Attachment 3

ASPEN Survey Explorer Update

The ASPEN Survey Explorer program has been updated to include the current CLIA regulations, published in the *Federal Register* on January 24, 2003. Because it has been a while since there has been an update for CLIA, we want to remind you of what is included in the ASPEN program:

- Only tagged regulation text, along with its associated interpretive guidelines and probes, are included in the ASPEN program. For example, the first "D tags" in the Interpretive Guidelines for Laboratories and Laboratory Services is D1000, §493.15(c) Certificate of waiver tests.

- Regulation text that is not tagged is not included in the ASPEN program. For example, none of the regulation text prior to D1000, or the associated interpretive guidelines and probes, is included in ASPEN.

Given that only tagged regulation text can be included in ASPEN, you may need to ensure that certain information is available to your State and Regional surveyors. For example:

- The Survey Process

- The Definitions section at §493.2

- The certificate sections at §§493.19, 493.20 and 493.25

- Subparts B, C, and D

In addition, due to the formatting of certain tables in the Interpretive Guidelines, the below listed tables are not included in ASPEN Explorer. Please ensure that your State and Regional surveyors either take these tables or bring a copy of the Interpretive Guidelines to the laboratory at the time of the survey. (The Interpretive Guidelines are available in (**http://www.cms.hhs.gov/clia**).) They are provided for you here.

The tables not in ASPEN Survey Explorer (but included in the Interpretive Guidelines) are:

- The NCCLS M2-A8 Antimicrobial Disk Diffusion Susceptibility (Bauer, Kirby, Sherris and Turk Method) appropriate control strains and the associated Table 3 quality control limits tables at D5507;

- The NCCLS M7-A6 Minimum Inhibitory Concentration (MIC) appropriate control strains and the associated Table 3 breakpoint tables at D5507;

- The table that defines the frequency and type of quality control to be performed for each container of antisera and reagent red cells in immunohematology testing at D5551;

- The table at Interpretive Guidelines §493.1276(b)(1)-(b)(3) in Clinical cytogenetics;

- The NCCLS M22-A2 Quality Assurance for Commercially Prepared Microbiological Culture Media; Approved Standard-Second Edition Table 2 at D5477.

ANTIMICROBIAL DISK DIFFUSION SUSCEPTIBILITY (BAUER, KIRBY, SHERRIS AND TURK METHOD)

Each new batch of medium and each new lot/shipment of antimicrobial disks must be checked as follows:

ANTIMICROBIAL DISK SUSCEPTIBILITY TEST

Appropriate Control Strain	*Each New Batch of Media and Disks*	*Each Day If Isolates Are:*
*S. aureus ATCC 25923 or equivalent***	*X*	*Staphylococcus spp.*
*E. coli ATCC 25922 or equivalent***	*X*	*Enterobacteriaceae*
*P. aeruginosa ATCC 27853 and E. coli ATCC 25922 or equivalent***	*X*	*Pseudomonas aeruginosa Acinteobacter spp.*

NOTE 1: Routine quality control testing of commercially prepared Mueller-Hinton agar for thymine and thymidine is not needed. However, if problems with quality control of sulfonamides and trimethoprim occur, the Mueller-Hinton agar should be checked with E. faecalis ATCC 29212 or alternatively, E. faecalis ATCC 33186 with trimethoprim-sulfamethoxazole disks. Satisfactory media will provide essentially clear distinct zones of inhibition 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

NOTE 2: If testing beta-lactam/beta-lactamase inhibitor antimicrobial agents (e.g., ampicillin-sulbactam, amoxicillin- clavulanic acid, piperacillin-tazobactam, or ticarcillin-clavulanic acid), the laboratory should test E. coli ATCC 35218 (beta-lactamase producing strain).

NOTE 3: If performing extended spectrum beta-lactamase (ESBL) tests, the laboratory should test Klebsiella pneumoniae ATCC 700603 (ESBL-producing strain).

Zone sizes must be recorded for each antimicrobial control and limits must be established.

***An equivalent strain is one which demonstrates reactivity similar to an ATCC strain and for which limits have been established. Organisms which manufacturers recommend or require for use in their systems are acceptable strains of control organisms.*

*Refer to Table 3A*** of the NCCLS Standard, "Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eighth Edition (M2-A8)" to determine the control strain to be used when performing antimicrobial disk susceptibility tests on isolates of Haemophilus spp., Neisseria gonorrhoeae, Streptococcus pneumoniae or other organisms as applicable.*

MINIMUM INHIBITORY CONCENTRATION (MIC)

Each new batch of macrodilution tubes, microdilution trays, or agar dilution plates must be checked as follows:

MINIMUM INHIBITORY CONCENTRATION (MIC)

Appropriate Control Strain	*Each New Batch of Media*	*Each Day If Isolates are:*
*S. aureus ATCC 29213 or equivalent***	*X*	*Staphylococcus spp.*
*E. coli ATCC 25922 or equivalent***	*X*	*Enterobacteriaceae*
*P. aeruginosa ATCC 27853 and E. coli ATCC 25922 or equivalent ***	*X*	*Non-Enterobacteriaceae to include Acinteobacter spp., Stenotrophomonas maltophilia, Pseudomonas spp. and other nonfastidious, glucose nonfermenting, gram-negative bacilli*
*E. faecalis ATCC 29212 or equivalent***	*X*	*Enterococcus spp.*

NOTE 1: To determine the suitability of the Mueller-Hinton broth for sulfonamide and trimethoprim tests, MICs may be performed with E. faecalis ATCC 29212. Routine quality control testing of commercially manufactured panels for thymine and thymidine is not needed. However, should problems with QC of sulfonamides and trimethoprim occur, an MIC test should be performed with E. faecalis ATCC 29212 with trimethoprim-sulfamethoxazole. If the MIC for trimethoprim-sulfamethoxazole is < 0.5/9.5 ug/ml, the medium may be considered adequate.

