

Clinical Laboratory Diagnostics for Invasive Aspergillosis
Contract No. HHSN266200700023C
Standard Operating Procedures

- SUBJECT:** **Comparative Studies for Nucleic Acid-Based Diagnostic Assays**
- PURPOSE:** To compare the performance of two assays using the same set of specimens with a confirmed clinical diagnosis (proven, probable or no invasive aspergillosis) based on MSG/EORTC definitions.
- LEVEL:** Principal Investigator/designee
Laboratory staff
- SUPPLIES/
EQUIPMENT:** Policy and Procedure Manual
Third party's Assay / Test Device

Third party's Assay Software
Third party's Test Procedure
AsTeC Consortium Laboratory Equipment
Comparator test reagents
Test samples
Controls
Pipettes and pipette tips
Computer
Printer
- REQUIREMENTS:** A specific SOP outlining test procedures according to third party's recommendations will be written for each molecular detection test method accepted for evaluation. The analyses described below will be conducted for each test method under evaluation following the individual test's specific SOP testing procedures.
- If multiple matrix specimen types (urine, serum, spinal fluid, whole blood, etc.) are to be assayed, separate analyses as described below for each specimen type will be performed.
- Familiarization Period*
An initial 5 day (minimum) familiarization period will be employed to reduce bias due to operator inexperience. During this time, the operator will become familiar with the test device and technology. Quality control material will be performed during this "break-in" period to establish stable results. Typically, the familiarization period will have been completed during the replication studies (refer to SOP 051.01) and will not need to be repeated.¹
- A. Chose specimens for testing

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Evaluate the test methods over the clinically meaningful range, i.e., where medical decisions are made. In general, this range extends from below to substantially above the expected reference range (analyte concentration interval claimed by the third party). Analyze at least 100 patient specimens distributed over the analytical measurement range to the extent possible including 10% of specimens below and 10% substantially above the expected reference range. At a minimum, at least 50 specimens should be positive (of which 10% should be substantially above the expected reference range when possible) and at least 50 specimens should be taken from patients who never developed an invasive fungal infection based on MSG/EORTC criteria during their course of surveillance (EP12-A page 7).

B. Analyze specimens

For test methods that measure the same analyte, specimens are tested in duplicate in order to measure bias between the two test methods. For tests that measure different analytes, specimens are tested only once rather than in duplicate. In both cases (the test methods measure the same analyte or the test methods measure different analytes), test the specimens over at least 10-20 (EP12 pg 7) operating days using both the test (new) method (Y) and the comparative (FDA-approved) method (X)(EP9A pg.1).² Analyze duplicates for each method within the same run for that method. Assign the first aliquot of the selected samples sequential positions in the run. Run the second or duplicate aliquots in reverse order to minimize the effects of carryover and drift. Select no more than 10 samples to be analyzed on a single day for each method (EP9A pg.6) to allow averaging of any between-day variability for either method.² Analysis by the two methods should occur within a time span that does not exceed the analyte's stability. When possible, assays by each method should be completed within 2 hours (not to exceed 4 hours) (EP9A pg.6 and EP15A pg 12.) of each other on the same day.^{2,3}

C. Quality Control

Test both kit controls and other recommended controls (e.g. whole organism preparations) during the experiment. Include negative extraction controls to monitor for contamination between specimens during pre-analytic processing, and to allow for prompt recognition of laboratory error that could adversely affect the performance characteristics of the test.⁴ Keep control charts and repeat any run that appears to be out of control on either method until the required number of samples is obtained. Document and retain a record of any situation that requires the rejection of data along with any discovered acceptable causes and problems (operator or device-indicated error).

D. Recording Results:

Record all data, including discrepant data that is not caused by operator or device-indicated error in Table 1. All data should be examined to detect any sources of analytical system or human errors. If it is determined that any results

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are due to explainable error, the error condition must be noted and the data not included in the data analysis. If a reason for a discrepancy cannot be determined, retain the original results in the data set.

E. Statistical Analyses

1. Bias estimation (For methods that measure the same analyte only. For assays that measure different analytes, proceed to Diagnostic Accuracy Estimation, Section E.2. below)

Outlier Test on Within-Method Duplicates

An initial test for within-method outliers will be performed. The analysis will be performed with all data points. Compute the mean absolute difference between duplicates for each method. Compute the “acceptability” limits (four times the mean absolute difference for each method). If any individual absolute difference exceeds the “acceptability” limit value, make an additional calculation for each method using normalized (relative) absolute differences (see EP9-A2 section 4.1 page 11 for calculations).² If a single data point falls outside the “acceptability” limits for both the range and relative range procedures, investigate why it did so and delete the samples from the data set. Continue analyzing the data after deleting all data for that sample.

If more than one sample has to be deleted, carry out an expanded investigation into the cause of the discrepancies. If the source of the problem can be identified and traced to the offending samples alone, replace those samples in the dataset and then document the cause of the problem. If it can be corrected but not traced to specific samples, the entire dataset must be recollected. If the cause of the problem cannot be found or corrected, evaluate the size of the maximum difference between duplicates relative to the allowable medical decision limits for precision of the method (refer to SOP 051.01).¹ If the limits are exceeded, stop the experiment and discuss the problem with the third party. If those limits are not exceeded, return the data and proceed with data analysis.

