CHALLENGE SET 2013

PARTICIPANT SUMMARY REPORT

May 2014

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, Tissue: *Yersinia pestis*

Submitter #: 2209		
	Your Laboratory's Results	**Intended Result**
Identification	***Yersinia pestis***	*Yersinia pestis*
Notify and refer Isolate to NDPHL	**CDC would be notified**	Yes - Would notify and refer isolate to NDPHL
Notify NDDoH; Division of Disease Control	**Yes**	Yes per ND Reportable Condition Rules
Methodology		
Gram stain	**Gram negative rods**	Gram negative bacilli
Oxidase	**Negative**	Negative
Indole	**Negative**	Negative
Urea	**Negative**	Negative
Motility	**Negative**	Negative

Goal:
The organism in specimen #1 was an avirulent strain of *Yersinia pestis*, specifically A1122. The goal was to exercise LRN Sentinel Laboratories' ability to rule-out and refer potential *Y. pestis* isolates to NDPHL (North Dakota's LRN Reference Laboratory). *Y. pestis* is a Category A bioterrorism organism; however, the strain used in this challenge set has been attenuated by removal of the virulence plasmid and, therefore, provides a safe alternative to work through the rule-out process.

We included the same organism in the 2007 Challenge Set. Although automatic instrument usage has decreased, we are still seeing the same problems identifying the organism. For example, *Y. pseudotuberculosis* could have been ruled out with indole – in 2007 35% of the labs indicated an indole result and in 2013, 73% indicated an indole result. Please follow the *Yersinia pestis* algorithm located in your *"NDLRN Bench Guide for Bioterrorism Agents"* or refer to the back of this *Y. pestis* write up for a list of simple biochemicals that your laboratory can use to aid in proper identification of *Y. pestis*. Again, we would like to stress that if Challenge Set bioterrorism organisms cannot be ruled out by your laboratory, the laboratory should immediately notify NDPHL. Call the main laboratory number during regular business hours (701-328-6272) or after hours call the laboratory 24/7 on-call phone number (701-400-2772).

Genus *Yersinia*:
The genus *Yersinia* is in the family *Enterobacteriaceae* and is comprised of over 10 species; most notable human pathogens are *Y. pestis, Y. enterolitica,* and *Y. pseudotuberculosis.* All other species are considered to be environmental isolates that are mostly non-pathogenic to humans. The members of this genus are Gram-negative, non-spore-forming bacilli that are facultative anaerobes capable of growth between 4-43 °C, but which grow optimally at 25-28 °C. Bipolar staining can be observed when specimens are stained with Giemsa or Wayson's dye. (3)

About *Yersinia pestis*:
The United States, Japan, and former USSR are known to have weaponized *Y. pestis,* during and after World War II and it is still considered a high-level biothreat agent. *Y. pestis* is a facultative, oxidase-negative, Gram-negative-staining bacillus that grows in a wide range of temperatures (4 to 43ºC) and is non-motile. Fresh clinical material likely to contain high concentrations of *Y. pestis* are blood, lymph

node tissue, and bronchial washes, and these sites offer the best specimens for recovery of the organism. Important characteristics of this organism are its Gram stain morphology, which is typically bi-polar, and negative urea and indole. (3)

Disease – Plague:
Yersinia pestis is the etiological agent of plague, one of the oldest recorded infectious diseases in history. For centuries, plague has caused devastating epidemics and, because of its historic and cultural context, a suspected plague outbreak could incite mass panic. There have been three plague pandemics associated with *Y. pestis*, each in a different part of the world and believed to be caused by different biovars. The earliest pandemic, the Justinian plague, took place during the 5th-7th centuries in Africa and was associated with biovar Antiqua. Biovar Medievalis, identified in Central Asia, was associated with the pandemic known as the Black Death during the 13th-15th centuries. Modern plague, originally associated with southern China and now worldwide, is attributed to biovar Orientalis. However, through microarray analysis, a fourth biovar, Microtus, was identified. This biovar, which includes strains previously included in Medievalis, was proposed based on biochemical, molecular, and pathogenicity differences. Biovar Microtus is pathogenic to voles, mice, and other small rodents but not to large animals, including humans. (3)

Plague is relatively rare in the U.S., with approximately 1,000 human cases occurring each year. Although endemic in North Dakota prairie dog populations, human plague does not occur naturally in North Dakota. It is endemic in the southwestern United States, with most human cases being reported in New Mexico, Arizona, Colorado, California, Oregon, and Nevada (CDC http://www.cdc.gov/plague/maps/index.html). Plague has recently reemerged as a significant human pathogen in eastern Africa and Madagascar. Between 1,000 – 2,000 cases of plague are reported to the World Health Organization (WHO) each year, although the actual number is likely much higher. WHO cites mortality rates around 8-10%, although this number is thought to be higher in endemic areas due to under-reporting and misdiagnosis. If not treated with antibiotics, death rates are as high as 66%.

There are three forms of plague: pneumonic, septicemic, and bubonic. Bubonic plague is the most common clinical presentation of *Y. pestis*. After the bite of an infected flea or a direct infection from handling infected animals, plague bacteria enter the body through breaks in the skin. *Y. pestis* then migrates through the blood and multiplies in regional lymph nodes. Following a 2-8 day incubation period, the affected lymph node becomes inflamed and enlarged, creating the characteristic “bubo”. From the bubo, the organisms can spread to other regions of the body to cause septicemic (blood) or pneumonic (lungs) plague. (3)

Pneumonic plague can arise as a rare complication of bubonic plague or can be the primary route of infection. In the latter case, inhalation of aerosolized organisms occurs either from contact with infected animals or during a bioterrorism event. Person to person transmission of aerosolized bacteria is also possible. After an incubation period of 1-6 days, symptoms develop and include high fever, cough with hemoptysis, and chest pain. Progression of pneumonic plague is rapid and can be fatal within 24 hours if untreated. Higher mortality rates are associated with this form of plague than with bubonic. (3)

Septicemic plague occurs when, after hematogenous inoculation through a flea bite, the organisms spread to the bloodstream without localizing in the regional lymph nodes. This form of the disease is more common in children and is rapidly fatal. Septicemic plague can also result from hematogenous spread of bubonic plague organisms that have not been adequately eliminated through treatment. (3)

Laboratory Identification of *Yersinia pestis*

Microscopic morphology:
The presence of small (1-2 µm x 0.5 µm), bipolar-staining, Gram-negative bacilli in direct smears, along with slow growth on plated media, **should trigger suspicion of *Y. pestis***. Direct microscopy of a tissue specimen stained with Wright, Giemsa, Wayson's, or methylene blue may help in presumptive identification due to **bipolar staining** of the organism. (3) Note: if Y. *pestis* is suspected, original specimens can be sent to NDPHL for rapid DFA testing and PCR. (2)

Growth Characteristics:
Y. pestis grows on most routine media, including blood, chocolate, and MAC, at 35-37°C. Ambient air is adequate for growth, though the use of 5% CO_2 is acceptable. After 48 hours, colonies are about 1-2 mm in diameter and demonstrate an irregular "hammered copper" appearance. No hemolysis is seen on blood agar and colonies viewed under a dissecting microscope have a "fried egg" appearance. (3)

Biochemical Characteristics:
As described in the attached algorithm, *Y. pestis* organisms are catalase-positive and oxidase-, urease-, indole-, and motility (at 25º and 35ºC)-negative. *Y. pestis* produces an alkaline slant and acid butt in triple sugar iron agar.

