CHALLENGE SET 2012

PARTICIPANT SUMMARY REPORT

May 2013

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

North Dakota LRN/MLS: Challenge Set 2012 Participant Summary Report

NDLRN Contacts;

Thank you for participating in the NDLRN/MLS 2012 Challenge Set. We hope this was a useful learning experience. The purpose of the Challenge Set program is to provide a learning tool for LRN Sentinel laboratorians to work through a variety of unusual, newly emerging, and "every day" bacterial organisms, as well as potential bioterrorism agents.

Participant Summary Report: On the cover page of this document is your facility's "submitter number", which has been permanently assigned to your facility and is tied to your laboratory's results. Following the title page you will find the Challenge Set Results Summary for each organism which contains the intended results alongside your laboratory's results. Pertinent information about each organism as well as statistics collated from North Dakota participating laboratories is provided for your consideration.

Competency questions: A set of questions with answers for each of this year's challenge set organisms has been provided so that you may use this summary as a competency in your laboratory. Please do not send your answers to the ND-PHL. The exam is intended to be used internally by your laboratory.

If there are any questions or comments regarding this Challenge Set, please feel free to contact me.

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Note: The North Dakota Department of Health, Public Health Laboratory (ND-PHL) does not support the substitution of these challenge sets for proficiency testing in which your laboratories currently participate.

Disclaimer: Names of vendors or manufacturers are provided for discussion purposes only; inclusion does not imply endorsement by the North Dakota Department of Health.

Specimen #1, Blood: *Francisella tularensis*

Submitter #: 2209		
	Your Laboratory's Results	**Intended Result**
Identification	***Francisella tularensis***	*Francisella tularensis*
Referral of Isolate	**We are the LRN reference lab. Our lab would refer this isolate for serotyping to CDC.**	Would refer isolate to ND LRN Reference Laboratory
Methodology		
Gram stain	**Gram negative coccobacilli**	Gram negative coccobacilli
β-lactamase	**Positive**	Positive
Oxidase	**Negative**	Negative
Satelliting	**No response given**	Negative
Urea	**Negative**	Negative
Catalase	**Positive**	Weakly positive or negative

Goal:
The organism in specimen #1 was a vaccine strain of *Francisella tularensis*. The goal was to exercise LRN Sentinel Laboratories' ability to rule-out and refer potential *F. tularensis* isolates to North Dakota Department of Health; Division of Laboratory Services (North Dakota's LRN Reference Laboratory). The Center for Disease Control & Prevention (CDC) has classified *F. tularensis* as a Category A bioterrorism agent and a Select Agent.

Genus *Francisella*:
The genus *Francisella*, family *Franicisellaceae*, is comprised of four species: *F. tularensis*, *F. philomiragia*, *F. novicida,* and *F. noatunensis*. *F. philomiragia* and *F. novicida* are rarely the cause of clinical disease and are more typically found in water animals and their environments (e.g. muskrats). The members of this genus are poorly staining tiny Gram-negative coccobacilli that are strict aerobes. Most members of the genus ferment only a few sugars (e.g. glucose, maltose, sucrose and glycerol). (6)

About *Francisella tularensis*:
F. tularensis, an intracellular pathogen, is a slow-growing, aerobic, oxidase-negative, tiny, faintly staining Gram-negative coccobacillus. Fresh clinical material likely to contain high concentrations of *F. tularensis* such as ulcers, wounds, and lymphoid tissue (liver, spleen, affected lymph node) are the best specimens for recovery of the organism. Important characteristics of this organism are its Gram stain morphology and negative urea and oxidase. (6)

F. tularensis has three subspecies: *tularensis* (type A), *holarctica* (type B), and *mediasiatica*. *F. tularensis* Type A is the most virulent subspecies with a 50% lethal dose of <10 cells for laboratory mice and rabbits; comparatively, *F. tularensis* Type B is less virulent with a 50% lethal dose of <1,000 cells. Type A almost exclusively occurs in North America where it is associated with infections in humans and rabbits. Because of the differences in virulence between Type A and Type B, it is important for clinical laboratories to refer isolates to their public health laboratories for typing. (6)

Tularemia:
G. W. McCoy described a plague-like disease in California (Tulane County) ground squirrels in 1911 and named the causative agent *Bacterium tularense* in 1912. Edward Francis recognized and described the human disease in 1922 and the agent was renamed *Francisella tularensis* in his honor. This illness was named "tularemia", and is also known as "Rabbit Fever" and "Deerfly Fever." (2,6)

There are several forms of tularemia: ulceroglandular, glandular, oculoglandular, pharyngeal, typhoidal, and pneumonic. Ulceroglandular infections are the most common naturally occurring form of the disease. Patients with this form usually acquire the disease through a tick or deerfly bite or by having some sort of contact with infected animals (typically rabbits). A non-healing lesion at the site of infection and regional

lymphadenopathy are the hallmarks of this form of the disease. Pneumonic tularemia is less common, much more deadly, and is the most likely form in the event of a bioterrorism incident. (6)

Tularemia is relatively rare in the U.S., with approximately 100 - 200 human cases occurring each year. Most cases are reported in south central states such as Arkansas, Missouri and Oklahoma. South Dakota also reported a high incidence in the years 1990-2000 when compared to the rest of the country. There are typically about 2 human cases of tularemia in North Dakota every spring/summer. In 2012 North Dakota had 3 human cases and no animal cases. In 2011 North Dakota reported 2 human cases and 5 animal cases (2 horses and 3 cats). There was 1 human case of tularemia in 2010 and 1 animal case (cat).

Martha's Vineyard is an island near Cape Cod, Massachusetts, where tularemia is endemic; it is one of the few places in the U.S. that have reported localized outbreaks of pneumonic tularemia. It is thought that *F. tularensis* was introduced onto the island in 1937 when more than 200,000 cottontail rabbits were released for sporting purposes from Missouri and Kansas. A study was conducted during an outbreak in the year 2000, where it was demonstrated that lawn mowing or brush-cutting were significant risk factors for acquiring pneumonic tularemia; supporting the hypothesis that these activities allowed for aerosolization and subsequent inhalation of the organism. (1,5)

Laboratory Identification of *Francisella tularensis*

Microscopic morphology:
The presence of very tiny (0.2-0.5 µm x 0.7-1.0 µm), faintly staining Gram-negative coccobacilli in direct smears, along with a slow-growing isolate on plated media, **should alone trigger the suspicion of *F. tularensis***. Note: if *F. tularensis* is suspected, original specimens can be sent to the North Dakota Department of Health; Division of Laboratory Services for rapid DFA testing and PCR. (2)

Growth Characteristics:
F. tularensis may grow on sheep blood agar (SBA) upon initial culturing, but will typically fail to grow on SBA with subsequent sub-culturing. The organism requires cysteine to grow, which may be found in certain formulations of Thayer-Martin agar, Chocolate agar (with IsoVitaleX), CHAB, BCYE agar, and thioglycollate broth. Incubation at 35-37°C in ambient air is adequate however the use of 5% CO_2 is acceptable. Primary plates should be examined for 5 days and up to 7 days if the patient had prior treatment with antibiotics, before reporting culture as "no growth." Colonies are usually too small to be seen at 24 hours incubation. After 48 hours, colonies are about 1-2 mm in diameter and grow as gray-white or bluish-gray, opaque colonies. There is no growth on MAC or EMB agar. (2)

Biochemical Characteristics:
As described in the attached algorithm, *F. tularensis* organisms are oxidase and urease negative; they are also β-lactamase positive and catalase weakly positive. Use of Commercial System for Identification: Use of an automated commercial identification system is not recommended with *F. tularensis* due to aerosol creation and subsequent exposures to laboratory personnel.

