§493.1261 Standard: Bacteriology.

(a) The laboratory must check the following for positive and negative reactivity using control organisms:

Interpretive Guidelines §493.1261(a)
When condition level deficiencies in Bacteriology are in any or all phases of testing, use D5002.

For direct antigen systems, laboratories may use bacterial cell suspensions to meet the requirement for control organisms since the cell suspensions are subjected to both the extraction and reaction phases of the test. However, a matrix similar to patient specimens is preferred. For example, for direct antigen tests for group A streptococcal antigen, ready prepared, dried (solid-shafted) swabs, one containing group A streptococcus (S. pyogenes) as a positive control and another with non-group A streptococcus and/or Staphylococcus aureus as a negative control may be used. Use D5453 for deficiencies related to the extraction process.

Additionally, if the manufacturer's instructions do not specify what the positive control contains, the laboratory should contact the manufacturer to ensure that the positive control contains a cell suspension of the organism. Otherwise, the laboratory must have an alternative mechanism for meeting this requirement (e.g., laboratory suspension stock ATTCC organism, commercially prepared organism controls).

For microbial identification systems utilizing two or more substrates, the laboratory must check each media using control organisms to verify positive and negative reactivity of each substrate. Use D5471.

If a laboratory utilizes primary isolation media (e.g., MacConkey, CLED, EMB), for presumptive identification of organisms, then the media should meet the quality control requirements at D5471 and D5477.

For bacitracin, catalase, coagulase plasma, desoxycholate, oxidase, PYR disks, spot indole, staphylococcal latex reagents, streptococcal latex grouping reagents, and X and V factor strips and disks, use D5471.

For bacteriology, XV discs or strips need only be checked with an organism that produces a positive reaction. Use 5471.

For guidelines for molecular amplification testing, use D5455.

D5501

§493.1261 Standard: Bacteriology.

(a)(1) Each day of use for beta-lactamase methods other than Cefinase™.

Interpretive Guidelines §493.1261(a)(1)
Beta-lactamase testing performed by acidometric, iodometric or chromogenic methodologies other than Cefinase™ must have positive and negative reactivity checked each day of use.

For Cefinase™, use D5471.

D5503

§493.1261 Standard: Bacteriology.

(a)(2) Each week of use for Gram stains.

D5505

§493.1261 Standard: Bacteriology.

(a)(3) When each batch (prepared in-house), lot number (commercially prepared), and shipment of antisera is prepared or opened, and once every 6 months thereafter.

Interpretive Guidelines §493.1261(a)(3)
In addition to Salmonella and Shigella antisera, antisera used for serotyping of homologous isolates, (i.e., streptococcal serotyping systems) must be checked for positive and negative reactivity. Polyvalent antisera should be tested with at least one organism from each polyvalent group.

Requirements for antisera QC apply to testing that has a direct impact on patient care.

D5507

§493.1261 Standard: Bacteriology.

(b) For antimicrobial susceptibility tests, the laboratory must check each batch of media and each lot number and shipment of antimicrobial agent(s) before, or concurrent with, initial use, using approved control organisms.
(b)(1) Each day tests are performed, the laboratory must use the appropriate control organism(s) to check the procedure.
(b)(2) The laboratory's zone sizes or minimum inhibitory concentration for control organisms must be within established limits before reporting patient results.

Interpretive Guidelines §493.1261(b)(1-2)
"Approved control organism(s)" means either an appropriate control strain or an equivalent strain as defined below.

The laboratory must ensure proper standardization of the inoculum (e.g., use a 0.5 McFarland standard or its optical equivalent, or follow manufacturer's instructions for a commercially available system). Use D5437.

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.*

When testing is performed daily, for each antimicrobial agent/organism combination, 1 out of every 20 consecutive results may be out of the acceptable range. Any more than 1 out-of-control result in 20 consecutive tests requires corrective action.

EXCEPTION: The laboratory may test each appropriate control strain a minimum of once each week, provided the following requirements are met:

The laboratory must document that appropriate control strains were tested for 20 or 30 consecutive test days. For each antimicrobial agent/organism combination, no more than one out of 20 or three out of the 30 zone diameters (i.e., zone diameters obtained from one antimicrobial agent-organism combination for 20 or 30 consecutive test days) may be

*outside established acceptable limits for quality control strains. These limits may be established by the laboratory, or the laboratory may use the acceptable ranges provided in Table 3*** and 3A*** of the NCCLS Standard, "Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eighth Edition (M2-A8)."*

NOTE: This procedure is to be used for demonstrating satisfactory performance for conversion from daily to weekly quality control of the antimicrobial disk diffusion test.

After a laboratory has implemented weekly quality control testing of the disk diffusion test:

- *Quality control testing must be performed whenever any reagent component of the test is changed (e.g., a new lot of agar or a new lot of disks);*
- *Corrective action is required if any of the weekly quality control results are outside of the established acceptable range;*
- *If a new antimicrobial agent is added, it must be tested for 20 or 30 consecutive days and have satisfactory performance documented before it can be tested on a weekly schedule; and*
- *If there is a major change on the method of reading test results, such as conversion from manual zone measurements to an automated zone reader, 20 or 30 days of testing is required.*

If a zone diameter is observed outside the established acceptable limits for quality control strains during weekly quality control testing, the following corrective action(s) are necessary:

- *If there is an obvious reason for the out-of-control result (e.g., use of the wrong disk, use of the wrong control strain, obvious contamination of the strain, inadvertent use of the wrong incubation temperature or conditions), document the reason and retest the strain on the day the error is observed. If the repeat result is within range, no further corrective action is necessary.*
- *If there is not an obvious reason for the out-of-control result, test the implicated antimicrobial agent/organism combination on the day the error is observed and test for a total of 5 consecutive test days. If all 5 zone diameter measurements for the antimicrobial agent/organism combination are within the established acceptable ranges, no additional corrective action is necessary.*

For the last item mentioned above, if any of the 5 zone diameter measurements are outside the established acceptable range, additional corrective action is necessary. Daily control of tests must be continued until final resolution of the problem is achieved. Once the problem is corrected, in order to return to weekly quality control testing, documentation of satisfactory performance for another 20 or 30 consecutive days is required.

Direct susceptibility testing is a modification of the standardized disk diffusion susceptibility testing method. Therefore, the laboratory must establish the interpretive zone diameters for patient specimens, as well as establish the zone diameters for quality control organisms. Since direct susceptibility testing is not a recommended NCCLS method, the laboratory may not go to weekly quality control, but must perform quality control daily using appropriate control organisms.

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.*

Each day the test is performed, the appropriate control strain(s) must be included to check the test system.

When testing is performed daily, for each antimicrobial agent/organism combination, 1 out of every 20 consecutive results may be out of the acceptable range. Any more than 1 out-of-control result in 20 consecutive tests requires corrective action.

*Refer to Table 3A*** of the NCCLS Standard, "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Sixth Edition (M7-A6)" to determine the control strain to be used when performing MIC tests on isolates of Campylobacter jejuni, Haemophilus spp., Neisseria gonorrhoeae, Streptococcus pneumoniae or other organisms as applicable.*

EXCEPTION: The laboratory may test each appropriate control strain(s) a minimum of once each test week if the following requirements are met:

*The laboratory must document that appropriate control strains are tested for a minimum of 20 or 30 consecutive test days. For each antimicrobial agent/organism combination, no more than one out of 20 or three out of 30 MICs values (i.e., MIC values obtained from one antimicrobial agent/organism combination for 20 or 30 consecutive test days) may be outside established acceptable limits for quality control strains. These limits may be established by the laboratory or the laboratory may use the acceptable range provided in Table 3*** and Table 3A*** of the NCCLS Standard, "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Sixth Edition (M7-A6)."*

NOTE: This procedure is to be used only for demonstrating satisfactory performance for conversion from daily to weekly quality control testing of the MIC test.

After a laboratory has implemented weekly quality control testing of the MIC test:

- *Quality control testing must be performed whenever any reagent component of the test is changed (i.e., a new lot of broth from the same manufacturer);*
- *Corrective action is required if any of the weekly quality control results is outside of the established acceptable range;*
- *If a new antimicrobial agent is added or a different broth manufacturer is used, it must be tested for 20 or 30 consecutive days and have satisfactory performance documented before it can be tested on a weekly schedule; and*
- *If there is a major change on the method of reading test results, such as conversion from a visual reading of MICs to an instrument reading or conversion in the type of panel used (i.e., changing from breakpoint to MIC panels), 20 or 30 days of testing is required.*

Whenever an MIC value is observed outside the established acceptable limits for quality control strains during weekly quality control testing, the following corrective actions are required:

- *If there is an obvious reason for the out-of-control result (e.g., use of the wrong control strain, obvious contamination of the strain or the medium, inadvertent use of the wrong incubation conditions), document the reason and retest the strain on the day the error is observed. If the repeat result is within range, no further corrective action is necessary.*
- *If there is not an obvious reason for the out-of-control result, test the implicated antimicrobial agent/organism combination on the day the error is observed and test for a total of five (5) consecutive test days. If all 5 MICs for the antimicrobial agent/organism combination are within the established acceptable range, no additional corrective action is necessary.*

For the last item mentioned above, if any of the 5 MICs is outside the established acceptable range, additional corrective action is necessary. Daily control of tests must be continued until final resolution of the problem is achieved. Once the problem is corrected, in order to return to weekly quality control testing, documentation of satisfactory performance for another 20 or 30 consecutive days is required.

NCCLS does not address performance issues or make recommendations about any commercial test system such as the E-test. The E-test is a quantitative technique test that determines antimicrobial susceptibility by using a predefined antibiotic gradient to determine the MIC of an individual antibiotic when tested on agar media by overnight incubation. Laboratories using the E-test should rely on the manufacturer to establish the appropriate accuracy control limits and may go to weekly quality control testing after meeting the requirements mentioned in the exception for the procedure to be used for demonstrating satisfactory performance for conversion from daily to weekly quality control testing of the MIC test.

****Permission to use portions of NCCLS publications M2-A8 (Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eighth Edition), and M7-A6 (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Sixth Edition), has been granted by NCCLS. Permission to reproduce additional copies or otherwise use the text of M2-A8, and M7-A6 to an extent not permitted under applicable Copyright Law must be obtained from NCCLS by written request. The quality control limits and the interpretive breakpoint tables for the M2-A8 and M7-A6 standards are related and are valid only if the methodology in these standards is used. NCCLS frequently updates the tables through new editions of the standards and supplements. Users should have the most recent editions. The current standards may be obtained from NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087; 610.688.0100, www.nccls.org.*

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 3A. Acceptable Limits for Quality Control Strains Used to Monitor Accuracy of Disk Diffusion Testing of Fastidious Organisms

Antimicrobial Agent	Disk Content	*Haemophilus influenzae* ATCC® 49247 [a]	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619 [b]
Amoxicillin-clavulanic acid	20/10 μg	15–23	–	–	–
Ampicillin	10 μg	13–21	–	–	30–36
Ampicillin-sulbactam	10/10 μg	14–22	–	–	–
Azithromycin	15 μg	13–21	–	–	19–25
Aztreonam	30 μg	30–38	–	–	–
Cefaclor	30 μg	–	25–31	–	24–32
Cefdinir	5 μg	–	24–31	40–49	26–31
Cefditoren	5 μg	25–34	–	–	27–35
Cefepime	30 μg	25–31	–	37–46	28–35
Cefetamet	10 μg	23–28	–	35–43	–
Cefixime	5 μg	25–33	–	37–45	16–23
Cefmetazole	30 μg	16–21	–	31–36	–
Cefonicid	30 μg	–	30–38	–	–
Cefotaxime	30 μg	31–39	–	38–48	31–39
Cefotetan	30 μg	–	–	30–36	–
Cefoxitin	30 μg	–	–	33–41	–
Cefpodoxime	10 μg	25–31	–	35–43	28–34
Cefprozil	30 μg	–	20–27	–	25–32
Ceftazidime	30 μg	27–35	–	35–43	–
Ceftibuten	30 μg	29–36	–	–	–
Ceftizoxime	30 μg	29–39	–	42–51	28–34
Ceftriaxone	30 μg	31–39	–	39–51	30–35
Cefuroxime	30 μg	–	28–36	33–41	–
Cephalothin	30 μg	–	–	–	26–32
Chloramphenicol	30 μg	31–40	–	–	23–27
Ciprofloxacin	5 μg	34–42	–	48–58	–
Clarithromycin	15 μg	11–17	–	–	25–31
Clinafloxacin	5 μg	34–43	–	–	27–34
Clindamycin	2 μg	–	–	–	19–25
Daptomycin[c]	30 μg	–	–	–	19–26
Dirithromycin	15 μg	–	–	–	18–25
Enoxacin	10 μg	–	–	43–51	–
Ertapenem	10 μg	20–28	27–33	–	28–35
Erythromycin	15 μg	–	–	–	25–30
Fleroxacin	5 μg	30–38	–	43–51	–
Garenoxacin	**5 μg**	**33-41**	**–**	**–**	**26–33**
Gatifloxacin	5 μg	33–41	–	45–56	24–31
Gemifloxacin	5 μg	30–37	–	–	28–34
Grepafloxacin	5 μg	32–39	–	44–52	21–28
Imipenem	10 μg	21–29	–	–	–
Levofloxacin	5 μg	32–40	–	–	20–25
Linezolid	30 μg	–	–	–	**25–34**
Lomefloxacin	10 μg	33–41	–	45–54	–
Loracarbef	30 μg	–	26–32	–	22–28
Meropenem	10 μg	20–28	–	–	28–35
Moxifloxacin	5 μg	31–39	–	–	25–31
Nitrofurantoin	300 μg	–	–	–	23–29
Norfloxacin	10 μg	–	–	–	15–21
Ofloxacin	5 μg	31–40	–	43–51	16–21
Oxacillin	1 μg	–	–	–	≤ 12[d]
Penicillin	10 units	–	–	26–34	24–30
Piperacillin-tazobactam	100/10 μg	33–38	–	–	–
Quinupristin-dalfopristin	15 μg	15–21	–	–	19–24
Rifampin	5 μg	22–30	–	–	25–30

Table 3A. (Continued)

Antimicrobial Agent	Disk Content	*Haemophilus influenzae* ATCC® 49247 [a]	*Haemophilus influenzae* ATCC® 49766	*Neisseria gonorrhoeae* ATCC® 49226	*Streptococcus pneumoniae* ATCC® 49619 [b]
Sparfloxacin	5 μg	32–40	–	43–51	21–27
Spectinomycin	100 μg	–	–	23–29	–
Telithromycin	15 μg	17–23	–	–	27–33
Tetracycline	30 μg	14–22	–	30–42	27–31
Trimethoprim-sulfamethoxazole	1.25/23.75 μg	24–32	–	–	20–28
Trospectomycin	30 μg	22–29	–	28–35	–
Trovafloxacin	10 μg	32–39	–	42–55	25–32
Vancomycin	30 μg	–	–	–	20–27

Disk Diffusion Testing Conditions for Clinical Isolates and Performance of Quality Control

Organism	Haemophilus influenzae	Neisseria gonorrhoeae	Streptococcus pneumoniae
Medium	Haemophilus *Test Medium*	*GC agar base and 1% defined growth supplement. The use of a cysteine-free growth supplement is not required for disk diffusion testing.*	*MHA supplemented with 5% defibrinated sheep blood*
Inoculum	*Direct colony suspension*	*Direct colony suspension*	*Direct colony suspension*
Incubation Characteristics	*5% CO_2; 16-18 hours; 35 °C*	*5% CO_2; 20-24 hours; 35 °C*	*5% CO_2; 20-24 hours; 35 °C*

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

Footnotes

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

b. Despite the lack of reliable disk diffusion interpretive criteria for S. pneumoniae *with certain β-lactams,* Streptococcus pneumoniae *ATCC® 49619 is the strain designated for quality control of all disk diffusion tests with all* Streptococcus *spp.*

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

d. Deterioration in oxacillin disk content is best assessed with QC organism Staphylococcus aureus ATCC® 25923, with an acceptable zone diameter of 18 to 24 mm.

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	–	–	–
Chloramphenicol	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 plates.

§493.1261 Standard: Bacteriology.

(c) The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1261(c)
QC records should include lot numbers, date prepared/opened, expiration dates, the actual measurements, reactions, and/or observations and demonstrate that controls were tested when shipments of reagents, disks, stains, or antisera for identification systems were opened or when the laboratory prepared these materials.

D5511

§493.1262 Standard: Mycobacteriology.

(a) Each day of use, the laboratory must check all reagents or test procedures used for mycobacteria identification with at least one acid-fast organism that produces a positive reaction and an acid-fast organism that produces a negative reaction.

Interpretive Guidelines §493.1262(a)
When condition level deficiencies in Mycobacteriology are in any or all phases of testing, use D5004.

For acid-fast stains (i.e., Ziehl-Neelsen, Kinyoun), use positive and negative stain controls each day of testing patient samples. Use D5473. For fluorochrome acid-fast stains, use positive and negative stain controls each time of use. Use D5475.

Controls for acid-fast and fluorochrome stains for clinical specimens can include previously processed specimens that contain confirmed acid-fast organisms such as Mycobacterium fortuitum or other non-tuberculous mycobacteria for the positive control, and a negative sputum seeded with Escherichia coli for a negative control. Control smears should be heat-fixed and stored in a protective box.

For controls when staining mycobacteriology cultures, use a previously confirmed acid-fast organism for the positive control, and a non-mycobacterial species such as Escherichia coli for the negative control.