<u>Use of Commercial System for Identification</u>: Use of an automated commercial identification system is not recommended with *Y. pestis* due to creation of aerosols and subsequent exposure of laboratory personnel. Additionally, since *Y. pestis* grows slowly and exhibits very few positive biochemical reactions, most conventional biochemical or commercial identification systems may have difficulty generating an ID. In this challenge, some participants who used a commercial ID system found that it produced accurate results with *Y. pestis*. This is in contrast with results from 2007 when the automated systems produced less reliable data. (2)

Identification data from automated commercial systems collected for this Challenge Set are represented in **Table 1**.

- In Challenge Set 2013, 41 labs used automated commercial ID systems versus 78 labs in 2007
- In 2013, as seen in previous data from 2007, all types of represented automated identification systems do not provide reliable identification. This is why the "NDLRN Bench Guide for Bioterrorism Agents" binder does not recommend using them.

Table 1: Identifications Obtained From Automated Instruments by System and Card Type (data from Challenge Set 2007 and 2013 for comparison, combination of MN and ND data)

	VITEK-2 GN		**VITEK-2 GNI +**		**PHOENIX NID**		**MICROSCAN – CONVENTIONAL OVERNIGHT GN**		**MICROSCAN RAPID GN**		**MALDI-TOF**	
	2007	2013	2007	2013	2007	2013	2007	2013	2007	2013	2007	2013
Total number of labs that used this system, card, or panel n = 82 (2007), 41 (2013) labs*	20	0	3	0	1	0	33	3	7	0	NA	0
Instrument Identifications												
Yersinia pestis	3	0	1	0	0	0	0	0	4	0	NA	0
Y. pseudotuberculosis	3	0	0	0	1	0	4	1	2	0	NA	0
Y. pestis/Y. pseudo (low discrimination)	3	0	0	0	0	0	0	0	0	0	NA	0

Yersinia spp.	0	0	1	0	0	0	1	1	0	0	NA	0
Pseudomonas luteola	8	0	0	0	0	0	0	0	0	0	NA	0
Acinetobacter lwoffii	0	0	0	0	0	0	13	0	0	0	NA	0
Shigella spp.	0	0	0	0	0	0	5	1	0	0	NA	0
No identification	2	0	1	0	0	0	10	0	1	0	NA	0

*Note: some labs used more than one type of ID system or card/panel, only the most common automated systems are shown.

LRN Sentinel Laboratory Algorithm:
The data obtained from this Challenge Set show that the LRN sentinel laboratory algorithm for the rule-out and referral of *Yersinia pestis* was not used consistently. Of those laboratories that reported a final identification of: "*Yersinia pestis*" (9 labs, 14%) or "Possible *Yersinia pestis*" (18 labs, 27%), a variety of methods used to obtain the results were reported, with six laboratories performing all five LRN Sentinel laboratory tests: motility, catalase, indole, oxidase, and urea. The data provided at the end of this document show that most laboratories did not perform most tests in the algorithm. We acknowledge that most laboratories are not capable of performing all of the biochemical tests required in the LRN algorithm; however, it is important to affirm that, if laboratories are capable of performing the recommended tests, they should do so. Nevertheless, as stated previously, **the presence of Gram-negative bacilli in direct smears, along with slow growth on plated media, should alone trigger suspicion of *Yersinia pestis*.**

Please refer to the flow chart from the "*NDLRN Bench Guide for Bioterrorism Agents*," for the appropriate *Y. pestis* rule-out procedures. These protocols are also available on the ASM website at: http://www.asm.org/

Laboratory Notification:
An essential component of the Laboratory Response Network (LRN) http://emergency.cdc.gov/lrn/ is notification of the LRN Reference Laboratory by the LRN Sentinel Laboratory when an isolate cannot be ruled out as a potential bioterrorism agent. Participating laboratories were asked to notify NDPHL (ND's LRN Reference Laboratory) as per Challenge Set instructions: "When appropriate, participants should follow the notification protocols outlined in the "NDLRN Bench Guide for Bioterrorism Agents " and call NDPHL." In order for a public health response to be initiated in a timely manner, **whenever an LRN Sentinel lab cannot rule out a potential agent of bioterrorism, a call should be made to NDPHL and the isolate should be referred.**

Laboratory Hazards:
Using an automated system to attempt identification of an organism suspected as *Y. pestis* is **not recommended** because of the potential for generating aerosols and subsequent exposure of laboratory personnel. BSL-2 practices and containment should be used when working with potentially infectious specimens. This organism is highly infectious; therefore, all manipulations should be performed in a Class II Biological Safety Cabinet and, if aerosolization may occur, isolates should be handled with BSL-3 practices and containment. (2)

Referral of Isolates:
If your laboratory is unable to rule-out *Y. pestis* in any specimen, **NDPHL must be called first** and the isolate must be referred to NDPHL and **not** to your regular reference laboratory. As an LRN Reference lab, NDPHL has rapid, validated LRN methods for confirming *Y. pestis* that regular commercial laboratories do not possess. Referral to a standard commercial reference laboratory will delay confirmation and subsequent public health response.

Call the NDPHL at 701-328-6272 or after hours call our On-Call Phone at 701-400-2772 and Refer Isolate if a majority of the following characteristics are noted:

1. Bipolar staining bacilli/rod on direct smear
2. Pinpoint colony at 24 h on Sheep Blood Agar
3. Non-lactose fermenter, growth may not be visible on MAC or EMB at 24 h
4. Oxidase, indole, motility (at 25º and 35º C), and urease negative

Reportable Disease Rule:
Plague *(Y. pestis)*, when found or suspected, must be reported **'immediately by telephone'** to NDDoH – Division of Disease Control and submission of clinical materials* to NDPHL is required as per the ND Communicable Disease Reporting Rule. For more information, 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:
Wild strains of *Y. pestis* are Tier One select agents per the Federal Select Agent Rule (Possession, Use and Transfer of Select Agents and Toxins; Final Rule 42 CFR part 1003). This rule dictates that isolates must be referred to a select agent registered laboratory or destroyed within 7 days of identification and appropriate documentation paperwork must be submitted. Since the NDPHL is no longer registered for Tier One select agents, you **cannot** transfer an isolate to the NDPHL **after** we have confirmed the isolate as a select agent. All isolate material must be transferred **prior** to confirmation or it must be destroyed at the Sentinel Laboratory after confirmation. The strain used in this challenge set was the attenuated vaccine strain, a strain excluded from this rule and, therefore, not a select agent. (4)

Upon identification of a select agent, Form 4 (Identification of a Select Agent or Toxin) must be completed and submitted to the CDC. If there is a suspected exposure to a select agent, such as working with the organism on an open bench top, Form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) must be filled out and sent as well. NDPHL can assist LRN Sentinel Laboratories with completing these forms. Electronic copies of the forms can be found online at www.selectagents.gov

Specimen #1, Tabulated Results

15/16 (93%) of labs performed tissue cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = ***Yersinia pestis*, with referral to NDPHL**

Acceptable Answers: 9/15 (60%)
8/15 (53%) Possible *Yersinia pestis*, with referral to NDPHL
1/15 (7%) *Yersinia pestis*, with referral to NDPHL

