Proposed Trace Elements Standards – Comments and Responses

Proposed Standards were made available to New York State permitted laboratories and laboratories in application for a permit on March 4th, 2020. The announcement was by e-mail to the facility and laboratory contact person's e-mail address and the Proposed Standards were posted to the CLEP website.

The comment period ended June 15th, 2020. Comments received from any regulated parties and responses are shown here.

Standards will be adopted July 13th, 2020 with an effective date of August 1st, 2020.

Trace Elements

Trace Elements	
Proposed Standard	***Proposed Guidance***
Trace Elements Standard of Practice 1 (TE S1): Method Detection Limit Calculation Initial validation of each trace element for each biological matrix must include calculation of the method detection limit (LOD) using a published protocol and must be based on ~~the average of~~ results ~~from at least seven (7) independent runs of~~ that includes an appropriate matrix blank or base level.	Calculation of the method LOD may be based on the ISO/IUPAC harmonized protocol of three (3) standard deviations (n≥ six (6)); or on the EPA procedure for environmental targets and 3.143 standard deviations (n≥ seven (7)); or another published procedure. If a matrix blank is unavailable, such as for essential nutrient elements, an alternative approach can be used (e.g., use of a low-level QC, matrix-matched calibration standard, reagent blank, etc.).

Trace Elements Standard of Practice 1 (TE S1): Method Detection Limit Calculation

COMMENT 1:

Reword to, Initial validation of each trace element for each biological matrix must include calculation of the method detection limit (LOD) and must be based on the average of results from 6 blank matrix or base level. It is preferred to have at least a minimum of 3 separate runs.
LOD can be done in many different ways: theoretical calculation, using a decision point, using the lowest non-zero calibrator,

estimating LOD using background noise or a reference material. There are many suggested ways and most guidance states use at least three separate runs. Seven is an odd number and has no precedence in any literature or statistical reason to be used. The FDA guides to use 6 blanks for chromatographic and 10 blanks for ligand binding assays. They do not even state that they should be separate runs. This is taken from their Bioanalytical Method Validation Guidance for Industry, May 2018. This rewording is a blending of guidance and standards across industry and is not as burdensome as seven independent runs of matrix blank.

RESPONSE 1:

We agree that there are several sound approaches to calculating the limit of detection (LOD), although a harmonized approach remains elusive. Laboratories are free to choose an alternate published approach as long as they follow the published approach closely and the data are well documented. The standard (TE S1) has been clarified to provide flexibility. The proposed guidance included a reference to the Harmonized IUPAC/ISO Harmonized Guidelines for Single Laboratory Validation of Methods of Analysis (Pure Appl. Chem., Vol. 74, No. 5, pp. 835–855, 2002.) that may be used. The IUPAC/ISO approach calls for a minimum of 6 complete determinations and recommends the inclusion of the sample matrix, which is important for clinical matrices. CDC relies on a well-known NIST authority (John Keenan Taylor, Quality Assurance of Chemical Measurements, doi.org/10.1201/9780203741610) to guide their QA policies on the LOD. Taylor's book recommends a minimum of 7 replicate measurements to establish the SD, which is multiplied by 3 to yield the method LOD. This is the same approach proposed in TE S1. Other approaches include CLSI EP17-A2 (2012), which calls for a minimal experimental design to include three separate runs performed over three days, and then 20 determinations within those runs. The EPA have also published updated guidance that is based on a minimum of 7 runs that includes the sample matrix. Other approaches have been proposed, which may be better suited to different types of analytical methods. However, it is important that the laboratory closely follow the approach chosen, and include the appropriate clinical matrix as part of the determination.

COMMENT 2:

What is the rationale for requiring "seven (7) independent runs of a matrix blank or base level"?

