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CLINICAL LABORATORY IMPROVEMENT AMENDMENTS (CLIA)

PROFICIENCY TESTING REGULATIONS RELATED TO ANALYTES AND ACCEPTABLE PERFORMANCE – A FINAL RULE

Introduction

The Clinical Laboratory Improvement Amendments (CLIA) have released new minimum performance specifications for Proficiency Testing (PT) in the form of a 'Final Rule' document, due to be implemented in July 2024. This final rule updates the PT regulations under CLIA 1988 to address changes in the requirements for existing analytes and novel technologies. This update also includes technical changes to PT regulations to bring them in line with the CLIA statute.

In 1988, US congress enacted CLIA 1988 to ensure the accuracy and reliability of testing in all laboratories that test human samples with the intention of providing information to aid in diagnosis, prevention and treatment of diseases, or the assessment of health in humans. While ground-breaking at the time, testing methodologies and technologies have evolved dramatically since these initial guidelines were published in 1992.

This final rule includes changes to definitions and procedures associated with PT relating to all laboratory specialities and several updates to existing regulations. The addition of many analytes to those regulated by CLIA and the changes in acceptable performance goals outlined in this update aim to improve laboratory testing proficiency across all laboratories and disciplines to improve patient outcomes and reduce the frequency of incorrect analysis, diagnosis, and treatment.

This guide details the major updates to PT regulations for immunology, routine chemistry, endocrinology, toxicology, and haematology including the updated acceptable performance criteria.

Changes to PT for Non-microbiology specialities and subspecialties

Additions to Regulated List of Analytes

Major proposals to non-microbiology specialities include amendments to acceptable limits (ALs) for certain analytes to include percentage limits with or without fixed ALs. Using a systematic approach, the following criteria were used to identify analytes which should be added to the list of regulated analytes:

1. Current availability of PT programmes and the number of PT programmes offering PT,
2. Volume of patient testing performed nationwide (US) and,
3. Cost and feasibility of implementation.

A full list of analytes for each speciality, along with the updated ALs can be seen in the *Updated List of Regulated Analytes and Acceptable Limits* section of this guide. Below is a list of the analytes added to the list regulated by CLIA:

- Anti-HBs
- Anti-HCV
- CRP (High sensitivity)
- BNP
- ProBNP
- CA 125
- CO2
- Carcinoembryonic antigen
- LDL Cholesterol – Direct
- Ferritin
- GGT
- HbA1c
- Phosphorus
- Total PSA
- Direct TIBC
- Troponin I
- Troponin T
- Estradiol
- Folate (serum)
- Follicle stimulating hormone
- Luteinizing hormone
- Progesterone
- Prolactin
- Parathyroid hormone
- Testosterone
- Vitamin B12
- Acetaminophen (serum)
- Salicylate
- Vancomycin

Deletions from the list of Regulated Analytes

Using the same systematic process and evaluation criteria as the previous section, CLIA compiled a list of analytes proposed for removal from the list of regulated analytes. This analysis was carried out to identify tests which are no longer frequently utilised, often due to emerging methods or novel markers. The impact of the removal of these analytes on public and patient health was assessed and reduced this list to 5 analytes:

- LDH isoenzymes
- Ethosuximide
- Quinidine
- Primidone
- Procainamide (and its metabolite N-acetyl procainamide)

"Criteria for Acceptable Performance"

This phrase is used throughout the regulations and CLIA recommended additional clarity be added to this terminology:

"Criteria for acceptable performance is meant for PT scoring only and not intended to be used to set acceptability criteria for a laboratory's verification or establishment of performance specifications."

This addition has been implemented as there are reports of many laboratories using these criteria for purposes other than that of PT.

Setting Target Values

In this section, CLIA have verified that PT providers should set target values for PT challenges according to peer-grouping, as many already do. However, the previous version of these regulations permitted PT providers to reference National Reference System for the Clinical Laboratory (NRCSL) or National Committee for Clinical Laboratory Standards (NCCLS) to set target values. This recommendation has now been removed.

Changing Acceptable Limits

"Update the ALs to reflect advancements in technology and analytical accuracy since the PT regulations were implemented in 1992."

ALs set in 1992 were calculated from the accuracy of the diagnostic methods available at that time. *In vitro* diagnostics have come a long way in the last few decades, seeing notable increases in accuracy and precision in that time. Therefore, it was considered appropriate to review and update, were necessary, the currently applicable ALs for non-microbiology specialities. Before deciding to adjust ALs, all available data was considered to limit the consequences of the new, more stringent ALs.