For the BACTEC NAP test, positive and negative control organisms must be tested each week of use. Controls should include M. tuberculosis ATCC 27294 and M. kansasii ATCC 35775. M. tuberculosis should be inhibited by NAP, while M. kansasii should have increasing growth index values in the presence of NAP.

For molecular amplification testing guidelines, use D5455.

Probes §493.1262(a)
How often are mycobacteriology cultures checked for growth prior to the issuance of final patient reports? How long are negative cultures held before a final patient report is issued (e.g., minimum of six weeks)? Use D5411 and D5413 as appropriate.

D5513

§493.1262 Standard: Mycobacteriology.

(b) For antimycobacterial susceptibility tests, the laboratory must check each batch of media and each lot number and shipment of antimycobacterial agent(s) before, or concurrent with, initial use, using an appropriate control organism(s).

Interpretive Guidelines §493.1262(b)
A susceptible control strain of Mycobacterium tuberculosis, such as H37Rv or other appropriate control strain, must be used to check the susceptibility procedure.

For automated mycobacterial susceptibility testing, organisms which manufacturers recommend or require for use in their systems are acceptable strains of control organisms.

Probes §493.1262(b)
Are quality control samples tested at the same time specimens are tested? For example, a growth control without antimycobacterial agent should be inoculated at the time of patient testing.

(b)(1) The laboratory must establish limits for acceptable control results.

Probes §493.1262(b)(1)
Which control strains are used and how did the laboratory establish acceptable control limits for susceptibility tests?

(b)(2) Each week tests are performed, the laboratory must use the appropriate control organism(s) to check the procedure.
(b)(3) The results for the control organism(s) must be within established limits before reporting patient results.

Interpretive Guidelines 493.1262(b)(3)
The laboratory must ensure that it performs and documents all corrective action(s) taken whenever the test results do not meet the laboratory control limits for susceptibility. Use D5781.

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§493.1262 Standard: Mycobacteriology.

(c) The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1262(c)
QC records should include lot numbers, date prepared/opened, expiration dates, the actual measurements, reactions, and/or observations and demonstrate that controls were tested when shipments of reagents, disks, stains, or antisera for identification systems were opened or when the laboratory prepared these materials.

D5517

§493.1263 Standard: Mycology.

(a) The laboratory must check each batch (prepared in-house), lot number (commercially prepared), and shipment of lactophenol cotton blue when prepared or opened for intended reactivity with a control organism(s).

Interpretive Guidelines §493.1263(a)
When condition-level deficiencies in Mycology are in any or all phases of testing, use D5006.

For non-culture identification systems (e.g., direct antigen) use D5449 and/or D5453 as appropriate.

A filamentous fungus such as Aspergillus species should be used to check staining of lactophenol cotton blue.

D5519

§493.1263 Standard: Mycology.

(b) For antifungal susceptibility tests, the laboratory must check each batch of media and each lot number and shipment of antifungal agent(s) before, or concurrent with, initial use, using an appropriate control organism(s).
(b)(1) The laboratory must establish limits for acceptable control results.

Probes §493.1263(b)(1)
Which control strains are used and how did the laboratory establish acceptable control limits for susceptibility tests?

(b)(2) Each day tests are performed, the laboratory must use the appropriate control organism(s) to check the procedure.

Probes §493.1263(b)(2)
Are quality control samples tested at the same time specimens are tested?

(b)(3) The results for the control organism(s) must be within established limits before reporting patient results.

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§493.1263 Standard: Mycology.

(c) The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1263(c)
QC records should include lot numbers, date prepared/opened, expiration dates, the actual measurements, reactions, and/or observations and demonstrate that controls were tested when shipments of reagents, discs, stains, or antisera for identification systems were opened or when the laboratory prepared these materials.

D5523

§493.1264 Standard: Parasitology.

(a) The laboratory must have available a reference collection of slides or photographs and, if available, gross specimens for identification of parasites and use these references in the laboratory for appropriate comparison with diagnostic specimens.

Interpretive Guidelines §493.1264(a)

When condition level deficiencies in Parasitology are in any or all phases of testing, use D5008.

The laboratory must have adequate reference material, but does not have to maintain several different reference systems. Textbooks with photographs, previously stained slide preparations, preserved specimens, or slides from proficiency testing programs are some acceptable systems.

If the laboratory uses zinc sulfate for concentration of fecal specimens for ova and parasite examinations, the acceptable specific gravity of the zinc sulfate solution is 1.18 for fresh fecal samples and 1.20 for formalinized fecal samples. Use D3007 or D5411 as applicable.

For non-culture identification systems (e.g., direct antigen) use D5449 and/or D5453 as appropriate.

D5525

§493.1264 Standard: Parasitology.

(b) The laboratory must calibrate and use the calibrated ocular micrometer for determining the size of ova and parasites, if size is a critical parameter.

Interpretive Guidelines §493.1264(b)
Check for the following:

- *Presence of an ocular micrometer for the microscope(s) used;*
- *Availability of a stage micrometer;*
- *Instructions for calibration. Use D5403;*
- *Records of the measurements and calculations used to show that each objective (high, oil, low) has been calibrated; and*
- *Criteria for the use of the micrometer for determining the size of ova and parasites. Use D5403.*

Probes §493.1264(b)
How has the laboratory determined the accuracy of the ocular calibration and that the staff has the knowledge for proper use?

D5527

§493.1264 Standard: Parasitology.

(c) Each month of use, the laboratory must check permanent stains using a fecal sample control material that will demonstrate staining characteristics.

Interpretive Guidelines §493.1264(c)
The fecal sample control may contain either parasites or added leukocytes sufficient to demonstrate staining characteristics. A commercially prepared quality control slide for intestinal parasites is also an acceptable control for checking permanent stains.

While a wet mount preparation may not be sufficiently sensitive to detect small numbers of ova or parasites in fecal specimens, or to render a final species identification, the regulations do not require use of concentrated and permanent stain techniques to identify fecal parasites. It is the laboratory's responsibility to assure that it can accurately and reliably identify the organisms it claims to be able to identify. Use D3007 and/or D5411

as applicable. Upon request, the laboratory must specify the method employed by the laboratory for screening fecal specimens and provide information to clients on the test report that may affect the interpretation of test results. Use D5805 and/or D5809 as applicable.

The working iodine solution is stable for approximately two weeks. If the laboratory does not prepare fresh working iodine solution at least every two weeks, it must assure that the iodine solution has not deteriorated by observing positive clinical specimens or formalin-fixed specimens. Use D5417. Protozoan cysts stained with iodine contain golden yellow cytoplasm, brown glycogen material and have refractile nuclei.

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§493.1264 Standard: Parasitology.

(d) The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1264(d)
QC records should include lot numbers, date prepared/opened, expiration dates, the actual measurements, reactions, and/or observations and demonstrate that controls were tested when shipments of reagents, disks, stains, or antisera for identification systems were opened or when the laboratory prepared these materials. QC records should also include documentation of the measurements and calculations for calibration of each objective (low, high, oil immersion) of the ocular micrometer, and demonstrate that permanent stain controls were tested with a fecal sample control material each month of use.

D5531

§493.1265 Standard: Virology.

(a) When using cell culture to isolate or identify viruses, the laboratory must simultaneously incubate a cell substrate control or uninoculated cells as a negative control material.

Interpretive Guidelines §493.1265(a)
When condition level deficiencies in Virology are in any or all phases of testing, use D5010.

Cell Culture
For commercially purchased cell culture media, the requirement for media quality control checks is satisfied by visually examining the media for sterility and assuring the ability of the media to sustain cell life. If the media is prepared or produced in the laboratory, use D5477:

- *Each component of cell culture media should be checked for sterility using bacterial culture techniques. In addition, fetal bovine serum must be checked for toxicity using cell culture systems;*
- *The combined product (e.g., Hanks, Eagles and Earles) should be checked for sterility using bacterial culture techniques and the ability to propagate growth with cell cultures; and*
- *Cell culture systems should be checked for mycoplasma contamination at regular intervals established by the laboratory.*

Non-culture Methods

1. *For other non-culture identification (e.g., antigen identification) systems that are used for viral identification, the laboratory is not required to maintain live viral cultures for quality control purposes. However, positive and negative controls are required to evaluate the detection phase, if such controls are available commercially or in the laboratory. Use D5449 and/or D5453 as appropriate.*
2. *If organism controls are not available, a previously extracted viral antigen as the positive control plus a previously confirmed negative control of the same matrix as the patient sample may be used. Use D5485. A positive organism control must be subjected to the extraction process if such a control is available in the laboratory. Use D5453.*
3. *For fluorescent stains, the control requirements are met by using virus-infected cells for a positive control among uninfected cells for a negative control. Use D5475.*

The intent of the regulations is for the laboratory to have methodologies available to isolate and identify the viruses that are etiologically related to the clinical disease for which services are offered. For example, if a laboratory offers services only for Herpes testing, it must have available host systems for the isolation and/or test methods for the identification of the Herpes virus. If the laboratory is not using the appropriate host system, use D3007.

"Host system" is defined as the animal, egg or cell culture model, which supports the propagation of viruses.

Clinical information important for the determination and selection of the proper host system should include (Use D5305):

- *Clinical symptoms of the patient;*
- *Age of the patient;*
- *Source of the specimen;*
- *Date of onset of clinical symptoms;*
- *Recent travel information of patient;*
- *Test request; and*
- *Date of specimen collection.*

Cell culture is the host system used most frequently. The specific cell line (type) is usually selected based upon its known sensitivity and susceptibility to different viruses. For example, the cell lines to be used as host systems for the following clinical specimens could be:

- *Upper respiratory infection specimens: Primary Monkey Kidney (PMK), Human Fetal Diploid Lung (HFDL), or equivalent;*
- *Enteric specimens: PMK, Human Fetal Diploid Kidney (HFDK), or equivalent;*
- *Urine specimens: HFDL, PMK, or equivalent;*
- *Genital specimens: Human Foreskin (HFD), Vero (Continuous Monkey Kidney), or equivalent;*
- *Vesicular lesions: HFDL, PMK, BSC-1 (Monkey Cell Line), or equivalent; and*
- *Tissues or Spinal fluids: PMK, Vero, BSC-1, HFDK or HFDL, or equivalent.*

Prior to the inoculation of the cell cultures, the laboratory should check the cell culture systems for the following:

- *The age of the cell culture monolayer (no more than 7-10 days post "seeding") (Use D5417);*
- *Maintenance media that is free from inhibitory substances (Use D5477); and*
- *Sterility (visual observation for turbidity) (Use D5477).*

Uninoculated cell substrate controls are used to determine whether the specificity of a test system has been assured. Generally, an uninoculated cell control for each cell line that is inoculated is used per inoculation day to determine whether the consequent cytopathic effect (CPE) in the cells inoculated with patient specimen was caused by specific etiologic agent(s), or caused by the nonspecific deterioration of the cells themselves. Often, as monolayer host cells age, the cells deteriorate, exhibiting "rounding" and "pulling-apart." This cell change may be confused with CPE if uninoculated cells are not available to compare with the inoculated cells.

Probes §493.1265(a)
How does the laboratory determine the specific cell line to be used as the host system? Use D3007 or D5411 as applicable.

When reviewing the laboratory's identification procedures for the clinical diseases for which services are offered, how does the laboratory rule out the presence of Clostridium difficile toxin in those cell cultures in which the patient specimen exhibits non-specific effects unrelated to viral cytopathic effect (CPE)? Use D3007 or D5411as applicable.

If presumptive reports are issued based on CPE, how does the laboratory confirm the identification reported? Use D3007 or D5411 as applicable.

For tests such as hemagglutination inhibition and viral neutralization in which antisera must be standardized, how has the laboratory determined the optimum dilution of the antisera to assure maximum sensitivity and specificity? Use D5437.

Neutralization Tests:
How does the laboratory standardize its dilution of the viral isolate and control virus to the appropriate Tissue Culture Dose 50 or equivalent, each time the test is performed? Use D5437.

How many varieties of uninoculated cell cultures does the laboratory use to check each new lot of anti-serum or serum pool for toxicity? Use D5477 or 5479 as applicable.

Hemagglutination Inhibition Tests:
After having determined the hemagglutination titer, how does the laboratory determine the working dilution of the viral isolate (i.e., usually 4 Hemagglutination units)? How does the laboratory ensure that this working dilution is correct for isolates and controls? Use D5421 or D5423 as applicable.

How often and for which hemagglutination inhibition tests does the laboratory include a serum/cell/buffer control and a cell/buffer control? Use D5425.

Does the laboratory include one known virus or viral antigen specific to each antisera used in the test procedure? Use D5449.

Direct Immunofluorescence Tests:
How does the laboratory determine which immune serum conjugate(s) to use when identifying viruses using antisera that react with viruses that are etiologically similar (e.g., an antigen test for specimens from patients with flu-like symptoms that identifies Respiratory Syncytial Virus, Influenza, and Parainfluenza)? How does the laboratory assure the specificity of this conjugate for the specific virus being identified? Use D5421 or D5423 as applicable.

How does the laboratory rule out non-specific reactivity for each conjugate used? Use D5421 or D5423 as applicable.

Indirect Immunofluorescence Tests:
Has the laboratory determined the optimum dilution of its anti-species [e.g., antibody to host system or cell culture (such as anti-PMK, conjugated immune serum)]? Use D5421 or D5423 as applicable.

Has the laboratory determined the optimum dilution of the virus specific immune serum? Use D5421 or D5423 as applicable.

Determine whether the laboratory is checking positive and negative reactivity using (Use D5475.):

- *Uninoculated cells plus immune serum plus anti-species conjugate (negative control); and*
- *Viral antigen or known virus infected cells plus immune serum plus anti-species conjugate (positive control).*

Determine whether the laboratory checks each new batch or shipment of conjugate using known virus infected cells plus PBS plus anti-species conjugate. Use D5471.

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§493.1265 Standard: Virology.

(b) The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1265(b)
QC records must identify the host cell cultures employed, the number of tubes or plates inoculated or uninoculated, maintenance medium used, the number of times the patient specimen was sub-cultured, the specific sub-culture or passage in which the virus was identified, the CPE observed, and post inoculation date of observations. If the deficiency is due to absence of dates of testing and observations, use D5787.

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§493.1267 Standard: Routine chemistry.

For blood gas analyses, the laboratory must perform the following:

Interpretive Guidelines §493.1267(a)-(d):
When condition level deficiencies in Routine Chemistry are identified in one or more phases of testing, use D5016.

Control materials generally are not available to verify the reportable range at the very high range of patient results. When necessary, the laboratory may verify the results by splitting patient samples and assaying them on two different blood gas analyzers.

Quality control records should include lot numbers, date prepared/opened, expiration dates, the actual measurements, reaction and/or observations and demonstrate that controls were tested as required.

Do not dictate the acceptable format for documentation.

Probes §493.1267(a)-(d):
For blood gas testing, do the records include barometric pressure and room temperature, as necessary?

Do the records of a laboratory that moves from testing site to testing site demonstrate the

performance of control samples following transport of equipment when such activity affects test performance specifications and/or instrument calibration?

D5535

§493.1267 Standard: Routine chemistry.

(a) Calibrate or verify calibration according to the manufacturer's specifications and with at least the frequency recommended by the manufacturer.

Interpretive Guidelines §493.1267(a)
For blood gas analysis, the laboratory must perform calibration and calibration verification in accordance with the manufacturer's instructions. If the laboratory meets the manufacturer's instructions, and the requirements at this section, the laboratory does not have to adhere to calibration and calibration verification requirements at §493.1255.

D5537

§493.1267 Standard: Routine chemistry.

(b) Test one sample of control material each 8 hours of testing using a combination of control materials that include both low and high values on each day of testing.

Interpretive Guideline§493.1267(b)
"Each 8 Hours of testing" is defined as each shift of 8 consecutive hours the laboratory is in operation, including "on-call" shifts. When documenting standards/controls results, the laboratory must identify the shifts in which controls are tested with patients.

For a laboratory that is only open 8 hours/day and the instrument autocalibrators, the laboratory must test both a low and high value in the eight hours to meet the requirement.

In addition to testing one control each eight hours, the combination of controls and calibrators used each day of testing must include a high and low value. Controls should be rotated to check normal, alkalosis and acidosis levels.

D5539

§493.1267 Standard: Routine chemistry.

(c) Test one sample of control material each time specimens are tested unless automated instrumentation internally verifies calibration at least every 30 minutes.

Interpretive Guidelines §493.1267(c)
If blood gas analysis is performed with an instrument that does not internally verify the calibration at least every thirty minutes, then a calibrator or control must be tested each time patient specimens are tested.

It is not the intent of this requirement to require the laboratory to maintain records of each auto-calibration.

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§493.1267 Standard: Routine chemistry.

(d) Document all control procedures performed, as specified in this section.

D5543

§493.1269 Standard: Hematology.

(a) For manual cell counts performed using a hemocytometer--
(a)(1) One control material must be tested each 8 hours of operation; and
(a)(2) Patient specimens and control materials must be tested in duplicate.

Interpretive Guidelines §493.1269(a)
When condition level deficiencies in Hematology are in any or all phases of testing, use D5024.

"Hours of operation" is defined as each shift of 8 consecutive hours the laboratory is in operation, including "on-call" shifts. When documenting standards/controls results, the laboratory must identify the shifts in which controls are tested with patients.

If the manufacturer of an instrument that performs automated differentials does not give criteria for when to perform a manual differential, the laboratory must establish criteria indicating when to perform a manual differential including instructions for reporting the results. Use D5423.

Control requirements for automated instruments that perform hemoglobin, hematocrit, red and white cell counts and differentials are found at §493.1256(d)(3)(i). Use D5447. The calibration verification exception for automated hematology cell counters is found at §493.1255(b). Use D5439.

