**ALERE DETERMINE TB LAM Ag METHOD VALIDATION PLAN**

|  |  |  |  |
| --- | --- | --- | --- |
| **Country:** | **Department/ Division:** | | **Unit/ Team:** |
| **Document Number:** | **Version Number:** | | **Effective Date:** |
| **Reviewed by (name, signature, and date):** | | **Approved by (names, signatures, and dates):** | |
|  | |

# **Method Validation Overview**

## **Purpose and Rationale**

To provide objective evidence that testing with the Abbott/ Alere DetermineTB LAM Ag test produces results that meet established acceptance criteria when performed according to manufacturer’s instructions [add current Package Insert as Appendix].

## **Method Description and Intended Use**

The Abbott/ Alere Determine TB LAM Ag test is an *in vitro,* point-of-care, qualitative assay that can detect the presence of mycobacterial lipoarabinomannan (LAM) in human urine. It is a lateral flow (LF) sandwich ELISA (enzyme linked immunosorbent assay) that detects the LAM antigen using polyclonal antibodies. This assay is used to aid in diagnosis of TB disease in eligible HIV-positive individuals as outlined in current WHO recommendations and/ or according to national guidelines.

[Enter latest WHO criteria or national guidelines].

## **Classification and Recommendation Status of Test Method**

CE-IVD

World Health Organization Recommended

## **Type of Validation**

Initial validation

Revalidation

Post market validation/ new lot testing

Modification to procedure

Change in test version/ formulation

Other\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## **Roles and Responsibilities**

[Insert name] is responsible for preparing the Method Validation Plan.

[Insert name] is responsible for review and approval of the Method Validation Plan prior to testing.

[Insert name/s] is/are responsible for performing the method validation testing.

[Insert name] is responsible for document management.

[Insert name] is responsible to review and approval of the Method Validation Summary upon completion.

## **Timeline**

Expected completion dates for each method validation task are shown in the table below, establishing an overall timeline for validation completion and approval of the test method for use of the tests and/ or reporting of clinical results.

|  |  |
| --- | --- |
| **Tasks** | **Expected Task Completion Dates [fill dates below]** |
| Plan Development |  |
| Plan Approval |  |
| Validation Testing |  |
| Analysis of Data |  |
| Development of Method Validation Summary |  |
| Approval of the Method Validation |  |

# **Sample Eligibility, Collection, Storage, and Preparation for Validation**

## **Sample Eligibility**

The validation will be performed using positive and negative quality-assured quality control samples. Samples may include human urine collected and stored according to the manufacturer’s instructions and characterized as positive or negative by a molecular WHO-recommended diagnostic (mWRD), commercially-provided panels, or locally produced external quality assurance specimens.

When using manufacturer and locally-produced samples, testing sites should ensure that they have been verified by the producer to accurately and reproducibly produce positive and negative results. When using urine, positive samples should be collected from patients with bacteriologically confirmed TB, while negative samples should be collected from patients bacteriologically confirmed to not have TB. mWRDs or TB culture may be used as reference methods to determine patient positivity or negativity according to WHO and national guidelines. Given the more rapid turnaround time associated with mWRD testing, use of these molecular reference tests may speed availability of results for urine sample characterization.

|  |  |
| --- | --- |
| **Positive Sample(s)** | * Manufacturer-provided and validated positive LF-LAM external quality control sample * Locally produced and validated positive LF-LAM external quality control sample * A urine specimen with a known positive Determine TB LAM Ag test result from a patient with bacteriologically confirmed TB by mWRD or culture on any sample type that is collected the same day as the urine. |
| **Negative Sample(s)** | * Manufacturer-provided and validated negative LF-LAM external quality control sample * Locally produced and validated negative LF-LAM external quality control sample * A urine specimen with a known negative Determine TB LAM Ag test result from a patient without TB as confirmed by mWRD or culture on all specimens collected the same day as the urine. |

## **Sample Collection**

External quality control samples procured from manufacturers or locally produced for validation testing should be sourced per provider instructions. For patient urine, according to manufacturer’s instructions as of 2023, a fresh, midstream sample will be collected in a sterile container after the urogenital area is cleaned with a sterile wipe. The type of urine collection container used should be appropriate for the collection site (standard urine collection cup with securable lid), storage (urine container, screw cap vial, or centrifuge tube), or transport (screw cap vial or centrifuge tube). Sufficient volume will be collected from each patient to, at a minimum, accommodate all validation tests and 10% contingency for any needed repeat testing, for each type of validation ([see **Section D**](#_Validation_Testing_Protocol)). A higher volume of urine per patient may be collected, as feasible, for storage and use as positive and negative external control material ([see **Sample Eligibility**](#_Sample_Eligibility)) in the future. Once collected, each urine container will be labelled with at least one unique specimen identifier, the date of specimen collection, the date the specimen was stored and the storage temperature.

**[Need for Informed Consent: If the test and/ or sample types are not already approved for programmatic use, indicate which patient consent procedures will be undertaken to ensure ethical collection and use of urine for validation purposes].**

## **Sample Storage**

All manufacturer provided and locally produced external quality control samples should be stored according to provider stability data and instructions for users. Similarly, urine samples will be acceptable for validation purposes only if they have been stored and transported under current manufacturer conditions. The table below outlines urine storage requirements for the Determine TB LAM Ag test as of [May 2022].

