

# **BACTERIOLOGY PROFICIENCY TESTING PROGRAM**

## **General Category**

**September 24, 2002**

This report summarizes the results of the proficiency test administered September 24, 2002 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. Failure of 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 90% 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 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 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%.

**SEPTEMBER 24, 2002 TEST EVENT**

**Number of Participating Laboratories**

**Receiving specimens            256**  
**Returning results                256    (100%)**

Grade Distribution		
Score	Number	Percent
100	205	80.1
90 - 99	5	1.9
80 - 89	40	15.6
70 - 79	2	0.8
60 - 69	4	1.6



**BACTERIOLOGY - GENERAL**  
**SEPTEMBER 24, 2002**  
**ANSWER KEY**

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

*Shigella flexneri*

**Specimen No. 2 – Urine (All Organisms Reported)**

*Burkholderia cepacia*

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

*Clostridium perfringens*

Group A *Streptococcus*

**Specimen No. 4 – Sputum (Pathogens Only) and Antibiotic Susceptibility**

*Klebsiella pneumoniae* (ESBL)

Susceptibility of *K. pneumoniae* to: Aztreonam - Resistant  
Cefotaxime – Not authenticated

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

*Haemophilus parainfluenzae*

**Educational Specimens – Blood**

A *Bacillus* species not *anthracis*

B *Bacillus* species not *anthracis*

**Chlamydia Specimen**

Positive for *Chlamydia trachomatis*

**Direct Antigen Detection**

A (Throat)

Positive for Group A *Streptococcus*

B (CSF)

*Haemophilus influenzae* b



## REFEREE LABORATORY RESULTS

<b>Specimen Number</b>	<b>Referee Laboratory Responses</b>	<b>Percent*</b>
<b>1</b>	<i>Shigella flexneri</i>	100
<b>2</b>	<i>Burkholderia cepacia</i>	100
<b>3</b>	<i>Clostridium perfringens</i>	100
	Group A <i>Streptococcus</i>	100
<b>4</b>	<i>Klebsiella pneumoniae</i>	100
<b>5</b>	<i>Haemophilus parainfluenzae</i>	100

\* Based on responses of 10 referee laboratories



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

This simulated stool specimen contained *Shigella flexneri*, serogroup B. All referee laboratories identified this organism. Of the participating laboratories that process stool cultures, 78% identified *Shigella flexneri*. An additional 10% of participants identified the organism as *Shigella* species, but would send the isolate to a reference laboratory for serogrouping. Twenty-three laboratories (approximately 9%) did not isolate *Shigella flexneri* and reported that this specimen contained normal enteric flora.

Additional organisms included in this specimen as normal flora were *Serratia marcescens* and *Escherichia coli*.

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

#### ***Shigella flexneri***

Vitek GNI	58
Dade Behring MicroScan	54
bioMerieux Vitek API 20E	49
Vitek ID-GNB	8
Two or more systems	8
Conventional biochemicals	6
BBL Crystal Enteric/Nonfermenter	3
BBL Enterotube II	1
bioMerieux Vitek API Rapid 20E	1
No information given	1
Remel RapID ONE	1
<b>TOTAL</b>	<b>190</b>

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

Dade Behring MicroScan	7
bioMerieux Vitek API 20E	5
bioMerieux Vitek GNI	5
BBL Crystal Enteric/Nonfermenter	4
bioMerieux Vitek ID-GNB	1
Dade Behring MicroScan	1
No information given	1
<b>TOTAL</b>	<b>24</b>

**No Enteric Pathogens / *Shigella flexneri* not isolated** 24

**Do not process stool specimens** 15

#### ***Shigella boydii***

bioMerieux Vitek GNI	1
Two or more systems	1
<b>TOTAL</b>	<b>2</b>

*Shigella dysenteriae*  
bioMerieux Vitek API 20E 1

\*\*\*\*\*

**Antisera used by laboratories reporting:**

*Shigella flexneri*  
BBL Shigella grouping antisera 74  
No antisera indicated 66  
Murex Wellcolex Colour Shigella 31  
Difco Shigella antisera 9  
Not specified 8  
Bacto *Shigella* 1  
Remel Shigellex 1  
TOTAL 190

*Shigella species*  
No antisera indicated 24

*Shigella boydii*  
No antisera indicated 2

*Shigella dysenteriae*  
BBL Shigella grouping antisera 1

Additional organisms reported in Specimen 1:  
*Burkholderia cepacia* 1

## ***Specimen No. 2 – Urine (All Organisms)***

This simulated urine specimen contained *Burkholderia cepacia*. All referee laboratories identified this organism. Of the participating laboratories that process urine specimens, approximately 95% identified *B. cepacia*. Those laboratories are still referring to this organism as *Pseudomonas cepacia* should note that the nomenclature changed in 1992 when *Pseudomonas cepacia* was transferred to the new genus *Burkholderia*.

Coeyne, T. et al. 2001. Taxonomy and Identification of the *Burkholderia cepacia* complex. J. Clin. Microbiol. 39(10): 3427-3436.

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

#### ***Burkholderia cepacia***

bioMerieux Vitek GNI	82
Dade Behring MicroScan	65
bioMerieux Vitek API 20E	34
bioMerieux Vitek API20NE	18
bioMerieux Vitek ID-GNB	12
Two or more systems	11
BBL Crystal Enteric/Nonfermenter	6
Conventional biochemicals	5
Remel RapID NF Plus	4
BBL Oxi/Ferm II	1
Difco Pasco	1
No information given	1
<b>TOTAL</b>	<b>240</b>

#### ***Pseudomonas cepacia***

Dade Behring MicroScan	4
bioMerieux Vitek API20NE	2
bioMerieux Vitek GNI	2
BBL Crystal Enteric/Nonfermenter	1
Remel RapID NF Plus	1
<b>TOTAL</b>	<b>10</b>

#### ***Burkholderia cepacia* complex**

Conventional biochemicals	1
Remel RapID NF Plus	1
<b>TOTAL</b>	<b>2</b>

**Not reported** 2

**Do not process urine specimens** 1

#### ***Pseudomonas luteola***

bioMerieux API 20NE	1
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**Additional organisms reported in Specimen 2:**

<i>Clostridium perfringens</i>	1
Group A Streptococcus	1
Other nonfermenting gram negative bacilli	1
<i>Shigella flexneri</i>	1

### ***Specimen No. 3 – Wound - Aerobic/Anaerobic (All Organisms)***

This simulated blood specimen, which was for both aerobic and anaerobic culture, contained *Clostridium perfringens* and Group A *Streptococcus*.

*Clostridium perfringens* was identified by all referee laboratories and by 95% of the participants that process wound specimens for anaerobic culture.

Group A *Streptococcus* was identified by all referee laboratories and by 99.6% of participants that process wound cultures.