Since the organism is slow-growing and has very few positive biochemical reactions, most conventional biochemical or commercial identification systems may have difficulty identifying this organism. In this challenge two participants attempted to use a commercial ID system but found that it did not produced reliable results. (2)

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 *Francisella tularensis* was not used consistently. Of those laboratories that reported a final identification of: *Francisella tularensis* (8 labs, 9%) or Possible *Francisella tularensis* (45 labs, 52%), a variety of methods were reported as used to obtain the results, with no laboratories performing all five LRN Sentinel laboratory tests. Those tests are: β-lactamase, oxidase, catalase, satelliting, 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 tests, they should do so. However, as stated previously, **the presence of very tiny, faintly staining Gram-negative coccobacilli in direct smears, along with a slow-growing isolate on plated media, should alone trigger the suspicion of *F. tularensis*.**

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 North Dakota LRN Reference Laboratory 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/guidelines/sentinel-guidelines

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. Participating laboratories were asked to notify the North Dakota Department of Health; Division of Laboratory Services (ND's LRN Reference Laboratory) as per challenge set instructions: "When appropriate, participants should follow the notification protocols outlined in the "Guidelines for LRN Advanced Sentinel Laboratories-2006" and call the ND LRN Reference Laboratory." Based on the identifications deemed "acceptable answers," (as stated below) in 2012 we would have expected 16/17 (94%) labs to notify the ND LRN Reference Laboratory, but only 15(94%) of those labs made a phone call. 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 ND LRN Reference Laboratory and then the isolate should be referred.**

Laboratory Hazards:
Using an automated system to attempt identification of an organism suspected as *F. tularensis* is **not** recommended because of the potential of generating aerosols and subsequent exposure to 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 *F. tularensis* in any specimen, **the ND LRN Reference Laboratory must be called first** and the isolate must be referred to the ND LRN Reference Laboratory and **not** to your regular reference laboratory. As an LRN Reference lab, the North Dakota Department of Health; Division of Laboratory Services has rapid, validated LRN methods for confirming *F. tularensis* that regular commercial laboratories do not have. Referral to a standard commercial reference laboratory will delay confirmation and subsequent public health response.

Call the ND LRN Reference Laboratory On-Call Phone at 701-400-2772 and Refer Isolate if a majority of the following characteristics are noted:

- Faintly staining, tiny Gram-negative coccobaccili on direct smear
- Pinpoint colony at 24 h on Chocolate Agar
- Satellite or XV test negative
- β-lactamase positive
- Oxidase and urease negative
- Catalase weak positive or negative

Reportable Disease Rule:
Tularemia *(F. tularensis)*, when found or suspected, must be reported **'immediately by telephone'** to the ND Department of Health; Division of Disease Control and submission of clinical materials* to the ND

Department of Health; Division of Laboratory Services is required as per the Communicable Disease Reporting ND Rule. For more information about the ND Communicable Disease Reporting Rule, please visit:
http://www.ndhealth.gov/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:
F. tularensis 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). This rule dictates that isolates must be referred to a select agent registered laboratory (i.e. ND Department of Health; Division of Laboratory Services) or destroyed within 7 days of identification and appropriate documentation paperwork must be submitted. The strain used in this challenge set was the vaccine strain, an excluded strain, therefore not a Select Agent. (3)

Upon identification of a select agent, a 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 on an open bench top, a Form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) must be filled out and sent as well. The ND Department of Health; Division of Laboratory Services can assist with completing these forms. Electronic copies of the forms can be found online at www.selectagents.gov

Specimen #2, Tabulated Results

16/17 (94%) of labs performed blood cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = ***Francisella tularensis,* with referral to ND LRN Reference Laboratory**

Acceptable Answers: 14/16 (88%)
11/16 (69%) Possible *Francisella tularensis*, with referral to ND LRN Reference Laboratory
1/16 (6%) *Francisella tularensis,* with referral to ND LRN Reference Laboratory
1/16 (6%) Gram-negative bacilli, NOS, with referral to ND LRN Reference Laboratory

Unacceptable Answers: 3/16 (18%)
1/16 (6%) Possible *Francisella tularensis*, without referral to ND LRN Reference Laboratory
1/16 (6%) *Brucella sp.*, without referral to ND LRN Reference Laboratory
1/16 (6%) Possible *Yersinia pestis*, without referral to ND LRN Reference Laboratory

ORGANISM CHARACTERISTICS

Gram Stain: intended result = **Tiny Gram-negative coccobacilli**
12/16 (75%) Tiny Gram-negative coccobacilli
3/16 (19%) Tiny Gram-negative bacilli/rods
1/16 (6%) Poorly staining tiny gram negative coccobacillus

β-lactamase: intended result = **Positive**
4/16 (25%) Laboratories did not indicate β-lactamase result
12/16 (75%) Laboratories reported β-lactamase as **positive**
0/16 (0%) Laboratories reported β-lactamase as **negative**

Nitrate: intended result = **Negative**
15/16 (94%) Laboratories did not indicate nitrate result
1/1 (100%) Laboratories reported as nitrate **negative**
0/0 (0%) Laboratories reported nitrate **positive**

Oxidase: intended result = **Negative**
1/16 (6%) Laboratories did not indicate oxidase result
15/16 (94%) Laboratories reported oxidase as **negative**
0/16 (0%) Laboratories reported oxidase as **positive**

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

Urea: intended result = **Negative**
2/16 (13%) Laboratories did not indicate urea result
14/16 (87%) Laboratories reported urea as **negative**

REFERENCES

1. Belding DL, Merrill M. Tularemia in imported rabbits in Massachusetts. N Engl J Med 1941; 1085-87.
2. Chu, C.C., R.B. Carey, and D.F. Welch, December 2001, Basic Protocols for Level A Laboratories for the Presumptive Identification of *Francisella tularensis*. ASM. *In* Guidelines for LRN Advanced Sentinel Laboratories 2006, Section 8 - *Francisella tularensis*, Minnesota Department of Health/Minnesota Laboratory System. St. Paul, Minnesota.
3. 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.
4. Farlow, J., D.M.Wagner, M. Dukerich, M. Stanley, M. Chu, K. Kubota, J. Petersen, and P. Keim. November 17, 2005, posting date. *Francisella tularensis* in the United States. Emerging Infectious Diseases. [Online.] http://www.cdc.gov/ncidod/EID/vol11no12/05-0728.htm
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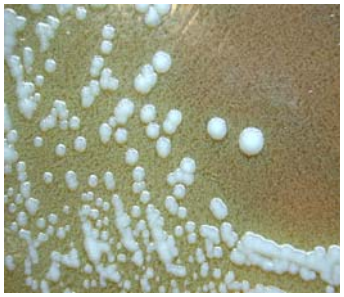
6. Peterson, J., M.E. Schriefer., and G.F. Araj. Francisella and Brucella, p. 751-769. *In* Versalovic, J., K.C.Carroll, J.H. Jorgensen, G. Funke, M.L. Landry, and D.W.Warnock (ed.), Manual of Clinical Microbiology, 10th Edition. Vol 1, ASM Press, Washington, DC.

