

BACTERIOLOGY PROFICIENCY TESTING PROGRAM

General Category

September 19, 2006

This report summarizes the results of the proficiency test administered September 19, 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

Update on Shiga toxin-producing *Escherichia coli*

The September 29, 2006 issue of the MMWR outlined the public health importance of identifying all serogroups of Shiga toxin-producing *E. coli* (STEC) from patient specimens. Included in this article are recommendations for clinical laboratories to submit positive Shiga toxin stool specimens to the state public health laboratory for isolation and identification of any O157 or non-O157 STEC from a patient specimen. Please refer to the CDC website, www.cdc.gov, for a copy of this article. Below, please find information regarding submission of specimens to the Wadsworth Center Bacteriology Laboratory.

Laboratories performing sorbitol MacConkey (SMAC) agar culture

If the patient specimen is NEGATIVE for O157 STEC and the treating physician has indicated that hemolytic uremic syndrome (HUS) or Shiga toxin mediated disease is suspected, you may submit the original stool specimen to the appropriate public health reference laboratory*.

Laboratories performing EIA or any Shiga toxin detecting assay

If the patient specimen is POSITIVE for Shiga toxin production, you may submit the original stool specimen along with the positive broth (please include the OD reading) to the appropriate public health reference laboratory*.

- OR -

Once you have identified a stool specimen that is POSITIVE for Shiga toxin, subculture the positive broth to a SMAC plate for isolation of O157 STEC. If this subculture is NEGATIVE for O157 STEC, you may submit the original stool specimen the appropriate public health reference laboratory*.

*For New York City Laboratories send specimens to the New York City Department of Mental Health and Hygiene. All other New York State Laboratories should send specimens to the Wadsworth Center Laboratory of the New York State Department of Health.

CLSI guidelines

The following standards have been updated for 2007 and will be available in January from the Clinical and Laboratory Standards Institute:

- **M100-S17** Performance Standards for Antimicrobial Susceptibility Testing: Sixteenth Informational Supplement. January 2007.
- **M11-A7** Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard – Seventh Edition. January 2007.

Clinical Laboratory and Standards Institute
940 West Valley Road, Suite 1400
Wayne, PA 19087-1898
(610) 688-0100
www.clsi.org

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. 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 tests because of inaccurate or outdated information on their Bacteriology Questionnaire. Grades will not be revised due to incorrect information on the questionnaire.**

Bacteriology Workshop

A workshop entitled "Just when you thought you had it covered" will be held April 24-26, 2007 at the Wadsworth Center Laboratories in Albany, NY. This workshop will include updates on diagnostic approaches for *Escherichia coli*, *Burkholderia cepacia* complex and *Staphylococcus aureus*. Check our website (<http://www.wadsworth.org/divisions/infdis/bacti/index.htm>) for information as it becomes available.

SEPTEMBER 19, 2006 TEST EVENT

Number of Participating Laboratories:

Receiving specimens 228
Returning results 227 (99.6%)

Grade Distribution		
Score	Number	Percent
100%	165	72.7
90 – 99%	18	7.9
80 – 89%	25	11.0
70 – 79%	11	4.8
60 – 69%	3	1.3
< 60%	5	2.2

REFEREE LABORATORY RESULTS

Specimen Number	Referee Laboratory Responses	Percent*
1	<i>Salmonella</i> group D	70
	<i>Salmonella</i> group D (not <i>typhi</i>)	20
	<i>Salmonella</i> serotype Enteritidis	10
2	<i>Haemophilus parainfluenzae</i>	90
	<i>Haemophilus influenzae</i>	10
3	<i>Clostridium sordellii</i>	100
	<i>Enterococcus faecium</i>	80
	<i>Enterococcus gallinarum</i>	10
	<i>Enterococcus</i> species	10
4	<i>Burkholderia cepacia</i> (complex)	100
5	<i>Morganella morganii</i>	100

* Based on responses of 10 referee laboratories

Specimen Number 1 - Stool (Pathogens Only)

This simulated stool sample contained *Salmonella*, serogroup D. This organism was identified by all referee laboratories. All participating laboratories that culture stool samples recovered this organism with 65.7% identifying the isolate as *Salmonella*, group D. Among those laboratories that do not perform serogrouping, 27% reported ‘*Salmonella* species’ and 4% reported ‘*Salmonella* species, not *typhi*’.

Escherichia coli and *Klebsiella pneumoniae* were included in this specimen as nonpathogenic flora.

