May 2024

Guidelines For Submission of Validation Packages for Approval of MALDI-TOF Mass Spectrometry (MALDI-TOF MS) For Bacterial and Fungal Identification

This guidance should be used for applications and/or modifications to assays/databases that are not included in the current FDA cleared tests. These guidelines should be used in conjunction with and not in lieu of the existing Microbiology Molecular Guidelines found on the Test Approval Page https://www.wadsworth.org/regulatory/clep/clinical-labs/obtain-permit/test-approval.

General Guidance:

- Submit a detailed SOPM including all relevant quality assurance information pertaining to this test. The SOPM must include appropriate controls including a negative and positive control, a testing algorithm, all expected reporting and reflex testing scenarios, and verification process for library updates. Specimen reports for each scenario must be included.
- If any alterations from the manufacturer's instructions or reporting guidelines (including acceptable score) are instituted, submission of additional validation data supporting this change would be required.
- All extraction methods that will be used in the laboratory must be included in the validation. At least 30 isolates for each extraction method must be included as part of the total number of representative isolates required in the validation. For modifications to FDA or CLEP approved assays, 20 isolates should be tested.
- If lab-developed or acquired databases will be used in addition to the library databases provided by the manufacturer, submit the criteria for isolate selection (why isolates were selected for library addition), confirmation method (how the organism was identified), spectral quality and number of spectra required for library creation.

For Blood Cultures:

- For validation of bacterial and fungal identification from blood cultures, include data from at least 30 positive blood culture specimens. These positives should include at least 10 of the major species typically identified in blood culture specimens in the laboratory. In addition, five negative blood culture specimens must be included in the validation study. A table of these results must include comparison identification information, identification score from MALDI-TOF MS, and final reporting information. If both bacterial and fungal identification are being validated, 30 samples for bacterial and 30 samples for fungal identification are required.
- The sample validation should utilize authentic clinical specimens. However, if the laboratory cannot acquire an adequate number of positive blood culture specimens within one month, then spiked specimens are acceptable to supplement the validation data. Minimum numbers apply as above for bacterial and yeast identifications. Please note that whole blood should be spiked with low numbers of yeast or bacterial agents before inoculating blood cultures. Blood cultures should include representative samples with a high white blood cell count.
- Reproducibility studies must be performed. For inter-assay reproducibility, at

least three authentic clinical samples or spiked clinical samples should be tested on three different days. For intra-assay reproducibility at least three authentic clinical samples or spiked clinical samples should be tested in triplicate. If both bacterial and fungal identification are being validated, separate reproducibility studies are needed with a minimum of three samples for each.

For Bacterial and Fungal Isolates:

- For assay modifications, the validation must include data from at least 20 representative isolates for each bacterial or fungal identification test, including the most common species isolated from different sources in the laboratory. At least 20 isolates for each extraction method must be included as part of the total number of representative isolates. No negative samples are required. A table of these results must include comparison identification information, identification score from MALDI-TOF MS, and final reporting information.
- Reproducibility studies must be performed. For inter-assay reproducibility, at least three clinical isolates should be tested on three different days. For intra-assay reproducibility, at least three clinical isolates should be tested in triplicate. If both bacterial and fungal identification are being validated, separate reproducibility studies are needed with a minimum of three isolates for each. Validations should be performed across different instruments, models, or technicians, when applicable.