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

##### ***Clostridium perfringens***

Remel RapID ANA II	106
bioMerieux Vitek ANI	38
bioMerieux Vitek API 20A	37
Dade MicroScan Rapid Anaerobe	20
Conventional biochemicals	8
Two or more systems	6
BBL Crystal Anaerobe	5
bioMerieux Vitek API Rapid ID 32A	4
bioMerieux Vitek API An-Ident	2
No information given	2
B-D Sceptor	1
<b>TOTAL</b>	<b>229</b>

**Do not isolate anaerobes** 12

##### ***Clostridium* species**

bioMerieux Vitek ANI	2
bioMerieux Vitek API 20A	1
bioMerieux Vitek API Rapid ID 32A	1
Remel RapID ANA II	1
<b>TOTAL</b>	<b>5</b>

**Anaerobic gram positive bacillus** 4

**No anaerobes isolated** 3

**Do not process wound specimens** 2

**No *Bifidobacterium* isolated** 1

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

**Group A *Streptococcus***

Murex Streptex	55
BBL Streptocard	52
DPC PathoDx Strep Grouping	43
Conventional biochemicals	24
Vitek GPI	21
Two or more systems	16
Dade Behring MicroScan	13
bioMerieux Vitek Slidex Strepto	6
bioMerieux Vitek API 20 Strep	6
Boule Phadebact Streptococcus	5
Meridian Meritec Strep	4
No information given	3
Lifesign Streptolex	2
The Binding Site Strep grouping	2
BBL Crystal Gram Positive	1
<b>TOTAL</b>	<b>253</b>

**Do not process wound specimens** 2

**Not reported** 1

**Additional organisms reported in Specimen 3:**

<i>Burkholderia cepacia</i>	1
Coagulase-negative <i>Staphylococcus</i>	1
Gram positive cocci	1
<i>Pseudomonas cepacia</i>	1

## ***Specimen No. 4 – Sputum (Pathogens Only) and Antibiotic Susceptibility***

The pathogenic organism included in this specimen was *Klebsiella pneumoniae*. All referee laboratories identified *Klebsiella pneumoniae* as did 99.6% of those participants that processed this test specimen. *Streptococcus sanguis* and *Neisseria mucosa* were included as additional flora in this specimen.

Antibiotic susceptibility testing was indicated for this specimen. The isolate of *K. pneumoniae* included in this specimen was ATCC 700603, which is the NCCLS – recommended quality control strain for extended spectrum beta-lactamase (ESBL) production. Ninety-four laboratories (approximately 55% of those that performed susceptibility testing with these antibiotics) recognized the presence of an ESBL in this isolate.

Extended-spectrum beta-lactamases were first described in the 1980's when decreased susceptibility to third-generation cephalosporins was noted among *Klebsiella* species. These  $\beta$ -lactamases are most commonly found in *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Escherichia coli* and confer resistance against the penicillins and aztreonam in addition to third-generation cephalosporins. Recently, extended-spectrum beta-lactamases have been detected in *Serratia marcescens*, *Proteus mirabilis*, *Morganella morgannii*, *Alcaligenes faecalis* and *Pseudomonas aeruginosa*.

Additionally, ESBL-producing organisms may appear susceptible *in vitro*, but are clinically resistant. Therefore, care should be taken when interpreting susceptibility results for those antibiotics listed above. NCCLS guidelines contain tables with information regarding screening for ESBL production to aid laboratories in the recognition of these strains. These guidelines recommend the use of at least two of the following antibiotics: cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone. Tables are provided with zone diameters and MIC values indicative of the presence of ESBL. Confirmatory testing is also described and involves the use of both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, which is a  $\beta$ -lactamase inhibitor. If the strain is an ESBL-producer, resistance is significantly decreased in the presence of clavulanic acid. It is recommended that all laboratories perform screening of *Klebsiella* species and *E. coli* for ESBL production.

This organism was resistant to aztreonam, with expected zone diameters in the range of 9-17 mm and an MIC of 64  $\mu$ g/ml. Ninety-seven percent of the participating laboratories that tested aztreonam reported this strain of *K. pneumoniae* as resistant.

The expected values for cefotaxime are zone diameters in the range of 17-25 mm and an MIC of 8  $\mu$ g/ml. While the NCCLS charts for testing of Enterobacteriaceae provide interpretations of intermediate or susceptible for these values, these results should not be reported without prior screening for ESBL production. Approximately 25% of participating laboratories reported this isolate as susceptible to cefotaxime without screening for ESBL production. However, approximately 58% of the laboratories that tested cefotaxime recognized this isolate as an ESBL-producer and interpreted the result as resistant. Unfortunately, a consensus was not reached for cefotaxime susceptibility, so this

antibiotic was not graded. However, these results illustrate the importance of ESBL screening.

Results of susceptibility testing on this organism can be found on pages 17 – 20.

Rasheed, J.K. et al. 2000. Characterization of the extended-spectrum  $\beta$ -lactamase reference strain, *Klebsiella pneumoniae* K6 (ATCC 700603), which produces the novel enzyme SHV-18. *Antimicrob. Agents Chemother.* 44(9): 2382-2388.

National Committee for Clinical Laboratory Standards. 2002. Performance standards for antimicrobial susceptibility testing; twelfth informational supplement, M100-S12. National Committee for Clinical Laboratory Standards, Wayne, PA.

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

bioMerieux Vitek GNI	94
Dade Behring MicroScan (all types)	81
bioMerieux Vitek API 20E	38
bioMerieux Vitek ID-GNB	13
BBL Crystal Enteric/Nonfermenter	5
Conventional biochemicals	4
Two or more systems	3
No information given	3
BBL Enterotube II	2
Remel RapID ONE	2
Cathra Autoreader	1
Difco Pasco	1
<b>TOTAL</b>	<b>247</b>

**Other reports:**

**Do not process sputum specimens** 8

***Klebsiella terrigena***

bioMerieux Vitek API 20E 1

**Results of Antimicrobial Susceptibility testing of *K. pneumoniae* with AZTREONAM by participating laboratories**

<b>Result</b>	<b>Method</b>	<b>MIC (µg/ml)</b>	<b>Zone diam. (mm)</b>	
<b>Resistant (169)</b>	MicroScan (70)	>=2 (1)		
		16 (1)		
		>16 (59)		
		Not given (9)		
	Vitek (50)	16 (2)		
		32 (1)		
		>=32 (29)		
		>32 (6)		
		>=64 (3)		
		>64 (4)		
		Not given (5)		
	Disk diffusion (42)		0 (1)	
			6 (1)	
			10 (7)	
			11 (2)	
			12 (19)	
			13 (5)	
			14 (5)	
			15 (1)	
			Not given (1)	
		No method indicated (3)		
		Agar dilution (1)	>16 (1)	
		B-D Pasco (1)	>16 (1)	
	E-test (1)	32 (1)		
	Sensititre (1)	Not given (1)		
<b>Aztreonam not tested (73)</b>				
<b>Do not process sputum cultures (8)</b>				
<b>Intermediate (3)</b>	Vitek (3)	16 (3)		
<b>Susceptible (2)</b>	Vitek (2)	<=2 (1)		
		<=8 (1)		
<b>Do not perform susceptibility testing (1)</b>				

**Results of Antimicrobial Susceptibility testing of *K. pneumoniae* with CEFOTAXIME by participating laboratories**

<b>Result</b>	<b>Method</b>	<b>MIC (µg/ml)</b>	<b>Zone diam. (mm)</b>	
<b>Cefotaxime not tested (95)</b>				
<b>Resistant (84)</b>	MicroScan (38)	>=2 (1)		
		<4 (3)		
		<=4 (2)		
		<8 (11)		
		<=8 (6)		
		8 (1)		
		>8 (1)		
		16 (2)		
		32 (3)		
		>32 (5)		
		Not given (3)		
	Vitek (20)	2 (1)		
		<4 (1)		
		<=4 (15)		
		4 (2)		
		8 (1)		
	Disk diffusion (16)			7 (1)
				16 (1)
				17 (1)
				18 (1)
			19 (3)	
			20 (2)	
			21 (3)	
			22 (1)	
			23 (1)	
			No given (2)	
E-test (4)	2 (1)			
	4 (2)			
	<8 (1)			
No method indicated (3)				
Agar dilution (1)	>32 (1)			
MIC (1)	<=4 (1)			
Sensititre (1)	Not given (1)			

Table continued on next page

**Antimicrobial Susceptibility testing of *K. pneumoniae* with CEFOTAXIME - con't**

<b>Result</b>	<b>Method</b>	<b>MIC (µg/ml)</b>	<b>Zone diam. (mm)</b>
<b>Susceptible (36)</b>	MicroScan (18)	<4 (3)	
		<=4 (1)	
		<8 (5)	
		<=8 (4)	
		Not given (5)	
	Vitek (15)	<4 (1)	
		<=4 (12)	
		<=16 (1)	
		Not given (1)	
	Disk diffusion (2)		19 (1)
		24 (1)	
Not given (1)			
<b>Intermediate (25)</b>	Disk diffusion (19)		16 (1)
			17 (5)
			18 (1)
			19 (4)
			20 (4)
			21 (2)
			22 (1)
			29 (1)
		MicroScan (6)	16 (1)
		32 (4)	
	Not given (1)		
<b>Do not process sputum cultures (8)</b>			
<b>No result reported (7)</b>			
<b>Do not perform susceptibility testing (1)</b>			