NOTE 2: If testing beta-lactam/beta-lactamase inhibitor antimicrobial agents (e.g., ampicillin-sulbactam, amoxicillin-clavulanic acid, piperacillin-tazobactam, or ticarcillin-clavulanic acid), the laboratory should test E. coli ATCC 35218.

NOTE 3: If performing extended spectrum beta-lactamase (ESBL) tests, the laboratory should test Klebsiella pneumoniae ATCC 700603 (ESBL-producing strain).

NOTE 4: If performing oxacillin salt agar screen tests, the laboratory should test S. aureus ATCC 29213 and 43300.

NOTE 5: If performing vancomycin BHI screen tests, the laboratory must test E. faecalis 29212 and 51299.

***An equivalent strain is one which demonstrates reactivity similar to an ATCC strain and for which limits have been established. Organisms which manufacturers recommend or require for use in their systems are acceptable strains of control organisms.*

Table 3. Acceptable Limits for Quality Control Strains Used to Monitor Accuracy of Disk Diffusion Testing of Nonfastidious Organisms (Using Mueller-Hinton Medium Without Blood or Other Supplements)

Antimicrobial Agent	Disk Content	*Escherichia coli* ATCC® 25922[b]	*Staphylococcus aureus* ATCC® 25923	*Pseudomonas aeruginosa* ATCC® 27853	*Escherichia coli* ATCC® 35218[f]
Amikacin	30 μg	19–26	20–26	18–26	–
Amoxicillin-clavulanic acid	20/10 μg	18–24	28–36	–	17–22
Ampicillin	10 μg	16–22	27–35	–	6
Ampicillin-sulbactam	10/10 μg	19–24	29–37	–	13–19
Azithromycin	15 μg	–	21–26	–	–
Azlocillin	75 μg	–	–	24–30	–
Aztreonam	30 μg	28–36	–	23–29	–
Carbenicillin	100 μg	23–29	–	18–24	–
Cefaclor	30 μg	23–27	27–31	–	–
Cefamandole	30 μg	26–32	26–34	–	–
Cefazolin	30 μg	21–27	29–35	–	–
Cefdinir	5 μg	24–28	25–32	–	–
Cefditoren	5 μg	22–28	20–28	–	–
Cefepime	30 μg	31–37	23–29	24–30	–
Cefetamet	10 μg	24–29	–	–	–
Cefixime	5 μg	23–27	–	–	–
Cefmetazole	30 μg	26–32	25–34	–	–
Cefonicid	30 μg	25–29	22–28	–	–
Cefoperazone	75 μg	28–34	24–33	23–29	–
Cefotaxime	30 μg	29–35	25–31	18–22	–
Cefotetan	30 μg	28–34	17–23	–	–
Cefoxitin	30 μg	23–29	23–29	–	–
Cefpodoxime	10 μg	23–28	19–25	–	–
Cefprozil	30 μg	21–27	27–33	–	–
Ceftazidime	30 μg	25–32	16–20	22-29	–
Ceftibuten	30 μg	27–35	–	–	–
Ceftizoxime	30 μg	30–36	27–35	12–17	–
Ceftriaxone	30 μg	29–35	22–28	17–23	–
Cefuroxime	30 μg	20–26	27–35	–	–
Cephalothin	30 μg	15–21	29–37	–	–
Chloramphenicol	30 μg	21–27	19–26	–	–
Cinoxacin	100 μg	26–32	–	–	–
Ciprofloxacin	5 μg	30–40	22–30	25–33	–
Clarithromycin	15 μg	–	26–32	–	–
Clinafloxacin	5 μg	31–40	28–37	27–35	–
Clindamycin	2 μg	–	24–30	–	–
Daptomycin[d]	30 μg	–	18–23	–	–
Dirithromycin	15 μg	–	18–26	–	–
Doxycycline	30 μg	18–24	23–29	–	–
Enoxacin	10 μg	28–36	22–28	22–28	–
Ertapenem	10 μg	29–36	24–31	13–21	–
Erythromycin	15 μg	–	22–30	–	–
Fleroxacin	5 μg	28–34	21–27	12–20	–
Fosfomycin[c]	200 μg	22–30	25–33	–	–
Garenoxacin	**5 μg**	**28-35**	**30-36**	**19-25**	**–**
Gatifloxacin	5 μg	30–37	27–33	20–28	–
Gemifloxacin	5 μg	29–36	27–33	19–25	–
Gentamicin[a]	10 μg	19–26	19–27	16–21	–
Grepafloxacin	5 μg	28–36	26–31	20–27	–
Imipenem	10 μg	26–32	–	20–28	–
Kanamycin	30 μg	17–25	19–26	–	–
Levofloxacin	5 μg	29–37	25–30	19–26	–
Linezolid	30 μg	–	**25–32**	–	–
Lomefloxacin	10 μg	27–33	23–29	22–28	–
Loracarbef	30 μg	23–29	23–31	–	–
Mecillinam	10 μg	24–30	–	–	–