Methods of identification used by laboratories reporting:

***Salmonella* serogroup D**

bioMerieux Vitek GNI +	56
Dade Behring MicroScan Gram Neg ID	42
bioMerieux API 20E	29
BD BBL Crystal Enteric/Nonfermenter	3
bioMerieux Vitek 2 GN	2
Two or more systems	2
Conventional biochemicals	1
bioMerieux API Rapid 20E	1
Unknown	1
Vitek	1
Vitek 2 GN, bioMerieux	1
Vitek GN	1
BD BBL Enterotube II	1
Not given	1
TOTAL	142

***Salmonella* species**

bioMerieux Vitek GNI +	25
Dade Behring MicroScan Gram Neg ID	16
bioMerieux API 20E	8
Two or more systems	3
Vitek	2
BD Phoenix	2
bioMerieux Vitek2 Compact GN	1
BD BBL Enterotube II	1
BD BBL Crystal Enteric/Nonfermenter	1
TOTAL	59

Specimen source (stool) not tested **11**

***Salmonella* species, not typhi**

Dade Behring MicroScan Gram Neg ID	4
bioMerieux Vitek GNI +	3
Conventional biochemicals	1
TOTAL	8

<i>Salmonella</i> group		
bioMerieux Vitek GNI +		2
<i>Salmonella</i> serotype Enteritidis		
Conventional biochemicals		2
Presumptive <i>Salmonella</i> species		
Two or more systems		1
<i>Salmonella</i> serotype Typhi		
Dade Behring MicroScan Gram Neg ID		1
<i>Salmonella sonnei</i>, Group D		
Dade Behring MicroScan Gram Neg ID		1

Specimen No. 2 – Peritoneal fluid (All Organisms)

This simulated peritoneal fluid specimen contained *Haemophilus parainfluenzae*. This organism was correctly identified by 90% of the referee laboratories and by 93% of participating laboratories that process this specimen source.

Methods of identification used by laboratories reporting:

Haemophilus parainfluenzae

Remel RapID NH	54
bioMerieux API NH	34
bioMerieux Vitek NHI	32
Conventional biochemicals	32
Dade Behring MicroScan HNID	25
Two or more systems	8
BD BBL Haemophilus ID Quad	7
Not given / unknown	3
BD BBL Crystal Neisseria/Haemophilus	2
Dade Behring MicroScan Gram Pos ID	1
Remel BactiCard Neisseria	1
Remel Haemophilus ID (HIDI) QUAD	1
Remel RapID CB Plus	1
Vitek	1
TOTAL	202

Specimen source (peritoneal fluid) not tested **10**

Haemophilus influenzae

bioMerieux Vitek NHI	2
Remel RapID NH	1
bioMerieux API NH	1
Remel (unspecified)	1
TOTAL	5

Haemophilus species, not influenzae

Conventional biochemicals	4
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Not reported **3**

Haemophilus species

Conventional biochemicals	1
Not given	1
TOTAL	2

Actinobacillus urinae

Unknown	1
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Also reported:

<i>Burkholderia cepacia</i> complex	1
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Specimen No. 3 – Abscess - Aerobic/Anaerobic (All Organisms)

This simulated abscess specimen contained *Clostridium sordellii* and *Enterococcus faecium*.

Clostridium sordellii was reported by all referee laboratories. Of the participating laboratories that isolate anaerobic organisms from abscess specimens, 89% identified *C. sordellii*. Additional reports included *Clostridium* species (3.7%) and *Clostridium* species not *perfringens* (2.8%).

Enterococcus faecium was identified by 80% of the referee laboratories. Of the participants that processed this specimen, 78.8% identified *Enterococcus faecium* with an additional 14.4% reporting *Enterococcus* species.

Methods of identification used by laboratories reporting:

Clostridium sordellii

Remel RapID ANA II	102
bioMerieux Vitek ANI	23
bioMerieux API 20A	22
Dade Behring MicroScan Rapid Anaerobe	17
Unknown / not given	7
Two or more systems	6
bioMerieux Rapid ID 32A	5
Remel MicroID	3
BD BBL Crystal Anaerobe	2
Conventional biochemicals	1
16S rDNA sequencing	1
Remel RapID NH	1
Vitek (unspecified)	1
TOTAL	191

***Clostridium* species**

Remel RapID ANA II	5
bioMerieux Vitek ANI	1
Conventional biochemicals	1
bioMerieux API 20A	1
TOTAL	8

Do not process anaerobic cultures 7

Clostridium* species, not *perfringens

bioMerieux Vitek ANI	2
Remel RapID ANA II	2
Conventional biochemicals	2
TOTAL	6

Specimen source (abscess) not tested 5

No report 2

<i>Clostridium bifermentans</i> bioMerieux API 20A	2
No <i>Bifidobacterium</i> isolated Conventional biochemicals	2
Anaerobic gram negative bacilli	1
Anaerobic gram positive spore forming bacilli	1
<i>Clostridium</i> species, not <i>perfringens</i> or <i>septicum</i> Conventional biochemicals	1
<i>Clostridium sporogenes</i> BD BBL Crystal Anaerobe	1

