages all laboratories to contact technical representatives of their automated systems to upgrade susceptibility cards and panels to newer versions that have lower MIC limits for the carbapenems.

There is debate among experts as to whether detection of KPC should be based on the combination of AST results for the third generation cephalosporins and elevated MIC’s to the carbapenems (as suggested by CLSI), or whether elevated carbapenem MICs alone are indicative. These issues were no doubt part of the impetus for the changes to the carbapenem breakpoints in the supplement to the CLSI M100-20S, estimated to be released in spring 2010 (see section below: “New CLSI Carbapenem Breakpoints”). (6) It is important that laboratories study these issues and develop internal protocols to enable detection of KPCs.

Confirmation of suspected carbapenemases: The M100-20S document also states that: **“It is not necessary to test an isolate for carbapenemase by the modified Hodge test when all of the carbapenems that are reported by a laboratory test either intermediate or resistant (ie, these carbapenem AST results should be reported as tested). However, the modified Hodge test may be useful in this case for infection control and epidemiological purposes.”** (2) For phenotypic confirmation of carbapenemase producing organisms, CLSI recommends the MHT using either ertapenem or meropenem disks. Please see pages 48-51 in the CLSI M100-S20 standards for the entire protocol for both screening and confirmation of carbapenemases.(2) CDC’s protocol for the MHT has also been included in this packet. If your laboratory detects an isolate with intermediate or resistant carbapenems, notify appropriate healthcare partners then proceed to the MHT to determine if it is a carbapenemase producer; if positive, please send an isolate to ND-PHL for banking. ND-PHL will refer the isolate to the Centers for Disease Control and Prevention or another reference laboratory for PCR when warranted. The MHT is capable of detecting carbapenemase production however it does not identify KPC as the specific mechanism. If your laboratory has isolated a *K. pneumoniae*

that is MHT positive (also designated as a CRE), it is statistically very likely that the isolate is a KPC producer. If the MHT is uninterpretable, please call ND-PHL to discuss the isolate further.

How to report AST results: When your laboratory discovers an isolate that tests susceptible by standard AST methods to a carbapenem, but positive by MHT or *bla*_{KPC}, then it is suggested by CLSI that you report the carbapenem MIC without an interpretation, with the following comment: **“This isolate demonstrates carbapenemase production. The clinical efficacy of the carbapenems has not been established for treating infections caused by *Enterobacteriaceae* that test carbapenem susceptible (eg, MIC ertapenem ≤ 2 µg/mL, imipenem ≤4 µg/mL, and/or meropenem ≤4 µg/mL) but demonstrate carbapenemase production in vitro.”** (2)

What to do if your lab identifies a Carbapenemase-producing *Enterobacteriaceae*: It is important that there is close collaboration between the laboratory, clinician, infection preventionist, and pharmacy. If your laboratory detects a carbapenem resistant *Enterobacteriaceae* (CRE) via MHT or a KPC via *bla*_{KPC} PCR, after notifying appropriate healthcare partners, please notify the North Dakota Department of Health; Division of Disease Control at: 1-800-472-2180 or 701-328-2378 and submit the isolate to the ND-PHL. In the past 18 months, MDH-PHL has confirmed 16 KPC isolates submitted from North Dakota and Minnesota laboratories, including 10 *K. pneumoniae* isolates, one *E. coli*, one *Citrobacter freundii*, and most recently four *Enterobacter cloacae*. Confirmation is done by performing the *bla*_{KPC} PCR and microbiological confirmation of the isolate identification.

While the North Dakota Department of Health and MDH will continue to monitor this emerging resistance issue and assist laboratories in characterizing appropriate isolates, all laboratories are strongly encouraged to institute the modified Hodge test or have access to a reference laboratory that can perform confirmatory testing. In this challenge, 23 laboratories performed the MHT with 22 (96%) of the labs correctly indicating a positive result.

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. In addition, the 3rd generation cephalosporins 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. Those laboratories providing no interpretation were also correct, based on the CLSI guidelines for reporting a KPC. See **Table 1** for carbapenem results and **Table 2** for 3rd generation cephalosporin results reported by participating laboratories.

Table 1: AST results from 87 laboratories reporting carbapenems

Antibiotic	Ertapenem (n=36)	Imipenem (n=76)	Meropenem (n=22)
Report = non-susceptible (I or R)	34 (94%)	60 (78%)	17 (77%)
Report = susceptible	1 (3%)	13 (19%)	2 (9%)
No interpretation provided	1 (3%)	3 (4%)	3 (14%)

Note: some labs reported more than one carbapenem

Table 2: AST results from 108 laboratories reporting 3rd Generation Cephalosporins

Antibiotic	Cefotaxime (n = 35)	Ceftazidime (n=65)	Ceftriaxone (n=88)
Report = resistant	24 (69%)	57 (88%)	69 (78%)
Report = intermediate	0	0	0
Report = susceptible	2 (6%)	0	8 (9%)
No interpretation provided	9 (26%)	8 (12%)	11 (13%)

Note: some labs reported more than one 3rd generation cephalosporin

Infection Prevention measures following identification of a KPC

Upon identification of the first CRE/KPC in your healthcare facility*:

- Lab: Alert infection prevention and clinical staff.
- Infection prevention and control: Place patient in Contact Precautions regardless of whether the isolate represents colonization or infection
- Clinical staff: Adjust treatment regimen as appropriate for clinical management
- Education: Inform staff, visitors, patients of infection prevention steps

Upon identification of subsequent CRE cases in your healthcare facility*:

- Lab and Infection Prevention & Control: follow measures above.
- Vigorously reinforce infection prevention measures for all healthcare personnel and visitors entering the patient's.
- Conduct a single round of active surveillance testing** of patients with epidemiological links to the most recent CRE case (e.g., those on the same unit or who shared healthcare staff)
 - If additional CRE case(s) are identified, repeat active surveillance cultures weekly until no new cases identified. Place all CRE patients in Contact Precautions
 - Once no new cases are identified, initiate periodic point prevalence surveys in high risk units.
- Simultaneously, review microbiology records for the previous 6 months preceding identification of the second CRE case to identify previously unrecognized CRE cases
 - If previously unrecognized cases are identified, conduct a single round of active surveillance cultures** in units with high-risk patients (e.g. units where CRE cases were previously identified).

*Additional infection prevention and control guidance can be found at:

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5810a4.htm?s_cid=mm5810a4_e

** The CDC "Laboratory Protocol for Detection of Carbapenem-Resistant or Carbapenemase-Producing, *Klebsiella* spp. and *E. coli* from Rectal Swabs" and the CDC "Modified Hodge Test for Carbapenemase Detection in *Enterobacteriaceae*" can be found under 'Latest Features' on the ND-PHL website

<http://www.ndhealth.gov/microlab/Default.aspx>.

Reportable Disease Rule: CRE isolates are not currently included as part of North Dakota's Mandatory Reportable Conditions, however, approval to include CRE isolates as part of the requirement is currently pending. The NDDoH requests that infection preventionists contact the Division of Disease Control during regular business hours to report these cases at 1-800-472-2180 or 701-328-2378. The NDDoH is available for consultation regarding laboratory testing and patient management, including surveillance and infection prevention and control measures. Furthermore, laboratories should submit isolates to the ND-PHL for banking and possible additional testing.

New CLSI Carbapenem Breakpoints: CLSI is expected to release a supplement to the M100-20S document in spring, 2010, which will lower the carbapenem breakpoints. This will theoretically eliminate the need to perform additional testing for carbapenemase production, such as the MHT, since most KPC producers would be interpreted as resistant using the lowered breakpoints. However, the new breakpoints are not without controversy and may be debated for some time. One argument is that clinicians should be informed when resistance is due specifically to carbapenemase production for patient management, infection control and epidemiologic reasons. In order to implement the new breakpoints, some clinical laboratories may choose to wait until manufacturers of their automated 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. See the new proposed carbapenem breakpoints for the CLSI M100-S20 Spring Supplement below in **Table 3**.

Table 3: Carbapenem breakpoints CLSI M100 S19 and S20 (spring supplement) (5)

	CLSI M100-S19 (2009)			CLSI M100-S20 (2010) - Supplement		
	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

A note about ESBLs: The isolate in this challenge set was also tested for ESBL in both the MDH-PHL and CDC laboratories. The results obtained demonstrate the incredible challenge phenotypic tests present when trying to accurately determine resistance. In the MDH-PHL the isolate screened positive for ESBL, but the CLSI disk confirmation test results were not clearly interpretable. The isolate was also tested at the CDC-AST laboratory with similar results. In MDH-PHL interactions with CDC, the CDC-AST laboratory personnel 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}, which was positive. With the highly resistant susceptibility pattern of this challenge isolate, the determination of ESBL is not necessary for clinical treatment.

It is important to note that the newest CLSI M100-S20 standards document has significantly revised breakpoints when testing *Enterobacteriaceae* for some of the cephalosporins and aztreonam. The breakpoints have been lowered, which may result in increased reporting of true resistance and a decreased need to perform phenotypic (double disk) testing for ESBL determination. See **Table 4** below for the new breakpoints.