D5545

§493.1269 Standard: Hematology.

(b) For all nonmanual coagulation test systems, the laboratory must include two levels of control material each 8 hours of operation and each time a reagent is changed.

Interpretive Guidelines §493.1269(b)-(c):
The laboratory performing non-manual coagulation tests subject to §493.1269 must either establish criteria or verify manufacturer's criteria for an acceptable range of performance as required in §493.1253(a). Use D5421 or D5423 as appropriate.

An automated (nonmanual) coagulation test system samples the plasma, combines the plasma with the reagents, detects the end point or clot formation and displays the test results without operator intervention.

The International Sensitivity Index (ISI) is the correction factor for variable sensitivities of thromboplastins. The International Normalized Ratio (INR) is a calculation primarily used for monitoring a patient's oral anticoagulant therapy. The INR corrects for the variability in Prothrombin Time (PT) results attributable to the ISI. Therefore, this allows all PT's to be corrected to the international standard.

INR Calculation
The INR is equal to the ratio of the patient's PT (in seconds) to the laboratory's established normal mean PT (in seconds), then raised to the power of the ISI.

INR = (Patient PT ÷ Mean Normal Range PT) ^{ISI}

NOTE: A scientific calculator is needed to calculate the INR.

Example:
Patient PT (in seconds)=18.5
Normal mean PT (in seconds)=12.9
ISI value (obtain from the package insert of the laboratory's current lot of thromboplastin reagent)=2.002

1. *18.5 ÷12.9 = 1.434 (Patient Ratio)*
2. *$1.434^{2.002}$ = 2.056 (INR Result)*
3. *Report the INR as: INR=2.1.*

For INR calculations, ensure that the laboratory:

- *Establishes a normal Prothrombin time mean with each new thromboplastin lot number, while testing patients with results in the therapeutic range and abnormal patients with the old lot and the new lot of reagents to verify consistency of results;*
- *Incorporates the current and pertinent normal Prothrombin time mean and ISI value for each lot of thromboplastin (manual, instrument, or LIS);*
- *Documents the manual check of the INR calculation for each new lot number; and*
- *Documents each thromboplastin lot number, with the normal Prothrombin time mean and the ISI value provided by the manufacturer (manual or instrument).*

<u>Probes §493.1269(b)-(c):</u>
Is the laboratory using the ISI value from the current manufacturer's package insert in calculating the INR values?

How does the laboratory ensure that the ISI values are changed with each change of thromboplastin lot number?

Has the laboratory established their own normal patient mean with each lot of thromboplastin?

For coagulation testing, do the records include timer checks, and temperature checks as necessary?

D5547

§493.1269 Standard: Hematology.

(c) For manual coagulation tests--
(c)(1) Each individual performing tests must test two levels of control materials before testing patient samples and each time a reagent is changed; and
(c)(2) Patient specimens and control materials must be tested in duplicate.

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§493.1269 Standard: Hematology.

(d) The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1269(d)
Quality control records should include lot numbers, date prepared/opened, expiration dates, the actual measurement(s) taken, reactions and/or observations and demonstrate that controls were tested when shipments of reagents or stains were opened or when the laboratory prepared these materials. However, do not dictate the acceptable format for documentation.

D5551

§493.1271 Standard: Immunohematology.

(a) Patient testing.
(a)(1) The laboratory must perform ABO grouping, D(Rho) typing, unexpected antibody detection, antibody identification, and compatibility testing by following the manufacturer's instructions, if provided, and as applicable, 21 CFR 606.151(a) through (e).

Interpretive Guidelines §493.1271(a)(1)
When condition level deficiencies in Immunohematology are in any or all phases of testing, use D5026.

There are no daily quality control requirements for reagent red cell panels used in antibody identification. Panel quality control is a combination of serological test results, such as: strength of reactions and patient phenotype; statistical probability, patient's medical history; and laboratory standard of practice (i.e., how the laboratory handles compatibility testing for patients with unexpected antibodies). However, the QC requirements pertaining to new batch, lot, shipment of identification systems at §493.1256(e)(1) must be met.

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 (at least one known antibody)	*Each day of use*	*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.

Blood banks may make their own reagents. If unlicensed reagents are used, the facility must follow FDA's Current Good Manufacturing Practices (CGMP) and additional standards found at:

- *21 CFR 606.65 (c), (d), (e);*
- *21 CFR 606.160 (b)(4), (5), (7);*
- *21 CFR 606.160(d);*
- *21 CFR 640.5(b), (c);*
- *and 21 CFR 660 subpart D.*

Rare reagents, e.g. anti-Jk^b, and anti-Le^b, etc. are sometimes used beyond the expiration date; this is acceptable only if adequate controls are used and the reactivity and specificity of the reagents are documented.

- *Determine the laboratory's policies regarding:*
- *Compatibility testing for patients with a history of a prior antibody;*
- *Compatibility testing for patients with no history of a prior antibody;*
- *Retyping, rescreening, and/or recrossmatching units which are not transfused at the original requested time; and*
- *Course of action to be taken for positive antibody screening and/or incompatible crossmatch.*

For deficiencies relating to director responsibilities use D6082.
For deficiencies relating to step-by-step procedure use D5403.

If the laboratory performs gel techniques for ABO, Rh or Antibody screening, ensure the laboratory maintains the proper centrifuge speed, centrifuge time, and pipet dispensing volume.

Probes §493.1271(a)(1)
If the patient has been previously tested, how are results of current testing compared with interpretations of previous testing? When the results of current testing are discrepant with results of previous testing, how has the laboratory resolved the difference? Use D5777.

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§493.1271 Standard: Immunohematology.

(a)(2) The laboratory must determine ABO group by concurrently testing unknown red cells with, at a minimum, anti-A and anti-B grouping reagents. For confirmation of ABO group, the unknown serum must be tested with known A1 and B red cells.

Interpretive Guidelines §493.1271(a)(2)
Determine if the laboratory has a policy to detect and resolve ABO discrepancies. If the laboratory does not have such procedures, use D5401. If the laboratory does not use patient records to confirm ABO group (i.e., current testing compared with historical records when available), use D5777.

§493.1271 Standard: Immunohematology.

(a)(3) The laboratory must determine the D(Rho) type by testing unknown red cells with anti-D (anti-Rho) blood typing reagent.

Interpretive Guidelines §493.1271(a)(3)
Determine if the laboratory has established a policy specifying when testing for weak D (Du) must be performed.

Probes §493.1271(a)(3)
Is the laboratory following this policy?

D5553

§493.1271 Standard: Immunohematology.

(b) Immunohematological testing and distribution of blood and blood products. Blood and blood product testing and distribution must comply with 21 CFR 606.100(b)(12); 606.160(b)(3)(ii) and (b)(3)(v); 610.40; 640.5(a); 640.5(b); 640.5(c); 640.5(e); and 640.11(b).

Interpretive Guidelines §493.1271(b)
NOTE: The focus of the FDA is on the manufacture of a safe and efficacious blood and blood product for transfusion and further manufacture, and on maintaining a healthy donor population. CLIA does not apply to quality control testing performed on specimens from the blood or blood products (e.g. pH, platelet count, hemolysis, volume). CLIA is concerned with the health of the individual donating, and the recipient of the blood product. CLIA is applicable to all testing performed in a donor situation because it is assessing the health of the donor or whether there is a potential for harm to the recipient if performed incorrectly (e.g., ABO, RH, antibody screen, HIV, Hepatitis, RPR, total protein, drug testing).

Refer to the current version of 21 CFR Parts 600-799 for the specified sections:

- *§606.100(b)(12) - Criteria for determining whether returned blood is suitable for reissue;*
- *§606.160(b)(3)(ii) – Visual inspection of whole blood and red blood cells during storage and immediately before distribution;*
- *§606.160(b)(3)(v) – Emergency release of blood, including signature of requesting physician obtained before or after release;*
- *§610.40 Testing for communicable diseases;*
- *§640.5(a) Syphilis testing;*
- *§640.5(b) Determination of Blood group;*

Interpretive Guidelines §493.1271(b)

- *§640.5(c) Determination of Rh factor;*
- *§640.5(e) Inspection of whole blood during storage and immediately prior to issue; and*
- *§640.11(b) Inspection of RBC during storage and at the time of issue.*

Probes §493.1271
If equipment and reagents are used in mobile or temporary testing sites, how are they protected from extreme temperature fluctuations when not in use (e.g., evenings, weekends, and holidays)?

D5555

§493.1271 Standard: Immunohematology.

(c) Blood and blood products storage. Blood and blood products must be stored under appropriate conditions that include an adequate temperature alarm system that is regularly inspected.
(c)(1) An audible alarm system must monitor proper blood and blood product storage temperature over a 24-hour period.
(c)(2) Inspections of the alarm system must be documented.

Interpretive Guidelines §493.1271(c)
Acceptable temperature ranges must be established and actual readings of temperature-controlled storage areas must be recorded during the time that blood or blood products for transfusion are stored. Whole Blood, Red Blood Cells, and Liquid Plasma should be stored between 1 and 6ºC; room temperature Platelets and Platelet Rich Plasma between 20 and 24ºC or 1-6ºC as indicated on the product label. Fresh Frozen Plasma, Plasma, and Cryoprecipitated AHF should be stored at -18ºC or colder. Temperatures continuously monitored by a recording thermograph or central monitoring system are acceptable. The charts or central monitoring system must be retained to document that temperatures are maintained within the FDA's acceptable limits.

Verify that the laboratory regularly inspects the alarm system(s) according to its established policy. When the facility performs alarm checks, the temperature at which the alarm sounds should be compared to the temperature on the recording chart. Verify that the alarm activates at the appropriate temperature(s).

FDA Reissue requirements are as follows: The container must have a tamper-proof seal which remains unbroken; and tamper-proof pilot tube or segment must be attached; records should indicate that the blood was maintained at 1 - 10ºC while outside the control of the establishment; and the unit must be inspected prior to reissue. Blood issued for transfusion to a ward or operating room and not refrigerated may be reissued if the unit is returned to the blood bank within 30 minutes.

Probes §493.1271(c)
Does the laboratory ensure that the freezer(s) used to store blood products is maintained at the recommended temperature(s) on a continuous basis?

Does the laboratory document and explain unacceptable storage temperatures? Use D5793.

What is the laboratory's criteria for determining blood or blood product suitable for reissue? Are they following their policy?

How are untested autologous units, potentially infectious units and reagents stored and segregated to prevent contamination?

If the laboratory does not have an emergency power source for the blood storage equipment and temperature alarm system, how does the laboratory ensure that blood is maintained at the appropriate temperature when a power failure occurs?

If the laboratory is not staffed 24 hours a day, seven days a week, how does it ensure prompt response to an activated alarm (evenings, weekends, and holidays)?

D5557

§493.1271 Standard: Immunohematology.

(d) Retention of samples of transfused blood. According to the laboratory's established procedures, samples of each unit of transfused blood must be retained for further testing in the event of transfusion reactions. The laboratory must promptly dispose of blood not retained for further testing that has passed its expiration date.

Interpretive Guidelines §493.1271(d)
There is no specific timeframe for retaining donor and recipient blood samples. However, it is common practice to keep these samples for a minimum of seven days after each transfusion in case there is a need for retesting.

D5559

§493.1271 Standard: Immunohematology.

(e) Investigation of transfusion reactions.
(e)(1) According to its established procedures, the laboratory that performs compatibility testing, or issues blood or blood products, must promptly investigate all transfusion reactions occurring in facilities for which it has investigational responsibility and make recommendations to the medical staff regarding improvements in transfusion procedures.
(e)(2) The laboratory must document, as applicable, that all necessary remedial actions are taken to prevent recurrences of transfusion reactions and that all policies and procedures are reviewed to assure they are adequate to ensure the safety of individuals being transfused.

Interpretive Guidelines §493.1271(e)(2):
Examine records of transfusion reaction investigations for completeness, accuracy, and promptness. Verify that investigations of transfusion reactions are conducted in accordance with the facility's established protocols. Records must include each step of the investigation, including conclusions and any follow-up.

Probes §493.1271(e)(2):
If problems or technical errors are identified during a transfusion reaction investigation, are corrective actions taken and, as applicable, procedures instituted to prevent a recurrence?

Did the laboratory assess the adequacy of the procedures implemented? Use D5793.

§493.1271 Standard: Immunohematology.

(f) *Documentation*. The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1271(f)
All non-transfusion related immunohematology QC records must be retained for at least 2 years. Use D3035

Transfusion-related immunohematology QC records, including but not limited to, donor processing, compatibility testing, and transfusion reaction investigations, must be retained for the timeframe stated at 21CFR 606.160(d).

D5601

§493.1273 Standard: Histopathology.

(a) As specified in §493.1256(e)(3), fluorescent and immunohistochemical stains must be checked for positive and negative reactivity each time of use. For all other differential or special stains, a control slide of known reactivity must be stained with each patient slide or group of patient slides. Reactions(s) of the control slide with each special stain must be documented.

Interpretive Guidelines §493.1273(a)
When condition level deficiencies in Histopathology are in any or all phases of testing, use D5028.

The laboratory must demonstrate that each reagent performs within the specifications established by the laboratory for the test procedure. Documentation of concurrent testing of reagents or acceptable quality control results will satisfy this requirement.

When the laboratory uses a manufacturer's kit, the reagents of the kit must not be combined, mixed, or replaced with components of another kit from a different lot number, unless otherwise permitted and specified by the manufacturer in the package insert. Use D5419.

Laboratories which use automated staining methodologies must follow the manufacturer's instructions. Use D5411.

Flow Cytometry
Staining controls for cell surface immunophenotyping by flow cytometry should consist of either normal, cultured or abnormal cells known to be positive for selected standard antigens and must verify the proper performance of reagents. Frozen or other preserved cells may be used. A negative reagent control must be run for each test cell preparation, and is to consist of monoclonal antibody(ies) of the same species and isotype or equivalent. Negative reagent controls will consist of:
a) For indirect stains, an irrelevant primary antibody and the same secondary antibody(ies) conjugated with the same fluorochrome(s) used in all relevant test combinations; and
b) For direct stains, an irrelevant antibody conjugated to the same fluorochrome and at the same fluorochromes: protein ratio used in all relevant test combinations.

Probes §493.1273(a)
For flow cell cytometric surface immunophenotyping, is a negative reagent control used to define a threshold for positive staining cells? If not, how does the laboratory define the threshold for positive staining cells?

For each staining run, is a quality control slide tested at the same time patient specimens are tested? Use D5611 for record keeping.

D5603

§493.1273 Standard: Histopathology.

(b) The laboratory must retain stained slides, specimen blocks, and tissue remnants as specified in §493.1105. The remnants of tissue specimens must be maintained in a

manner that ensures proper preservation of the tissue specimens until the portions submitted for microscopic examination have been examined and a diagnosis made by an individual qualified under §§493.1449(b), (l), or (m).

D5605

§493.1273 Standard: Histopathology.

(c) An individual who has successfully completed a training program in neuromuscular pathology approved by HHS may examine and provide reports for neuromuscular pathology.

Interpretive Guidelines §493.1273(c)
HHS approves the American Academy of Neurology Committee for Neuromuscular Pathology Training Program.

D5607

§493.1273 Standard: Histopathology.

(d) Tissue pathology reports must be signed by an individual qualified as specified in paragraph (b) or, as appropriate, paragraph (c) of this section. If a computer report is generated with an electronic signature, it must be authorized by the individual who performed the examination and made the diagnosis.

Interpretive Guidelines §493.1273(d)
The laboratory must ensure that only those individuals qualified to evaluate histopathology specimens can release his or her electronic signature for reporting purposes.

In the event of a computer-generated signature, the laboratory must ensure that the system is protected from use by unauthorized individuals.

D5609

§493.1273 Standard: Histopathology.

(e) The laboratory must use acceptable terminology of a recognized system of disease nomenclature in reporting results.

Interpretive Guidelines §493.1273(e)
"SNOMED®" - Systemized Nomenclature of Medicine is an example of a recognized system of disease nomenclature.

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§493.1273 Standard: Histopathology.

(f) The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1273(f)
QC records should include lot numbers, date prepared/opened, expiration dates, the actual measurements, reactions, and/or observations and demonstrate that controls were

tested when shipments of reagents, stains, or kits were opened or when the laboratory prepared these materials.

D5613

§493.1274 Standard: Cytology.

(a) Cytology slide examination site. All cytology slide preparations must be evaluated on the premises of a laboratory certified to conduct testing in the subspecialty of cytology.

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§493.1274 Standard: Cytology.

(b) Staining. The laboratory must have available and follow written policies and procedures for each of the following, if applicable:

D5615

§493.1274 Standard: Cytology.

(b)(1) All gynecologic slide preparations must be stained using a Papanicolaou or modified Papanicolaou staining method.

Interpretive Guidelines §493.1274(b)(1)
The Papanicolaou staining procedure is a polychrome method that enhances differences in cellular morphology. The procedure utilizes a nuclear stain, hematoxylin and two cytoplasmic counterstains, OG-6 and EA. The Papanicolaou method is used for staining cytologic preparations because it provides well-defined nuclear detail, stains cytoplasm of various cell types different colors, and renders transparent cytoplasm. There are a variety of formulas for making hematoxylin, OG-6, and EA stains. The actual staining technique may vary among laboratories depending on the type of stains used and the laboratories' modification of the staining method. Modifications of the staining procedure must include the four main steps of the standard Papanicolaou staining method: fixation, nuclear staining, cytoplasmic staining, and clearing.