Antibiotic Susceptibility Results - Participating & Referee Labs <i>Klebsiella pneumoniae</i>				
	Aztreonam		Cefotaxime	
	Referee <sup>a</sup>	Participant <sup>b</sup>	Referee <sup>a</sup>	Participant <sup>b</sup>
Susceptible	0	2	1	34
Intermediate	1	2	0	25
Resistant	6	163	5	79
Not Tested <sup>c</sup>	3	70	4	91
Do not process source <sup>d</sup>	0	8	0	8
No result reported	0	0	0	7
Not performed <sup>e</sup>	0	1	0	1

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

<sup>b</sup>Other Participating Laboratories (246 labs total)

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

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

<sup>e</sup>Do not perform antimicrobial susceptibility testing

## ***Specimen No. 5 – Blood (All Organisms)***

This simulated blood culture contained *Haemophilus parainfluenzae*. All referee laboratories correctly identified *H. parainfluenzae*. Of the participating laboratories that processed this specimen, approximately 93% reported *H. parainfluenzae*.

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

Conventional biochemicals	55
Remel RapID NH	52
bioMerieux Vitek NHI	39
Dade Behring MicroScan HNID	34
bioMerieux Vitek API NH	24
Two or more systems	14
BBL <i>Haemophilus</i> ID Quad Plated	5
BBL Crystal <i>Neisseria/Haemophilus</i>	3
Remel RapID NF Plus	2
<b>TOTAL</b>	<b>228</b>

### **Other reports:**

**Do not process blood cultures** 11

### ***Haemophilus* species**

No system indicated	2
Conventional biochemicals	1
Dade Behring MicroScan HNID	1
<b>TOTAL</b>	<b>4</b>

### ***Haemophilus influenzae***

Remel RapID NH	2
Dade Behring MicroScan HNID	1
<b>TOTAL</b>	<b>3</b>

### ***Haemophilus* species, not *influenzae***

Conventional biochemicals	2
Remel RapID NH	1
<b>TOTAL</b>	<b>3</b>

### ***Haemophilus parahaemolyticus***

Remel RapID NH	2
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### ***Haemophilus aegyptius***

Remel RapID NH	1
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### ***Haemophilus influenzae* II**

Remel RapID NH	1
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<b><i>Haemophilus influenzae</i> IV</b>	
Remel RapID NH	<b>1</b>
<b><i>Haemophilus influenzae</i>, not type b</b>	
bioMerieux Vitek NHI	<b>1</b>
<b>No growth</b>	<b>1</b>

## ***Educational Specimen – Blood – Aerobic / Anaerobic (All Organisms)***

Two educational samples were included in this proficiency test event to provide laboratories with experience in screening specimens for possible *Bacillus anthracis*. Both contained species of *Bacillus* that could be safely handled in all clinical laboratories. Laboratories were asked to report results for tests such as gram stain reaction, colony morphology and hemolysis, motility and catalase.

### **Educational Sample A**

Educational Sample A contained *Bacillus pumilus*. Colonies produced by this organism were nonhemolytic, flat and gray with an irregular margin. In contrast to colonies of *B. anthracis*, these colonies did not have a ground glass appearance. The organism was motile and was reported as such by approximately 86% of participants. Based on a positive motility result, it was possible to rule out *B. anthracis* in this specimen and the result should have been reported as “*Bacillus* species not *anthracis*”. Of those laboratories that processed this specimen, approximately 80% indicated that this specimen was negative for *B. anthracis*. Approximately 6% were unable to rule out *B. anthracis* in this specimen. An additional 10% of participants reported a result of “*Bacillus* species”. This is not an appropriate result since there is no indication of whether *B. anthracis* had been ruled out. If *B. anthracis* cannot be ruled out the results should be reported as “Non-hemolytic, non-motile *Bacillus* species was isolated; referred to a reference laboratory for identification”.

### **Educational Sample B**

Educational Sample B contained *Bacillus* species. The organism was a rarely encountered environmental species and 16S rDNA sequencing yielded results of either *Bacillus macroides* or *Bacillus simplex*. Colonies were nonhemolytic, round and raised with a somewhat ground-glass appearance. This organism was weakly motile. Of the participating laboratories that processed this specimen, 57% indicated that they were unable to rule out the presence of *B. anthracis* while 23% reported that the specimen contained *Bacillus* species not *anthracis*. Approximately 9% simply reported a result of *Bacillus* species and the remainder reported various identifications which were not *B. anthracis*. The weak motility result which may have been interpreted as non-motile is the most likely reason that laboratories were unable to rule out *B. anthracis*. Approximately 84% of participating laboratories reported that the isolate was non-motile and the vast majority (94%) were unable to rule out *B. anthracis*.

Photographs of colonies and gram stains of both educational specimens and *Bacillus anthracis* are included on the next pages.

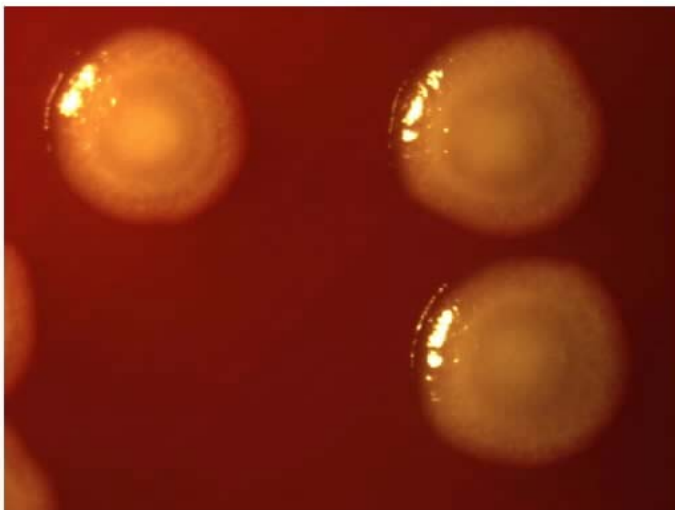
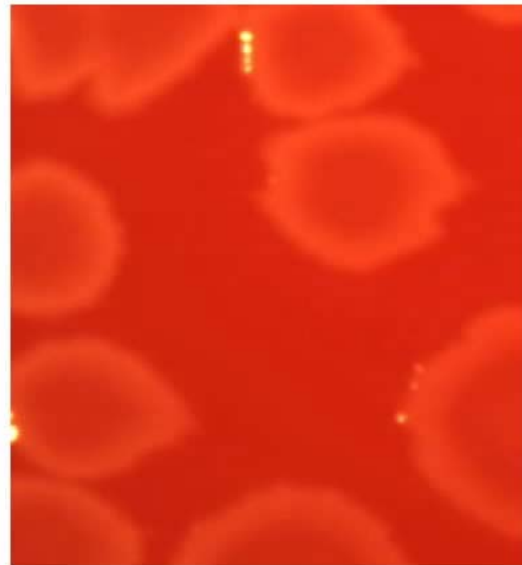
It can be difficult to interpret motility test media results for *Bacillus* species. The following suggestions may assist in the interpretation of results: use of a motility tube medium with a lower agar concentration such as Remel’s motility S medium; re-incubation of questionable results for an additional 24 hours; incorporation of an un-inoculated tube (or aseptically inoculated) and a weakly motile organism as additional controls. Motility testing can be performed by wet mount but misinterpretation of these results can occur if the test organism is sluggish/slower than the positive control and may be confused with Brownian movement.

