

Example Verification Protocol for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Nucleic Acid Amplification Testing of Rectal Swabs

Compiled by the APHL/STD Steering Committee - October 15, 2009

This document is intended to serve as an example only. Laboratories are encouraged to consider clearing the study design including specimen numbers and acceptance criteria with your local CLIA inspector prior to initiation of the verification study.

Objective:

Through the documentation of performance characteristics, this verification is performed to demonstrate that the [insert NAAT test name], which is currently FDA cleared for cervical, urethral, urine, and vaginal specimens, can be expanded to include rectal swabs for testing in [insert your Laboratory Name].

Study Design:

The following performance characteristics will be assessed:

Accuracy – 30 previously tested rectal swabs will be obtained from a laboratory that currently has validated this NAAT test for rectal swabs or as a panel of well-characterized specimens obtained through CDC. The 30 specimens should include 15 negative specimens and a combination of 15 positive specimens that are positive for Chlamydia only, positive for GC only, and contain at least one specimen that is co-infected with both CT and GC. The negative specimens should include some specimens that contain (or are spiked with) commensal *Neisseria spp.*, such as *N. cinerea*, *N. sicca* or *N. lactamica*.

Precision – These 30 rectal specimens will be run in duplicate on the same run, and then again on a subsequent run to demonstrate intra-run and inter-run reproducibility. The quantitative measure of the assay [RLU for GenProbe APTIMA, MOTA score for BD ProbeTec] will only be captured for the purpose of comparing the repeat specimen results, and then in very rough groupings (high positives, low positives). Patient results will only be reported qualitatively.

Analytical Sensitivity – This has been demonstrated as part of the package insert, and does not need further verification when performing matrix expansion, except to demonstrate that verified positive specimens will test positive.

Analytical Specificity – There may be concern that organisms present in the rectum may cause cross reactivity. Testing negative specimens from a prescribed panel of specimens that contain commensal *Neisseria spp.* will address this performance characteristic.

Reportable Range of Test Results – Verification of the reportable range of patient test results was demonstrated during the initial verification of this FDA approved assay, and does not require further verification when performing matrix expansion.

Reference Intervals – This is a qualitative test, and will be reported as presence or absence, based on the package insert. No additional verification is necessary when performing matrix expansion, as the reference interval remains unchanged.

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Other Applicable Performance Characteristics – none

Calibration and Control Procedures – The frequency of quality control and other control procedures will remain unchanged from that established during the verification of the FDA approved assay.

Materials and Methods:

30 previously tested and blinded rectal specimens (15 positive, 15 negative) will be tested in duplicate on the same run, and run in singlet on a subsequent run.

After all testing is completed, results are unblinded, and results of this verification testing will be compared to the result obtained from the originating laboratory.

Failure to meet acceptance criteria would require additional verification. This may entail a review of current procedures to ensure the test is performing according to package insert specifications, and/or increasing the sample size by obtaining an additional panel of 30 specimens for testing.

Acceptance Criteria:

97% of the known negative specimens tested in triplicate, counting each triplicate as one test, must test negative to demonstrate accuracy, the lack of cross reactivity and analytical specificity.

97% of the known positive specimens tested in triplicate, counting each triplicate as one test, must test positive to demonstrate accuracy and analytical sensitivity.

To verify with-in run (intra-run) precision or reproducibility, each duplicate quantitative measure [RLU or MOTA score] on the same day's assay run must agree within a certain range. Specimens that test in the lower range of detection [150 – 500 RLU range for Gen-Probe APTIMA or similar MOTA score for BD ProbeTec] should duplicate in that range. Specimens that test in the upper range of detection [500 – 1000 or more RLU for Gen-Probe APTIMA or similar MOTA score for BD ProbeTec] should duplicate in that range.

To verify run-to-run (inter-run) precision or reproducibility, the specimen result from the previous day's run should compare to the next day's run within a certain range. Specimens that test in the lower range of detection [150 – 500 RLU range for Gen-Probe APTIMA or similar MOTA score for BD ProbeTec] should duplicate in that range. Specimens that test in the upper range of detection [500 – 1000 or more RLU for Gen-Probe APTIMA or similar MOTA score for BD ProbeTec] should duplicate in that range.

Using the RLU or MOTA scores for verification of precision may be problematic, as there is variation in these values due to the lack of homogeneity in the specimen, and the nature of the testing methodology. It may be necessary to only evaluate the presence/absence of target.

Evaluation and Conclusions:

A line listing of the specimens showing specimen source, quantitative measure of the result [RLU or MOTA score] for the intra-run (in duplicate) and inter-run testing, and comparison to the known expected value will be attached, with a brief summary. The conclusion should state whether the testing met the above listed acceptance criteria, and whether the [insert NAAT test] can be expanded for testing rectal swabs in your laboratory.

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The final documentation should include this protocol, the line listing of specimen results, the narrative summary conclusion, the revised Standard Operating Procedure, including any additional Quality Assurance measures, documentation of staff training, and final written review of the documentation and approval by the Laboratory Director or Designee.