

## **BD ProbeTec™ ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays**

### **I. INTENDED USE**

The **BD ProbeTec™** ET *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) Amplified DNA Assays, when tested with the **BD ProbeTec** ET System, use Strand Displacement Amplification (SDA) technology for the direct, qualitative detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA in endocervical swabs, male urethral swabs, and in female and male urine specimens as evidence of infection with *C. trachomatis*, *N. gonorrhoeae*, or of co-infection with both *C. trachomatis* and *N. gonorrhoeae*. Specimens may be from symptomatic or asymptomatic females and males. A separate Amplification Control is an option for inhibition testing (**BD ProbeTec** ET CT/GC/AC Reagent Pack). The **BD ProbeTec** ET CT/GC assays may be performed using either the **BD ProbeTec** ET System or a combination of the **BD ProbeTec** ET System and **BD Viper™** instrument.

### **II. PRINCIPLES OF THE PROCEDURE**

The **BD ProbeTec** ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays are based on the simultaneous amplification and detection of target DNA using amplification primers and a fluorescent labeled detector probe.<sup>1,2</sup> The SDA reagents are dried in two separate disposable microwell strips. The processed sample is added to the Priming Microwell that contains the amplification primers, fluorescent labeled detector probe, and other reagents necessary for amplification. After incubation, the reaction mixture is transferred to the Amplification Microwell, which contains two enzymes (a DNA polymerase and a restriction endonuclease) necessary for SDA. The Amplification Microwells are sealed to prevent contamination and then incubated in a thermally controlled fluorescent reader that monitors each reaction for the generation of amplified products. The presence or absence of CT and GC is determined by relating the **BD ProbeTec** ET MOTA (Method Other Than Acceleration) scores for the sample to pre-determined cutoff values. The MOTA score is a metric used to assess the magnitude of signal generated as a result of the reaction.

This procedure describes the test procedures for two assay kit configurations - the CT/GC Reagent Pack and the CT/GC/AC Reagent Pack. If the CT/GC Reagent Pack is used, each sample and control is tested in two discrete microwells: one for *C. trachomatis* and one for *N. gonorrhoeae*. If the CT/GC/AC Reagent Pack is used, each sample and control is tested in three discrete microwells: *C. trachomatis*, *N. gonorrhoeae*, and the Amplification Control. The purpose of the Amplification Control is to identify a sample that may inhibit the SDA reaction.

### **III. REAGENTS**

Each **BD ProbeTec** ET CT/GC Reagent Pack contains:

- *Chlamydia trachomatis* (CT) Priming Microwells, 4 x 96:  
4 Oligonucleotides  $\geq$  7 pmol; dNTP  $\geq$  35 nmol; Detector probe  $\geq$  25 pmol; with buffers and stabilizers.
- *Neisseria gonorrhoeae* (GC) Priming Microwells, 4 x 96:

4 Oligonucleotides  $\geq 7$  pmol; dNTP  $\geq 35$  nmol; Detector probe  $\geq 25$  pmol; with buffers and stabilizers.

- *Chlamydia trachomatis* (CT) Amplification Microwells, 4 x 96:  
Restriction enzyme  $\geq 30$  Units; DNA Polymerase  $\geq 25$  Units; dNTP's  $\geq 80$  nmol; with buffers and stabilizers.
- *Neisseria gonorrhoeae* (GC) Amplification Microwells, 4 x 96:  
Restriction enzyme  $\geq 15$  Units; DNA Polymerase  $\geq 2$  Units; dNTP's  $\geq 80$  nmol; with buffers and stabilizers.

In addition to the above reagents, the **BD ProbeTec** ET CT/GC/AC Reagent Pack also contains:

- Amplification Control (AC) Priming Microwells, 4 x 96:  
4 Oligonucleotides  $\geq 7$  pmol; dNTP  $\geq 35$  nmol; Detector probe  $\geq 25$  pmol;  $\geq 1,000$  copies per reaction of pGC10 linearized plasmid; with buffers and stabilizers.
- Amplification Control (AC) Amplification Microwells, 4 x 96:  
Restriction enzyme  $\geq 15$  Units; DNA Polymerase  $\geq 2$  Units; dNTP's  $\geq 80$  nmol; with buffers and stabilizers.

**NOTE:** Each microwell pouch contains one desiccant bag.

Accessories: Priming Covers; Amplification Sealers, 40 each; Disposal Bags, 20 each.

Each **BD ProbeTec** ET (CT/GC) Control Set contains:

- 20 CT/GC Positive Controls (50  $\mu$ L dried) containing 750 copies per reaction of pCT16 linearized plasmid\* and 250 copies per reaction of pGC10 linearized plasmid\* with  $\geq 5$   $\mu$ g Salmon testes DNA.
- 20 CT/GC Negative Controls (50  $\mu$ L dried) with  $\geq 5$   $\mu$ g Salmon testes DNA.

**BD ProbeTec** ET CT/GC Diluent Tubes:

- 400 tubes each containing 2 mL of Sample Diluent, which contains potassium phosphate, DMSO, glycerol, Polysorbate 20, and 0.03% Proclin™ (preservative).

**BD ProbeTec** ET Diluent (CT/GC):

- 225 mL Sample Diluent which contains potassium phosphate, DMSO, glycerol, Polysorbate 20, and 0.03% Proclin (preservative).

\*The concentration of this DNA was determined spectrophotometrically at 260 nm.

#### **Storage and Handling Requirements:**

Reagents may be stored at 2–33°C. Unopened Reagent Packs are stable until the expiration date. Once a pouch is opened, the microwells are stable for 4 weeks if properly sealed or until the expiration date, whichever comes first. Do not freeze.

#### **IV. MATERIALS REQUIRED BUT NOT PROVIDED**

- Centrifuge capable of 2000 x g
- Vortex mixer
- Gloves

- Pipettes capable of delivering 1 mL, 2 mL and 4 mL
- 1% (v/v) sodium hypochlorite with Alconox™\* or ELIMINase™ or DNA AWAY™\*\*
- Clean container suitable for holding aliquotted Diluent
- Timer and absorbent paper
- Sterile urine specimen collection cups

\*Mix 200 mL of bleach with 800 mL of warm water. Add 7.5 g of Alconox and mix. Prepare fresh daily.

\*\* Use undiluted.

## V. WARNINGS:

For *in vitro* Diagnostic Use

1. This reagent pack is for testing endocervical and male urethral swabs and male and female urine specimens with the **BD ProbeTec** ET System.
2. For collection of endocervical swab specimens, only the **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/GC) Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit and the **BD ProbeTec** ET CT/GC Amplified DNA Assay Collection Kit for Endocervical Specimens have been validated.
3. For collection of male urethral swab specimens, only the **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/GC) Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit and the **BD ProbeTec** ET CT/GC Amplified DNA Assay Collection Kit for Male Urethral Specimens have been validated.
4. For urine specimens, only the **BD ProbeTec** ET Urine Processing Pouch (UPP), the **BD ProbeTec** Urine Preservative Transport (UPT) and unpreserved (neat) urine have been validated.
5. The **BD ProbeTec** Urine Preservative Transport (UPT) Kit contains **NAP Guard™** ( $\geq 742.5$  mM  $K_2EDTA$ ). **NAP Guard** may be irritating to the eyes, skin and respiratory system. In case of contact with eyes, rinse opened eye immediately with plenty of water and seek medical advice if symptoms persist. After contact with skin, wash immediately with plenty of soap and water. If inhaled, seek medical attention in case of problems.
6. Laboratories may validate other swab or urine collection and transport devices for use with the **BD ProbeTec** ET CT/GC assays according to the "Verification and Validation Procedure in the Clinical Microbiology Laboratory." Cumitech 31, B.L. Elder et al., American Society for Microbiology, Washington D. C., February, 1997.
7. Do not test the CT/GC Diluent tube from the **BD ProbeTec** ET CT/GC Amplified Assay Collection Kits if received in the laboratory without the swab present. A false negative test result may occur.
8. Use only the **BD ProbeTec** ET Pipettor and **BD ProbeTec** ET Pipette tips for the transfer of processed samples to Priming Microwells and the transfer of samples from the Priming Microwells to the Amplification Microwells.
9. Do not interchange or mix kit reagents from kits with different lot numbers.

10. Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions”<sup>3-6</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.
11. Use established laboratory practices when disposing of used pipette tips, sample tubes, Priming Microwells and other disposables. Discard disposables carefully. Seal and dispose of waste containers when they are 3/4 full or daily (whichever comes first).
12. The **BD ProbeTec** ET Diluent (CT/GC) and CT/GC Diluent tube contain dimethyl sulfoxide (DMSO). DMSO is harmful by inhalation, contact with skin or if swallowed. Avoid contact with eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water.
13. Reagent pouches containing unused Priming Microwells and Amplification Microwells **MUST** be carefully resealed after opening. Verify that a desiccant is present prior to resealing the reagent pouches.
14. The plate containing the Amplification Microwells **MUST** be properly sealed with the Amplification sealer prior to moving the plate from the **BD ProbeTec** ET Priming and Warming Heater to the **BD ProbeTec** ET Instrument. Sealing ensures a closed reaction for amplification and detection and is necessary to avoid contamination of the instrument and work area with amplification products. **Do not remove sealing material from microwells at any time.**
15. Priming Microwells with residual fluid (after transfer of fluid from the Priming Microwells to the Amplification Microwells) represent a source of target contamination. Carefully seal Priming Microwells with plate sealer prior to disposal.
16. To prevent contamination of the work environment with amplification products, use the disposal bags provided in the Reagent Packs to dispose of tested Amplification Microwells. Make sure the bags are properly closed before disposal.
17. Although dedicated work areas are not required because the **BD ProbeTec** ET design reduces the possibility of amplicon contamination in the testing environment, other precautions for controlling contamination, particularly to avoid contamination of specimens during processing, are necessary.
18. Because of the potential for false positivity with some non-gonococcal *Neisseria* found in the respiratory tract (see “Limitations of the Procedure” #20), contamination of reagents and specimens with respiratory aerosols should be avoided.
19. **CHANGE GLOVES** after removing and discarding caps from lysed samples and controls to avoid cross-contamination of specimens. If gloves come in contact with specimen or appear to be wet, immediately change gloves to avoid contaminating other specimens. Change gloves before leaving work area and upon entry into work area.
20. In the event of contamination of the work area or equipment with samples or controls, thoroughly clean the contaminated area with ELIMINase, DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox and rinse thoroughly with water. Allow surface to dry completely before proceeding.
21. In case of a spill on the Lysing Rack: The rack may be immersed in ELIMINase, DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox for 1-2 min. Do not exceed 2 min. Thoroughly rinse the rack with water and allow to air dry.
22. Clean the entire work area - counter tops and instrument surfaces - with ELIMINase,

DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox on a daily basis. Thoroughly rinse with water. Allow surfaces to dry completely before proceeding with additional testing.

23. Contact Technical Services in the event of an unusual situation, such as a spill into the **BD ProbeTec** ET instrument or DNA contamination that cannot be removed by cleaning.

## VI. SAMPLE COLLECTION AND TRANSPORT

The **BD ProbeTec** ET System is designed to detect the presence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in endocervical swabs, male urethral swabs and male and female urine specimens using the appropriate collection method.

