

Susceptibility Testing for Fastidious Organisms: CLSI M45-A2 update

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History

- CLSI has provided testing and interpretive criteria for common organisms
- Labs occasionally need to test "infrequently isolated or fastidious" species
- In absence of guidelines, labs have used various methods and applied breakpoints from other groups of organisms
- There is a need to provide better guidance for use of standard methods for these unusual species
- M45-A Published 2006; M45-A2 2010
- Janet Hindler and Jim Jorgensen spearheaded the project

Update - M45 A2

- **Forward** - statement that commercial devices that are FDA approved may be suitable for testing.
- Includes antibiotic gradient method or the Etest
- However, this document addresses only generic reference procedures that can be used routinely or to evaluate commercial devices

The original "orphan" organisms

- Abiotrophia and *Granulicatella* spp
- *Aeromonas hydrophila* complex
- *Bacillus* spp.
- *Campylobacter jejuni/coli*
- *Corynebacterium* spp.
- *Erysipelothrix rhusiopathiae*
- HACEK group
- *Lactobacillus* species
- *Leuconostoc* species
- *Listeria monocytogenes*
- *Moraxella catarrhalis*
- *Pasteurella multocida*
- *Pediococcus* species
- *Vibrio* species

New additions

- Agents of Bioterrorism imported from M100 with additional interpretive criteria tables, antimicrobials for primary testing
- *Vibrio* spp. imported from M100 with additional interpretive criteria tables and antimicrobials to consider for primary testing.
- *Helicobacter pylori* imported from M100

Introduction - additions

- Updated taxonomy
- Resistance mechanisms in GP-rods
- Added list of abbreviations and acronyms
- Added definition of non-susceptible - only S interpretive criteria due to absence or rare occurrence of resistant strains
- More information on QC strains

Traditional factors used for MIC breakpoint definitions

- MIC distribution in wild type
- MICs with known resistance mechanisms
- Pharmacokinetics and pharmacodynamics
- Monte Carlo simulations - dose vs maintenance of drug level vs MIC
- Clinical and bacteriological response from clinical trial data
- Animal models

Challenges of the "orphans"

- Relatively few strains available
- Resistant strains may be rare
- No clinical trials
- Most clinical data from case reports
- Lab data from centers doing surveys and studies
- Guidelines for breakpoints cannot be as stringent as standards for common species

New CLSI working group established - M45

- Working group 2004 - microbiologists with expertise for each genus
- Interpretive criteria based on review of literature, PK/PD information, microbiological data to establish MIC distribution
- Methods similar to M2 and M7
- QC parameters using current strains
- Publication of M45-A 2006
- M45-A2 in 2010

M45 A2- Format for tables

- Box - Testing conditions- medium, inoculum, incubation
- Box - Minimum QC recommendation
- Box - Agents to consider for primary testing
- General comments - e.g. very fastidious, require CO₂
- Antimicrobial agents - S-I-R - comments
- Supplemental information - resistance, reasons for testing, derivation of interpretive criteria

Abiotrophia and Granulicatella

No changes

- Test as *S. pneumoniae* except supplement with 1ug/ml pyridoxal HCl
- QC *S. pneumoniae* ; interpretive criteria adapted from M100 for *Streptococcus*
- Very fastidious - requires cysteine or pyridoxal; may grow as satellite colonies around staph
- May demonstrate diminished susceptibility to pcn; fluoroquinolone-resistant strain reported

Table 1. Abiotrophia spp. and Granulicatella spp. (Formerly Known as Nutritionally Deficient or Nutritionally Variant Streptococci)—Information and Interpretive Criteria for Broth Microdilution Susceptibility Testing