<i>Enterococcus faecium</i>	
Dade Behring MicroScan Gram Pos ID	67
bioMerieux Vitek GPI	58
Conventional biochemicals	13
bioMerieux API 20 Strep	12
Two or more systems	7
Remel RapID STR	6
bioMerieux Vitek 2 GP	5
Not given / unknown	3
BD Phoenix	2
Dade Behring MicroScan Rapid Gram Pos	1
Vitek	1
TOTAL	175

<i>Enterococcus species</i>	
Conventional biochemicals	19
Dade Behring MicroScan Gram Pos ID	5
bioMerieux Vitek GPI	4
bioMerieux API 20 Strep	1
EY Labs: Strep-a-check	1
PML Microbiologicals Identicult AE	1
Remel BactiCard Strep	1
TOTAL	32

<i>Enterococcus gallinarum</i>	
bioMerieux Vitek GPI	4
BD Phoenix	1
TOTAL	5

Specimen source (abscess) not tested **5**

<i>Enterococcus Group D</i>	
Conventional biochemicals	3
bioMerieux Vitek GPI	1
TOTAL	4

Gamma-hemolytic <i>Streptococcus</i>	
Conventional biochemicals	1
Not given	1
TOTAL	2

<i>Enterococcus casseliflavus</i>	
Dade Behring MicroScan Gram Pos ID	1

<i>Enterococcus durans</i>	
bioMerieux API 20 Strep	1

No report **1**

Streptococcus faecium
bioMerieux Vitek GPI **1**

Also reported:
Corynebacterium species 1
Haemophilus species 1
Nonfermenter 1

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

The pathogenic organism included in this sample was *Burkholderia cenocepacia*, genomovar III of the *Burkholderia cepacia* complex. All referee laboratories identified *Burkholderia cepacia* or *B. cepacia* complex. Of the participating laboratories that process sputum specimens, 79% identified this organism as *B. cepacia* / *B. cepacia* complex.

Members of the *Burkholderia cepacia* complex are causative agents of ventilator-associated pneumonias in otherwise healthy adults and are associated with pneumonia and pulmonary decline in patients with cystic fibrosis (CF). *Burkholderia cenocepacia* was formerly named *Burkholderia cepacia* genomovar III. There are 9 genomovars of *Burkholderia cepacia* that constitute the *Burkholderia cepacia* complex. Now, all 9 genomovars have their own species designation. Most commercial identification systems are able to distinguish *Burkholderia* species from other organisms such as *Pseudomonas* species and *Acinetobacter* species. However, these systems will categorize all species of *Burkholderia* related to the former genomovars of *cepacia* under one name, *Burkholderia cepacia* complex. This designation is due to the fact that the 9 species in this complex are difficult to distinguish when only limited conventional biochemical analysis is used for identification.

Key reactions in the identification of the *Burkholderia cepacia* complex include a positive oxidase reaction that may be weak or delayed, positive lysine and ONPG (both can be variable), lack of both a fruity odor and fluorescent pigment, resistance to polymyxin B, and acid production from OF glucose. In addition, use of a selective agar such as OFPBL (oxidative-fermentative base, polymyxin b, bacitracin and lactose), BCSA (*Burkholderia cepacia* selective agar) or PC (*Pseudomonas cepacia*) is helpful in isolation of *Burkholderia* species while inhibiting most strains of *Pseudomonas aeruginosa*. However, there is no single biochemical reaction that will rule-in or rule-out *Burkholderia cepacia* complex organisms. Diligent attention to the patient presentation and growth characteristics of the organism will help guide identification.

For patients with suspected ventilator-associated pneumonia, the identification of *Burkholderia cepacia* complex is enough information for a physician to treat the patient appropriately. However, in patients with CF, *Burkholderia cenocepacia* infection has been implicated in a rapid decline of lung function. Infection with this organism has serious consequences, one of which is subsequent ineligibility of the patient for a lung transplant. Therefore, in this population, it is very important to be aware of these consequences and have any *Burkholderia cepacia* complex isolates sent to a reference laboratory for species identification. Beginning in 2007, the Wadsworth Center Bacteriology Laboratory will have full biochemical and DNA analysis available for speciation of these organisms.