Cytology laboratories may receive reagents, solutions, and stains from a manufacturer in large volume stock containers. For ease in handling, portions of these reagents are usually decanted into smaller working containers, which must be labeled in accordance with §493.1252(c). Some manufacturers do not label stain or reagent containers with the expiration date; however, lot numbers and package inserts refer to this information. (Use D5417 if the laboratory uses materials beyond the expiration dates or the materials have deteriorated.)

If the laboratory uses a manufacturer's kit, the reagents of the kit must not be combined, mixed, or replaced with components of another kit from a different lot number, unless otherwise permitted and specified by the manufacturer in the package insert (use D5419). Laboratories which use automated staining methodologies must follow the manufacturers instructions (use D5411).

The cytology laboratory must document the expiration date of stock reagents, working stains, and solutions made in the laboratory. Use D5415.

Laboratories may use staining procedures, other than the Papanicolaou method, for staining nongynecologic specimens.

Review the written staining procedure for staining gynecologic specimens. Confirm that the written procedures reflect:

- *Stains used (i.e., Harris, Gill or other type of hematoxylin, OG-6, modified OG-6, EA36, EA50, EA65, modified EA) or the identity of a combination counterstain;*
- *Solutions used (water, alcohol, clearing reagent, acid and bluing agent);*
- *Concentration of each solution used (i.e., percentage (%) of alcohol, acid, ammonium hydroxide or lithium carbonate solution);*
- *Length of time or number of dips slides are placed in each stain or solution;*
- *The staining dishes must be labeled to reflect content (not just lids); and*
- *Procedure for coverslipping slides.*

Current time frames must be specified in the procedure manual for each step in the staining of cytology specimens using the Papanicolaou staining method. Adjustments to time frame changes must be documented.

Step-by-step written procedures must be available and followed to prepare nongynecologic specimens.

Use D5403 if any of the above findings is not met.

The laboratory must ensure that the gynecologic and non-gynecologic stains have been tested to ensure predictable staining characteristics on a daily basis. Use D5473.

NOTE: Any fixatives, reagents, or preservatives intended to be used on one liquid-based manufacturer's instrument must not be used on another manufacturer's instrument.

D5617

§493.1274 Standard: Cytology.

(b)(2) Effective measures to prevent cross-contamination between gynecologic and nongynecologic specimens during the staining process must be used.

Interpretive Guidelines §493.1274(b)(2)
The laboratory must develop its own policies and procedures for the prevention of cross-contamination between gynecologic and nongynecologic specimens. The majority of gynecologic specimens are fixed prior to transport to the laboratory. Staining times may differ between gynecologic and nongynecologic specimens. Commonly used methods include separate staining dishes for various specimens (i.e., gynecologic specimens, CSF, sputa, other body fluids), or separate staining times (i.e., gynecologic specimens in the morning and nongynecologic specimens in the afternoon), with the staining dishes washed and stains filtered between staining times.

Probes §493.1274(b)(2)
What does the laboratory do to ensure that cross-contamination between gynecologic and nongynecologic specimens does not occur?

D5619

§493.1274 Standard: Cytology.

(b)(3) Nongynecologic specimens that have a high potential for cross-contamination must be stained separately from other nongynecologic specimens, and the stains must be filtered or changed following staining.

Interpretive Guidelines §493.1274(b)(3)
A monochromatic stain such as toluidine blue may be used to determine the cellularity of nongynecologic specimens. Once a specimen has been concentrated, usually by centrifugation, a small drop of specimen is placed on a slide. A drop of stain is placed next to the specimen, allowed to mix, and coverslipped. Cellularity is evaluated microscopically. Highly cellular specimens have a high potential for cross-contamination. One option would be for the laboratory to stain these specimens after routine staining has been completed.

Laboratories which use automated staining methodologies must follow the manufacturer's instructions. Use D5411.

Probes §493.1274(b)(3)
How is the cellularity of nongynecologic specimens checked prior to cytopreparation (staining)?

What procedure does the laboratory use to determine which specimens must be stained separately?

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§493.1274 Standard: Cytology.

(c) Control procedures. The laboratory must establish and follow written policies and procedures for a program designed to detect errors in the performance of cytologic examinations and the reporting of results. The program must include the following:

D5621

§493.1274 Standard: Cytology.

(c)(1) A review of slides from at least 10 percent of the gynecologic cases interpreted by individuals qualified under §§493.1469 or 493.1483, to be negative for epithelial cell abnormalities and other malignant neoplasms (as defined in paragraph (e)(1) of this section).

Interpretive Guidelines §493.1274(c)(1)
The 10 percent rescreen of negative cases is not required for a one-person laboratory consisting of a technical supervisor or a laboratory which only employs pathologists qualified as technical supervisors. However, these laboratories must establish and follow a program to detect errors. This program must include, but is not limited to, cytologic/histologic correlations, retrospective review of negative cases, documentation of initial and rescreening results, and statistics [(c)(2)-(5) of this section].

The laboratory must review all slides from each case selected for rescreen.

(c)(1)(i) The review must be performed by an individual who meets one of the following qualifications:
(c)(1)(i)(A) A technical supervisor qualified under §§493.1449(b) or (k).
(c)(1)(i)(B) A cytology general supervisor qualified under §493.1469.
(c)(1)(i)(C) A cytotechnologist qualified under §493.1483 who has the experience specified in §493.1469(b)(2).

Interpretive Guidelines §493.1274(c)(1)(i)
The laboratory must document which individual(s) are qualified to conduct the 10 percent rescreen. Slides reviewed as part of the 10 percent rescreen must be included in the workload limit of the cytology general supervisor or the cytotechnologist performing the review. Use D5639.

(c)(1)(ii) Cases must be randomly selected from the total caseload and include negatives and those from patients or groups of patients that are identified as having a higher than average probability of developing cervical cancer based on available patient information.

Interpretive Guidelines §493.1274(c)(1)(ii)
The laboratory must have a procedure to determine which slides are rescreened. This procedure should ensure that individuals screening the slides do not know which slides will be chosen for rescreen.

The laboratory must establish criteria to ensure that random negative gynecological cases selected for rescreening include, when possible, cases from patients that are identified as having a higher than average probability for developing cervical cancer.

(c)(1)(iii) The review of those cases selected must be completed before reporting patient results.

D5623

§493.1274 Standard: Cytology.

(c)(2) Laboratory comparison of clinical information, when available, with cytology reports and comparison of all gynecologic cytology reports with a diagnosis of high-grade squamous intraepithelial lesion (HSIL), adenocarcinoma, or other malignant neoplasms with the histopathology report, if available in the laboratory (either on-site or in storage), and determination of the causes of any discrepancies.

Interpretive Guidelines §493.1274(c)(2)
The laboratory must compare clinical information with cytology final reports. For example, an atrophic smear (usually characteristic of a post menopausal woman) from a 21-year-old female with an LMP (last menstrual period) of 2-weeks-ago constitutes inconsistent findings and must be resolved.

The laboratory must define criteria to determine a discrepancy between a final cytological diagnosis of High Grade Squamous Intraepithelial Lesion (HSIL) or squamous carcinoma, adenocarcinoma or other malignant neoplasias and the correlating histology report.

Cases considered HSIL include: moderate and severe dysplasia, carcinoma in-situ (CIS)/Cervical Intraepithelial Neoplasia (CIN) 2 and CIN 3 or with features suspicious for invasion.

Probes §493.1274(c)(2)

How does the laboratory identify and resolve discrepancies for:

- *Clinical information vs. cytology report; and*
- *Gynecologic cytology report vs. histopathology report?*

D5625

§493.1274 Standard: Cytology.

(c)(3) For each patient with a current HSIL, adenocarcinoma, or other malignant neoplasm, laboratory review of all normal or negative gynecologic specimens received within the previous 5 years, if available in the laboratory (either on-site or in storage). If significant discrepancies are found that will affect current patient care, the laboratory must notify the patient's physician and issue an amended report.

Probes §493.1274(c)(3)
How does the laboratory track previous cases on an individual patient?

What criteria does the laboratory use to determine discrepancies when reviewing normal or negative slides from the past five years? How does the laboratory document the review?

How does the laboratory use the retrospective review to assess the analytic system and communicate findings to the appropriate staff? Use D5793

D5627

§493.1274 Standard: Cytology.

(c)(4) Records of initial examinations and all rescreening results must be documented.

D5629

§493.1274 Standard: Cytology.

(c)(5) An annual statistical laboratory evaluation of the number of –
(c)(5)(i) Cytology cases examined;
(c)(5)(ii) Specimens processed by specimen type;
(c)(5)(iii) Patient cases reported by diagnosis (including the number reported as unsatisfactory for diagnostic interpretation);
(c)(5)(iv) Gynecologic cases with a diagnosis of HSIL, adenocarcinoma, or other malignant neoplasm for which histology results were available for comparison;
(c)(5)(v) Gynecologic cases where cytology and histology are discrepant; and
(c)(5)(vi) Gynecologic cases where any rescreen of a normal or negative specimen results in reclassification as low-grade squamous intraepithelial lesion (LSIL), HSIL, adenocarcinoma, or other malignant neoplasms.

Interpretive Guidelines §493.1274(c)(5)(vi)
Low-grade Squamous Intraepithelial Lesions (LSIL) encompasses all lesions that demonstrate cellular changes consistent with human papillomavirus, mild dysplasia, or CIN 1.

D5631

§493.1274 Standard: Cytology.

(c)(6) An evaluation of the case reviews of each individual examining slides against the laboratory's overall statistical values, documentation of any discrepancies, including reasons for the deviation, and, if appropriate, corrective actions taken.

Probes §493.1274(c)(6)
How does the laboratory evaluate each individual's case reviews against the overall laboratory statistics?

What corrective actions are taken to resolve discrepancies? Use D5751.

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§493.1274 Standard: Cytology.

(d) Workload limits. The laboratory must establish and follow written policies and procedures that ensure the following:

D5633

§493.1274 Standard: Cytology.

(d)(1) The technical supervisor establishes a maximum workload limit for each individual who performs primary screening.

Interpretive Guidelines §493.1274(d)(1)
The maximum workload limit established by the technical supervisor must be based on each individual's capabilities. A generic workload limit for the laboratory as a whole does not meet this requirement.

Probes §493.1274(d)(1)
What criteria does the technical supervisor use to determine the slide limit for each person who examines slides?

D5635

§493.1274 Standard: Cytology.

(d)(1)(i) The workload limit is based on the individual's performance using evaluations of the following:

Interpretive Guidelines §493.1274(d)(1)(i)
The technical supervisor maintains documentation of the slide performance and provides feedback.

Probes §493.1274(d)(1)(i)
What records are maintained to document the technical supervisor's evaluation of the slide performance of each individual?

(d)(1)(i)(A) Review of 10 percent of the cases interpreted as negative for the conditions defined in paragraph (e)(1) of this section.

(d)(1)(i)(B) Comparison of the individual's interpretation with the technical supervisor's confirmation of patient smears specified in paragraphs (e)(1) and (e)(3) of this section.

Probes §493.1274(d)(1)(i)(B)
How does the technical supervisor ensure that feedback is provided on slide examination performance to each person evaluating slides?

What mechanism is used to allow individuals an opportunity to discuss instances of misdiagnosis?

D5637

§493.1274 Standard: Cytology.

(d)(1)(ii) Each individual's workload limit is reassessed at least every 6 months and adjusted when necessary.

Probes §493.1274(d)(1)(ii)
What criteria does the technical supervisor use to determine when a workload adjustment is needed?

How are records maintained to document that workload records are reassessed at least every six months and adjusted when necessary?

D5639

§493.1274 Standard: Cytology.

(d)(2) The maximum number of slides examined by an individual in each 24-hour period does not exceed 100 slides (one patient specimen per slide; gynecologic, nongynecologic, or both) irrespective of the site or laboratory. This limit represents an absolute maximum number of slides and must not be employed as an individual's performance target. In addition—

Interpretive Guidelines §493.1274(d)(2)
The maximum total number of slides an individual may screen is 100 per 24 hours regardless of site or laboratory. Although the regulation establishes this maximum number, not every individual will be able to accurately examine 100 slides in 24 hours. The laboratory must establish how many slides can be screened per day for each individual. Refer to §493.1274(d)(1) to insure that the technical supervisor has established a maximum number of slides that each individual is capable of evaluating. The laboratory must ensure that persons employed at other sites or locations do not exceed the maximum of 100 slides in 24 hours .

This 100-slide limit is also applicable to those technical supervisors who examine previously unevaluated cytology specimens.

Probes §493.1274(d)(2)
How does the laboratory ensure that each individual examining slides (cytotechnologists, cytology general supervisors and technical supervisors in cytology, as applicable) examines no more that 100 slides in a 24-hour period regardless of site or location?

(d)(2)(i) The maximum number of 100 slides is examined in no less than an 8-hour workday;

Probes §493.1274(d)(2)(i)
What records are used to verify that the maximum number of 100 slides is examined in no less than 8 hours, especially in the situation in which individuals screen slides at different sites or locations?

D5641

§493.1274 Standard: Cytology.

(d)(2)(ii) For the purposes of establishing workload limits for individuals examining slides in less than an 8-hour workday (includes full-time employees with duties other than slide examination and part-time employees), a period of 8 hours is used to prorate the number of slides that may be examined.

The formula--

$$\frac{\text{Number of hours examining slides X 100}}{8}$$

is used to determine maximum slide volume to be examined;

D5643

§493.1274 Standard: Cytology.

(d)(2)(iii) Nongynecologic slide preparations made using liquid-based slide preparatory techniques that result in cell dispersion over one-half or less of the total available slide may be counted as one-half slide; and

Interpretive Guidelines §493.1274(d)(2)(iii)
Nongynecologic slide preparations made using automated, semi-automated or other liquid-based slide preparatory techniques include specimens prepared by centrifugation, cytocentrifugation, filtering techniques or monolayering techniques. Any instrument used to assist in the adherence of cells to the slide is considered to meet this requirement. This requirement refers to slide preparatory techniques, not liquid based coverslips. Slides prepared by traditional methods (usually smears prepared by hand) are not included.

Maximum Workload Limits for Nongynecologic Specimens

Traditional Smear Technique	*100 Slides*
Automated, Semi-Automated, Liquid-Based	*200 Slides*
Combination of Techniques	*100 - 200 Slides*
(Based on Prorated Time)	

(d)(2)(iv) Technical supervisors who perform primary screening are not required to include tissue pathology slides and previously examined cytology slides (gynecologic and nongynecologic) in the 100 slide workload limit.

D5645

§493.1274 Standard: Cytology.

(d)(3) The laboratory must maintain records of the total number of slides examined by each individual during each 24-hour period and the number of hours spent examining slides in the 24-hour period irrespective of the site or laboratory.

Interpretive Guidelines §493.1274(d)(3)
Verify that the laboratory monitors the number of slides examined by each individual and the number of hours spent examining slides.

D5647

§493.1274 Standard: Cytology.

(d)(4) Records are available to document the workload limit for each individual.

Probes §493.1274(d)(4)
What records are maintained of each individual's workload limit when various types of slides are evaluated?

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§493.1274 Standard: Cytology.

(e) Slide examination and reporting. The laboratory must establish and follow written policies and procedures that ensure the following:

D5649

§493.1274 Standard: Cytology.

(e)(1) A technical supervisor confirms each gynecologic slide preparation interpreted to exhibit reactive or reparative changes or any of the following epithelial cell abnormalities:
(e)(1)(i) Squamous Cell

Interpretive Guidelines §493.1274(e)(1)(i)
Note: This requirement is in addition to the review and confirmation by a technical supervisor of all nongynecologic preparations as described under §493.1274(e)(3).

Probes §493.1274(e)(1)(i)
How does the laboratory ensure that the technical supervisor confirms every slide containing cells exhibiting reactive, reparative, atypical squamous/glandular cells, LSIL, HSIL, and all carcinomas?

(e)(1)(i)(A) Atypical squamous cells of undetermined significance (ASC-US) or cannot exclude HSIL (ASC-H).
(e)(1)(i)(B) LSIL-Human papillomavirus (HPV)/mild dysplasia/cervical intraepithelial neoplasia 1 (CIN 1).
(e)(1)(i)(C) HSIL-moderate and severe dysplasia, carcinoma in situ (CIS)/CIN 2 and CIN 3 or with features suspicious for invasion.
(e)(1)(i)(D) Squamous cell carcinoma.
(e)(1)(ii) Glandular Cell
(e)(1)(ii)(A) Atypical cells not otherwise specified (NOS) or specified in comments (endocervical, endometrial, or glandular).
(e)(1)(ii)(B) Atypical cells favor neoplastic (endocervical or glandular).
(e)(1)(ii)(C) Endocervical adenocarcinoma in situ.

(e)(1)(ii)(D) Adenocarcinoma endocervical, adenocarcinoma endometrial, adenocarcinoma extrauterine, and adenocarcinoma NOS.
(e)(1)(iii) Other malignant neoplasms.

D5651

§493.1274 Standard: Cytology.

(e)(2) The report of gynecologic slide preparations with conditions specified in paragraph (e)(1) of this section must be signed to reflect the technical supervisory review or, if a computer report is generated with signature, it must reflect an electronic signature authorized by the technical supervisor who performed the review.

Interpretive Guidelines §493.1274(e)(2)
The laboratory must ensure that the technical supervisor is the only individual to release his or her electronic signature for reports requiring technical supervisory review.

If an electronic signature is used, the laboratory must ensure that the system is protected from use by unauthorized individuals.

D5653

§493.1274 Standard: Cytology.

