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Center for Clinical Standards and Quality/Quality, Safety & Oversight Group

Ref: QSO-22-21-CLIA

DATE: July 11, 2022

TO: State Survey Agency Directors

FROM: Director, Quality, Safety & Oversight Group (QSOG)

SUBJECT: Final Rule - Clinical Laboratory Improvement Amendments of 1988 (CLIA) Proficiency Testing - Analytes and Acceptable Performance Final Rule (CMS-3355-F)

Memorandum Summary

- **Publication of Final Rule: CMS-3355-F was published on July 11, 2022.** In this final rule we implement revised regulations to update those that the Centers for Medicare & Medicaid Services (CMS) has identified as unnecessary, obsolete, or excessively burdensome on laboratories under the Clinical Laboratory Improvement Amendments of 1988 (CLIA).
- **Effective Date:** The regulations §§ 493.2 and 493.801 through 493.959 are effective two years after publication in the *Federal Register* [July 11, 2024]*;* amendments to 42 CFR §§ 493.20 and 493.25 related to laboratories performing tests of moderate complexity and high complexity testing that also perform waived testing and proficiency testing enrollment will be effective 30 days after the publication date of this final rule, and are effective August 10, 2022.

Background:

Clinical laboratory testing has evolved significantly since 1992 when the CLIA regulations were implemented, and technology is now more accurate and precise than the methods in use at that time. In addition, many tests for analytes not included in the CLIA PT regulations are now in routine clinical use. For example, tests for cardiac markers such as troponins (which are used to diagnose heart attacks) and hemoglobin A1c (a test used to monitor glycemic control in persons with diabetes) were not routinely performed before 1992. Because PT requirements are specified in the regulation, rulemaking is required to update them.

Participation in PT is required under the CLIA statute for laboratories that perform moderate or high complexity testing. PT evaluates a laboratory's performance by testing of unknown samples just as it would test patient samples. An HHS-approved PT program sends unknown samples to a laboratory for analysis. After testing, the laboratory reports its results to the PT program, which grades the results using the CLIA grading criteria and provides the laboratory with its scores. PT is crucial to maintaining the quality of laboratory testing because it independently verifies the accuracy and reliability of laboratory testing, including the competency of testing personnel. As an example, glucose (blood sugar) and white blood cell count measurements are analyte test procedures required as part of PT programs.

The final rule may be viewed at https://www.federalregister.gov/.

Discussion:

A. Microbiology

Microbiology regulations have been modified to remove the types of services listed for each microbiology subspecialty and to add categories of testing (that is, replace the list with broader categories of organisms) for each microbiology subspecialty as described in the bullets below. We believe that the revised microbiology PT regulations better reflect current practices in microbiology.

1. Subspecialty Requirements:
   - For bacteriology, the categories required include, as applicable: Gram stain including bacterial morphology; direct bacterial antigen detection; bacterial toxin detection; detection and identification of bacteria which includes one of the following: detection of the presence or absence of bacteria without identification, or identification of bacteria; and antimicrobial susceptibility testing of select bacteria.
   - The bacteriology annual PT program content described must include representatives of the following major groups of medically important aerobic and anaerobic bacteria if appropriate for the sample sources: Gram-negative bacilli; Gram-positive bacilli; Gram-negative cocci; and Gram-positive cocci.
   - For mycobacteriology, the categories for which PT is required include, as applicable: acid fast stain; and detection and identification of mycobacteria which includes one of the following: detection of the presence or absence of mycobacteria without identification, or identification of mycobacteria.
   - The annual mycobacteriology PT program content must include *Mycobacterium tuberculosis complex* and *Mycobacterium* other than tuberculosis (MOTT), if appropriate for the sample sources.
   - For mycology, the categories for which PT is required include, as applicable: direct fungal antigen detection; and detection and identification of fungi and aerobic actinomycetes which includes one of the following: detection of the presence or absence of fungi and aerobic actinomycetes without identification, or identification of fungi and aerobic actinomycetes.
   - The annual mycology PT program content must include the following major groups of medically important fungi and aerobic actinomycetes if appropriate for the sample sources: yeast or yeast like organisms; molds that include dematiaceous fungi, dermatophytes, hyaline hyphomycetes, and mucormycetes; and aerobic actinomycetes.
   - For parasitology, the categories for which PT is required include, as applicable: direct parasite antigen detection; and detection and identification of parasites which includes one of the following: detection of the presence or absence of parasites without identification, or identification of parasites.
   - The annual parasitology PT program content must include intestinal parasites and blood and tissue parasites, if appropriate for the sample sources.
   - For virology, the categories for which PT is required include, as applicable: viral antigen detection; and detection and identification of viruses.

