**Verification of Patient Self-Collected Extragenital Nucleic Acid Amplification Tests for Diagnosis of Neisseria gonorrhoeae (GC) and Chlamydia trachomatis (CT)**

**Objective:**

Through the documentation of performance characteristics, this verification is performed to demonstrate that the Gen-Probe APTIMA, (Hologic Gen-Probe, San Diego, CA) which is currently FDA cleared for cervical, urethral, urine, and vaginal specimens, can be expanded to include patient self-collected rectal swabs for testing in INSERT LAB NAME Laboratory.

**Study Methods:**

**Specimen collection:** For the time period INSERT TIME PERIOD patients at NAME OF MEDICAL CENTER receiving testing for rectal GC/CT will obtain an additional self-collected specimen at the same visit. The clinician-collected specimen will be sent to INSERT LAB NAME in the usual fashion. The self-collected specimens will be labeled in the usual fashion and an additional colored label or mark will be added to the original label to distinguish the specimen as self-collected. These patient-collected specimens will be held at the Medical Center at room temperature until requested by INSERT LAB NAME for the verification procedure.

**Accuracy** – INSERT LAB NAME has previously validated the accuracy of clinician-collected rectal swabs for nucleic acid amplification tests for gonorrhea and chlamydia in YEAR. (Insert name of protocol binder, page XXX for reference) Therefore, 70 previously tested, clinician-collected rectal swabs from INSERT LAB NAME will be utilized for this current validation of patient-collected specimens. The 70 clinician-collected specimens used as the reference standard will include 30 negative specimens and a combination of 20 specimens that are positive for CT, 20 specimens that are positive for GC, and among the positive specimens, contain at least 2 specimens that are co-infected with both CT and GC.

Once the panel of 70 reference specimens has been established, patient-collected specimens corresponding to the 70 reference specimens (i.e., collected at the same visit) will also be tested according to the manufacturer’s package insert. Laboratorians will be blinded to the results of the reference specimens at the time that testing of the patient-collected specimens is performed.

**Precision** – The 70 patient-collected 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 APTIMA assay, relative light units [RLU], will only be captured for the purpose of comparing the repeat specimen results. Results will only be reported qualitatively as recommended by the manufacturer.

**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, however documentation of analytical specificity was performed during the initial validation of clinician-collected rectal specimens in NAME YEAR. Additional verification is not needed when performing matrix expansion to patient-collected specimens.

**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.

**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.

**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.

**Analysis:**

After all testing is completed of the 70 clinician-collected specimens and the corresponding 70 patient-collected specimens, results of this verification testing will be compared using the clinician-collected results as the reference standard.

Additional verification may be required if acceptance criteria outlined below are not met. 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:**

* At least 95% of the patient-collected specimens will correspond to the results from the clinician-collected specimens. (i.e. at least 38/40 will test positive and 29/30 will test negative)
* At least 97% of the patient-collected negative specimens tested in triplicate, counting each triplicate as one test, must test negative. (demonstrate accuracy, the lack of cross reactivity and analytical specificity)
* At least 95% of the patient-collected positive specimens tested in triplicate, counting each triplicate as one test, must test positive (demonstrates accuracy and analytical sensitivity)

To verify with-in run (intra-run) precision or reproducibility, each duplicate quantitative measure [RLU] 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] should duplicate in that range. Specimens that test in the upper range of detection [500 – 1000 or more RLU for Gen-Probe APTIMA] should duplicate in that range.

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

Using the RLU 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] 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 Gen-Probe APTIMA can be expanded for testing rectal swabs at the laboratory.

The final documentation will 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, INSERT NAME.