(e)(3) All nongynecologic preparations are reviewed by a technical supervisor. The report must be signed to reflect technical supervisory review or, if a computer report is generated with signature, it must reflect an electronic signature authorized by the technical supervisor who performed the review.

Interpretive Guidelines §493.1274(e)(3)
The laboratory must ensure that the technical supervisor:

- *Is the only individual to release his or her electronic signature for reports requiring technical supervisory review; and*
- *Reviews all nongynecologic cytological preparations.*

If an electronic signature is used, the laboratory must ensure that the system is protected from use by unauthorized individuals.

D5655

§493.1274 Standard: Cytology.

(e)(4) Unsatisfactory specimens or slide preparations are identified and reported as unsatisfactory.

Interpretive Guidelines §493.1274(e)(4)
The report should clearly specify when the slide is unsatisfactory for evaluation. Unsatisfactory slide preparations should not be reported as negative or normal. Use D5805.

Probes §493.1274(e)(4)
What criteria have been developed for categorizing a slide preparation as unsatisfactory (e.g., scant cellularity, obscuring blood, obscuring inflammation, or lack of endocervical component)?

D5657

§493.1274 Standard: Cytology.

(e)(5) The report contains narrative descriptive nomenclature for all results.

Interpretive Guidelines §493.1274(e)(5)
In cytology, great variation exists among the systems and terms a laboratory may use to report patient results on cytology reports. The laboratory must specify the descriptive nomenclature used for reporting patient results. This nomenclature must define the criteria used to classify patient results in a particular category in a clear and concise manner to ensure that all employees report patient results in a uniform, consistent manner. Use of the Papanicolaou numerical system without narrative description is not acceptable.

The Bethesda System is an example of a recognized system of narrative descriptive nomenclature for gynecologic cytology.

Probes §493.1274(e)(5)
When cytology evaluations are recorded on worksheets in "code" how does the laboratory ensure that the correct interpretation is used in reporting the results? Use D5801.

D5659

§493.1274 Standard: Cytology.

(e)(6) Corrected reports issued by the laboratory indicate the basis for correction.

Probes §493.1274(e)(6)
How does the laboratory indicate that the report is a corrected report (to avoid confusion with the initial report)? Use D5821.

How does the laboratory include the cause or reason for the correction in the report?

§493.1274 Standard: Cytology.

(f) Record and slide retention.
(f)(1) The laboratory must retain all records and slide preparations as specified in §493.1105.

D5661

§493.1274 Standard: Cytology.

(f)(2) Slides may be loaned to proficiency testing programs in lieu of maintaining them for the required time period, provided the laboratory receives written acknowledgment of the receipt of slides by the proficiency testing program and maintains the acknowledgment to document the loan of these slides.
(f)(3) Documentation of slides loaned or referred for purposes other than proficiency testing must be maintained.

D5663

§493.1274 Standard: Cytology.

(f)(4) All slides must be retrievable upon request.

Probes §493.1274(f)(4)
If the laboratory loans slides, what protocol has been established to ensure prompt return of slides, when necessary?

D5665

§493.1274 Standard: Cytology.

(g) Automated and semi-automated screening devices. When performing evaluations using automated and semi-automated screening devices, the laboratory must follow manufacturer's instructions for preanalytic, analytic, and postanalytic phases of testing, as applicable, and meet the applicable requirements of this subpart K.

Interpretive Guidelines §493.1274(g)
Some automated devices, such as instruments where only a portion of the slide is reviewed, may have a higher workload limit than 100 slides. This must be stated in the manufacturer's product insert to be applicable. However, the maximum workload limit for those slides which require 100% manual review (as a result of automated or semi-automated analysis OR in the routine workload) remains 100 slides.

Probes §493.1274(g)
When technology (automated/semi-automated devices) is introduced into the laboratory, how does the laboratory ensure its operation is within the specifications of previous methods used by the laboratory?

Some automated devices remove a percentage of the slides from the workload. How does the laboratory ensure that the correct slides are archived?

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§493.1274 Standard: Cytology.

(h) *Documentation*. The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1274(h)
QC records should include lot numbers, date prepared/opened, expiration dates, the actual measurements, reactions, and/or observations and demonstrate that controls were tested when shipments of reagents, stains, or kits were opened or when the laboratory prepared these materials.

The actual measurements(s) taken, reactions and/or observations must be recorded. However, do not dictate the acceptable format for documentation.

The laboratory must maintain documentation to demonstrate that ten percent of the negative cases were rescreened.

All QC records must be maintained for two years, for example: five year retrospective review, 10 percent rescreens, cytology/histology correlations, cytotechnologist's

performance evaluations, individual's and laboratory's statistics (use D3031). Use D3043 for retention of glass slides and D3041 for retention of patient test reports.

The laboratory must document the evaluation of quality control data and ensure that corrective actions are effective. Use D5793.

Probes §493.1274(h)
What information is documented on the quality control records?

What records does the laboratory maintain to document that stains are filtered or changed when necessary?

D5681

§493.1276 Standard: Clinical cytogenetics.

(a) The laboratory must have policies and procedures for ensuring accurate and reliable patient specimen identification during the process of accessioning, cell preparation, photographing or other image reproduction technique, photographic printing, and reporting and storage of results, karyotypes, and photographs.

Interpretive Guidelines §493.1276(a)
When condition level deficiencies in Clinical Cytogenetics are in any or all phases of testing, use D5034.

Determine which of the following services may be provided:

- *Tissue Cultures (e.g., skin, lung, product of conception);*
- *Bone Marrow Cultures;*
- *Solid Tumors;*
- *Lymph Nodes;*
- *Chorionic Villus Samples (CVS);*
- *Peripheral Lymphocyte Cultures;*
- *Amniotic Fluid Cultures;*
- *High resolution chromosome analysis;*
- *Special techniques (e.g., Fragile "X" Studies, Chromosome Breakage analysis);*
- *Karyotype analysis (photographic and/or computer methods);*
- *Transplant studies;*
- *Chromosome staining (banding techniques) such as:*
  - *Quinacrine fluorescence (Q Banding);*
  - *Giesma/trypsin (G Banding);*
  - *Sodium phosphate/acridine or giesma/heat (R Banding);*
  - *Barium hydroxide/heat (C Banding);*
  - *Nuclear Organizing Region - Silver Stain (NOR);*
  - *Distamycin A/4-6-diamidino-2-phenylindole (DA/DAPI); or*
  - *Giemsa 11 (pH 11.0 for heterochromatin) (G 11).*

NOTE: The above listing is not intended to be all-inclusive.

Review a sample of patient case files to determine if it is possible to go from the accession number to the patient's file with karyotypes, report and observation records, the microscope slide, photographs or requisition forms.

Probes §493.1276(a)

When photographs are taken, are the coordinates of the microscope noted for each cell selected? If not, how does the laboratory identify the cell for future reference?

What system does the laboratory use to ensure that records reflect accurate patient identification when:

- *Photographing chromosome spreads;*
- *Using computer systems to assist in karyotyping; or*
- *Storing photographic images of chromosomes and chromosomes spreads?*

D5683

§493.1276 Standard: Clinical cytogenetics.

(b) The laboratory must have records that document the following:
(b)(1) The media used, reactions observed, number of cells counted, number of cells karyotyped, number of chromosomes counted for each metaphase spread, and the quality of the banding
(b)(2) The resolution is appropriate for the type of tissue or specimen and the type of study required based on the clinical information provided to the laboratory.
(b)(3) An adequate number of karyotypes are prepared for each patient.

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.

A number of factors may influence the quality of the metaphase spreading (e.g., humidity, air flow, cell concentration, and cell storage conditions).

An analysis of at least 50 cells is recommended when:

- *Single trisomic cells are found during a study;*
- *Mosaicism is suspected on the basis of a phenotype not correlating with the karyotype during the study; or*
- *Sex chromosome abnormalities are suspected.*

Additionally, when mosaicism is suspected, ensure that adequate number of cells or nuclei are scored.

- *Follow manufacturer's instructions for the use of the probe in accordance with the FDA requirements for "Analyte Specific Reagents (ASR)."*

- *Establish or verify test system performance using each new probe and each new lot of probe in accordance with D5421 or D5423; thereafter the laboratory must ensure test methodology performance in accordance with D5411.*

- *Establish criteria for scoring the number of probe signals and the number of cells to be examined. Use D5425.*

<u>For fragile X analysis</u>:

- *Males - at least 50-100 cells should be scored for negative analysis.*
- *Females - at least 100-150 cells should be scored for negative analysis.*

The presence of the Xq27.3 fragile site should be confirmed with chromosome banding.

Fragile X studies require low folate medium and media which includes treatment with an antimetabolite such as fluorodeoxyuridine (FUdR), methotrexate, excess thymidine, fluordeoxycytidine (FdC) or other proven induction systems.

<u>General guidance:</u>

Examine the karyotypes and a slide from among the laboratory cases and determine if the quality of banding and resolution was sufficient to render the reported interpretation. Examination of the long arm on the 18th chromosome should demonstrate at least two distinct dark staining G-bands at the 400 band level.

Verify that the laboratory's policy establishes a specific band level of resolution that would be dependent upon the study requested.

High resolution chromosome analysis should refer to studies done above the 550 band stage. (Above 650 band stage for an unfocused study. A focused study should be done at a level of resolution at which the band in question is clearly separated from surrounding bands in one member of the homologous pair in question.) Use D5683.

Probes §493.1276(b)(1)-(b)(3)
For fragile X analysis, if a folate deficient medium is not used as described above, how does the laboratory assure the validity of the test system and the accuracy of results? Use D5411 or D5413, as applicable.

How many photographic and/or computerized karyotypes are prepared from each cell line? (A minimum of 2 is recommended.)

What band level of resolution is used by the laboratory to rule out structural defects (i.e., routine or 400-500 band stage, or high resolution or 650-850 band stage)?

D5685

§493.1276 Standard: Clinical cytogenetics.

(c) Determination of sex must be performed by full chromosome analysis.

D5687

§493.1276 Standard: Clinical cytogenetics.

(d) The laboratory report must include a summary and interpretation of the observations, number of cells counted and analyzed, and use the International System for Human Cytogenetic Nomenclature.

Probes §493.1276(d)
Does the laboratory report include:

- *Type of banding method used, if applicable;*
- *Stage of cell mitosis when banded;*
- *Number of cells counted and analyzed microscopically;*
- *Number of cells from which photographic or computerized karyotypes were prepared; and*
- *Estimate of the banding resolution achieved?*

Does the laboratory, where appropriate, ensure that FISH clinical interpretations are made in conjunction with standard cytogenetic analyses and evaluated against patient medical history and other diagnostic test results?

Preliminary reports of karyotypes based on less than full analysis are acceptable if the diagnosis is clear.

For what types of cultures are preliminary reports issued? These may include, but are not limited to, the following:

- *Bone marrow analysis (within 14 days);*
- *Unstimulated blood cultures (within 14 days); and*
- *Lymphocytes from newborns (within 7 days).*

What is the average length of time for reporting (use D5801or D5815, as appropriate):

- *Amniotic fluid cell cultures (90% of prenatal diagnosis cases should be signed out in 21 days);*
- *Routine lymphocyte cultures (approximately 4-5 weeks); and*
- *Fibroblast cultures (approximately 2-3 months)?*

Do records document:

- *Observations made concurrently with the performance of each step in the examination of specimens/cultures (use D5683); and*
- *The number of cases reviewed, signed out and/or the frequency of failed or sub-optimal cultures?*

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§493.1276 Standard: Clinical cytogenetics.

(e) The laboratory must document all control procedures performed, as specified in this section.

Probes §493.1276(e)
Each day of use, does the laboratory test the positive and negative reactivity of staining materials to ensure predictable staining characteristics? Use D5473.

Does the laboratory, concurrent with the initial use, check each batch of media for pH (amniotic cell cultures should be kept between pH 6.8 and 7.8), sterility, and ability to support growth? Use D5477.

Does the laboratory employ an alternative procedure for the immediate assessment and monitoring of all testing over time? For example: Control materials are not routinely available to demonstrate chromosome abnormalities for linkage, breakage or translocation, but the laboratory must demonstrate an alternative mechanism for detecting chromosome abnormalities to be analyzed. Use 5485.

An alternative procedure might include spit sample with another laboratory, repeat patient specimen, special stains, FISH assays, and/or molecular assays.

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§493.1278 Standard: Histocompatibility.

(a) *General*. The laboratory must meet the following requirements.

Probes: §493.1278(a):
When condition level deficiencies in Histocompatibility are in any or all phases of testing, cite D5038.

D5729

§493.1278 Standard: Histocompatibility.

(a)(1) An audible alarm system must be used to monitor the storage temperature of specimens (donor and recipient) and reagents. The laboratory must have an emergency plan for alternate storage.

Interpretive Guidelines §493.1278(a)(1)-(a)(2):
Ultra low (-80^{o}C) freezers and liquid nitrogen (LN2) reservoirs are common in these laboratories. LN2 reservoirs should be monitored to ensure adequate supply of LN2 at all times.

Verify that the laboratory has an audible alarm system for freezers and refrigerators where critical patient specimens and test reagents are stored. The laboratory should

have established the temperature at which the audible alarm will activate. Determine if the laboratory has an emergency power source for this alarm system in the event of an electrical failure. If emergency power is not available, the laboratory should have policies/procedures on how to ensure a prompt response to an activated alarm, 24 hours a day, 7 days a week, including holidays.
An emergency plan for alternate storage of historic patient serum specimens necessary for pre-transplant crossmatching is critical. Verify that the laboratory has an emergency plan for alternate storage appropriate for its operational needs.

D5731

§493.1278 Standard: Histocompatibility.

(a)(2) All patient specimens must be easily retrievable.

Interpretive Guidelines §493.1278(2):
Patient specimens needed for pre-transplant testing should be stored on-site.

D5733

§493.1278 Standard: Histocompatibility.

(a)(3) Reagent typing sera inventory prepared in-house must indicate source, bleeding date and identification number, reagent specificity, and volume remaining.

D5735

§493.1278 Standard: Histocompatibility.

(a)(4) If the laboratory uses immunologic reagents (for example, antibodies, antibody-coated particles, or complement) to facilitate or enhance the isolation of lymphocytes, or lymphocyte subsets, the efficacy of the methods must be monitored with appropriate quality control procedures.

Interpretive Guidelines 493.1278(a)(4):
Lymphocytes can be isolated from peripheral blood, lymph nodes and spleen. These cells can be further separated into subsets such as T cells and B cells. Examples of commonly used commercial immunologic reagents include immunomagnetic beads and monoclonal reagents. The laboratory should determine the quality (cell viability), the quantity (final yield), subset specificity (T cell, B cell, etc.), and purity (contaminating cells removed) of the final cell preparation. The laboratory should have policies and/or procedures for assessment of the efficacy of these reagents to include criteria for acceptability. For deficiencies related to the procedure, use D5403; for control material acceptability, use D5469.

The subset specificity of each lot of immunomagnetic beads should be verified with antiserum specific for each cell type (e.g., T cell beads with anti-T-lymphocyte serum).

D5737

§493.1278 Standard: Histocompatibility.

(a)(5) Participate in at least one national or regional cell exchange program, if available, or develop an exchange system with another laboratory in order to validate interlaboratory

reproducibility.

Interpretive Guidelines §493.1278(a)(5):
Programs offered by proficiency testing companies and cell exchanges for histocompatibility laboratories are readily available. An example of a regional exchange program is the Southeastern Organ Procurement Foundation (SEOPF). UCLA provides an international monthly exchange program with sera, cells and DNA. The College of American Pathologists (CAP) and the American Society for Histocompatibility and Immunogenetics (ASHI) each offer programs that assess the primary areas of testing in histocompatibility laboratories by test techniques (i.e., antibody screening and identification, HLA typing for Class I (HLA-A, B, C) and Class II (HLA-DR, DQ), lymphocyte crossmatching (T cell and B cell)).

Laboratories participating in a local exchange should record information concerning the frequency of exchange and the grading system.

Cite a deficiency if the laboratory is not enrolled in a cell exchange program or is enrolled in a program, but fails to return the results. A laboratory's performance in a regional or national exchange program should be evaluated against a peer group performing the same technique.

D5739

§493.1278 Standard: Histocompatibility.

(b) *HLA typing.* The laboratory must do the following:

Interpretive Guidelines §493.1278(b):
HLA (Human Leukocyte Antigens) typing is the identification of histocompatibility antigens and/or alleles. HLA typing is performed by serologic or molecular methods.

Serologic typing is usually performed by incubating viable lymphocytes with antisera of known HLA specificities. Antibodies will bind cells with the corresponding HLA antigen(s) on their surface. When complement is added to an immune complex, it binds to the complex causing cell death. The surface of the lymphocyte becomes permeable to stains and this positivity is determined microscopically.

HLA typing using nucleic acid (DNA) and primers and/or probes involves using the polymerase chain reaction (PCR) to amplify HLA sequences of interest which are detected by gel electrophoresis, ELISA or by fluorescence detection using flow cytometry.

(b)(1) Use a technique(s) that is established to optimally define, as applicable, HLA Class I and II specificities.

Interpretive Guidelines §493.1278(b)(1):
HLA CLASS I specificities include HLA-A, B, Cw.

HLA CLASS II specificities include HLA-DR, DQ, and DP.

Verify that the laboratory has validated the reagents and methods it uses. For deficiencies related to verification of methods, use D5421; for establishment of methods, use D5423.

(b)(2) HLA type all potential transplant recipients at a level appropriate to support clinical

transplant protocol and donor selection.