Table 4: ESBL revised breakpoints CLSI M100 S19 and S20 (5)

	CLSI M100-S19 (2009)			CLSI M100-S20 (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

Specimen #1, Tabulated Results

118/118 (100%) = Laboratories performed urine cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = *Klebsiella pneumoniae*

Acceptable Answers (116/118 = 98%)

95/116 (82%) *Klebsiella pneumoniae*

2/116 (2%) *Klebsiella pneumoniae*; carbapenemase producer

5/116 (4%) *Klebsiella pneumoniae*; possible carbapenemase producer

4/116 (3%) *Klebsiella pneumoniae*; carbapenemase producer; Extended Spectrum β-lactamase (ESBL) producer

3/116 (3%) *Klebsiella pneumoniae*; possible carbapenemase producer; ESBL producer

3/116 (3%) *Klebsiella pneumoniae*; possible ESBL producer

2/116 (2%) *Klebsiella pneumoniae*; possible carbapenemase producer; possible ESBL producer

2/116 (2%) *Klebsiella pneumoniae*; Extended Spectrum β-lactamase (ESBL) producer

Unacceptable Answers (2/118 = 2%)

1/2 (50%) *Enterobacter aerogenes*

1/2 (50%) *Legionella* sp.

Antibiotics reported by participating labs

[*Carbapenems **3rd Generation Cephalosporins]

Antimicrobial Agent	MIC					KB disk diffusion				
	# of labs reporting antibiotic	No interp	S	I	R	# of labs reporting antibiotic	No interp	S	I	R
Amikacin	55	1	52	1	1					
Amoxicillin	1		-	-	1					
Amox-Clav	66	3	-	-	63	1	-	-	-	1
Ampicillin	96	6	-	-	90	2	-	-	-	2
Amp-Sulbactam	71	3	-	-	68	1	-	-	-	1
Aztreonam	31	7	-	-	24					
Carbenicillin	3	-	-	-	3					
Cefazolin	98	6	-	-	92	2	-	-	-	2
Cefepime	59	5	-	2	52					
Cefotaxime**	35	9	2	-	24					
Cefotax-Clav	3	-	-	-	3					
Cefotetan	10	-	4	2	4					
Cefoxitin	41	-	-	-	41	1	-	-	-	1
Cefpodoxime	2	-	-	-	2					
Ceftazidime**	63	8	-	-	55	2	-	-	-	2
Ceftaz-Clav	2	2	-	-	-					
Ceftizoxime**	1	-	-	-	1					
Ceftriaxone**	86	11	-	8	67	2	-	-	-	2
Cefuroxime	41	4			37					
Cephalothin	18	3	-	-	15	1	-	-	-	1
Chloramphenicol	2	-	-	-	2					
Ciprofloxacin	104	3	-	-	101	2	-	-	-	2
Ertapenem*	35	1	1	-	33	1	-	-	-	1
Gatifloxacin	5	-	-	1	4					
Gemifloxacin	4	-	-	1	3					
Gentamicin	108	4	18	69	17	3	-	2	1	-
Imipenem*	77	3	14	21	39	1	-	-	-	1
Levofloxacin	82	3	-	-	89					
Meropenem*	20	3	3	-	14	2	-	-	1	1
Minocycline	2	-	1	-	1					
Moxifloxacin	4	1	-	-	3					
Nalidixic Acid	2	-	-	-	2					
Nitrofurantoin	95	7	-	5	83	3	-	-	-	3
Norfloxacin	5	-	-	-	5					
Piperacillin	9	-	-	-	9					
Pip-Tazobactam	80	4	-	-	76	1	-	-	-	1
Tetracycline	46	1	20	24	1	1	-	-	1	-
Ticarcillin	1	-	-	-	1					
Ticar-Clav	30	-	-	-	30	1	-	-	-	1
Tobramycin	68	2	-	20	46	1	-	-	-	1
Trimethoprim	7	-	-	-	7					
TMP/SXT	91	6	5	-	80	1	-	-	-	1

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Specimen #2 Blood: *Haemophilus influenzae* serotype f (Hif)

Submitter #:		
	Your Laboratory's Results	Intended Result
Identification	Not Performed	<i>Haemophilus influenzae</i>
Referral of Isolate	Not Performed	Would refer isolate to ND-PHL
Gram stain		Gram-negative bacilli or coccobacilli
Factors X and V		Requires both X and V Factors
Urea		Positive
β-lactamase		Positive

Goal: The organism in specimen #2 was *Haemophilus influenzae* serotype f (Hif). This β-lactamase positive organism was chosen to exercise LRN Sentinel Laboratories' ability to rule-out or refer potential bioterrorism organisms (such as *Francisella tularensis* and *Brucella* species) to the North Dakota Department of Health -PHL. This organism was also chosen to highlight the public health significance of *H. influenzae* serotypes other than serotype b (Hib) in the context of widespread Hib vaccination.

The genus *Haemophilus*: Most species of the genus *Haemophilus* are commensal gram-negative, non-motile rods that are adapted solely to mucosal membranes of the human upper respiratory tract, where they make up about 10% of the normal bacterial flora (4,7). Microscopically, *Haemophilus* may have a pleomorphic appearance ranging from tiny coccobacilli to long, filamentous rods, which can lead to misinterpretation of direct Gram stains such as CSF and blood culture smears (4,10). All species of *Haemophilus* are facultative anaerobes, but have various aerobic requirements for hemin (Factor X) and nicotinamide adenine dinucleotide (NAD, or Factor V) (10). The name "*Haemophilus*" originates from the *in vitro* growth requirement of these two factors, which are found in blood (4,10).

About *Haemophilus influenzae*: *Haemophilus influenzae* is a component of the normal oropharyngeal flora in healthy humans, which are the only known host for the organism. Colonization of the upper respiratory tract occurs in early childhood through person-to-person droplet transmission or by direct contact with respiratory secretions (4,10). The organism exists as both encapsulated and non-encapsulated forms. Non-encapsulated strains are primarily associated with otitis media, community-acquired pneumonia, sinusitis, and conjunctivitis, particularly in children aged 6 months to 5 years (10).

Strains expressing a polysaccharide capsule can be classified into six antigenically distinct serotypes designated 'a' through 'f'. *H. influenzae* serotype b (Hib) is the serotype most closely associated with significant disease in humans, causing bacterial meningitis, septicemia, and primary pneumonia (4,10). Hematogenous spread of both non-encapsulated and encapsulated strains, including Hib, can also result in septic arthritis, osteomyelitis, cellulitis, and pericarditis (4). Mortality from invasive Hib disease is highest among children under the age of 5 years, especially those between four and 18 months (11). While prompt and appropriate treatment results in only a 5% mortality rate in Hib meningitis cases, 15-35% of survivors develop permanent sequelae, including mental retardation and hearing loss (10,14).

Hib Vaccination: A polysaccharide-conjugate vaccine directed against Hib has been widely available in the U.S. since the early 1990s (11). Carriage rates among unvaccinated children previously ranged from 4-6%; with the introduction of the Hib vaccine, the carrier state for Hib has been all but eliminated from the vaccinated population (4). As a result, fewer than 100 cases of serious Hib disease occur annually in the U.S., and all occur in unvaccinated or incompletely vaccinated children (4). However, Hib still causes more than 3 million cases of serious disease and an estimated 386,000 deaths globally each year among unvaccinated children (14). Minnesota attracted national attention in 2008 when five confirmed cases of invasive Hib disease, the highest number since the beginning of widespread Hib vaccination, were reported to MDH. All five patients were children ages 5 months to 3 years; three families had declined

Hib primary vaccination, including a child that died (5). A survey in 2009 by the MDH Infectious Disease Epidemiology, Prevention, and Control division and the Centers for Disease Control and Prevention did not find Hib carriage in children, but confirmed a decrease in both Hib primary immunization coverage and Hib booster vaccination, during a temporary vaccine shortage from December 2007 through July 2009. These declines in vaccination may have led to decreased herd immunity to a level sufficient for transmission of Hib during this period, which subsequently placed unvaccinated and vulnerable children at increased risk of invasive Hib disease. The same survey did find carriage of other *H. influenzae* serotypes and nontypeable *H. influenzae* among young children. (2)

Serotypes other than Hib: As vaccination continues to diminish the number of cases of serious Hib disease, other nonencapsulated and encapsulated strains (particularly *H. influenzae* serotype f, or Hif) have emerged as significant causes of disease. Hif has been linked to serious invasive disease in pediatric and adult patients with underlying health conditions such as malignancy, alcoholism, and HIV infection (1,4,13). Studies have indicated that the highest rates of invasive Hif disease, and a subsequent increase in mortality, occur in adult patients over the age of 65 with predisposing conditions, followed by patients under the age of 5 years (3,13). While the proportion of invasive disease caused by Hif has increased following the introduction of the Hib vaccine, the overall incidence of invasive Hif disease remains low, suggesting that Hif has a decreased virulence compared to that of Hib (13).