Testing Conditions	Minimal QC Recommendations	Agents to Consider for Primary Testing	
Medium: Cation-adjusted Mueller-Hinton broth with lysed horse blood (2.5-6% v/v) and 0.01% (v/v, 1 µg/mL) pyridoxal hydrochloride. Inoculum: 0.5 McFarland standard. Incubation: 35 °C, ambient air, 20-24 hours.	<i>Streptococcus pneumoniae</i> ATCC™ 49619	Penicillin Vancomycin Clindamycin or clindamycin	
General Comments			
(1) Growth characteristics on routine media: Very fastidious; requires cysteine or pyridoxal for growth. Some strains may grow marginally on enriched chocolate agar or ampicillin agar formulations supplemented with added cysteine. CO ₂ 20-24 hours.			
(2) For some organism/antimicrobial combinations, the absence of one occurrence of resistant strains precludes defining any results category other than "susceptible." For strains yielding results suggestive of a "resuscitator" category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory for confirmation.			
NOTE: Information in baseline type is considered tentative for one year.			
Antimicrobial Class	Antimicrobial Agent	MIC (µg/mL) Interpretive Criteria	Comments
PENICILLINS	Penicillin	≤12 0.25-2 24	
	Ampicillin	≤125 0.5-4 16	
CEPHALOSPORINS	Ceftriaxone	≤1 2 24	
	Cefuroxime	≤1 2 24	
CARBAPENEMS	Meropenem	≤1 1 24	
	Ertapenem	≤1 1 24	
GLUCOSAMINES	Linezolid	≤1 1 24	See comment (2)

Table 1. *Aerobius* spp. and *Granulicatella* spp. (Continued)

Antibacterial Class	Antibacterial Agent	MIC (μg/ml)			Comments
		S	I	R	
MACROLIDES	Erythromycin	100.00	0.0	0.0	
	Clarithromycin	100.00	0.0	0.0	
Glycosylamines	Chlortetracycline	100.00	0.0	0.0	
	Doxycycline	100.00	0.0	0.0	
	Lincosamide	100.00	0.0	0.0	
PHENACOLS	Chloramphenicol	100.00	0.0	0.0	
	Flucloxacillin	100.00	0.0	0.0	
LINCOSAMIDES	Clindamycin	100.00	0.0	0.0	
	Clindamycin	100.00	0.0	0.0	

ATCC® is a registered trademark of the American Type Culture Collection.

Supplemental Information

Resistance: *Aerobius* spp. and *Granulicatella* spp. may demonstrate diminished susceptibility to penicillins, resulting in greater difficulty in treatment of patients with endocarditis, in one case, fluoroquinolone resistance was reported in an isolate from an immunosuppressed patient.

Reasons for Testing/Not Testing: For isolates from respiratory sources or wounds, testing is usually not necessary. Testing of isolates from normally sterile sources (blood cultures, deep tissue, implanted prosthetic devices) may be warranted, especially in immunocompetent patients.

Formulation of Interpretive Criteria: Interpretive criteria are adapted from those for *Streptococcus* spp. as published in the current edition of CLSI document M100.018. For *Aerobius* spp. and *Granulicatella* spp. interpretation of susceptibility breakpoints include references 2, 3, 11.

Testing Notes: Many laboratories cannot readily distinguish *Aerobius* spp. from *Granulicatella* spp. or determine species level identification. The requirement for systems or protocols in the medium or suitable growth characteristics for both *Aerobius* and *Granulicatella*. Many laboratories may not do additional tests to separate *Aerobius* from *Granulicatella* or to obtain a species identification.

Aeromonas spp

- Includes members of *A. caviae* complex, *A. hydrophila* complex, and *A. veronii* complex (17 valid species)
- CAMHB, direct colony suspension, 35C ambient air, DD-16-18h, BMD 16-20h
- Pleisiomonas included because of similarity
- Testing usually for extraintestinal sites

Aeromonas hydrophila infection

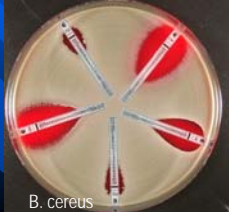
- Acute Post op infection with *Aeromonas* from leech therapy
- 5 male patients with trauma injuries
- Post op - venous congestion
- Medical leeches applied
 - Passive bleeding after bite
 - Superficial skin perfusion after bite
 - Anti-coagulant effect of saliva
- All developed infection with *A. hydrophila*
- Leech guts are colonized with *Aeromonas*



Schwartz et al. Arch Orthop Trauma Surg 2010

Bacillus spp. - not B. anthracis

- Added fluoroquinolones for primary testing
- Testing conditions - same as staph
- QC - *S. aureus*
- Reasons for testing - normally sterile sites esp in immunodeficient pts
- Beta-lactamase testing is unreliable
- Interpretive criteria derived from M100 Staphylococcus
- Do not test oxacillin!