Approximately 5-10% of the participants reported beta-hemolytic colonies for Educational samples A and B. Both organisms are non-hemolytic on sheep blood agar. Colonies of *B. anthracis* are not hemolytic, but weak hemolysis may be observed under areas of confluent growth in aging cultures and should not be interpreted as beta-hemolysis. This observation also applies to other *Bacillus* species.



*Bacillus anthracis* colonies on blood agar (CDC photos).

Educational sample A colonies on blood agar.

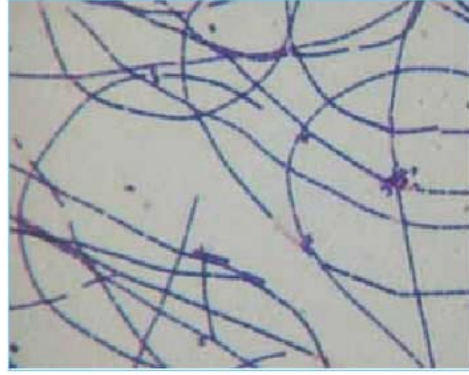


Educational sample B colonies on blood agar.

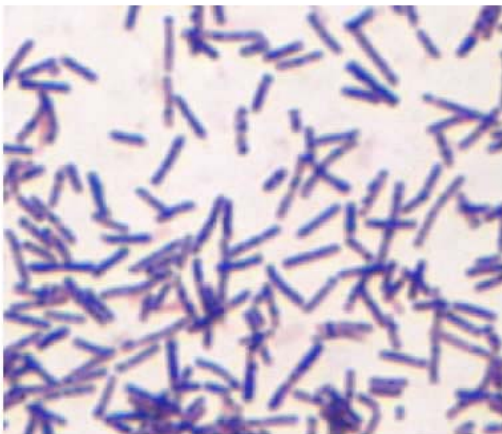




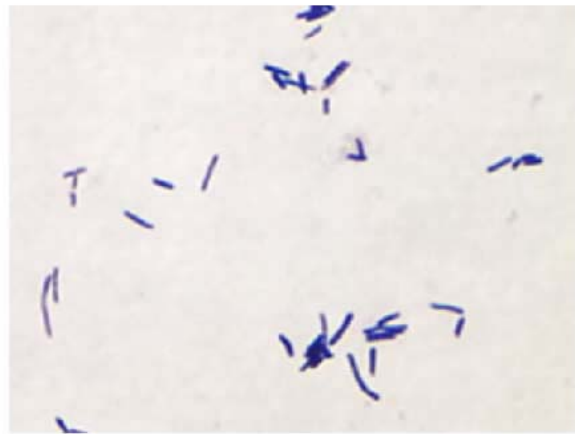
*Bacillus anthracis* gram stain from primary specimen (CDC photo).



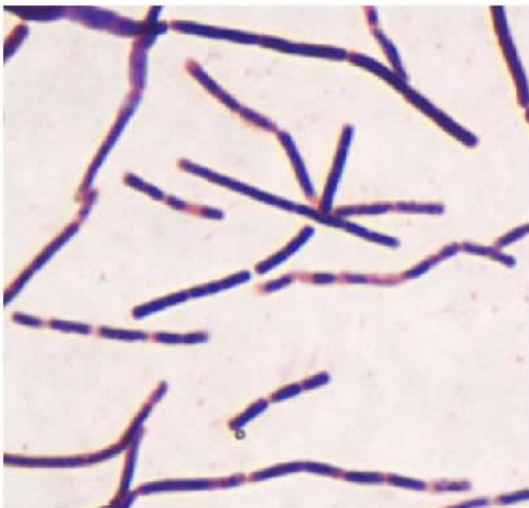
*Bacillus anthracis* gram stain from broth culture.



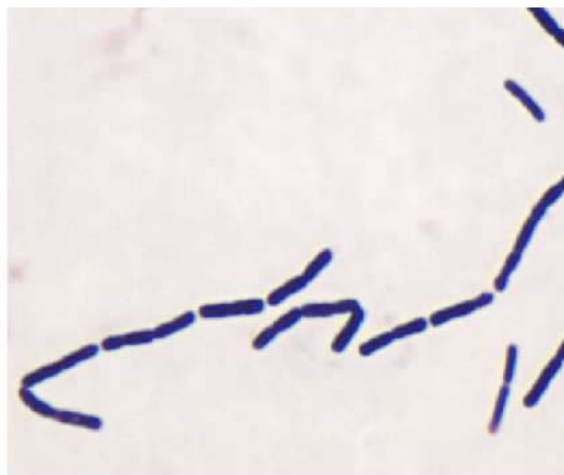
Educational sample A gram stain from solid media.



Educational sample A gram stain from broth culture.



Educational sample B gram stain from solid media.



Educational sample B gram stain from broth culture.



## Key Tests to rule out *Bacillus anthracis*

The key tests that should be performed to rule out *B. anthracis* are listed below. Perform these tests when:

- 1) Specimens are submitted specifically to rule out anthrax (e.g., non-formalized biopsies of eschar tissue; and nasal swabs submitted from patients thought to be exposed to aerosols containing anthrax spores)
- 2) Aerobic, gram-positive bacillus is isolated from a normally sterile body site
- 3) Aerobic, gram positive bacillus is the predominant organism in other types of clinical specimens

Key test results indicative of possible *Bacillus anthracis* (No single test result should be used to rule out *B. anthracis*):

- ✓ Colony morphology: 2-5 mm in diameter; flat or slightly convex colonies are irregularly round, with edges that are slightly undulate (irregular, wavy border), and have a ground-glass appearance. There may be comma-shaped projections from the colony edge, producing the “Medusa-head” colony.
- ✓ Gram stain: large gram-positive rod (1-1.5 X 3-5 um); *B. anthracis* forms oval, central-to-subterminal spores (1 X 1.5 um) on SBA (sheep blood agar) that do not cause significant swelling of the cell; frequently occur as long chains of bacilli. However, cells from growth on SBA regardless of the incubation conditions (ambient atmosphere or CO<sub>2</sub> enriched) are not encapsulated.
- ✓ Motility: Nonmotile
- ✓ Catalase: Positive

If *B. anthracis* cannot be ruled out, refer the isolate to a Level B or Level C laboratory for identification confirmation. This testing is available at the Level C Wadsworth Center Bacteriology Laboratory, Albany, NY. Level B reference laboratories will soon be available as part of the CDC Laboratory Response Network.

## Reporting

The wording of the report after performing key tests to rule out *B. anthracis* is critical. The LRN Level A laboratory key tests cannot presumptively identify *B. anthracis*. Neither can many additional conventional tests performed in Level A laboratories (sugars, egg yolk, citrate, urea, etc.). LRN Level B tests need to be performed to confirm the identity of *B. anthracis* (e.g. gamma phage, DFA). Approximately, 28% of labs that submitted SOPs noted that their report would state “presumptive *B. anthracis*” and an additional 8% would report “possible *B. anthracis*.” **A report stating “presumptive” or “possible *B. anthracis*” is not appropriate and can be very misleading to the medical community and first responders.**

To ensure standardized reporting for all New York State’s Level A laboratories, the following reports and notification actions are strongly recommended and should be incorporated in the laboratory’s SOP:

1. If Level A lab test results can not rule out *B. anthracis*, the report should read:  
**“Non-hemolytic, non-motile *Bacillus* species was isolated; referred to a reference laboratory for identification.”**

**Immediately notify physician/infection control according to internal policies if *B. anthracis* cannot be ruled out.**

2. If *B. anthracis* is ruled out, the report should read:

**“*Bacillus* species, not *anthracis*, was isolated/identified.”**

The laboratory can proceed with identification using established procedures.

The notification protocol and the name and address of the reference lab should also be included in the SOP.