Interpretive Guidelines §493.1278(b)(2):
The laboratory should be an active participant of the transplant center's clinical program. It should provide the technical assistance and pertinent data necessary to help establish transplant protocols for solid organ, tissue and cellular transplants and transfusions. Each protocol should specify what HLA specificities should be identified and at what level this testing needs to be performed. HLA Class I and Class II typing must be performed in accordance with the protocol.

(b)(3) HLA type cells from organ donors referred to the laboratory.
(b)(4) Use HLA antigen terminology that conforms to the latest report of the World Health Organization (W.H.O.) Committee on Nomenclature. Potential new antigens not yet approved by this committee must have a designation that cannot be confused with W.H.O. terminology.
(b)(5) Have available and follow written criteria for the following:

D5741

§493.1278 Standard: Histocompatibility.

(b)(5)(i) The preparation of cells or cellular extracts (for example, solubilized antigens and nucleic acids), as applicable to the HLA typing technique(s) performed.

Interpretive Guidelines §493.1278(b)(5)(i):
The laboratory's procedure manual should contain cell and /or DNA isolation procedures for each type of specimen it uses (e.g., peripheral blood, lymph nodes and spleen, cell cultures, filter paper blood spots, buccal swabs).

Laboratories should assess pretest viability of cells prior to dotting on typing trays. They may use trypan blue stain, wet preps, etc. Verify that the laboratory maintains records of this activity. For most techniques, viability should exceed 80%.

Determine if the laboratory has verified their extraction method. Use D5421.

D5743

§493.1278 Standard: Histocompatibility.

(b)(5)(ii)Selecting typing reagents, whether prepared in-house or commercially.

Interpretive Guidelines §493.1278(b)(5)(ii)
For HLA complement dependent lymphocytotoxicity typing, each batch of complement must be tested to determine that it mediates cytotoxicity (cell death) in the presence of a specific HLA antibody, but is not cytotoxic in the absence of a specific antibody. The test should ensure that it is maximally active at least one dilution beyond that intended for use. The test should be carried out with at least two antibodies known to react with at least two different cells (positive control), and at least one cell which should not react (negative control). A strong and a weak antibody should be selected for the test. Serial dilutions of a single serum may also be used. Verify that the laboratory has performed complement quality control and that an optimum dilution has been selected and documented. Complement is temperature sensitive (labile) and should be retitered periodically to ensure its activity. Determine if the laboratory has complement retitering policies/procedures.

The results of each batch/lot of reagents (typing trays) whether commercially made or prepared in-house must be reviewed to determine which sera failed to react as expected (false negative reactions) and which sera had unexpected reactions (false positive reactions). Future tray preparation and interpretation of commercially purchased trays should be evaluated and revised based on the results of these reviews.

Probes §493.1278(b)(5)(ii):
What criteria were used to determine the acceptability of each batch of complement for HLA serologic assays?

How does the laboratory select the typing trays it uses for each patient?

D5745

§493.1278 Standard: Histocompatibility.

(b)(5)(iii) Ensuring that reagents used for typing are adequate to define all HLA-A, B and DR specificities that are officially recognized by the most recent W.H.O. Committee on Nomenclature and for which reagents are readily available.

Interpretive Guidelines §493.1278(b)(5)(iii):
Antisera for less frequent and rare specificities may be unavailable to laboratories. It is good laboratory practice for each HLA antigen to be defined by at least two operationally monospecific sera. Typing for (HLA) class I or class II antigens must employ a sufficient number of anitsera or monoclonal antibodies to clearly define all the antigens for which the laboratory tests. For example: If multispecific sera must be used, at least three partially non-overlapping sera should be used to define each HLA-antigen. For each HLA-DR and HLA-DQ antigen to be defined, at least 3 operationally monospecific sera should be used. If multispecific sera must be used, at least 5 partially non-overlapping sera should be used.

The laboratory should demonstrate that typing sera reactions are recorded, reviewed and used to modify locally prepared typing trays and interpret commercial tray specificities.

Primer and/or probe sequence, specificity and sensitivity should be defined with reference material (previously typed DNA). For typing methods using probe technology, verify whether optimum hybridization temperatures have been verified or established for each probe.

The laboratory should demonstrate that reference material testing is recorded regularly, reviewed and used to modify locally prepared reagents, as well as interpret commercial primer and/or probe specificities.

Probes §493.1278(b)(5)(iii):
How are the specificities of new typing sera, primers and probes (whether local or commercial) verified, e.g., by parallel testing with known cells or DNA?

How does the laboratory report HLA typings performed by serology and DNA (i.e., follow the W.H.O. nomenclature list)? Are antigens and alleles reported appropriately?

D5747

§493.1278 Standard: Histocompatibility.

(b)(5)(iv) The assignment of HLA antigens.

Interpretive Guidelines §493.1278(b)(5)(iv):
Criteria for antigen and/or allele assignment must take into account basic principles of genetic inheritance.

Examples:
*1. No more than 2 antigens or alleles per HLA-A, B, and DR locus can be assigned to any patient; e.g., antigens HLA-A2, A24; B46, B61; DR8, 14; alleles HLA-A*02XX, 24XX; B*4002, 4601; DRB1*0803, 1401. Public specificities may be observed; i.e. for HLA-B, additional specificities of Bw4 and/or Bw6 are reported, for Class II antigens, additional gene products of DR51, DR52 and/or DR53 are reported.*
2. When family studies are performed, typing interpretations should be in accordance with genetic relationships (i.e., haplotype assignments, determination of homozygosity at a particular locus).

Verify that the laboratory has established acceptability criteria for assignment of HLA antigens and/or alleles. Examples for alleles include signal intensity, band clarity and migration, specificity, and procedures to resolve ambiguous alternative combinations.

Determine if testing personnel follow the scoring and reporting system defined in the procedure manual. Two independent interpretations are recommended for each DNA analysis. Determine if the laboratory has validated computer software for the analysis of antigens and/or alleles.

D5749

§493.1278 Standard: Histocompatibility.

(b)(5)(v) When antigen redefinition and retyping are required.

Interpretive Guidelines §493.1278(b)(5)(v):
Verify that the laboratory has policies and procedures for antigen and/or allele redefinition and retyping. Records should indicate that results from redefinition and retyping are evaluated and that patient typings are updated accordingly. Discrepancies identified as the result of this activity should be documented and resolved. Use D5775.

D5751

§493.1278 Standard: Histocompatibility.

(b)(6) Check each HLA typing by testing, at a minimum the following:
(b)(6)(i) A positive control material.
(b)(6)(ii) A negative control material in which, if applicable to the technique performed, cell viability at the end of incubation is sufficient to permit accurate interpretation of results. In assays in which cell viability is not required, the negative control result must be sufficiently different from the positive control result to permit accurate interpretation of results.
(b)(6)(iii) Positive control materials for specific cell types when applicable (that is, T cells, B cells, and monocytes).

Interpretive Guidelines §493.1278(b)(6):
Each HLA-A, B, C or supplemental Class I typing tray must include at least one positive control serum, previously shown to react with all lymphocytes, and one negative control serum which has been demonstrated to be non-cytotoxic. HLA-DR and DQ typing trays must include a positive control serum, previously shown to react with only B cells, and

one negative control serum which has been demonstrated to be non-cytotoxic.

Cell controls must be tested with each batch/lot/shipment of typing trays. Typing results are invalid if controls fail to react as expected. The negative control should either be one previously shown to lack antibody or should be from a healthy male with no history of blood transfusion. Cell viability in the negative control well at the end of the incubation must be sufficient to permit accurate interpretation of results. For most techniques, viability should exceed 80%. However, when less than optimal specimens, such as cadaver and mailed specimens, this threshold may not be met.

For DNA typing, negative control wells or wells with no DNA should not give a positive result (the presence of a band), however, internal controls should give a positive result. DNA reference material must be tested with each lot of typing reagents. Primers and/or probes must be tested for allele specificity with reference material.

D5753

§493.1278 Standard: Histocompatibility.

(c) *Disease-associated studies.* The laboratory must check each typing for disease-associated HLA antigens using control materials to monitor the test components and each phase of the test system to ensure acceptable performance.

Interpretive Guidelines §493.1278(c):
Disease association studies are single or limited antigen typings usually performed by serologic typing methods and more rarely performed by flow cytometric methods.

Positive and negative controls must be run with each test.

Control cells must be tested with each lot and shipment of reagents. Use D5469.

For serologic typings, the control cells should include at least two cells known to express the specified antigen and two cells known to express cross-reacting antigens that might be confused with the specific antigen. Control cells should also include at least two cells lacking the specific and cross-reacting antigen.

For typing sera acceptability, use D5745.

D5755

§493.1278 Standard: Histocompatibility.

(d) *Antibody Screening.* The laboratory must do the following:
(d)(1) Use a technique(s) that detects HLA-specific antibody with a specificity equivalent or superior to that of the basic complement-dependent microlymphocytotoxicity assay.
(d)(2) Use a method that distinguishes antibodies to HLA Class II antigens from antibodies to Class I antigens to detect antibodies to HLA Class II antigens.
(d)(3) Use a panel that contains all the major HLA specificities and common splits. If the laboratory does not use commercial panels, it must maintain a list of individuals for fresh panel bleeding.

Interpretive Guidelines §493.1278(d)(1)-(d)(3):
An antibody screen is performed to identify whether a patient's serum contains antibodies to one or more HLA antigens. This is accomplished by screening the serum against target antigens from a suitable panel appropriate for the population served, i.e., a variety

of ethnic groups. Results are expressed as percent reactive antibodies (PRA).

The panel of antigens used must include all of the HLA antigens to which the most common HLA antibodies are formed. Cell panels of known HLA type must be available to prove the specificity of new antibodies. The serum cell panel should be consistent from month to month and from lot to lot. Verify that the frequency of each antigen represented does not vary significantly.

An example of PRA differences from panel to panel:
If a patient demonstrates a HLA-A2 antibody and the cell panel contains 15 A2 positive cells out of 100, the patient's PRA on this tray will be 15%. If the same patient is tested against a panel where there are 37 A2 positive cells out of 100, the patient's PRA will increase to 37%. The number of A2 positive cells on this laboratory's cell panel should reflect the frequency observed in the population it serves; e.g., 15-20% of the local population possess the HLA-A2 antigen.

If the laboratory tests for antibodies to Class II antigens, the laboratory should have a procedure for removing Class I antibodies or should use purified Class II antigens. Class II antigens (HLA-DR, DQ) are found only on the B cell subset of lymphocytes. B cells also have a high density of Class I antigens (HLA-A, B, C), which are found on all nucleated cells. If a patient has a significant titer of Class I antibodies, it may result in a false positive Class II antibody test result. Platelet absorption is one method of removing the Class I antibodies.

Verify that the laboratory's antibody screening technique is as sensitive as the crossmatch method it uses to ensure optimum compatibility.

D5757

§493.1278 Standard: Histocompatibility.

(d)(4) Make a reasonable attempt to have available monthly serum specimens for all potential transplant recipients for periodic antibody screening and crossmatch.
(d)(5) Have available and follow a written policy consistent with clinical transplant protocols for the frequency of screening potential transplant recipient sera for preformed HLA-specific antibodies.

Interpretive Guidelines §493.1278(d)(4)-(d)(5):
A recipient's antibody profile should be evaluated when the individual is entered on the transplant waiting list. Determine whether the laboratory obtains specimens at initial typing for antibody screening and for pre-transplantation auto crossmatches.

The laboratory should have clearly defined and appropriate screening protocols for potentially sensitizing events such as transfusion, transplant loss, pregnancy or infection. Verify that the laboratory obtains and tests patient specimens to determine if there have been changes in the antibody profiles as defined by the transplant center's protocols. Determine when the laboratory verifies that the antibodies in the serum have been characterized against HLA antigens.

Probes §493.1278(d)(4)-(d)(5)
What policies and procedures has the laboratory implemented in an effort to procure monthly serum specimens for potential transplant recipients?

What is the laboratory's frequency for screening potential transplant recipient sera for preformed HLA-specific antibodies?

D5759

§493.1278 Standard: Histocompatibility.

(d)(6) Check each antibody screening by testing, at a minimum the following:
(d)(6)(i) A positive control material containing antibodies of the appropriate isotype for the assay.
(d)(6)(ii) A negative control material.

Interpretive Guidelines §493.1278(d)(6):
For serologic antibody screening, each tray must include at least one positive control serum previously shown to react with all lymphocytes and one negative control serum which has been demonstrated to be non-cytotoxic or lack antibody. Results are invalid if controls fail to react as expected. Cell viability in the negative control well at the time of reading must be sufficient to permit accurate interpretation of results. Viability should exceed 80%. The positive control must contain antibodies of the appropriate isotype (e.g., IgG and/or IgM). If the frozen cell tray is specific for Class II (HLA-DR or DQ) antibody testing, the laboratory must ensure B cells are being tested and have a mechanism to distinguish Class II antibodies from antibodies to Class I antigens that are also found on B cells.

Laboratories using ELISA and/or flow cytometric techniques must include one positive control serum and one negative control serum. Reagent controls for non-specific binding of antibody should be included with all ELISA testing. The negative control for flow cytometers should demonstrate non-reactivity and the positive control should be specific for HLA antigens. Again, the positive control for both techniques must contain antibodies of the appropriate isotype (i.e., IgG and/or IgM).

Verify that the laboratory uses a negative control and the appropriate isotype for its positive control.

Verify that the laboratory has established acceptability criteria for each control and for each method it uses.

D5761

§493.1278 Standard: Histocompatibility.

(d)(7) As applicable, have available and follow written criteria and procedures for antibody identification to the level appropriate to support clinical transplant protocol.

Probe §493.1278(d)(7):
Does the laboratory's policies specify when antibody reactivity (positive antibody screen) will be further characterized, (i.e., identification of antibody directed against specific HLA antigens) and the procedures to be used for antibody identification?

D5763

§493.1278 Standard: Histocompatibility.

(e) *Crossmatching*. The laboratory must do the following:
(e)(1) Use a technique(s) documented to have increased sensitivity in comparison with the basic complement-dependent microlymphocytotoxicity assay.

Interpretive Guidelines §493.1278(e)(1):
The minimum technique for crossmatching for transplantation must be more sensitive than the basic lymphocytotoxicity test (standard complement dependent or NIH procedure). A technique that enhances sensitivity must be used (e.g., increased incubation time, additional wash steps, antihumanglobulin (AHG) augmentation, ELISA testing, flow cytometry testing).

D5765

§493.1278 Standard: Histocompatibility.

(e)(2) Have available and follow written criteria for the following:
(e)(2)(i) Selecting appropriate patient serum samples for crossmatching.

Interpretive Guidelines §493.1278(e)(2)(i):
The laboratory must have clearly defined protocols for selection of serum for crossmatch testing. There are numerous acceptable protocols for the selection of crossmatch samples which vary from transplant center to center. However, every effort should be made to procure a specimen at the time of transplant or unless the laboratory can clearly establish that the patient did not receive a blood transfusion or other alloimmunizing event between the times of specimen collection and transplant date.

Review patient transplant records for lymphocyte crossmatch results. Verify serum selected for crossmatching against antibody screening/identification records. Verify if the serum is tested at an optimal dilution. Crossmatches are performed with donor T cells (T lymphocytes) or unseparated lymphocytes. Crossmatches with donor B cells (B lymphocytes) may be performed.

Probes §493.1278(e)(2)(i):
Does the laboratory's policies and procedures specify which patient serum samples are to be used for crossmatching (e.g., renal, pancreas, heart, lung, small intestine or liver transplants)?

(e)(2)(ii) The preparation of donor cells or cellular extracts (for example, solubilized antigens and nucleic acids), as applicable to the crossmatch technique(s) performed.

Interpretive Guidelines §493.1278(e)(2)(ii):
There are various techniques for the isolation of donor cells for use in crossmatching e.g., immunomagnetic beads, monoclonal antibody preparations, density gradient (ficoll hypaque). Crossmatching techniques utilizing cellular extracts (solubilized antigens and nucleic acid) are not well documented in the clinical setting.

Determine if the laboratory follows manufacturer's product insert procedures. Use D5479.

Verify that the laboratory has established procedures and criteria for cell preparation viability, purity and quantity (i.e. peripheral blood, lymph node, spleen).

D5767

§493.1278 Standard: Histocompatibility.

(e)(3) Check each crossmatch and compatibility test for HLA Class II antigenic differences using control materials to monitor the test components and each phase of the test system

to ensure acceptable performance.

Interpretive Guidelines §493.1278(e)(3):
The mixed leukocyte (lymphocyte) culture (MLC) is used by a small number of laboratories and it may be used in conjunction with other cellular assays such as cell mediated lympholysis (CML), primed lymphocyte typing (PLT) or homozygous typing cell (HTC) to determine donor recipient pair compatibility in renal or tissue transplants.

The MLC method may vary from micro, macro, one way or both one way, and two way. Data expressed in counts per minute of tritiated thymidine (H3) are used to calculate the stimulation index (SI) or the relative response (RR). Controls include: a negative control (responder cells stimulated with autologous cells), positive controls (responder cells stimulated with cells from unrelated individuals with known Class II antigen differences or fresh or frozen cell pool). If the laboratory performs MLCs, review their criteria for accepting or rejecting a run and a narrative report on donor recipient compatibility. Confirm that all combinations of any given stimulator is tested against any given responder.

Verify that the laboratory has established criteria for defining positive and negative crossmatches.

Example1:
Basic crossmatch technique: (includes increased incubation time testing or wash(es))
1) Each crossmatch tray must include one positive control serum previously shown to react with all cells and one negative control serum which demonstrates non-cytotoxic activity. Additional controls may include antisera against specific cell lines and reagent controls.
2) Each serum is tested undiluted and at one or more dilutions.