B. cereus

Serious Bacillus infections

- Nosocomial central catheter-related bacteremia following cholecystectomy in an immunocompetent patient
- B. cereus* bacteremia in preterm neonate (review)
- Bacillus* spp. and hematological malignancies -3.4% of episodes

(Herrnaiz et al, CMI 2003 9:973) ; (Hilliard et al, JCM 2003 41:3441)
(Ozkocaman et al, JHI 2006 64:169)

Bacillus spp. susceptibility to antimicrobial agents

- B. cereus* group - most R to pcn; 2 of 29 R to cipro; R to erythro sporadic; 1 strain R to doxy
- Other *Bacillus* spp - including *B. subtilis*, *B. pumilus*, *B. firmus*. Most S to cipro, many to pcn, most S to erythro

(Citron et al JCM 2006 44:3814)

Campylobacter jejuni/coli

- Added azithromycin and clarithromycin
- Clarified incubation conditions for disk and MIC tests
- Test conditions - CAMHD + LHB
- Incubation - 36-37C for 48h, or 42c for 24h. Microaerophilic atmosphere. Use gas generating sachets or compressed gas mixture. Sealed pouches or bags not recommended.
- Resistance known to occur with erythro, but more problematic with cipro.
- Use of disks - no zone as resistance screen for cipro and erythro. Any zone of inhibition requires MIC determination.

Table 4. *Campylobacter jejuni/coli*—Information and Interpretive Criteria for Broth Microdilution and Disk Diffusion Susceptibility Testing

Testing Conditions

Medium: Casein-adjusted Mueller-Hinton broth with yeast extract base (2.5-5% w/v) as appropriate. Mueller-Hinton agar with 5% sheep blood (MHSA) for disk diffusion testing.

Incubation: Direct colony suspension, equivalent to a 0.5 McFarland standard.

Incubation: 36-37 °C for all tests or 42 °C for 24 hours (incubation at less than 36 °C or greater than 42 °C may not yield satisfactory growth). Microaerophilic atmosphere required for 10% CO₂, 5% O₂ and 85% N₂. Use of a compressed gas incubator is preferable. However, acceptable performance may be achieved using microaerophilic gas-generating sachets. Sealed plastic bags or pouches do not result in reproducible data, and are not recommended.

Minimal QC Recommendations

Media: CAMHD, ATCC 33960, 36-37 °C for 48 hours or 42 °C for 24 hours (see Table 18-B)

Disk Diffusion: Streptomycin (S), Erythromycin (E), Ciprofloxacin (C), Azithromycin (AZ), Clarithromycin (CL)

Agents to Consider for Primary Testing: Erythromycin, Ciprofloxacin

General Comments

(1) Growth characteristics on media media. *Campylobacter jejuni/coli* will grow on 5% sheep blood, requires a microaerophilic atmosphere (10% CO₂, 5% O₂, and 85% N₂), 36-37 °C for 48 hours or 42 °C for 24 hours.

NOTE: Information on broth tests is contained elsewhere in the guide.

