

# **BACTERIOLOGY PROFICIENCY TESTING PROGRAM**

## **General Category**

**May 2, 2006**

This report summarizes the results of the proficiency test administered May 2, 2006 to laboratories in the General Bacteriology category.

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# ***Bacteriology Proficiency Testing Program***

## ***GENERAL INFORMATION***

**The Bacteriology Proficiency Testing Program.** Three proficiency testing events are given annually, each consisting of a minimum of five specimens. In order to successfully complete a test event, participating laboratories must achieve a score of 80% or greater. Unsuccessful performance in the testing program is defined as a score of less than 80% on two of three consecutive test events.

**Authentication.** The presence and identity of the organism(s) in each specimen must be confirmed by at least 80% of the referee or participating laboratories. Referee laboratories are selected from New York State participating laboratories (located throughout the State) with acceptable and reproducible levels of performance. Sample vials are subjected to extensive quality control testing in our laboratory during preparation and storage.

**Grading System.** Laboratories are to process proficiency test specimens in the same manner as patient specimens. Thus, laboratories are responsible for identifying test isolates to the same level as performed on patient isolates. If your laboratory speciates an organism on special request, then you must also speciate it in the proficiency test; consider speciation to have been requested on all reportable isolates. In addition, laboratories are not responsible for culturing any test samples from specimen sources which they do not process. Information regarding your laboratory's reporting protocol was provided to us in the questionnaire previously distributed to all laboratories. Any changes in reporting protocol must be received by our office prior to the mailout date for proficiency testing for that information to be considered in grading.

Our testing format is in compliance with HCFA CMS guidelines as specified in the regulations of CLIA '88. One-half of our samples require identification of all organisms present. The other half requires that only the pathogenic organism(s) be reported. We recognize the potential for any organism to be pathogenic depending on the clinical condition of the patient. However, our samples are designed so that only well-established pathogens should be reported.

Tests are graded in strict adherence to HCFA CMS guidelines, as specified in the regulations of CLIA '88. Each of the specimens receives a score as determined by the following formula:

$$(a + b)/(c + d + e) \times 100\%$$

a = # correct identifications

b = # correct antibiotic susceptibility results (if applicable)

c = # possible identifications

d = # possible antibiotic susceptibility results (if applicable)

e = # additional organisms reported

Grades for each sample are then averaged to determine the final grade for this testing event. The minimum passing grade for each test event is 80%.

**Disclaimer**

The use of brand and/or trade names in this report does not constitute an endorsement of the products on the part of the Wadsworth Center or the New York State Department of Health.

## *Notes of Interest*

### **Bacteriology Questionnaires**

Please make sure that the information on your laboratory's Bacteriology Questionnaire is accurate. If you need a copy of your questionnaire for review, please contact our office at 518-474-4177 or email us at [bacti@wadsworth.org](mailto:bacti@wadsworth.org). Please note that proficiency test results are graded in accordance with information on the questionnaire. **Recently, there have been several instances where laboratories have lost credit on proficiency test results because of inaccurate or outdated information on their Bacteriology Questionnaire. Grades will not be revised due to incorrect information on the questionnaire.**

### **National Laboratory Training Network**

The National Laboratory Training Network (NLTN) is a valuable educational resource for clinical laboratories. The NLTN offers continuing education in a wide variety of areas and addresses timely issues such as the latest changes in antimicrobial susceptibility testing, bioterrorism preparedness, and packaging and shipping of infectious agents. Training options include audioconferences and study modules. In addition, workshops are given at various locations throughout the country. Their website is: <http://www.phppo.cdc.gov/nltm/default.aspx> or they can be reached at 800-536-NLTN (6586).

**MAY 2, 2006 TEST EVENT**

**Number of Participating Laboratories:**

**Receiving specimens**           **232**  
**Returning results**           **232**   **(100%)**

Grade Distribution		
Score	Number	Percent
100%	113	48.7
90 – 99%	51	21.9
80 – 89%	41	17.7
70 – 79%	14	6.0
60 – 69%	6	2.6
< 60%	7	3.0

**BACTERIOLOGY - GENERAL**  
**MAY 2, 2006**  
**ANSWER KEY**

**Specimen No. 1 - Stool (Pathogens Only)**

*Shigella flexneri*, serogroup B

**Specimen No. 2 – Cervix (Pathogens Only)**

*Neisseria gonorrhoeae*

**Specimen No. 3 – Blood - Aerobic / Anaerobic (All Organisms Reported)**

*Bacteroides vulgatus*

*Staphylococcus aureus*

**Specimen No. 4 – Joint aspirate (All Organisms) and Antibiotic Susceptibility**

*Staphylococcus lugdunensis*

Susceptibility of *S. lugdunensis* to: Gentamicin - Susceptible

Oxacillin - Susceptible

**Specimen No. 5 – Urine (All Organisms Reported)**

*Klebsiella oxytoca*

**Educational A – Blood**

*Bacillus cereus* (non-hemolytic, motile)

**Educational B – Blood**

*Bacillus cereus* (non-hemolytic, non-motile)

***Chlamydia* Specimen**

Negative for *Chlamydia trachomatis*

**Direct Antigen Detection**

A (Throat)

Positive for Group A *Streptococcus*

C (Genital)