FRANCISELLA TULARENSIS

Cysteine Heart Agar - 72 hrs.



Chocolate Agar - 72 hrs.

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 #2, Knee joint fluid: *Neisseria gonorrhoeae*

Submitter #: 2209		
	Your Laboratory's Results	**Intended Results**
Identification	***Neisseria gonorrhoeae***	*Neisseria gonorrhoeae*
Gram stain	**Gram negative diplococci**	Gram negative diplococci
Oxidase	**Positive**	Positive

Goal:
The organism in specimen #2 was *Neisseria gonorrhoeae* in pure culture. This isolate was chosen to represent an organism that is uncommon in this source but an important pathogen of public health concern. The goal was to exercise laboratories' ability to detect and identify this organism from this source and to discuss the public health importance of *N. gonorrhoeae,* including new CDC guidelines for treatment.

Genus *Neisseria*:
Members of the genus *Neisseria* are coccal or rod-shaped, gram-negative organisms, that do not form spores, and are non-motile. They may appear in short chains and have a 'coffee bean' shape. Most members are inhabitants of the mucous membrane surfaces of warm-blooded hosts. Most grow optimally at 35-37ºC and are stimulated by CO_2 and humidity. All members are oxidase positive. Pathogenic species of Neisseria are somewhat more demanding in their nutrition and require cysteine and a useable energy source such has glucose, pyruvate, or lactate. (1)

About *Neisseria gonorrhoeae*:
Although most *N. gonorrhoeae* infections cause an acute urethritis in males and infection of the endocervix in females, it is possible to acquire pharyngeal, anorectal, ocular, and disseminated gonococcal infections (DGI) as well.

Mode of Infection:
The overriding risk factor for acquiring gonorrhea is sexual intercourse with an infected partner. The risk of transmission of gonorrhea from an infected woman to the urethra of her male partner is approximately 20% per episode of unprotected intercourse and increases 60-80% after four or more exposures. Although less studied, the risk of male to female transmission is approximately 50-70% per contact. (2)

Clinical Significance:
Infections caused by *N. gonorrhoeae* can range in severity from acute urethritis to septicemia. *N. gonorrhoeae* can also cause disseminated disease, referred to as disseminated gonococcal infection (DGI), in 0.5 to 3% of individuals following infection of a genital site that spreads to other sites such as knee, elbow, wrist, finger, or ankle joints. DGI is characterized by low-grade fever, chills, hemorrhagic skin lesions, tenosynovitis, migratory polyarthralgias, and arthritis. Skin lesions are often painful and appear as papules that turn into necrotic pustules, most often found on the extremities. Complications of DGI include endocarditis and meningitis. DGI endocarditis usually involves the aortic valve and follows a rapid, destructive course. Pericarditis, pericardial effusions, and adult respiratory distress syndrome may also be complications of DGI. Thirty to forty percent of septic cases will localize to one or more joints to cause gonococcal arthritis. Joint involvement is usually asymmetric (1). Repeat bouts of DGI may occur in individuals with complement component deficiencies (C7, 8, or 9), AIDS, or lupus. (1)

Public Health Surveillance:
Screening of sexually active people is the number one pre-emptive measure to prevent gonorrhea and other treatable sexually transmitted diseases (STDs). Because there was increased emphasis on STD screening in the mid-70's, there was a large decrease nationwide in gonorrhea cases after the mid-1970s. Sexually active women younger than age 25 and older women with risk factors should be screened annually. Sexually active men who have sex with men (MSM) should be tested routinely for gonococcal infection depending on anatomical sites exposed. Reporting of cases by health care providers is important for epidemiological monitoring and helps to target control efforts. Public education and personal counseling to encourage healthy sexual behavior and barrier contraceptives are essential to STD control. After any STD diagnosis, patients should be screened for other common sexually transmitted infections. (2)

Incidence and Epidemiology:
In the United States as well as North Dakota, the highest incidence rates of genital gonorrhea occur in 15-24 year old men and women and incidence is strikingly higher among African-Americans compared to Caucasians. More cases of gonorrhea are reported in men than in women reflecting both a greater ease of diagnosis and incidence of infection in MSM than in heterosexual men and women. Demographic factors of gonorrhea throughout the world are similar to those in the United States. (2)

Treatment:
Although antimicrobial susceptibilities vary greatly over geographical areas, populations, and time, recommended therapy for arthritis is ceftriaxone or cefotaxime for 7 days (3). Similarly, DGI endocarditis recommendations are ceftriaxone or equivalent cephalosporin for 4 weeks. Meningitis is treated with a 10-14 day dose of ceftriaxone. (2)

In August, 2012, CDC released new treatment guidelines stating that **oral** cephalosporins were no longer recommended for treating gonoccocal infections. It has been several years since penicillin, tetracyclines, and fluoroquinolones have not been recommended for treatment for gonorrhea in the U.S. The Gonoccoccal Isolate Surveillance Project (GISP), a CDC supported sentinel surveillance system that MDH-PHL has been involved in since 1986, was established to monitor *N. gonorrhoeae* antimicrobial susceptibilities and is the only source in the U.S. of such data.

In 2007, analysis of GISP data prompted CDC officials to no longer recommend fluorquinolones for treatment due to emerging resistance, leaving cephalosporins as the only remaining recommended drug class. In 2010, to ensure treatment of co-pathogens (e.g. *Chlamydia trachomatis*) and address concerns about emerging gonoccocal resistance, CDC's treatment guidelines recommended combination therapy of a cephalosporin (ceftriaxone or cefixime) for *N. gonorrhoeae* plus either azithromycin or doxycycline. Current GISP data and reports from Europe suggest that the effectiveness of cefixime might be waning. CDC officials believe that as cefixime becomes less effective, continued use might hasten the development of resistance to ceftriaxone. Maintaining the effectiveness of ceftriaxone for as long as possible is of the utmost importance, therefore CDC no longer recommends the routine use of cefixime as a first-line regimen for treatment of gonorrhea. Currently, the CDC recommends dual therapy with ceftriaxone 250 mg **intramuscularly** as a single dose plus either azithromycin 1 gram orally as a single dose or doxycycline 100 mg orally twice a day for 7 days as the most effective treatments. (4, 6)

A key feature of the new guidelines and one that directly affects microbiology laboratories is the recommendation that patients diagnosed with gonorrhea having persistent symptoms that do not resolve within 3-5 days post-treatment should be cultured for *N. gonorrhoeae* and any subsequent isolate tested for cephalosporin resistance. (4) Test of cure should be performed one week after re-treatment. The obvious challenge is that the number of laboratories performing culture for *N. gonorrhoeae* has rapidly declined after the widespread adoption of nucleic acid amplification testing (NAAT) for gonorrhea diagnosis and the capacity to perform antimicrobial susceptibility testing (AST) on *N. gonorrhoeae* is even more limited.– If your laboratory has isolated *N. gonorrhoeae* that requires AST, and it is not

offered by your normal reference laboratory, please contact the North Dakota Department of Health; Division of Laboratory Services to discuss susceptibility testing options. (6)

Laboratory Identification of *Neisseria gonorrhoeae*

Specimen Collection and Organism Isolation:
Transport of specimens already inoculated onto growth media has several advantages over swab transport systems. Commercially available systems such as JEMBEC plates and the Gono-Pak protect the organism in a CO_2-rich environment. Media is inoculated with the specimen from a swab and placed into an impermeable plastic bag with a bicarbonate-citric acid pellet which when in contact with water, generates CO_2.