Example 2:
Anti-human globulin augmentation:
1) Each crossmatch tray must include one positive control serum previously shown to react with all cells and one negative control serum which demonstrates non-cytotoxic activity. Additional controls may include antisera against specific cell lines and reagent controls.
2) Each serum is tested undiluted and at one or more dilutions.
3) Verify that AHG has been titered for optimum test performance.

Example 3:
Flow cytometry:
1) Each crossmatch must include one positive control serum and one negative control serum. The positive control should be human serum of the appropriate isotype and specific for HLA antigens shown to react with all cells. The negative control should demonstrate non-reactivity against lymphocytes.
2) Verify that the laboratory has established a threshold for determining a positive reaction (e.g., mean channel shifts, quantitative fluorescence measurements).
3) The laboratory should be running an optical standard (lens focusing and alignment) and fluorescent standard (adequate signal amplification) with each use of the instrument.
4) Verify that the laboratory has established an optimum serum/cell ratio (standard number of cells to a fixed volume of serum).
5) A multi color technique should be used to ensure the purity of the cell population being tested.

Probes §493.1278(e)(3):
What is the laboratory's control acceptance criteria for MLC testing?

D5769

§493.1278 Standard: Histocompatibility

(f) *Transplantation.* Laboratories performing histocompatibility testing for transfusion and transplantation purposes must do the following:
(f)(1) Have available and follow written policies and protocols specifying the histocompatibility testing (that is, HLA typing, antibody screening, compatibility testing and crossmatching) to be performed for each type of cell, tissue or organ to be transfused or transplanted. The laboratory's policies must include, as applicable--
(f)(1)(i) Testing protocols for cadaver donor, living, living-related, and combined organ and tissue transplants;
(f)(1)(ii) Testing protocols for patients at high risk for allograft rejection; and
(f)(1)(iii)The level of testing required to support clinical transplant protocols (for example, antigen or allele level).

Interpretive Guidelines §493.1278(f):
In conjunction with the transplantation center the laboratory establishes written policies on the testing protocols it performs in support of the clinical transplant program. Policies should address when HLA testing and final crossmatches are required for patients that have demonstrated presensitization. For organs such as liver and heart (non-renal), it is not uncommon for laboratories to perform retrospective crossmatches if the patient demonstrates the absence of preformed antibodies by prior screening. Failure to perform a crossmatch prior to transplant is not a deficiency provided emergency transplant circumstances are documented.

For solid organ transplants (renal, heart, liver, lung, small intestine):
1. Determine what tests are performed for potential kidney and pancreas recipients.
2. Determine what tests are performed on living-related or unrelated donors and cadaver donors referred to the laboratory.
3. Determine if the laboratory performs HLA typing using complement dependent lymphocytotoxicity testing (antigen level) and/or DNA testing (allele level);
4. Compare policies for pre-sensitized patients with laboratory antibody screening and identification protocols for consistency;
5. Verify that the laboratory is using a crossmatch technique with increased sensitivity; and
6. Deviations from the established protocols should be documented by the laboratory, indicating the reason for the deviation, e.g., transplant physician request, emergency transplant.

For transfusions (platelet support of refractory patients):
1. Determine what tests are performed on recipients and donors. Recipients are usually HLA-A and HLA-B typed, e.g., platelets do not have Class II (HLA-DR, DQ) antigens on their surface. Donors may be typed by the laboratory, a blood center or a donor program laboratory. HLA typing may be performed using complement dependent lymphocytotoxicity testing (antigen level) and/or DNA testing (allele level).
2. Determine if the laboratory performs antibody screening/identification on the recipient. Compare with the laboratory protocol for antibody screening and identification.
3. Determine if the laboratory performs Class I crossmatch testing.

For tissue transplant (bone marrow/stem cells, etc.)
1. Determine what level of HLA typing is performed on recipients and donors. For bone marrow/stem cell transplantation, recipients are at a minimum HLA-A and HLA-B typed by complement dependent lymphocytotoxicity and/or DNA testing. Recipients should be HLA-DR typed by high resolution DNA typing (allele level). Donors may be typed by the

laboratory or a donor program laboratory.
2. Determine if the laboratory performs crossmatch testing, when a selected potential donor has an HLA mismatch. Determine if the laboratory performs Class II compatibility to evaluate Class II identity by either MLC testing, high resolution DNA typing, or a family study.

§493.1278(f) Probes:
What is the laboratory's policy/protocol on referring patient specimens for testing at another laboratory?

What is the laboratory's policy/protocol on accepting HLA typing results obtained at another laboratory (i.e., does the laboratory reconfirm (repeat) testing)?

D5771

§493.1278 Standard: Histocompatibility.

(f)(2) For renal allotransplantation and combined organ and tissue transplants in which a kidney is to be transplanted, have available results of final crossmatches before the kidney is transplanted.

Probes §493.1278(f)(2):
If the laboratory performs cadaveric renal transplant testing, what are the staffing policies and how do they ensure 24-hour coverage of qualified testing personnel and supervision for technical review?

D5773

§493.1278 Standard: Histocompatibility.

(f)(3) For nonrenal transplantation, if HLA testing and final crossmatches were not performed prospectively because of an emergency situation, the laboratory must document the circumstances, if known, under which the emergency transplant was performed, and records of the transplant must reflect any information provided to the laboratory by the patient's physician.

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§493.1278 Standard: Histocompatibility.

(g) The laboratory must document all control procedures performed, as specified in this section.

§493.1278(g) Guidelines:
All QC records must be maintained for two years including instrument charts, graphs, printouts, transcribed data, manufacturer's assay information sheet for control and calibration materials and reagents to include typing trays, primers and/or probes. Do not dictate the acceptable format for documentation.

D5775

§493.1281 Standard: Comparison of test results.

(a) If a laboratory performs the same test using different methodologies or instruments, or performs the same test at multiple testing sites, the laboratory must have a system that

twice a year evaluates and defines the relationship between test results using the different methodologies, instruments, or testing sites.

Interpretive Guidelines §493.1281(a)-(c):
The laboratory must have a system to monitor and evaluate all testing it performs. Examples of materials that may be used to evaluate the same test performed by different methodologies, at multiple locations, and/or on multiple instruments in the same laboratory are proficiency testing samples, split samples or "blind" testing of materials with known values.

A laboratory that performs the same test at multiple locations or on more than one instrument must have written criteria for acceptable differences in test values (e.g., between different or identical models of an instrument from the same manufacturer, between instruments from different manufacturers).

If the laboratory performs calibration verification as specified in §493.1255(b), it may use the calibration verification to meet the requirements at §493.1281(a), provided the 3 levels of materials used for calibration verification meet the laboratory's criteria for acceptable differences in test values.

D5777

§493.1281 Standard: Comparison of test results.

(b) The laboratory must have a system to identify and assess patient test results that appear inconsistent with the following relevant criteria, when available:
(b)(1) Patient age.
(b)(2) Sex.
(b)(3) Diagnosis or pertinent clinical data.
(b)(4) Distribution of patient test results.
(b)(5) Relationship with other test parameters.

Interpretive Guidelines §493.1281(b):
Verify that the laboratory has a system in place to monitor and evaluate test results for inconsistencies with patient information, and for correlation between test results. For example, a laboratory could multiply the hemoglobin result by a factor of 3, to see if the result is equal to the hematocrit. If the laboratory has auto-validation in it's Laboratory Information System (LIS), verify that the laboratory is taking steps to reduce the likelihood of sample-switching errors, for example, when the creatinine result is significantly different from the patient's previous creatinine test results, or if the MCV is significantly different from the patient's previous test results and the patient did not receive a blood transfusion.

For automated laboratories, inconsistent patient results may be evaluated through the use of verified LIS supported logic, patient distribution test results, verified automated test comparison logic programs and individual test repeat criteria.

Probes §493.1281(b):
How does the laboratory obtain sufficient information to enable an evaluation of test results with clinically relevant patient information?

Does the laboratory have procedures to assess and evaluate patient test results for inconsistencies?

For example:

- *Hemoglobin and Hematocrit (MCHC value exceeds reference range);*
- *BUN and Creatinine comparison;*
- *Albumin and Total Protein;*
- *Correlation of urine culture with urine microscopic; and*
- *Alkaline phosphatase with orthopedic surgical patients and/or pediatric patients; and*
- *Correlation of microscopic sediment findings with macroscopic results, such as, the presence of protein with casts, positive occults blood with red cells, and positive leukocyte esterase with white cells.*

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§493.1281 Standard: Comparison of test results.

(c) The laboratory must document all test result comparison activities.

Interpretive Guidelines §493.1281(c):
The actual measurement(s) of test results and comparison activities must be recorded. Acceptable formats for documentation may vary. Cite documentation deficiencies at §493.1281(a) or §493.1281(b). Use D5775 or D5777, as appropriate.

D5779

§493.1282 Standard: Corrective actions.

(a) Corrective action policies and procedures must be available and followed as necessary to maintain the laboratory's operation for testing patient specimens in a manner that ensures accurate and reliable patient test results and reports.

Interpretive Guidelines §493.1282(a):
Corrective action must be taken when unacceptable differences in test values occur with testing performed using different methodologies or instruments or with the same test performed at multiple testing sites.

Probes §493.1282(a):
When test results do not correlate with patient information (e.g., age, sex, submitted diagnosis) what actions are taken by the laboratory to confirm test results or patient information?

D5781

§493.1282 Standard: Corrective actions.

(b) The laboratory must document all corrective actions taken, including actions taken when any of the following occur:
(b)(1) Test systems do not meet the laboratory's verified or established performance specifications, as determined in §493.1253(b), which include but are not limited to--
(b)(1)(i) Equipment or methodologies that perform outside of established operating parameters or performance specifications;
(b)(1)(ii) Patient test values that are outside of the laboratory's reportable range of test results for the test system; and
(b)(1)(iii) When the laboratory determines that the reference intervals (normal values) for a test procedure are inappropriate for the laboratory's patient population.

Interpretive Guidelines §493.1282(b)(1):
The laboratory's corrective action records should contain sufficient information to resolve the problem if it reoccurs.

Probes §493.1282(b)(1):
When equipment malfunctions or a test method problem exists, how does the laboratory identify and solve the problem?

What corrective actions are taken if patient test results exceed the laboratory's reportable range of patient test results?

If a dilution procedure is used when patient results exceed the test system's reportable range, how does the laboratory assure the appropriate diluent is used for each type of specimen? Use D5401.

How does the laboratory verify and document the accuracy of the results for diluted specimens? Use D5421 or D5423 as appropriate.

D5783

§493.1282 Standard: Corrective actions.

(b)(2) Results of control or calibration materials, or both, fail to meet the laboratory's established criteria for acceptability. All patient test results obtained in the unacceptable test run and since the last acceptable test run must be evaluated to determine if patient test results have been adversely affected. The laboratory must take the corrective action necessary to ensure the reporting of accurate and reliable patient test results.

Interpretive Guidelines §493.1282(b)(2):
When an internal control fails to fall within the defined limits of acceptability, the laboratory must identify the reason for the failure and correct the problem before resuming testing of patients. The laboratory must evaluate all patients test results since the last acceptable external control.

Probes §493.1282(b)(2):
When suboptimal staining or improper coverslipping are identified through quality control procedures, what corrective actions does the laboratory take?

What actions does the laboratory take when controls reflect an unusual trend or are outside of the acceptable limits and other means of assessing and correcting unacceptable control values have failed to identify and correct the problem?

D5785

§493.1282 Standard: Corrective actions.

(b)(3) The criteria for proper storage of reagents and specimens, as specified under §493.1252(b), are not met.

Probes §493.1282(b)(3):
What actions does the laboratory take if the storage temperature for a test system's reagents has been exceeded?

D5787

§493.1283 Standard: Test records.

(a) The laboratory must maintain an information or record system that includes the following:
(a)(1) The positive identification of the specimen.
(a)(2) The date and time of specimen receipt into the laboratory.
(a)(3) The condition and disposition of specimens that do not meet the laboratory's criteria for specimen acceptability.
(a)(4) The records and dates of all specimen testing, including the identity of the personnel who performed the test(s).

Interpretive Guidelines §493.1283(a):
The regulations provide laboratories the flexibility to establish a system that ensures positive patient identification through specimen accessioning and storage, testing and reporting of test results. This may include a system that involves labeling the specimen container and request slip or the patient's medical record or chart with a unique patient identification number, but does not preclude the use of other mechanisms to assist in patient identification and tracking of specimens throughout the testing and reporting processes. The patient's name may be used as part of the identification system.

Ensure that work records reflect all the tests and dates of performance of in-house patient testing. For example, in bacteriology, each step from media inoculation to organism isolation and identification must be documented on worksheet records either manually or in a computer system.

Corrections of laboratory results include the corrected result, incorrect result (noted as such), the date of the correction, and the initials of the person making the correction. Laboratory records should not be documented in pencil and the use of whiteout is not acceptable for making corrections.

Probes §493.1283(a):
Do the records reflect all patient testing and the dates of their performance?

If handwritten values were reported, can the laboratory demonstrate the analytic source of those results?

If the laboratory has not retained the appropriate test records, cite D3031, D3033, or D3035.

D5789

§493.1283 Standard: Test records.

(b) Records of patient testing including, if applicable, instrument printouts, must be retained.

Interpretive Guidelines §493.1283(b):
The regulations do not require that instrument printouts be posted directly in the patient's medical record or chart. However, these printouts must be maintained as part of the laboratory's record retention requirements specified throughout the regulations.

Probes §493.1283(b):
Are the original analytic work records complete (e.g., in a randomly chosen sample, is there an instrument printout for every day of the month on which testing was performed)? Are the original, as opposed to transcribed and/or edited work records, being retained? If the laboratory fails to retain the records for the appropriate amount of time, use D3031.

D5791

§493.1289 Standard: Analytic systems quality assessment.

(a) The laboratory must establish and follow written policies and procedures for an ongoing mechanism to monitor, assess, and when indicated, correct problems identified in the analytic systems specified in §§493.1251 through 493.1283.

Interpretive Guidelines §493.1289(a)-(c):
Quality Assessment (QA) is an ongoing review process that encompasses all facets of the laboratory's technical and non-technical functions at all location/sites where testing is performed. QA also extends to the laboratory's interactions with and responsibilities to patients, physicians, and other laboratories ordering tests, and the non-laboratory areas or the facility of which it is a part.

When the laboratory discovers an error or identifies a potential problem, actions must be taken to correct the situation. This correction process involves identification and resolution of the problem, and development of policies that will prevent recurrence. Policies for preventing problems that have been identified must be written as well as communicated to the laboratory personnel and other staff, clients, etc., as appropriate. Over time, the laboratory must monitor the corrective action(s) to ensure the action(s) taken have prevented recurrence of the original problem.

All pertinent laboratory staff must be involved in the assessment process through discussions or active participation.

QA of the Analytic System includes assessing:

- *Test procedures;*
- *Accurate and reliable test systems, equipment, instruments, reagents, materials, and supplies;*
- *Specimen and reagent storage condition;*
- *Equipment/instrument/test/system maintenance and function checks;*
- *Establishment and verification of method performance specifications;*
- *Calibration and calibration verification;*
- *Control procedures;*
- *Comparison of test results;*
- *Corrective actions; and*
- *Test records.*

For Clinical Cytogenetics, cases, the laboratory should identify increases in or excessive culture failure rates, determine the contributing factors, document efforts to reduce or eliminate these factors and assess the effectiveness of actions taken. (i.e., a decrease in the culture failure rate).

Review assessment policies, procedures and reports to verify that the laboratory has a system in place to ensure continuous improvement. Corrective action reports are one indication that the laboratory is monitoring and evaluating laboratory performance and the quality of services.

Select a sample of abnormal cytology patient reports and determine that, when available, the histopathology and cytology comparison was performed and the cytology 5-year retrospective review was performed. Ensure the laboratory documents any discrepancies and performs corrective action.

Review quality control records to determine if the laboratory's monitoring efforts are detecting control failures, shifts, and trends. If the surveyor identifies previously undetected quality control failures or omission, then the laboratory's system for monitoring and evaluating quality control may not be adequate.

For International Normalized Ratio (INR) calculation, ensure the laboratory:

- *Periodically verifies, for each thromboplastin lot number in use, the correct normal prothrombin time mean and (the International Sensitivity Index (ISI) value are being used for calculating the INR value.*
- *Periodically verifies the accuracy of the INR calculation (manual, instrument or LIS).*

To verify Prothrombin time testing with INR calculations:

- *Check the accuracy of normal Prothrombin time mean calculation (manual, instrument or LIS).*
- *Verify the ISI used in the calculation correlates with the ISI specified in the reagent package insert. Select an abnormal low or abnormal high prothrombin time result and verify the calculation.*

Probes §493.1289(a):
For clinical cytogenetics cases, does the laboratory monitor the frequency of culture failures and sub-optimal analyses?

Does the laboratory add additional maintenance procedures and/or function checks, when needed, to ensure accurate and reliable test results?

What is the laboratory's system for monitoring and evaluating test results for inconsistencies with patient information?

D5793

§493.1289 Standard: Analytic systems quality assessment.

(b) The analytic systems quality assessment must include a review of the effectiveness of corrective actions taken to resolve problems, revision of policies and procedures necessary to prevent recurrence of problems, and discussion of analytic systems quality assessment reviews with appropriate staff.