Positive for Group B *Streptococcus*

## REFEREE LABORATORY RESULTS

Specimen Number	Referee Laboratory Responses	Percent*
1	<i>Shigella flexneri</i> , group B	90
	<i>Shigella</i> species	10
2	<i>Neisseria gonorrhoeae</i>	100
3	<i>Bacteroides vulgatus</i>	100
	<i>Staphylococcus aureus</i>	100
4	<i>Staphylococcus lugdunensis</i>	80
	<i>Staphylococcus</i> , coagulase negative <sup>1</sup>	10
	<i>Staphylococcus haemolyticus</i>	10
5	<i>Klebsiella oxytoca</i>	100

\* Based on responses of 10 referee laboratories

<sup>1</sup> This laboratory does not identify coagulase-negative staphylococci to the species level

## ***Specimen Number 1 - Stool (Pathogens Only)***

This simulated stool sample contained *Shigella flexneri*, group B. This organism was identified by 90% of the referee laboratories. Among participating laboratories that culture stool samples for *Shigella*, 85% successfully isolated the organism. Of these, 83% fully identified the isolate as *Shigella flexneri*, group B. An additional 14% did not perform serogrouping and reported ‘*Shigella species*’.

*Enterobacter cloacae* and *Citrobacter freundii* were included in this specimen as nonpathogenic flora.

### **Methods of identification used by laboratories reporting:**

#### ***Shigella flexneri*, group B**

bioMerieux Vitek GNI +	48
Dade Behring MicroScan Gram Neg ID	45
bioMerieux API 20E	30
Two or more	13
Conventional biochemicals	4
bioMerieux API Rapid 20E	2
Test method not indicated	2
Wellcolex Colour <i>Shigella</i>	2
BD BBL Crystal Enteric/Nonfermenter	3
Biolog MicroLog Gram Negative	1
BD BBL Enterotube II	1
bioMerieux Vitek 2 GN	1
Remel RapID ONE	1
Dade Behring MicroScan Rapid Gram Neg	1
<b>TOTAL</b>	<b>154</b>

**No enteric pathogens isolated** **33**

#### ***Shigella species***

bioMerieux Vitek GNI +	11
Dade Behring MicroScan Gram Neg ID	7
bioMerieux API 20E	5
Two or more test methods	2
bioMerieux Vitek 2 GN	1
<b>TOTAL</b>	<b>26</b>

**Specimen source (stool culture) not tested** **13**

#### ***Shigella group***

bioMerieux Vitek GNI +	2
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#### ***Shigella boydii*, group C**

bioMerieux Vitek GNI +	1
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<i>Shigella boydii/flexneri</i>	
bioMerieux Vitek GNI +	1
<b><i>Shigella Group D</i></b>	
Dade Behring MicroScan Gram Neg ID	1
<i>Shigella species, not sonnei</i>	
bioMerieux Vitek GNI +	1

***Specimen No. 2 – Cervix (Pathogens Only)***

This simulated cervical culture contained *Neisseria gonorrhoeae*. All referee laboratories correctly identified this organism as did 99% of participating laboratories that processed this specimen source.

*Staphylococcus hominis* and *Corynebacterium xerosis* were included as additional nonpathogenic flora in this sample.

**Methods of identification used by laboratories reporting:**

***Neisseria gonorrhoeae***

Remel RapID NH	65
bioMerieux API NH	37
bioMerieux Vitek NHI	30
Dade Behring MicroScan HNID	24
Conventional biochemicals	23
Two or more test methods	21
Remel BactiCard <i>Neisseria</i>	5
Boule Diagnostics Phadebact <i>Streptococcus</i>	3
GenProbe (not further specified)	3
Gen-Probe AccuProbe for <i>Neisseria gonorrhoeae</i>	3
BD BBL Crystal <i>Neisseria/Haemophilus</i>	2
BD BBL GonoGen II	2
Gen Probe Pace 2 GC	2
EY Laboratories Gonocheck	1
<b>TOTAL</b>	<b>221</b>

**Specimen source (cervical culture) not tested** 9

***Gardnerella vaginalis***

Remel RapID NH	1
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**No pathogens isolated** 1

**Additional organism reported in Specimen 2:**

<i>Staphylococcus aureus</i>	1
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### **Specimen No. 3 – Blood - Aerobic/Anaerobic (All Organisms)**

This simulated blood culture contained *Bacteroides vulgatus* and *Staphylococcus aureus*.

*Bacteroides vulgatus* was reported by all referee laboratories. Of the participating laboratories that isolate anaerobic organisms from blood cultures, 80% identified *B. vulgatus*. Additional reports included *Bacteroides* species (3.6%) and *Bacteroides fragilis* group (1.4%). The following misidentifications were reported: *Prevotella melaninogenica* (6%), *Prevotella* species (1.8%) and *Prevotella denticola* (1.4%). For those laboratories that misidentified this organism, the following characteristics are useful in differentiating the *B. fragilis* group from the pigmented *Prevotella* species:

	<i>Bacteroides fragilis</i> group, including <i>B. vulgatus</i>	Pigmented <i>Prevotella</i> sp, including <i>P. melaninogenica</i> and <i>P. denticola</i>
Growth in 20% bile	+	-
Pigmented colonies (brown or black)	-	+
Brick-red fluorescence under UV light	-	+
Growth on Bacteroides Bile Esculin Agar	+	-

Reference: Mangels, J.I. (ed.) Anaerobic Bacteriology. In: Isenberg, H.D. (ed.) *Clinical Microbiology Procedures Handbook*, 2<sup>nd</sup> edition. ASM Press, Washington, DC. 2004.

*Staphylococcus aureus* was identified by all referee laboratories. Of the participants that processed this specimen, 97% identified *Staphylococcus aureus* and the remaining 3% reported ‘*Staphylococcus* coagulase positive’.