Stuart's or Amies buffered semisolid transport media can be used for the transport of swabs with suspect *N. gonorrhoeae*. Some swab transport systems use sponges while others use a semisolid medium with or without activated charcoal. These systems may preserve the viability of gonococci for as long as 48 hours. Semisolid transport media are superior to media-soaked sponges because sponges may contain substances that can inhibit or injure fastidious organisms. In order to prevent loss of viability, specimens in transport medium should not be refrigerated and should be inoculated onto growth medium within 6 hours of collection. (1)

For non-genital sources, during the arthritis-dermatitis state, gonococci can often be recovered by blood culture, but synovial fluid if obtained usually contains less than 200,000 leukocytes/ml and is sterile. Gonococci can often be seen by immunohistochemical methods in biopsy specimens of skin lesions, but cultures are sometimes sterile. In septic gonococcal arthritis, synovial fluid contains more than 50,000 leukocytes/ml and is often positive with gonococci, but at this stage blood cultures are often negative. A minimum of 3 blood cultures is suggested because bacteremia is intermittent with DGI. (2)

Microscopic Morphology:
N. gonorrhoeae is a Gram-negative diplococci on Gram-stained smears and has a "coffee bean" appearance, due to the flattened adjacent sides of the organism. If the strain is one that is heavily encapsulated, pink halos may be observed around the cells. (1)

Culture and Characterization:
After 18-24 hours incubation, *N. gonorrhoeae* colonies produce variable colony morphologies ranging from about 0.5-1.0 mm in diameter, glistening, and have a raised surface. Multiple colony morphologies on one plate may give the appearance of a mixed culture. Some *N. gonorrhoeae* have the ability to grow on sheep blood agar after proper incubation, while others only grow on chocolate agar. (1)

Biochemical Characteristics:
N. gonorrhoeae is oxidase and catalase positive, and produces acid from glucose, pyruvate, and lactate. Identification can be made using carbohydrate acidification, the ability to grow on Modified Thayer Martin agar and/or nutrient agar, chromogenic enzyme substrate tests, immunological tests, multitest identification systems, and DNA probe tests. (1)

Use of Commercial Systems for Identification:
Over half (11/16= 69%) of participants used automated commercial identification systems (Table 1**)**. All systems generally identified the organism very well at least to the genus level with only 1 lab not being able to identify the organism from the automated method.

Table 1: Identifications Obtained From Automated Instruments by System and Card Type

	VITEK GN-ID	VITEK NHI	Microscan HNID	MICROSCAN CONVENTIONAL OVERNIGHT GN	REMEL RAPID ID NF PLUS
Total number of labs that used this system, card, or panel n = 11	2	1	6	1	1
Instrument Identifications					
N. gonorrhoeae	2	1	6		1
No identification				1	

Antimicrobial Susceptibilities:
Please refer to current Clinical and Laboratory Standards Institute (CLSI) guidelines for most current susceptibility recommendations. Susceptibilities to spectinomycin, extended-spectrum cephalosporins, fluoroquinolones, and azithromycin should be determined for surveillance purposes. (1)

With the increase testing done via NAAT on genital isolates, which does not produce a cultured bacterial isolate, monitoring of resistance has become more difficult, an important issue for public health monitoring of drug resistance. (1)

Referral of Isolates:
Gonorrhea *(N. gonorrhoeae)*, when found in any source, must be reported within seven working days to ND Department of Health: Division of Disease Control as per the North Dakota Communicable Disease Reporting Rule. For more information, please visit:
http://www.ndhealth.gov/Disease/Document/Reportable Conditions.pdf
Routine submission of isolates is not required but, if antimicrobial susceptibility testing is necessary to understand a treatment failure in a patient, please call the ND Department of Health; Division of Laboratory Services to discuss referral of the isolate for testing 701-328-6272.

Specimen #2, Tabulated Results

16/17 (94%) of labs performed synovial fluid cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = ***Neisseria gonorrhoeae***

Acceptable Answers: 74/82 (90%)
11/16 (69%) *Neisseria gonorrhoeae*
2/16 (12%) *Neisseria sp.*
2/16 (12%) Gram-negative coccobacilli, NOS

Unacceptable Answers: 8/82 (10%)
1/16 (6%) Possible *B. pseudomallei*

ORGANISM CHARACTERISTICS

Gram Stain: Intended result = **Gram-negative diplococcus**
14/16 (88%) Gram negative diplococcus
2/6 (12%) Gram negative coccobacilli

Oxidase: Intended result = **positive**
16/16 (100%) Laboratories reported oxidase **positive**
0/16 (0%) Laboratories reported oxidase **negative**

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Specimen #3, Wound: Vancomycin Intermediate MRSA

Submitter #: 2209	**Your Result**	**Intended Result**
Identification	***Staphylococcus aureus*, methicillin resistant (MRSA)**	*Staphylococcus aureus*, methicillin resistant (MRSA)
Referral of Isolate	**Our lab would notify the NDDoH; Division of Disease Control.**	Would notify NDDoH; Division of Disease Control and refer isolate to NDDoH; Division of Laboratory Services
Susceptibilities		
Cefoxitin*	**No response given**	Positive
Clindamycin	**Susceptible**	Susceptible
Daptomycin	**No response given**	Nonsusceptible
Erythromycin	**Resistant**	Resistant
Linezolid	**Susceptible**	Susceptible
Oxacillin	**Resistant**	Resistant
Penicillin	**No response given**	Resistant
Rifampin	**No response given**	Resistant
Tetracycline**	**Susceptible**	Susceptible
Trimethroprim-Sulfa	**Susceptible**	Susceptible
Vancomycin	**Intermediate**	Intermediate

* Cefoxitin is used as a surrogate for oxacillin. Report oxacillin susceptible or resistant based on the cefoxitin result. (7)
** Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. (7)

Challenge Set Organism Characteristics:
The isolate sent out in this Challenge Set was a Methicillin Resistant *Staphylococcus aureus* (MRSA) and therefore resistant to oxacillin. The isolate was beta lactamase positive. It was negative for inducible clindamycin resistance, therefore the clindamycin was reported as sensitive. However, most importantly this isolate was vancomycin intermediate with an MIC of 4-8µg/ml. Vancomycin intermediate *S. aureus* (VISA) isolates should be submitted to NDDoH-Division of Laboratory Services and reported to the NDDoH; Division of Disease Control to support disease surveillance efforts.