Antimicrobial susceptibility testing was indicated for this specimen using meropenem and minocycline. A large number of laboratories reported that they do not test either of these antibiotics. Of those laboratories submitting susceptibility results, 76% reported that the isolate was resistant to meropenem and 16% reported it as intermediate; therefore a result of either resistant or intermediate was considered correct for meropenem. Minocycline was reported as susceptible by 92% of participants who reported results for this antibiotic.

In their 2004 guidelines, CLSI (then NCCLS) introduced specific incubation conditions and

interpretive criteria for disk diffusion testing of *Burkholderia cepacia*. The 2006 guidelines include separate tables for both disk diffusion and broth dilution testing of *Burkholderia cepacia* isolates. The interpretive criteria in these tables apply to all species or genomovars of the *Burkholderia cepacia* complex.

Coenye T, P. Vandamme, J.R.W. Govan, and J.J. LiPuma. 2001. Taxonomy and Identification of the *Burkholderia cepacia* complex. J. Clin. Micro. 39(10):3427-3436.

Henry, D.A., E. Mahenthalingam, P. Vandamme, T. Coenye, and D.P. Speert. 2001. Phenotypic Methods for Determining Genomovar Status of the *Burkholderia cepacia* complex. J. Clin. Micro. 39(3):1073-1078.

Isenberg, Henry D. 2004. *Clinical Microbiology Procedures Handbook*, 2nd edition. ASM Press, Washington, DC.

LiPuma JJ. 2005. Update on the *Burkholderia cepacia* complex. Curr. Opin. Pulm. Med. 11:528-533.

McMenamin, J.D., T.M. Zacccone, T. Coeyne, P. VanDamme, and JJ LiPuma. 2000. Misidentification of *Burkholderia cepacia* in US Cystic Fibrosis Treatment Centers. Chest. 117: 1661-1665.

National Committee for Clinical Laboratory Standards, 2004. Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement, M100-S14. National Committee for Clinical Laboratory Standards, Wayne, PA.

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:

***Burkholderia cepacia/Burkholderia cepacia* complex**

Dade Behring MicroScan Gram Neg ID	53
bioMerieux API 20NE	36
bioMerieux API 20E	24
Two or more systems	19
Remel RapID NF Plus	14
bioMerieux Vitek GNI +	14
Conventional biochemicals	4
16S rDNA sequencing	2
BD BBL Crystal Enteric/Nonfermenter	1
Vitek	1
Unknown	1
Microscan Gram Neg ID/API 20NE	1
Dade Behring MicroScan Rapid Gram Neg	1
bioMerieux API Rapid 20E	1
BD Phoenix	1
bioMerieux API NH	1
TOTAL	174
Specimen source (sputum) not tested	8
No pathogens isolated	7

<i>Pseudomonas aeruginosa</i>	
bioMerieux Vitek GNI +	4
bioMerieux Vitek2 Compact GN	1
Unknown	1
Vitek GN	1
TOTAL	7
<i>Burkholderia species</i>	
Dade Behring MicroScan Gram Neg ID	2
Remel RapID NF Plus	1
Conventional biochemicals	1
TOTAL	4
Gram negative bacillus	3
<i>Pseudomonas cepacia</i>	
Dade Behring MicroScan Gram Neg ID	2
Conventional biochemicals	1
TOTAL	3
Non-fermenting gram negative bacillus	
bioMerieux Vitek GNI +	1
Two or more systems	1
TOTAL	2
<i>Pseudomonas fluorescens/putida group</i>	
bioMerieux Vitek GNI +	2
<i>Pseudomonas species</i>	
bioMerieux Vitek GNI +	1
Dade Behring MicroScan Gram Neg ID	1
TOTAL	2
<i>Acinetobacter baumannii</i>	
Conventional biochemicals	1
<i>Acinetobacter species</i>	
bioMerieux API 20E	1
<i>Alcaligenes xylooxidans</i>	
Vitek	1
<i>Burkholderia gladioli</i>	
BD BBL Crystal Enteric/Nonfermenter	1
<i>Burkholderia species /Ralstonia species</i>	
BD Phoenix	1

<i>Flavobacterium</i> species		
bioMerieux API 20E		1
Gram negative bacillus, not <i>P. aeruginosa</i>		
Two or more systems		1
No report		1
Non fermenter, not <i>P. aeruginosa</i>		
Not given		1
Possible <i>Burkholderia</i> species		
Dade Behring MicroScan Gram Neg ID		1
<i>Pseudomonas fluorescens</i>		
bioMerieux Vitek GNI +		1
<i>Pseudomonas putida</i>		
bioMerieux Vitek GNI +		1
<i>Pseudomonas</i> species, not <i>aeruginosa</i>		
bioMerieux Vitek GNI +		1
<i>Sphingobacterium</i> species		
Conventional biochemicals		1
Unable to ID		
Conventional biochemicals		1