#### **Methods of identification used by laboratories reporting:**

##### ***Bacteroides vulgatus***

Remel RapID ANA II	84
bioMerieux Vitek ANI	28
bioMerieux API 20A	24
Dade Behring MicroScan Rapid Anaerobe	16
bioMerieux API Rapid ID 32A	7
Two or more test methods	7
BD BBL Crystal Anaerobe	4
Conventional biochemicals	3
Test method not indicated	2
16s rDNA sequencing	1
<b>TOTAL</b>	<b>176</b>

##### ***Prevotella melaninogenica***

Remel RapID ANA II	13
Two or more test methods	1
<b>TOTAL</b>	<b>14</b>

<b>Specimen source (blood culture) not tested</b>	<b>8</b>
<b><i>Bacteroides</i> species</b>	
Remel RapID ANA II	3
bioMerieux Vitek ANI	2
bioMerieux API 20A	1
Dade Behring MicroScan Rapid Anaerobe	1
Two or more test methods	1
<b>TOTAL</b>	<b>8</b>
<b>Do not perform anaerobic cultures</b>	<b>5</b>
<b>No anaerobic organisms</b>	<b>4</b>
<b><i>Prevotella</i> species</b>	
Remel RapID ANA II	4
<b><i>Prevotella denticola</i></b>	
Remel RapID ANA II	3
<b>Anaerobic gram negative bacillus</b>	<b>3</b>
<b><i>Bacteroides fragilis</i> group</b>	
Remel RapID ANA II	2
Conventional biochemicals	1
<b>TOTAL</b>	<b>3</b>
<b>No <i>Bifidobacterium</i></b>	<b>2</b>
<b>No report</b>	<b>2</b>
<b><i>Bacteroides</i> species, not <i>fragilis</i></b>	
Two or more test methods	1
<b>Gram positive bacillus</b>	<b>1</b>

***Staphylococcus aureus***

Dade Behring MicroScan Gram Pos ID	59
Murex Staphaurex	42
Conventional biochemicals	37
Two or more test methods	26
BD BBL Staphyloslide	18
bioMerieux Vitek GPI	12
Remel BactiStaph	11
Fisher Healthcare SureVue Color Staph	4
bioMerieux API Staph	3
Sanofi Diagnostics Pasteur Pastorex Staph-Plus	2
bioMerieux Vitek 2 GP	1
bioMerieux Vitek Slidex Staph	1
Hardy Diagnostics StaphTex	1
Pro-Lab Diagnostics Prolex Staph latex	1
<b>TOTAL</b>	<b>218</b>

**Specimen source not tested** **8**

***Staphylococcus, coagulase positive***

Murex Staphaurex	3
bioMerieux Vitek Slidex Staph	1
Conventional biochemicals	1
Dade Behring MicroScan Gram Pos ID	1
<b>TOTAL</b>	<b>6</b>

## ***Specimen No. 4 – Joint Aspirate (All Organisms) and Antibiotic Susceptibility***

This simulated joint aspirate contained a pure culture of *Staphylococcus lugdunensis*. Eighty percent of the referee laboratories identified this organism as did 81% of participating laboratories that would fully identify coagulase negative staphylococci from this specimen source. This organism was first included in our proficiency test in January 2003. At that time, 37% of all laboratories that processed the specimen source identified *S. lugdunensis*. This test event shows an increase in the number of laboratories reporting this organism with 59% of all laboratories that processed this specimen identifying *S. lugdunensis*. However, there still remains a large percentage of laboratories (30%) that do not distinguish this pathogen from other coagulase negative staphylococci.

Identification of *Staphylococcus lugdunensis* is important both because of this organism's pathogenicity and because correct identification is needed for proper susceptibility testing. *Staphylococcus lugdunensis* was first described in 1988. Although a member of the coagulase negative staphylococci, the course of disease caused by this organism resembles that of *Staphylococcus aureus*. Infections caused by this pathogen can be rapidly progressive and include endocarditis with rapid destruction of heart valves, meningitis secondary to shunt infections, peritonitis, breast abscesses, osteomyelitis, prosthetic joint infections, septic arthritis, as well as skin and post-surgical wound infections.<sup>1</sup>

A simple screening test for *Staphylococcus lugdunensis* incorporating PYR (pyrrolidonyl-arylamidase), ornithine decarboxylase and mannose was published by Schnitzler et al in 1998. Based upon this scheme, isolates of coagulase-negative staphylococci that are PYR positive, ornithine positive and mannose positive can be identified as *S. lugdunensis*.<sup>2</sup>

Antimicrobial susceptibility testing was indicated for this specimen using gentamicin and oxacillin. This isolate was reported as susceptible to gentamicin by all referee laboratories and by more than 99% of participants that tested this antibiotic. However, oxacillin susceptibility testing for this specimen was problematic for many laboratories. While all referee laboratories reported that this organism was susceptible to oxacillin, only 66% of participants reported it as such.