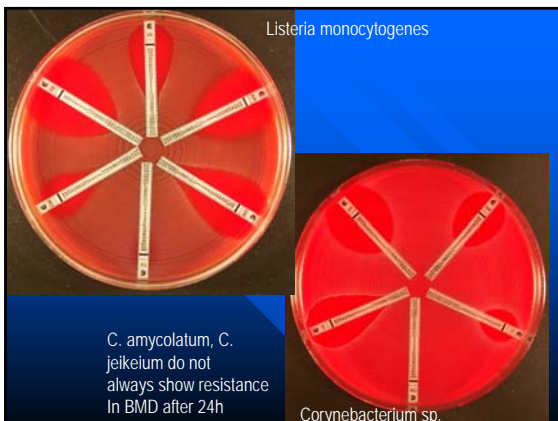
Antibiotic Class	Antibiotic Agent	Disk Diameter	Zone diameters (mm)					MIC (µg/ml)	Comments
			S	E	C	AZ	CL		
MACROLIDE	Erythromycin	15 mm	1	1	1	1	1	1	A disk diffusion zone of 8 mm (growth up to the edge of a 16 mm disk) indicates resistance. (S), erythromycin; (E), erythromycin base; (C), ciprofloxacin; (AZ), azithromycin; (CL), clarithromycin. For accurate interpretation of any zone of inhibition result requires MIC determination for accurate interpretation of susceptibility.
	Clarithromycin	15 mm	1	1	1	1	1	1	A disk diffusion zone of 8 mm (growth up to the edge of a 16 mm disk) indicates resistance. (S), erythromycin; (E), erythromycin base; (C), ciprofloxacin; (AZ), azithromycin; (CL), clarithromycin. For accurate interpretation of any zone of inhibition result requires MIC determination for accurate interpretation of susceptibility.
QUINOLONE	Ciprofloxacin	6 mm	1	1	1	1	1	1	A disk diffusion zone of 8 mm (growth up to the edge of a 16 mm disk) indicates resistance. (S), erythromycin; (E), erythromycin base; (C), ciprofloxacin; (AZ), azithromycin; (CL), clarithromycin. For accurate interpretation of any zone of inhibition result requires MIC determination for accurate interpretation of susceptibility.
	Levofloxacin	6 mm	1	1	1	1	1	1	A disk diffusion zone of 8 mm (growth up to the edge of a 16 mm disk) indicates resistance. (S), erythromycin; (E), erythromycin base; (C), ciprofloxacin; (AZ), azithromycin; (CL), clarithromycin. For accurate interpretation of any zone of inhibition result requires MIC determination for accurate interpretation of susceptibility.
TETRACYCLINE	Doxycycline	15 mm	1	1	1	1	1	1	A disk diffusion zone of 8 mm (growth up to the edge of a 16 mm disk) indicates resistance. (S), erythromycin; (E), erythromycin base; (C), ciprofloxacin; (AZ), azithromycin; (CL), clarithromycin. For accurate interpretation of any zone of inhibition result requires MIC determination for accurate interpretation of susceptibility.
	Tetracycline	15 mm	1	1	1	1	1	1	A disk diffusion zone of 8 mm (growth up to the edge of a 16 mm disk) indicates resistance. (S), erythromycin; (E), erythromycin base; (C), ciprofloxacin; (AZ), azithromycin; (CL), clarithromycin. For accurate interpretation of any zone of inhibition result requires MIC determination for accurate interpretation of susceptibility.

Corynebacterium and other coryneforms

- Modified vanco breakpoints
- Clarified scope of other GPRs
- Test conditions - CAMHB +LHB
- QC - *S. pneumoniae*
- Some strains fastidious - require blood supplementation
- Some species may exhibit resistance to multiple drug classes
- Interpretive criteria for pcn and erythro based on MIC distributions from testing large numbers of isolates; ceph from Strep spp; linezolid from Enterococcus; remainder from Staph (M100) Do not test oxacillin!
- Report resistant results after 24h; if B-lactams appear sens, reincubate for 48h

Recent reports of infections with *Corynebacterium* spp

- Septic arthritis - *C. striatum*, *C. kitcheri*
- Encrusted cystitis - *C. urealyticum*
- Toxigenic *C. ulcerans* - mimics diphtheria
- Granulomatous mastitis - *C. amycolyticum*, *C. accolens*, *C. striatum*, *C. Kroppenstedtii*
- Exacerbation of COPD-serious resp inf - *C. striatum* (3 deaths with pure cult)
- Pancreatic abscess - *Corynebacterium coyleae* mimicking malignant neoplasm.
- Urinary tract infection - *Corynebacterium pseudogenitalium*