Unacceptable Answers: 6/15 (40%)
1/15 (7%) *Yersinia pseudotuberculosis*
1/15 (7%) *Pseudomonas luteola*
1/15 (13%) Possible *Burkholderia mallei*
1/15 (7%) *Shigella* species
1/15 (7%) *Yersinia* species, without referral to NDPHL
1/15 (7%) *Burkholderia mallei*

ORGANISM CHARACTERISTICS

Gram Stain: intended result = **Gram-negative bacilli**

12/15 (80%) Gram-negative bacilli
2/15 (13%) Gram-negative coccobacilli
1/15 (7%) Tiny Gram-negative bacilli/rods

Motility: intended result **= Negative**
5/15 (33%) Laboratories did not perform motility
10/10 (100%) Laboratories reported motility as **negative**

Oxidase: intended result = **Negative**
1/15 (7%) Laboratories reported oxidase as **positive**
14/15 (93%) Laboratories reported oxidase as **negative**

Indole: intended result **= Negative**
4/15 (27%) Laboratories did not indicate indole result
11/15 (73%) Laboratories reported indole as **negative**

Urea: intended result **= Negative**
2/15 (13%) Laboratories did not indicate urea result
13/15 (87%) Laboratories reported urea as **negative**

REFERENCES

1. Department of Health and Human Services. March 2005. *Possession, Use and Transfer of Select Agents and Toxins*, Final Rule 42 CFR part 1003, Federal Register. Vol. 70, No. 52. National Archives and Records Administration.

2. Plague: Maps and Statistics. Apr 23 2013, posting date. CDC. [Online.] http://www.cdc.gov/plague/maps/index.html

3. Schriefer, M. E. and Peterson, J. p. 627-638. *In* Versalovic, J., K.C.Carroll, J.H. Jorgensen, G. Funke, M.L. Landry, and D.W.Warnock (ed.), Manual of Clinical Microbiology, 10th Edition. Vol 1, ASM Press, Washington, DC.3.

4. Sharpe, S.E. and M. A. Sauboulle, June 2010, Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Disease: *Yersinia pestis*, ASM. *In* Guidelines for LRN Advanced Sentinel Laboratories 2013, Section 9 – *Yersinia pestis*, Minnesota Department of Health/Minnesota Laboratory System. St. Paul, Minnesota.

Specimen #2, Throat swab: *Corynebacterium diphtheriae*

Submitter #: 2209		
	Your Laboratory's Results	**Intended Result**
Identification	***Corynebacterium diphtheriae***	*Corynebacterium diphtheriae*
Referral of Isolate	**Our lab would notify disease control.**	Would refer isolate to NDPHL
Notify NDDoH; Division of Disease Control	**Yes**	Yes per ND Reportable Condition Rules
Methodology		
Gram stain	**Gram positive bacilli/rods**	Gram positive bacilli
Catalase	**Positive**	Positive
Nitrate	**Not Performed**	Positive
CAMP	**Not Performed**	Negative
Motility	**Not performed**	Negative

Goal:
The organism in specimen #2 was a rare, avirulent strain of *Corynebacterium diphtheriae* for routine throat culture from a two-year old male with sore throat, fever, and cough. The goal was to exercise the laboratories' ability to identify and refer potential *C. diphtheriae* isolates to NDPHL. NDPHL acknowledges that not all facilities perform routine throat cultures and coryneform bacteria are not typically identified unless *C. diphtheriae* is suspected or requested by a clinician. Instructions should have indicated clinical suspicion of diphtheria by physician. Therefore most labs (74%) did not provide an acceptable answer with referral to NDPHL.

Genus *Corynebacterium*:
The genus *Corynebacterium* is comprised of 67 species, 40 of which are medically relevant. A Gram stain of corynebacteria will show slightly curved, pleomorphic Gram-positive rods with characteristic club-shaped ends. Corynebacteria are non-spore formers. If corynebacteria cells are examined from liquid media, they appear as a mixture of single cells, pairs, V forms, palisades, and clusters with a so-called "Chinese letter" appearance. Corynebacteria are always catalase-positive, and the medically relevant species are all non-motile. The genus includes both fermenting and non-fermenting species. Many species of corynebacteria are part of the normal flora of the skin and mucous membranes in humans and mammals. *Corynebacterium* grow well between 30-37º C in a CO_2-enriched environment. (2)

About *Corynebacterium diphtheriae*:
Toxin producing *C. diphtheriae* cause an acute bacterial disease primarily involving the mucous membrane of the upper respiratory tract (nose, tonsils, pharynx, larynx) skin, or rarely other mucous membranes. *C. diphtheriae* is capable of causing several clinical manifestations of disease. Respiratory diphtheria usually occurs as a membranous nasopharyngitis or obstructive laryngotracheitis. Local infections are associated with a low-grade fever and gradual onset of symptoms over 1-2 days. Cutaneous diphtheria is more common in tropical areas and among urban homeless. Serious complications of diphtheria include severe neck swelling accompanied by upper airway obstruction caused by extensive membrane formation, myocarditis, and peripheral neuropathies. (4)

Disease – Diphtheria:
Diphtheria remains endemic in many parts of the developing world including some countries of the Caribbean and Latin America, Eastern Europe, Southeast Asia, and Africa. In the 1990s, a large epidemic occurred in the former Soviet Union where diphtheria had previously been well-controlled. During the past few years, many developing countries have achieved high childhood immunization coverage with

DTP/DTaP vaccine resulting in a significant decrease in diphtheria incidence. Sporadic cases and outbreaks still occur among population subgroups, particularly among adolescents and adults who have not received routine childhood vaccinations or boosters. (1)

Toxin Testing

All biotypes of *C. diphtheriae* may be either toxigenic or non-toxogenic. Toxigenic strains express an exotoxin that consists of an enzmyatically active A domain and binding B domain, which promotes the entry of A into the cell. The toxin gene, *tox*, is carried by a family of related coynebacteria phages. The toxin inhibits protein synthesis in all eukaryotic cells, including myocardial, renal, and peripheral nerve cells, resulting in myocarditis, acute tubular necrosis, and delayed peripheral nerve condition. Non-toxigenic stains of *C. diphtheriae* can cause sore throats and other invasive infections including endocarditis. (3)

It is recommended that at least 10 colonies of *C. diphtheriae* be tested for diphtheria toxin by qualified testing personnel. Isolation of *C. diphtheriae* may not always be possible due to the administration of antibiotics before diagnosis is considered. A positive PCR test in the absence of a positive toxin producing culture does not meet the laboratory criteria for classifying a case as a confirmed case because other strains (*C. ulcerans* and *C. pseudotuberculosis)* may also harbor the bacteriophage which carries the diphtheria toxin gene. (3)

Public Health Significance

Early recognition and reporting of the disease is important to ensure timely, appropriate treatment with *C. diphtheriae* antitoxin, to obtain necessary laboratory specimens before antibiotic or antitoxin treatment eliminates the organism from the patient, to identify and evaluate contacts, and to provide necessary antibiotic prophylaxis to prevent further spread. The outcome of *C. diptheriae* infection improves with timely, appropriate treatment. (1)

Because patient conditions can deteriorate quickly, a single dose of IV equine antitoxin should be given on the basis of clinical diagnosis before culture results become available. Tests for sensitivity to horse serum should be performed prior to antitoxin (as allergic reactions to horse serum are observed in 5-20% of patients). The site and size of the membrane, the degree of toxic effects, and the duration of illness are guides for estimating the dose of antitoxin. Antitoxin can be obtained from the CDC. Doses range from 20,000 U for pharyngeal disease of 48 hours or less to 120,000 U in extensive disease of 3 days duration or more. (3)