Below are the test results submitted by participants for each of the educational samples:

**Specimen A**

**Gram Stain Results**

Gram positive bacillus	228
No answer provided	17
Gram variable bacillus	4
Gram positive/variable bacillus	3
Gram negative bacillus	1
Spores present in gram stain	23
(subterminal – 4; oval, subterminal – 1; subterminal and central – 1; round, central – 1)	
Chains visible on gram stain	3

**Other descriptions of cell morphology**

large	28
small	11
short	10
medium	6
thin	3
long	2
Other (1 lab each)	25

**Colony morphology**

Gray	106
Flat	100
Irregular	51
Large	49
Some form of white	45
Dry	31
Smooth	29
Round	18
Medium	15
Spreading	15
Moist/mucoid	15
Shiny	14
ground glass	7
Dull	5

**Hemolysis**

None	209
Beta	21
No answer	17
Alpha	4
slight alpha	1
slight Beta	1

**Motility**

Motile	198
Non-motile	20
No answer	18
Weakly motile	13
Motile on wet mount; negative/weak in media	2
Not performed	1
Few motile	1

**Motility by method**

Media – total	73
Motile	56
Non-motile	13
weakly motile	4
Wet mount - total	114
Motile	104
Weakly motile	6
Non-motile	4
Both - total	45
Motile	38
Non-motile	3
Weakly motile	2
Motile in wet mount; Neg/weak in media	2

**Catalase**

Positive	229
No answer	22
Negative	1
Not performed	1

**Other tests performed**

Penicillin sensitivity	61
Various sugars	22
Egg yolk/lecithinase	22
India Ink/capsule	16
Anaerobic growth	14
PEA	7
A large variety of others.	

**Result**

<i>Bacillus</i> species, not <i>anthracis</i>	157
<i>Bacillus</i> species	25
No result given	17
<i>Bacillus pumilus</i>	13
<i>Bacillus</i> species, refer for ID or to rule out <i>anthracis</i>	6
<i>Bacillus</i> species, not <i>anthracis</i> or <i>cereus</i>	5
<i>Bacillus</i> species, possible <i>anthracis</i>	3
<i>Bacillus subtilis</i>	3
No <i>Bacillus anthracis</i>	3
<i>Bacillus</i> species, not <i>anthracis</i> , <i>cereus</i> or <i>subtilis</i>	2
Gram positive bacillus	2
Not tested	2
Unable to rule out <i>Bacillus anthracis</i>	2
Anaerobic gram positive bacillus, not anthrax	1
<i>Bacillus cereus</i>	1
<i>Bacillus</i> (possible) <i>firmus</i>	1
<i>Bacillus</i> species, cannot rule out <i>anthracis</i>	1
<i>Bacillus</i> species, non-motile	1
<i>Bacillus</i> species, not <i>cereus</i>	1
<i>Bacillus</i> species, subgroup 1, not <i>anthracis</i>	1

<i>Bacillus stearothermophilus</i>	1
Diphtheroids	1
Gram positive, non-spore-forming bacillus	1
<i>Listeria monocytogenes</i>	1

### **Specimen B**

#### **Gram stain results**

Gram positive bacillus	229
No answer	20
Gram variable bacillus	2
Gram positive/variable bacillus	1
Gram negative bacillus	1
Spores present in gram stain (terminal - 3; subterminal - 4; terminal & subterminal - 1; oval, swollen - 1; cylindrical, oval - 1; cylindrical - 1; oval - 1; oval, terminal - 1)	44
Chains visible on gram stain	43

#### **Other descriptions of cell morphology**

large	60
long	40
fat	5
boxcar	2
filamentous	2
Other ( 1 lab each)	12

#### **Colony morphology**

Gray	98
Large	69
Round	42
Flat	35
Ground glass	33
Irregular	25
Dry	23
Some form of white	23
Smooth	21
Some form of yellow	18
Moist/mucoid	16
Fried-egg	15
Spreading	11

#### **Hemolysis**

None	214
No answer	18
Alpha	13
Beta	6
slight alpha	2

#### **Motility**

Non-motile	194
No answer	21
Weakly motile	19
Motile	18
Not performed	1

Presumptive for <i>B. anthracis</i>	1
Sent to reference lab	1

#### **Motility by method**

Wet Mount- total	110
Non-motile	93
Weakly motile	11
Motile	5
Media - total	72
Non-motile	60
Motile	8
Weakly motile	4
Both - total	51
Non-motile	41
Motile	5
Weakly motile	4
No answer	1

#### **Catalase**

Positive	226
No answer	26
Negative	1

#### **Other tests performed**

Penicillin sensitivity	68
Egg yolk/lecithinase	27
some type of sugar	26
India Ink/capsule	19
Anaerobic growth	17
PEA	5
A large variety of others.	

#### **Result**

<i>Bacillus</i> species, Send to ref lab to ID/ rule out <i>B. anthracis</i>	56
<i>Bacillus</i> species, not <i>anthracis</i>	46
<i>Bacillus</i> species, cannot rule out <i>anthracis</i>	45
<i>Bacillus</i> species	21
No response	21
<i>Bacillus</i> species, possible/presumptive <i>anthracis</i>	20
<i>Bacillus</i> species, not <i>anthracis</i> or <i>cereus</i>	6
<i>Bacillus sphaericus</i>	5
Unable to rule out <i>B. anthracis</i>	5
<i>Bacillus megaterium</i>	3
<i>Bacillus mycoides</i>	3
Send to reference lab	3
Gram positive bacillus	2
Aerobic gram positive bacillus, not anthrax	1

Aerobic, gram-positive, non-motile		<i>Bacillus thermodenitrificans</i>	1
<i>Bacillus</i> species	1	<i>Brevibacillus</i> species	1
<i>Bacillus alvei</i>	1	Colonies resembling <i>Bacillus</i> species	1
<i>Bacillus cereus</i>	1	<i>Corynebacterium jeikeium</i>	1
<i>Bacillus circulans</i>	1	Do not test for <i>Bacillus</i>	1
<i>Bacillus</i> (possible) <i>sphaericus</i>	1	Non-hemolytic, non-motile	
<i>Bacillus</i> species, non-motile	1	<i>Bacillus</i> species	1
<i>Bacillus</i> species, not <i>anthracis</i> ,		Non-motile <i>Bacillus</i> , suspicious of	
<i>subtilis</i> or <i>cereus</i>	1	<i>B. anthracis</i>	1
<i>Bacillus</i> species, subgroup 2		? <i>Bacillus anthracis</i>	1
(not <i>anthracis</i> )	1		



## Laboratory Response Network

The New York State Laboratory Response Network (LRN) is part of a nationwide laboratory network developed by the CDC and APHL to provide a coordinated, timely and effective response to bioterrorism threats and public health emergencies involving infectious agents. There are four LRN laboratory levels involved in this network: 1) Level A – clinical microbiology labs; 2) Level B – public health labs; 3) Level C – public health labs that perform molecular testing (NYS Bacteriology Laboratory); 4) Level D – CDC lab with BSL 4 containment.

The Level A laboratory acts as the first detector of infectious organisms and select agents (biothreat organisms). These laboratories analyze clinical specimens only and have the microbiological capability to rule out select agents. All clinical microbiology laboratories that maintain a NYS General Bacteriology Laboratory permit and test specimens for select agents are designated as LRN Level A Laboratories. The NYS LRN program strongly recommends that these Level A labs incorporate the standardized LRN procedure for ruling out *Bacillus anthracis* in their SOP's. The testing, reporting, and referral procedures are included below.

### Environmental Samples

Environmental samples (swabs, swipes, powders, etc.) should not be processed in Level A Laboratories without NYS Environmental Laboratory Approval Program (ELAP) certification. One protocol submitted by a participating laboratory included instructions for the handling of powder samples. Powders should ONLY be processed in an ELAP approved Biosafety Level 3 (BSL 3) laboratory.