Plotting the Data

Plots will be made of the data as described in EP9A2 Document, Section 4.2.² The plots will be checked for a linear relationship between the comparative method and the test method throughout the measured range. If there appears to be a satisfactory linear relationship, then examine the data for visually obvious outliers. If outliers exist, perform analyses to assess for *Between-Method Outliers* as described in EP9A2 Document Section 4.4.² If more than 2.5% of the data are identified as outliers by this test, investigate possible interferences. If obvious causes cannot be determined and if differences resulting between the values exceeds the bounds of medical significance, stop the evaluation and discuss with the

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AsTeC Review Committee. A decision will be made as to whether 100 additional specimens should be analyzed.

If evaluation of the plots reveals evidence of a non-linear relationship, visually determine whether the data contains a linear portion. If the non-linear relationship occurs at the extremes of the concentration values, truncate the data points where they begin to be nonlinear and decide if the remaining portion is wide enough to cover the medically useful range. If so, analyze additional specimens within that range to replace the excluded specimens. The new data will need to be reanalyzed, beginning with *Outlier Tests on Within-Method Duplicates*. If no linear portion is evident or if the range is too small, stop the evaluation and notify the AsTeC Review Committee. A decision will be made as to whether to begin the experiment again with new specimens.

The correlation coefficient (r) will be calculated to determine if the range of the data from the comparative test (X) is sufficiently wide to proceed with regression analyses. As a general rule, the range of X can be considered adequate if $r \geq 0.975$ ($r^2 \geq 0.95$). If this is true, then proceed with simple linear regression. If $r^2 < 0.95$, then the range of the data must be extended by assaying additional specimens. Then begin examining the entire dataset again.

Visual Check for Constant Scatter

Examine the scatter and bias plots for constant scatter. If the data appear to exhibit reasonably constant scatter, perform linear regression to compute average bias. If nonconstant scatter is suspected then discuss with the AsTeC Review Committee to decide whether to add more specimens or to proceed with data analysis using transformed data.

Linear Regression

If the data have passed uniform range and adequate scatter checks, perform linear regression as described in EP9-A2 Section 6.1, to compute predicted bias and its 95% confidence interval (CI) using residuals and their respective standard error of estimates.²

Interpreting Results

Compare the 95%CI of predicted bias with the definition of acceptable error at the medical decision point. If the 95%CI for predicted bias includes the defined acceptable bias, then the data do not show that the bias of the candidate method is different from the acceptable bias. Proceed with analysis for diagnostic accuracy comparisons between the two methods.

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If the 95%CI for expected bias does not contain the defined acceptable bias, then one of the two following decisions can be made.

- a.) If the acceptable bias is less than the lower limit of the 95% CI of the predicted bias then the performance of the Test Method is NOT equivalent to the Comparative Method. Consult the AsTeC Review Committee before proceeding with comparative testing.
- b.) If the acceptable bias is greater than the higher limit of the 95% CI of the predicted bias then the performance of the Test Method is equivalent to the Comparative Method. Proceed with analysis for diagnostic accuracy comparisons between the two methods.

2. *Diagnostic Accuracy Estimation*

For assays that measure the same analyte, the first result for each sample obtained by the new and comparative tests will be used to assess and compare the diagnostic accuracy for the assays under study. For assays that measure different analytes, the single test result will be used.

Receiver operating characteristic (ROC) plots will be developed to assess differences in sensitivity and specificity due to the choice of cut off (the test method and comparative method performance represent two different points on the same ROC plot) versus real differences in diagnostic performance (the test method and comparative method have two different ROC plots).⁶ ROC plots will also be used to assess the accuracy of the test compared to the clinical status of the patient and to compare overall accuracy between the two methods. Sensitivity, specificity, and their respective 95% score CI will be calculated for each method as described in EP12-A Section 9.0, using MSG/EORTC definitions as the “reference method” for diagnostic certainty.⁵ A three way comparison between the new test method, old test method, and MSG/EORTC diagnosis category (proven versus proven and probable IA) will be performed to determine estimated differences in sensitivity and specificity and their respective 95% CI⁵.

Interpreting Results

If the confidence limits for the estimated difference in sensitivity and specificity include zero, there is evidence for statistically significant difference between the two methods.

F. After completing the comparative study, a final GLP compliant report will be prepared and submitted to the Project Officer for review and approval.

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REFERENCES:

1. Clinical Laboratory Diagnostics for Invasive Aspergillosis-Contract No. HHSN266200700023C--Standard Operating Procedures SOPPM 051.01 (09 07)
2. NCCLS. Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition. NCCLS document EP9-A2. 2002. NCCLS, Wayne, PA.
3. NCCLS. User Demonstration of Performance for Precision and Accuracy; Approved Guideline. NCCLS document EP15-A. 2001. NCCLS, Wayne, PA.
4. FDA Guidance Document 1560 –*Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens* December 08, 2005. U.S. Department of Health and Human Services Food and Drug Administration Center for Devices and Radiological Health.
5. NCCLS. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline. NCCLS document EP12-A. 2002. NCCLS, Wayne, PA.
6. NCCLS. Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots; Approved Guideline. NCCLS document GP10. 1995. NCCLS, Wayne, PA.

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ORIGINAL IMPLEMENTATION DATE: _____

APPROVED BY NIH NIAID Project Officer: _____ DATE _____

APPROVED BY PI/designee: _____ DATE _____

APPROVED BY Laboratory Coordinator: _____ DATE _____