The only devices that have been validated for collecting swab specimens for testing on the **BD ProbeTec** ET Instrument are:

- **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/GC) Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit
- **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/GC) Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit
- **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/GC) Amplified DNA Assay Collection Kit for Endocervical Specimens
- **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/GC) Amplified DNA Assay Collection Kit for Male Urethral Specimens

For U.S. and international shipments, specimens should be labeled in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and etiologic agents/infectious substances. Time and temperature conditions for storage must be maintained during transport.

Urine specimens must be collected in a sterile, plastic, preservative-free, specimen collection cup. For urine specimens, only the **BD ProbeTec** ET Urine processing Pouch (UPP), the **BD ProbeTec** Urine Preservative Transport (UPT), and unpreserved (neat) urine have been validated.

### Swab Specimen Collection

#### Endocervical Swab Specimen Collection using **BD ProbeTec** ET CT/GC Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit:

1. Remove excess mucus from the cervical os with the large-tipped cleaning swab provided in the **BD ProbeTec** ET CT/GC Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit and discard.
2. Insert the Endocervical Specimen Collection and DRY TRANSPORT swab into the cervical canal and rotate for 15-30 s.
3. Withdraw the swab carefully. Avoid contact with the vaginal mucosa.
4. Immediately place the cap/swab into the transport tube. Make sure the cap is tightly secured to the tube.
5. Label the tube with patient information and date/time collected.
6. Transport to laboratory.

### **Endocervical Swab Specimen Collection using BD ProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Endocervical Specimens**

1. Remove the cleaning swab from packaging.
2. Using cleaning swab, remove excess mucus from the cervical os.
3. **Discard** the used cleaning swab.
4. Remove the collection swab from packaging.
5. Insert the collection swab into the cervical canal and rotate for 15-30 s.
6. Withdraw the swab carefully. Avoid contact with the vaginal mucosa.
7. Uncap the CT/GC Diluent tube.
8. Fully insert the collection swab into the CT/GC Diluent tube.
9. Break the shaft of the swab at the score mark. Use care to avoid splashing of contents.
10. **Tightly** recap the tube.
11. Label the tube with patient information and date/time collected.
12. Transport to laboratory.

### **Male Urethral Swab Specimen Collection using BD ProbeTec ET CT/GC Amplified DNA Assay Male Urethral Collection and DRY TRANSPORT Kit:**

1. Insert the Male Urethral Collection and DRY TRANSPORT swab 2-4 cm into the urethra and rotate for 3-5 s.
2. Withdraw the swab and place the cap/swab into the transport tube. Make sure the cap is tightly secured to the tube.
3. Label the tube with patient information and date/time collected.
4. Transport to laboratory.

### **Male Urethral Swab Specimen Collection using BD ProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Male Urethral Specimens.**

1. Remove the swab from packaging.
2. Insert the swab 2-4 cm into the urethra and rotate for 3 -5 s.
3. Withdraw the swab.
4. Uncap the CT/GC Diluent tube.
5. Fully insert the swab into the CT/GC Diluent tube.
6. Break the shaft of the swab at the score mark. Use care to avoid splashing of contents.
7. **Tightly** recap the tube.
8. Label the tube with patient information and date/time collected.
9. Transport to laboratory.

### **Swab Storage and Transport**

After collection, the endocervical swabs and the male urethral swabs must be stored and transported to the laboratory and/or test site at 2-27°C within 4-6 days. Storage up to 4 days has been validated with clinical specimens; storage up to 6 days has been demonstrated with seeded specimens. Refer to “Performance Characteristics.”

**NOTE:** If specimens cannot be transported directly to the testing laboratory under ambient temperatures (15-27°C) and must be shipped, an insulated container with ice should be used with either an overnight or 2-day delivery vendor.

### Urine Specimen Collection, Storage and Transport

Collect urine specimens in a sterile, preservative-free collection cup. Urine specimens may be stored and transported in three ways- (1) unpreserved (neat), (2) using the **BD ProbeTec** Urine Preservative Transport (UPT) and (3) using the **BD ProbeTec** ET Urine Processing Pouch (UPP). The following chart provides a summary of storage and transport conditions for neat urine, UPT and UPP.

Urine Specimen Type to be Processed	NEAT			UPT			UPP		
				Urine Stored at 2-30°C- Transfer to UPT Within 8 hrs of Collection	Urine stored at 2-8 °C- Transfer to UPT Within 24 hours of Collection		UPP Added at Specimen Collection Site	UPP Added at Testing Site	
<b>Temperature Condition for Transport to Test Site and Storage</b>	2-30°C	2-8 °C	-20 °C	2-30°C	2-30°C	-20 °C	Transport to Lab at 2-8 °C	Transport to Lab at 15-27 °C	Transport to Lab at 2-8 °C
<b>Process Specimen According to Instructions</b>	Within 30 hours of collection	Within 7 days of collection	Within 2 months of collection	Within 30 days after transfer to UPT	Within 30 days after transfer to UPT	Within 2 months after transfer to UPT	Within 4-6 days of collection	Within 2 days of collection	Within 4-6 days of collection

### Unpreserved (Neat) Urine

#### Collection

1. The patient should not have urinated for at least 1h prior to specimen collection.
2. Collect the specimen in a sterile, preservative-free specimen collection cup.
3. The patient should collect the first 15-60 mL of voided urine (the first part of the stream- not midstream) into a urine collection cup.
4. Cap and label the urine collection cup with patient identification and date/time collected.

## Storage and Transport

1. Store and transport neat urine from the collection site to the test site at 2-30°C.
2. Sample processing must be completed within 30 h of collection if stored at 2-30°C or within 7 days of collection if stored at 2-8 °C.

### NOTE:

Specimens must be shipped in an insulated container with ice using either an overnight or 2-day delivery vendor. Storage up to 7 days at 2-8 °C has been demonstrated with seeded specimens.

## Using BD ProbeTec Urine Preservative Transport (UPT)

### Collection

1. The patient should not have urinated for at least 1 h prior to specimen collection.
2. Collect the specimen in a sterile, preservative-free specimen collection cup.
3. The patient should collect the first 15-60 mL of voided urine (the first part of the stream-not midstream) into a urine collection cup.
4. Cap and label the urine collection cup with patient identification and date/time collected.

### Urine Transfer to UPT

**NOTES:** Urine should be transferred from the collection cup to the UPT within 8 h of collection provided the urine has been stored at 2-30°C. Urine can be held for up to 24 h prior to transfer to the UPT provided the urine has been stored at 2-8°C.

Wear clean gloves when handling the UPT and the urine specimen. If gloves come in contact with the specimen, immediately change gloves to prevent contamination of other specimens.

1. After the patient has collected the urine sample, label the urine collection cup.
2. Open the Urine Preservative Transport Kit and remove the UPT and the transfer pipette. Label the UPT with the patient identification and date/time collected.
3. Hold the UPT upright and firmly tap the bottom of the tube on a flat surface to dislodge any large drops from inside the cap. Repeat if necessary.
4. Uncap the UPT and use the transfer pipette to transfer urine into the tube. The correct volume of urine has been added when the fluid level is between the black lines on the fill window on the UPT label. This volume corresponds to approximately 2.5-3.45 mL of urine. DO NOT overfill or under fill the tube.
5. Discard the transfer pipette. NOTE: The transfer pipette is intended for use with a single specimen.
6. Tighten the cap securely on the UPT.
7. Invert the UPT 3-4 times to ensure that the specimen and reagent are well mixed.

### UPT Storage and Transport

Store and transport urine specimens in UPT at 2-30°C and process within 30 days of collection. Specimens may be stored at -20°C for up to two months.

## Using the BD ProbeTec ET Urine Processing Pouch (UPP)

The Urine Processing Pouch (UPP) can be added at either the test site or at the collection site.

Instructions are provided for each option.

**Urine Collection (UPP Added at Test Site)**

1. The patient should not have urinated for at least 1 h prior to specimen collection.
2. Collect the specimen in a sterile, plastic, preservative-free specimen collection cup.
3. The patient should collect the first 15-20 mL of voided urine (the first part of the stream, not midstream) into a urine collection cup.

**NOTE:** During the clinical evaluation, testing urine volumes up to 60 mL was included in the performance estimates.

4. Cap and label the urine collection cup with patient identification and date/time collected.

**Urine Storage and Transport (Addition of UPP at Test Site):**

**NOTE:** Specimens must be shipped in an insulated container with ice using either an overnight or 2-day delivery vendor. Storage up to 4 days has been validated with clinical specimens; storage up to 6 days has been demonstrated with seeded specimens. Refer to “Performance Characteristics.”

1. Store and transport urine specimens to the test site at 2-8°C within 4-6 days of collection.
2. Add the UPP to the urine specimen collection cup. Wear clean gloves when handling the UPP and urine specimen.

**NOTE:** Do not place UPP on any work surface. Remove UPP from pouch with a freshly gloved hand or with clean, sterile forceps. Add UPP carefully to avoid splashing.

3. Cap the collection cup and swirl gently to ensure that the UPP is completely submerged in urine.
4. The UPP must be in contact with the urine specimen for at least 2 h prior to processing.
5. Do not freeze the urine specimen.

**Urine Collection (UPP Added at Collection Site)**

1. The patient should not have urinated for at least 1 h prior to specimen collection.
2. Collect specimen in a sterile, plastic, preservative-free specimen collection cup.
3. The patient should collect the first 15-20 mL of voided urine (the first part of the stream, NOT midstream).

**NOTE:** During the clinical evaluation, testing urine volumes up to 60 mL was included in the performance estimates.

4. Immediately add the UPP to the specimen collection cup. Wear clean gloves when handling the UPP and urine specimen.

**NOTE:** Do not place UPP on any work surface. Remove UPP from pouch with a freshly gloved hand or with clean, sterile forceps. Add UPP carefully to avoid splashing.

5. Cap collection cup and swirl gently to ensure that the UPP is completely submerged in urine.
6. Label with patient identification and date/time collected.

### Urine Storage and Transport (UPP Added at Collection Site):

**NOTE:** If specimens cannot be transported directly to the testing laboratory under ambient temperatures (15-27°C) and must be shipped, an insulated container with ice should be used with either an overnight or 2-day delivery vendor. Storage up to 4 days has been validated with clinical specimens; storage up to 6 days has been demonstrated with seeded specimens. Refer to “Performance Characteristics.”

1. Store and transport urine specimens containing a UPP to the laboratory or test site at 2-8°C within 4-6 days of collection or at 15-27°C within 2 days of collection.
2. Do not freeze the urine specimen.
3. The UPP must be in contact with the urine specimen for at least 2 h prior to processing.

## VII. TEST PROCEDURE

Refer to the **BD ProbeTec** ET System User’s Manual for specific instructions for operating and maintaining the components of the system. The optimum environmental conditions for the CT/GC assay were found to be 18-23°C at 25-75% Relative Humidity and 23-28°C at 25-50% Relative Humidity. The performance of the CT/GC assay at temperatures in excess of 28°C is not recommended.

### A. Instrument Preparation:

1. Instrument power must be on and instruments allowed to warm-up prior to beginning the assay.
  - a. The Lysing Heater and the Priming and Warming Heater require approximately 90 min for warm-up and stabilization.