Results of Antimicrobial Susceptibility Testing – *B. cepacia* with meropenem

Result	Method	MIC - µg/ml	Zone - mm
Resistant (62)	Disk diffusion (32)		0 (7)
			6 (5)
			8 (1)
			9 (4)
			10 (4)
			11 (3)
			12 (3)
			13 (2)
			14 (3)
	Microscan (12)	>8 (7)	
		16 (1)	
		8 (4)	
	A-B Biodisk E-test (8)	32 (4)	
		>32 (4)	
	BioMerieux Vitek (5)	>=16 (3)	
		>16 (1)	
		Not given (1)	
	Sensititre (3)	16 (1)	
		>16 (1)	
		8 (1)	
Trek (1)	>8 (1)		
Intermediate (13)	Microscan (9)	8 (8)	
		Not given (1)	
	BioMerieux Vitek (2)	8 (1)	
		Not given (1)	
	Trek (1)	8 (1)	
Susceptible (7)	Microscan (5)	<=4 (3)	
		4 (1)	
		Not given (1)	
	Agar dilution	<=4 (1)	
	Disk diffusion (1)		26 (1)
Meropenem not tested (137)			
Specimen source (sputum) not tested (8)			

Number of laboratories reporting each result indicated in ()

Results of Antimicrobial Susceptibility Testing – *B. cepacia* with minocycline

Result	Method	MIC - µg/ml	Zone - mm	
Susceptible (36)	Disk diffusion (29)		20 (3)	
			21 (2)	
			22 (2)	
			23 (3)	
			24 (5)	
			25 (2)	
			27 (3)	
			28 (1)	
			29 (2)	
			30 (6)	
	A-B Biodisk E-test (3)	4 (2)	3 (1)	
	Agar dilution (1)	4 (1)		
Broth Microdilution	2 (1)			
Sensititre	2 (1)			
Trek	1 (1)			
Resistant (3)	Microscan (2)	>8 (2)		
	Disk diffusion (1)		10 (1)	
Minocycline not tested (180)				
Specimen source (sputum) not tested (8)				

Number of laboratories reporting each result indicated in ()

Antibiotic Susceptibility Results - Participating & Referee Labs <i>Burkholderia cepacia</i>				
	Meropenem		Minocycline	
	Referee ^a	Participant ^b	Referee ^a	Participant ^b
Susceptible	0	7	4	32
Intermediate	0	13	0	0
Resistant	4	58	0	3
Not Tested ^c	6	131	6	174
Do not process source ^d	0	8	0	8

^aReferee Laboratories (10 labs total)

^bOther Participating Laboratories (217 labs total)

^cAntibiotic not tested / reported for this organism

^dDo not process specimen source

Specimen No. 5 – Urine (All Organisms)

This simulated urine culture sample contained *Morganella morganii*. All referee laboratories as well as all participants that process urine cultures correctly identified this organism.

Methods of identification used by participating laboratories reporting:

Morganella morganii

bioMerieux Vitek GNI +	94
Dade Behring MicroScan Gram Neg ID	75
bioMerieux API 20E	32
BD BBL Crystal Enteric/Nonfermenter	4
Vitek (unspecified)	3
Two or more systems	3
BD Phoenix	3
bioMerieux Vitek 2 GN	3
BD BBL Enterotube II	2
Dade Behring MicroScan Rapid Gram Neg	2
bioMerieux API Rapid 20E	1
Vitek GN	1
Unknown	1
Microscan Gram Neg ID	1
Conventional biochemicals	1
TOTAL	226
Specimen source (urine) not processed	1

Educational Specimens A and B – Sputum cultures for Legionella sp.

Educational samples A and B were both simulated sputum specimens indicated for *Legionella* culture. Specimen A contained *Legionella micdadei* and Specimen B contained *Legionella pneumophila*. Only 30% of the participating laboratories reported results for these samples since the vast majority do not perform culture for *Legionella*.

Specimen A contained *Legionella micdadei*. Of the laboratories that processed this sample, approximately 65% reported that the sample contained *Legionella* species or *Legionella* species not *pneumophila*. An additional 10% identified the organism as *Legionella micdadei*. However, 20% of the laboratories did not isolate an organism from this specimen. Please refer to the discussion below for information on the growth characteristics of this organism and instruction on the proper media for culture.

Specimen B contained *Legionella pneumophila* serogroup 1. Of the laboratories that reported results for this sample, 37% identified *Legionella pneumophila* while 48% reported that the specimen contained *Legionella* species. Approximately 8% were unable to isolate this organism.