- The annual virology PT program content must include respiratory viruses, herpes viruses, enterovirus, and intestinal viruses, if appropriate for the sample sources.

2. Miscellaneous microbiology requirements:
   - Laboratories are required to report PT results for microbiology organism identification to the highest level that they report results on patient specimens.
   - Bacterial morphology is required for Gram stains.
   - The mixed culture requirement has been lowered from 50 percent to 25 percent for bacteriology, mycobacteriology, and mycology. There are no mixed culture requirements for parasitology or virology.
   - Antimicrobial susceptibility testing
     - Bacteriology: A least two PT samples per event for susceptibility testing. The program must annually provide samples that include Gram-positive organisms and Gram-negative organisms that have a predetermined pattern of susceptibility to common antimicrobial agents.

B. Non-Microbiology Analytes

CMS and CDC determined which analytes should be added to, or deleted from, Subpart I. In addition, criteria for acceptable performance, which include the target values and acceptance limits (ALs) have been updated or established by CMS and CDC. All new and currently required analytes' criteria for acceptable performance were evaluated and amended to include percentages with or without fixed ALs.

A percentage-based criterion can be unnecessarily stringent at low concentrations – either because of technical feasibility or because medical needs at the low concentration do not require such tight precision. As a result, analytes have percentage based ALs with or without additional fixed ALs.

- 29 New Analytes Finalized in subpart I

CLIA Regulation	Analytes
General Immunology § 493.927	Anti-HBs Anti-HCV C-reactive protein (high sensitivity)
Routine Chemistry § 493.931	B-natriuretic peptide (BNP) ProBNP Cancer antigen (CA) 125 Carbon dioxide Carcinoembryonic antigen Cholesterol, low density lipoprotein, direct measurement Ferritin Gamma glutamyl transferase Hemoglobin A1c Phosphorus Prostate specific antigen, total Total iron binding capacity (TIBC), direct measurement Troponin I Troponin T
Endocrinology § 493.933	Estradiol Folate, serum Follicle stimulating hormone Luteinizing hormone Progesterone Prolactin Parathyroid hormone Testosterone Vitamin B12
Toxicology § 493.937	Acetaminophen, serum Salicylate Vancomycin

- Five analytes deleted from subpart I
  The following analytes have been deleted from subpart I: LDH isoenzymes, ethosuximide, quinidine, primidone, and procainamide (and its metabolite, N acetyl procainamide).
- Criteria for Acceptable Performance – see Attachment 1

C. Definitions

- *Acceptance limit* means the symmetrical tolerance (plus and minus) around the target value.
- *Peer group* means a group of laboratories whose testing process utilizes similar instruments, methodologies, and/or reagent systems and is not to be assigned using the reagent lot number level.
- *Target value* for quantitative tests means:
  (1) If the peer group consists of 10 participants or greater:
  (i) The mean of all participant responses after removal of outliers (that is, those responses greater than three standard deviations from the original mean, as applicable);
  (ii) The mean established by a definitive method or reference methods; or
  (iii) If a definitive method or reference methods are not available, the mean of a peer group; or
  (2) If the peer group consists of fewer than 10 participants, the mean of all participant responses after removal of outliers (as defined in paragraph (1) of this definition) unless

acceptable scientific reasons are available to indicate that such an evaluation is not appropriate.

D. Other Finalized Changes

- Require a minimum of 10 laboratory participants for each specialty, subspecialty, and analyte or test for which the proficiency testing program is seeking reapproval;**.**
- A contractor performing technical and scientific responsibilities as described in this section and § 493.903 (including, but not limited to, processes for selecting appropriate target values to be included in challenges as part of the annual PT program or grading PT results, determining target values, reporting scores to CMS, and determining organisms included in microbiology PT samples) must be a private nonprofit organization or a Federal or State agency, or an entity acting as a designated agent for the Federal or State agency.
- HHS may require on-site visits for all initial proficiency testing program applications for CMS approval and periodically or when problems are encountered for previously HHS-approved proficiency testing programs.
- HHS may require a proficiency testing program to reapply for approval using the process for initial applications if significant problems are encountered during the reapproval process.
- If a proficiency testing program is determined by HHS to fail to meet any criteria contained in §§ 493.901 through 493.959 for approval of the proficiency testing program, CMS will notify the program of its withdrawal of approval. Approval of the PT program remains in effect for 60 days from the date of notification. The proficiency testing program must notify all of its participating laboratories of the withdrawal of approval within 30 days from the date of notification. CMS may disapprove any proficiency testing program that provides false or misleading information with respect to any information that is necessary to meet any criteria contained in §§ 493.901 through 493.959 for approval of the proficiency testing program.
- Any proficiency testing program that is dissatisfied with a determination to disapprove the program may request that CMS reconsider the determination, in accordance with subpart D of part 488.