Table 3. (Continued)

Antimicrobial Agent	Disk Content	*Escherichia coli* ATCC® 25922[b]	*Staphylococcus aureus* ATCC® 25923	*Pseudomonas aeruginosa* ATCC® 27853	*Escherichia coli* ATCC® 35218[f]
Meropenem	10 μg	28-34	29–37	27–33	–
Methicillin	5 μg	–	17–22	–	–
Mezlocillin	75 μg	23–29	–	19–25	–
Minocycline	30 μg	19–25	25–30	–	–
Moxalactam	30 μg	28–35	18–24	17–25	–
Moxifloxacin	5 μg	28–35	28–35	17–25	–
Nafcillin	1 μg	–	16–22	–	–
Nalidixic acid	30 μg	22–28	–	–	–
Netilmicin	30 μg	22–30	22–31	17–23	–
Nitrofurantoin	300 μg	20–25	18–22	–	–
Norfloxacin	10 μg	28–35	17–28	22–29	–
Ofloxacin	5 μg	29–33	24–28	17–21	–
Oxacillin	1 μg	–	18–24	–	–
Penicillin	10 units	–	26–37	–	–
Piperacillin	100 μg	24–30	–	25–33	12–18
Piperacillin-tazobactam	100/10 μg	24–30	27–36	25–33	24–30
Quinupristin-dalfopristin	15 μg	–	21–28	–	–
Rifampin	5 μg	8–10	26–34	–	–
Sparfloxacin	5 μg	30–38	27–33	21–29	–
Streptomycin[a]	10 μg	12–20	14–22	–	–
Sulfisoxazole[e]	250 μg or 300 μg	15–23	24–34	–	–
Teicoplanin	30 μg	–	15–21	–	–
Telithromycin	15 μg	–	24–30	–	–
Tetracycline	30 μg	18–25	24–30	–	–
Ticarcillin	75 μg	24–30	–	21–27	6
Ticarcillin-clavulanic acid	75/10 μg	24–30	29–37	20–28	21–25
Tobramycin	10 μg	18–26	19–29	19–25	–
Trimethoprim[e]	5 μg	21–28	19–26	–	–
Trimethoprim-sulfamethoxazole[e]	1.25/23.75 μg	23–29	24–32	–	–
Trospectomycin	30 μg	10–16	15–20	–	–
Trovafloxacin	10 μg	29–36	29–35	21–27	–
Vancomycin	30 μg	–	17–21	–	–

NOTE: Information in boldface type is considered tentative for one year.

Footnotes

a. For control limits of gentamicin 120-μg and streptomycin 300-μg disks, use *Enterococcus faecalis* ATCC® 29212 (gentamicin: 16 to 23 mm; streptomycin: 14 to 20 mm).

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

c. The 200-μg fosfomycin disk contains 50 μg of glucose-6-phosphate.

d. Some lots of Mueller-Hinton agar are deficient in calcium and give small zones.

e. These agents can be affected by excess levels of thymidine and thymine. See M2, Section 4.1.4 for guidance should a problem with quality control occur.

f. Careful organism maintenance is required; refer to M2, Section 10.3.

Table 3. Acceptable Limits for Quality Control Strains Used to Monitor Accuracy of Minimal Inhibitory Concentrations (MICs) (μg/mL) of Nonfastidious Organisms (Using Mueller-Hinton Medium Without Blood or Other Supplements)

Antimicrobial Agent	*Staphylococcus aureus* ATCC® 29213[a]	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Escherichia coli* ATCC® 35218[b]
Amikacin	1–4	64–256	0.5–4	1–4	–
Amoxicillin-clavulanic acid	0.12/0.06–0.5/0.25	0.25/0.12–1.0/0.5	2/1–8/4	–	4/2–16/8
Ampicillin	0.5–2	0.5–2	2–8	–	–
Ampicillin-sulbactam	–	–	2/1–8/4	–	8/4–32/16
Azithromycin	0.5–2	–	–	–	–
Azlocillin	2–8	1–4	8–32	2–8	–
Aztreonam	–	–	0.06–0.25	2–8	–
Carbenicillin	2–8	16–64	4–16	16–64	–
Cefaclor	1–4	–	1–4	–	–
Cefamandole	0.25–1	–	0.25–1	–	–
Cefazolin	0.25–1	–	1–4	–	–
Cefdinir	0.12–0.5	–	0.12–0.5	–	–
Cefditoren	0.25–2	–	0.12–1	–	–
Cefepime	1–4	–	0.016–0.12	1–8	–
Cefetamet	–	–	0.25–1	–	–
Cefixime	8–32	–	0.25–1	–	–
Cefmetazole	0.5–2	–	0.25–2	> 32	–
Cefonicid	1–4	–	0.25–1	–	–
Cefoperazone	1–4	–	0.12–0.5	2–8	–
Cefotaxime	1–4	–	0.03–0.12	8–32	–
Cefotetan	4–16	–	0.06–0.25	–	–
Cefoxitin	1–4	–	2–8	–	–
Cefpodoxime	1–8	–	0.25–1	–	–
Cefprozil	0.25–1	–	1–4	–	–
Ceftazidime	4–16	–	0.06–0.5	1–4	–
Ceftibuten	–	–	0.12–0.5	–	–
Ceftizoxime	2–8	–	0.03–0.12	16–64	–
Ceftriaxone	1–8	–	0.03–0.12	8–64	–
Cefuroxime	0.5–2	–	2–8	–	–
Cephalothin	0.12–0.5	–	4–16	–	–
Chloramphenicol	2–8	4–16	2–8	–	–
Cinoxacin	–	–	2–8	–	–
Ciprofloxacin	0.12–0.5	0.25–2	0.004–0.016	0.25–1	–
Clarithromycin	0.12–0.5	–	–	–	–
Clinafloxacin	0.008–0.06	0.03–0.25	0.002–0.016	0.06–0.5	–
Clindamycin	0.06–0.25	4–16	–	–	–
Daptomycin[c]	0.25–1	1–8	–	–	–
Dirithromycin	1–4	–	–	–	–
Doxycycline	–	–	0.5–2	–	–
Enoxacin	0.5–2	2–16	0.06–0.25	2–8	–
Ertapenem	0.06–0.25	4–16	0.004–0 .016	2–8	–
Erythromycin	0.25–1	1–4	–	–	–
Fleroxacin	0.25–1	2–8	0.03–0.12	1–4	–
Fosfomycin[d]	0.5–4	32–128	0.5–2	2–8	–
Garenoxacin	**0.004-0.03**	**0.03-0.25**	**0.004-0.03**	**0.5-2**	**–**
Gatifloxacin	0.03–0.12	0.12–1.0	0.008–0.03	0.5–2	–
Gemifloxacin	0.008–0.03	0.016–0.12	0.004–0.016	0.25–1	–
Gentamicin[e]	0.12–1	4–16	0.25–1	0.5–2	–
Grepafloxacin	0.03–0.12	0.12–0.5	0.004–0.03	0.25–2.0	–
Imipenem	0.016–0.06	0.5–2	0.06–0.25	1–4	–
Kanamycin	1–4	16–64	1–4	–	–
Levofloxacin	0.06–0.5	0.25–2	0.008–0.06	0.5–4	–
Linezolid	1–4	1–4	–	–	–