Recently, there have been several important changes made by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) regarding susceptibility testing of oxacillin for *Staphylococcus lugdunensis*. In their 2005 guidelines, CLSI established separate criteria for the interpretation of oxacillin susceptibility results for *Staphylococcus lugdunensis* as opposed to other coagulase negative staphylococci. These cutoffs are now the same as those used to interpret oxacillin susceptibility for *Staphylococcus aureus*. The CLSI 2005 guidelines also recommended that disk diffusion testing for oxacillin susceptibility be performed using a 30 µg cefoxitin disk instead of a 1 µg oxacillin disk since the zones produced by cefoxitin are easier to read. In addition, CLSI indicated in the 2006 guidelines that oxacillin disks should no longer be tested for *S. lugdunensis* and that cefoxitin disks must instead be tested as a surrogate. Effective with the 2006 guidelines, there are no longer interpretive criteria for evaluating results obtained with oxacillin disks for *S. lugdunensis*. For laboratories performing MIC testing, it should be noted that the cefoxitin disk is more reliable for detecting oxacillin-susceptible strains of coagulase-negative staphylococci (including *S. lugdunensis*) than oxacillin MIC testing.<sup>3,4</sup>

**These changes in the recent CLSI guidelines and their impact on susceptibility testing underscore the importance of laboratories having the most up-to-date guidelines.**

Correct testing for oxacillin susceptibility on this sample required laboratories to be aware of these significant changes to the CLSI guidelines. Of the laboratories that identified this isolate as *S. lugdunensis* and tested for oxacillin susceptibility, 86% correctly reported it as susceptible to oxacillin. Use of outdated guidelines and/or the inability to identify *S. lugdunensis* resulted in incorrect interpretations for oxacillin susceptibility.

Due to the pathogenic nature of *Staphylococcus lugdunensis* and the impact of its identification on interpretation of susceptibility results, laboratories are strongly encouraged to screen coagulase negative staphylococci isolated from sterile body sites for this species. For those laboratories that are unable to identify this organism, an interpretation for oxacillin susceptibility cannot be determined and should not be reported. The isolate should be forwarded to a reference laboratory for full identification and susceptibility testing if required.

**Below is a summary of the 2006 CLSI guidelines regarding oxacillin susceptibility testing for staphylococci:**

	<b>Resistant</b>	<b>Susceptible</b>	
<b>Disk diffusion</b> using 30 µg cefoxitin disk	≤ 19mm	≥ 20mm	For <i>S. aureus</i> and <i>S. lugdunensis</i>
	≤ 24mm	≥ 25mm	For coag neg staph other than <i>S. lugdunensis</i>
<b>MIC</b> using oxacillin	≥ 4	≤ 2	For <i>S. aureus</i> and <i>S. lugdunensis</i>
	≥ 0.5	≤ 0.25	For coag neg staph other than <i>S. lugdunensis</i>

<sup>1</sup> Hellbacher, C. et al. *Staphylococcus lugdunensis*: clinical spectrum, antibiotic susceptibility, and phenotypic and genotypic patterns of 39 isolates. Clin Microbiol Infect, 2006; 12: 43-49.

<sup>2</sup> Schnitzler, N. et al. *Staphylococcus lugdunensis*: Report of a case of peritonitis and an easy-to-perform screening strategy. J Clin Microbiol, 1998; 36: 812-813.

<sup>3</sup> Clinical and Laboratory Standards Institute. 2005. Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement, M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.

<sup>4</sup> Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement, M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.

**Methods of identification used by laboratories reporting:**

***Staphylococcus lugdunensis***

Dade Behring MicroScan Gram Pos ID	46
bioMerieux Vitek GPI	21
Conventional biochemicals	21
Two or more test methods	21
bioMerieux API Staph	17
bioMerieux Vitek 2 GP	2
Murex Staphaurex	2
BD Phoenix	1
ID 32 Staph	1
BD BBL Staphyloslide	1
<b>TOTAL</b>	<b>133</b>

***Staphylococcus, coagulase negative***

Murex Staphaurex	17
Conventional biochemicals	16
Two or more test methods	12
BD BBL Staphyloslide	9
Dade Behring MicroScan Gram Pos ID	4
Fisher Healthcare SureVue Color Staph	2
bioMerieux Vitek GPI	2
Remel BactiStaph	2
bioMerieux RAPIDEC Staph	1
bioMerieux Vitek Slidex Staph	1
Sanofi Diagnostics Pasteur Pastorex Staph-Plus	1
<b>TOTAL</b>	<b>67</b>

***Staphylococcus haemolyticus***

Dade Behring MicroScan Gram Pos ID	14
BD BBL Staphyloslide	1
<b>TOTAL</b>	<b>15</b>

**Specimen source not tested** **8**

***Staphylococcus hominis***

bioMerieux Vitek GPI	2
Two or more test methods	1
<b>TOTAL</b>	<b>3</b>

***Staphylococcus species, not aureus***

Dade Behring MicroScan Gram Pos ID	1
Murex Staphaurex	1
<b>TOTAL</b>	<b>2</b>

<i>Staphylococcus epidermidis</i>	
bioMerieux Vitek GPI	1
Two or more test methods	1
<b>TOTAL</b>	<b>2</b>