### SOP Review

In addition to reporting the results of the educational samples, laboratories were asked to submit their laboratory standard operating procedure (SOP) for handling aerobic gram positive spore-forming bacilli. The protocols were assessed for the clinical laboratory's ability to handle potential anthrax specimens. Most labs performed all the tests on the Educational Specimen Survey form, but the review of the submitted SOPs showed that many of the protocols were incomplete (see table below). Note: This review assumed that quality control was performed as per CLEP regulations, and did not require QC to be included as part of the SOP.

The Laboratory Response Network (LRN) protocols for Level A laboratories (NYS CLEP General Bacteriology Permit labs) include the key tests (gram stain, colony morphology, motility, catalase) to rule out *B. anthracis*. Of the participants that submitted SOPs, 84%-88% included the key tests in their procedures (except catalase – only 42%). Therefore, procedures for these key tests should be included in each laboratory's SOP for handling aerobic, gram-positive, spore-forming bacilli. The LRN Level A Laboratory procedures for rule out *B. anthracis* are included below.

## NYS Laboratory SOP Evaluation

SOP Submission		
	Number	Percentage
Respondents	252	
Samples not tested	9	3.6
No SOP Submitted	71	28.2
SOP Submitted	172	68.2

Number of Laboratories including procedure in SOP		
Procedure	Number	Percentage
Colony morphology	132	77
Gram Stain	148	86
Hemolysis	144	84
Catalase	73	42
Motility	152	88
Reporting	91	53
Referral	127	74
Notification	84	49

Included on the following pages is a protocol that may be used by level A laboratories to rule out *B. anthracis* in clinical samples.

**Laboratory Response Network (LRN) Level A Laboratory Procedure  
(As adapted by NYS Bacteriology Laboratory – January 2003)**

**Rule Out *Bacillus anthracis***

**I. Principle**

The Level A Laboratory procedures (updated 2002) described below function to rule out *B. anthracis* from clinical specimens or isolates. These procedures should be performed using Biological Safety Level 2 (BSL 2) practices. The growth of *B. anthracis* from primary specimens may be detectable within 24 hours following incubation. Level A procedures may be completed in an additional 24 hours.

**II. Clinical Significance**

*B. anthracis* is extremely virulent for humans and is the causative agent of anthrax. The production of the antiphagocytic capsule and potent exotoxins (i.e., edema toxin and lethal toxin) mediate cell and tissue destruction.

There are three forms of anthrax:

- cutaneous anthrax occurs at the site of spore penetration 2 to 5 days after exposure and is manifested by progressive stages from an erythematous papule to ulceration and finally to formation of a black scar (i.e., eschar).
- pulmonary anthrax follows inhalation of spores and progresses from malaise with mild fever and nonproductive cough to respiratory distress, massive chest edema, cyanosis, and death.
- gastrointestinal anthrax follows ingestion of spores and affects either the oropharyngeal or the abdominal area. It is characterized by abdominal distress followed by fever, septicemia and (rarely) death.

**III. Recommended General Safety Precautions:**

1. Handling of specimens

For safety considerations, analysis of specimens for biological threat agents should be performed within a certified Class II biological safety cabinet (BSC). Procedures requiring removal of items from a BSC, such as slides for microscopy, should follow published microbiological practices and precautions. When using a BSC, be sure that the cabinet does not contain unnecessary items that will interfere with proper airflow and function. Do not use an open flame. As for any procedure involving infectious materials, standard personal protective gear should be used. This includes, but is not necessarily limited to, latex gloves and lab coats or disposable over-garments. Once a biological agent has been identified, modifications in handling of samples can then be considered depending on the identification.

2. Disinfection

Commercially available household bleach solutions contain 5.25% hypochlorite and when diluted 1:10, are effective in routine decontamination of surfaces and instruments after working with *B. anthracis*. Contaminated items such as pipettes, needles, loops, and microscope slides should be immersed in decontamination solution until autoclaving. Work surfaces, such as a biological safety cabinet (BSC), should be wiped down before and after use with decontamination solution. Spills that involve fresh cultures, samples known to have spores, organic matter, or which occur in areas maintained at a temperature below ambient (refrigerators,

freezers) should be exposed to decontamination solution for at least one hour before cleanup. Personnel involved in the cleanup of any spill should wear gloves, safety glasses, and a laboratory coat or gown during the cleanup process. Respiratory protection should be considered for spills in which aerosolization is suspected.

#### **IV. Collection and Transport of Clinical Specimens**

##### Acceptable specimens

1. Cutaneous anthrax
  - a. Vesicular stage: Aseptically collect vesicular fluid on sterile swabs from previously unopened vesicles. Note: The anthrax bacilli are most likely to be seen by Gram stain in the vesicular stage.
  - b. Eschar stage: Collect eschar material by carefully lifting the eschar's outer edge; insert a sterile swab, then slowly rotate for 2-3 sec beneath the edge of the eschar without removing it.
2. Inhalational anthrax
  - a. Blood cultures: Collect appropriate blood volume and number of sets per laboratory protocol.
  - b. Sputum: Collect >1 ml of a lower respiratory specimen into a sterile container. Inhalational anthrax usually does not result in sputum formation.
3. Gastrointestinal anthrax
  - a. Blood cultures: Collect appropriate blood volume and number of sets per laboratory protocol. In later stages of disease (2-8 days post-exposure) blood cultures may yield the organism, especially if obtained before antibiotic treatment.
  - b. Stool: Transfer approximately 5g of stool directly into a clean, dry, sterile, wide-mouth, leak-proof container.
  - c. Rectal swab: For patients unable to pass a specimen, obtain a rectal swab by carefully inserting a swab 1 inch beyond the anal sphincter.

Rejection criteria: Use standard laboratory criteria.

##### Specimen transport and storage

1. Swabs: Transport directly to laboratory at room temperature. For transport time >1h, transport at 2-8°C.
2. Stool: Transport unpreserved stool to laboratory within 1 h. For transport time >1, transport at 2-8°C; Cary-Blair or equivalent transport media is acceptable.
3. Sputum: Transport in sterile, screw-capped container at room temperature when transport time is <1h. For transport time >1h, transport at 2-8°C.
4. Blood culture: Transport directly to laboratory at room temperature.

#### **V. Materials**

##### **Reagents**

1. Gram stain reagents
2. Catalase reagent (3% hydrogen peroxide)
3. Motility media (or slide, coverslips, saline for wet mount)
4. India ink (an optional test)
5. Sterile saline

### **Media**

1. 5% sheep blood agar (SBA) or equivalent
2. Chocolate agar (CA)
3. MacConkey agar (MAC)
4. Phenyl ethyl alcohol agar (PEA)
5. Blood culture bottles
6. Tubed motility media
7. Tryptic soy broth (TSB), or equivalent
8. Thioglycollate broth or equivalent

### **Equipment**

1. Blood culture instrument (optional)
2. Light microscope with 10X, 40X and 100X objectives and 10X eyepiece
3. Microscope slides and coverslips
4. Disposable bacteriological inoculating loops, needles
5. Incubator, 35-37°C, ambient preferred (CO<sub>2</sub> enriched is acceptable)

**VI. Quality Control:** Document all quality control results for the following tests per standard laboratory procedure

### **VII. Level A Laboratory Procedure**

#### **Gram Stain**

1. Procedure: Perform Gram stain procedure/QC per standard laboratory protocol.
2. Interpretation
  - a. *B. anthracis* is a large gram-positive rod (1-1.5 X 3-5 um).
  - b. Blood and impression smears: Vegetative cells seen on Gram stain are in short chains of 2-4 cells that are encapsulated, which may be seen on the Gram stain as clear zones around the bacilli. Spores are not present in clinical samples unless exposed to low CO<sub>2</sub> levels, such as those found in the atmosphere; higher CO<sub>2</sub> levels within the body inhibit sporulation. The presence of large encapsulated gram-positive rods in the blood is strongly presumptive for *B. anthracis* identification.
  - c. Growth on Sheep Blood Agar (SBA) or equivalent medium: *B. anthracis* forms oval, central to subterminal spores (1 X 1.5 um) on SBA that do not cause significant swelling of the cell and frequently occur as long chains of bacilli. Cells from growth on SBA, regardless of the incubation conditions (ambient atmosphere or CO<sub>2</sub> enriched) are not encapsulated.