Since it was first identified after the American Legion Convention in Philadelphia, PA in 1976, members of the genus *Legionella* have been widely associated with hospital-acquired and community-acquired pneumonia. The spectrum of illness can range from a flu-like illness (Pontiac Fever) to a severe, often fatal disease, although the most common presentation is pneumonia. There are more than 40 named species, and about half of these have been implicated in human illness. However, most cases of legionellosis are caused by *L. pneumophila* serogroup 1. Cases frequently have risk factors, such as cigarette smoking or chronic lung disease, and transplant patients are at higher risk for nosocomial infection. Legionnaire's Disease cannot be distinguished clinically from pneumonia caused by other agents. Clinicians should maintain heightened awareness for legionellosis for facility-associated illness in patients with increased risk. Transmission does not occur person to person. Access to the respiratory tract is by aspiration of contaminated water and is frequently associated with potable water or aerosol-generating devices, such as cooling towers, respiratory therapy equipment, showers, and whirlpools. It is important to reduce the level of *Legionella* in water systems through super-heating, hyper-chlorination, copper-silver ionization or other eradication methods. A guidance document regarding hospital-acquired legionellosis was issued to hospital administrators in July 2005 by the NYSDOH. This document provides useful information regarding prevention of this disease in healthcare facilities. This document can be found at: http://www.health.state.ny.us/nysdoh/infection/docs/doc050714_0.pdf.

For most laboratories, culturing for these organisms can be challenging and time consuming. *Legionella* does not grow on standard microbiologic media. Buffered charcoal yeast extract (BCYE) with ACES buffer, α -ketoglutarate, L-cysteine and ferric pyrophosphate is the standard media for isolation. Variations of this media contain antibiotics and dyes to enhance recovery and identification. It is important to note that not all *Legionella* species grow in the presence of the antimicrobial agents. For example, *L. micdadei* does not grow in the presence of cefemandole, and so specimens should be cultured on both selective and non-selective media. Since *Legionella* infect the lower respiratory tract, appropriate specimens include sputum, tracheal aspirates, bronchoscopy specimens, pleural fluid and lung tissue. Colonies that exhibit

characteristic ground glass morphology, have a thin-gram negative rod appearance, and exhibit a growth requirement for L-cysteine as determined by subculture to BCYE and 5% sheep blood agar (or BCYE without L-cysteine) can be presumptively identified as *Legionella* and should be forwarded to a reference laboratory for speciation and serogrouping. The development of the urinary antigen test to detect *Legionella* antigens in a urine specimen is a less invasive test and has been widely implemented in the clinical laboratory. However, the antigen test is not reliable for *Legionella* spp. other than *L. pneumophila* serogroup 1 and does not allow for organism isolation and identification.

It is recommended that clinical laboratories maintain their proficiency in the identification of *Legionella* species and be able to perform culture on request.

Participating laboratory responses for educational samples:

Educational A

Do not test for *Legionella* 130

No report 26

***Legionella* species**

Conventional biochemicals	16
Not given	3
Oxoid Diagnostic Reagent	1
DFA	1
Zeus <i>Legionella</i> IFA Direct FA	1
SciMedx DFA	1
Wampole Polyvalent FITC	1
Polymerase chain reaction	1
TOTAL	25

Presumptive *Legionella* species

Conventional biochemicals	12
BioRad Monofluo IFA Test Kit	1
TOTAL	13

No *Legionella* isolated 7

No growth 6

Legionella micdadei

M Tech DFA	2
SciMedX DFA	1
Zeus <i>Legionella</i> IFA Direct FA	1
Conventional biochemicals	1
16S rDNA sequencing	1
TOTAL	6

Legionella* species, not *pneumophila

BioRad Monofluo IFA Test Kit	2
SciMedX DFA	1
MarDx <i>Legionella</i> FA	1
TOTAL	4

Legionella pneumophila

BioRad Monofluo IFA Test Kit	1
Conventional biochemicals	1
DFA	1
Zeus <i>Legionella</i> IFA Direct FA	1

TOTAL	4
Presumptive <i>Legionella</i> species, not <i>pneumophila</i>	
BioRad Monofluo IFA Test Kit	2
<i>Legionella pneumophila</i>, not serogroup 1	
Zeus <i>Legionella</i> IFA Direct FA	1
Suspicious for <i>Legionella</i> species	
Not given	1
<i>Legionella</i> species, possible <i>micdadei</i>	
<i>Legionella</i> DFA test system	1
No pathogens isolated	1