<i>Klebsiella oxytoca</i>	
bioMerieux Vitek GNI +	1

<i>Staphylococcus intermedius</i>	
Two or more test methods	1

**Results of Antimicrobial Susceptibility Testing – *S. lugdunensis* with Gentamicin**

<b>Result</b>	<b>Method</b>	<b>MIC - µg/ml</b>	<b>Zone - mm</b>	
<b>Susceptible (204)</b>	Dade Behring MicroScan (76)	<1 (6)		
		≤1 (24)		
		<4 (12)		
		≤4 (31)		
		Not indicated (3)		
	BioMerieux Vitek (67)	<0.5 (1)		
		≤0.5 (26)		
		<2 (5)		
		≤2 (33)		
		≤4 (1)		
		Not indicated (1)		
	Disk diffusion (52)			13 (1)
				20 (2)
				21 (1)
				22 (1)
				23 (1)
				26 (4)
				27 (6)
				28 (5)
				29 (4)
				30 (8)
				31 (1)
				32 (5)
				33 (5)
				34 (2)
				35 (3)
		36 (1)		
		Not indicated (2)		
	A-B Biodisk E-test (2)	0.047 (1)		
		0.32 (1)		
	Trek Sensititre (2)	0.12 (1)		
		≤2 (1)		
	B-D Phoenix (1)	≤1 (1)		
CEI (1)	≤0.5 (1)			
Microdilution (1)	0.12 (1)			
Mini broth dilutions (1)	<4 (1)			
Two methods (1)	≤0.12 (1)			
<b>Resistant (1)</b>	Disk diffusion (1)		31 (1)	
<b>Gentamicin not tested (19)</b>				
<b>Joint aspirate cultures not performed (8)</b>				

Number of laboratories reporting each result indicated in ( )

**Results of Antimicrobial Susceptibility Testing – *S. lugdunensis* with Oxacillin**

<b>Result</b>	<b>Method</b>	<b>MIC (µg/ml)</b>	<b>Zone (mm)</b>	
<b>Susceptible (141)</b>	Dade Behring MicroScan (58)	<0.25 (5)		
		≤0.25 (14)		
		<0.5 (1)		
		0.5 (15)		
		≤1 (1)		
		1 (19)		
		Not indicated (3)		
	Disk diffusion (42)  * most of these laboratories reported that a 30 µg cefoxitin disk was used			0 (1)
				12 (1)
				13 (8)
				14 (3)
				15 (1)
				18 (2)
				19 (1) *
				21 (1) *
				22 (1) *
				25 (1) *
				26 (1) *
				27 (1) *
				28 (5) *
				29 (3) *
			30 (7) *	
			31 (3) *	
			32 (2) *	
	bioMerieux Vitek (31)		≤0.25 (1)	
			0.5 (20)	
			1 (6)	
		<2 (1)		
		2 (3)		
A-B Biodisk E-test (2)		0.5 (1)		
		1.5 (1)		
Trek Sensititre (2)		≤0.25		
		0.25		
Two methods (2)		1 (2)		
Agar dilution (1)		1 (1)		
BD Phoenix (1)		0.5 (1)		
Microdilution (1)		0.5 (1)		
Test method not indicated (1)				

**Results of Antimicrobial Susceptibility Testing – *S. lugdunensis* with Oxacillin – con't**

<b>Result</b>	<b>Method</b>	<b>MIC (µg/ml)</b>	<b>Zone (mm)</b>	
<b>Resistant (70)</b>	BioMerieux Vitek (36)	0.5 (30)		
		1 (4)		
		2 (2)		
	Dade Behring MicroScan (16)	0.5 (10)		
		1 (5)		
		>2 (1)		
	Disk diffusion (16)			0 (2)
				10 (3)
				7 (1)
				9 (1)
				11 (1)
12 (3)				
14 (3)				
16 (2)				
Mini broth dilution (1)	1 (1)			
Test method not indicated (1)	0.5 (1)			
<b>Intermediate (3)</b>	Disk diffusion (3)		11 (1)	
			12 (1)	
			14 (1)	
<b>Oxacillin not tested (10)</b>				
<b>Joint aspirate cultures not performed (8)</b>				

Number of laboratories reporting each result indicated in ( )

Antibiotic Susceptibility Results - Participating & Referee Labs <i>Staphylococcus lugdunensis</i>				
	Gentamicin		Oxacillin	
	Referee <sup>a</sup>	Participant <sup>b</sup>	Referee <sup>a</sup>	Participant <sup>b</sup>
Susceptible	10	194	10	131
Intermediate	0	0	0	3
Resistant	0	1	0	70
Not Tested <sup>c</sup>	0	19	0	10
Do not process source <sup>d</sup>	0	8	0	8

<sup>a</sup>Referee Laboratories (10 labs total)

<sup>b</sup>Other Participating Laboratories (222 labs total)

<sup>c</sup>Antibiotic not tested / reported for this organism

<sup>d</sup>Do not process specimen source

***Specimen No. 5 – Urine (All Organisms)***

This simulated urine culture contained *Klebsiella oxytoca*. All referee laboratories as well as 98% of participants that process urine cultures correctly identified this organism.

**Methods of identification used by participating laboratories reporting:**

***Klebsiella oxytoca***

bioMerieux Vitek GNI +	92
Dade Behring MicroScan Gram Neg ID	79
bioMerieux API 20E	28
Two or more test methods	8
BD BBL Crystal Enteric/Nonfermenter	5
bioMerieux Vitek 2 GN	2
BD Phoenix	2
BD BBL Enterotube II	2
Conventional biochemicals	2
bioMerieux API Rapid 20E	1
Biolog MicroLog Gram Negative	1
bioMerieux-Vitek GNI	1
Dade Behring MicroScan Rapid Gram Neg	1
Remel RapID ONE	1
Unknown	1
<b>TOTAL</b>	<b>226</b>

***Klebsiella pneumoniae***

bioMerieux Vitek GNI +	4
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**Specimen source not tested** 1

***Staphylococcus, coagulase negative***

Conventional biochemicals	1
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**Additional organisms reported in Specimen 5:**

<i>Staphylococcus aureus</i>	1
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## ***Educational Specimens A and B – Blood cultures***

Both educational specimens were simulated blood cultures containing *Bacillus cereus*. Sample A contained a *B. cereus* that was non-hemolytic and motile. Sample B contained a *B. cereus* that was non-hemolytic and non-motile. These specimens were designed to see how laboratories handle *Bacillus* species from blood cultures that have characteristics similar to *Bacillus anthracis*.