RESPONSE 2:

The rationale for "seven (7) independent runs of a matrix blank or base level" was based in part on the well-known NIST authority on the subject (John Keenan Taylor, Quality Assurance of Chemical Measurements, doi.org/10.1201/9780203741610) and on the US EPA's revised definition and procedure for the determination of the method detection limit (EPA 821-R-16-006). Taylor's book recommends a minimum of 7 replicate measurements to establish the SD, which is multiplied by 3 to yield the method LOD. This is the same approach proposed in TE S1. The inclusion of the matrix blank or base level is important for clinical matrices that can strongly affect the instrument signal in trace element analysis. There are several sound approaches to calculating the limit of detection (LOD), although a harmonized approach remains elusive. Laboratories are free to choose an alternate published approach as long as they follow the published approach closely and the data are well documented. The standard (TE S1) has been clarified to provide flexibility.

Trace Elements	
Proposed Standard	***Proposed Guidance***
Trace Element Standard of Practice 2 (TE S2): Materials Contamination Control The laboratory must implement procedures to ensure that materials distributed for specimen collection and supplies used for processing in the laboratory are free from significant contamination for each element tested.	To ensure that tubes or containers are free from contamination for each element tested, specimen collection tubes ~~should~~ may be lot-tested and certified as free from significant trace element contamination, or manufacturer-certified for trace element use. Where appropriate, laboratory supplies (e.g., flasks, autosampler tubes, and pipet tips), used for trace element analysis ~~must~~ may need to be pre-checked for contamination and/or acid-washed (e.g., with dilute nitric acid), and certified as fit for purpose. Disposable plastic ware can be verified as contamination-free by randomly checking materials by lot number. Monitoring of reagent blank data is appropriate to documenting contamination of the system (e.g., pipette tips and autosampler sample cups).

Trace Element Standard of Practice 2 (TE S2): Materials Contamination Control

COMMENT:

I believe TE S2 to be excessive. If we had a contamination problem we would see it in our blanks, delta checks, CAP surveys and or repeat samples. We do check our plates and our sample tubes already for contamination, but to check lot to lot of pipette tips, sample containers, and other labware that we have used for years, seems excessive.

RESPONSE:

The use of reagent blank data is a reasonable approach to documenting contamination of the system that includes pipette tips and autosampler sample cups. Of course, large blanks would warrant a corrective action that may include lot checking individual supplies to determine root cause. Some items such as uncertified blood collection tubes and urine collection cups may still need to be checked to ensure freedom from significant contamination, as neither reagent blanks nor CAP surveys will detect that problem. The proposed guidance has been modified to include monitoring of reagent blank data.

Trace Elements	
Proposed Standard	***Proposed Guidance***
Trace Elements Standard of Practice 6 (TE S6): Quality Control The laboratory must: a) ensure that at least two (2) levels of quality control (QC) are included in each test run for all non-essential toxic elements, e.g., normal and abnormal-high concentration; b) ensure three (3) levels of QC for the essential trace elements, QC must include ~~abnormal~~ low (if available), ~~normal~~ intermediate and ~~abnormal high~~ elevated that covers the analytical range of values reported; c) use matrix-matched QC materials; d) run at least one (1) level of QC at the end of each batch of specimens; and e) adjust the frequency of instrument re-calibration based on quality control data.	b) For the limited purposes of TE S6b, the definition of "essential" applies only to copper (Cu), zinc (Zn) and selenium (Se) in blood, serum or plasma, and to iodine (I) in urine. d) ~~a~~An analytical batch is the maximum number of samples that can be run with an autosampler (ICP-MS) or carousel tray (GFAAS).

Trace Elements Standard of Practice 6 (TE S6): Quality Control

COMMENT 1:

Reword section b) QC must include low, intermediate and elevated levels of controls that appropriately covers the AMR of the test. It may be impractical to produce an abnormal low QC for trace elements in an appropriate matrix, when there are normal levels within the matrix. Diluting the matrix to reach lower levels may have other unwarranted effects on the control.

RESPONSE 1:

We agree with the proposed rewording of section b. We also agree that diluting the matrix to reach lower levels may have other unwarranted effects on the control.

COMMENT 2:

Clarify which elements are considered essential trace elements so that there is no confusion as to which elements require 3 levels of QC compared to the 2 stated in item a of this standard.