Interpretive Guidelines §493.1289(b):
Verify that the laboratory has a system in place to monitor and evaluate test results for inconsistencies with patient information, and for correlation between test results. For example, a laboratory could multiply the hemoglobin result by a factor of 3, to see if the result is equal to the hematocrit. If the laboratory has auto-validation in it's Laboratory Information System (LIS), verify that the laboratory is taking steps to reduce the likelihood of sample-switching errors, for example, when the creatinine result is significantly different from the patient's previous creatinine test results, or if the MCV is significantly different from the patient's previous test results and the patient did not receive a blood transfusion.

Probes §493.1289(b):
How does the laboratory address multiple failed or sub-optimal cultures that have been submitted from one client?

How does the laboratory use the review of all normal or negative gynecologic specimens received within the previous 5 years to assess the analytic system and communicate findings to the staff?

(c) The laboratory must document all analytic systems assessment activities.

Interpretive Guidelines §493.1289(c):
The steps taken by the laboratory to identify and correct problems and prevent their recurrence must be documented. All laboratory policies amended due to its QA activities must also be noted.

D5800

Postanalytic Systems

§493.1290 Condition: Postanalytic systems.

Each laboratory that performs nonwaived testing must meet the applicable postanalytic systems requirements in §493.1291 unless HHS approves a procedure, specified in Appendix C of the State Operations Manual (CMS Pub. 7) that provides equivalent quality testing. The laboratory must monitor and evaluate the overall quality of the postanalytic systems and correct identified problems as specified in §493.1299 for each specialty and subspecialty of testing performed.

Interpretive Guidelines §493.1290:
Significant deficiencies cited under this condition may indicate deficiencies under personnel responsibilities. Use D5800 when deficiencies are identified that are: significant and have the potential to, or adversely affect, patient testing, are systemic and pervasive throughout the laboratory, and are not limited to any one specialty or subspecialty.

D5801

§493.1291 Standard; Test report.

(a) The laboratory must have an adequate manual or electronic system(s) in place to ensure test results and other patient-specific data are accurately and reliably sent from the point of data entry (whether interfaced or entered manually) to final report destination, in a timely manner. This includes the following:

Interpretive Guidelines §493.1291(a):
The regulations apply to manual as well as automated record systems, i.e., a laboratory information system or LIS. However, the regulations do not specify the mechanism or frequency for which a laboratory should evaluate its record storage and retrieval system(s).

Probes §493.1291(a):
How does the laboratory ensure that transmitted reports are legible and the information received at the final destination was the same data sent by the laboratory?

If the laboratory uses a LIS or facsimile, what security measures have been instituted to ensure that transmitted reports go directly from the device sending reports only to the authorized individual, their location or electronic system?

§493.1291 Standard: Test report.

(a)(1) Results reported from calculated data.
(a)(2) Results and patient-specific data electronically reported to network or interfaced systems.
(a)(3) Manually transcribed or electronically transmitted results and patient-specific information reported directly or upon receipt from outside referral laboratories, satellite or point-of-care testing locations.

Interpretive Guidelines §493.1291(a)(3):
Manually transcribed or electronically transmitted results from an outside referral laboratory or from within the laboratory system (e.g., satellite or point-of-care testing locations) must be periodically verified for accuracy and timely reporting.

D5803

§493.1291 Standard: Test report.

(b) Test report information maintained as part of the patient's chart or medical record must be readily available to the laboratory and to CMS or a CMS agent upon request.

Interpretive Guidelines §493.1291(b):
The test report information should be legible, understandable, and complete.

D5805

§493.1291 Standard: Test report.

(c) The test report must indicate the following:
(c)(1) For positive patient identification, either the patient's name and identification number, or a unique patient identifier and identification number.

Interpretive Guidelines §493.1291(c)(1)- (c)(6):
Use D5203 for deficiencies related to specimen identification problems.

When used on the test report, the patient's name must be accompanied by an identification or accession number. When for confidentiality purposes a patient's name is not used or when the identity of the person is not known, a unique patient identifier and identification or accession number must be used on the report.

(c)(2) The name and address of the laboratory location where the test was performed.

Interpretive Guidelines §493.1291(c)(2):
Laboratories having a single certificate for multiple sites/locations must have a system in place to identify which tests were performed at each site.

A code to identify the name and address of the laboratory performing testing is acceptable as long as the code is clearly annotated on the patient test report. This may be accomplished by using abbreviated indicators (e.g., asterisks) as long as they are identified and apparent to the individual receiving the report. This or a similar system

may be seen on cumulative reports. The name and address of the reference laboratory may also be defined on a subsequent page or on the back of the report. Laboratories have latitude to develop other formats to meet this requirement.

§493.1291 Standard: Test report.

(c)(3) The test report date.

Interpretive Guidelines §493.1291(c)(3):
The date of the test report is the date results were generated as a final report and must not change on copies generated at a later date.

(c)(4) The test performed.

Interpretive Guidelines §493.1291(c)(4):
Laboratories using manufacturer's instruments, kits or test systems labeled for "investigational use only" or "research use only" must clearly state that the test results are not to be used for treatment or diagnostic purposes. If results of such tests are being reported without a disclaimer statement, or are being used by the provider for patient care, they are in the same category as in-house developed tests and the laboratory must establish performance specifications in accordance with §493.1253.

The disclaimer for Analyte Specific Reagents (ASR) should state ["This test was developed and its performance characteristics determined by (Laboratory Name). It has not been cleared or approved by the U.S. Food and Drug Administration"]. The ASR disclaimer on the test report is required by the FDA under 21 CFR, Part 809.30 "Restrictions on the sale, distribution and use of analyte specific reagents."

(c)(5) Specimen source, when appropriate.

Interpretive Guidelines §493.1291(c)(5):
Some examples of source of the specimen needed by the laboratory to accurately perform testing and report results would be: site of culture; type of body fluid; whether a submitted separated specimen is plasma, serum, urine, etc.

§493.1291 Standard: Test report.

(c)(6) The test result and, if applicable, the units of measurement or interpretation, or both.

Interpretive Guidelines §493.1291(c)(6):
If the laboratory prints normal ranges on the patient test report, verify that "sex and/or age specific" normal ranges are printed by the LIS on the patient test report.

"Less than" is used for reporting test results (qualitative or quantitative) that are below the laboratory's detection limits for an analyte. (Detection limits must be established through method verification as described in §493.1253.) "Equivalent designation" is used to report test results for those methods that yield results below a clinically significant level (e.g., for a quantitative immunology test, patient results may be clinically negative at a 1:8 titer and test results may be reported as "1:8 negative". The normal range is 1:8 or less.) "Greater than" is used for reporting test results (qualitative or quantitative) that are above the laboratory's detection limits for an analyte. If patient test results exceed the laboratory's reportable range, the laboratory must report the result as greater than the highest detection limit, reassay a diluted patient specimen and report the calculated result, or send the specimen to a reference laboratory.

For flow cytometry, to interpret results, staff should have access to the complementary

clinical picture of the patient. This may include such results as white cell count, cell differential, cell morphology, and cytogenetics.

Flow cytometry patient data files should include any gating analysis regions used to obtain reported test results.

For genetic tests, the laboratory should include the test method(s) employed and any mutations on the test report.

Probes §493.1291(c)(6):
When additional information is critical for the interpretation of test results (e.g., screening vs. confirmatory procedures), how does the laboratory convey this information to the individual ordering or using test results?

If the laboratory does not print normal ranges on the test report, how does the laboratory notify the client that reported results are abnormal for the patient due to their particular sex and/or age?

§493.1291 Standard: Test report.

(c)(7) Any information regarding the condition and disposition of specimens that do not meet the laboratory's criteria for acceptability.

Interpretive Guidelines §493.1291(c)(7):
If the laboratory functions as a reference laboratory, how does it notify the referring laboratory or client of unacceptable specimens in a timely manner? Use D5801 to cite timeliness deficiencies. Use D5805 to cite the referring laboratory's failure to notify the appropriate individual concerning the unacceptable specimen.

D5807

§493.1291 Standard: Test report

(d) Pertinent "reference intervals" or "normal" values, as determined by the laboratory performing the tests, must be available to the authorized person who ordered the tests and, if applicable, the individual responsible for using the test results.

Interpretive Guidelines §493.1291(d):
The laboratory must ensure the "reference intervals" or "normal" values it provides to its clients are accurate, include appropriate units of measurement, and reflect the method performed and the patient population (if applicable).

D5809

§493.1291 Standard: Test report.

(e) The laboratory must, upon request, make available to clients a list of test methods employed by the laboratory and, as applicable, the performance specifications established or verified as specified in §493.1253. In addition, information that may affect the interpretation of test results, for example test interferences, must be provided upon request. Pertinent updates on testing information must be provided to clients whenever changes occur that affect the test results or interpretation of test results.

Interpretive Guidelines §493.1291(e):
When the laboratory changes methods, establishes a new procedure or refers tests to

another laboratory, the laboratory must provide the client with necessary updated information concerning parameters such as patient preparation, preservation of specimens, specimen collection or new "normal" values.

§493.1291(e) Probes:
How does the laboratory keep its clients informed about tests offered, methods used, and specimen requirements?

What means does the laboratory use to provide interpretation of results to its clients?

D5811

§493.1291 Standard: Test report.

(f) Test results must be released only to authorized persons and, if applicable, the individual responsible for using the test results and the laboratory that initially requested the test.

Probes §493.1291(f):
What security measures have been instituted to ensure that reports go directly from the device sending reports (e.g., LIS, facsimile) to only the individual ordering the test or utilizing the test results?

D5813

§493.1291 Standard: Test report.

(g) The laboratory must immediately alert the individual or entity requesting the test and, if applicable, the individual responsible for using the test results when any test result indicates an imminently life-threatening condition, or panic or alert values.

Interpretive Guidelines §493.1291(g):
The laboratory records should document the date, time, test results, and person to whom the test results were reported.

Probes §493.1291(g):
What means does the laboratory use to ensure the person ordering a test or the caregiver is alerted in a timely manner to critical or panic test results?

D5815

§493.1291 Standard: Test report.

(h) When the laboratory cannot report patient test results within its established time frames, the laboratory must determine, based on the urgency of the patient test(s) requested, the need to notify the appropriate individual(s) of the delayed testing.

Interpretive Guidelines §493.1291(h):
If a delay in reporting patient test results may negatively impact patient care, the laboratory should have an alternative method for reporting patient results when the LIS or test system is down.

Cite deficiencies only when the laboratory has failed to notify its client(s) when delays in

testing patient specimens have the potential for or are adversely affecting patient care.

Probes §493.1291(h):
What criteria has the laboratory established for notifying the appropriate individual of the delay in testing? Use D5403.

How will the laboratory report patient test results if the LIS or test system is down?

D5817

§493.1291 Standard: Test report.

(i) If a laboratory refers patient specimens for testing--
(i)(1) The referring laboratory must not revise results or information directly related to the interpretation of results provided by the testing laboratory;

Interpretive Guidelines §493.1291(h)(i)(1):
If the laboratory transcribes results from the reference laboratory report, the test results, interpretation and information directly related to the interpretation must be copied exactly as reported by the reference laboratory. The report must adhere to the requirements in §§493.1291(c)(1)–(c)(7) and 493.1291(d).

(i)(2) The referring laboratory may permit each testing laboratory to send the test result directly to the authorized person who initially requested the test. The referring laboratory must retain or be able to produce an exact duplicate of each testing laboratory's report; and

Interpretive Guidelines §493.1291(h)(i)(2):
An "exact duplicate" is an exact copy of the information sent to the individual requesting the test or using the test result(s), and includes the name and address of the laboratory performing the test. The exact copy need not be paper, it may be retrieved from a computer system, microfilm or microfiche record, as long as it contains the exact information in the same format as sent to the individual ordering the test or utilizing the test results. For tests requiring an authorized signature or containing personnel identifiers (e.g., Pathology), the exact duplicate must include the signatures or identifiers. "Pathology" includes all of its subspecialties (i.e., Histopathology, Oral pathology, Cytology).

A "preliminary report" means a test result that has been reported to the authorized person or laboratory that initially requested the test before the final test result is completed. Frequently, a preliminary report will contain significant, but not definitive information (e.g., a urine culture preliminary report of >100,000 Gram-negative bacilli after 24 hours incubation or a beta subunit preliminary report of >200 miu/ml). It should be noted on the report when the result is a preliminary result and that a final report will follow.

A "partial report" means multiple tests are ordered on the same specimen or patient. If partial reports are issued for only those tests that have been completed, then the report date will be the date when all tests have been completed. However, the laboratory should be able to identify the date that each new test is appended to the report.

The laboratory must have a system for retaining copies of all reports including original, preliminary, corrected, and final reports. This includes computer-generated reports.

(i)(3) The authorized person who orders a test must be notified by the referring laboratory of the name and address of each laboratory location where the test was performed.

Interpretive Guidelines §493.1291(h)(i)(3):
Test report forms may include codes to identify the name and address of the laboratory that performed the test, provided the interpretations of the codes are available to the authorized person using the test results.

D5819

§493.1291 Standard: Test report.

(j) All test reports or records of the information on the test reports must be maintained by the laboratory in a manner that permits ready identification and timely accessibility.

D5821

§493.1291 Standard: Test report.

(k) When errors in the reported patient test results are detected, the laboratory must do the following:
(k)(1) Promptly notify the authorized person ordering the test and, if applicable, the individual using the test results of reporting errors.

Interpretive Guidelines §493.1291(k)(1):
When determining whether the laboratory gave prompt notification of test and/or reporting errors to the authorized person(s) consider:

- *When the error was identified and when the authorized person was notified; and*
- *Extent of error (e.g., clinically significant results reported on the wrong patient).*

Probes §493.1291(k)(1):
What mechanism(s) does the laboratory use for notifying the authorized person of the corrected values?

§493.1291 Standard: Test report.

(k)(2) Issue corrected reports promptly to the authorized person ordering the test and, if applicable, the individual using the test results.

Interpretive Guidelines §493.1291(k)(2):
Corrected reports must clearly indicate that they are corrected reports and the corrected result must also be indicated. Use D5821. For corrected reports in Cytology, use D5659.

How does the laboratory ensure that incorrect original results are not reissued verbally, in writing or electronically?

§493.1291 Standard: Test report.

(k)(3) Maintain duplicates of the original report, as well as the corrected report.

Interpretive Guidelines §493.1291(k)(3):
The laboratory must have a system for maintaining copies of the original and corrected reports. Computer-generated reports or electronically stored copies are acceptable.

Copies of all reports, including corrected reports, provided by the referral laboratory must be maintained by both the referral and referring laboratories for the required time periods.

Probes §493.1291(k)(3):
For laboratories that maintain the patient's medical record as the test reports, what is the mechanism for differentiating between the incorrect original report and the corrected report?

D5891

§493.1299 Standard: Postanalytic systems quality assessment.

(a) The laboratory must establish and follow written policies and procedures for an ongoing mechanism to monitor, assess and, when indicated, correct problems identified in the postanalytic systems specified in §493.1291.

Interpretive Guidelines §493.1299(a)-(c):
Quality Assessment (QA) is an ongoing review process that encompasses all facets of the laboratory's technical and non-technical functions and all locations/sites where testing is performed. QA also extends to the laboratory's interactions with and responsibilities to patients, physicians, and other laboratories ordering tests, and non-laboratory areas or departments of the facility of which it is a part.

When the laboratory discovers an error or identifies a potential problem, actions must be taken to correct the situation. This correction process involves investigation, identification and resolution of the problem, and development of policies that will prevent recurrence. Policies for preventing problems that have been identified must be written as well as communicated to the laboratory personnel and other staff, clients, etc., as appropriate. Over time, the laboratory must monitor the corrective action(s) to ensure the action(s) taken have prevented recurrence of the original problem.

All pertinent laboratory staff must be involved in the assessment process through discussions or active participation.

*QA of the **Postanalytic System** includes assessing practices/issues related to test reports. Examples include monitoring and evaluating the accuracy and completeness of the laboratory's test reports (i.e., patient information, test results, normal ranges, and the disposition of unacceptable specimens), and the laboratory's turn-around times and procedures for notification of test results e.g., routine tests, STATS, abnormal or panic values.*

Review a cross-section of patient test reports for accuracy of patient information, test results and normal ranges to verify that the laboratory is effectively monitoring and evaluating the quality and accuracy of the information supplied to its clients.

Verify that the laboratory has a system in place to monitor and evaluate its established reporting time frames and procedures for notification of test results, routine tests, STATS, abnormal or panic values.

If the laboratory uses an LIS, the laboratory must have a mechanism to periodically verify the accuracy of:

- *its calculated data;*
- *its results sent to interfaced systems; and*
- *patient specific data.*

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§493.1299 Standard: Postanalytic systems quality assessment.

(b) The postanalytic systems quality assessment must include a review of the effectiveness of corrective actions taken to resolve problems, revision of policies and procedures necessary to prevent recurrence of problems, and discussion of postanalytic systems quality assessment reviews with appropriate staff.

Interpretive Guidelines §493.1299(b):
Review assessment policies, procedures and reports to verify that the laboratory has a system in place to ensure continuous improvement. Corrective action reports are one indication that the laboratory is monitoring and evaluating laboratory performance and the quality of services.

(c) The laboratory must document all postanalytic systems quality assessment activities.

Interpretive Guidelines §493.1299(c):
The steps taken by the laboratory to identify and correct problems, and prevent their recurrence must be documented. All laboratory policies amended due to its QA activities must be noted.

Probes §493.1299(a)-(c):
What mechanism does the laboratory use to update and correlate the information to clients (e.g., client reference manuals), procedure manuals, reporting systems (e.g., LIS) when the laboratory introduces a new test system with different normal/reference range?