Changes to Percentage Acceptable Limits

53 analytes were identified, for which CLIA have proposed new percentage based ALs. Of those, 34 analytes are now tighter than or equal to biological variation. The limits proposed for the remaining 19 analytes are wider than biological variation. This decision was reached as, in some cases, the current testing capabilities cannot reach the accuracy required to achieve biological variation limits.

Adding Fixed Concentration Units to Fixed Percentage Units

For some assays, an AL in the form of a percentage may be unnecessarily stringent at the low end of the assay. This may be due to technical limitations or because the medical requirements at low concentrations do not need to be as tight. To resolve this, CLIA has updated many of the recommended ALs to include both a fixed percentage AL and a fixed concentration AL. This helps attain ALs which can be considered fair and more achievable.

Tightening Existing Percentage Acceptable Limits

There have been significant improvements in reported PT performance across all laboratory disciplines. This has motivated CLIA to tighten many of its acceptable performance criteria for existing analytes to bring them more in line with analytical accuracy goals. Analytical accuracy goals are calculated based on biological variation and simulation data.

Other Non-microbiology updates

The following are some additional updates of note:

"Annual PT programs must provide samples that cover the full range of values that could occur in patient specimens. We proposed this amendment so that PT programs must provide samples across a toxicology sample's entire reportable range rather than just provide samples within a sample's therapeutic range."

It is crucial for PT to cover the full clinically relevant range to provide a challenge to the full range of values which may be present in patient samples. This inclusion brings the CLIA regulations in line with many other internationally recognised standards.

"We propose changing the criteria for acceptable performance for "cell identification" from 90% to 80%."

This update is proposed as the requirement of 5 samples per event does not allow for a score of 90%, each sample being worth 20%.

"We propose changing the criteria for acceptable performance for unexpected antibody detection from 80% accuracy to 100% accuracy."

This update has been included to reinforce the importance of identifying unexpected antibodies when cross matching blood in order to protect public health and reduce negative impacts on patient outcomes.

Additional Changes

The table below shows updates to definitions and terminology included in the 2024 CLIA update:

Term	Definition / Changes
Target value	Removal of the reference to NRSCL and NCCLS and retaining the other options for setting target values in this final rule.
Acceptance Limit	Symmetrical tolerance (plus and minus) around the target value.
Unacceptable Score	PT results that are outside the criteria for acceptable performance for a single challenge or sample.
Peer group	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. PT programs should assign peer groups based on their own policies and procedures and not based on direction from any manufacturer.

Data Submission

"PT programs must not change or add any information on the PT result submission for any reason, including, but not limited to, the testing methodology, results, data, or units."

This has been included in the update as a result of PT programmes editing submission data by adding or changing data omitted by the laboratory.

Updated List of Regulated Analytes and Acceptable Limits

General Immunology[1]

Analyte	Criteria for Acceptable Performance
Alpha-1 antitrypsin	Target value ± 20%
Alpha-fetoprotein	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-HIV	Reactive (positive) or non-reactive (negative)
Complement C3	Target value ± 15%
Complement C4	Target value ± 20% or ± 5mg/dL
C-reactive protein (HS)	Target value ±30% or ±1mg/dL
HBsAg	Reactive (positive) or non-reactive (negative)

Anti-HBc	Reactive (positive) or non-reactive (negative)
HBeAg	Reactive (positive) or non-reactive (negative)
Anti-HBsAg	Reactive (positive) or non-reactive (negative)
Anti-HCV	Reactive (positive) or non-reactive (negative)
Anti-HIV	Reactive (positive) or non-reactive (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 non immune

Endocrinology[1]

Analyte	Criteria for Acceptable Performance
CA 125	Target value ± 20%
Carcinoembryonic antigen	Target value ± 15% or ± 1ng/dL
Cortisol	Target value ± 20%
Estradiol	Target value ± 30%
Folate (serum)	Target value ± 30% or ± 1ng/dL
Follicle stimulating hormone	Target value ± 18% or ± 2IU/L
Free Thyroxine	Target value ± 15% or ± 0.3ng/dL
Human Chronic Gonadotropin	Target value ± 30% or ± 3mUI/mL 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 ± 20ng/dL
T3 uptake	Target value ± 18%
Triiodothyronine	Target value ± 30%
Thyroid-stimulating hormone	Target value ± 20% or ± 0.2mUI/mL
Thyroxine	Target value ± 20% or ± 1 mcg/dL
Vitamin B12	Target value ± 25% or ± 30 pg/ml

Routine Chemistry[1]