Educational B	
Do not test for <i>Legionella</i>	130
No report	26
<i>Legionella pneumophila</i>	
BioRad Monofluo IFA Test Kit	8
Conventional biochemicals	6
DFA	2
Not given	2
SciMedX DFA	2
Zeus <i>Legionella</i> IFA Direct FA	1
DFA M-TECH	1
16S rDNA sequencing	1
MarDx <i>Legionella</i> FA	1
TOTAL	26
<i>Legionella species</i>	
Conventional biochemicals	15
Not given	3
SciMEDx DFA	1
Zeus <i>Legionella</i> IFA Direct FA	1
Polymerase chain reaction	1
Wampole Polyvalent FITC	1
TOTAL	22
Presumptive <i>Legionella species</i>	
Conventional biochemicals	11
Not given	1
TOTAL	12
No growth	3
<i>Legionella pneumophila serogroup 1</i>	
BioRad Monofluo IFA Test Kit	1
Oxoid Diagnostic Reagent	1
TOTAL	2
Negative for <i>Legionella</i>	2
No pathogens isolated	1
Positive for <i>Legionella</i>	
Conventional biochemicals	1
<i>Legionella pneumophila, not serogroup 1</i>	
Zeus <i>Legionella</i> IFA Direct FA	1

<i>Legionella</i> species, not <i>pneumophila</i>	
Conventional biochemicals	1
Suspicious for <i>Legionella</i> species	
Not given	1
<i>Legionella</i> species, possible <i>pneumophila</i>	
<i>Legionella</i> DFA test system	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. Currently, 111 of 227 participating laboratories (49%) perform direct detection testing for *Chlamydia*.

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	41
Gen-Probe Aptima Combo 2	16
Roche Diagnostics COBAS Amplicor CT/NG	14
BD ProbeTec ET CT or CT/GC	13
Test method not indicated	7
bioMerieux VIDAS	7
Unknown	3
Digene Hybrid Capture hc2 CT/GC	3
Beckman Coulter Access Chlamydia EIA	2
BioRad Chlamydia EIA plate	1
BioStar Chlamydia OIA	1
Real-time PCR	1
Roche Diagnostics Amplicor CT/NG	1
Viper	1
TOTAL	111

No report **1**

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

Test kits used by laboratories reporting Specimen A as:

Negative for Group A *Streptococcus*:

BD Directigen EZ Strep	13
BioStar Aceava Strep A	11
Genzyme OSOM Ultra Strep A	8
Abbott Signify Strep A Dipstick	7
Quidel QuickVue + Strep A	7
Test method not indicated / unknown	7
BD Chek Strep A	5
BioStar Strep A OIA Max	4
Quidel QuickVue Inline Strep A	4
Fisher Healthcare Sure-Vue Strep A	3
SP Strep A Cassette	3
Abbott Signify Strep A Cassette	2
Fisher Sureview SELECT	2
LifeSign Status Accustrep A	2
Meridian Bioscience ImmunoCard STAT Strep A	2
Remel PathoDx Strep A	2
SP Strep A Dipstick	2
Applied Biotech SureStep Strep A	1
Gen-Probe Group A Strep	1
Mainline Confirms Strep A	1
Polymedco Poly Stat Strep A	1
Remel RIM A.R.C. Strep A	1
Sacks Medical Corp RefuAH Strep A	1
Wampole Clearview Strep A Extract	1
TOTAL	91

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	7
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BACTERIAL IDENTIFICATION BY PARTICIPATING LABORATORIES

	<u>Number Reported</u>	<u>%</u>
SPECIMEN NUMBER 1		
<i>Salmonella</i> serogroup D	142	62.6
<i>Salmonella</i> species	59	26.0
Specimen source not tested	11	4.8
<i>Salmonella</i> species, not <i>typhi</i>	8	3.5
<i>Salmonella</i> group	2	0.9
<i>Salmonella</i> serotype Enteriditis	2	0.9
Presumptive <i>Salmonella</i> species	1	0.4
<i>Salmonella</i> serotype Typhi	1	0.4
<i>Salmonella sonnei</i> , group D	1	0.4

SPECIMEN NUMBER 2		
<i>Haemophilus parainfluenzae</i>	202	88.9
Specimen source not tested	10	4.4
<i>Haemophilus influenzae</i>	5	2.2
<i>Haemophilus</i> species, not <i>influenzae</i>	4	1.8
Not reported	3	1.3
<i>Haemophilus</i> species	2	0.9
<i>Actinobacillus urinae</i>	1	0.4