Vaccination

Diphtheria is a vaccine preventable disease. Primary diphtheria vaccination with diphtheria-tetanus toxoids-acellular pertussis vaccine (DTaP) is recommended for all people at least 6 weeks old but less than 7 years of age and without a history of complications. (1)

Laboratory Identification of *C. diphtheriae*

Specimen Collection:

The diagnosis of diphtheria is primarily a clinical one and the physician should notify the receiving clinical laboratory immediately if diphtheria is suspected. In case of respiratory diphtheria, material for culture should be obtained on a swab from the inflamed areas in the nasopharynx. Specimens should be obtained from the nose, throat, or any mucous or cutaneous lesion. Material should also be obtained from under the membrane or the membrane itself should be submitted for culture. The laboratory should be notified that *C. diphtheriae* is suspected. If needed, collected specimens can be placed in any transport medium or sterile container and transported to a reference laboratory. (3)

NDPHL recognizes that not all facilities carry the specific media recommended for the growth of *C. diphtheriae.*

Growth Characteristics:
Although relatively resistant to drying and moderate temperature changes, coryneform-suspect cultures should be plated as soon as possible on appropriate media. The sample should be plated on one selective medium such as Cystine-Tellurite blood agar (CTBA) or Tindsdale medium. If CTBA or Tinsdale media are not available, CNA plates are recommended. Tellurite inhibits the growth of many non-coryneform bacteria and some corynebacterium produce small, black colonies. The best medium for direct culturing of *C. diphtheriae* is Tinsdale medium. On Tinsdale plates, both tellurite reductase activity (as demsonstrated by black colonies) and cystinase activity (as shown by a brown halo around colonies) can be observed. (2)

Biochemical Characteristics:
Von Graevenitz and Funke's biochemical identification system for coryneform bacteria ~~which~~ was based on previous results from the CDC's Special Bacteriology Reference Laboratory. This system includes testing for catalase, fermentation or oxidation, motility, nitrate reduction, urea hydrolysis, esculin hydrolysis, acid production from glucose, maltose, sucrose, mannitol, and xylose, lack of a CAMP reaction with a beta-hemolysin-producing strain of *Staphylococcus aureus* (*C. diphtheriae* does not produce an arrowhead compared to other coryneform strains), negative PZA and alkaline phosphatase, and lipophilia. Fully realizing that most labs do not keep these tests or media on hand, manually performed identification panels such as API or RapID CB Plus systems can also be used with reliable results. (2)

Microscopic Characteristics
A methylene blue stain of *C. diphtheriae* grown on nonselective Loeffler's media highlights the condition of metachromasia. It appears as deeply stained granules surrounded by lighter blue stained cytoplasm. (2)

Laboratory Hazards:
BSL-2 practices and containment should be used when working with potentially infectious specimens. This organism is highly infectious; therefore, all manipulations should be performed in a Class II Biological Safety Cabinet. (2)

Reportable Disease Rule:
Diphtheria *(C. diphtheriae)*, when found or suspected, must be reported **'immediately by telephone'** to NDDoH; Division of Disease Control and submission of clinical materials* to NDPHL is required as per the Communicable Disease Reporting ND Rule. For more information, please visit: http://www.health.state.nd.us/Disease/Documents/ReportableConditions.pdf Prompt suspicion and reporting of the disease is important to ensure early, appropriate treatment with diphtheria antitoxin; to obtain necessary laboratory specimens before antibiotic or antitoxin treatment; to identify and evaluate contacts; and to provide necessary antimicrobial prophylaxis to prevent further spread. The outcome of diphtheria infection improves with early, appropriate treatment. (1)
**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.*

Specimen #2, Tabulated Results

16/16 (100%) of labs performed routine throat cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = ***C. diphtheriae*, with referral to NDPHL**

Acceptable Answers: 7/16 (44%)
2/16 (13%) Possible *Corynebacterium diphtheriae*, with referral to NDPHL
2/16 (13%) *Corynebacterium* species, with referral to NDPHL
1/16 (6%) Diphtheroid bacillus, NOS, with referral to NDPHL
2/16 (13%) Gram-positive bacilli NOS, with referral to NDPHL
Unacceptable Answers: 63/16 (56%)

3/16 (19%) Normal flora, no pathogens
2/16 (13%) Possible *Corynebacterium diphtheriae*, without referral to NDPHL
1/16 (13%) Gram-positive bacilli NOS, without referral to NDPHL
1/16 (6%) Possible *B. anthracis*
1/16 (6%) *Corynebacterium diphtheriae*, without referral to NDPHL
1/16 (6%) Possible *Yersinia pestis*

ORGANISM CHARACTERISTICS

Gram Stain: intended result = **Gram-positive bacilli**
15/16 (94%) Gram positive bacilli
1/16 (6%) Gram negative bacilli

Catalase: intended result = **positive**
3/16 (19%) Laboratories did not indicate catalase result
13/13 (100%) Laboratories reported catalase as **positive**

CAMP: intended result = **Negative**
15/16 (94%) Laboratories did not indicate CAMP result
1/1 (100%) Laboratories reported CAMP as **negative**

Nitrate: intended result **= Positive**
14/16 (88%) Laboratories did not indicate nitrate result
1/2 (50%) Laboratories reported nitrate as **negative**
1/2 (50%) Laboratories reported nitrate as **positive**

Motility: intended result **= Negative**
11/16 (69%) Laboratories did not indicate motility result
5/5 (100%) Laboratories reported motility as **negative**

REFERENCES

1. Centers for Disease Control and Prevention. Manual for the surveillance of vaccine-preventable diseases. Centers for Disease Control and Prevention, Atlanta, GA, 2008.

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Specimen #3, Wound culture: *Plesiomonas shigelloides*

Submitter #: 2209		
	Your Laboratory's Results	**Intended Result**
Identification	***Plesiomonas shigelloides***	*Plesiomonas shigelloides*
Referral of Isolate	**Our lab would not refer this isolate for confirmation**	Not a reportable isolate
Notify NDDoH; Division of Disease Control	**No**	No – Not a reportable condition
Methodology		
Gram stain	**Gram negative rods**	Gram negative bacilli
Oxidase	**Positive**	Positive
Indole	**Positive**	Positive

Goals:
Specimen 3 contained an isolate of *Plesiomonas shigelloides*. The primary objective for this isolate was to ensure that ND laboratories were able to correctly identify *P. shigelloides*, an uncommonly isolated enteric pathogen. This organism was selected to test laboratories on their ability to properly identify an atypical pathogen from a wound culture. Overall, of the laboratories that performed wound cultures, 14/16 (88%) correctly identified *P. shigelloides* from the provided sample, indicating most laboratories were successful in identifying this organism.