E. Testing of PT Samples, PT Referral for Waived Tests

The finalized regulations reflect that if moderate and high complexity laboratories also perform waived tests, compliance with § 493.801(a) and (b)(7) are not applicable for the waived tests. However, compliance with § 493.801(b)(1) through (6) is applicable to waived testing, which does not exclude waived tests from the ban on improper PT referral.

Effective Date of Final Rule: The effective date of the revisions to proficiency testing (§§ 493.2 and 493.801 through 493.959) will be delayed until two years after the publication of the final rule in the *Federal Register*. The effective date of these requirements will be July 11, 2024. The delayed effective date reflects the timeframe that we believe PT programs will need to produce or acquire PT samples to meet the revised regulations and incorporate any updates to PT reporting requirements. In addition, laboratories will need time to enroll in PT to meet the new requirements after the samples are available from the PT programs.

The regulations related to laboratories performing tests of moderate complexity and high complexity testing that are subject to PT enrollment and also perform waived testing (§§ 493.20

and 493.25) will be effective 30 days [August 10, 2022] after the publication date of the final rule.

Contact:
For questions or concerns relating to this memorandum, please contact LabExcellence@cms.hhs.gov.

Effective Date:
Please communicate to all appropriate staff within 30 days. See "Effective Date of Final Rule" for effective dates of the final rule.

/s/
David R. Wright
Director, Quality, Safety & Oversight Group

Attachment - Criteria for Acceptable Performance

Criteria for Acceptable Performance

§ 493.927 General immunology.

Analyte or test	Criteria for acceptable performance
Alpha-1 antitrypsin	Target value ± 20%.
Alpha-fetoprotein (tumor marker).	Target value ± 20%.
Antinuclear antibody (ANA).....	Target value ±2 dilutions or positive or negative.
Antistreptolysin O	Target value ±2 dilutions or positive or negative.
Anti-Human Immunodeficiency virus. (HIV)	Reactive (positive) or nonreactive (negative).
Complement C3	Target value ±15%.
Complement C4	Target value ±20% or ±5 mg/dL (greater).
C-reactive protein (HS)	Target value ±30% or ±1 mg/L (greater).
HBsAg.....	Reactive (positive) or nonreactive (negative).
Anti-HBc.....	Reactive (positive) or nonreactive (negative).
HBeAg.....	Reactive (positive) or nonreactive (negative).
Anti-HBs……	Reactive (positive) or nonreactive (negative).
Anti-HCV.....	Reactive (positive) or nonreactive (negative).
IgA	Target value ±20%.
IgE	Target value ±20%.
IgG	Target value ±20%.
IgM	Target value ±20%.
Infectious mononucleosis	Target value ±2 dilutions or positive or negative.
Rheumatoid factor	Target value ±2 dilutions or positive or negative.
Rubella	Target value ±2 dilutions or positive or negative or immune or nonimmune.

§ 493.931 Routine chemistry.

Analyte or test	Criteria for acceptable performance
Alanine aminotransferase (ALT/SGPT)	Target value ±15% or ±6U/L (greater).
Albumin	Target value ±8%.

Alkaline phosphatase	Target value ±20%.
Amylase	Target value ±20%.
Aspartate aminotransferase (AST/SGOT)	Target value ±15% or ±6U/L (greater).
Bilirubin, total	Target value ±20% or ±0.4 mg/dL (greater).
Blood gas pCO2	Target value ±8% or ±5 mm Hg (greater).
Blood gas pO2	Target value ±15% or ±15 mmHg (greater).
Blood gas pH	Target value ±0.04.
B-natriuretic peptide (BNP).....	Target value ±30%.
Pro B-natriuretic peptide (proBNP)…	Target value ±30%.
Calcium, total	Target value ±1.0 mg/dL.
Carbon dioxide	Target value ±20%.
Chloride	Target value ±5%.
Cholesterol, total	Target value ±10%.
Cholesterol, high density lipoprotein (HDL)	Target value ±20% or ±6 mg/dL (greater).
Cholesterol, low density lipoprotein (LDL), direct measurement	Target value ±20%.
Creatine kinase (CK)	Target value ±20%.
CK-MB isoenzymes	Target value ± 25% or ±3 ng/mL (greater) or MB elevated (presence or absence).
Creatinine	Target value ±10% or ±0.2 mg/dL (greater).
Ferritin	Target value ±20%.
Gamma glutamyl transferase.	Target value ±15% or ±5 U/L (greater).
Glucose (excluding measurement devices cleared by FDA for home use.)	Target value ±8% or ±6 mg/dL (greater).
Hemoglobin A1c	Target value ±8%.
Iron, total	Target value ±15%.
Lactate dehydrogenase (LDH)	Target value ±15%.
Magnesium	Target value ±15%.
Phosphorus	Target value ± 10% or ±0.3 mg/dL (greater).
Potassium	Target value ±0.3 mmol/L.
Prostate Specific Antigen, total.	Target value ±20% or ±0.2 ng/mL (greater).
Sodium	Target value ±4 mmol/L.
Total Iron Binding Capacity (TIBC). (direct measurement).	Target value ±20%.
Total Protein	Target value ±8%.