Goals and Objectives:
The isolate in this specimen was a vancomycin intermediate *Staphylococcus aureus* (VISA). The goal in sending this organism was to reinforce the need for clinical laboratories and their healthcare teams to maintain vigilant detection, reporting, and infection prevention strategies in order to prevent and control VISA and vancomycin resistant *S. aureus* (VRSA). The objectives were to assess the ability of ND sentinel site laboratories to recognize the decreased susceptibility of vancomycin in this isolate; evaluate changes in antimicrobial susceptibility testing practices among ND sentinel site laboratories; and to provide information to assist laboratories in the detection of VISA and VRSA.

Importance of VISA:
Detection and notification of VISA is important for both therapeutic and infection prevention and control reasons. Vancomycin has broad-spectrum activity and is often used empirically for potentially life-threatening infections such as sepsis. It is also used for treating infections caused by gram-positive cocci that are multi-drug resistant (i.e. *Enterococcus* spp. resistant). Therefore, if vancomycin-intermediate or resistant organisms are present in a healthcare setting there are significant limitations to the treatment options for the most critically ill patients. Studies regarding patients with a VISA show poor clinical outcomes, increased hospital expenditures, and increased mortality. Collectively, these features demonstrate the significance of rapid laboratory detection and reporting of VISA to clinicians and infection preventionists so that infection prevention and control measures can be implemented. Any VISA, regardless of resistance mechanism, is

clinically significant and should trigger implementation of infection prevention and control measures, including implementation of contact precautions. The earlier VISA is identified and reported, the sooner the patient can be placed on appropriate treatment and the more likely that spread throughout the healthcare facility will be prevented. (4)

Most infections caused by VISA/VRSA are healthcare-associated or occur in patients that have other underlying conditions leaving them vulnerable to disease. Fortunately, there are other drug options for treating patients having VISA/VRSA, as of October 2010, all VISA and VRSA isolates have been susceptible to other FDA-approved antibiotics. (3)

A Healthcare Team Approach in Response to VISA:
A team approach, which includes laboratorians, clinicians, infection preventionists, and pharmacists, is necessary to effectively identify and control VISA. This approach will not only help to prevent transmission, but will reinforce better antibiotic stewardship and overall prevention of healthcare associated infections. For specific infection prevention and control guidelines please see the NDDoH MRSA website at: http://www.ndhealth.gov/disease/Info/MRSAfacts.aspx

Identification of *Staphylococcus aureus*
In this challenge set, nearly all participants (94%) correctly identified this organism, which is readily identified by commercial methods. Eighty-two percent (43% by Vitek and 57% by Microscan) of participants used automated methods to identify this specimen. Classical methods were also used by some facilities to identify this organism, such as Gram stain (100%), catalase (94%), coagulase (47%), and latex agglutination (71%).

Vancomycin and Resistance Mechanisms:
Vancomycin, the only glycopeptide marketed for clinical use in the United States, was originally used against penicillin-resistant staphylococcus. It has since become the most powerful antibiotic against methicillin-resistant staphylococcus and is used in patients allergic to penicillins or cephalosporins. Glycopeptides inhibit peptidoglycan synthesis in the bacterial cell wall by complexing with the D-alanyl-D-alanine portion of the cell wall precursor. (1)

Vancomycin resistance can occur. All VRSA isolates detected in the country to date contain the *vanA* vancomycin resistance gene. The *vanA* gene is usually found in enterococci and typically confers high-level vancomycin resistance. Most VRSA patients had infections by both VRE containing *vanA* and MRSA. It's likely that the *vanA* determinant was transferred via plasmid or transposon from the VRE to the MRSA strain, resulting in the VRSA. The mechanism of reduced vancomycin susceptibility in VISA strains is not fully understood however it is thought to be due to change in the cell wall versus acquisition of the *vanA* gene. Vancomycin must reach the cell membrane and bind to the growing cell wall complex to inhibit cell growth. (2)

Prevalence of Vancomycin Resistance:
There have been 13 known cases of patients with vancomycin resistant *S. aureus* (VRSA) infections in the U.S. to date. There have been no cases of VISA/VRSA to date in North Dakota. Since antimicrobial resistance does not recognize borders, it's important to note that Minnesota has reported 2 cases of VISA so far in 2013. VISA/VRSA infections continue to be rare but a few existing factors seem to predispose patients to becoming infected: prior MRSA and *Enterococcus* infections or colonization, underlying health conditions, and previous treatment with vancomycin. (6)

Laboratory Challenges in the Detection of VISA:
There are several challenges in detecting VISA. Not all susceptibility testing methods detect VISA/VRSA isolates. According to CLSI, vancomycin should only be tested by minimum inhibitory concentration (MIC) as disk diffusion does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-

intermediate isolates. (7) VRSA isolates can be detected by reference methods such as broth microdilution, agar dilution, Etest, MicroScan, Phoenix, TREK Sensititre, Vitek 2, and vancomycin screen agar. Methods that confirm VISA are typically non-automated MIC methods including reference broth microdilution, agar dilution, and Etest.

Interpretive criteria for staphylococcus has evolved, with interpretive criteria changes in 2006 – see Table 1.

Table 1: CLSI - MIC Interpretive criteria for Staphylococcus aureus 2005 and 2006 – Present (1)

Agent	Susceptible	Intermediate	Resistant
Vancomycin – CLSI 2005	≤4 µg/ml	8 – 16 µg/ml	≥32 µg/ml
Vancomycin – CLSI 2006 - present	≤2 µg/ml	4 – 8 µg/ml	≥16 µg/ml

Reliable detection of VISAs may require a non-automated MIC method, therefore all labs should develop a procedure for detecting VISA/VRSA specifically for their laboratory. All *S. aureus* isolates for which the vancomycin MIC ≥ 4 µg/ml are unusual and should not be discarded. The susceptibilities should then be repeated using a second validated method, the MICs confirmed, the isolate determined pure, and the genus and species confirmed. If retesting confirms a VISA/VRSA, infection control should be notified as well as NDDoH-Division of Disease Control. (4) VISA and VRSA isolates are required to be submitted to NDDoH-Division of Laboratory Services per the Disease Reporting Rule. At NDDoH-Division of Laboratory Services confirmatory broth microdilution and Etest can be utilized to confirm antimicrobials. If the vancomycin MIC is found to be ≥ 8 µg/ml then an isolate is forwarded to CDC.

Most commercial automated susceptibility methods (e.g. Vitek, Microscan, etc.) are validated for VRSA detection and no longer require the use of vancomycin screening agar. However, a few laboratories – 4 in this challenge set, still utilize the vancomycin screening agar. The vancomycin screen agar uses commercially prepared brain heart infusion agar, containing 6µg/ml of vancomycin, to screen pure staphylococci isolates for vancomycin resistance. An inoculum of 1-10 µl of a 0.5 McFarland suspension is spotted onto the agar surface; the plate is then incubated for a full 24 hours at 35°C in ambient air. A positive result is defined as 2 or more colonies of growth. All staphylococci that grow on this plate should be checked for purity and an MIC method should be run for confirmation of resistance. (1) Vancomycin screen agar plates usually detect isolates for which vancomycin MICs are 8 µg/ml, but may provide variable results for MICs that are 4 µg/ml. (4)

In this Challenge Set, 14 of the 17 participants performing wound cultures used a commercial automated method to perform susceptibility testing on this isolate. Eight used Microscan, 5 used Vitek, and 1 Trek Sensititre. Manual methods reported were 3 labs used E test and 2 labs used vancomycin screening plates.