Genus *Plesiomonas*:
The genus *Plesiomonas* was originally classified as a member of the family *Vibrionaceaeae*, but was later reclassified as belonging to the family *Enterobacteriaceae.* (4,10,12) It is unique among the *Enterobacteriaceae* in that it is the only member to be oxidase positive. In spite of this difference, *Plesiomonas* species, like the rest of the *Enterobacteriaceae*, are facultatively anaerobic Gram negative rods that grow well at a temperature range of 25-37ºC, prefer low salinity (0-4%), and moderate pH range (4.0-8.0) (1). The genus contains only a single species, *P. shigelloides*. It is most closely related to *Edwardsiella tarda* within the *Enterobacteriaceae.* (12,14)

About *Plesiomonas shigelloides*:
Plesiomonas shigelloides was formerly known as *Aeromonas shigelloides* (2), and indeed shares common features with the *Aeromonadaceae*. Notable similarities include: distribution in a wide range of aquatic ecosystems (freshwater, estuarine, marine), Gram negative rods that produce a positive oxidase reaction, and implication as a cause of diarrheal illness. (1) It is predicted to be the cause of approximately 5% of reported cases of "Traveler's diarrhea." (12) *P. shigelloides* is thought to have a high infective dose (>1 million organisms). It is not considered a human commensal and is most often associated with a self-limiting gastroenteritis. (9,18)

P. shigelloides possesses a few potential virulence factors, including heat-stable and heat-labile enterotoxins, cholera-like toxins, and a hemolysin. (1,9,13) Most isolates are motile, which may contribute to invasiveness during infection. Expression of DNase is also a common feature of *P. shigelloides* isolates, which may enable it to better evade the innate immune response (13). From several studies, it is clear that *P. shigelloides* undergoes rapid genetic recombination at rates greatly accelerated above those of other *Enterobacteriaceae*, which often creates difficulties in strain tracking during public health investigations (14). Established PCR assays for amplification of the 23S rRNA and *hugA* genes are capable of identifying *P. shigelloides* from environmental and clinical sources. (6,7) Most recently, MALDI-TOF MS protein profiles have been developed that increase strain typing resolution. (8)

Disease and Risk Factors:
Disease caused by *P. shigelloides* is most often characterized by acute, self-limiting episodes of watery, non-mucoid, non-bloody diarrhea that resolves within 1 to 7 days. (1,9,18) Additional sequelae may include: low grade fever, chills, abdominal pain, cramping, nausea, and vomiting. (9,18) Onset of symptoms is generally within 20-50 hours of consumption or contact with a contaminated source (water, seafood, etc.). (9) However, in some cases, diarrhea can last up to 21 days. Occasionally, a dysenteric form of diarrhea (bloody, greenish-yellow, foamy, and may contain mucus and polymorphonuclear neutrophils) is reported. (1,9,18) Dehydration may be an issue with either form of diarrhea. Hospitalizations for gastroenteritis usually only occur for instances of severe disease or in patients with underlying conditions. (1,9)

P. shigelloides is also capable of causing a variety of extraintestinal diseases, though incidence is rare. Described conditions attributable to *P. shigelloides* infection include: septicemia (in adults), bacteremia, meningitis, septic arthritis, osteomyelitis, peritonitis, cellulitis, and pneumonia. As the organism is hypothesized to require a high infectious dose to cause disease, the elderly, the very young, the immunocompromised, and those afflicted with cancer, blood disorders, or hepatobiliary disease are thought to be the most at risk for *P. shigelloides* infection. (9,18)

Public Health Surveillance:
There is limited data on the incidence of *P. shigelloides*, both worldwide and nationally. However, Minnesota reported seeing an unusually high number of cultures positive for *P. shigelloides* during the summer of 2012. Effective September 10, 2012 Minnesota began requiring reporting of disease within Minnesota Disease Reporting Rules under the category of "unusual or increased case incidence of any suspect infectious illness". *P. shigelloides* however, is not reportable in North Dakota at this time.

Due to the self-limiting nature of *P. shigelloides* gastroenteritis, knowledge of the true prevalence of *P. shigelloides* is limited. It is known to be free-living primarily in freshwater habitats worldwide, can be present in a variety of animal species, including seafood, and is most commonly reported in tropical or sub-tropical environments. (1,9) The most common route of human infection is ingestion or exposure to contaminated water (aquatic habitats, ornamental fish tanks) or consumption of undercooked seafood (fish, shellfish, crabs) from a contaminated water source. (1,9,16) Summer and autumn are the months of highest incidence. (9,18) Vegetables cleaned with contaminated water have also been implicated as a source of transmission in tropical climates. (11) Zoonotic transmission from asymptomatic animal sources (mammals, birds, fish, water-dwelling reptiles and amphibians) is believed possible, though there is limited evidence to support this hypothesis. (1,5, 9) A recent literature survey (Shah, N., 2009) shows that the occurrence of *P. shigelloides*-associated "Traveler's Diarrhea" is higher in Asia than in other sites reported. Human to human transmission is through the fecal-oral route. (18)

Laboratory Identification of *Plesiomonas shigelloides*

Collection and Transport:
For a stool culture: *P. shigelloides* organisms are fragile outside of the host or aquatic environment, therefore, transport time is critical; if fresh stool specimen is received >2 hours from collection it may be detrimental to *P. shigelloides*. If immediate plating is not practical, many of the commercially available transport media (e.g., Modified Cary-Blair) are satisfactory to use for recovery of this organism and collected specimens may be refrigerated. (1)

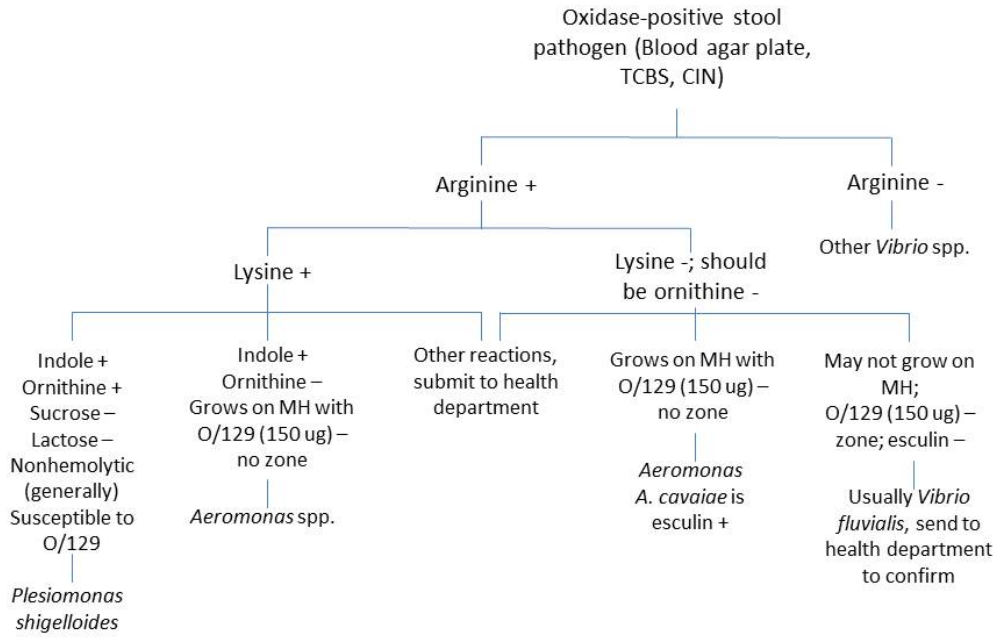
Enrichment, Isolation, and Screening Methods:
P. shigelloides will readily grow on a variety of selective and non-selective enteric media, and appears non-lactose and non-sucrose fermenting on common enteric media. In addition to the standard

MacConkey agar, it is recommended that *P. shigelloides* be grown on a medium suitable for oxidase testing such as blood agar, as the oxidase test is a key biochemical differential for this organism. Unfortunately, 28% of laboratories did not perform an oxidase test with this specimen. Unlike some *Vibrio* spp., *P. shigelloides* does not require salt for growth. (1)