**Determine TB LAM Ag Test Sample Storage Requirements [Review and confirm or revise for currency]**

|  |  |  |  |
| --- | --- | --- | --- |
| **Temperature** | Room temperature  (18 to 24 °C) | Refrigerated temperature  2 to 8 °C\* | Frozen temperature  ≤ -20 °C\* |
| **Storage times** | ≤ 8 hours | 3 days | 3 years# |

\* Samples stored at ≤ -20° C and 2-8° C must be brought to room temperature (18-24° C) one hour before use.

# Samples may only undergo freeze thaw three times prior to testing. Once thawed, the sample will be frozen immediately after use

and the date of each freeze thaw will be recorded along with the other details associated with the sample.

## **Sample Preparation for Validation**

All sample types will be inspected for acceptability prior to use (sufficient volume, maintenance of appropriate temperature, appropriate labelling, physically intact). If the specimens meet acceptance criteria, they may be used for validation testing. Preparation of manufacturer and locally produced sample types should be done according to the provided procedures. Refrigerated and frozen urine samples will be brought to room temperature one hour prior to testing. Frozen urine samples may contain aggregates (small clumps or sediments from solution that form during the freezing process); therefore, all thawed samples will be centrifuged at 10,000 x g for 5 minutes at room temperature. After centrifugation, 60 µL of the sample will be carefully collected from the clear supernatant for each test. For other sample types, 60 µL of the urine samples will be pipetted directly to the sample pad.

# **Training and Competency Assessment of Validation Testers**

[This general guideline may be adapted according to the laboratory’s policies and procedures].

## **Initial Training Requirements**

Laboratory personnel performing the validation testing are required to complete and document training prior to validation. The training may include the following tasks:

|  |  |  |
| --- | --- | --- |
| **Task Check List** [check all relevant tasks] | | |
| 1. | Read and understand the standard operating procedure |  |
| 2. | Read and understand the validation plan |  |
| 3. | Review of the test |  |
| 4. | Competency assessment |  |
| 5. | Other trainings required as required by the participating laboratory’s policies |  |

Note*:* Per national policy, initial training occurs when a new or current employee is trained to perform a new test or when a new assay is introduced. Retraining may occur when periodic competency assessments are unacceptable. Training updates are required whenever changes are implemented to a procedure or when additions are made to the training document, such as new learning objectives. Competency assessments should be conducted routinely after initial training is completed ([**Competency Assessment Requirements**](#_Competency_Assessment_Requirements)).

The following lab personnel are trained, and documentation is complete:

|  |  |
| --- | --- |
| **Personnel** | **Date Completed** |
| [Name] | [Date] |
| [Name] | [Date] |
| [Name] | [Date] |

## **Competency Assessment Requirements**

As per College of American Pathologists (CAP) recommendations for point-of-care testing, laboratory personnel already trained in a testing method are required to complete and document competency assessment (CA) prior to patient testing **and validation testing**. Testing personnel are assessed for CA semiannually during their first year and annually thereafter.

|  |  |
| --- | --- |
| **Competency assessments include** | |
| 1. | Reading and understanding the standard operating procedure |
| 2. | Direct observation of test performance |
| 3. | Monitoring of the recording and reporting of test results |
| 4. | Review of intermediate test results or worksheets, quality control records, external quality assurance testing results (if available), and preventive maintenance records |
| 5. | Direct observation of performance of instrument maintenance and function checks, if applicable |
| 6. | Assessment of test performance through testing previously analyzed specimens; internal blind testing samples, or external quality assurance samples (if available) |
| 7. | Assessment of problem-solving skills (e.g., 2 written questions). |

Not every type of assessment needs to be performed for each area being reviewed, and the type of assessment tool used from the list above will be selected based on whether it will provide an accurate reflection of the performance and competence of the worker.

# **Validation Testing Protocol**

## **Accuracy Validation Plan**

Known positive and known negative samples (see [Sample Eligibility](#_Sample_Eligibility)) will be tested for presence or absence of LAM using the Determine TB LAM Ag test. For Initial Validations, at least 10 positive and 10 negative samples will be tested. A lower number of samples may be used for other types of validation (see [Type of Validation](#_Type_of_Validation)). If done by more than one tester, each tester will perform the test independently. All testers will be blinded to expected results. Validation test results for accuracy will be recorded in the [Accuracy Table](#_Accuracy_Table) of the [Sample Validation Summary](#_Sample_Validation_Summary). Evaluation of acceptability will be done using all valid test results. Any invalid test results will be repeated using the same sample and the final valid result used for the accuracy analysis. [**NOTE:** The same sample numbers may be used for validation when using other commercially available or lab developed EQA material]

|  |  |
| --- | --- |
| Number of Testers |  |
| Number of Positive Samples\* |  |
| Number of Negative Samples\* |  |

\*<10 samples may be used for validation types (see [**Section A**](#_Type_of_Validation)) other than the Initial Validation.