Antibiotic resistance: While wild-type isolates of *H. influenzae* are sensitive to most antimicrobials, the spread of conjugative plasmids has led to increasing rates of antibiotic resistance in clinical isolates, particularly to the β -lactam class. In some countries, up to 60% of clinical isolates of *H. influenzae* can be resistant to ampicillin (4). Among Hif isolates from Western countries, 26-33% have been reported to express β -lactamase, a trend that mirrors the well-established problem of β -lactam resistance in Hib (1,13). In addition, an increasing number of Hif isolates have demonstrated resistance to macrolides (erythromycin, clarithromycin), tetracycline, trimethoprim-sulfamethoxazole, rifampin, and chloramphenicol (1). Multiple drug resistance is still uncommon, but does occur in strains that have accumulated multiple resistance plasmids (1). Isolation of *H. influenzae* from any normally sterile site is always clinically significant; therefore, antimicrobial susceptibility testing (AST) would be indicated for this isolate (4). Among participating laboratories 43% (49/113) reported that this blood isolate would routinely undergo AST, 50% (57/113) reported that AST would not be performed on this isolate, and 6% (7/113) did not indicate whether AST would be performed. This isolate is also positive for β -lactamase activity; of the 43 laboratories that reported testing for β -lactamase, 100% indicated the correct response.

Laboratory Identification of *H. influenzae*

Collection and Transport: When invasive *H. influenzae* disease is suspected, the preferred specimens for culture are blood and cerebrospinal fluid (CSF) (4). However, *H. influenzae* may be isolated from a variety of clinical specimens, including sterile body fluids, sputum, purulent discharge from infected eyes, inner ear aspirates, and throat and nasopharyngeal swabs. In all cases, prompt transport to the laboratory for processing or direct plating of the specimen at the time of collection is preferred since *Haemophilus* is susceptible to desiccation. In addition, timely processing of CSF and blood specimens allows the fastest possible diagnosis for guiding patient treatment decisions (4).

Laboratory Safety: Due to the risk of invasive disease, clinical specimens and cultures known or suspected to contain *H. influenzae* should be handled in a BSL2 laboratory (11). Any procedure that may produce infectious aerosols (such as catalase testing, vortexing of bacterial suspensions, or sampling from blood culture bottles) should be performed inside a biosafety cabinet (12). Cultures suspected of containing *Francisella tularensis* or for which *F. tularensis* cannot be ruled out by LRN Sentinel

laboratory methods, represent a significant risk of laboratory exposure and should be handled only in a biosafety cabinet under BSL3 conditions and practices (12).

Gram stain: The pleomorphic appearance and weak staining characteristics of some *Haemophilus influenzae* strains are similar to *Francisella tularensis* and other small, gram-negative organisms, especially in blood cultures. While its microscopic morphology is not diagnostic, *F. tularensis* tends to appear as tiny/small, uniform coccobacilli rather than pleomorphic coccobacilli or rods during growth in liquid culture media (11). One hundred ten of the 113 participating laboratories performed Gram stain on this isolate, and 89% (98/110) correctly categorized the isolate as gram-negative bacilli or coccobacilli.

Biochemical Identification: Traditional biochemical tests for differentiating *H. influenzae* from other *Haemophilus* species include a requirement for both factors X (hemin) and V (NAD); lack of hemolysis on sheep or horse blood agar; fermentation of glucose, but not sucrose, lactose, or mannose; and a lack of porphyrin production in the presence of δ -aminolevulinic acid (ALA). *H. influenzae* is also catalase positive, β -galactosidase (ONPG) negative, and H₂S negative. Almost all strains of Hif give positive reactions for indole production, urease, and ornithine decarboxylase (4). In suspected cases of tularemia, *H. influenzae* may be differentiated from *F. tularensis* on the basis of X and V requirements (*F. tularensis* does not require either), and a positive urease reaction (*F. tularensis* is negative). While primary cultures of *F. tularensis* may initially demonstrate growth on sheep blood agar (SBA), many strains will fail to grow on SBA after sub-culture due to cysteine requirements (9).

Thirteen of the 113 participating laboratories (12%) indicated that this isolate would be sent to a reference laboratory for identification. Of the 59 laboratories that performed only classical identification in-house, only 54% (32/59) performed testing for X and V requirements; 78% (27/32) gave the correct response. The remaining 27 laboratories that did not test for X and V requirements were of particular interest: eight of these labs indicated that identification was based on a single biochemical reaction; eight indicated that identification was based on two reactions; and eight provided no biochemical results to support their reported identification. Eight laboratories performed classical urea testing, with seven laboratories (88%) correctly identifying the isolate as urea positive. Considering the similarities between *H. influenzae* and *F. tularensis*, accurate and adequate biochemical testing is essential to obtaining the correct identification and ruling out *F. tularensis* according to Laboratory Response Network (LRN) protocols – Please refer to the attached “North Dakota Flow Chart for Rule-out and Referral of *Francisella tularensis*.” The algorithm and protocols can be found in the NDDoH PHL-issued “Bench Guide for Bioterrorism Agents.” The protocols are also found on the ASM Sentinel Level Clinical Microbiology Laboratory Guidelines website at: http://www.asm.org/index.php?option=com_content&view=article&id=6342&Itemid=639

Commercial Identification Systems: Specialized quadrant agar plates are available commercially for determination of factor X and V requirements, in addition to several commercial kits for biochemical identification of *H. influenzae*. However, many of these kits use hemolysis as the sole differentiating test between *H. influenzae* and *H. haemolyticus*, another organism commonly found in the human upper respiratory tract. Many strains of *H. haemolyticus* are not hemolytic, which can result in misidentification when using commercial identification kits (10). Commercial kits and some automated platforms may also be used for identification, although they often do not provide sufficient information to reliably confirm species identification without additional tests (4, 10). Traditional biochemical tests or molecular DNA sequencing can be used to confirm any unusual results obtained from a commercial identification system (10). Forty-two of the 113 participating laboratories (37%) utilized at least one commercial identification system for testing this isolate, and 88% (37/42) gave correct identifications. Quadrant agar plates were used by six laboratories; four of those laboratories (67%) correctly identified the isolate as *H. influenzae*.

Reportable Disease Rule: In North Dakota, state statute requires that all cases of invasive disease caused by *H. influenzae* be reported to the North Dakota Department of Health; Division of Disease Control within seven days of identification due to the significant clinical and epidemiologic impact of *H. influenzae* disease and the need to identify unvaccinated or incompletely vaccinated patients for epidemiologic purposes. In addition to case reporting, culture isolates from invasive cases of *H. influenzae* should be sent to the North Dakota Department of Health Public Health Laboratory for biochemical culture confirmation and serotyping by slide agglutination using commercially available antisera. Among participating laboratories, 35% (39/113) either reported that this isolate would not be referred to the PHL, or did not indicate whether the isolate would be referred. This was also true when the isolate was incorrectly identified as possible *Brucella* or *Francisella* species. Suspected or confirmed cases of tularemia or brucellosis must be reported to the North Dakota Department of Health immediately by telephone. **Whenever an LRN Sentinel lab cannot rule out a potential agent of bioterrorism, a call should be made to North Dakota Department of Health-PHL and the isolate submitted.** 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

113/118 (96%) Laboratories performed blood cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = *Haemophilus influenzae*, with referral to PHL

Acceptable Answers (63/113 = 56%)

41/63 (65%) *Haemophilus influenzae*; with referral to PHL

16/63 (25%) *Haemophilus* species; with referral to PHL

2/63 (3%) Possible *Haemophilus* species; with referral to PHL

4/63 (7%) Gram-negative coccobacilli, refer for identification; with referral to PHL

Unacceptable Answers (50/113 = 44%)

17/50 (34%) Gram-negative coccobacilli, refer for identification; without referral to MDH-PHL

11/50 (22%) *Haemophilus influenzae*; without referral to either MDH-PHL or NDPHL

3/50 (6%) Gram-negative bacilli, refer for identification; without referral to MDH-PHL

2/50 (4%) Gram-negative coccobacilli, no further identification; without referral to MDH-PHL

2/50 (4%) Possible *Haemophilus* species; without referral to MDH-PHL

2/50 (4%) Possible *Francisella* species; with referral to MDH-PHL

1/50 (2%) Gram-positive bacilli, refer for identification; without referral to NDPHL

1/50 (2%) Gram-negative coccobacilli, refer for identification; referral to MDH-PHL not indicated

1/50 (2%) *Haemophilus parainfluenzae*; with referral to MDH-PHL

1/50 (2%) *Neisseria* species; without referral to MDH-PHL

1/50 (2%) *Pasteurella* species; with referral to MDH-PHL

1/50 (2%) Possible *Brucella* species; with referral to MDH-PHL

1/50 (2%) Possible *Brucella* species; without referral to MDH-PHL

1/50 (2%) Possible *Brucella* species; referral not indicated

1/50 (2%) *Francisella* species, not *F. tularensis*; with referral to NDPHL

4/50 (8%) Identification not indicated

ORGANISM CHARACTERISTICS

Gram stain: intended result = **gram-negative bacilli or coccobacilli**

66/113 (59%) Laboratories reported tiny, small, or medium **gram-negative coccobacilli**