Table 3. (Continued)

Antimicrobial Agent	*Staphylococcus aureus* ATCC® 29213[a]	*Enterococcus faecalis* ATCC® 29212	*Escherichia coli* ATCC® 25922	*Pseudomonas aeruginosa* ATCC® 27853	*Escherichia coli* ATCC® 35218[b]
Lomefloxacin	0.25–2	2–8	0.03–0.12	1–4	
Loracarbef	0.5–2	–	0.5–2	>8	
Mecillinam	–	–	0.03–0.25[f]	–	–
Meropenem	0.03–0.12	2–8	0.008–0.06	0.25–1	–
Methicillin	0.5–2	>16	–	–	–
Mezlocillin	1–4	1–4	2–8	8–32	–
Minocycline	0.06–0.5	1–4	0.25–1	–	–
Moxalactam	4–16	–	0.12–0.5	8–32	–
Moxifloxacin	0.016–0.12	0.06–0.5	0.008–0.06	1–8	–
Nafcillin	0.12–0.5	2–8	–	–	–
Nalidixic acid	–	–	1–4	–	–
Netilmicin	≤ 0.25	4–16	≤ 0.5–1	0.5–8	–
Nitrofurantoin	8–32	4–16	4–16	–	–
Norfloxacin	0.5–2	2–8	0.03–0.12	1–4	–
Ofloxacin	0.12–1	1–4	0.015–0.12	1–8	–
Oxacillin	0.12–0.5	8–32	–	–	–
Penicillin	0.25–2	1–4	–	–	–
Piperacillin	1–4	1–4	1–4	1–8	–
Piperacillin-tazobactam	0.25/4–2/4	1/4–4/4	1/4–4/4	1/4–8/4	0.5/4–2/4
Quinupristin-dalfopristin	0.25–1	2–8	–	–	–
Rifampin	0.004–0.016	0.5–4	4–16	16–64	–
Sparfloxacin	0.03–0.12	0.12–0.5	0.004–0.016	0.5–2	–
Sulfisoxazole[g]	32–128	32–128	8–32	–	–
Teicoplanin	0.25–1	0.06–0.25	–	–	–
Telithromycin	0.06–0.25	0.016–0.12	–	–	–
Tetracycline	0.12–1	8–32	0.5–2	8–32	–
Ticarcillin	2–8	16–64	4–16	8–32	–
Ticarcillin-clavulanic acid	0.5/2–2/2	16/2–64/2	4/2–16/2	8/2–32/2	**8/2–32/2**
Tobramycin	0.12–1	8–32	0.25–1	0.25–1	–
Trimethoprim[g]	1–4	≤ 1	0.5–2	>64	–
Trimethoprim-sulfamethoxazole	≤ 0.5/9.5	≤ 0.5/9.5	≤ 0.5/9.5	8/152–32/608	–
Trospectomycin	2–16	2–8	8–32	–	–
Trovafloxacin	0.008–0.03	0.06–0.25	0.004–0.016	0.25–2	–
Vancomycin[h]	0.5–2	1–4	–	–	–

NOTE 1: These MICs were obtained in several reference laboratories by broth microdilution. If four or fewer concentrations are tested, quality control may be more difficult.

NOTE 2: Information in boldface type is considered tentative for one year.

NOTE 3: For four-dilution ranges, results at the extremes of the acceptable range(s) should be suspect. Verify control validity with data from other control strains.