RESPONSE 2:

We agree that this is an important question so as to avoid confusion. For the limited purposes of this standard (TE 6b), the definition of “essential” applies only to copper (Cu), zinc (Zn) and selenium (Se) in blood, serum or plasma, and to iodine (I) in urine. The guidance for TE 6b has been updated accordingly.

COMMENT 3:

Suggest the following change
b) ensure three (3) levels of QC for the essential trace elements, QC must include abnormal low (<u>if available</u>), normal and abnormal high. Rationale: We are concerned an abnormal low is not always available.

RESPONSE 3:

The concern is justified. The standard has been updated to reflect the potential lack of availability of an abnormal low and guidance has been updated to limit TE S6b to copper (Cu), zinc (Zn) and selenium (Se) in blood, serum or plasma, and to iodine (I) in urine

Trace Elements	
Proposed Standard	***Proposed Guidance***
Trace Elements Standard of Practice 8 (TE S8): Repeat Analysis All trace element results that are above the laboratory's definition of ~~abnormally high~~ elevated (or below the definition of ~~abnormally~~ low) must be verified by repeat analysis. The laboratory must: a) define trace element concentrations for ~~abnormal-high~~ elevated and, where appropriate, ~~abnormal-~~low; b) define critical call values for trace elements where appropriate; c) establish reportable protocols for lead, cadmium, mercury and arsenic consistent with the requirements of 10NYCRR Parts 22.6 and 22.7 (NYS Heavy Metals Registry) and report results, as applicable, according to Public Health Reporting Standard of Practice 1; d) establish criteria for the maximum discrepancy allowable on duplicate measurements that are consistent with the expected method repeatability; and e) perform a third analysis (triplicate) when the discrepancy between the first two (2) results exceeds the maximum allowed in (~~c~~d) above.	A new aliquot from the original specimen should be used when a repeat analysis is performed. A clinical action threshold is defined as that level where clinical intervention would be recommended. Where no action threshold has been established (e.g., biomonitoring studies), the laboratory may define elevated based on published or laboratory derived data. Repeat analysis is not normally required for values that fall within the reference range. ~~For non-essential trace elements such as mercury and arsenic, only values that exceed the upper threshold need to be repeated, while for essential elements, values that are either above the upper threshold (abnormal-high) or below the lower threshold (abnormal-low), must be repeated. Note that a lower threshold (abnormal-low) is not required for "non-essential" trace elements.~~ The laboratory must define elevated (or low) for repeat analysis purposes, while recognizing that these may not always be equivalent to the upper (or lower) limit of the reference interval.

Trace Elements Standard of Practice 8 (TE S8): Repeat Analysis

COMMENT 1:

Reword section e) perform a third analysis (triplicate) when the discrepancy between the first two (2) results exceeds the maximum allowed in (d)above.

There may be a typo here by referring to "(c)" when it seems likely it should be "(d)", which mentions establishing criteria for maximum allowed discrepancy on duplicate measurements.

REPONSE 1:

The Standard has been revised based on the comment received.

COMMENT 2:

Please clarify the definition of abnormal in the TE S8 proposed standard. Based on clinical diagnostic convention, "Measured values outside reference interval" does not equate to "abnormal".

RESPONSE 2:

The definition of the reference interval yields an upper limit (typically the 97.5th percentile) and for some analytes a lower limit (e.g., 2.5th percentile), which implies test results outside the reference interval are not “normal”. Yet we accept that for some test results, being outside the reference interval may have no immediate clinical consequences. Should every test result that is flagged as elevated be repeated? It will depend on the specific analyte. For example, a blood lead test result of 6 µg/dL is elevated but no clinical intervention is warranted. Yet it should be confirmed by repeat analysis if the blood sample is venous and sufficient sample volume remains. For comparison purposes, a blood cadmium value of 2 µg/L might fall above the 95th percentile (NHANES), yet this might not be considered abnormal based on clinical diagnostic convention. Clearly, the laboratory must define what is meant by abnormally high (or abnormally low) for repeat analysis purposes, while recognizing that these may not always be equivalent to the upper (or lower) limit of the reference interval. The guidance has been updated to provide this clarification.