The CDC website provides abundant information about VISA/VRSA. You can access the links for procedures and information by visiting: http://www.cdc.gov/HAI/organisms/visa_vrsa/visa_vrsa.html. Information about VISA/VRSA can also be found on the North Dakota Department of Health; Division of Disease Control website at: http://www.ndhealth.gov/disease/Info/MRSAfacts.aspx.

About Oxacillin:

The isolate in this challenge was oxacillin resistant and therefore a MRSA. Per the CLSI M100-S23: "In most staphylococcal isolates, oxacillin resistance is mediated by *mec*A, encoding the pencillin-binding protein 2a (PBP 2a, also called PBP2′). Isolates that test positive for *mec*A or PBP 2a should be reported as oxacillin resistant. Isolates that test resistant by oxacillin MIC, cefoxitin MIC, or cefoxitin disk test should also be reported as oxacillin resistant. Mechanisms of oxacillin resistance other than *mec*A are rare and include a novel *mec*A homologue, *mec*C. MICs for strains with *mec*C are typically in the resistant range for cefoxitin and/or oxacillin; *mec*C resistance cannot be detected by tests directed at *mec*A or PBP 2a." (7)

About Ceftaroline:
New in the CLSI M100-S23 - Ceftaroline was added to Test Report Group B with the note for *S. aureus* only including methicillin-resistant *S. aureus* (MRSA). (7) No laboratories reported ceftaroline in their challenge set data.

Reportable Disease Rule:
Staphylococcus aureus in the following circumstances when found or suspected must be reported 'immediately' to NDDoH – Division of Disease Control: vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA), and death or critical illness due to community-associated *S. aureus* in a previously healthy individual. In addition, submission of clinical materials from these cases to NDDoH-Division of Laboratory Services is required. http://www.ndhealth.gov/Disease/Document/Reportable Conditions.pdf

Among the 16 laboratories that correctly identified the organism in this challenge, 8 (50%) indicated that they would notify NDDoH-Division of Disease Control and refer the isolate to the NDDoH-Division of Laboratory Services, 4 labs (25%) would refer to their normal reference lab, and 4 laboratories (25%) would take no further action.

How to Submit an Isolate to NDDoH-Division of Laboratory Services:
If a *S. aureus* isolate is found to be intermediate (4-8 µg/mL) or resistant (≥16 µg/mL) to vancomycin it must be reported and an isolate sent to NDDoH-Division of Laboratory Services for confirmatory testing. Prior to sending to NDDoH-Division of Laboratory Services, the susceptibility should be repeated. When sending, please ensure that the isolate is pure and genus and species is confirmed. Please include with the submitted isolate:

- A copy of a printed report from the commercial AST method (please include any antibiotics that may normally be suppressed on the patient report)
- Results from any other supplemental AST methods

Specimen #3, Tabulated Results

17/17 (100%) = Laboratories performed wound cultures

ORGANISM IDENTIFICATION

Identification: Intended answer = *Staphylococcus aureus,* methicillin resistant (MRSA), with referral to NDDoH-Division of Laboratory Services

Acceptable Answers: 8/17 (47%)
8/17 (47%) = *Staphylococcus aureus,* methicillin resistant (MRSA), with referral to NDDoH-Division of Laboratory Services

Unacceptable Answers: 9/17 (33%)
4/17 (23%) = *Staphylococcus aureus,* methicillin resistant (MRSA), without referral to NDDoH-Division of Laboratory Services
4/17 (23%) = *Staphylococcus aureus,* without referral to NDDoH-Division of Laboratory Services
1/17 (6%) = *Staphylococcus* sp.

Susceptibility Results Reported by Participating Labs

Antibiotic	Vancomycin (n = 15)	Oxacillin (n= 15)	Clindamycin (n = 14)
Report = resistant	[MIC = 16] - 1 labs (7%)	15 labs (100%)	0 labs (0%)
Report = intermediate	[MIC = 4] - 8 labs (53%)	-	-
	[MIC = 8] - 6 labs (40%)	-	-
Report = susceptible	0 labs (0%)	0 labs (5%)	14 labs (100%)
No interpretation provided	2 labs (1%)	0 labs (0%)	0 labs (0%)

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Specimen #4, Stool: *Shigella sonnei* (Group D)

Submitter #: 2209		
	Your Laboratory's Results	**Intended Result**
Identification	***Shigella sonnei* (Group D)**	*Shigella sonnei* (Group D)
Referral of Isolate	**Since we are the NDDoH-Division of Laboratory Services, we would notify Disease Control.**	Would refer isolate to NDDoH-Division of Laboratory Services

Goal:
The organism in specimen #4 was *Shigella sonnei* (Group D). 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 invasive disease. This organism was also selected to emphasize the importance of submitting specimens to NDDoH-Division of Laboratory Services according to the North Dakota Communicable Disease Reporting to assist in the public health response.

The genus *Shigella:*
Members of the genus *Shigella* are gram-negative, non- motile, facultatively anaerobic members of the family Enterobacteriaceae. There are four subgroups of *Shigella* that historically have been treated as species: *Shigella dysenteriae* (subgroup A), *Shigella flexneri* (subgroup B), *Shigella boydii* (subgroup C), and *Shigella sonnei* (subgroup D). Within the 4 major subgroups, more than 40 serotypes have been identified based on the O antigen. From a genetic standpoint, the four species of *Shigella* and together with *E. coli* represent a single genomospecies, with >75% nucleotide similarity. Therefore, genetically, the four species of *Shigella* would be regarded as serologically defined non-gas producing, lactose negative biotypes of *E. coli*. Enteroinvasive *E. coli* (EIEC), a somewhat biochemically inert *E. coli* biotype, shares virulence genes with *Shigella spp*., and is responsible for disease indistinguishable from shigellosis. The current nomenclature of *Shigella* is retained mainly for medical purposes because of the useful association of the genus with the distinctive disease caused by these organisms. (1)

Shigella species are a common cause of bacterial gastroenteritis and an important cause of morbidity among children, particularly in day-care settings. Humans and other large primates are the only natural reservoirs of *Shigella* bacteria. *Shigella* has a very low infective dose with fewer than 100 viable cells readily producing disease in healthy adults (2). Therefore, most transmission is through person-to-person spread, although infection can also be caused by ingestion of contaminated food or water. Most infections in the United States, North Dakota, and other developed countries are caused by *S. sonnei*, with *S. flexneri* being the second most common serogroup.