33/113 (29%) Laboratories reported tiny or small **gram-negative bacilli**

10/113 (9%) Laboratories reported tiny or small gram-negative cocci or diplococci

1/113 (1%) Laboratories reported gram-positive bacilli

3/113 (2%) Laboratories did not report Gram stain results

X and V requirement: intended result = **Requires both X and V Factors**

27/113 (24%) Laboratories reported isolate **requires X and V Factors**

3/113 (2%) Laboratories reported isolate **requires Factor V only**

2/113 (2%) Laboratories reported isolate **requires Factor X only**

1/113 (1%) Laboratories reported isolate requires neither Factor X nor Factor V

70/113 (62%) Laboratories did not perform testing for X and V requirement

10/113 (9%) Laboratories did not indicate results for X and V requirement

Urea: intended result = **Positive**

7/113 (6%) Laboratories reported urea as **positive**

1/113 (1%) Laboratories reported urea as **negative**

105/113 (93%) Laboratories did not indicate urea result

β -lactamase: intended result = **Positive**

43/113 (38%) Laboratories reported β -lactamase as **positive**

70/113 (62%) Laboratories did not indicate results for β -lactamase

BLOOD CULTURE METHODS

59/113 (52%) Laboratories utilized BACTEC systems

36/113 (32%) Laboratories utilized BacT/Alert systems

10/113 (8%) Laboratories utilized Septi-check systems

2/113 (2%) Laboratories utilized Oxoid SIGNAL

2/113 (2%) Laboratories utilized Versa Trek

2/113 (2%) Laboratories utilized manual subculture methods

2/113 (2%) Laboratories did not indicate blood culture method

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FRANCISELLA TULARENSIS

Cysteine Heart Agar - 72 hrs.
hrs.

Chocolate Agar - 72



Tiny, gram negative coccobacilli. Poor staining. Poor growth on SAB after 48 hrs. Blue-white to gray, flat, smooth, shiny on Chocolate Agar.



YES

SATELLITE OR XV TEST: Negative
OXIDASE: Negative
CATALASE: Weak Positive
B-LACTAMASE: Positive
UREASE: Negative

NO-
Features NOT
Present

Francisella tularensis
RULED OUT

YES-
Features
Present

CANNOT RULE
OUT *Francisella tularensis*
Contact NDPHL

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 (701.328.9921 or 800.472.2121) or call our on-call microbiologist
directly at 701-400-2772

Specimen #3 Stool: *Salmonella enterica* subspecies IV (*houtenae*)

Submitter #:		
	Your Laboratory's Results	Intended Result
Identification	Not Performed	<i>Salmonella</i> species
Referral of Isolate	Not Performed	Would refer isolate to ND-PHL

Goal: The organism in specimen #3 was *Salmonella enterica* subspecies IV (*houtenae*) serotype 44:z4z32:--. This isolate was chosen to allow laboratories to test their ability to identify this stool culture pathogen, which is a significant cause of diarrheal disease and occasionally causes invasive disease. This organism was also selected to emphasize the importance of submitting specimens to ND-PHL to assist in the public health response.

The genus *Salmonella*: Members of the genus *Salmonella* are gram-negative, facultatively anaerobic bacilli that exist as commensals or pathogens in a wide range of human and animal hosts, including wild and domesticated mammals, birds and poultry, and reptiles and amphibians. Until relatively recently, the genus was taxonomically divided into multiple species. However, current taxonomy based on genetic comparison includes only two species: *Salmonella enterica* and *Salmonella bongori* (12). *S. enterica*, which includes six subspecies (I, II, IIIa, IIIb, IV, and VI) and *S. bongori* (subspecies V) can be further classified into over 2500 serotypes based on phenotypic variations in the somatic O antigen, the capsular Vi antigen, and the flagellar H antigen. The heat-stable somatic O antigens were originally divided into serogroups that were assigned letter designations that are still commonly used today. Serogroups A, B, C1, C2, D, and E account for >99% of human *Salmonella* infections (12). Serotype names are abbreviated by genus and serotype, and often correspond to the geographic location where they were first identified (e.g. *Salmonella enterica* subspecies I serotype Dublin may be abbreviated as *Salmonella* Dublin). Although detection of certain serotypes, like *Salmonella* Typhi, can be helpful in diagnosing specific clinical syndromes, the system for determining serotypes in salmonellae is primarily used in public health epidemiologic studies and for tracking outbreaks (12). In this challenge, 18% (12/67) of laboratories performing stool cultures also performed serotyping.

Salmonellae are also classified as typhoidal or non-typhoidal based on the type of disease with which they are associated. The vast majority of *Salmonella* strains are non-typhoidal and typically cause self-limited intestinal illness (diarrhea, fever, abdominal cramping) lasting a week or more, although immunocompromised patients, infants, and the elderly may develop serious invasive disease, including meningitis and sepsis (6). In the U.S., approximately 40,000 cases of laboratory-confirmed salmonellosis are reported to the CDC each year; however, since symptoms may be mild or absent, the number of undiagnosed infections is estimated to be as high as 1.2 million each year (3). Typhoidal strains cause serious bloodstream infections in humans (typhoid or enteric fever). This differentiation is significant because patients with typhoid fever rarely present with intestinal illness, and only 26% of typhoid patients have positive stool cultures (12). Although only about 400 cases of typhoid fever are reported to the CDC annually, *Salmonella* Typhi causes an estimated 21.5 million cases globally each year, making it a significant public health threat in developing countries and endemic areas (4). Due to its extremely low incidence rate in the U.S., any suspected or confirmed cases of *Salmonella* Typhi infection are highly significant and should be reported to MDH as soon as possible.

Rates of salmonellosis reported in North Dakota increased by more than 25% between 2007 and 2008. A total of 27 distinct serotypes were identified between July 2008 and June of 2009, but 70% of salmonellosis cases were attributed to only three of those serotypes: *S. Enteritidis* (17 cases), *S. Typhimurium* (34 cases), and *S. Montevideo* (47 cases). In recent years, there have been several well-

publicized outbreaks involving contaminated food items including commercially distributed peanut butter, fresh produce, and pre-packaged frozen foods (7,8,9). However, most of the documented cases of salmonellosis could not be definitively linked to an outbreak. In North Dakota a significant local outbreak of *Salmonella* occurred in mid June through July 2009 from three separate events resulting in the analysis of twenty food samples, nineteen environmental samples and numerous clinical specimens. A common unlicensed caterer was identified in all three events and laboratory analysis was able to identify four contaminated food sources. Forty five clinical isolates, in addition to the 4 food isolates, were matched by PFGE to these three events. The pulsed-field gel electrophoresis (PFGE) subtype pattern was the same pattern associated with a regional outbreak of *Salmonella Montevideo* in baby chicks in 2007 that continued through 2008. Investigation of the outbreak included collection and testing of fecal/environmental samples from chicks owned and handled by the caterer. Some cases were a result of secondary transmission and contributed to an extensive investigation by the ND Department of Health; Division of Disease Control with support from the ND-PHL.

The ND-PHL was also actively involved in the national King Nut *Salmonella Typhimurium* outbreak in 2009. ND-PHL received numerous samples (both peanut butter and clinical) between November 2008 and February 2009. Eighteen human isolates were matched to the PFGE codes linked to this outbreak. North Dakota was one of the states with the highest per capita incidence of infection resulting from this foodborne outbreak.

Reptile-associated *Salmonella*: While *Salmonella* is ubiquitous in animal populations, some serotypes such as *Salmonella Typhi* (human) and *Salmonella Gallinarum* (poultry) are restricted to specific groups of hosts, or a single host species. Subspecies I accounts for the majority of serotypes that are known to be pathogenic to humans, including *Salmonella Typhimurium* and *Salmonella Enteritidis* (12). Most infections with subspecies I serotypes are caused by eating contaminated meat, poultry, or eggs. In contrast, serotypes primarily associated with reptiles and amphibians, including members of subspecies IV, account for roughly 40% of identified *Salmonella* serotypes yet only an estimated 6% of sporadic salmonellosis cases nationwide each year (2,6). When infections with reptile-associated serotypes are identified, they are more likely to be associated with invasive disease, hospitalization, and involve infants than other *Salmonella* infections (6).

The link between reptiles and salmonellosis is well established (1,2,5,6). However, most distributors and owners of pet reptiles (e.g. lizards, snakes, and turtles) remain unaware that reptile contact places them and their family members, especially small children, at increased risk for infection (5). Most reptiles carry *Salmonella* asymptomatically in their intestinal tracts and intermittently shed the organism in their feces (5,6). The ability of *Salmonella* to survive for prolonged periods on contaminated surfaces increases the risk of infection due to even minimal indirect contact with reptiles. Attempts to treat reptiles with antibiotics to clear *Salmonella* carriage have been unsuccessful and may result in increased antibiotic resistance (5,6).