The two *Bacillus* species of greatest clinical concern are *B. anthracis* and *B. cereus*. The naturally occurring form of *B. anthracis* generally results in cutaneous infections from working with contaminated goats or cattle. Pulmonary infections rarely occur and are the result of inhalation of bacillus spores. This occurs most often in endemic areas where workers process animal hides in poorly ventilated factories. Even though anthrax is not endemic to the United States (prior to 2001 only 18 cases of pulmonary anthrax were reported) laboratories need to be prepared for natural cases imported into the U.S. from other countries, e.g. illness associated with processing imported animal hides. The gastrointestinal form of anthrax is rare in the U.S. but occurs after consumption of contaminated meat from infected animals. Anthrax is also an agent of bioterrorism and clinical laboratories are the first place that an intentional event might be noticed.

*Bacillus cereus* is a common agent of food poisoning, eye infections after traumatic penetration, wound infections (especially in IV drug users), and umbilical infections in neonates. Infections with this organism are common and care should be given to isolates recovered from blood cultures to rule out this organism as well. Therefore, it is important for laboratories to be aware that not all *Bacillus* species recovered from a blood culture are contaminants and a phone call to the attending physician for a patient's history may help when determining the importance of the isolate.

*Bacillus* species are common in the environment and often represent contaminants when isolated in a single blood culture. However, before an isolate is disregarded as a contaminant, attention must be given to a few key characteristics. If a laboratorian observes a small (2-5 mm) white or gray colony that is non-hemolytic and on Gram stain appears gram-positive with vacuoles consistent with spores, *B. anthracis* should be considered. The colonies of the educational samples were medium-sized, gray and moist with Specimen B appearing to have a slight ground glass appearance, so keep in mind that colony morphology may not always be "textbook". If *Bacillus* species is suspected based on colony morphology and gram stain, a motility test should then be performed in a biosafety cabinet. The simplest test to perform at this point is a tube motility. It is recommended not to perform a hanging drop to assess motility when attempting to rule out *B. anthracis* to avoid aerosolization of the organism. There are commercially available motility media, some containing indicators such as TTC (triphenyl-tetrazolium chloride) which can aid laboratorians in detecting motility. If the tube motility is negative, the isolate should be referred to the Wadsworth Center or your regional LRN reference laboratory for definitive testing to rule out *B. anthracis*. If the motility is positive, biochemical analysis can be performed in-house for speciation or the isolate can be referred to a reference laboratory if needed. Conventional biochemicals and automated systems are able to identify *B. cereus* due to specific sugar fermentation and nitrate reactions, but it is recommended to assess organism motility in order to rule out *B. anthracis* before continuing on to species identification.

Acceptable answers for Specimen A: *Bacillus cereus* (6.4% of labs) or *Bacillus* species, not *anthracis* (52% of labs). The acceptable answer for Specimen B: *Bacillus* species, unable to r/o *anthracis* – sent to reference laboratory (40% of labs). While a specimen report of *B. cereus* for Specimen B is the correct identification of the organism, the laboratory should have stopped work-up once it was noticed that the organism in question was non-hemolytic and non-motile. Also, reporting *Bacillus cereus* group is not sufficient for Specimen A since *B. anthracis* is part of that group. The report should reflect that *B. anthracis* was tested for and ruled out. Motility was a key criterion for the process of identification of these organisms and the variety of answers (such as *Bacillus thuringiensis*, *Bacillus coagulans*, *Klebsiella oxytoca*, “Not a *Bacillus* species”, *Bacillus* species not *cereus* or *anthracis* etc.) submitted by over 50% of the laboratories was unexpected. If you experienced problems with motility regarding either of these specimens, please call the number given below for guidance and assistance.

### Reminders:

- Test all suspected *Bacillus* species isolates for hemolysis and motility prior to work-up or dismissal.
- All specimen processing that might result in aerosol production should be performed in a class II biological safety cabinet.
- Please call the Wadsworth Center Bacteriology or Biodefense Laboratories at (518) 474-4177 or your regional LRN reference laboratory if you have any questions about how to rule out anthrax. Additional information and rule-out algorithms for select agents can be found on the Health Provider Network (HPN) LRN homepage. Laboratorians can gain access through their HPN coordinator or through the NYSDOH. For further information, contact the NYS Laboratory Response Network a 518-457-9795. Also, reference materials for ruling out anthrax are available at [www.asm.org](http://www.asm.org).
- “B-SAFE” bench top reference materials outlining procedures for handling select agents in the clinical laboratory are in production. These will be distributed by the LRN in the near future.