Public Health Surveillance:
In North Dakota, outbreaks of foodborne illnesses are detected through two primary mechanisms. North Dakota residents can call the NDDoH; Division of Disease Control at 701.328.2378 or 1.800.472.2180 to report suspected cases of foodborne illness. However, the most reliable mechanism for detecting bacterial foodborne disease outbreaks, including *Shigella*, is the submission of isolates by clinical microbiology laboratories to the NDDoH; Division of Laboratory Services for additional characterization as part of the North Dakota Disease Reporting Rule. All reported cases of suspected or confirmed *Shigella* infections are investigated by the NDDoH; Division of Diseases Control in conjunction with further testing of isolates submitted to the NDDoH; Division of Laboratory Services. Additional testing performed at the NDDoH; Division of Laboratory Services may include pulsed field gel electrophoresis (PFGE), a process in which the bacterial DNA is fragmented and separated in an agarose gel to generate specific patterns or

"fingerprints." These patterns are compared to those of other *Shigella* isolates to track disease, detect outbreaks, and prevent additional cases.
During 2012, 8 culture-confirmed *Shigella* cases were reported to the ND Department of Health; Division of Disease Control. *S. sonnei* accounted for 5 (62.5%) cases, *S. flexneri* 2 (25%), and one unknown species (12.5%). In contrast, for 2011, 2 culture-confirmed cases of *Shigella* infection (0.3 per 100,000 population) were reported. The two cases in 2011 were an increase from zero cases reported in 2010, but a 87% decrease from the median number of cases reported annually from 2001 to 2010 (median, 15.5; range, 0 to 235). In 2011, *S. sonnei* accounted for one of the cases, the other was an unknown species. Neither of the cases were ≤5 years of age nor were they hospitalized. The North Dakota Department of Health; Division of Laboratory Services submits every 20th isolate to the CDC as part of the National Antimicrobial Resistance Monitoring System (NARMS), so that resistance trends can be monitored nation-wide (http://www.cdc.gov/narms/about.html).

Laboratory Safety:
Like all enteric pathogens, clinical specimens and cultures suspected or known to contain *Shigella* 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 (1).

Collection and Transport:
Shigella organisms are extremely fragile, therefore, transport time is critical; if fresh stool specimen is received >2 hours from collection it may be detrimental to most *Shigella* spp. (2) 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. For cases of suspected *Shigella* 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), it is very important for the recovery of *Shigella* sp. (as well as *Campylobacter* sp.) that they be refrigerated at 4°C in an approved transport medium such as Cary-Blair, Stuart's, or Amies medium. There are data showing that transport at ambient temperature may adversely affect the recovery of *Shigella* sp. (1).

Enrichment, Isolation, and Screening Methods:
All stools submitted for culture of bacterial enteric pathogens should be examined for *Shigella*. There is no reliably effective enrichment medium for <u>all</u> *Shigella* isolates, but GN broth and selenite broth can be used. For optimal isolation of *Shigella*, two different selective media should be used: a general-purpose plating medium of low selectivity (*e.g.,* MAC) and a more selective agar medium (*e.g.,* XLD or HE). Salmonella-*Shigella* agar (SS) should be used with caution, because it may inhibit the growth of some strains of *Shigella* (1).

Table 1 – Media used by laboratories to set up routine stool cultures (n = 14)

Medium	# and % of labs using media	Medium	# and % of labs using media
Campylobacter blood agar (CVA)	9 (64%)	Hektoen Enteric agar	9 (64%)
CHROMagar O157	0 (0%)	MacConkey agar	12 (86%)
CHROMagar Salmonella	0 (0%)	MacConkey broth	2 (14%)
CIN	4 (29%)	MacConkey Sorbitol agar (SMAC)	13 (93%)
CNA	1 (7%)	Selenite broth	4 (29%)
CT-SMAC	1 (7%)	Sheep Blood agar	12 (86%)
EMB	11 (79%)	SS agar (salmonella *Shigella* agar)	1 (7%)
GN broth	5 (36%)	XLD	6 (43%)

Laboratory Identification of *Shigella:*

Compared with *Escherichia*, *Shigella* strains are less active in their use of carbohydrates. On TSI slants, *Shigella* characteristically produce an alkaline slant and an acid butt (K/A) but do not produce gas or H_2S. Motility, urea and lysine decarboxylase tests are all negative. Rare strains of *S. boydii* are ornithine negative, and may be misidentified. Due to the low biochemical reactivity of these organisms, automated methods may have problems identifying *Shigella*. Confirmation of *Shigella* requires both biochemical and serologic identification and labs that do not perform both types of tests should send *Shigella* isolates to a reference laboratory (1).

- Of the 14 labs that did stool cultures in this Challenge Set, 71% (10/14) of the laboratories do not perform serotyping.

Biochemical Identification:

Shigella and inactive *E. coli* are frequently biochemically hard to distinguish because *Shigella* is lysine decarboxylase, motility, gas, acetate, mucate, and lactose negative.

- In Challenge Set 2012, 13/14 (92%) of laboratories used commercial identification methods to aid in identification.

Serotyping:

Shigella somatic antigens of most serotypes are either identical or closely related to those of *E. coli*, suspicious colonies should be investigated further. Serologic identification is usually performed by slide agglutination with polyvalent somatic (O) antigen grouping sera. Three of the four subgroups A (*S. dysentariae*), B (*S. flexneri*), and C (*S boydii*) are made up of numerous serotypes. Group D (*S. sonnei*) is made up of a single serotype. Serotyping is essential. (1)

- Of the laboratories that do serotyping, only 21% (3/14) test specifically for *Shigella.*

Antimicrobial Susceptibility Testing:

For most individuals, antibiotic treatment reduces the number of symptomatic days and length of shedding, but to limit the development of resistance, some providers treat only the most severe infections. (1) Because of the widespread antimicrobial resistance among *Shigella* strains, all isolates should undergo susceptibility testing. Fecal isolates should be tested against ampicillin and trimethoprim-sulfamethoxazole. Strains may produce sensitive AST results for fluoroquinolones, but if they are resistant to nalidixic acid, treatment may result in a delayed clinical response or treatment failure. *Shigella* should not be reported as sensitive to narrow-spectrum and extended-spectrum cephalosporins and cephamycins or to aminoglycosides because these drugs are not effective clinically. (1)

- In this challenge, 10 of the 14 labs (71%) doing stool cultures stated they routinely performed susceptibility testing on *Shigella* isolates.