Triglycerides	Target value ±15%.
Troponin I	Target value ± 30% or ±0.9 ng/mL (greater).
Troponin T	Target value ±30% or ±0.2 ng/mL (greater).
Urea nitrogen	Target value ±9% or ±2 mg/dL (greater).
Uric acid	Target value ±10%.

§ 493.933 Endocrinology.

Analyte or test	Criteria for acceptable performance
Cancer antigen (CA) 125	Target value ±20%.
Carcinoembryonic antigen (CEA)	Target value ±15% or ±1 ng/dL (greater).
Cortisol	Target value ±20%.
Estradiol	Target value ±30%.
Folate, serum	Target value ±30% or ±1 ng/mL (greater).
Follicle stimulating hormone.	Target value ±18% or ±2 IU/L (greater).
Free thyroxine.....	Target value or ±15% or ±0.3 ng/dL (greater).
Human chorionic gonadotropin (excluding urine pregnancy tests done by visual color comparison categorized as waived tests).	Target value ±18% or ±3 mIU/mL (greater) or positive or negative.
Luteinizing hormone	Target value ±20%.
Parathyroid hormone	Target value ±30%.
Progesterone	Target value ±25%.
Prolactin	Target value ±20%.
Testosterone	Target value ±30% or ±20 ng/dL (greater).
T3 uptake	Target value ±18%.
Triiodothyronine	Target value ±30%.
Thyroid-stimulating hormone.	Target value ±20% or ±0.2 mIU/L (greater).
Thyroxine	Target value ±20% or ±1.0 mcg/dL (greater).
Vitamin B12……	Target value ±25% or ±30 pg/mL (greater).

§ 493.937 Toxicology.

Analyte or test	Criteria for acceptable performance
Acetaminophen	Target value ±15% or ±3 mcg/mL (greater).
Alcohol, blood	Target Value ±20%.
Blood lead	Target Value ±10% or ±2 mcg/dL (greater).
Carbamazepine, total.....	Target Value ±20% or ±1.0 mcg/mL (greater).
Digoxin, total	Target Value ±15% or ± 0.2 ng/mL (greater).
Gentamicin	Target Value ±25%.
Lithium	Target Value ±15% or ±0.3 mmol/L (greater).
Phenobarbital	Target Value ±15% or ±2 mcg/mL (greater).
Phenytoin total.....	Target Value ±15% or ± 2 mcg/mL (greater).
Salicylate	Target Value ±15% or ±2 mcg/mL (greater).
Theophylline	Target Value ±20%.
Tobramycin	Target Value ±20%.
Valproic Acid, total.....	Target Value ±20%.
Vancomycin	Target Value ±15% or ±2 mcg/mL (greater).

§ 493.941 Hematology (including routine hematology and coagulation).

Analyte or test	Criteria for acceptable performance
Cell identification	80% or greater consensus on identification.
White blood cell differential ...	Target ±3SD based on the percentage of different types of white blood cells in the samples
Erythrocyte count	Target ±4%.
Hematocrit (Excluding spun hematocrit).	Target ±4%.
Hemoglobin	Target ±4%.
Leukocyte count	Target ±10%.
Platelet count	Target ±25%.
Fibrinogen	Target ±20%.
Partial thromboplastin time	Target ±15%.

If a laboratory reports a prothrombin time in both INR and seconds, the INR should be reported to the PT provider program.

Prothrombin time (seconds or INR) ...	Target ±15%.

§ 493.959 Immunohematology.

Analyte or test	Criteria for acceptable performance
ABO group	100% accuracy.
D (Rho) typing	100% accuracy.
Unexpected antibody detection.	100% accuracy.
Compatibility testing	100% accuracy.
Antibody identification	80%+ accuracy.