Footnotes

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

b. Careful organism maintenance is required; refer to M7, Section 12.4. .

c. QC ranges reflect MICs obtained when Mueller-Hinton broth is supplemented with calcium to a final concentration of 50 μg/mL.

d. The approved MIC susceptibility testing method is agar dilution. Agar media should be supplemented with 25 μg/mL of glucose-6-phosphate. Broth dilution should not be performed.

e. For control organisms for gentamicin and streptomycin high-level aminoglycoside screen tests for enterococci, see Table 2D.

f. This test should be performed by agar dilution only.

g. Very medium-dependent, especially with enterococci.

h. For control organisms for vancomycin screen test for enterococci, see Table 2D.

Table 3A. Acceptable Limits for Quality Control Strains Used to Monitor Accuracy of Minimal Inhibitory Concentrations (MICs) (µg/mL) of Fastidious Organisms

Antimicrobial Agent	*Haemophilus influenzae* ATCC® 49247 [a]	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619	*Helicobacter pylori* ATCC® 43504	*Campylobacter jejuni* ATCC® 33560 [b] 36 °C/48 hours	*Campylobacter jejuni* ATCC® 33560 [b] 42 °C/24 hours
Amoxicillin	–	–	–	0.03–0.12	0.016–0.12	–	–
Amoxicillin-clavulanic	2/1–16/8	–	–	0.03/0.016–0.12/0.06	–	–	–
Ampicillin	2–8	–	–	0.06–0.25	–	–	–
Ampicillin-sulbactam	2/1–8/4	–	–	–	–	–	–
Azithromycin	1–4	–	–	0.06–0.25	–	–	–
Aztreonam	0.12–0.5	–	–	–	–	–	–
Cefaclor	–	1–4	–	1–4	–	–	–
Cefamandole	–	0.25–1	–	–	–	–	–
Cefdinir	–	0.12–0.5	0.008–0.03	0.03–0.25	–	–	–
Cefditoren	0.06–0.25	–	–	0.016–0.12	–	–	–
Cefepime	0.5–2	–	0.016–0.06	**0.03–0.25**	–	–	–
Cefetamet	0.5–2	–	0.016–0.25	0.5–2	–	–	–
Cefixime	0.12–1	–	0.004–0.03	–	–	–	–
Cefmetazole	2–16	–	0.5–2	–	–	–	–
Cefonicid	–	0.06–0.25	–	–	–	–	–
Cefotaxime	0.12–0.5	–	0.015–0.06	0.03–0.12	–	–	–
Cefotetan	–	–	0.5–2	–	–	–	–
Cefoxitin	–	–	0.5–2	–	–	–	–
Cefpirome	0.25–1	–	–	–	–	–	–
Cefpodoxime	0.25–1	–	0.03–0.12	0.03–0.12	–	–	–
Cefprozil	–	1–4	–	0.25–1	–	–	–
Ceftazidime	0.12–1	–	0.03–0.12	–	–	–	–
Ceftibuten	0.25–1	–	–	–	–	–	–
Ceftizoxime	0.06–0.5	–	0.008–0.03	0.12–0.5	–	–	–
Ceftriaxone	0.06–0.25	–	0.004–0.016	0.03–0.12	–	–	–
Cefuroxime	–	0.25–1	0.25–1	0.25–1	–	–	–
Cephalothin	–	–	–	0.5–2	–	–	–
Chloramphen-icol	0.25–1	–	–	2–8	–	–	–
Ciprofloxacin	0.004–0.03	–	0.001–0.008	–	–	**0.12-1**	**0.06-0.5**
Clarithromycin	4–16	–	–	0.03–0.12	0.016–0.12	–	–
Clinafloxacin	0.001–0.008	–	–	0.03–0.12	–	–	–
Clindamycin	–	–	–	0.03–0.12	–	–	–
Daptomycin [c]	–	–	–	0.06–0.5	–	–	–
Dirithromycin	8–32	–	–	0.06–0.25	–	–	–
Doxycycline	–	–	–	–	–	**0.5–2**	**0.25–2**
Enoxacin	–	–	0.016–0.06	–	–	–	–
Ertapenem	–	0.016–0.06	–	0.03–0.25	–	–	–
Erythromycin	–	–	–	0.03–0.12	–	**1–8**	**1–4**
Fleroxacin	0.03–0.12	–	0.008–0.03	–	–	–	–
Garenoxacin	**0.002-0.008**	**–**	**–**	**0.016–0.06**	**–**	–	–
Gatifloxacin	0.004–0.03	–	0.002–0.016	0.12–0.5	–	–	–
Gemifloxacin	0.002–0.008	–	–	0.008–0.03	–	–	–
Gentamicin	–	–	–	–	–	**0.5–2**	**0.5–4**
Grepafloxacin	0.002–0.016	–	0.004–0.03	0.06–0.5	–	–	–
Imipenem	–	0.25–1	–	0.03–0.12	–	–	–
Levofloxacin	0.008–0.03	–	–	0.5–2	–	–	–
Linezolid	–	–	–	0.5–2	–	–	–
Lomefloxacin	0.03–0.12	–	0.008–0.03	–	–	–	–
Loracarbef	–	0.5–2	–	2–8	–	–	–
Metronidazole	–	–	–	–	64–256	–	–
Meropenem	–	0.03–0.12	–	0.06–0.25	–	**0.004–0.015**	**0.008–0.03**
Moxifloxacin	0.008–0.03	–	–	0.06–0.25	–	–	–

Table 3A. (Continued)

