CHLAMYDIA TRACHOMATIS AND NEISSERIA GONORRHOEAE

I. Introduction

Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC) testing are performed using BD ProbeTec™ ET System. The assay uses a Strand Displacement Amplification (SDA) technology for the direct, quantitative detection of CT and GC DNA in endocervical swabs, male urethral swabs, and urine specimens.

II. Specimen Collection and Processing

Use Culturette™ Direct Swab for collecting endocervical specimen, and Mini-Tip Culturette™ for urethral specimen. The swabs can be stored between 4 to 6 days at 2^0C to 27^0C before testing.
15-20 ml of the first voided urine can be collected in a preservative free, sterile container. The specimen should be forwarded to the Virology laboratory as soon as possible. Upon arrival in the laboratory, a UPP(Urine Processing pouch) is added to the specimen for at least 2 hours, which will remove inhibitors of SDA. Urine can be stored for 2 days at 15-27^0C or 4 to 6 days at 2-8^0C.

III. Procedure

Reagents:

BD ProbeTec™ ET CT/GC reagent pouches containing:
CT priming and Amplification microwells
GC priming and Amplification microwells

Reagent pouches may be stored at 2 to 33^0C.Once a pouch is opened, the microwells are stable for 4 weeks if sealed properly or until expiry date, whichever comes first.

BD ProbeTec™ ET control set
CT/GC Positive Controls
CT/GC Negative Controls

1. BD ProbeTec™ ET Sample Dilution Tubes
2. BD ProbeTec™ ET System supplies:
   BD ProbeTec™ ET Instrument
   Microcell plates
   Lysing Heater and Lysing Rack
   Primary and warming rack
   Pipettor and Power Supply
   Urine Processing Kit (UPP)
   Sample tubes
   Pipette tips

ii) Other Materials:

Centrifuge
Vortex mixer
Gloves (powder free)
Transfer Pipette
1%(V/V) Sodium hypochlorite
Timer
Absorbent Paper (roll)

iii) Sample Processing

1. **Swabs:**

a) Label and remove cap from sample diluent tube.
b) Insert swab and swirl in diluent for 5-10 seconds.
c) Express liquid from swab and discard.
d) Tightly recap the tube and vortex for 5 seconds.

2. **Urine:**

a) UPP must be in contact with specimen for a minimum of two hours.
b) Mix urine by swirling and pipetting 4 ml into empty sample tube.
c) Cap tube and centrifuge at 2000xg for 30 minutes.
d) Decant tubes. End decanting motion with a gentle "flick" of the wrist to remove the residual fluid from the tube.
e) Add 2 ml of sample diluent.
f) Recap tubes and vortex 5 seconds.

3. **Controls:**

a) Use 1 Positive Control and 1 Negative control for each run.
b) Add 2 ml of sample diluent to each tube.
c) Recap tubes and vortex 5 seconds.

Note: It is important to dispense liquid against the inside wall **of the microwells to insure complete specimen addition and to avoid cross-contamination.**

iv) Sample Lysing:

d) Use plate layout template to place samples and controls in correct position in lysing rack.
e) Place rack in lysing heater for 30 minutes. Remove rack and let samples cool for at least 15 minutes.

iv) Assay Procedure:

1. Allow samples to cool at room temperature for at least 15 minutes (may be held up to 6 hours) after lysing is completed.
2. Remove and discard caps from tubes.
3. Change gloves to avoid contamination
4. Using the Plate Layout Report for the run, preparing the Priming Microwell Plate. Microwells must be in vertical correct order with CT, and follow by GC.
5. Using Program #3, expand pipettor by pulling the handle all the way out.
6. Aspirate samples from the first vertical column of tubes.

   Note: It is important to dispense liquid against the inside of the microwells to insure complete specimen addition and to avoid cross-contamination.

7. Gently collapse the pipettor and dispense 150 ul into each of the corresponding column of Priming Microwell Plate.

   Note: Recap lysed specimen tubes; hold these until run is completed.

8. Cover the Priming Microwell Plate with the priming cover and let the plate sit at room temperature for at least 20 minutes.

   Note: The plate may sit up to 6 hours before proceeding with the assay.

9. Prepare the Amplification Microwell Plate by Configuring the Amplification Microwell Plate to match the Plate Layout Report.

10. Remove the cover from the Priming Microwell Plate.

11. Check the temperature of the Priming/Warming Heater:

    Priming Heater: 72-73^{0}C
    Warming Heater: 53.5-54.5^{0}C

12. Place the Priming Microwell Plate into the Priming Heater.
13. **Immediately** place the Amplification Microwell Plate into the Warming Heater
14. Set the timer for 10 minutes.
15. At the end of the 10 minutes incubation, select Program'5' on the pipettor.
16. Pick up new tips and transfer 100 ul from the column 1 of the Priming Microwell Plate to column 1 of the Amplification Microwell Plate.
    Allow pipette tips to touch sides of microwells
    After dispensing the liquid, allow pipettor to automatically mix the liquid in the wells, and discard tips.
    Continue transferring from column to column using new tips each time
17. When the last column has been transferred, seal the Amplification Microwell Plate using an Amplification Plate Sealer.
18. Place the Amplification Microwell Plate into the BD ProbeTec™ ET Instrument for analysis. The Amplification Microwell Plate must be loaded into the instrument within 30 seconds of transferring the last column of samples.
    Timing is extremely critical. Do Not Delay entry of plate into the instrument.
19. Test results will be printed out as soon as the testing is finished.
20. See Appendix VIII for Workflow Overview.

II. Validation:

MOTA Score is a metric used to assess the magnitude of signal generated as a result of the reaction. The magnitude of the MOTA Score is not indicative of the level of organism in the specimen.

MOTA Score for CT or GC :

CT/NG Positive Control : > or = 2000
CT/NG Negative Control : < 2000

If any of these two controls fails, the test is invalid. Should inform Senior/Charge technologists and repeat testing.

V. Interpretation of Results:

MOTA score for CT/NG is > or = 2000 is considered as Positive, reported as Positive for Chlamydia trachomatis / Neisseria gonorhoeae by Nucleic Acid Amplification
MOTA Score for CT/NG is < 2000 is considered as Negative, reported as Negative for Chlamydia trachomatis / Neisseria gonorhoeae by Nucleic Acid Amplification

VI. Reporting

Positive report:
Positive for Chlamydia trachomatis by Nucleic Acid Amplification
Positive for Neisseria gonorhoeae by Nucleic Acid Amplification
Negative report:
Negative for Chlamydia trachomatis by Nucleic Acid Amplification
Negative for Neisseria gonorhoeae by Nucleic Acid Amplification

VII. Quality Control

One Positive Control and one Negative Control must be included in each run.
Run external control (Accurun 341)) every second week. Result filed in Reagent Lot Binder.

External proficiency testing is provided by CAP and QMP-LS .

Maintenance QC:
Enter temperature checks on lysing heater, priming heater, warming heater, and instrument in System Maintenance Log every time the test is performed.
Clean/replace Air Filter and verify BD ProbeTec ET temperature monthly.
Wipe checks for Analyser,Bench,Lysing rack,Pipette,Priming heater ,Lysing heater are done every two weeks (see appendix#) .

VIII. Reference

Package insert for BD ProbeTec™ ET CT/GC Amplified DNA Assay.
Becton, Dickinson and Company, 7 Loveton Circle, Sparks, MD 21152,USA.