Public Health Surveillance: The North Dakota Public Health Laboratory (ND-PHL) performs full biochemical, serological and molecular analysis on each isolate of *Salmonella* received as part of the North Dakota Disease Reporting Rule. Once the identification of *Salmonella* has been confirmed and the serotype determined, isolates are subjected to analysis by pulsed-field gel electrophoresis (PFGE) in order to determine their DNA “fingerprints.” ND-PHL then reports PFGE results to the ND Department of Health; Division of Disease Control, which uses the data for outbreak investigation. PFGE patterns are also submitted to CDC’s national database called PulseNet, which is used by CDC and state public health laboratories across the nation to detect outbreaks and identify potential sources of infection through comparison of PFGE patterns.

Laboratory Identification of *Salmonella*

Collection and Transport: For cases of suspected non-typhoidal *Salmonella* infection, the ideal specimen is freshly passed stool collected in a sterile container. If specimens cannot be processed immediately (i.e. within 1-2 hours of collection), they should be refrigerated or frozen at -70°C in an approved transport medium such as Cary-Blair, Stuart's, or Amies medium (10). If typhoid or enteric fever is suspected, blood and bone marrow are the specimens of choice (12).

Laboratory Safety: Like all enteric pathogens, clinical specimens and cultures suspected or known to contain *Salmonella* should be handled under BSL2 conditions. Any procedure that may produce infectious aerosols (such as sampling from blood culture bottles) should be performed inside a biosafety cabinet (11).

Enrichment, Isolation, and Screening Methods

Primary culture and enrichment media used by participating laboratories for routine stool cultures are summarized in **Table 1**. A wide variety of primary isolation media are available for detection of *Salmonella*. Although the vast majority of isolates are lactose negative, it is important to note that a very small percentage of *Salmonella* isolates (~1%) are lactose positive. The use of media such as Salmonella-Shigella, Hektoen enteric, or xylose-lysine-deoxycholate (XLD) agars allows the detection of rare isolates that both ferment lactose and produce H₂S, a characteristic shared by virtually all *Salmonella* isolates (10,12). The small percentage of lactose-positive *Salmonella* isolates will not be detected when using MacConkey agar alone without additional selective media (12). Lysine iron agar (LIA) is another useful screening medium since most isolates, including those that are lactose positive, will be positive for lysine decarboxylase and produce H₂S (10).

Table 1 – Media used by laboratories to set up routine stool cultures (n = 81)

Medium	# and % of labs using media	Medium	# and % of labs using media
Blood/EMB bi-plates	1 (1%)	MacConkey agar	56 (69%)
CAMP-BAP w/ 10% sheep blood	1 (1%)	MacConkey broth	5 (6%)
Campylobacter blood agar (CVA)	70 (86%)	MacConkey Sorbitol agar (SMAC)	68 (84%)
Campy CSM (BBL)	1 (1%)	Mannitol Salt	1 (1%)
CHROM agar O157	2 (2%)	PEA	3 (4%)
CIN	16 (20%)	Selenite broth	30 (37%)
CNA	10 (12%)	Sheep Blood agar	59 (73%)
CT-SMAC	6 (7%)	SS agar (salmonella shigella agar)	11 (14%)
EMB	14 (17%)	Tergitol 7	1 (1%)
GN broth	24 (21%)	XLD	25 (31%)
Hektoen agar	66 (81%)	Yersinia selective agar	1 (1%)

Biochemical Identification: Like other members of the *Enterobacteriaceae*, *Salmonella* species are facultative, gram-negative rods that ferment glucose to acid, reduce nitrates, and are oxidase negative (12). With the exception of *Salmonella* Gallinarum-Pullorum, all salmonellae are motile, and most do not ferment lactose. *Salmonella* is H₂S positive and will yield a K/A result with gas on triple sugar iron (TSI) agar slant, with one exception: *Salmonella* Typhi characteristically does not produce gas from glucose fermentation and produces a very small amount of H₂S at the stab site (10). A variety of commercial identification systems, both manual and automated, will readily identify *Salmonella* species. The organism in this challenge was strongly H₂S positive, lactose negative, and positive for lysine

decarboxylase. Among participating laboratories, 94% (76/81) used commercial identification systems; all but one of these labs obtained the correct identification.

Serotyping: Typing antisera are commercially available for the detection of the most common O antigen serogroups of *Salmonella enterica* subspecies I (A, B, C1, C2, D, and E) by slide agglutination. Determination of O antigens usually begins with the use of pooled antisera specific for multiple serogroups (i.e. poly A-I), followed by testing with individual antisera for the six most common serogroups in the pool. While O antigen determination is adequate for culture confirmation, further characterization is typically unnecessary for clinical management of *Salmonella* infections (10). Most commercial serotyping kits also include antisera for the capsular Vi antigen, which is variably expressed by the typhoidal strains of *Salmonella*, and can mask detection of the somatic O antigens. Since the non-typhoidal isolate in this challenge is a member of subspecies IV, it does not react with individual antisera for detection of the common O antigen serogroups or the Vi antigen, however it will react in polyvalent antisera directed against the O antigen. Among participating laboratories that perform serotyping (15 out of 81), 80% (12/15) correctly reported positive reactions in polyvalent antisera against serogroups A-I. Thirteen of the 15 serotyping labs (87%) also reported negative reactions for each of the individual serogroups A through E. Six labs (50%) incorrectly reported observing positive reactions when testing with Vi antiserum.

Antimicrobial Susceptibility Testing: Antibiotic treatment of uncomplicated non-typhoidal intestinal illness caused by *Salmonella* is not recommended in most patients since the disease is typically self-limiting. However, in cases of invasive illness and typhoidal infections, antimicrobial susceptibility testing can be crucial to prompt and accurate treatment. The case fatality rate for untreated typhoid fever is >10%; therefore, susceptibility results from suspected cases should be reported as soon as possible (10). As with many other organisms, emerging drug resistance has become a significant problem in *Salmonella* as multiple-drug resistant strains such as *Salmonella* Agona and *Salmonella* Newport continue to spread both nationally and globally (10). Thirty-three of 81 participating laboratories (41%) reported that they would routinely perform susceptibility testing on a *Salmonella* isolate from this specimen.

Reportable Disease Rule: In North Dakota, state statute requires that all cases salmonellosis, including typhoid fever, be reported to the North Dakota Department of Health within seven days due to the significant clinical and epidemiologic impact of salmonellosis. In addition to case reporting, culture isolates from all cases should be sent to ND-PHL for complete serotyping and molecular analysis by PFGE. For more information about the ND Communicable Disease Reporting Rule, please visit: <http://www.health.state.nd.us/Disease/Documents/ReportableConditions.pdf>

Specimen #3, Tabulated Results

81/118 (69%) Laboratories performed stool cultures for pathogenic enteric bacteria

ORGANISM IDENTIFICATION

Identification: Intended answer = *Salmonella* species, with referral to PHL

Acceptable Answers (79/81 = 98%)

64/81 (79%) *Salmonella* species; with referral to either ND-PHL or MN-PHL

11/81 (11%) *Salmonella* species; no *Campylobacter*, *Shigella*, or *E. coli* O157 isolated; with referral to ND-PHL or MDH-PHL

2/81 (2%) *Salmonella* species, untypeable; with referral to ND-PHL or MDH-PHL

1/81 (1%) *Salmonella* species, not *S. Typhi*; with referral to MDH-PHL

1/81 (1%) Possible *Salmonella* species; with referral to ND-PHL

Unacceptable Answers (2/81 = 2%)

- 1/2 (50%) Gram-negative bacillus, refer for identification; without referral to MDH-PHL
 1/2 (50%) Identification not indicated

USE OF NON-CULTURE METHODS

Table 2 reflects data collected between 2007 and 2009 on the use of non-culture tests for stool pathogens by MLS laboratories. The use of these methods, which include rapid, non-culture based, and non-visual based tests, continues to be monitored by MDH due their potential impact on isolate submission and disease surveillance.

Table 2 – The Use of Non-culture-based Methods on Direct Stool Specimens

Test	Number and % of labs performing test - 2007 n = 107	Number and % of labs performing test - 2008 n = 104	Number and % of labs performing test - 2009 n = 100
<i>C. difficile</i> toxin A	61 (57%)	64 (62%)	66 (66%)
<i>C. difficile</i> toxin B	50 (47%)	62 (60%)	65 (66%)
<i>Campylobacter</i> species	n/a	n/a	3 (3%)
<i>Cryptosporidium</i> sp.	37 (35%)	37 (36%)	37 (37%)
<i>E. coli</i> O157	1 (<1%)	10 (10%)	3 (3%)
<i>Giardia</i> species	39 (36%)	42 (40%)	40 (40%)
Rotavirus	19 (18%)	25 (24%)	23 (23%)
<i>Salmonella</i> species	n/a	n/a	1 (1%)
Shigatoxin producing <i>E. coli</i> other than O157	5 (5%)	14 (13%)	15 (15%)

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Specimen #4, Blood Culture: *Bacillus anthracis*, Sterne strain

Submitter #:	Your Laboratory's Results	Intended Result
Identification		<i>Bacillus anthracis</i>
Referral of Isolate		Would refer isolate to NDPHL
Notify ND-PHL		Yes - would notify NDPHL
Methodology		
Gram stain		Large Gram-positive Bacilli/rod
Hemolysis on SBA		Gamma-hemolysis
Motility		Negative
Catalase		Positive

Goal: The isolate in this specimen was the Sterne strain of *Bacillus anthracis*. This organism was selected to exercise the ability of LRN Sentinel Laboratories to rule-out and refer potential *B. anthracis* isolates to NDPHL (North Dakota's LRN Reference Laboratory), as well as notify NDPHL of the potential bioterrorism agent.