The Setpoint for the Lysing heater is 114°C.

The Setpoint for the Priming component of the Priming and Warming Heater is 72.5°C.

The Setpoint for the Warming component of the Priming and Warming Heater is 54°C.
  - b. The **BD ProbeTec** ET instrument is under software control and requires approximately 30 min to warm-up.
2. Heater temperatures must be checked prior to beginning the assay.
  - a. Lysing Heater  
Remove the plastic cover and allow temperature to equilibrate for 15 min.  
The thermometer must read between 112-116°C.
  - b. Priming and Warming Heater  
The Priming heater thermometer must read between 72-73°C.  
The Warming heater thermometer must read between 53.5-54.5°C.
3. Check the temperature displayed on the **BD ProbeTec** ET screen. The temperature must read 47.5-55.0°C.

## B. Pipettor:

**NOTE:** Refer to the **BD ProbeTec** ET System User's Manual for detailed explanations on **BD ProbeTec** ET Pipettor programming and other keypad functions.

1. The following programs are required for performing the CT/GC assays:
  - **Program 2** is used with the CT/GC Reagent Pack. It transfers liquid from the processed samples to the CT/GC Priming Microwells.
  - **Program 3** is used with the CT/GC/AC Reagent Pack. It transfers liquid from the processed samples to the CT/GC and AC Priming Microwells.
  - **Program 5** transfers liquid from the Priming Microwells to the Amplification Microwells.

## C. Plate Layout:

The Plate Layout Report is generated from the **BD ProbeTec** ET instrument after the assay type, specimen identification, control lot numbers, and kit lot numbers are logged into the system. The Plate Layout Report shows the physical layout of specimens and controls for each plate to be tested. The system software groups adjacent plate locations for the wells required for a specific assay. For the CT/GC Amplified DNA Assay, columns are assigned as follows: CT/GC. For the CT/GC/AC Amplified Assay, columns are assigned as follows: CT/GC/AC. This orientation is used for both the Priming Microwell plate and the Amplification Microwell plate.

**Priming Microwells** are the **solid** colored microwell strips (CT - solid green; GC - solid yellow; AC - solid black, if applicable).

**Amplification Microwells** are the **striped** microwell strips (CT - striped green; GC - striped yellow; AC - striped black, if applicable).

## D. Swab Processing:

Swab specimens must be processed within 4-6 days of collection if stored at 2-27°C.

**NOTE:** Swabs and CT/GC Diluent Tubes should be at room temperature prior to use.

**Processing Procedure for swabs collected with the BD ProbeTec ET CT/GC Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit or the BD ProbeTec ET CT/GC Amplified DNA Assay Male Urethral Specimen Collection Kit and DRY TRANSPORT:**

1. Label a CT/GC Diluent Tube for each swab specimen to be processed.
2. Remove the cap from the tube and insert the swab. Mix by swirling the swab into Diluent for 5 -10 s.
3. Express the specimen swab along the inside of the tube so that the liquid runs back into the bottom of the tube.
4. Remove swab carefully to avoid splashing.

**NOTE:** Droplets may cause contamination of work area.

5. Place swab back into transport tube and discard.
6. Tightly replace the cap on the CT/GC Diluent tube.
7. Vortex tube for 5 s.
8. Using the Plate Layout Report, place tube in order in the Lysing Rack.
9. Repeat steps 1-8 for additional swab specimens.
10. Lock the samples into place in the Lysing Rack.
11. Specimens are ready to be Lysed.

**NOTE:** Alternatively, if a multi-tube vortexer is available, skip step 7 and vortex the entire Lysing Rack for 15-20 s after step 10 and before Lysing.

**NOTE:** Specimens processed, but not yet lysed, may be stored at room temperature for up to 6 h or overnight at 2-8°C.

**Processing Procedure for swabs collected with the BD ProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Endocervical Specimens or the BD ProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Male Urethral Specimens (Wet Transport).**

1. Vortex the CT/GC Diluent Tube for 5 s.

**NOTE:** Alternatively, if a multi-tube vortexer is available, perform steps 2 and 3; then vortex the entire Lysing Rack for 15-20 s and proceed to step 4.

2. Using the Plate Layout Report, place sample and control tubes in order in the Lysing Rack.
3. Lock the samples into place in the Lysing Rack.
4. Specimens are ready to be lysed.

**E. Urine Processing:**

**Processing Procedure for Unpreserved (Neat) Urine Specimens**

Neat urine specimens must be processed within 30 h of collection if stored at 2-30°C, within 7 days of collection if stored at 2-8°C, and within 2 months from the date of collection if stored at -20°C.

**NOTES:**

**BD ProbeTec** ET Diluent should be at room temperature before use.

Aliquot the needed quantity of **BD ProbeTec** Diluent (CT/GC) into a clean container. To estimate the quantity needed, multiply the number of samples by 2 and add an additional 1-2 mL for pipetting ease. **To avoid contamination of the Diluent-Do Not Pour Leftover Diluent back into the Bottle.**

1. Label an empty **BD ProbeTec** ET Sample Tube for each urine to be processed.
2. Swirl the container to mix the urine and open carefully.

**NOTE:**

Open carefully to avoid spills or droplets which may cause contamination of work area.

Frozen urine specimens must be thawed and mixed completely before transfer to the Sample Tube.

3. Pipette 4.0 mL of urine into the appropriately labeled tube and tightly recap the tube.
4. Repeat steps 2-3 for additional neat urine samples. Use a new pipette or pipette tip for each sample.
5. Insert sample tubes into the **BD ProbeTec** ET Lysing Rack.
6. Insert the Lysing Rack into the Lysing Heater to pre-warm the samples.
7. Heat the samples for 10 minutes.
8. After 10 min, remove the Lysing Rack from the Lysing Heater and cool tubes at room temperature for a minimum of 15 min, or a maximum of 6 h.

**NOTE:** Do not refrigerate or freeze the sample tubes after the 10 min pre-warm.

9. Centrifuge the tubes at 2000 x g for 30 min.
10. At the end of centrifugation, carefully remove the tubes from the centrifuge.
11. Uncap the first tube and carefully decant the supernatant. End the decanting motion with a gentle "flick" of the wrist to remove residual fluid from the tube.

**NOTE:** This is a critical step - excess residual specimen may cause inhibition. Tubes should be individually blotted on a separate sheet of absorbent paper to enhance removal of residual urine.

12. Place the cap loosely on the tube.
13. Repeat steps 11-12 for each centrifuged urine specimen.
14. Pipette 2.0 mL of Diluent into each tube. Use a new pipette or pipette tip for each tube.
15. Tightly recap the sample tubes and vortex 5 s to completely resuspend the sediment in the Diluent.
16. Samples are ready to be lysed.

**NOTE:** Specimens processed, but not yet lysed, may be stored at room temperature for up to 6 h or overnight at 2-8°C.

### **Processing Procedure Using Urine Specimens Collected Using the BD ProbeTec Urine Preservative Transport Kit (UPT)**

#### **NOTES:**

UPT samples may be stored at 2-30°C and processed within 30 days of collection or frozen at -20°C and processed within 2 months of collection.

**BD ProbeTec** ET Diluent (CT/GC) should be at room temperature before use.

Aliquot the needed quantity of **BD ProbeTec** Diluent (CT/GC) into a clean container. To estimate the quantity needed, multiply the number of samples by 2 and add an additional 1-2 mL for pipetting ease. **To avoid contamination of the Diluent-Do Not Pour Leftover Diluent back into the Bottle.**

**Make sure the urine volume in each tube falls between the lines indicated on the tube label. Under filling and over filling the tube may affect assay performance**

1. Insert UPT tubes into the **BD ProbeTec** ET Lysing Rack.

**NOTE:** If specimens were frozen, make sure they are thawed completely and mixed by inversion prior to heating.

2. Insert the Lysing Rack into the Lysing Heater to pre-warm the samples.
3. Heat the samples for 10 minutes.
4. After 10 min, remove the Lysing Rack from the Lysing Heater and cool tubes at room temperature for a minimum of 15 min, or a maximum of 6 h.

**NOTE:** Do not refrigerate or freeze the sample tubes after the 10 min pre-warm.

5. Centrifuge the tubes at 2000 x g for 30 min.
6. At the end of centrifugation, carefully remove the tubes from the centrifuge.
7. Uncap the first UPT and carefully decant the supernatant. End the decanting motion with a gentle "flick" of the wrist to remove residual fluid from the tube and blot the tube on a separate piece of absorbent paper.
8. Place the cap loosely on the tube.
9. Repeat steps 7-8 for each centrifuged urine specimen.
10. Pipette 2.0 mL of Diluent into each tube. Use a new pipette or pipette tip for each tube.
11. Tightly recap the UPT tubes and vortex 5 s to completely resuspend the sediment in the Diluent.
12. Samples are ready to be lysed.

**NOTE:** Specimens processed, but not yet lysed, may be stored at room temperature for up to 6 h or overnight at 2-8°C.

### **Processing Procedure for Specimens Collected Using the BD ProbeTec ET Urine Processing Pouch (UPP)**

Urine specimens must be processed within 4-6 days of collection if stored at 2-8°C (UPP added at either collection or testing site) or within 2 days of collection if stored at 15-27°C (UPP added at collection site).

#### **NOTES:**

**BD ProbeTec** ET Diluent (CT/GC) should be at room temperature before use.

Urine must be in contact with the UPP for at least 2h before processing.

Aliquot the needed quantity of **BD ProbeTec** Diluent (CT/GC) into a clean container. To estimate the quantity needed, multiply the number of samples by 2 and add an additional 1-2 mL for pipetting ease. **To avoid contamination of the Diluent-Do Not Pour Leftover Diluent back into the Bottle.**

1. Label an empty **BD ProbeTec** ET Sample Tube for each urine to be processed.
2. Swirl the container to mix the urine and open carefully.

**NOTE:** Open carefully to avoid spills or droplets which may cause contamination of work area.

3. Pipette 4.0 mL of urine into the appropriately labeled tube and tightly recap the tube.

4. Repeat steps 2-3 for additional neat urine samples. Use a new pipette or pipette tip for each sample.
5. Centrifuge the tubes at 2000 x g for 30 min.
6. At the end of centrifugation, carefully remove the tubes from the centrifuge.
7. Uncap the first tube and carefully decant the supernatant. End the decanting motion with a gentle "flick" of the wrist to remove residual fluid from the tube.

**NOTE:** This is a critical step - excess residual specimen may cause inhibition. Tubes should be individually blotted on a separate sheet of absorbent paper to enhance removal of residual urine.

8. Place the cap loosely on the tube.
9. Repeat steps 7-8 for each centrifuged urine specimen.
10. Pipette 2.0 mL of Diluent into each tube. Use a new pipette or pipette tip for each tube.
11. Tightly recap the sample tubes and vortex 5 s to completely resuspend the sediment in the Diluent.
12. Samples are ready to be lysed.