P. shigelloides cannot be cultivated on thiosulfate-citrate-bile salts-sucrose (TCBS) agar but will grow on cefsulodin-irgasan-novobiocin (CIN) agar, commonly used to select for *Yersinia*. On CIN agar, *P. shigelloides* appears as opaque colonies lacking a pink center. It can be differentiated on this medium from *Aeromonas* spp. in that *Aeromonas* spp. exhibit a pink center with an opaque apron. (1)

Biochemical Identification:
P. shigelloides is Gram negative, and described as "straight rods of sizes similar to other *Enterobacteriaceae.*" (1) It is catalase and oxidase positive, does not produce gas from glucose, is susceptible to the O/129 vibriostatic agent, and is indole positive. (1,3) While *P. shigelloides* produces a hemolysin, a study has shown that hemolysis on blood agar is dependent on the type of erythrocytes used. A stronger hemolytic reaction will be observed on blood agar supplemented with calcium and iron. (13) Of the labs reporting a hemolytic result for *P. shigelloides*, 13/13 (100%) described gamma-hemolysis. While hemolysis is not a characteristic that is regularly reported for stool cultures, it may be noted for isolates from a wound culture as was the case here.

P. shigelloides possesses a somatic antigen that is similar to the smooth antigen from *Shigella* and has been known to cross react with *Shigella* serological tests. (1) Following differentiation of *P. shigelloides* from other members of the *Enterobacteriaceae*, the organism can be further differentiated from *Aeromonas* spp. by reactions in Moeller's lysine, ornthine, and arginine, and fermentation of *m*-inositol. (1,3) The primary difficulty in rapid identification of *P. shigelloides* is likely to be the result of low clinical incidence, which reinforces the need for education about this uncommon pathogen. A flowchart showing biochemical differentiation of *P. shigelloides* using standard test methods is included below.



This flowchart based on Figure 3.8.1-1 in the Manual of Clinical Microbiology Laboratory Procedures, 3rd Edition.

Antimicrobial Susceptibility Testing:
Due to the generally self-limiting nature of *P. shigelloides* infection, regular antimicrobial susceptibility testing (AST) from fecal culture isolates is not recommended. (1,18) However, in the event of extraintestinal infection or more severe disease in an immunocompromised patient, AST may be necessary. *P. shigelloides* is naturally resistant to penicillin antibiotics (1,18) and variably resistant to most aminoglycosides and tetracycline. (1) Cephalosporins, quinolones, carbapenems, and β-lactamase inhibitor combinations show good activity against this organism. (1,18) Several agents are recommended for primary antimicrobial susceptibility testing: amoxicillin-clavulanic acid, broad spectrum/extended-specturm cephalosporins, fluoroquinolones, and TMP-SMX. (1) For *P. shigelloides* AST testing methods, please refer to current CLSI documents.

Laboratory Safety:
Like all enteric pathogens, clinical specimens and cultures suspected or known to contain *P. shigelloides* should be handled under BSL2 conditions. Any procedure that may produce infectious aerosols (such as sampling from a fecal collection container) should be performed inside a biosafety cabinet. (17)

Reportable Disease Rule:
P. shigelloides historically has not been reportable to NDDoH. However, as with any disease, if an unusual increase in incidence is observed in your laboratory, please notify NDDoH; Division of Disease Control.

Specimen #3, Tabulated Results

16/16 (100%) of labs performed wound cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = ***Plesiomonas shigelloides***

Acceptable Answers: 14/16 (88%)

14/16 (88%) *Plesiomonas shigelloides*

Unacceptable Answers: 3/84 (4%)

1/16 (6%) Possible *B. pseudomallei*
1/16 (6%) Gram negative bacilli, NOS

ORGANISM CHARACTERISTICS

Gram Stain: intended result = **Gram-negative bacilli**

11/15 (73%) Gram-negative bacilli
2/15 (13%) Tiny Gram-negative bacilli/rods
2/15 (13%) Gram-negative coccobacilli

Oxidase: intended result = **Positive**

2/16 (13%) Laboratories did not indicate oxidase result
14/14 (100%) Laboratories reported oxidase as **positive**

Indole: intended result = **Positive**

9/15 (60%) Laboratories did not indicate an indole result
7/7 (100%) Laboratories reported indole as **positive**

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Specimen #4, Stool: *Vibrio parahaemolyticus*

Submitter #: 2209		
	Your Laboratory's Results	**Intended Result**
Identification	***Vibrio parahaemolyticus***	*Vibrio parahaemolyticus*
Referral of Isolate	**Our lab would notify disease control.**	Would refer isolate to NDPHL
Notify NDDoH; Division of Disease Control	**Yes**	Yes per ND Reportable Condition Rules
Methodology		
Oxidase	**Positive**	Positive

Goals:
The organism in Specimen 4 was *Vibrio parahaemolyticus*; it was chosen with the goal of raising awareness of this uncommonly isolated organism, and demonstrating the potential for illness in susceptible patient populations. The objectives were to assess the ability of North Dakota's laboratories to identify this unusual stool pathogen and to determine the current standard of practice among North Dakota laboratories for referral of this isolate to NDPHL and notification of NDDoH; Division of Disease Control in accordance with the Communicable Disease Reporting Rule.

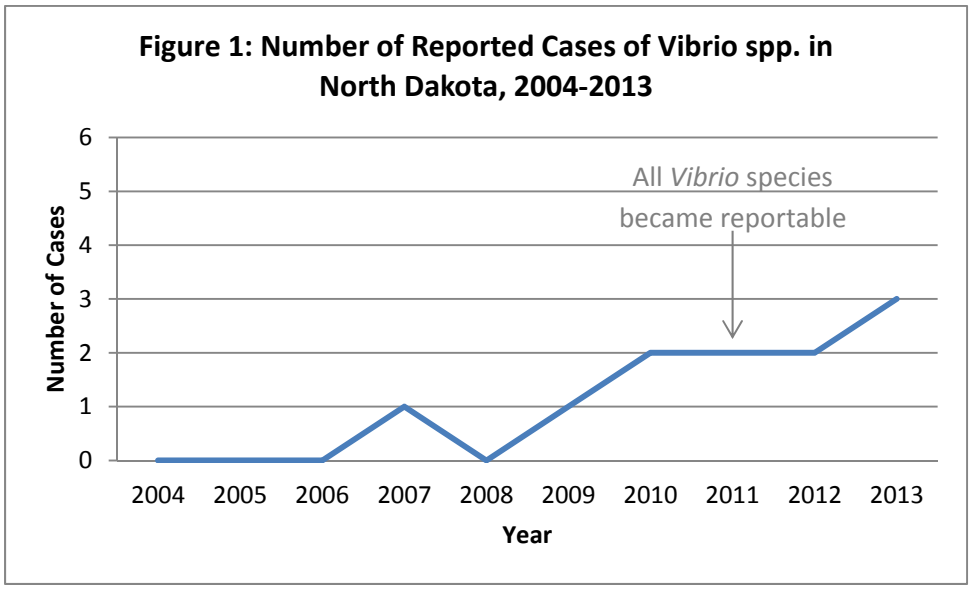
Genus *Vibrio*:
The genus *Vibrio* consists of several well-described human pathogens, including the causative agent of cholera (toxigenic *Vibrio cholerae*) and other potentially fatal human infections. The halophilic (salt-loving) *Vibrio* species typically require sodium concentrations between 0.029 and 4.1% for growth. (1) *Vibrio* species are most commonly isolated in aquatic environments and their distribution depends on water temperature, salt concentration, and the availability of other nutrients. Non-halophilic *Vibrio* species (e.g. *V. cholerae* and *V. mimicus*) may be found in freshwater rivers and lakes, as well as marine environments. *Vibrio* concentrations in aquatic sources typically peak during the summer months. (1)