Genus *Bacillus*: Members of the genus *Bacillus* are large, regular, gram-positive bacilli that characteristically produce endospores when grown under aerobic conditions. These endospores are resistant to a variety of environmental conditions, allowing them to germinate into viable organisms after many years of dormancy. The 1986 version of Bergey's Manual of Systematic Bacteriology listed only 40 species of *Bacillus*; however, through the use of new molecular taxonomic methods, the genus now contains more than 100 species. The *Bacillus* species most commonly isolated from clinical specimens belong to either the *B. subtilis* group or the *B. cereus* group. *Bacillus* species cause a wide variety of diseases, including toxin-associated food poisoning and a variety of opportunistic infections. *B. anthracis*, a member of the *B. cereus* group, causes the most clinically significant infections (5).

About *Bacillus anthracis* (anthrax): *Bacillus anthracis* is the causative agent of anthrax, which is predominately a disease of domestic and wild animals. Like other *Bacillus* species, *B. anthracis* produces highly resistant endospores that can persist in the soil for extremely long periods. Spores of *B. anthracis* can be found in the soil throughout the U.S. (including North Dakota) and worldwide. Spores are typically deposited in a cyclical pattern in which animals ingest spores from the soil and, upon death, shed the vegetative organism back into the soil (1). Once exposed to air, these vegetative cells sporulate and can remain viable for decades, providing a potential reservoir for future infections. Environmental contamination with *B. anthracis* spores is extremely difficult to remove and will resurface during periods of floods, wind or other environmental disturbances (7). Until the introduction of a veterinary vaccine in the 1930's, anthrax was the leading cause of death worldwide in cattle, sheep, goats and horses. Anthrax remains a problem in developing countries (5).

In humans, anthrax may appear in three forms: inhalational, cutaneous and gastrointestinal. Cutaneous is by far the most common type, as it accounts for 99% of naturally acquired human cases and is often associated with occupational exposure (5). Cutaneous anthrax begins with a lesion at the site of infection that quickly forms a dark black scab or eschar. The coal-black color of the scab is the basis for the name "anthrax." Gastrointestinal anthrax is often seen in developing countries where infected cattle are butchered and eaten. Symptoms begin with fever, nausea and vomiting and progress to abdominal pain and bloody diarrhea. Inhalational anthrax is the deadliest form of the disease and causes many non-specific symptoms such as fever, malaise, nausea, vomiting and non-productive cough, but quickly progresses to bacteremia and toxemia (2). The virulence of *B. anthracis* is due to the production of two extracellular toxins and a capsule that enables the organism to evade phagocytosis (7). Since the potentially fatal symptoms of

anthrax are toxin-mediated and can persist even after clearing the organism, rapid antibiotic treatment for all three forms is essential.

In the early 1900's, approximately 130 cases of anthrax were reported each year in the United States (1). These infections were mainly due to occupational exposure to infected animals or animal hides contaminated with spores. While there are still 2,000-20,000 cases of anthrax reported annually worldwide, it is extremely rare in the United States where only one to two cases are reported each year (3).

The Centers for Disease Control & Prevention (CDC) has classified *B. anthracis* as a Category A Bioterrorism agent. Since the spores of *B. anthracis* are environmentally stable and form highly infectious aerosols, *B. anthracis* is thought to be one of the most likely agents to be intentionally released. As a result, inhalational anthrax would be the most likely form of anthrax to appear in patients following a terrorist attack. Many countries have had biologic warfare programs that include the manufacturing of weapons grade *B. anthracis* spores. Most recently, *B. anthracis* was used in the bioterrorism attacks of 2001 when several letters containing weaponized *B. anthracis* spores were delivered through the U.S. mail. While the FBI recently closed the official investigation into these attacks, there is still much controversy in the scientific community regarding the perpetrator. Twenty-two cases of anthrax were associated with the contaminated letters and five patients died as a result (6). A clinical laboratory in Florida was the first to recognize the potential agent and quickly forwarded the sample to their LRN Reference Laboratory. This rapid recognition and subsequent referral was extremely important in immediately spearheading an investigation into the source. The ASM Sentinel laboratory protocols provide procedures and algorithms for LRN sentinel laboratories to rule-out or refer potential bioterrorism agents to the nearest LRN Reference Laboratory. The protocols can be found on the ASM Sentinel Level Clinical Microbiology Laboratory Guidelines website at:

http://www.asm.org/index.php?option=com_content&view=article&id=6342&Itemid=639

You may also refer to the attached "North Dakota Flow Chart for Rule-out and Referral of *Bacillus anthracis*." The algorithm and protocols can be found in the ND-PHL-issued "Bench Guide for Bioterrorism Agents."

Since 2001, a handful of anthrax cases have been associated with new emerging risk factors rather than overt acts of bioterrorism. A number of recent cases have been associated with exposure to drums made with imported animal hides that were contaminated with *B. anthracis* spores (3). In each of these cases, environmental sampling found that contamination with anthrax spores had spread to the homes of the drum owners. There is also an ongoing outbreak of anthrax in Scotland among heroin users. There have been 26 cases associated with the outbreak thus far with 10 deaths, but there is potential for additional cases (9). The outbreak investigation has been complicated by the difficulty in identifying the distribution network of the drug and the source of the contamination. These examples illustrate the ongoing threat posed by *B. anthracis*, even without being released intentionally. They also demonstrate the need for LRN sentinel laboratories to remain vigilant and competent in their ability to recognize *B. anthracis*.

Laboratory Identification of *Bacillus anthracis*

Collection and Transport: The specimen of choice for detecting *B. anthracis* depends on the clinical presentation of disease. Blood cultures are the specimen of choice for both inhalational anthrax and gastrointestinal anthrax. Stool or rectal swab may also be collected for suspect gastrointestinal anthrax. For suspected cases of cutaneous anthrax, vesicular fluid or eschar material should be collected on a sterile Dacron swab. It is important to note that collecting specimens prior to antibiotic delivery is very important, as *B. anthracis* is very susceptible to the proper antibiotics. (2).

Laboratory Safety: BSL-2 practices and containment should be used when working with clinical specimens. This organism is highly infectious; therefore, all manipulations should be performed in a Class II Biological Safety Cabinet. Perform activities with high potential for aerosolization using BSL-3 practices and containment. (8)

Microscopic morphology: *B. anthracis* is a large gram-positive rod (1-1.5 x 3-5µm) with oval, central to subterminal spores, which do not cause significant swelling of the cell. When grown in a liquid medium, cells are typically seen in long chains (2).

Growth Characteristics: Growth is rapid on sheep blood agar (SBA). After 16-18 hours incubation, white/gray colonies are 2-5 mm in diameter, irregularly shaped, and rough with a “ground glass” appearance. There may be comma-shaped projections from the edges of the colonies, producing the classic “Medusa head” appearance. Colonies are non-hemolytic on SBA and are “sticky”, adhering to the agar (2).

Motility: *Bacillus anthracis* is non-motile. Most other *Bacillus* species are motile and typically display sluggish motility that may not be detected through the use of semi-solid motility media. Wet mounts of colonies from solid growth media (such as SBA) will rarely demonstrate motility and may be misinterpreted as negative. The best method to determine the motility of *Bacillus* sp. is to incubate the organism in a broth, such as tryptic soy, overnight at room temperature and perform a wet mount using the “hanging drop” method.

Hanging Drop Wet Mount Motility Procedure⁵

1. Suspend suspect colony from a 12-20 hour culture into 0.5 ml of Tryptic Soy Broth or equivalent.
2. Incubate at room temperature 18-24 hrs. Motile organisms may be seen after 8 hrs of incubation.
3. Using a wooden applicator stick prepare a “hanging drop” slide using petroleum jelly to mark out a box the size of a cover slip on a microscope slide.
4. Transfer approximately 10 µl of the suspension to a cover slip then flip it over onto the prepared microscope slide. Alternatively, a drop can be placed directly on the slide and then cover slipped.
5. Examine the slide for motility, under a microscope with reduced light using 40X objective.

In this year’s challenge set, 65% (74/113) of labs performed motility testing; half of these used the hanging drop method. Eighty-eight percent (68/77) of labs that did motility reported the result correctly as negative. This is increased significantly from Challenge Set 2 (the last time *Bacillus* sp. was sent out) when 22% of labs reported the motility correctly. In 2009, ND-PHL received 20 isolates from NDLRN Sentinel laboratories to rule-out *B. anthracis*; 35% of the referred isolates were ruled out by ND-PHL by positive motility using the hanging drop method. In comparison, nearly 80% of *B. anthracis* rule-out isolates received at ND-PHL prior to Challenge Set 2 (2004) were motile using the same method. While ND-PHL continues to encourage the referral of potential *B. anthracis* isolates that cannot be ruled out, the use of hanging drop motility is a simple, inexpensive, and more sensitive means of detecting motility in *Bacillus* isolates. Accurate interpretation of this test has the potential to reduce unnecessary referrals and the associated expense of packaging and shipping to ND-PHL.