#### **India Ink** (Primary clinical specimens only)

1. Purpose: India ink is used to improve visualization of encapsulated *B. anthracis* in clinical specimens (blood, blood culture bottles, or cerebrospinal fluid).
2. Perform India ink procedure/QC per standard laboratory protocol.
3. Clinical specimen with encapsulated (visualized with India ink), gram-positive rods is strongly a rule in for *B. anthracis* identification. Please refer specimen to a Level B or C laboratory for confirmation.
4. Every effort should be made to obtain an isolate for continued testing and referral to reference laboratory.

5. Limitations
  - a. Interpretation of results requires trained/experienced staff.
  - b. **A negative test result should not be used to rule out *B. anthracis*.**

### ***Culture set up and incubation***

1. Inoculation and plating of primary clinical specimens: inoculate and streak the following media for isolation of the respective specimen types. Note: standard media should be used according to normal laboratory procedures.
  - a. Blood cultures: Process following routine laboratory protocol.
  - b. Cutaneous swab specimens: Plate directly on media used routinely for surface wounds, such as SBA, MAC, PEA, and broth enrichment, and prepare smears for staining. Note: *B. anthracis* does not grow on MAC.
  - c. Stool specimen: Plate directly on appropriate media, such as PEA, SBA, and MAC.
  - d. Sputum specimens: Plate directly on media used routinely, such as SBA, MAC, PEA, and CA, and prepare smears for staining.
2. Incubation
  - a. Temperature: 35-37°C
  - b. Atmosphere: Ambient preferred
  - c. Length of incubation: Hold primary plates for at least 3 days; read daily. Examine plates within 18-24 h of incubation. Growth of *B. anthracis* may be observed as early as 8 h after incubation.

### ***Colonial morphology of B. anthracis***

1. After incubation of SBA plates for 18-24 h at 35-37°C, well isolated colonies of *B. anthracis* are 2-5 mm in diameter. The flat or slightly convex, white/gray colonies are irregularly round, with edges that are slightly undulate (irregular, wavy border, and have a ground-glass appearance. There may be comma-shaped projections from the colony edge, producing the “Medusa-head” colony.
2. *B. anthracis* colonies are not beta-hemolytic. However weak hemolysis may be observed under areas of confluent growth in aging cultures and should not be confused with beta-hemolysis.
3. *B. anthracis* colonies on SBA usually have a tenacious consistency. When teased with a loop, the growth will stand up like beaten egg white.
4. *B. anthracis* grows rapidly; heavily inoculated areas may show growth within 6-8 h and individual colonies may be detected within 12-15 h. This trait can be used to isolate *B. anthracis* from mixed cultures containing slower-growing organisms.

### ***Motility test: Wet mount or motility medium***

Purpose: Used to determine motility of suspected isolates; *B. anthracis* is nonmotile.

#### **Wet mount procedure**

1. Deliver 2 drops (approximately 0.1 ml) of Tryptic Soy Broth (TSB), or equivalent, into a sterile tube. Using an inoculating loop, transfer a portion of the suspect colony from a 12-20 h culture and suspend the growth in the broth medium.
2. Alternatively, a loopful of medium from a fresh broth culture can be used.
3. Transfer 10 ul of the suspension to a microscope slide and overlay with a coverslip.

4. Examine slide under a microscope using the 40X objective (total magnification 400X; may also be viewed at 1000X with oil objective).
5. Discard slide(s) following standard laboratory procedures, such as into 0.5% hypochlorite solution.

### **Motility medium test procedure**

1. Using a sterile inoculating needle, remove a portion of growth from an isolated, suspect colony after 18-24 h incubation.
2. Inoculate the motility medium by carefully stabbing the needle 3-4 cm into the medium and then drawing the needle directly back out so that a single line of inoculum can be observed.
3. Incubate the tube at 35-37°C in ambient atmosphere for 18-24 h.

### **Interpretation of motility results**

Lack of motility is unusual among *Bacillus* species and is therefore useful in the LRN rule out procedures.

#### **Wet mount**

1. Positive result: Motile organisms will be observed moving throughout the suspension. Observe that the movement may be sluggish/slower than that of the positive controls.
2. Negative result: Nonmotile organisms either do not move or move with Brownian motion.

#### **Motility medium test**

1. Positive result: Motile organisms will form a diffuse growth zone around the inoculum stab.
2. Negative result: Nonmotile organisms, such as *B. anthracis*, will form a single line of growth that does not deviate from the original inoculum stab.

### **Quality control**

1. Positive control strain: *Pseudomonas aeruginosa* ATCC 35032 or laboratory validated equivalent will demonstrate motility.
2. Negative control strain: *Acinetobacter* species 49139 or laboratory validated equivalent will show no motility.
3. Control strains should be assayed on each day of testing. Perform the test with fresh cultures of the control strains using the same method as the unknowns.

#### **Catalase**

Purpose: Differentiate *Bacillus* species (most are catalase positive) from aerotolerant *Clostridium* species, *Lactobacillus* species and a few non-sporeforming, gram-positive bacilli that are catalase negative. *B. anthracis* is catalase positive.

1. Perform catalase procedure/QC according to standard laboratory protocol.

### **VIII. Interpretation**

- **Rule out *B. anthracis*:** Isolates can be ruled out if they are motile, hemolytic, and lack characteristic Gram stain morphology. Isolates should not be ruled out based on any single test.
- Potential *B. anthracis*

1. Direct smears from clinical specimens, such as blood, CSF, or skin lesion (eschar) material: Encapsulated gram-positive rods
2. Growth on SBA or equivalent media: Large gram-positive rods (may stain gram-variable after 72 h of culture). Spores may be found in culture, under non-CO<sub>2</sub> atmosphere (but not on direct examination). Spores are nonswelling and oval-shaped.
3. Rapid, aerobic growth, and tenacious colonies on sheep blood agar.
4. Catalase positive
5. Nonmotile: In addition to *B. anthracis*, *B. cereus* var. *mycoides* is nonmotile.
6. Nonhemolytic on SBA, ground glass appearance of colonies.

### IX. Reporting and Specimen Retention

To ensure standardized reporting for all New York State's Level A laboratories, the following reports and notification actions are strongly recommended and should be incorporated in the laboratory's SOP:

1. If Level A lab test results can not rule out *B. anthracis*, the report should read:  
**“Non-hemolytic, non-motile *Bacillus* species was isolated; referred to a reference laboratory for identification.”**

Immediately notify physician/infection control according to internal policies if *B. anthracis* cannot be ruled out. Call the NYS Bacteriology Laboratory at 518-474-4177 before submitting isolates/specimens. Retain the original sample and isolates in a secured area until the Level B/C lab testing is complete. If results are negative, dispose of accordingly. If the results are positive, contact the Wadsworth Center (518-474-4177) to determine the appropriate disposition of the original sample and/or isolates.

2. If *B. anthracis* is ruled out, the report should read:  
**“*Bacillus* species, not *anthracis*, was isolated/identified.”**  
 The laboratory can proceed with identification using established procedures.

