Effective Date: 02-2023

# COLA Technical Bulletin

## 2023-3: Molecular Diagnostic Testing

COLA periodically reviews the criteria for accreditation and makes changes or additions for several reasons:

- To clarify language, so that the intent of the criterion is clear
- To incorporate new information in response to changes in technology or regulatory emphasis

The 2023 COLA Accreditation Manual will be published in February 2023. This edition includes the revision and addition of several Molecular Diagnostics (**MDT**) criteria, to guide COLA laboratories in best practices. The purpose of this Technical Bulletin is to provide advance notice of these changes.

See additional 2023 Technical Bulletins for information on other criteria changes and additions.

## Revisions to COLA Criteria

Significant changes or clarifications to existing COLA criteria are underlined below.

**MDT 6 R**

**For each molecular diagnostics test that is non-FDA approved, has the laboratory established requirements for acceptability of the primary and extracted specimen, including storage temperature and specimen age requirements, prior to testing?**

Specimen stability studies must be included with the laboratory method validation studies, and must be performed for each specimen type. Test procedures must include criteria for specimen rejection, including specimen transport, storage and age limitation criteria validated by the laboratory and approved by the Laboratory Director. The storage and stability of the extracted specimen is not required for laboratories that test specimens immediately after extraction.

The reference materials used to validate storage stability must be representative of the targets to be identified, and cannot be performed with nongenomic reference materials (e,g, plasmids, oligonucleotides). For FDA approved molecular methods, refer to the VER section for verification of performance requirements.

**MDT 11 R**

**For non-FDA approved molecular genetic testing, does the laboratory perform Quality Control with each patient run, including:**

**a) A minimum of a positive control, and a non-template control?**

**Plus, as applicable:**

**b) A positive control, non-template control, and an extraction control if the test involves a manual extraction step? An extraction control if the test process involves an extraction step?**

**c) A reverse transcriptase control if the test process involves a reverse transcription step?**

**d) An internal control if reaction inhibition is a significant source of false negative results?**

*The Quality Control Plan must include acceptability criteria for each type of control, as well as steps to take when controls do not yield expected results. If a manual extraction step is included, at least one control must go through the entire testing process, including the extraction step.*

*Separate control materials for each of the above are not required. For example: the positive control can also serve as the extraction control if the control material undergoes the extraction process.*

*The positive control is not required to contain every target that is tested by the laboratory, but the target(s) must be rotated periodically.*

## New COLA Criteria

**MDT 14 R**

**For non-FDA approved molecular testing, has the laboratory's validation included the use of suitable reference materials?**

*The laboratory must select reference materials that as closely as possible resemble the complexity of genomic DNA/RNA to be identified in the patient sample. These include whole, intact human cells or organisms; genomic DNA; and genomic RNA. Nongenomic reference materials, such as plasmids and synthetic oligonucleotides, are acceptable for use only when suitable materials are not readily available. The material to be used to routinely monitor the performance of the assay cannot be used to prove the performance specifications.*

***MDT 14 (continued)***

*Genomic DNA (gDNA) – total DNA from an organism or a cell, which includes the chromosomes within the nucleus (CLSI MM17)*

*Individual assay validations which are performed solely with nongenomic reference materials must include a detailed justification, approved by the Laboratory Director, for not using more suitable reference materials, as well as a plan to confirm the assay's accuracy after the initiation of patient testing. This may include confirming the first few positive patient samples with another CLIA-certified laboratory or test method.*

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**MDT 15 R**

**Has the laboratory established each extraction method to be used?**

*If the accuracy studies were not performed using reference materials that validate the extraction process, an additional study is required. The reference materials used to validate extraction must be representative of the targets to be identified. Nongenomic reference materials, such as plasmids and synthetic oligonucleotides, cannot be used to validate the extraction process.*

**MDT 16 R**

**Has the laboratory established the limit of detection (LoD) for each target and sample matrix?**

*The limit of detection must be established through the testing of two or more serial dilutions of a sample which contains the target of interest. The study must include at least one concentration that achieves the laboratory's criteria for establishing limit of detection (e.g. 95% detectability based on a defined cycle threshold (Ct) cutoff), and at least one concentration that does not achieve the laboratory's criteria.*

*Alternatively, the laboratory may establish the cycle threshold (Ct) cutoff based on the testing of a sample with a concentration at the desired limit of detection.*

*This criterion does not apply to assays testing for germline mutations.*

**MDT 17 R**

**Does the laboratory's validation include a summary of the in silico cross-reactivity for each assay?**

*All organisms that have the potential to cross-react with an assay must be evaluated to determine clinical impact. Action must be taken for any organisms which cross-react and may have a clinical impact on the patient.*

**MDT 18 R**

**Does the laboratory procedure for each analyte reported using molecular techniques include specific identification criteria approved by the Laboratory Director?**

*The procedure must include the laboratory's criteria for evaluating molecular results, based upon credible reference materials and the laboratory's own validation studies. Identification criteria may include as applicable: cycle threshold (Ct), quantification cycle (Cq), amplification score, Cq confidence, fluorescence threshold, and baseline. Identification criteria specific to a particular reported analyte must be defined.*

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**MDT 19 R**

**Are new lots and shipments of critical components of the test procedure evaluated for comparable performance prior to use with patient testing?**

*The laboratory's procedure must define all critical components, how the laboratory will determine comparable performance to the components currently in use, and the actions to take if a component does not provide comparable performance.*

*One method for evaluating comparable performance is to test previously tested patients and/or controls using the new lot/shipment concurrently with the current lot, and ensure observed cycle threshold (Ct) or quantification cycle (Cq) values are comparable.*