About *Vibrio parahaemolyticus*:
Vibrio parahaemolyticus is halophilic and can be found free-living in coastal marine environments. During the warm seasons of the year, *V. parahaemolyticus* exists as either a free-swimming form in an estuarine water source or as a sessile form attached to marine organisms commonly consumed as seafood (i.e. shellfish). As the temperature decreases during winter months, *V. parahaemolyticus* numbers decline and some cells settle into the marine silt until temperatures again become favorable. (2) This species possesses two distinct antigens, O and K; over a dozen O antigen types and over 60 K antigen types have been identified. (2) In 1997 an unusual serotype of *V. parahaemolyticus*, O3:K6, emerged in Taiwan and caused higher than normal foodborne disease outbreaks from1996-1999. (1) Since then, the O3:K6 serotype and several serovariants have spread through much of the world. Intriguingly, the O3:K6 serovariants have not demonstrated the same capacity to disseminate and cause severe disease. (1)

Pathogenic strains of *V. parahaemolyticus* generally produce themostable direct hemolysins (TDH) and/or thermostable direct-related hemolysin (TRH), both toxins capable of inducing diarrhea in humans. DNA gene probes targeting the *tdh* or *trh* genes are typically used to determine if these virulence factors are present in clinical isolates. (2) Of note, strains lacking the *tdh* and *trh* genes are still capable of causing human disease, indicating that *V. parahaemolyticus* possesses other, as of yet unidentified, virulence factors. (6)

Disease and Risk Factors:
In the U.S., *V. parahaemolyticus* is primarily found in seafood harvested from coastal areas. Consumption of raw or undercooked seafood, particularly clams and oysters, may lead to gastroenteritis, typically of moderate severity, that lasts 1-7 days and is generally self-limiting. (2) Symptoms include watery diarrhea, abdominal cramping, nausea, vomiting, fever, and headache; about 25% of patients may experience bloody and/or mucoid stools with a high white blood cell count and a high fever. (2) Septicemia can be a complication in individuals with pre-existing medical conditions. (6) Immunosuppressed individuals as well as individuals with diabetes, liver disease, peptic ulcers, and a decreased gastric acidity are more susceptible to acquiring infection. (2) In more rare cases, *Vibrio parahaemolyticus* may cause wound infections when abraded tissue is contaminated with the organism (typically by exposure to contaminated seawater).

Public Health Surveillance:
Since vibriosis became reportable to the North Dakota Department of Health in 2011, there have been seven cases reported. Prior to 2011, only *vibrio cholera* species were reportable in North Dakota. Figure 1 shows the number of *Vibrio* illness reported in North Dakota from 2004-2013. Isolates since 2011 have been identified as *V. parahaemolyticus* (2), *V. mimicus* (1), and non 01/0139 *V. cholera* (4). The incidence of *Vibrio* infection is low in North Dakota.



Laboratory Identification of *Vibrio parahaemolyticus*

Laboratory Safety:
Like all enteric pathogens, clinical specimens and cultures suspected or known to contain *Vibrio parahaemolyticus* should be handled under BSL2 conditions. Any procedure that may produce infectious aerosols (such as vortexing bacterial suspensions, or manipulating blood culture bottles) should be performed inside a Class II biosafety cabinet. (3)

Collection and Transport:
Specimens should be collected as quickly as possible after the onset of symptoms and before any antibiotic treatment has begun. (1) The ideal specimen from gastroenteritis infections is freshly passed stool collected in a sterile container, although rectal swabs with visible fecal staining or vomitus are also acceptable from patients with acute illness. If specimens cannot be processed immediately (i.e. within 2-4 hours of collection), then they should be collected in an approved transport medium such as Cary-Blair,

Stuart's, or Amies medium. (1) Wound or blood cultures do not require any specialized collection procedures since *Vibrio* species are typically found in pure culture from these sites and most commercial media contain enough salt to support growth.

Enrichment, Isolation, and Screening Methods: *Vibrio parahaemolyticus* will grow on most commercially available media, therefore specialized enrichment or isolation media are not routinely necessary. However, thiosulfate-citrate-bile salts-sucrose (TCBS) agar can be a useful screening medium for *Vibrio* species in stool cultures due to the selective properties of the bile salts and thiosulfate, and the differential properties of sucrose. *V. parahaemolyticus* will most often produce green colonies on TCBS since most strains do not ferment sucrose. (1) CHROMagar™ Vibrio, a chromogenic agar, may be used to recover *V. parahaemolyticus* while inhibiting the growth of other enteric organisms. (1) *Vibrio* colonies on this agar range from white to pale blue or violet. On non-selective media, *Vibrio* species in pure culture may produce a variety of colony morphologies, giving the appearance of a mixed culture. (1)

Biochemical Characteristics:
Vibrio species are Gram-negative straight, curved, or comma-shaped rods that are catalase and oxidase positive. (1) They are lactose negative on MacConkey agar and. motile.Some *V. parahaemolyticus* isolates will demonstrate swarming activity on solid media. *Vibrio* species also require various concentrations of sodium ions for growth, which, in addition to the oxidase reaction, can be helpful in differentiating them from *Enterobacteriaceae.* Susceptibility to O129 (2,4-diamino-6,7-diisopropyl-pteridine phosphate), a vibriostatic compound, as well as a positive string test can further differentiate *Vibrio* from *Aeromonas* spp.

Moreover, *V. parahaemolyticus* ferments glucose (without gas production) produces a negative Voges-Proskauer reaction and a positive lysine decarboxylase reaction when these media are supplemented with NaCl (1). This organism can also be identified by commercial identification systems, although there may be difficulty with some systems due to the organism's requirement for salt. (1)

Antimicrobial Susceptibility Testing:
Though *Vibrio parahaemolyticus* is susceptible to several classes of antibiotics, there is evidence that more antimicrobial-resistant strains are emerging. *Vibrio parahaemolyticus* is resistant to ampicillin (4), and more recent studies have demonstrated resistance to cefazolin, amikacin, and cefuroxime along with increasing resistance in other antimicrobial classes. (5)
Gastrointestinal illness caused by *Vibrio parahaemolyticus* is typically self-limiting, and treatment with antibiotics has not been shown to decrease the severity of the disease. However, treatment may be required for wound infections and rare cases of septicemia. Effective antimicrobials include tetracycline, third generation cephalosporins, aminoglycosides, and fluoroquinolones, though appropriate antibiotic treatment should be selected based upon the susceptibilities of the particular organism.

Reportable Disease Rule:
Rapid and accurate diagnosis by clinical laboratories is crucial for the detection of outbreaks, timely public health interventions, and detection of *Vibrio* species. In North Dakota, all suspected or confirmed cases of *Vibrio* infection, regardless of species or source, must be reported to NDDoH; Division of Disease Control within seven working days of identification due to the significant clinical and epidemiologic impact of Vibrio disease. In addition to case reporting, culture isolates from all cases must be sent to NDPHL for complete characterization.