### X. References

- **Brachman, P.S., and A.M. Friedlander.** Anthrax, p. 729-739. In S.A. Plotkin and E.A. Mortimer, Jr. (ed), Vaccines. W.B. Saunders, Philadelphia, PA.
- **Cieslak, T.J. and E.M. Eitzen, Jr.** 1999. Clinical and epidemiologic principles of Emerg. Infect. Dis. 5:552-555.
- **Dutz, W. and E. Kohout.** 1971. Anthrax. Pathol. Annu. 6:209-248.
- **Gilchrist, M.J.R., W.P. McKinney, J.M. Miller, and A.S. Weissfeld.** 2000. Cumitech 33, Laboratory safety, management, and diagnosis of biological agents associated with bioterrorism. Coordinating ed., J.W. Snyder, ASM Press, Washington D.C.
- **Lew, D.P.** 2000. *Bacillus anthracis* (Anthrax). P. 2215-2220. In G.L. Mandell, J.E. Bennett, and R. Dolin (ed), Principles and Practice of Infectious Disease, 5<sup>th</sup> ed. Churchill Livingstone, Philadelphia, PA.
- **Logan, N.A. and P.C. Turnbull.** 1999. *Bacillus* and recently derived genera, p. 357-369. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed), Manual of Clinical Microbiology, 7<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.

## **XI. Additional website resources**

- American Society for Microbiology (ASM) - [www.asm.org/pasrc/bioprep.htm](http://www.asm.org/pasrc/bioprep.htm)
- Centers for Disease Control and Prevention (CDC) - [www.bt.cdc.gov](http://www.bt.cdc.gov)
- Association for Professionals in Infection Control and Epidemiology, Inc. (APIC) - [www.apic.org/bioterror](http://www.apic.org/bioterror)
- National Laboratory Training Network (NLTN) - [www.nltn.org](http://www.nltn.org)

## **XII. Contacts**

### **New York State Department Of Health**

#### **Wadsworth Center**

- Biodefense Laboratory – 518-474-4177
- Bacteriology Laboratory – 518-474-4177
- Education - 518-474-6196

#### **Epidemiology**

- Epidemiology – 518-473-4436
- Duty officer (nights, weekends, holidays) – 518-465-9720
- Health Provider Network (HPN) information – 518-473-4432



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

This simulated cervical swab specimen was positive for *Chlamydia trachomatis*. This specimen was appropriate for laboratories that test for *Chlamydia* using direct testing methods but was not suitable for culture. Approximately 99% of the laboratories that processed this specimen reported it as positive for *Chlamydia*.

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

Gen-Probe PACE 2	59
Abbott LCx <i>C. trachomatis</i> assay	13
Becton-Dickinson Probe Tec	9
bioMerieux Vitek VIDAS	6
Roche Diagnostics COBAS	6
Beckman Coulter Access <i>Chlamydia</i> EIA	4
Roche Diagnostics AMPLICOR CT/NG	4
Trinity Biotech MicoTrak II <i>Chlamydia</i> EIA	2
GenProbe Aptima	1
Wampole MicroTrak II <i>Chlamydia</i> EIA	1
<b>TOTAL</b>	<b>105</b>

### **Other reports:**

#### **Positive screen for *C. trachomatis* and/ or *N. gonorrhoeae***

Gen-Probe PACE 2 CT/GC	1
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#### **Negative for *Chlamydia trachomatis***

Gen Probe PACE 2	1
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## ***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 simulated CSF to be tested for bacterial antigens. 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*. Ninety-eight percent of participating laboratories that processed this specimen reported it as positive.

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

Becton-Dickinson Directigen 1-2-3 Grp A Strep	18
Abbott Signify Strep A	13
Thermo BioStar Strep A OIA Max	7
Thermo BioStar Aceava Strep A	7
Fisher Sure-Vue Strep A	4
Pacific Biotech Cards Q.S. Strep A	4
DPC PathoDx Strep A	3
Remel RIM A.R.C. Strep A	3
Beckman-Coulter Icon Fx Strep A	2
Becton-Dickinson Link 2 Strep A	2
GenProbe Group A Strep	2
Lifesign Status AccuStrep A	2
Quidel Quick Vue + Strep A	2
Quidel Quick Vue Inline Strep A	2
Applied Biotech Signify Strep A	1
Applied Biotech SureStep Strep A	1
Meridian Immunocard Stat Strep A	1
Murex Reveal Colour Strep A	1
Polymedco PolyStat Strep A	1
Wampole Clearview Strep A	1
No test kit indicated	1
<b>TOTAL</b>	<b>78</b>

#### **Other Responses :**

##### **Negative for Group A *Streptococcus***

Pacific Biotech Cards Q.S. Strep A	1
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**Specimen B - Source: CSF**

This specimen contained *Haemophilus influenzae* b. Of the participating laboratories that tested this specimen, all reported it as positive for *H. influenzae* b.

**Test kits used by laboratories reporting Specimen B as positive for *Haemophilus influenzae* b:**

Murex Wellcogen Bacterial Antigen kit	25
B-D Directigen Meningitis Combo test	23
No test kit indicated	3
16S rDNA identification	1
<b>TOTAL</b>	<b>52</b>

**Note:**

The inclusion of specimens for direct antigen testing does not reflect any endorsement by the New York State Department of Health of use of these tests in the clinical laboratory.

**BACTERIAL IDENTIFICATION BY PARTICIPATING LABORATORIES**

	<u>Number Reported</u>	<u>%</u>
<b>SPECIMEN NUMBER 1</b>		
<i>Shigella flexneri</i>	190	74.2
<i>Shigella</i> species	24	9.4
No Enteric Pathogens / <i>Shigella flexneri</i> not isolated	24	9.4
Do not process stool specimens	15	5.9
<i>Shigella boydii</i>	2	0.8
<i>Shigella dysenteriae</i>	1	0.4
*****		

<b>SPECIMEN NUMBER 2</b>		
<i>Burkholderia cepacia</i>	240	93.8
<i>Pseudomonas cepacia</i>	10	3.9
<i>Burkholderia cepacia</i> complex	2	0.8
Not reported	2	0.8
Do not process urine specimens	1	0.4
<i>Pseudomonas luteola</i>	1	0.4
*****		

<b>SPECIMEN NUMBER 3</b>		
<i>Clostridium perfringens</i>	229	86.4
Do not isolate anaerobes	12	4.7
<i>Clostridium</i> species	5	1.9
Anaerobic gram positive bacillus	4	1.6
No anaerobes isolated	3	1.2
Do not process wound specimens	2	0.8
No <i>Bifidobacterium</i> isolated	1	0.4
Group A <i>Streptococcus</i>	253	98.8
Do not process wound specimens	2	0.8
Not reported	1	0.4
*****		

<b>SPECIMEN NUMBER 4</b>		
<i>Klebsiella pneumoniae</i>	247	96.5
Do not process sputum specimens	8	3.1
<i>Klebsiella terrigena</i>	1	0.4

**SPECIMEN NUMBER 5**

<i>Haemophilus parainfluenzae</i>	228	89.1
Do not process blood cultures	11	4.3
<i>Haemophilus</i> species	4	1.6
<i>Haemophilus influenzae</i>	3	1.2
<i>Haemophilus</i> species, not <i>influenzae</i>	3	1.2
<i>Haemophilus parahaemolyticus</i>	2	0.8
<i>Haemophilus aegyptius</i>	1	0.4
<i>Haemophilus influenzae</i> II	1	0.4
<i>Haemophilus influenzae</i> IV	1	0.4
<i>Haemophilus influenzae</i> , not type b	1	0.4
No growth	1	0.4

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**CHLAMYDIA SPECIMEN**

Positive for <i>Chlamydia trachomatis</i>	105	98.1
Positive screen for <i>C. trachomatis</i> and/ or <i>N. gonorrhoeae</i>	1	0.9
Negative for <i>Chlamydia trachomatis</i>	1	0.9

\*\*\*\*\*

**DIRECT ANTIGEN SPECIMEN**

A. Positive for Group A <i>Streptococcus</i>	78	98.7
Negative for Group A <i>Streptococcus</i>	1	1.3
B. <i>Haemophilus influenzae</i> b	52	100.0