Antimicrobial Agent	*Haemophilus influenzae* ATCC® 49247 [a]	*Haemophilus influenzae* ATCC 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619	*Helicobacter pylori* ATCC® 43504	*Campylobacter jejuni* ATCC® 33560 [b] 36 °C/48 hours	*Campylobacter jejuni* ATCC® 33560 [b] 42 °C/24 hours
Nitrofurantoin	–	–	–	4–16	–	–	–
Norfloxacin	–	–	–	2–8	–	–	–
Ofloxacin	0.016–0.06	–	0.004–0.016	1–4	–	–	–
Penicillin	–	–	0.25–1	0.25-1	–	–	–
Piperacillin-tazobactam	0.06/4–0.5/4	–	–	–	–	–	–
Quinupristin-dalfopristin	2-8	–	–	0.25-1	–	–	–
Rifampin	0.25–1	–	–	0.015–0.06	–	–	–
Sparfloxacin	0.004–0.016	–	0.004–0.016	0.12–0.5	–	–	–
Spectinomycin	–	–	8–32	–	–	–	–
Telithromycin	1–4	–	–	0.004–0.03	0.06–0.5	–	–
Tetracycline	4–32	–	0.25–1	0.12–0.5	0.12–1.0	–	–
Trimethoprim-sulfamethoxazole	0.03/0.59-0.25/4.75	–	–	0.12/2.4–1/19	–	–	–
Trospectomycin	0.5–2	–	1–4	1–4	–	–	–
Trovafloxacin	0.004–0.016	–	0.004–0.016	0.06–0.25	–	–	–
Vancomycin	–	–	–	0.12–0.5	–	–	–

Testing Conditions for Clinical Isolates and Performance of Quality Control

Organism	*Haemophilus influenzae*	*Neisseria gonorrhoeae*	*Streptococcus pneumoniae*	*Helicobacter pylori*	***Campylobacter* spp.**
Medium	Broth dilution: Haemophilus Test Medium (HTM) broth	Agar dilution: GC agar base and 1% defined growth supplement. The use of a cysteine-free supplement is required for agar dilution tests with carbapenems and clavulanate. Cysteine-containing defined growth supplements *do not* significantly alter dilution test results with other drugs.	Broth dilution: Cation-adjusted Mueller-Hinton broth with lysed horse blood (2-5% v/v).	Agar Dilution: Mueller-Hinton agar with aged (≥ 2-week-old) sheep blood (5% v/v).	**Agar dilution: Mueller-Hinton agar with 5% defibrinated sheep blood**
Inoculum	Direct colony suspension, **equivalent to a 0.5 McFarland standard**	Direct colony suspension, **equivalent to a 0.5 McFarland standard**	Direct colony suspension, **equivalent to a 0.5 McFarland standard**	See footnote d, below.	**Direct colony suspension, equivalent to a 0.5 McFarland standard**
Incubation Characteristics	35 °C; ambient air; 20-24 hours	35 °C; 5% CO_2; 20-24 hours	35 °C; ambient air; 20-24 hours	35 °C; 3 days; microaerobic atmosphere produced by gas-generating system suitable for campylobacters.	**36 °C /48 hours or 42 °C/ 24 hours; 10% CO_2, 5% O_2 and 85% N_2 or a microaerophilic environment**

NOTE 1: Information in boldface type is considered tentative for one year.

NOTE 2: For four-dilution ranges, results at the extremes of the acceptable range(s) should be suspect. Verify control validity with data from other control strains.

Footnotes

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

b. Since some isolates of *C. jejuni* ssp. *doylei*, *C. fetus* and *C. lari* may not grow at 42 °C, susceptibility testing of these isolates should be performed at 36 °C.

c. QC ranges reflect MICs obtained when Mueller-Hinton broth is supplemented with calcium to a final concentration of 50 μg/mL.

d. The inoculum for testing of *Helicobacter pylori* should be as follows: a saline suspension equivalent to a 2.0 McFarland standard (containing $1x10^7$ to $1x10^8$ CFU/mL), to be prepared from a 72-hour-old subculture from a blood agar plate. The inoculum (1 to 3 μL per spot) is replicated directly on the antimicrobial agent-containing agar dilution plate

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Interpretive Guidelines §493.1271(a)(1)

The following table defines the frequency and the type of quality control to be performed for each container of antisera and reagent red cells use for immunohematology testing:

Reagent	***Positive Control***	***Negative Control***
ABO Antisera	*Each day of use*	*N/A*
Rh Antisera	*Each day of use*	*Each day of use*
Other Anti-sera	**Each day of use*	*Each day of use*
**Anti-human globulin sera*	**Each day of use*	**Each day of use*
ABO Reagent red cells	*Each day of use*	*N/A*
Antibody Screening cells	*Each day of use (at least one known antibody)*	*N/A*

In daily quality control testing, it is sufficient to test antiglobulin serum for IgG only. Anticomplement activity can be checked, if desired, against complement coated RBC's but this need not be a routine procedure.

**This requirement is satisfied by checking the antihuman immune globulin (Coombs Serum) in one of the following ways:*

- *React anti-human globulin with a pre-sensitized reagent red blood cell which is either prepared commercially or by the laboratory;*
- *Perform the quality control for antibody detection using a known antibody which is demonstrated by the addition of antihuman globulin; or*
- *Add a pre-sensitized reagent red blood cell to all negative antiglobulin tests (direct antiglobulin, indirect antiglobulin, antibody detection and identification test) to indicate that antiglobulin serum present in the test was not inactivated by*

Interpretive Guidelines §493.1271(a)(1)

unbound globulins or diluted by excess residual saline, and that the negative results reflect true absence of reactivity in the test. Using green antiglobulin serum does not substitute for this control.