**NOTE:** Specimens processed, but not yet lysed, may be stored at room temperature for up to 6 h or overnight at 2-8°C.

#### **F. Quality Control Preparation:**

**NOTE:** The **BD ProbeTec** ET (CT/GC) Controls and Diluent should be at room temperature prior to use.

1. For each run (plate) to be tested, prepare one CT/GC Negative Control Tube and one CT/GC Positive Control Tube. If a plate contains more than one Reagent Pack lot number, controls must be tested with each lot.
2. Remove the cap from the CT/GC Negative Control Tube. Using a new pipette tip or pipette, add 2.0 mL of Diluent.
3. Recap the tube and vortex for 5 s.
4. Remove the cap from the CT/GC Positive Control Tube. Using a new pipette tip or pipette, add 2.0 mL of Diluent.
5. Recap the tube and vortex for 5 s.
6. Controls are ready to be lysed.

#### **G. Lysing the Samples and Controls**

1. After placing processed Controls and samples into the Lysing Rack, insert the Lysing Rack into the Lysing Heater.
2. Heat the samples for 30 min.
3. After 30 min, remove the Lysing Rack from the Lysing Heater and allow to cool at room temperature for at least 15 min.

**NOTE:** After lysing samples:

- a. They may be stored at 18-30°C for up to 6 h and may be tested without re-lysing.
- b. They may be stored up to 5 days at 2-8°C. Samples must be vortexed and re-lysed prior to testing.
- c. They may be stored up to 98 days at -20°C or colder. Samples must be thawed at room temperature, vortexed and re-lysed prior to testing. Lysed samples may be frozen and thawed twice.

### H.1 Testing Procedure for the CT/GC Reagent Pack

**NOTE:** The Priming and Amplification Microwells should be at room temperature prior to use.

1. For specimens collected with the **BD ProbeTec ET CT/GC Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit** or the **BD ProbeTec ET CT/GC Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit**, remove and discard the caps from the lysed and cooled samples and controls.
2. For swabs collected with the **BD ProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Endocervical Specimens** or the **BD ProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Male Urethral Specimens (WET TRANSPORT)**, do the following:
  - a. Uncap the tube and gently press the swab against the side of the tube to remove excess fluid.
  - b. Pull the cap/swab out of the tube. Do not press against the wall of tube to avoid splattering droplets which may cause cross-contamination.
  - c. Discard the cap/swab.
3. For processed urine specimens, uncap the tube and discard the cap.

**Critical Step: Change gloves before proceeding to avoid contamination.**

4. Using the Plate Layout Report, prepare the priming plate. **The Priming Microwells must be placed in a plate in the following order:** CT (solid green microwells) then GC (solid yellow microwells). Repeat until plate is configured like the Plate Layout Report.
5. Re-seal the Microwell pouches as follows.
  - a. Place pouch on flat surface. Hold the open end flat with one hand.
  - b. While applying pressure, slide finger across outside of seal moving from one edge of pouch to other.
  - c. Inspect to ensure pouch is sealed.
6. Select **Program 2** on the **BD ProbeTec ET Pipettor**.
7. Pick up pipette tips. Expand the pipettor by pulling the spacing knob all the way out.

**NOTE:** Make sure tips are fitted securely on the pipettor to prevent leakage.

8. Aspirate 400 µL from the 1st column of samples.
9. Gently collapse the pipettor, touch pipette tips to sides of wells and dispense 150 µL into each of the 2 corresponding columns of Priming Microwells (1 A-H; 2 A-H).

**NOTE:** Do not collapse pipettor over samples or microwells as this may cause contamination. Abrupt movements may cause droplets or aerosols.

**NOTE:** It is important to dispense liquids against the inside wall of microwells to assure accuracy and precision and to avoid cross-contamination.

10. Discard tips. Depress the pipetting trigger to reset the pipettor.

**NOTE:** Discard tips carefully to avoid droplets or aerosols which may contaminate the work area.

11. Pick up new tips and aspirate 400  $\mu\text{L}$  from the 2nd column of samples.
12. Gently collapse the pipettor, touch pipette tips to sides of wells and dispense 150  $\mu\text{L}$  into each of the 2 corresponding columns of Priming Microwells (3 A-H; 4 A-H).
13. Discard tips.
14. Continue transferring the remaining samples for the run.
15. Cover the Priming Microwell plate with the Priming cover and let plate incubate at room temperature for at least 20 min. (May incubate up to 6 h.)

**NOTE:** Recap the processed samples with new caps to retain the sample tubes.

16. At the end of the priming incubation prepare the amplification plate. Configure the Amplification Microwells in a plate to match the Plate Layout Report (same as the priming plate). Reseal the microwell pouches as described in step #5.
17. Remove the cover from the Priming Microwell plate and place the plate in the Priming Heater. **IMMEDIATELY** place the Amplification Microwell plate in the Warming Heater to pre-warm.
18. Set timer for 10 min.

**NOTE:** This step is time critical.

19. At the end of the 10 min (+/- 1 min) incubation, select **Program 5** on the pipettor.
20. Pick up tips and transfer 100  $\mu\text{L}$  from column 1 of the Priming Microwell plate to column 1 of the Amplification Microwell plate. Allow pipette tips to touch sides of wells and dispense the liquid. After dispensing, allow pipettor to automatically mix the liquid in the wells. Carefully lift the pipettor away from the plate. Avoid touching other wells.
21. Discard tips. Pick up new tips and continue to transfer the reaction mixture from the Priming Microwells to the Amplification Microwells, column by column, using new tips for each column.
22. When the last column has been transferred, remove the backing from an Amplification Sealer (remove one half of the sealer backing if 6 or fewer columns are occupied by microwells; remove the entire backing if 7 or more columns are occupied by microwells). Hold the sealer by the edges and center over the plate. Use the guides on the Warming Heater to assist you in centering the sealer. The sealer will extend over the microwells on both sides of the plate. Press downward on sealer to ensure that all microwells are completely sealed.
23. At the **BD ProbeTec** ET user interface, move the carrier out and open the doors. **IMMEDIATELY** (within 30 s) transfer the **sealed** Amplification Microwell plate to the **BD ProbeTec** ET Instrument and initiate the run. (Refer to the **BD ProbeTec** ET System User's Manual for detailed instructions.)
24. After initiating the run, complete the following portion of the clean-up procedure:

- a. Seal the Priming Microwells with an Amplification Sealer and remove plate from the Priming and Warming Heater.

**WARNING:** Temperature is in excess of 70°C. Use the heat resistant glove to remove the plate.

- b. Allow the plate to cool on the bench for 5 min.
  - c. Remove the sealed Priming Microwells from the plate by holding the top and bottom of the sealer and lifting the wells straight up as a unit. Place the Microwells into a Disposal Bag and seal.
  - d. Clean the metal plate:  
Rinse the plate with ELIMINase, DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox solution.  
Rinse the plate with water.  
Wrap plate in a clean towel and allow to completely dry prior to re-use.
25. When the run is complete, a printout of the test results will be generated.
  26. Move the plate carrier out of the stage, open the door, and remove the plate. Close the door and return the plate stage to the inside of the instrument.
  27. Remove the sealed Amplification Microwells from the plate.

**CAUTION: Do Not Remove Sealing Material from Microwells.** The sealed microwells may be easily removed as a unit by holding the sealer at the top and bottom and lifting straight up and out of the plate. Place the sealed microwells into the Disposal Bag. Seal the bag.

28. Clean the metal plate:
  - a. Rinse the plate with ELIMINase, DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox solution.
  - b. Rinse the plate with water.
  - c. Wrap plate in a clean towel and allow to completely dry prior to re-use.
29. After the last run of the day, perform the following clean-up procedures:
  - a. Saturate paper towels or gauze with ELIMINase, DNA AWAY or the 1% (v/v) sodium hypochlorite with Alconox solution and apply to countertops and the exterior surfaces of the Lysing Heater, Priming and Warming Heater, and the **BD ProbeTec** ET Instrument. Allow the solution to remain on surfaces for 2-3 min. Saturate paper towels or gauze pads with water and remove cleaning solution. Change towels or gauze frequently when applying cleaning solution and when rinsing with water. Dampen paper towels or gauze pads with ELIMINase, DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox and wipe the Pipettor handle (**ONLY THE HANDLE**). After 2-3 min wipe the handle with paper towels or gauze pads dampened with water.
  - b. Immerse the Lysing Rack, Lysing Rack base, Lysing Rack cover and plates in ELIMINase, DNA AWAY or 1% sodium hypochlorite with Alconox for 1-2 min. Rinse thoroughly with water and allow to air dry.
  - c. Recharge the Pipettor.
  - d. Dispose of sealed Disposal Bag and biohazard bag according to established procedures for disposal of contaminated biological waste material.

## H.2. Testing Procedure for the CT/GC/AC Reagent Pack

**NOTE:** The Priming and Amplification Microwells should be at room temperature prior to use.

1. For specimens collected with the **BD ProbeTec ET CT/GC Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit** or the **BD ProbeTec ET CT/GC Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit**, remove and discard the caps from the lysed and cooled samples and controls.
2. For swabs collected with the **BD ProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Endocervical Specimens** or the **BD ProbeTec ET CT/GC Amplified DNA Assay Collection Kit for Male Urethral Specimens (WET TRANSPORT)**, do the following:
  - a. Uncap the tube and gently press the swab against the side of the tube to remove excess fluid.
  - b. Pull the cap/swab out of the tube. Do not press against the wall of tube to avoid splattering droplets which may cause cross-contamination.
  - c. Discard the cap/swab.
3. For Processed urine specimens, uncap the tube and discard the cap.

**Critical Step: Change gloves before proceeding to avoid contamination.**

4. Using the Plate Layout Report, prepare the priming plate. The Priming Microwells must be placed in a plate in the following order: CT (solid green microwells), GC (solid yellow microwells), and AC (solid black microwells). Repeat until plate is configured like the Plate Layout Report.
5. Re-seal the Microwell pouches as follows.
  - a. Place pouch on flat surface. Hold the open end flat with one hand.
  - b. While applying pressure, slide finger across outside of seal moving from one edge of pouch to other.
  - c. Inspect to ensure pouch is sealed.
6. Select **Program 3** on the **BD ProbeTec ET Pipettor**.
7. Pick up pipette tips. Expand the pipettor by pulling the spacing knob all the way out.

**NOTE:** Make sure tips are fitted securely on the pipettor to prevent leakage.

8. Aspirate 600  $\mu$ L from 1st column of samples.
9. Gently collapse the pipettor, touch pipette tips to sides of wells and dispense 150  $\mu$ L into each of the 3 corresponding columns of Priming Microwells (1 A-H; 2 A-H; 3 A-H).

**NOTE:** Do not collapse pipettor over samples or microwells as this may cause contamination. Abrupt movements may cause droplets or aerosols.

**NOTE:** It is important to dispense liquids against the inside wall of microwells to assure accuracy and precision and to avoid cross-contamination.

10. Discard tips. Depress the pipetting trigger to reset the pipettor.

**NOTE:** Discard tips carefully to avoid droplets or aerosols which may contaminate the work area.

11. Pick up new tips and aspirate 600  $\mu$ L from the 2nd column of samples.

12. Gently collapse the pipettor, touch pipette tips to sides of wells and dispense 150  $\mu$ L into each of the 3 corresponding columns of Priming Microwells (4 A-H; 5 A-H; 6 A-H).
13. Discard tips.
14. Continue transferring the remaining samples for the run.
15. Cover the Priming Microwell plate with the Priming cover and let plate incubate at room temperature for at least 20 min. (May incubate up to 6 h.)