**Participating laboratory responses for educational samples:**

**Educational A**

***Bacillus* species, not *anthracis***

Conventional biochemicals	108
Test method not indicated	13
Two or more test methods	1
<b>TOTAL</b>	<b>122</b>

***Bacillus* species**

Conventional biochemicals	41
Test method not indicated	3
Two or more test methods	3
<b>TOTAL</b>	<b>47</b>

***Bacillus* species, unable to r/o *anthracis***

Conventional biochemicals	17
Test method not indicated	2
<b>TOTAL</b>	<b>19</b>

***Bacillus cereus***

Conventional biochemicals	13
MIDI Sherlock Microbial ID System	1
Two or more test methods	1
<b>TOTAL</b>	<b>15</b>

**No report** 10

**Specimen source (blood culture) not tested** 9

***Bacillus cereus* group**

Conventional biochemicals	4
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***Bacillus thuringiensis***

BD Phoenix	1
Two or more test methods	1
<b>TOTAL</b>	<b>2</b>

***Bacillus* species, not *cereus* or *anthracis***

Conventional biochemicals	2
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**Gram positive bacillus** 1

<i>Bacillus coagulans</i>	
Conventional biochemicals	<b>1</b>
<b>Additional organisms reported in Educational A:</b>	
<i>Klebsiella oxytoca</i>	1
*****	
<b>Educational B</b>	
<b><i>Bacillus species, unable to r/o anthracis</i></b>	
Conventional biochemicals	85
Test method not indicated	9
<b>TOTAL</b>	<b>94</b>
<b><i>Bacillus species</i></b>	
Conventional biochemicals	47
Test method not indicated	5
Dade Behring MicroScan Gram Pos ID	1
<b>TOTAL</b>	<b>53</b>
<b><i>Bacillus species, not anthracis</i></b>	
Conventional biochemicals	40
Test method not indicated	5
Two or more test methods	1
<b>TOTAL</b>	<b>46</b>
<b>No report</b>	<b>11</b>
<b>Specimen source (blood culture) not tested</b>	<b>9</b>
<b><i>Bacillus cereus</i></b>	
Conventional biochemicals	3
BD Phoenix	1
<b>TOTAL</b>	<b>4</b>
<b>Not <i>Bacillus species</i></b>	
Conventional biochemicals	2
<b><i>Bacillus mycoides</i></b>	
Conventional biochemicals	1
Two or more test methods	1
<b>TOTAL</b>	<b>2</b>

<b><i>Bacillus</i> species, not <i>cereus</i> or <i>anthracis</i></b>	
Conventional biochemicals	2
<b><i>Bacillus thuringiensis</i></b>	
Two or more test methods	1
Conventional biochemicals	1
<b>TOTAL</b>	<b>2</b>
<b>Gram positive bacilli</b>	<b>2</b>
<b><i>Bacillus cereus/thuringiensis</i></b>	
Vitek 2 Compact BCL card	1
<b><i>Bacillus cereus</i> group, not <i>anthracis</i></b>	
Conventional biochemicals	1
<b><i>Bacillus coagulans</i></b>	
Conventional biochemicals	1
<b><i>Bacillus licheniformis</i></b>	
Conventional biochemicals	1
<b><i>Bacillus</i> species not <i>cereus</i> group</b>	
Conventional biochemicals	1
<b>Additional organisms reported in Educational B:</b>	
<i>Klebsiella oxytoca</i>	1

### ***Chlamydia – cervical swab for direct testing***

This simulated cervical swab was provided to laboratories that test for *Chlamydia* using direct detection methods. This sample contains non-viable organisms and is not suitable for laboratories performing *Chlamydia* culture.

This sample was negative for *Chlamydia* and was reported as such by all of the participating laboratories that tested this specimen.

#### **Test kits used by laboratories reporting this specimen as:**

##### **Negative for *Chlamydia trachomatis***

Gen-Probe PACE 2 CT or CT/GC	52
BD ProbeTec C. trachomatis assay	16
Gen-Probe Aptima Combo 2	13
Roche Diagnostics COBAS Amplicor CT/NG	13
BioMerieux VIDAS	5
Beckman Coulter Access Chlamydia EIA	3
Test method not indicated	3
PCR	3
Trinity Biotech C. trachomatis direct test	1
Roche Diagnostics Amplicor CT/NG	1
BioStar Chlamydia OIA	1
BioRad Pathfinder	1
BioRad Chlamydia Microplate	1
Digene	1
<b>TOTAL</b>	<b>114</b>
<b>No report</b>	<b>1</b>

## ***Direct Antigen Detection***

All participating laboratories which perform direct antigen testing received either a simulated throat swab to be tested for Group A *Streptococcus* or a genital swab to be tested for Group B *Streptococcus*. Information provided in the Bacteriology Questionnaire was used to determine which type of specimen to send to each laboratory.

### **Specimen A - Source: Throat for Group A *Streptococcus***

This specimen was positive for Group A *Streptococcus*. All of the participating laboratories that processed this specimen reported it as positive.

#### **Test kits used by laboratories reporting Specimen A as: Positive for Group A *Streptococcus*:**

BD Directigen EZ Strep	14
BioStar Aceava Strep A	13
Abbott Signify Strep A	10
Genzyme OSOM Ultra Strep A	8
Quidel QuickVue + Strep A	6
BioStar Strep A OIA Max	4
Fisher Healthcare Sure-Vue Strep A	4
Quidel QuickVue Inline Strep A	4
Test method not indicated	4
BD Chek Strep A	3
BD Q Test Strep	2
Gen-Probe Group A Strep	2
LifeSign Status Accustrep A	2
Meridian Bioscience ImmunoCard STAT Strep A	2
Remel PathoDx Strep A	2
Remel RIM A.R.C. Strep A	2
Wampole Clearview Strep A Extract	2
Applied Biotech SureStep Strep A	1
BD Link 2 Strep A	1
Beckman Coulter Icon SC	1
Cardinal Strep A Cassette	1
Fisher Sureview SELECT	1
Mainline Confirms Strep A	1
Polymedco Poly Stat Strep A	1
Quidel QuickVue Dipstick Strep A	1
Sacks Medical Corp RefuAH Strep A	1
SP Strep A Dipstick	1
SP Strep A Cassette	1
<b>TOTAL</b>	<b>95</b>

**Specimen C – Source: Genital for Group B *Streptococcus***

This specimen was positive for Group B *Streptococcus*. All laboratories that tested this sample reported it as positive.