Reportable Disease Rule:

In North Dakota, the Communicable Disease Reporting Rule requires that all cases of shigellosis be reported to NDDoH; Division of Disease Control within seven working day of identification due to the significant clinical and epidemiologic impact of shigellosis. In addition to case reporting, culture isolates or clinical material from all cases are required to be sent to the NDDoH; Division of Laboratory Services. For more information regarding the ND Communicable Disease Reporting Rule go to: http://www.ndhealth.gov/Disease/Documents/ReportableConditions.pdf

Specimen #4, Tabulated Results

14/17 (71%) labs performed stool cultures

ORGANISM IDENTIFICATION

Identification: Intended result = ***Shigella sonnei* (Group D), with referral to NDDoH-Division of Laboratory Services**

Acceptable Answers = 10/12 (83%)
11/12 (75%) *Shigella sonnei* (Group D), with referral to NDDoH-Division of Laboratory Services
1/12 (8%) *Shigella spp,* with referral to NDDoH-Division of Laboratory Services

Unacceptable Answers = 2/12 (17%)
2/12 (17%) *Shigella sonnei* (Group D), without referral to NDDoH-Division of Laboratory Services

Bacterial Enteric Pathogens Included in Routine Stool Cultures

The number of laboratories that culture for the following organisms in routine stool cultures (n=14):

Pathogen	# of labs culturing for pathogen	% of labs culturing for pathogen
Shigella	14	100
Salmonella	14	100
Campylobacter	12	86
E. coli O157: H7	12	86
Aeromonas	13	93
Yersinia	10	71
Vibrio	11	79
Pleisiomonas	11	79

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NDLRN/MLS: Challenge Set 2012
Self-Study Quiz

Specimen #1 – *Francisella tularensis*

1. Notification of a *Francisella* isolate must be (chose all that apply):
   a. Reported prior to sending the isolate
   b. Done by calling the ND Department of Health; Division of Laboratory Services on-call phone 24/7 at 701-400-2772
   c. As soon as *Francisella* 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 *F. tularensis* (choose all that apply)?
   a. Very tiny Gram-negative coccobacillus
   b. Takes 48 hours of incubation to show good growth
   c. Oxidase negative
   d. β-lactamase positive
   e. all of the above

3. *F. tularensis* is most commonly misidentified as (choose all that apply):
   a. *Pseudomonas aeruginosa*
   b. *Haemophilus influenzae*
   c. *Actinobacillus species (beta-lactamase negative)*
   d. *Yersinia pestis*

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

Specimen #2 – *Neisseria gonorrhoeae*

1. Which of the following are characteristic of *N. gonorrhoeae* (choose all that apply)?
   a. Gram-negative diplococci
   b. Readily grow on chocolate agar
   c. Readily grow on blood agar
   d. Oxidase positive

2. Which usable energy forms are fermented by *Neisseria gonorrhoeae* (choose all that apply)?
   a. Glucose
   b. Pyruvate
   c. Lactate
   d. Sucrose
   e. All of the above

3. **True** **False** *N. gonorrhoeae* is primarily a human pathogen.
4. **True** **False** *N. gonorrhoeae* requires special transport media.
5. **True** **False** ND Department of Health; Division of Laboratory Services should be notified of gonorrhea (both genital and non-genital sources) according to the Reportable Disease Rule.

Specimen #3 – Vancomycin Intermediate *Staph aureus* (VISA)

1. According to CLSI, which of the following MICs is considered intermediate for vancomycin *Staph aureus* ?
   a. ≤2 ug/ml
   b. 4-8 ug/ml
   c. 8-16 ug/ml
   d. ≥16 ug/ml

2. Vancomycin screen agar is made of BHI agar and ____ ug/ml of vancomycin
   a. 2
   b. 4
   c. 6
   d. 8

3. **True** **False** VISA patients should be isolated according to facility infection control procedures.
4. **True** **False** VISA must be reported to ND Department of Health; Division of Disease Control as part of the Reportable Disease Rule.
5. **True** **False** A vancomycin intermediate *Staph aureus* should be confirmed by another validated MIC method.

Specimen #4 – *Shigella sonnei*

1. Serotype designation of Shigella is based on:
   a. The O antigen
   b. The X antigen
   c. Motility
   d. Hemolysis

2. Which serotype is *Shigella sonnei*?
   a. A
   b. B
   c. C
   d. D

3. Which of the following tests is performed at ND Department of Health; Division of Laboratory Services to aid in epidemiologic investigations of *Shigella sonnei* outbreaks? (choose one):
   a. Voges-Proskauer test (VP)
   b. Pulsed-field gel electrophoresis (PFGE)
   c. High-pressure liquid chromatography (HPLC)
   d. DNA hybridization probes

4. **True** **False** Salmonella-Shigella agar (SS agar) is the best agar to use when isolating Shigella.
5. **True** **False** All Shigella isolates, regardless of source, should be submitted to the ND Department of Health: Division of Laboratory Services.

Specimen #1 – *Francisella tularensis*

1. Notification of a *Francisella* isolate must be (chose all that apply):
   a. Reported prior to sending the isolate
   b. Done by calling the ND Department of Health; Division of Laboratory Services on-call phone 24/7 at 701-400-2772
   c. As soon as *Francisella* 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 *F. tularensis* (choose all that apply)?
   a. Very tiny Gram-negative coccobacillus
   b. Takes 48 hours of incubation to show good growth
   c. Oxidase negative
   d. β-lactamase positive
   e. all of the above

3. *F. tularensis* is sometimes misidentified as (choose all that apply):
   a. *Pseudomonas aeruginosa*
   b. *Haemophilus influenzae*
   c. *Actinobacillus species (beta-lactamase negative)*
   d. *Yersinia pestis*

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

Specimen #2 – *Neisseria gonorrhoeae*

1. Which of the following are characteristic of *N. gonorrhoeae* (choose all that apply)?
   a. Gram-negative diplococci
   b. Readily grow on chocolate agar
   c. Readily grow on blood agar
   d. Oxidase positive

2. Which usable energy forms are fermented by *Neisseria gonorrhoeae* (choose all that apply)?
   a. Glucose
   b. Pyruvate
   c. Lactate
   d. Sucrose
   e. All of the above

3. **True** **False** *N. gonorrhoeae* is primarily a human pathogen.
4. **True** **False** *N. gonorrhoeae* requires special transport media.
5. **True** **False** ND Department of Health; Division of Disease Control should be notified of gonorrhea (both genital and non-genital sources) according to the Reportable Disease Rule.

Specimen #3 – Vancomycin Intermediate *Staph aureus* (VISA)

1. According to CLSI, which of the following MICs is considered intermediate for vancomycin *Staph aureus* ?
   a. ≤2 ug/ml
   b. 4-8 ug/ml
   c. 8-16 ug/ml
   d. ≥16 ug/ml

2. Vancomycin screen agar is made of BHI agar and ____ ug/ml of vancomycin
   a. 2
   b. 4
   c. 6
   d. 8

3. **True** **False** VISA patients should be isolated according to facility infection control procedures.
4. **True** **False** VISA must be reported to ND Department of Health; Division of Disease Control as part of the Reportable Disease Rule.
5. **True** **False** A vancomycin intermediate *Staph aureus* should be confirmed by another validated MIC method.

Specimen #4 – *Shigella sonnei*

1. Serotype designation of Shigella is based on:
   a. The O antigen
   b. The X antigen
   c. Motility
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2. Which serotype is *Shigella sonnei*?
   a. A
   b. B
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   d. D

3. Which of the following tests is performed at ND Department of Health; Division of Laboratory Services to aid in epidemiologic investigations of *Shigella sonnei* outbreaks? (choose one):
   a. Voges-Proskauer test (VP)
   b. Pulsed-field gel electrophoresis (PFGE)
   c. High-pressure liquid chromatography (HPLC)
   d. DNA hybridization probes

4. **True** **False** Salmonella-Shigella agar (SS agar) is the best agar to use when isolating Shigella.
5. **True** **False** All Shigella isolates, regardless of source, should be submitted to the North Dakota Department of Health; Division of Laboratory Services.