HACEK organisms

- *Aggregatibacter* new name for *Actinobacillus*
- Testing conditions - CAMHB + LHB
- Incubation - 35C; CO₂, 24-48h
- QC - *S. pneumoniae*
- Growth characteristics - very fastidious, most will grow on BAP or CHOC in CO₂. Some will not grow in supplemented broths. Do not attempt to interpret results if insufficient growth in control well.
- Etest more reliable than BMD
- Routine testing for B-lactamase recommended

Helicobacter pylori

- Imported from M100
- Agar dilution method using 2 week old sheep blood
- Inoculum - turbidity = to No. 2 McFarland standard
- Incubation - microaerobic environment
- Test group - clarithromycin

Lactobacillus spp

- Added breakpoints for dapto and linezolid
- Added I and R breakpoints for imipenem
- Modified clinda and vanco breakpoints
- Test conditions- CAMHB + LHB
- 35 C with 5% CO₂ 24-48h
- Test PCN, ampicillin
- L. casei group are intrinsically Van-R
- L. acidophilus group - Van-S



Lactobacillus gasseri

- Elderly lady admitted from nursing home with urosepsis
- Several blood culture sets yielded large GPB
- S to vanco, catalase-neg
- ID by 16S rDNA seq.



Mary York

Lactobacillus Bacteremia

- Vancomycin-resistant group
 - L. rhamnosus 53.5%
 - L. fermentum 14%
 - L. casei 14%
- Vancomycin-susceptible
 - Other species 18.6 %
 - » L. jensenii
 - » L. salivarius
 - » L. gasseri
- All have PCN MICs \leq 4 ug/ml

Sallinen et al CID 2006;42

Leuconostoc

- Added comment for adding gent to pcn or amp for serious infections
- Testing conditions - CAMHB + LHB
- QC- S. pneumoniae
- Incubation - ambient, 20-24h
- Test pcn, amp; intrinsically vanco-R
- Interpretive criteria from staph (GM), Enterococcus for other drugs



Leuconostoc

Moraxella catarrhalis

- Modified MIC breakpoints for amox/clav (S only), azithro, clarithro, erythro
- Added disk diffusion breakpoints for amox/clav, azithro, clarithro, erythro, tetra, SMX
- Updated derivation of interpretive criteria

Pasteurella species

- Clarified incubation conditions
- CAMHB + LHB
- MHA w/5% SB for disk diffusion
- QC - *S. pneumoniae* BMD; *S. aureus* for disk
- Fastidious - requires blood
- Rare isolates B-lactamase pos; pcn MICs >0.5
- Testing not needed for bite isolates; testing from normally sterile sites and respiratory infections
- Interpretive criteria based on MIC distributions from large numbers of isolates from human animal bite infections
- Testing *S. aureus* 25923 on BMHA - use M100, Table 3 ranges



Pasteurella infections not associated with bites

- Pneumonia and septicemia in immunocompetent patient without any evidence of cutaneous lesion - only exposure to dog.
- Septic shock, sinusitis, pneumonia in 20 yo female with close contact with cat, but only minor scratches on arm
- Other reports of meningitis, peritonitis, osteoarticular infections, endocarditis

Pakenyte et al, Med Mal Inf 2007 37:354;
Ryosuke et al, J Inf Chemother 2004 10:250

Pediococcus

- Test conditions - CAMHB + LHB
- Incubate - 35C ambient 20-24h
- QC - *S. pneumoniae*
- Non-fastidious
- Intrinsically vanco-resistant
- Test isolates from normally sterile sites
- Interpretive criteria adapted from Enterococcus, M100

Vibrio spp. including V. cholera

- Imported table from M100
- Clarified incubation conditions
- Listed agents for testing *V. cholera* vs others
- CAMHB for broth microdilution
- MHA for disk diffusion
- Direct colony suspension in saline
- Test isolates from extra intestinal sites
- Test tetra, cefotaxime, fluoroquinolones
- Usually resistant to PCN, older cephs, sulfonamides
- Interpretive criteria adapted from enterobacteriaceae

Vibrio vulnificus infections

- Case 1- 27 yo man struck by lightning while windsurfing. Found pulseless in water and was resuscitated. Developed resp failure, nec fasc, pos blood cultures. Treated with abx and recovered.
- Case 2- 43 yo Asian oyster shucker with redness, swelling and photophobia in R eye. DX - corneal ulcer with pos culture