Laboratory Response Network (LRN) Sentinel Laboratory Algorithm: The data obtained from this challenge set show that the use of the LRN sentinel laboratory algorithm for the rule-out and referral of *B. anthracis* was used fairly consistently. Laboratories that performed Gram stain, hemolysis, catalase and motility testing were correct in not being able to rule-out *B. anthracis*. However, laboratories were inconsistent in their indication of referral of the isolate to ND-PHL and of actual notification of ND-PHL in this exercise. **The presence of large, gram-positive bacilli/rods that are gamma-hemolytic, catalase positive and non-motile, should trigger suspicion of *B. anthracis* and elicit a phone call to ND-PHL.** Please refer to the attached NDLRN “Bench Guide for Bioterrorism Agents”-*Bacillus anthracis*.

Laboratory Notification: An essential component of the LRN is the notification of the LRN Reference Laboratory by the LRN Sentinel Laboratory when an isolate cannot be ruled out as a potential bioterrorism agent. Challenge Set 6 (2008), for the first time, asked participating laboratories to exercise notification of ND-PHL (the LRN Reference Laboratory for North Dakota). This years’ challenge set instructions also stated: “**ALL** participants should follow the notification protocols outlined in the NDLRN “Bench Guide

for bioterrorism Agents”-*Bacillus anthracis*” and call ND-PHL when appropriate.” Among the 67 laboratories that gave acceptable organism identification responses, all were expected to notify their PHL; however, only 42/67 (63%) of those labs contacted their PHL. Notification was improved from Challenge Set 6 in 2008, when only 49% of expected labs contacted their PHL to report the suspected bioterrorism agent *Francisella tularensis*. Prompt notification of the LRN Reference Laboratory is critical for a timely public health response to any potential bioterrorism agent. One factor that may explain the lower notification rate in 2008 is that the instruction to notify was new last year and laboratories may be more capable of ruling-out and referring *B. anthracis* than *F. tularensis*.

Referral of Isolates: If your laboratory is unable to rule-out *B. anthracis* in any specimen, the isolate must be referred to the ND-PHL and **not** to your regular reference laboratory. As an LRN Reference lab, ND-PHL uses rapid, validated LRN methods for confirming *B. anthracis* that are unavailable to clinical laboratories. Referral to a commercial reference laboratory will delay confirmation and subsequent public health response.

Call the ND-PHL (LRN Reference Lab) at 701-328-6272 and Refer Isolate if the following characteristics are noted:

1. Large gram-positive rod, **and**
2. Rough, ground glass colony morphology; **and**
3. Non-hemolytic; **and**
4. Catalase positive; **and**
5. Motility negative

Reportable Disease Rule: Anthrax, when found or suspected, must be reported ‘**immediately by telephone**’ to the North Dakota Department of Health: Division of Disease Control. Submission of isolates or clinical material to ND-PHL is required as per the North Dakota Communicable Disease Reporting Rule. For more information about the ND Communicable Disease Reporting Rule, please visit: <http://www.health.state.nd.us/Disease/Documents/ReportableConditions.pdf>

*Clinical materials: Submit isolate or, if an isolate is not available, submit material containing the infectious agent in the following order of preference: a patient specimen; nucleic acid; or other laboratory material.

Select Agent Rule: *B. anthracis* is a select agent per the Federal Select Agent Rule (Possession, Use and Transfer of Select Agents and Toxins; Final Rule 42 CFR part 1003 Internet: <http://www.selectagents.gov>). This rule dictates that isolates must be referred to a select agent registered laboratory (i.e. ND-PHL) or destroyed within 7 days of identification and appropriate documentation paperwork must be submitted. The Sterne strain of *B. anthracis* used in this challenge set is not a select agent (4).

Specimen #2, Tabulated Results

113/118 (96%) Laboratories performed blood cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = *Bacillus anthracis*, with referral to ND-PHL

Acceptable Answers (78/113 = 69%)

46/78 (59%) Possible *Bacillus anthracis*; with referral to ND-PHL or MDH-PHL

16/78 (21%) *Bacillus* sp.; with referral to ND-PHL or MDH-PHL

12/78 (15%) Gram-positive bacillus; with referral to ND-PHL or MDH-PHL

4/78 (5%) Gram-positive, spore forming bacillus; with referral to ND-PHL or MDH-PHL

Unacceptable Answers (35/113 = 31%)

10/35 (29%) Gram-positive bacillus; no referral to ND-PHL or MDH-PHL

7/35 (20%) *Bacillus* sp, not anthracis; no referral to ND-PHL or MDH-PHL

2/35 (6%) Gram-positive, spore forming bacillus; no referral to ND-PHL or MDH-PHL

- 2/35 (6%) *Bacillus* sp.; no referral to ND-PHL or MDH-PHL
- 1/35 (3%) *Bacillus* sp, not anthracis; with referral to MDH-PHL
- 1/35 (3%) Gram-positive bacillus; referral not indicated.
- 1/35 (3%) Possible *Bacillus anthracis*; no referral to MDH-PHL
- 1/35 (3%) Pseudomonas sp; no referral to MDH-PHL
- 1/35 (3%) Gram-negative bacillus; with referral to MDH-PHL
- 1/35 (3%) Brucella sp; with referral to MDH-PHL
- 8/35 (23%) ID not indicated

ORGANISM CHARACTERISTICS

Gram Stain: intended result = **Large Gram-positive Bacilli/rod (with or without spores)**

- 53/113 (47%) Large Gram-positive Bacilli/rod
- 40/113 (35%) Large Gram-positive Bacilli/rod – with spores
- 4/113 (4%) Large Gram-positive Bacilli/rod – bipolar
- 2/113 (2%) Large Gram-variable Bacilli/rod – with spores
- 1/113 (<1%) Large Gram-variable Bacilli/rod – bipolar
- 1/113 (<1%) Large Gram-negative Bacilli/rod
- 1/113 (<1%) Large Gram-positive
- 3/113 (3%) Medium Gram-positive Bacilli/rod
- 2/113 (2%) Medium Gram-positive Bacilli/rod – with spores
- 1/113 (<1%) Medium Gram-variable Bacilli/rod
- 1/113 (<1%) Small Gram-positive Bacilli/rod
- 1/113 (<1%) Bacilli/rod
- 1/113 (<1%) Gram-positive Bacilli/rod
- 2/113 (2%) Gram-positive Bacilli/rod – with spores
- 1/113 (<1%) Gram stain result not indicated

Hemolysis: intended result = **Gamma-hemolysis**

- 92/113 (81%) Laboratories reported **gamma-hemolysis**
- 2/113 (2%) Laboratories reported **alpha-hemolysis**
- 1/113 (<1%) Laboratories reported **beta-hemolysis**
- 15/113(13%) Laboratories did not perform hemolysis testing
- 3/113 (3%) Laboratories did not indicate hemolysis results

Motility: intended result = **Negative**

- 68/113 (60%) Laboratories reported motility as **negative**
- 4/113 (4%) Laboratories reported motility as **positive**
- 3/113 (3%) Laboratories reported motility as **tumbling or twitching**
- 2/113 (2%) Laboratories reported motility as **sluggish**
- 32/113 (28%) Laboratories did not perform motility testing
- 4/113 (4%) Laboratories did not indicate motility result

Motility: (of the 77 labs that did motility) intended result = **Negative**

- 68/77 (88%) Laboratories reported motility as **negative**
- 9/77 (12%) Laboratories reported motility as **positive**

Motility method used: preferred method = **Hanging drop wet prep**

- 34/77 (44%) Laboratories reported **Hanging drop wet prep**
- 25/77 (32%) Laboratories reported **Direct wet prep**
- 11/77 (14%) Laboratories reported **Motility media**