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Interpretive Guidelines §493.1276(b)(1)-(b)(3)

Culture Type	***Minimum Number of Spreads Counted per Patient***	***Minimum Number of Cells Analyzed per Patient***
Amniotic Fluid		
Flasks	*15 cells from at least 2 independent primary cultures*	*5 cells from at least 2 independent primary cultures*
in situ	*15 cells from at least 10 colonies from 2 independent primary cultures*	*5 cells from different colonies and split between different primary cultures*

Many laboratories use a combination of the flask and in situ culture methods or use the flask method as a backup for the in situ method.

Chorionic Villus		
Direct	*15 cells*	*5 cells*
Culture	*as in amniotic fluid, flask technique*	
Peripheral Blood		
Constitutional	*20 cells*	*5 cells*
Possible sex chromosome abnormality	*30 cells (total count)*	*5 cells*

Culture Type	***Minimum Number of Spreads Counted per Patient***	***Minimum Number of Cells Analyzed per Patient***
Blood (cancer)	*20 cells*	*20 cells*
Bone Marrow (cancer)	*20 cells*	*20 cells*
Tissue Fibroblasts	*15 cells from 2 independent cultures*	*5 cells split between 2 independent cell cultures*

For confirmation of chromosomally abnormal amniotic fluid results, or familial chromosome abnormality, examination of fewer cells is permitted.

Table 2. Manufacturers' Quality Assurance Procedure for Commercially Prepared Media

Medium	Atmosphere, Length of Incubation[1]	Control Organisms (ATCC No.)[2]	Expected Results
Anaerobic sheep blood and laked blood agar media (non-selective)	Anaerobic, 24–48 h	*B. fragilis* (25285) *C. perfringens* (13124) *F. nucleatum* (25586) *P. anaerobius* (27337)	Growth Growth, beta hemolysis Growth Growth
Anaerobic broths - see Thioglycollate medium			
Blood Agar-nonselective sheep blood agar media	Aerobic or CO_2, 24 h	*S. pyogenes* (19615) *S. pneumoniae* (6305) *S. aureus* (25923) *E. coli* (25922)	Growth, beta hemolysis Growth, alpha hemolysis Growth Growth
Blood Agar-CAMP Test (TSA with sheep blood only)	Aerobic, 24 h	*S. aureus* (33862) or *S. aureus* (25923) *S. agalactiae* (12386) *S. pyogenes* (19615)	Positive reaction (arrowhead area of clearing) Negative reaction (no arrowhead formation)
Blood Agar-Selective sheep blood agar media (Columbia CNA Agar, phenylethyl alcohol agar)	Columbia CNA CO_2, 24–48h	*S. pyogenes* (19615) *S. pneumoniae* (6305) *S. aureus* (25923) *P. mirabilis* (12453)	Growth, beta hemolysis Growth, alpha hemolysis Growth Inhibition (partial)
	Phenylethyl alcohol agar CO_2, 24–48h	*S. pyogenes* (19615) *S. aureus* (25923) *P. mirabilis* (12453)	Growth Growth Inhibition (partial)
Blood culture media (This applies to BHI, TSB, and Thiol-based media. Other media for blood culture are exempt from user performance testing provided that manufacturers certify that additional organisms appropriate for their intended use have been tested.)	Anaerobic (nonvented) within 5 days	*B. fragilis* (25285) *S. pneumoniae* (6305)	Growth Growth
	Aerobic (vented) within 5 days	*P. aeruginosa* (27853) *S. pneumoniae* (6305)	Growth Growth
Campylobacter agar *(User quality control required.)*	Reduced O_2, enriched with CO_2, 42 °C, 48 h	*C. jejuni* (33291) *E. coli* (25922)	Growth Inhibition (partial)

Table 2. Manufacturers' Quality Assurance Procedure for Commercially Prepared Media (Continued)