Vibrio vulnificus infections

- Case 3 - 46 yo man with abdominal pain, nausea, chills, bullous lesions on legs. Developed DIC, and cultures from legs grew *V. vulnificus*. He ate raw oysters 3 days previously.
- Case 4 - 32 yo woman with HIV, HepC, with cirrhosis, presented with fever, chills, productive cough, and red spots on her extremities and buttocks. Blood cultures pos for *V. vulnificus*. Treated with abx and recovered.

Potential agents of bioterrorism

- Includes *B. anthracis*, *Y. pestis*, *B. mallei*, *B. pseudomallei*, *F. tularensis*, and *Brucella* sp.
- Broth microdilution media
- Incubation conditions for each
- Agents to test
- Interpretive criteria
- QC strains for each
- Precautions - recommend BL-2 practices
- Notify Public Health dept.

Table 17. Summary of Testing Conditions and Quality Control Recommendations for Infrequently Isolated or Fastidious Bacteria

Table No.	Organism/Organism Group	Broth Microdilution MIC Test Medium	Broth Microdilution MIC Incubation Conditions	Disk Diffusion Test Medium/Incubation Conditions	Quality Control
1	<i>Abiotrophia</i> spp., <i>Granulicatella</i> spp.	CAMHB-LHB (2.0%-5% v/v) + 0.001% pyridoxal HCl	35 °C; ambient air; 20-24 h	NA ^a	<i>S. pneumoniae</i> ATCC [®] 49619
2	<i>Aeromonas hydrophila</i> complex, <i>Photobacterium phagebolicum</i>	CAMHB	35 °C; ambient air; 16-20 h	MHA (unsupplemented)/35 °C; ambient air; 16-18 h	<i>E. coli</i> ATCC [®] 25922 <i>E. coli</i> ATCC [®] 35218 ^b
3	<i>Bacillus</i> spp. (not <i>B. anthracis</i>)	CAMHB	35 °C; ambient air; 16-20 h	NA	<i>S. aureus</i> ATCC [®] 29213
4	<i>Campylobacter jejuni</i> complex	CAMHB-LHB (2.0%-5% v/v)	36-37 °C/6 h or 42 °C/24 h, 95% CO ₂ , 5% O ₂ , 80% N ₂ (Prepackat)	MHA with 5% sheep blood/36-37 °C/6 h or 42 °C/24 h; 5% CO ₂ , 5% O ₂ , 80% N ₂ (Prepackat)	<i>C. jejuni</i> ATCC [®] 35069 for microdilution <i>S. aureus</i> ATCC [®] 25922, MR0458-07 ^c for 16-18 h in ambient air for disk diffusion
5	<i>Corynebacterium</i> spp.	CAMHB-LHB (2.0%-5% v/v)	35 °C; ambient air; 24-48 h	NA	<i>S. pneumoniae</i> ATCC [®] 49619 <i>E. coli</i> ATCC [®] 25922 for gentamicin
6	<i>Erysipelothrix rhusopathiae</i>	CAMHB-LHB (2.0%-5% v/v)	35 °C; ambient air; 20-24 h	NA	<i>S. pneumoniae</i> ATCC [®] 49619 <i>E. coli</i> ATCC [®] 35218 ^b
7	HACEK group	CAMHB-LHB (2.0%-5% v/v)	35 °C; 5% CO ₂ ; 24-48 h	NA	<i>S. pneumoniae</i> ATCC [®] 49619 <i>E. coli</i> ATCC [®] 35218 ^b
8	<i>Haemophilus pylori</i>	Agar dilution: MHA and 5% sheep blood (5% v/v)	35 °C; 5% CO ₂ ; 24 to 48 hours	NA	<i>Haemophilus pylori</i> ATCC [®] 43094
9	<i>Lactobacillus</i> spp.	CAMHB-LHB (2.0%-5% v/v)	35 °C; ambient air; 24 to 48 hours	NA	<i>S. pneumoniae</i> ATCC [®] 49619 <i>E. coli</i> ATCC [®] 25922 for gentamicin
10	<i>Leuconostoc</i> spp.	CAMHB-LHB (2.0%-5% v/v)	35 °C; ambient air; 20-24 h	NA	<i>S. pneumoniae</i> ATCC [®] 49619 <i>E. coli</i> ATCC [®] 25922 for gentamicin
11	<i>Listeria monocytogenes</i>	CAMHB-LHB (2.0%-5% v/v)	35 °C; ambient air; 20-24 h	NA	<i>S. pneumoniae</i> ATCC [®] 49619