7/77 (10%) Laboratories did not indicate motility method

Catalase: intended result = **Positive**

84/113 (74%) Laboratories reported catalase as **positive**

1/113 (<1%) Laboratories reported catalase as **negative**

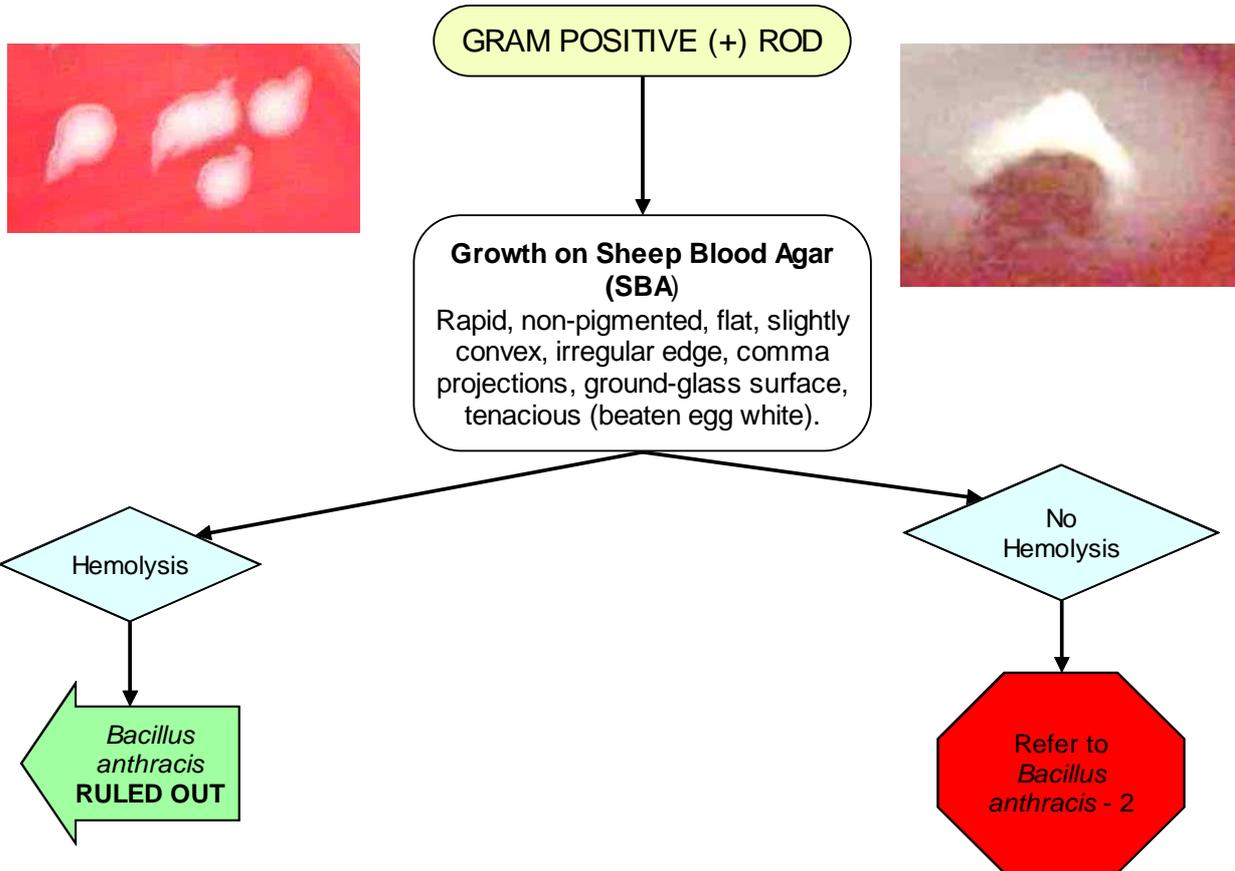
1/113 (<1%) Laboratories reported catalase as **weak positive**

27/113 (24%) Laboratories did not indicate catalase result

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NDLRN Bench Guide for Bioterrorism Agents - *Bacillus anthracis*



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 (701.328.9921 or 701-400-2772)



ND-PHL/MLS: Challenge Set Seven (2009)
Competency Exam
March 2010

Specimen #1 – *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 carbapenemase resistant *Klebsiella pneumoniae* is an urgent situation that requires immediate notification of the clinician and/or infection prevention staff.
6. Which of the following is a phenotypic confirmatory test for carbapenemase-producing *Enterobacteriaceae* (CRE)?
 - a. D-test
 - b. Modified Hodge test
 - c. Cefoxitin disk test
 - d. None of the above

Specimen #2 – *Haemophilus influenzae* serotype f (Hif)

1. All of the following statements regarding *Haemophilus influenzae* are true, **except** (choose one):
 - a. *H. influenzae* is a small, pleomorphic, facultative Gram-negative coccobacillus
 - b. *H. influenzae* can be differentiated from other Gram-negative organisms by its lactose reaction on MacConkey agar
 - c. *H. influenzae* is often a component of the normal flora of the human upper respiratory tract
 - d. Hib is the serotype most closely associated with serious human disease, causing meningitis, septicemia, and primary pneumonia
2. Which of the following is characteristic of *H. influenzae*?
 - a. Does not require Factors X (hemin) and V (NAD) for growth
 - b. Requires only Factor X (hemin) for growth
 - c. Requires only Factor V (NAD) for growth
 - d. Requires both Factor X (hemin) and Factor V (NAD) for growth

3. Fewer than 100 cases of invasive Hib disease occur in the U.S. each year, primarily because (choose one):
 - a. Accumulated mutations in the Hib genome have led to a change in its host specificity
 - b. Antibiotic prophylaxis programs in school children have reduced rates of carriage
 - c. The introduction of a polysaccharide-conjugate vaccine has virtually eliminated Hib from the vaccinated population
 - d. Routine use of surgical masks in hospitals has reduced droplet transmission of the organism
4. **True False** In suspected cases of tularemia, *H. influenzae* can be differentiated from *Francisella tularensis* on the basis of Gram stain alone
5. **True False** Culture isolates from cases of invasive *H. influenzae* disease are required to be submitted to ND-PHL under the North Dakota Communicable Disease Reporting Rule.

Specimen #3 – *Salmonella enterica* subspecies IV (*houtenae*) serotype 44:z4z32:--

1. A very small percentage (~1%) of *Salmonella* isolates will not be detected when MacConkey agar is used as the only stool culture screening agar without additional media (i.e. Salmonella-Shigella, Hektoen Enteric, or XLD agar) because they are _____.
 - a. Negative for lysine decarboxylase
 - b. Positive for lactose fermentation
 - c. Positive for H₂S production
 - d. Resistant to ampicillin
2. While *Salmonella enterica* subspecies I accounts for the majority of salmonellosis outbreaks, reptile associated strains of subspecies IV are responsible for what percentage of sporadic cases each year?
 - a. <1%
 - b. 5%
 - c. 6%
 - d. 10%
3. Serotyping of *Salmonella* isolates is based on which of the following antigens?
 - a. Capsular Vi antigen
 - b. Somatic O antigen
 - c. Flagellar H antigen
 - d. All of the above
4. An estimated 40,000 cases of salmonellosis are reported to the CDC each year. How many cases go undetected or unreported?
 - a. 120,000
 - b. 500,000
 - c. 1.2 million
 - d. 2.4 million
5. **True False** The preferred specimen for suspected cases of typhoid or enteric fever is freshly passed stool in a sterile container, or stool collected in an appropriate transport medium and refrigerated or frozen to -70°C.

Specimen #4 – *Bacillus anthracis*

1. Which of the following statements most accurately describes anthrax? (choose one)
 - a. A disease caused by a spore-forming gram-positive rod
 - b. A highly contagious infection that is easily spread person to person
 - c. Most commonly associated with exposure to small rodents, such as prairie dogs
 - d. A disease that is always caused by an act of bioterrorism
 - e. None of the above

2. In humans, anthrax may appear in which of the following forms? (choose all that apply)
 - a. Ulcerative
 - b. Inhalational
 - c. Cutaneous
 - d. Gastrointestinal

3. Which of the following biochemical reactions is not included in the LRN Sentinel protocol for *Bacillus anthracis*? (choose one)
 - a. Beta hemolytic on sheep blood agar
 - b. Arginine decarboxylase positive
 - c. Catalase positive
 - d. Motility negative

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

5. **True False** The “hanging drop” method is more sensitive than direct wet mounts or semi-solid media for detection of motility.



ND-PHL/MLS: Challenge Set Seven (2009)
Answers to the Competency Exam
March 2010

Specimen #1 – *Klebsiella pneumoniae* – carbapenemase producer

1. **True**
2. **d.** Northeast
3. **b.** Aztreonam
4. **a.** Imipenem
5. **True**
6. **b.** Modified Hodge test

Specimen #2 – *Haemophilus influenzae* serotype b

1. **b.** Lactose reaction on MacConkey (*H. influenzae* will not grow on MacConkey agar)
2. **d.** Requires both X and V factors
3. **c.** Polysaccharide-conjugate vaccine has virtually eliminated carriage
4. **False:** Both organisms have similar microscopic morphologies and require biochemical tests to differentiate.
5. **True**

Specimen #3 – *Salmonella enterica* subspecies IV (*houtenae*)

1. **b.** Positive for lactose fermentation
2. **c.** 6 %
3. **d.** All of the above (somatic O, capsular Vi, and flagellar H antigens)
4. **c.** 1.2 million
5. **False:** Blood or bone marrow are preferred for suspected typhoid or enteric fever

Specimen #4 – *Bacillus anthracis* Sterne strain

1. **a.** Caused by a spore-forming, gram-positive rod
2. **b.** Inhalational; **c.** Cutaneous; **d.** Gastrointestinal
3. **b.** Arginine decarboxylase positive
4. **False:** Isolates that cannot be ruled out should be sent to MDH as the LRN Reference Lab for MN.
5. **True**