A sensitivity and specificity of ≥90% will be considered acceptable. The acceptability criteria were set at <100% to account for variable and/ or low concentration(s) of the LAM molecule in the previously tested samples (see [Qualitative Analyses](#_Qualitative_Analyses:_Analytic)).

## **Precision Validation Plan**

One known positive and one known negative sample (see [Sample Eligibility](#_Sample_Eligibility)) will be tested three separate times (replicates). The matrix below may be used to indicate how many replicates will be tested by each tester. Like accuracy testing, all tester(s) should remain blinded to expected results.

[Check appropriate box to indicate the number of precision testers. If two testers are available, check the box in the middle column to indicate whether tester Replicate 2 will be tested by Tester 1 or Tester 2].

|  |  |  |  |
| --- | --- | --- | --- |
| **Number of testers available** | **1 Tester** | **2 Testers** | **3 Testers** |
| **Assay will be performed by** | | |
| Positive sample replicate 1  Negative sample replicate 1 | Tester 1 | Tester 1 | Tester 1 |
| Positive sample replicate 2  Negative sample replicate 2 | Tester 1 | Tester 1 *OR*  Tester 2 | Tester 2 |
| Positive sample replicate 3  Negative sample replicate 3 | Tester 1 | Tester 2 | Tester 3 |

Results will be recorded in the Precision Table of the Sample Validation Summary below (see [Precision table](#_Precision_Table))*.* Complete agreement (100%) among all tests and testers will be considered valid and meet acceptability criteria for precision. Any invalid test results will be repeated by the initial tester and the valid result used in the final set of results. The acceptable criteria for precision will be 100% (see [Precision table](#_Precision_Table))*.* i.e., the tester(s) need to obtain true positive or negative results on all the samples tested.

## **Qualitative Analyses: Analytic Sensitivity, Specificity and Precision**

|  |  |
| --- | --- |
| **Specification** | **Planned Calculations** |
| Sensitivity | No. of true positive  X 100  No. of true positive + No. of false negative |
| Specificity | No. of true negative  X 100  No. of true negatives + No. of false positive |
| Precision | No. of replicates in agreement  X 100  Total no. of results |

## **Validation Protocol Approval (Prior to Testing)**

|  |  |  |  |
| --- | --- | --- | --- |
| Name: | Title: Quality Manager | Signature: | Date: |
| Name: | Title: Lab Supervisor | Signature: | Date: |
| Name: | Title: | Signature | Date: |

# **Validation Plan Summary and Approval**

After completion of validation testing, raw data and results will be compiled and filed with quality assurance documentation. All validation records will be retained according to the institution’s Records retention policy. The validation summary (see [Sample Validation Summary](#_Sample_Validation_Summary)) is completed with the signature of the Laboratory Director, indicating the validation has been reviewed and approved or requires investigation and corrective action prior to finalization.

## **Sample Validation Summary**

**Test:** Abbott/Alere DetermineTB LAM Ag

## **Accuracy Table**

[Number of samples] samples were tested on [date] by [Name]

|  |  |  |  |
| --- | --- | --- | --- |
| Number of true-positive results: \_\_\_  Number of false-negative results: \_\_\_ | Sensitivity (%): \_\_\_\_\_ | Acceptance Criteria:  ≥ 90% | **Result:**  Acceptable  Not Acceptable |
| Number of true-positive results: \_\_\_  Number of false-negative results: \_\_\_ | Specificity (%): \_\_\_\_\_ | Acceptance Criteria:  ≥ 90% | **Result:**  Acceptable  Not Acceptable |

## **Precision Table**

[Number of samples] (in triplicate) were tested on [dates] by [Name/s]

|  |  |  |  |
| --- | --- | --- | --- |
| No. of replicate results in agreement: \_\_\_  Total no. of results: \_\_\_ | Precision (%): \_\_\_\_\_ | Acceptance criteria: 100% | **Result:**  Acceptable  Not Acceptable |

I have reviewed the validation data for the Abbott/ Alere DetermineTB LAM Ag test and test performance is:

☐ Acceptable for test distribution, use and patient testing.

☐ **NOT** acceptable for test distribution and patient testing and requires the following further action(s):

☐ Investigation into unacceptable results. Findings from investigation are outlined here:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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☐ Definition and completion of corrective action(s), including:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

☐ Repeat validation testing by [Date]

☐ Other action(s)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

## **Validation Plan Approval**

**Approved**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Title** | **Signature** | **Date** |
|  |  |  |  |

**NOT Approved**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Title** | **Signature** | **Date** |
|  |  |  |  |

# **Related Documents**

|  |  |  |  |
| --- | --- | --- | --- |
| **Document Name** | **Document Number** | **Revision #** | **Effective date** |
| Abbott/ Alere DetermineTB LAM Ag Test Standard Operating Procedure (site-specific) |  |  |  |
| Method Validation Policy and Procedure |  |  |  |
| Personnel Training Policy and Procedure |  |  |  |
| Personnel Competency Assessment Policy and Procedure |  |  |  |
| Abbott/ Alere DetermineTB LAM Ag Test package insert |  |  |  |