Analyte	Criteria for Acceptable Performance
Alanine aminotransferase	Target value ± 15% or ± 6U/L
Albumin	Target value ± 8%
Alkaline phosphatase	Target value ± 20%
Amylase	Target value ± 20%
Aspartate aminotransferase	Target value ± 15% or ± 6U/L
Bilirubin, total	Target value ± 20% or ± 0.4mg/dL
Blood gas pCO_2	Target value ± 8% or ± 5mmHg
Blood gas pO2	Target value ± 15% or ± 15mmHg
Blood gas pH	Target value ± 0.04
B-natriuretic peptide	Target value ± 30%
proBNP	Target value ± 30%
Calcium, total	Target value ± 1.0mg/dL
CO_2	Target value ± 20%
Chloride	Target value ± 5%
Cholesterol, total	Target value ± 10%
Cholesterol, HDL	Target value ± 20% or ± 6mg/dL

Cholesterol, LDL direct	Target value ± 20%
Creatine Kinase	Target value ± 20%
CK-MB isoenzymes	Target value ± 25% or ± 3ng/mL or MB elevated
Creatinine	Target value ± 10% or ± 0.2mg/dL
Ferritin	Target value ± 20%
Gamma glutamyl transferase	Target value ± 15% or ± 5U/L
Glucose	Target value ± 8% or ± 6mg/dL
HbA1c	Target value ± 8%
Iron, total	Target value ± 15%
Lactate dehydrogenase	Target value ± 15%
Magnesium	Target value ± 15%
Phosphorus	Target value ± 10% or ± 0.3mg/dL
Potassium	Target value ± 0.3mmol/L
Prostate specific antigen, total	Target value ± 20% or ± 0.2ng/mL
Sodium	Target value ± 4mmol/L
TIBC, direct	Target value ± 20%
Total protein	Target value ± 8%
Triglycerides	Target value ± 15%
Troponin I	Target value ± 30% or ± 0.9ng/mL
Troponin T	Target value ± 30% or ± 0.2ng/mL
Urea Nitrogen	Target value ± 9% or ± 2mg/dL
Uric Acid	Target value ± 10%

Toxicology[1]

Analyte	Criteria for Acceptable Performance
Acetaminophen	Target value ± 15% or ± 3mcg/mL
Alcohol, blood	Target value ± 20%
Blood lead	Target value ± 10% or ± 2mcg/mL
Carbamazepine, total	Target value ± 20% or ± 1mcg/mL
Digoxin, total	Target value ± 15% or ± 0.2ng/mL
Gentamicin	Target value ± 25%
Lithium	Target value ± 15% or ± 0.3mmol/L
Phenobarbital	Target value ± 15% or ± 2mcg/mL
Phenytoin, total	Target value ± 15% or ± 2mcg/mL
Salicylate	Target value ± 15% or ± 2mcg/ml
Theophylline	Target value ± 20%
Tobramycin	Target value ± 20%
Valproic acid, total	Target value ± 20%
Vancomycin	Target value ± 15% or ± 2mcg/ml

Haematology / Immunohematology[1]

Analyte	Criteria for Acceptable Performance
Cell identification	80% or greater consensus on identification
White blood cell differential	Target ± 3SD based on percentage of different types of white blood cells in the samples
Erythrocyte count	Target value ± 4%
Haematocrit	Target value ± 4%
Haemoglobin	Target value ± 4%
Leukocyte count	Target value ± 10%

Platelet count	Target value ± 25%
Fibrinogen	Target value ± 20%
Partial thromboplastin time	Target value ± 15%
Prothrombin time	Target value ± 15%
ABO group	100% accuracy
D (Rho) typing	100% accuracy
Unexpected antibody detection	100% accuracy
Compatibility testing	100% accuracy
Antibody identification	80% accuracy

Conclusion

The final rule for CLIA 1988 has been introduced with the aim to improve the accuracy of laboratory testing and update the regulation to bring them in line with the CLIA statute. This guide details many of the updates in relation to this final rule but is not comprehensive. It is recommended that laboratories in the US, and those who are CAP or JC accredited, should carry out an analysis of their current PT/EQA provider and their programmes to ensure they will achieve the requirements necessary to comply with these new CLIA regulations.

The introduction of these updated regulations in July 2024 will help to validate and improve the accuracy of methodologies used in diagnosis, prevention and treatment of patients and reduce the frequency of errors in the laboratory which ultimately leads to incorrect or delayed diagnosis and treatment.

References

1. Becerra X. Clinical Laboratory Improvement Amendments of 1988 (CLIA) Proficiency Testing Regulations Related to Analytes and Acceptable Performance. Federal Register. 2022;87(131):41194-41242.

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