Specimen #4 Tabulated Results

ORGANISM IDENTIFICATION
11/16 (69%) of labs performed stool cultures

ORGANISM IDENTIFICATION
Identification: Intended answer = ***Vibrio parahaemolyticus*, with referral to NDPHL**

Acceptable Answers: 7/10 (64%)
5/11 (45%) *Vibrio parahaemolyticus*, with referral to NDPHL
3/11 (27%) *Vibrio* species, with referral to NDPHL

Unacceptable Answers: 6/54 (11%)
1/11 (9%) *Vibrio parahaemolyticus*, without referral to NDPHL
1/11 (9%) *Shigella sp.*
1/11 (9%) No growth

ORGANISM CHARACTERISTICS

Oxidase: intended result = **positive**
7/15 (33%) Laboratories did not indicate oxidase result
8/8 (100%) Laboratories reported oxidase as **positive**

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NDLRN/MLS: Challenge Set 2013
Self-Study Quiz

Specimen #1 – *Yersinia pestis*

1. Notification of a possible *Yersinia pestis* isolate must be (chose all that apply):
   a. Reported prior to sending the isolate
   b. Done by calling the ND-PHL 701-328-6272 Monday-Friday 8:00 AM to 5:00 PM or 701-400-2772 after hours.
   c. As soon as *Yersinia pestis* could not be ruled out
   d. You do not have to notify the PHL – just send it

2. Which of the following are characteristic of *Y. pestis* (choose all that apply)?
   a. Gram negative bacillus
   b. Hammered copper colonies
   c. Bipolar staining
   d. Motility negative
   e. all of the above

3. *Y. pestis* is sometimes misidentified as (choose all that apply):
   a. *Shigella* species
   b. *Salmonella* species
   c. *Acinetobacter* species
   d. *Yersinia pseudotuberculosis*
   e. Any of the above

4. **True** **False** Working with *Yersinia pestis* on an open bench is considered a potential exposure.
5. **True** **False** Using an automated method is an appropriate way to identify *Yersinia pestis*.

Specimen #2 – *Corynebacterium diphtheria*

1. Which of the following are characteristic of *C. diphtheria* (choose all that apply)?
   a. Gram-negative bacillus
   b. Forms black colonies on Tinsdale media
   c. Catalase negative
   d. CAMP positive

2. Which usable energy forms are fermented by *C. diphtheriae* (choose all that apply)?
   a. Glucose
   b. Maltose
   c. Sucrose
   d. Mannitol
   e. Xylose
   f. All of the above

3. **True** **False** Medically relevant species of Corynebacteria are all motile.
4. **True** **False** Diphtheria is a vaccine preventable disease.
5. **True** **False** NDDoH; Division of Disease Control should be notified of diphtheria according to the Reportable Disease Rule.

Specimen #3 – *Plesiomonas shigelloides*

1. *P. shigelloides* is _____ and _____ positive.
   a. PYR, indole
   b. Oxidase, indole
   c. Beta-lactamase, oxidase
   d. ONPG, PYR

2. *P. shigelloides* can be differentiated from *Aeromonas* spp. by (choose all that apply).
   a. Lysine
   b. Ornithine
   c. Arginine
   d. M-inositol

3. **True** **False** *P shigelloides* is motile.
4. **True** **False** *P. shigelloides* must be reported to NDDoH; Division of Disease Control as part of the Reportable Disease Rule.
5. **True** **False** Expression of DNase in *P. shigelloides* isolates help it evade the innate immune response.

Specimen #4 – *Vibrio parahaemolyticus*

1. *V. parahaemolyticus* can cause:
   a. Diarrhea
   b. Septicemia
   c. Wound infections
   d. All of the above

2. *Vibrio spp.* requires _____ for growth.
   a. Glucose
   b. Lacose
   c. NaCl
   d. ONPG

3. Which two distinct antigens does *V. parahaemolyticus* possess? (choose two):
   a. O
   b. S
   c. K
   d. N

4. **True** **False** In North Dakota, the majority of *Vibrio* isolates were *V. parahaemolyticus*.
5. **True** **False** All *Vibrio* isolates, regardless of source, should be submitted to the North Dakota Department of Health; Division of Laboratory Services.

NDLRN/MLS: Challenge Set 2013
Self-Study Quiz

Specimen #1 – *Yersinia pestis*

1. Notification of a *Yersinia pestis* isolate must be (chose all that apply):
   a. Reported prior to sending the isolate
   b. Done by calling the ND-PHL
   c. As soon as *Yersinia pestis* could not be ruled out
   d. You do not have to notify the PHL – just send it

2. Which of the following are characteristic of *Y. pestis* (choose all that apply)?
   a. Gram negative bacillus
   b. Hammered copper colonies
   c. Bipolar staining
   d. Motility negative
   e. all of the above

3. *Y. pestis* is sometimes misidentified as (choose all that apply):
   a. *Shigella* species
   b. *Salmonella* species
   c. *Acinetobacter* species
   d. *Yersinia pseudotuberculosis*
   e. Any of the above

4. **True** **False** Working with *Yersinia pestis* on an open bench is considered a potential exposure.
5. **True** **False** Using an automated method is an appropriate way to identify *Yersinia pestis*.

Specimen #2 – *Corynebacterium diphtheria*

1. Which of the following are characteristic of *C. diphtheria* (choose all that apply)?
   a. Gram-negative bacillus
   b. Forms black colonies on Tinsdale media
   c. Catalase negative
   d. CAMP positive

2. Which usable energy forms are fermented by *C. diphtheriae* (choose all that apply)?
   a. Glucose
   b. Maltose
   c. Sucrose
   d. Mannitol
   e. Xylose
   f. All of the above

3. **True** **False** Medically relevant species of Corynebacteria are all motile.
4. **True** **False** Diphtheria is a vaccine preventable disease.
5. **True** **False** NDDoH; Division of Disease Control should be notified of diphtheria according to the Reportable Disease Rule.

Specimen #3 – *Plesiomonas shigelloides*

1. *P. shigelloides* is _____ and _____ positive.
   a. PYR, indole
   b. Oxidase, indole
   c. Beta-lactamase, oxidase
   d. ONPG, PYR

2. *P. shigelloides* can be differentiated from *Aeromonas* spp. by (choose all that apply).
   a. Lysine
   b. Ornithine
   c. Arginine
   d. M-inositol

3. **True** **False** *P shigelloides* is motile.
4. **True** **False** *P. shigelloides* must be reported to NDDoH; Division of Disease Control as part of the Reportable Disease Rule.
5. **True** **False** Expression of DNase in *P. shigelloides* isolates help it evade the innate immune response.

Specimen #4 – *Vibrio parahaemolyticus*

1. *V. parahaemolyticus* can cause:
   a. Diarrhea
   b. Septicemia
   c. Wound infections
   d. All of the above

2. *Vibrio spp.* requires _____ for growth.
   a. Glucose
   b. Lacose
   c. NaCl
   d. ONPG

3. Which two distinct antigens does *V. parahaemolyticus* possess? (choose two):
   a. O
   b. S
   c. K
   d. N

4. **True** **False** In North Dakota, the majority of *Vibrio* isolates were *V. parahaemolyticus*.
5. **True** **False** All *Vibrio* isolates, regardless of source, should be submitted to the North Dakota Department of Health; Division of Laboratory Services-Microbiology (ND-PHL).