**NOTE:** Recap the processed samples with new caps to retain the sample tubes.

16. At the end of the priming incubation prepare the amplification plate. Configure the Amplification Microwells in a plate to match the Plate Layout Report (same as the priming plate). Reseal the microwell pouches as described in step #5.
17. Remove the cover from the Priming Microwell plate and place the plate in the Priming Heater. **IMMEDIATELY** place the Amplification Microwell plate in the Warming Heater to pre-warm.
18. Set timer for 10 min.

**NOTE:** This step is time critical.

19. At the end of the 10 min (+/- 1 min) incubation, select **Program 5** on the pipettor.
20. Pick up tips and transfer 100  $\mu$ L from column 1 of the Priming Microwell plate to column 1 of the Amplification Microwell plate. Allow pipette tips to touch sides of wells and dispense the liquid. After dispensing, allow pipettor to automatically mix the liquid in the wells. Carefully lift the pipettor away from the plate. Avoid touching other wells.
21. Discard tips. Pick up new tips and continue to transfer the reaction mixture from the Priming Microwells to the Amplification Microwells, column by column, using new tips for each column.
22. When the last column has been transferred, remove the backing from an Amplification sealer (remove one half of the sealer backing if 6 or fewer columns are occupied by microwells; remove the entire backing if 7 or more columns are occupied by microwells). Hold the sealer by the edges and center over the plate. Use the guides on the Warming Heater to assist you in centering the sealer. The sealer will extend over the microwells on both sides of the plate. Press downward on sealer to ensure that all microwells are completely sealed.
23. At the **BD ProbeTec** ET user interface, move the carrier out and open the doors. **IMMEDIATELY** (within 30 s) transfer the **sealed** Amplification Microwell plate to the **BD ProbeTec** ET Instrument and initiate the run. (Refer to the **BD ProbeTec** ET System User's Manual for detailed instructions.)
24. After initiating the run, complete the following portion of the clean-up procedure:
  - a. Seal the Priming Microwells with an Amplification Sealer and remove plate from the Priming and Warming Heater.

**WARNING:** Temperature is in excess of 70°C. Use the heat resistant glove to remove the plate.

- b. Allow the plate to cool on the bench for 5 min.

- c. Remove the sealed Priming Microwells from the plate by holding the outer edges of the sealer and lifting the wells straight up as a unit. Place the Microwells into a Disposal Bag and seal.
  - d. Clean the metal plate:  
Rinse the plate with ELIMINase, DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox solution.  
Rinse the plate with water.  
Wrap plate in a clean towel and allow to completely dry prior to re-use.
25. When the run is complete, a printout of the test results will be generated.
  26. Move the plate carrier out of the stage, open the door, and remove the plate. Close the door and return the plate stage to the inside of the instrument.
  27. Remove the sealed Amplification Microwells from the plate.

**CAUTION: Do Not Remove Sealing Material from Microwells.** The sealed microwells may be easily removed as a unit by holding the top and bottom of the sealer and lifting straight up and out of the plate. Place the sealed microwells into the Disposal Bag. Seal the bag.

28. Clean the metal plate:  
Rinse the plate with ELIMINase, DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox solution.  
Rinse the plate with water.  
Wrap plate in a clean towel and allow to completely dry prior to re-use.
29. After the last run of the day, perform the following clean-up procedures:
  - a. Saturate paper towels or gauze with ELIMINase, DNA AWAY or the 1% (v/v) sodium hypochlorite with Alconox solution and apply to countertops and the exterior surfaces of the Lysing Heater, Priming and Warming Heater, and the **BD ProbeTec** ET Instrument. Allow the solution to remain on surfaces for 2-3 min. Saturate paper towels or gauze pads with water and remove cleaning solution. Change towels or gauze frequently when applying cleaning solution and when rinsing with water. Dampen paper towels or gauze pads with ELIMINase, DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox and wipe the Pipettor handle (**ONLY THE HANDLE**). After 2-3 min wipe the handle with paper towels or gauze pads dampened with water.
  - b. Immerse the Lysing Rack, Lysing Rack base, Lysing Rack cover and plates in ELIMINase, DNA AWAY or 1% (v/v) sodium hypochlorite with Alconox for 1-2 min. Rinse thoroughly with water and allow to air dry.
  - c. Recharge the Pipettor.
  - d. Dispose of sealed Disposal Bag and biohazard bag according to established procedures for disposal of contaminated biological waste material.

## VIII. QUALITY CONTROL

The **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* positive and negative control set is provided separately. One positive and one negative control must be included in each assay run and for each new reagent kit lot number. Controls may be randomly positioned. The CT/GC

positive control will monitor for substantial reagent failure only. The CT/GC negative control monitors for reagent and/or environmental contamination.

The positive control has both cloned CT and GC target regions that are not necessarily representative of organism target DNA detected by the assay nor do they represent specimen matrices (urine and epithelial cell suspensions) indicated for use with the **BD ProbeTec ET System**. These controls may be used for internal quality control or users may develop their own internal quality control material, as described by NCCLS C24-A2.<sup>7</sup> Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Refer to NCCLS C24-A2 for additional guidance on appropriate internal quality control testing practices. The positive control contains 750 copies per reaction of pCT16 linearized plasmid and 250 copies per reaction of pGC10 linearized plasmid. Both organisms have multiple copies of the target. The **BD ProbeTec ET** amplification reaction volume is 100 µL of rehydrated control.

Because the CT/GC positive control is used for both CT and GC testing, correct positioning of the microwell strips is important for proper result reporting. Refer to Section H of the "Test Procedure" for correct microwell strip positioning.

The CT/GC positive and CT/GC negative control must test as positive and negative, respectively, in order to report patient results. If controls do not perform as expected, the assay run is considered invalid and patient results will not be reported by the instrument. If the QC does not meet the expected results, repeat the entire run using a new set of controls, new microwells, and the processed specimens. If the repeat QC does not provide the expected results, contact Technical Services. (See "Interpretation of Results.")

Refer to Section F of the "Test Procedure" for directions on preparing the controls. Once the controls have been prepared, continue with testing as described in Section G of the "Test Procedure."

A separate Amplification Control (AC) is an option for inhibition testing and is available in the CT/GC/AC Reagent Pack. When the CT/GC/AC Reagent Pack is used, the AC must be included for each patient sample and control. The Amplification Control microwells contain  $\geq 1000$  copies per reaction of pGC10 linearized plasmid that should be amplified in the sample matrix. The amplification control is designed to identify samples that may contain amplification inhibitors that could prevent detection of CT or GC DNA if present. (See "Interpretation of Results.")

### **Interpretation of Control Results:**

#### **Control Interpretation without the AC:**

	<b>CT or GC MOTA Score</b>	<b>Result</b>
<b>CT/GC Positive Control</b>	<b>MOTA <math>\geq 2000</math></b>	<b>Acceptable</b>
<b>CT/GC Negative Control</b>	<b>MOTA <math>&lt; 2000</math></b>	<b>Acceptable</b>

### Control Interpretation with the AC:

	CT or GC MOTA Score	AC MOTA Score*	Result
CT/GC Positive Control	MOTA $\geq$ 2000	MOTA $\geq$ 1000	Acceptable
CT/GC Negative Control	MOTA $<$ 2000	MOTA $\geq$ 1000	Acceptable

**\*If the AC fails (MOTA  $<$  1000), the control fails.**

### Specimen Processing Controls:

Specimen processing controls may be tested in accordance with the requirements of appropriate accrediting organizations. A positive control should test the entire assay system. For this purpose, known positive specimens can serve as controls by being processed and tested in conjunction with unknown specimens. Specimens used as processing controls must be stored, processed, and tested according to the package insert. As an alternative to using positive specimens, specimen processing controls simulating urine processing can be prepared as described below.

#### ***Chlamydia trachomatis*:**

If a known positive specimen is not available, another approach is to assay a stock culture of *C. trachomatis* LGV2 (ATCC™# VR-902B) prepared as described below:

1. Thaw a vial of *C. trachomatis* LGV2 cells received from ATCC.
2. Prepare 10-fold serial dilutions to a  $10^5$  dilution (at least 5 mL final volume) in phosphate buffered saline (PBS).
3. Place 4 mL of  $10^5$  dilution in a **BD ProbeTec** ET sample tube.
4. Process as a urine sample starting at Section E, step 5 of the “Test Procedure.”
5. After processing, lyse sample as described in Section G of the “Test Procedure.”
6. Continue testing as described in Section H of the “Test Procedure.”

#### ***Neisseria gonorrhoeae*:**

If a known positive specimen is not available, another approach is to assay a stock culture of *N. gonorrhoeae* (available from the ATCC, strain # 19424) prepared as described below:

1. Thaw a vial of *N. gonorrhoeae* stock culture, received from ATCC and immediately inoculate a chocolate agar plate.
2. Incubate at 37°C in 3-5% CO<sub>2</sub> for 24-48 h.
3. Resuspend colonies from the chocolate agar plate with phosphate buffered saline (PBS).
4. Dilute cells in PBS to a 1.0 McFarland turbidity standard (approximately  $3 \times 10^8$  cells/mL).
5. Prepare 10-fold serial dilutions to a  $10^5$  dilution of the McFarland (at least 5 mL final volume) in PBS.
6. Place 4 mL of the  $10^5$  dilution in a **BD ProbeTec** ET sample tube.
7. Process as a urine sample starting at Section E, step 5 of the “Test Procedure.”
8. After processing, lyse sample as described in Section G of the “Test Procedure.”
9. Continue testing as described in Section H of the “Test Procedure.”

## Monitoring for the Presence of DNA Contamination

At least monthly, the following test procedure should be performed to monitor the work area and equipment surfaces for the presence of DNA contamination. Environmental monitoring is essential to detect contamination prior to the development of a problem.

1. For each area to be tested, use a clean collection swab from either of the **BD ProbeTec ET** Endocervical specimen collection and transport systems and a CT/GC Diluent tube. [Alternatively, a sample tube containing 2 mL of Diluent (CT/GC), may be used.]
2. Dip the swab into the CT/GC Diluent and wipe the first area using a broad sweeping motion.

**NOTE:** Recommended areas to test include: surface of the Lysing Heater, Lysing Rack, Priming and Warming Heater, black microwell trays, pipettor handle, instrument touch keys, instrument keyboard, instrument door release (teal key), centrifuge drum, and work bench(es) including sample processing areas.

3. Express the swab in the CT/GC Diluent tube. Recap the tube and vortex for 5 s.
4. Repeat for each desired area.
5. After all swabs have been collected, expressed in Diluent and vortexed, the tubes are ready to be lysed (Section G) and assayed (Section H) according to the “Test Procedure.”

If an area gives a positive result, clean the area with fresh ELIMINase, DNA AWAY, or 1% (v/v) sodium hypochlorite with Alconox. Make sure the entire area is wetted with the solution and allowed to remain on the surface for at least two minutes or until dry. If necessary, remove excess cleaning solution with a clean towel. Wipe the area with a clean towel saturated with water and allow the surface to dry. Retest the area. Repeat until negative results are obtained. If the contamination does not resolve, contact Technical Services for additional information.