**Test kits used by laboratories reporting Specimen C as:  
Positive for Group B *Streptococcus***

BioStar Strep B OIA

2

**BACTERIAL IDENTIFICATION BY PARTICIPATING LABORATORIES**

	<u>Number Reported</u>	<u>%</u>
<b>SPECIMEN NUMBER 1</b>		
<i>Shigella flexneri</i> , group B	154	66.4
No enteric pathogens isolated	33	14.2
<i>Shigella</i> species	26	11.2
Specimen source (stool culture) not tested	13	5.6
<i>Shigella</i> group	2	0.9
<i>Shigella boydii</i> , group C	1	0.4
<i>Shigella boydii/flexneri</i>	1	0.4
<i>Shigella</i> group D	1	0.4
<i>Shigella</i> species, not <i>sonnei</i>	1	0.4
*****		

<b>SPECIMEN NUMBER 2</b>		
<i>Neisseria gonorrhoeae</i>	221	95.3
Specimen source (cervical culture) not tested	9	3.9
<i>Gardnerella vaginalis</i>	1	0.4
No pathogens isolated	1	0.4
*****		

<b>SPECIMEN NUMBER 3</b>		
<i>Bacteroides vulgatus</i>	176	75.9
<i>Prevotella melaninogenica</i>	14	6.0
Specimen source (blood culture) not tested	8	3.4
<i>Bacteroides</i> species	8	3.4
Do not perform anaerobic cultures	5	2.2
No anaerobic organisms	4	1.7
<i>Prevotella</i> species	4	1.7
<i>Prevotella denticola</i>	3	1.3
Anaerobic gram negative bacillus	3	1.3
<i>Bacteroides fragilis</i> group	3	1.3
No <i>Bifidobacterium</i>	2	0.9
<i>Bacteroides</i> species, not <i>fragilis</i>	1	0.4
Gram positive bacillus	1	0.4
<i>Staphylococcus aureus</i>	218	93.9
Specimen source (blood culture) not tested	8	3.4
<i>Staphylococcus</i> , coagulase positive	6	2.6
*****		

<b>SPECIMEN NUMBER 4</b>		
<i>Staphylococcus lugdunensis</i>	133	57.3
<i>Staphylococcus</i> , coagulase negative	67	28.9
<i>Staphylococcus haemolyticus</i>	15	6.5
Specimen source (joint aspirate) not tested	8	3.4
<i>Staphylococcus hominis</i>	3	1.3

<i>Staphylococcus</i> species, not <i>aureus</i>	2	0.9
<i>Staphylococcus epidermidis</i>	2	0.9
<i>Klebsiella oxytoca</i>	1	0.4
<i>Staphylococcus intermedius</i>	1	0.4
*****		

**SPECIMEN NUMBER 5**

<i>Klebsiella oxytoca</i>	226	97.4
<i>Klebsiella pneumoniae</i>	4	1.7
Specimen source (urine culture) not tested	1	0.4
<i>Staphylococcus</i> , coagulase negative	1	0.4
*****		

**EDUCATIONAL A**

<i>Bacillus</i> species, not <i>anthracis</i>	122	52.6
<i>Bacillus</i> species	47	20.3
<i>Bacillus</i> species, unable to r/o <i>anthracis</i>	19	8.2
<i>Bacillus cereus</i>	15	6.5
No report	10	4.3
Specimen source (blood culture) not tested	9	3.9
<i>Bacillus cereus</i> group	4	1.7
<i>Bacillus thuringiensis</i>	2	0.9
<i>Bacillus</i> species, not <i>cereus</i> or <i>anthracis</i>	2	0.9
Gram positive bacillus	1	0.4
<i>Bacillus coagulans</i>	1	0.4
*****		

**EDUCATIONAL B**

<i>Bacillus</i> species, unable to r/o <i>anthracis</i>	94	40.5
<i>Bacillus</i> species	53	22.8
<i>Bacillus</i> species, not <i>anthracis</i>	46	19.8
No report	11	4.7
Specimen source (blood culture) not tested	9	3.9
<i>Bacillus cereus</i>	4	1.7
Not <i>Bacillus</i> species	2	0.9
<i>Bacillus mycoides</i>	2	0.9
<i>Bacillus</i> species, not <i>cereus</i> or <i>anthracis</i>	2	0.9
<i>Bacillus thuringiensis</i>	2	0.9
Gram positive bacillus	2	0.9
<i>Bacillus cereus</i> / <i>thuringiensis</i>	1	0.4
<i>Bacillus cereus</i> group, not <i>anthracis</i>	1	0.4
<i>Bacillus coagulans</i>	1	0.4
<i>Bacillus licheniformis</i>	1	0.4
<i>Bacillus</i> species, not <i>cereus</i> group	1	0.4
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**CHLAMYDIA SPECIMEN**

Negative for <i>Chlamydia trachomatis</i>	114	99.1
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No report	1	0.9
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**DIRECT ANTIGEN SPECIMENS**

A. Positive for Group A <i>Streptococcus</i>	95	100.0
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C. Positive for Group B <i>Streptococcus</i>	2	100.0
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