SPECIMEN NUMBER 3		
<i>Clostridium sordellii</i>	191	84.1
<i>Clostridium</i> species	8	3.5
Do not process anaerobic cultures	7	3.1
<i>Clostridium</i> species not <i>perfringens</i>	6	2.6
Specimen source not tested	5	2.2
No report	2	0.9
<i>Clostridium bifermentans</i>	2	0.9
No <i>Bifidobacterium</i> isolated	2	0.9
Anaerobic gram negative bacilli	1	0.4
Anaerobic gram positive spore forming bacilli	1	0.4
<i>Clostridium</i> species, not <i>perfringens</i> or <i>septicum</i>	1	0.4
<i>Clostridium sporogenes</i>	1	0.4
<i>Enterococcus faecium</i>	175	77.1
<i>Enterococcus</i> species	32	14.1
<i>Enterococcus gallinarum</i>	5	2.2
Specimen source not tested	5	2.2
<i>Enterococcus</i> group D	4	1.8
Gamma-hemolytic <i>Streptococcus</i>	2	0.9
<i>Enterococcus casseliflavus</i>	1	0.4
<i>Enterococcus durans</i>	1	0.4
No report	1	0.4

<i>Streptococcus faecium</i>	1	0.4
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SPECIMEN NUMBER 4

<i>Burkholderia cepacia/Burkholderia cepacia</i> complex	174	76.7
Specimen source not tested	8	3.5
No pathogens isolated	7	3.1
<i>Pseudomonas aeruginosa</i>	7	3.1
<i>Burkholderia</i> species	4	1.8
Gram negative bacillus	3	1.3
<i>Pseudomonas cepacia</i>	3	1.3
Non-fermenting gram negative bacillus	2	0.9
<i>Pseudomonas fluorescens/putdia</i> group	2	0.9
<i>Pseudomonas</i> species	2	0.9
<i>Acinetobacter baumannii</i>	1	0.4
<i>Acinetobacter</i> species	1	0.4
<i>Alcaligenes xylosoxidans</i>	1	0.4
<i>Burkholderia gladioli</i>	1	0.4
<i>Burkholderia</i> species / <i>Ralstonia</i> species	1	0.4
<i>Flavobacterium</i> species	1	0.4
Gram negative bacillus, not <i>P. aeruginosa</i>	1	0.4
No report	1	0.4
Nonfermenter, not <i>P. aeruginosa</i>	1	0.4
Possible <i>Burkholderia</i> species	1	0.4
<i>Pseudomonas fluorescens</i>	1	0.4
<i>Pseudomonas putida</i>	1	0.4
<i>Pseudomonas</i> species, not <i>aeruginosa</i>	1	0.4
<i>Sphingobacterium</i> species	1	0.4
Unable to ID	1	0.4

SPECIMEN NUMBER 5

<i>Morganella morganii</i>	226	99.6
Specimen source not processed	1	0.4

EDUCATIONAL A

Do not test for <i>Legionella</i>	130	57.3
No report	26	11.5
<i>Legionella</i> species	25	11.0
Presumptive <i>Legionella</i> species	13	5.7
No <i>Legionella</i> isolated	7	3.1
No growth	6	2.6
<i>Legionella micdadei</i>	6	2.6
<i>Legionella</i> species, not <i>pneumophila</i>	4	1.8
<i>Legionella pneumophila</i>	4	1.8
Presumptive <i>Legionella</i> species, not <i>pneumophila</i>	2	0.9
<i>Legionella pneumophila</i> , not serogroup 1	1	0.4

<i>Legionella</i> species, possible <i>micdadei</i>	1	0.4
Suspicious for <i>Legionella</i> species	1	0.4
No pathogens isolated	1	0.4

EDUCATIONAL B

Do not test for <i>Legionella</i>	130	57.3
No report	26	11.5
<i>Legionella pneumophila</i>	24	10.6
<i>Legionella</i> species	22	9.7
Presumptive <i>Legionella</i> species	12	5.3
No growth	3	1.3
<i>Legionella pneumophila</i> serogroup 1	2	0.9
Negative for <i>Legionella</i>	2	0.9
No pathogens isolated	1	0.4
Positive for <i>Legionella</i>	1	0.4
<i>Legionella pneumophila</i> , not serogroup 1	1	0.4
<i>Legionella</i> species, not <i>pneumophila</i>	1	0.4
Suspicious for <i>Legionella</i> species	1	0.4
<i>Legionella</i> species, possible <i>pneumophila</i>	1	0.4

CHLAMYDIA SPECIMEN

Negative for <i>Chlamydia trachomatis</i>	111	99.1
No report	1	0.9

DIRECT ANTIGEN SPECIMENS

A. Negative for Group A <i>Streptococcus</i>	91	100.0
C. Positive for Group B <i>Streptococcus</i>	7	100.0