## IX. INTERPRETATION OF TEST RESULTS

The **BD ProbeTec ET** *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assay uses fluorescent energy transfer as the detection method to test for the presence of *C. trachomatis* and *N. gonorrhoeae* in clinical specimens. All calculations are performed automatically by the instrument software.

The presence or absence of *C. trachomatis* and *N. gonorrhoeae* is determined by relating the **BD ProbeTec ET** MOTA scores for the specimen to pre-determined cutoff values. The 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. If assay controls are not as expected, patient results should not be reported. See QC section for expected control values. Reported results are determined as follows.

**For the CT/GC Reagent Pack:**

***C. trachomatis* and *N. gonorrhoeae* Result Interpretation without AC**

CT or GC MOTA Score	Result	Report	Interpretation
≥ 10,000	Positive <sup>1</sup>	<i>C. trachomatis</i> plasmid DNA and/or <i>N. gonorrhoeae</i> DNA detected by SDA	Positive for <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> . <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.
2,000-9,999	Low Positive <sup>1,2,3</sup>	<i>C. trachomatis</i> plasmid DNA and/or <i>N. gonorrhoeae</i> DNA detected by SDA	<i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> likely. Supplemental testing may be useful for verifying presence of <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> . <sup>2</sup>
< 2,000	Negative	<i>C. trachomatis</i> plasmid DNA and/or <i>N. gonorrhoeae</i> DNA not detected by SDA	Presumed negative for <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> . A negative result does not preclude <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> infection because results are dependent on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.

<sup>1</sup> According to CDC guidelines, “consideration should be given to routine additional testing for persons with positive *C. trachomatis* or *N. gonorrhoeae* screening tests when risk-factor information or actual surveys indicate that the prevalence is low, resulting in a lower PPV (e.g., <90%).” Regardless of the screening method used (e.g. NAAT, DFA, EIA, Nucleic Acid Probe), “all positive screening tests should be considered presumptive evidence of infection.”<sup>8</sup> Refer to CDC guidelines for details on additional testing and patient management after a positive screening test.

<sup>2</sup> Refer to cutoff description below and Figures 2 and 3 in “Performance Characteristics” in the Package Insert for additional information on the distribution of CT and GC MOTA values by specimen type observed in the clinical trials.

<sup>3</sup> The magnitude of the MOTA score is not indicative of the level of organism in the specimen.

**For the CT/GC/AC Reagent Pack:**

***C. trachomatis* and *N. gonorrhoeae* Result Interpretation with AC**

CT or GC MOTA Score	AC MOTA Score	Result	Report	Interpretation
≥ 10,000	Any	Positive <sup>1</sup>	<i>C. trachomatis</i> plasmid DNA and/or <i>N. gonorrhoeae</i> DNA detected by SDA	Positive for <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> . <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> organism viability and/or infectivity cannot be inferred since target DNA may persist in the absence of viable organisms.
2,000 – 9,999	Any	Low Positive <sup>1,2,3</sup>	<i>C. trachomatis</i> plasmid DNA and/or <i>N. gonorrhoeae</i> DNA detected by SDA	<i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> likely. Supplemental testing may be useful for verifying presence of <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> . <sup>2</sup>

CT or GC MOTA Score	AC MOTA Score	Result	Report	Interpretation
< 2,000	≥ 1,000	Negative	<i>C. trachomatis</i> plasmid DNA and/or <i>N. gonorrhoeae</i> DNA not detected by SDA	Presumed negative for <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> . A negative result does not preclude <i>C. trachomatis</i> and/or <i>N. gonorrhoeae</i> infection because results are dependent on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.
< 2000	< 1000	Indeterminate	Amplification control inhibited. • Repeat test. <sup>4</sup>	Repeatedly inhibitory specimen. <i>C. trachomatis</i> or <i>N. gonorrhoeae</i> , if present, would not be detectable using SDA. Submit another specimen for testing.

<sup>1</sup> According to CDC guidelines, “consideration should be given to routine additional testing for persons with positive *C. trachomatis* or *N. gonorrhoeae* screening tests when risk-factor information or actual surveys indicate that the prevalence is low, resulting in a lower PPV (e.g., <90%).” Regardless of the screening method used (e.g. NAAT, DFA, EIA, Nucleic Acid Probe), “all positive screening tests should be considered presumptive evidence of infection.”<sup>8</sup> Refer to CDC guidelines for details on additional testing and patient management after a positive screening test.

<sup>2</sup> Refer to cutoff description below and Figures 2 and 3 in “Performance Characteristics” in the Package Insert for additional information on the distribution of CT and GC MOTA values by specimen type observed in clinical trials.

<sup>3</sup> The magnitude of the MOTA score is not indicative of the level of organism in the specimen.

<sup>4</sup> Repeat **BD ProbeTec** ET test. For urines, repeat from the original specimen. If original specimen is not available, repeat from the processed sample tube. For swabs, repeat from the processed sample tube. If repeat result is either positive or negative, interpret as described above. If results repeat as indeterminate, a new specimen should be requested.

### Determination of CT/GC/AC Cutoff:

The assay and amplification control cutoffs for CT and GC specimen results were determined based on Receiver Operating Characteristic (ROC) curve analysis of MOTA values obtained with patient specimens (male urethral swab, female endocervical swab, male and female urine) tested using both the **BD ProbeTec** ET CT/GC assay and another amplified method during preclinical studies. The cutoffs were confirmed in clinical studies by using the **BD ProbeTec** ET CT/GC assay and culture, Direct Fluorescence Antibody (DFA) (CT only) and another amplified method. These studies show that for the majority of the time, CT and/or GC MOTA values greater than 2,000 will indicate the presence of *C. trachomatis* and/or *N. gonorrhoeae*. CT and/or GC MOTA values less than 2000 correlate with negative *C. trachomatis* and/or *N. gonorrhoeae* culture results the majority of the time. Male urethral swab, female endocervical swab and male urine specimens with CT MOTA values between 2,000 and 4,000 had a decreased likelihood of being true positive compared to results with MOTA values above 4,000. For female urine specimens, CT positive results with MOTA values between 2,000 and 10,000 also had a decreased likelihood of being true positive compared to results with MOTA values above 10,000. GC positive results with MOTA values between 2,000 and 10,000 also had a decreased likelihood of being true positive compared to results with MOTA values above 10,000. Refer to Figures 2 and 3 in the Package Insert\* for the distribution of CT and GC MOTA values by specimen type observed in the clinical study.

Depending on the types of specimens tested, populations sampled, and laboratory practices, supplemental testing for specimens with MOTAs values between 2,000-10,000 may be useful. Refer to CDC guidelines for details on additional testing and patient management after a positive screening test.

*N. cinerea* has been shown to cross-react in the **BD ProbeTec** ET GC assay and other *Neisseria* species may also cause false positive results. In settings with a high prevalence of sexually transmitted disease, positive assay results have a high likelihood of being truly positive. In settings with a low prevalence of sexually transmitted disease, or in any setting in which a patient's clinical signs and symptoms or risk factors are inconsistent with gonococcal or chlamydial urogenital infection, positive results should be carefully assessed and the patient retested by other methods (e.g., culture for GC) if appropriate.

## X. LIMITATIONS OF THE PROCEDURE

1. This method has been tested only with endocervical swabs, male urethral swabs, and male and female urine specimens. Performance with other specimen types has not been assessed.
2. Optimal performance of the test requires adequate specimen collection and handling. Refer to the "Sample Collection and Transport" sections of this procedure.
3. Endocervical specimen adequacy can only be assessed by microscopic visualization of columnar epithelial cells in the specimens.
4. Collection and testing of urine specimens with the **BD ProbeTec** ET *Chlamydia trachomatis* /*Neisseria gonorrhoeae* Amplified DNA Assay is not intended to replace cervical exam and endocervical sampling for diagnosis of urogenital infection. Cervicitis, urethritis, urinary tract infections and vaginal infections may result from other causes or concurrent infections may occur.
5. The **BD ProbeTec** ET *Chlamydia trachomatis*/*Neisseria gonorrhoeae* Amplified DNA Assay for male and female urine testing should be performed on first catch random urine specimens (defined as the first 15-20 mL of the urine stream when using the UPP). During the clinical evaluation, testing urine volumes up to 60 mL was included in the performance estimates. Dilutional effects of larger urine volumes may result in reduced assay sensitivity. The effects of other variables such as mid-stream collection have not been determined. Performance has not been established when the UPP is added to the collection cup prior to collection.
6. The effects of other potential variables such as vaginal discharge, use of tampons, douching, and specimen collection variables have not been determined.
7. A negative test result does not exclude the possibility of infection because test results may be affected by improper specimen collection, technical error, specimen mix-up, concurrent antibiotic therapy, or the number of organisms in the specimen which may be below the sensitivity of the test.
8. As with many diagnostic tests, results from the **BD ProbeTec** ET *Chlamydia trachomatis*/*Neisseria gonorrhoeae* Amplified DNA Assay should be interpreted in conjunction with other laboratory and clinical data available to the physician.
9. The **BD ProbeTec** ET *Chlamydia trachomatis*/*Neisseria gonorrhoeae* Amplified DNA Assay does not detect plasmid-free variants of *C. trachomatis*.

10. The **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* Amplified DNA Assay should not be used for the evaluation of suspected sexual abuse or for other medico-legal indications. Additional testing is recommended in any circumstance when false positive or false negative results could lead to adverse medical, social, or psychological consequences.
11. The **BD ProbeTec** ET system cannot be used to assess therapeutic success or failure since nucleic acids from *Chlamydia trachomatis* and *Neisseria gonorrhoeae* may persist following antimicrobial therapy.
12. The **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* Amplified DNA Assay provides qualitative results. No correlation can be drawn between the magnitude of MOTA score and the number of cells in an infected sample.
13. The predictive value of an assay depends on the prevalence of the disease in any particular population. See Tables 1 and 2 in the Package Insert\* for hypothetical predictive values when testing varied populations.
14. Because the CT/GC positive control is used for both CT and GC testing, correct positioning of the microwell strips is important for final results reporting. Refer to Section H of the “Test Procedure” for correct microwell strip positioning.
15. Use of the **BD ProbeTec** ET *Chlamydia trachomatis/Neisseria gonorrhoeae* Amplified DNA Assay is limited to personnel who have been trained in the assay procedure and the **BD ProbeTec** ET system.
16. In laboratory studies, blood > 5% (v/v) was shown to cause indeterminate (inhibitory) results in both urine and swab specimens (with AC) and false negative results in urine specimens (with and without AC). Blood > 5% (v/v) may cause false negative results in swab specimens (with and without AC). Specimens with moderate to gross blood may interfere with **BD ProbeTec** ET CT/GC Assay results. Refer to “Performance Characteristics” for specific performance of female swab specimens with observed blood.
17. The presence of highly pigmented substances in urine, such as bilirubin (10 mg/mL) and Phenazopyridine (10 mg/mL), may cause indeterminate or false negative results.
18. Leukocytes in excess of 250,000 cells/mL (swab specimens) may cause indeterminate or false negative results.
19. The presence of serum, feminine deodorant sprays, or talcum powder may cause false negative results (urine specimens).
20. The **BD ProbeTec** ET *C. trachomatis/N. gonorrhoeae* Amplified DNA Assays may cross-react with *N. cinerea* and *N. lactamica*. Refer to “Performance Characteristics” (Analytical Specificity) for further information.
21. The reproducibility of the **BD ProbeTec** ET CT/GC Assay was established using seeded swab specimens and seeded buffer to simulate urine specimens. These specimens were inoculated with both *C. trachomatis* and *N. gonorrhoeae*. Reproducibility when testing urine samples and samples with *C. trachomatis* only and *N. gonorrhoeae* only has not been determined.
22. Performance characteristics for detecting *N. gonorrhoeae* in males are based on testing patients with infection rates of 0-43%; the male populations sampled were primarily from STD clinics where the prevalence of GC is higher than in other clinical settings. In males, 16 gonococcal infections were identified in the low prevalence setting (0-8% prevalence). Likewise, the majority of females in the study with GC infections were from STD clinics. In females, only six gonococcal infections were identified in the low prevalence setting (1.2% prevalence). **Positive**

**results in low prevalence populations should be interpreted carefully in conjunction with clinical signs and symptoms, patient risk profile, and other findings with the understanding that the likelihood of a false positive may be higher than a true positive.**

23. Testing urine specimens from female patients as the sole test for identifying chlamydial or gonococcal infections may miss infected individuals (17/100 or 17% of females with CT-positive cultures and 11/80 or 13.8% of females with GC-positive cultures had negative results when urine only was tested) with the **BD ProbeTec** ET CT/GC Assay.
24. Because the AC uses GC target, the efficacy of the AC for detecting inhibition is reduced in GC infected samples. Refer to “Performance Characteristics” for results with co-infected patients.
25. Performance has not been established for UPT fill volumes other than volumes falling within the black lines on the fill window (approximately 2.5 mL to 3.45 mL).