Table No.	Organism/Organism Group	Broth Microdilution MIC Test Medium	Broth Microdilution MIC Incubation Conditions	Disk Diffusion Test Medium/Incubation Conditions	Quality Control
12	<i>Moraxella catarrhalis</i>	CAMHB	35 °C; ambient air; 20-24 h	MHA (unsupplemented)/35 °C; 5% CO ₂ ; 24-24 h	<i>S. aureus</i> ATCC [®] 29213 <i>E. coli</i> ATCC [®] 35218 ^b
13	<i>Pasteurella</i> spp.	CAMHB-LHB (2.0%-5% v/v)	35 °C; ambient air; 16-24 h	MHA with 5% sheep blood/35 °C; ambient air; 16-18 h	<i>S. pneumoniae</i> ATCC [®] 49619 <i>E. coli</i> ATCC [®] 35218 ^b <i>S. aureus</i> ATCC [®] 25922 for disk diffusion with selected drugs
14	<i>Pedibacillus</i> spp.	CAMHB-LHB (2.0%-5% v/v)	35 °C; ambient air; 20-24 h	NA	<i>S. pneumoniae</i> ATCC [®] 49619 <i>E. coli</i> ATCC [®] 25922 for gentamicin
15	Witro spp. (including <i>V. cholerae</i>)	CAMHB	35 °C; ambient air; 16-20 h	MHA (unsupplemented)/35 °C; ambient air; 16-18 h ^d	<i>E. coli</i> ATCC [®] 25922 <i>E. coli</i> ATCC [®] 35218 ^b
Potential Bacterial Agents of Bioterrorism					
16	<i>Bacillus anthracis</i>	CAMHB	35 °C; ambient air; 16-20 h	NA	<i>E. coli</i> ATCC [®] 25922 <i>S. aureus</i> ATCC [®] 29213
16	<i>Brevibacterium</i> spp.	Unsupplemented Bivocita broth pH adjusted to 7.1 ± 0.1	35 °C; ambient air; 48 h	NA	<i>E. coli</i> ATCC [®] 25922 <i>S. pneumoniae</i> ATCC [®] 49619
16	<i>Burkholderia mallei</i>	CAMHB	35 °C; ambient air; 16-20 h	NA	<i>E. coli</i> ATCC [®] 25922 <i>Pseudomonas aeruginosa</i> ATCC [®] 27853
16	<i>Burkholderia pseudomallei</i>	CAMHB	35 °C; ambient air; 16-20 h	NA	<i>E. coli</i> ATCC [®] 25922 <i>E. coli</i> ATCC [®] 35218 ^b <i>P. aeruginosa</i> ATCC [®] 27853
16	<i>Francisella tularensis</i>	CAMHB + 2% defined growth supplement	35 °C; ambient air; 48 h	NA	<i>E. coli</i> ATCC [®] 25922 <i>S. aureus</i> ATCC [®] 29213 <i>P. aeruginosa</i> ATCC [®] 27853
16	<i>Yersinia pestis</i>	CAMHB	35 ± 2 °C; ambient air; 24 h, and 16-20 h growth in the control well; reincubate an additional 24 h	NA	<i>E. coli</i> ATCC [®] 25922

Footnotes

^aNA, not applicable.
^b*E. coli* ATCC[®] 35218 is used for QC when testing *B. anthracis*/lactamase inhibitor combination drugs.

Conclusion

- CLSI welcomes comments, questions, suggestions
- Especially important for this document because of the derivation of information
- www.clsi.org