Medium	Atmosphere, Length of Incubation[1]	Control Organisms (ATCC No.)[2]	Expected Results
Chocolate agar	CO_2, 24 and 48 h	*N. gonorrhoeae* (43069 or 43070) *H. influenzae* (10211)	Growth Growth
CIN Agar	Aerobic, 24–48 h 25 °C	*Y. enterocolitica* (9610) *E. coli* (25922) *P. aeruginosa* (27853) *E. faecalis* (29212)	Growth; deep red center, transparent border (bulls eye) Inhibition (partial to complete) Inhibition (partial to complete) Inhibition (partial to complete)
CLED Agar	Aerobic, 24–48 h	*E. coli* (25922) *P. vulgaris* (8427) *S. aureus* (25923)	Growth; yellow centers Growth; bluish, spreading inhibited (partial) Growth; uniform deep yellow
CYE/BCYE Agar	Aerobic, 48–72 h	*L. pneumophilia* (33152) *L. bozemanii* (33217)	Growth; yellow-green fluorescence under long-wave u.v. light Growth; blue-white fluorescence under long-wave u.v. light
Enrichment broths for enterics (GN Broth, Selenite Broths)	Aerobic, up to 24 h	*S. typhimurium* (14028) *S. sonnei* (9290) *E. coli* (25922)	Growth on subculture Growth on subculture (may be inhibited by Selenite media) Inhibition (partial to complete) on subculture. Growth on subculture from GN broth
Eosin methylene blue media (Levine EMB Agar; EMB Agar, modified)	Aerobic, 24 h	*S. typhimurium* (14028) *E. coli* (25922) *E. faecalis* (29212)	Growth, colorless to amber colonies Growth, blue-black colonies w/green metallic sheen Inhibition (partial)
Hektoen enteric agar	Aerobic, 24 h	*S. typhimurium* (14028) *S. flexneri* (12022) *E. faecalis* (29212) *E. coli* (25922)	Growth, colonies blue to green-blue with black centers Growth, colonies green to blue-green Inhibition (partial; colonies yellow) Inhibition (partial to complete; colonies yellow to salmon colored)
MacConkey agar	Aerobic, 24 h	*E. coli* (25922) *P. mirablis* (12453) *S. typhimurium* (14028) *E. faecalis* (29212)	Growth, pink colonies Growth, colorless colonies, inhibition of swarming (partial) Growth, colorless colonies Inhibition (partial)
Mannitol salt agar	Aerobic, 24 and 48 h	*S. aureus* (25923) *S. epidermidis* (12228) *P. mirabilis* (12453)	Growth, colonies have yellow zones at 48 h Growth, colonies have red zones at 48 h Inhibition (partial)

Table 2. Manufacturers' Quality Assurance Procedure for Commercially Prepared Media (Continued)

Medium	Atmosphere, Length of Incubation[1]	Control Organisms (ATCC No.)[2]	Expected Results
Mycobacteria agar media (Lowenstein–Jensen and Middlebrook)	CO_2 up to 21 days	*M. tuberculosis* H37Ra (25177) *M. kansasii* Group I (12478) *M. scrofulaceum* Group II (19981) *M. intracellulare* Group III (13950) *M. fortuitum* Group IV (6841) *E. coli* (25922)	Growth Growth Growth—May be inhibited on selective L-J and selective Middlebrook media Growth—May be inhibited on selective L-J and selective Middlebrook media Growth Inhibition (partial to complete)—Use only for selective mycobacteria media
Sabouraud dextrose agar	Aerobic, up to 7 days 25–35 °C	*C. albicans* (60193 or 10231) *T. mentagrophytes* (9533)	Growth Growth
Salmonella–Shigella (SS) agar	Aerobic, 24 h	*S. typhimurium* (14028) *S. flexneri* (12022) *E. faecalis* (29212) *E. coli* (25922)	Growth, colonies colorless with or without black centers Growth, colorless colonies Inhibition (complete) Inhibition (partial to complete; colonies pink to rose-red with precipitate)
Selective mycology media (media containing cycloheximide and chloramphenicol, excluding inhibitory mold agar)	Aerobic, up to 7 days 25 °C	*A. niger* (16404) *C. albicans* (10231) *T. mentagrophytes* (9533) *E. coli* (25922)	Inhibition (partial to complete) on media containing cycloheximide Growth Growth Inhibition (partial to complete) on media containing chloramphenicol
Selective media for pathogenic *Neisseria* spp. *(User quality control required.)*	CO_2, 24–48 h	*N. gonorrhoeae* (43069 or 43070) *N. meningitidis* (13090)[3] *P. mirabilis* (43071) *E. coli* (25922)[3] *N. sicca* (9913)[3] *C. albicans* (60193)[3] *S. epidermidis* (12228)	Growth Growth Inhibition (partial)—use only for media containing trimethoprim Inhibition (partial) Inhibition (partial) Inhibition (partial) Inhibition (partial)
Selective media for enterococci, with azide	Aerobic, 24 and 48 h	*E. faecalis* (29212) *S. pyogenes* (19615) *E. coli* (25922)	Growth, blackening around colonies Inhibition (partial to complete) Inhibition (partial)—Colorless colonies on bile esculin agar
Selective media for enterococci, without azide	Aerobic, 24 and 48 h	*E. faecalis* (29212) *S. pyogenes* (19615)	Growth, blackening around colonies Inhibition (partial to complete)

Table 2. Manufacturers' Quality Assurance Procedure for Commercially Prepared Media (Continued)

Medium	Atmosphere, Length of Incubation[1]	Control Organisms (ATCC No.)[2]	Expected Results
Thioglycolate medium, with or without indicator	Aerobic, 48 h (tightened cap)	*B. fragilis* (25285) *S. aureus* (25923)	Growth Growth
Thioglycolate medium, enriched with vitamin K and hemin	Aerobic, 48 h (tightened cap)	*P. anaerobius* (27337) *B. vulgatus* (8482) *C. perfringens* (13124)	Growth Growth Growth
Tubed media (BHI and Tryptic Soy Broth)	Aerobic, 24-48 h	*E. coli* (25922) *S. aureus* (25923)	Growth Growth
XLD (xylose lysine desoxycholate) Agar	Aerobic, 24 h	*S. typhimurium* (14028) *S. flexneri* (12022) *E. faecalis* (29212) *E. coli* (25922)	Growth—Colonies red with black centers Growth—Colonies red Inhibition (partial) Inhibition (partial to complete; colonies yellow to yellow-red)

[1]Temperature is 35 °C unless otherwise specified.
[2]ATCC is a registered trademark of the American Type Culture Collection.
[3]Required for commercial manufacturers; not necessary for testing by users.