**\*BD ProbeTec™ ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays Package Insert**

## **XI. EXPECTED RESULTS**

### **A. Prevalence**

The prevalence of positive *C. trachomatis* or *N. gonorrhoeae* specimens in patient populations depends on: clinic type, age, risk factors, gender, and test method. The prevalence observed with the **BD ProbeTec** ET CT/GC amplified DNA assay during a multi-center clinical trial ranged from 4.5 to 28.6% for CT (Table 6 in the Package Insert\*) and from 0 to 42.9% for GC (Table 12 in the Package Insert\*). Co-infections ranged from 0% to 5.4%.

### **B. Positive and Negative Predictive Values**

Hypothetical positive and negative predictive values (PPV & NPV) for the **BD ProbeTec** ET CT/GC amplified DNA assays are shown in Tables 1 and 2 in the Package Insert.\* These calculations are based on hypothetical prevalence and overall CT sensitivity and specificity (as compared to the patient infected status) of 90.7 and 96.6%, respectively, and overall GC sensitivity and specificity of 96.0 and 98.8%, respectively. In addition, PPV and NPV based on actual prevalence, sensitivity and specificity are shown in Table 6 and Table 12 in the Package Insert\*.

### **C. MOTA Score Frequency Distribution**

A total of 5119 specimens collected from clinics in nine different geographic locations were assayed with the **BD ProbeTec** ET system for *C. trachomatis* and/or *N. gonorrhoeae* in seven clinical laboratories. A frequency distribution of the initial MOTA scores for AC is shown in Figure 1 in the Package Insert by specimen type\*.

A total of 4108 **BD ProbeTec** ET *C. trachomatis* results were evaluated at seven clinical sites. A frequency distribution of the initial MOTA scores for CT is shown in Figure 2 in the Package Insert\*. The distribution of uniquely **BD ProbeTec** ET false positive (test which was positive in the **BD ProbeTec** ET but not positive by cell culture, DFA or AMP1 in either specimen type) and false negative results are shown in Figure 2 in the Package Insert\*.

A total of 5093 **BD ProbeTec** ET *N. gonorrhoeae* results were evaluated at nine clinical sites. A frequency distribution of the initial MOTA scores for GC is shown in Figure 3 in the Package Insert\*. The distribution of uniquely **BD ProbeTec** ET false positive (test which was positive in the

**BD ProbeTec** ET but not positive by culture or AMP1 in either specimen type) and false negative results are shown in Figure 3 in the Package Insert\*.

\***BD ProbeTec**<sup>TM</sup> ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays Package Insert

#### D. Controls

During the clinical evaluation, CT/GC positive control failures were observed in 22 of 518 CT and GC assay runs. For the negative CT/GC control, failures were observed in 19 of 518 CT assay runs and in 12 of 518 GC assay runs. Eight of these observed CT and GC control failures occurred as a result of the operator switching the positive and negative control.

The CT/GC positive and negative control MOTA scores observed in the clinical trials are shown in the following table.

Control	Range	5 <sup>th</sup> Percentile	MOTA Mean Score	Median	95 <sup>th</sup> Percentile
CT Negative	0-499	0	113	109.5	262
CT Positive	2055-67281	8222	26816	24681	52725
GC Negative	0-800	0	90	71.5	245
GC Positive	2013-54240	7404	22452	21228	41405

## XII. PERFORMANCE CHARACTERISTICS:

### Clinical Performance

Performance characteristics for the **BD ProbeTec** ET *C. trachomatis* and *N. gonorrhoeae* (CT/GC) Amplified DNA Assay were established in a multi-center study at seven geographically diverse clinical sites. Each site was required to pass a proficiency panel prior to enrolling patients in the study. The study included 4131 specimens collected from 2109 patients attending sexually transmitted disease (STD) clinics, OB/GYN Clinics, Family Planning Clinics, Adolescent Clinics, and Emergency Rooms. A total of 22 CT results were excluded from the data analysis due to cell culture contamination. One additional specimen was excluded due to a missing DFA result. A total of 26 GC results were excluded from the data analysis. Of these 26, 15 were excluded due to culture contamination and 11 were excluded due to failure to collect a swab for culture. Therefore, a total of 4108 CT and 4105 GC results from 2109 patients were used in the final data analysis. Paired specimens (swab and urine) were collected from 2020 of the 2109 patients. The majority of these were from patients in STD and family planning clinics. Four endocervical swabs and one urine specimen were collected from female patients. The swabs were tested by cell culture for CT, culture for GC, the **BD ProbeTec** ET assay, and a commercially available amplification method (AMP1). The endocervical swab collection order was rotated throughout the study to minimize effects of collection order. For males, two urethral swabs and one urine specimen were collected. The first swab was used for GC culture and then the **BD ProbeTec** ET assay. The second swab was used for CT cell culture. The UPP was added to the urine at the collection site prior to transporting to the laboratory.

*C. trachomatis* was detected by cell culture of endocervical and male urethral swabs. Positivity was based on detecting at least one inclusion-forming unit (IFU) in either first or second passage. Female and male urine **BD ProbeTec** ET results were compared to culture results of endocervical

and male urethral swab specimens, respectively. In addition, a commercially available amplification assay (AMP1) was performed on all endocervical swabs and urine specimens. If cell culture was negative but either amplification assay was positive, a DFA test was performed from the cell culture transport medium. For male urethral swab specimens, testing included cell culture but not the AMP1 method. If the cell culture was negative, but **BD ProbeTec** ET (swab or urine) and/or the AMP1 CT urine test were positive, DFA was performed from the cell culture transport medium. A different commercially available amplification assay (AMP2) was performed from culture transport medium for those male patients who had a positive urine AMP1 test and the corresponding swabs were culture negative.

*N. gonorrhoeae* was detected by recovery of gram-negative, oxidase-positive colonies on agar. Culture identification was confirmed by two methods, one biochemical and one either immunological or fluorometric. **BD ProbeTec** ET assay results were compared to culture and a commercially available amplification assay (AMP1). All GC cultures were incubated between 48-72 hours prior to reporting a final result.

Performance characteristics for CT and GC were calculated both with and without the amplification control (AC). All data are presented without the amplification control. Assay interpretation differences resulting from use of the amplification control are footnoted at the bottom of each table\*. For true CT and/or GC positives, the target level is generally high enough to overcome the inhibitory effects of the specimen matrix. These specimens are interpreted as positive by the instrument algorithm even if the AC is negative (MOTA < 1000). All initially indeterminate results were repeated. Performance was calculated based on the results of repeat testing. Specimens were classified as positive, negative or indeterminate. Repeatedly inhibitory specimens were considered uninterpretable and excluded from sensitivity and specificity calculations. To calculate performance without the AC, indeterminate results (results with negative AC) were interpreted as negative for CT and/or GC. The numbers of initial and final indeterminate results by patient infected status are shown in Table 3 (CT)\* and Table 4 (GC) in the Package Insert\*. The numbers of initial and final indeterminate results by specimen type are shown in Table 5 (CT) and Table 11 (GC) in the Package Insert\*.

In the previous multi-center study, the seven sites collected 183 and 184 asymptomatic male GC swab and urine specimens, respectively. To supplement this data, a similar study was conducted at three clinical sites; one of which participated in the original evaluation. The study included specimens collected from two STD clinics and a teaching hospital. The male patients attending the STD clinics may have had a prior STD infection, an infected partner, or attending for a routine visit. A total of 560 patients were enrolled in the study, 41 of which were excluded from the data analysis due to noncompliance issues (e.g. patients enrolled prior to proficiency completed, symptomatic patients enrolled, GC culture not performed). From the remaining 519 patients, 1038 paired specimens (swab and urine) were collected. A total of 50 specimens were excluded for various reasons (e.g. urine frozen prior to testing, incomplete proficiency testing, specimens older than six days). Therefore, a total of 988 specimens collected from 519 patients were used in the final data analysis. The swab specimen was used for GC culture and then the **BD ProbeTec** ET assay. The urine specimen was tested using both the **BD ProbeTec** ET assay and a commercially available amplification assay (AMP1). The UPP was added to the urine at the test site. The **BD ProbeTec** ET urine results were compared to the culture results of the male urethral swab specimens. Results were combined with the data collected in the original multicenter study and are

included in the data presented in Figures 1 and 3 and Tables 2, 4, 11, 12, 13, 14, and 16 in the Package Insert\*.

In a prospective clinical agreement study, four geographically diverse clinical sites evaluated the performance of neat urine specimens and urine specimens processed with the UPT for both CT and GC against urine specimens processed with the UPP. These urine specimens were collected from both symptomatic and asymptomatic males and females. A total of 1183 compliant CT urine specimens and 1181 compliant GC urine specimens were collected and divided between neat urine, the UPT and the UPP and were included in the indeterminate analysis. For performance without the AC, a total of 1182 compliant CT neat/UPP and UPT/UPP paired specimens and 1181 compliant GC neat/UPP and UPT/UPP paired specimens were included. Performance with the Amplification Control was calculated for 1171 compliant CT neat/UPP paired specimens and 1169 compliant GC neat/UPP paired specimens. Performance with the AC was calculated for 1164 complaint CT UPT/UPP paired specimens and 1162 compliant GC UPT/UPP paired specimens. The agreement results of neat urine compared to UPP for CT and GC, both with and without the AC, are summarized in Table 23 in the Package Insert\*.

**\*BD ProbeTec™ ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays**  
Package Insert

### ***C. trachomatis***

**BD ProbeTec** ET *C. trachomatis* results were compared to culture and patient infected status. Performance estimates for each specimen type and symptomatic status are shown in Table 5 in the Package Insert\*. A patient was considered infected if (1) the culture was positive, or (2) positive results were obtained for both AMP1 (in either the swab or urine) and DFA, or (3) AMP1 was positive in both swab and urine paired specimens. Data on pregnant females are footnoted at the bottom of Table 5 in the Package Insert\*. Of the 1,419 female swab specimens tested in the clinical evaluations by the **BD ProbeTec** ET CT Assay, 101 (7.1%) were classified as grossly bloody and 242 (17.1%) as moderately bloody. Assay performance with moderately to grossly bloody swabs was not statistically different than assay performance with non-bloody or lightly bloody swabs. Table 6 in the Package Insert\* shows performance estimates for the **BD ProbeTec** ET CT assay as compared to patient infected status for each clinical site differentiated by specimen type.

In the clinical trial, the AMP1 assay was performed on all endocervical swabs and urine specimens (males and females). A comparison of the **BD ProbeTec** ET assay and AMP1 CT assay to culture and DFA (on culture negative, assay positive specimens) is presented in Table 7 in the Package Insert\*. Table 8 in the Package Insert\* shows the percent agreement between **BD ProbeTec** ET CT results and AMP1 results.

A summary of test results on paired specimens is contained in Tables 9 (females) and 10 (males) in the Package Insert\*. Patient infected status is also shown in these tables\*.

### ***N. gonorrhoeae***

**BD ProbeTec** ET *N. gonorrhoeae* results were compared to culture and patient infected status. Performance estimates for each specimen type and symptomatic status are shown in Table 11 in the Package Insert\*. A patient was considered infected if (1) the culture was positive or (2) in females, if AMP1 was positive in both swab and urine (paired specimens). Data on pregnant females are footnoted at the bottom of Table 11 in the Package Insert\*. Of the 1,411 female swab specimens

tested in the clinical evaluations by the **BD ProbeTec** ET GC assay, 102 (7.2%) were classified as grossly bloody and 242 (17.2%) as moderately bloody. Assay performance with moderately to grossly bloody swabs was not statistically different than assay performance with non-bloody or lightly bloody swabs. Table 12 in the package Insert\* shows performance estimates for the **BD ProbeTec** ET GC assay as compared to patient infected status for each clinical site differentiated by specimen type.

In the clinical trial the AMP1 assay was performed on all endocervical swabs and urine specimens (males and females). A comparison of the **BD ProbeTec** ET assay and AMP1 GC assay against culture is presented in Table 13 in the Package Insert\*. Table 14 in the Package Insert\* shows the percent agreement between **BD ProbeTec** ET GC results and AMP1 results.

A summary of test results on paired specimens is contained in Tables 15\* (females) and 16 (males) in the Package Insert\*. Patient infected status is also shown in these tables\*.

### ***C. trachomatis* and *N. gonorrhoeae* co-infection**

In the clinical trial, both **BD ProbeTec** ET CT and GC results were available for 4082 specimens. A summary of **BD ProbeTec** ET performance for detecting both CT and GC in specimens from patients considered co-infected by the patient infected status is presented in Table 17 in the package Insert\*.

**\*BD ProbeTec™ ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays Package Insert**

### **Analytical Studies**

**NOTE:** The **BD ProbeTec™** ET CT/GC amplification reaction volume is 100 µL of processed sample.

#### **Precision**

Precision of the **BD ProbeTec** ET CT/GC Amplified DNA Assays was demonstrated by testing a five-member panel consisting of four dilutions co-inoculated with *C. trachomatis* and *N. gonorrhoeae* in Diluent (CT/GC) and a negative (uninoculated Diluent). The five member panel was made up of samples containing 0-100 *C. trachomatis* Elementary Bodies per reaction (EBs/rxn) and 0-100 *N. gonorrhoeae* cells/rxn. This precision panel was run at two clinical sites and internally. Six replicates of each panel were run twice a day for three days. Because no significant run-to-run or site-to-site variability was observed, the data were combined and presented in Table 18 in the Package Insert\*. No positive or negative CT/GC control failures were observed in the Precision study.

#### **Proficiency / Reproducibility**

Prior to data collection for the clinical trial, each technologist processed and performed two proficiency panels. One panel consisted of seeded swab specimens; the other panel consisted of seeded buffer to simulate testing urine specimens. Each 30-member swab panel contained 12 replicates of a level seeded with both 500 EBs/rxn (CT) and 500 cells/rxn (GC), 12 replicates of a level seeded with both 50 EBs/rxn (CT) and 30 cells/rxn (GC) and six unseeded samples. Each 30 member urine panel contained 12 replicates of a level seeded with both 600 EBs/rxn (CT) and 500 cells/rxn (GC), 12 replicates of a level seeded with both 115 EBs/rxn (CT) and 100 cells/rxn (GC) and six unseeded samples.

Results from this proficiency study were combined across 23 operators and across all sample levels (negative, low level, high-level) to estimate reproducibility. Reproducibility estimates are presented in Table 19 in the Package Insert\* as percent correct versus expected results. No positive or negative CT/GC control failures were observed in the Proficiency/Reproducibility study. At three of the clinical sites designated technologists with various levels of experience ran panels twice in one day to show that multiple runs in the same room do not adversely affect results. No decrease in correct results was seen between first and second runs. Separate chi-square tests were performed to compare the two runs for swab and urine samples. No statistical differences were observed (p-value for swab samples: 0.1769; p-value for urine samples: 0.7691).

### **Specimen Stability Studies**

Transport and storage of specimens for testing were evaluated using the information collected during the clinical studies as well as by conducting internal analytical studies. The majority of the clinical specimens were transported to the laboratory within one day and held refrigerated or at room temperature and tested within four days of collection.

Recommendations to support an additional two days of specimen stability at 2-8°C were based on in-house studies that were conducted by seeding swabs and human urine with approximately 200 CT EBs and 200 GC cells per reaction. Both seeded and unseeded swabs and urine were held at refrigerated conditions and tested on Days 0,1,2,4,5 and 6. Each positive and negative sample was tested in triplicate for a total of 18 positive and nine negative data points on each day. The data demonstrated that both swabs and urines were stable up to Day 6. Recommendations to support an additional two days of swab specimen stability at 15-27°C were based on in-house studies that were conducted as described above. The data demonstrated that the swabs were stable up to Day 6.

In addition, a separate stability study was conducted at two clinical sites to verify room temperature stability with clinical swabs and urine specimens. Five swabs were collected from female patients (one for AMP1 and four for **BD ProbeTec ET**). Urine specimens were collected from both male and female patients. Baseline (Day 0) specimens were processed within 24 h of collection.

Additional samples were held at room temperature and processed on Days 2, 4 and 5. Each timepoint was compared to **BD ProbeTec ET** results on Day 0. **CT Results:** Of the 101 swab specimens, 29 were positive and 57 negative at each time point. The remaining 15 specimens (14.9%) were variable from day to day. Of the 107 urine specimens, 27 were positive and 68 negative at each time point. The remaining 12 urine specimens (11.2%) varied between days. **GC Results:** Of the 101 swab specimens, 28 were positive and 67 negative at each time point. The remaining 7 swab specimens (6.9%) were variable from day to day. Of the 107 urine specimens, 30 were positive and 69 negative at all time points. The remaining 8 specimens (7.5%) varied from day to day.

### **Conclusion:**

Day to day variability for both swabs and urines ranged from 5.6-10.9% for CT and 1.9-5.9% for GC. It is unknown whether specimens stored at 2-8°C would have less variability in day to day testing.

### **Analytical Sensitivity**

The analytical sensitivity (Limit of Detection or LOD) of the **BD ProbeTec ET** *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assay was determined by diluting 15 *C. trachomatis* serovars and 39 *N. gonorrhoeae* strains in Diluent (CT/GC). Quantitated CT cultures were diluted to 0,5,15,35,70 and 200 EBs per reaction for each serovar. Quantitated GC

cultures were diluted to 0,5,10,15 and 25 cells per reaction for each strain. Samples were processed and assayed in triplicate.

The LOD of the *C. trachomatis* serovars ranged from 5-200 EBs per reaction with a median of 35 EBs per reaction. The 15 CT serovars, with the corresponding LOD for each in parentheses (expressed as EBs/reaction) are as follows: A (15), B (35), Ba (35), C (5), D (70), E (35), F (200), G (35), H (15), I (200), J (70), K (200), LGV-1 (35), LGV-2 (15), LGV-3 (35). Quantitation of *C. trachomatis* (CT) based on EBs was found to be more accurate and reproducible than quantitation by inclusion-forming units (IFU). Quantitation of IFU tends to be variable and consistently gives a lower number when compared with direct (DFA) quantitation of EBs. To determine the correlation between quantitation by DFA and IFU titers, all 15 CT serovars were grown in tissue culture, then the EBs were collected and quantitated by both DFA and IFU. The ratio of the EB counts (from DFA) to IFU titers for each serovar was calculated. The mean EB to IFU ratio for the 15 CT serovars (A through LGV-3) was determined to be 167 EBs per IFU. For the STD group (CT serovars D-K), the mean ratio was 317 EBs per IFU. These ratios are representative of the variation found between serovars. With these conversions, the analytical sensitivity of the CT assay would be < 1 IFU.

The LOD of the 39 *N. gonorrhoeae* strains ranged from 5-25 cells per reaction with a median of 10 cells per reaction. These strains included 14 ATCC strains (including six different *N. gonorrhoeae* auxotypes) and 25 clinical isolates obtained from geographically diverse sites.

### **Analytical Specificity**

Table 20 (see page 130 in the Package Insert)\* identifies the bacteria, viruses, and yeasts evaluated using the **BD ProbeTec** ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assays. Bacterial isolates were tested using at least 10<sup>8</sup> Colony Forming Units (CFU)/mL or equivalent copies of genomic DNA except as indicated. Viruses were tested using at least 10<sup>8</sup> Plaque Forming Units (PFU)/mL or equivalent copies of genomic DNA. The tested organisms include those commonly found in the urogenital tract as well as others.

For *Chlamydia trachomatis*, all results were negative as expected.

Three *N. cinerea* strains were tested in the **BD ProbeTec** ET GC assay. Of these, two were repeatedly positive. Sixteen *N. subflava* strains were tested in triplicate. Two strains were positive in one of three replicates. When the two strains were prepared and tested again, all results were negative. Eight *N. lactamica* strains were tested in triplicate. One strain was positive in one of the three replicates. When that strain was prepared and tested again, all results were negative.

### **Interfering Substances**

Potential interfering substances which may be encountered in swab and/or urine specimens were tested with the **BD ProbeTec** ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Amplified DNA Assay. Potential interfering substances were evaluated in the absence of target or with 200 CT EBs per reaction (i.e., 1000 EBs/mL of urine or 4000 EBs per swab) and 200 GC cells per reaction (i.e., 1000 cells/mL of urine or 4000 cells per swab). Results are summarized in Table 21 in the Package Insert\*.

## AVAILABILITY

The following **BD ProbeTec™** ET products are also available:

<b>Cat. No.</b>	<b>Description</b>
220142	<b>BD ProbeTec™</b> ET <i>Chlamydia trachomatis/Neisseria gonorrhoeae</i> (CT/GC) Amplified DNA Assay Collection Kit for Endocervical Specimens, 100 units.
220143	<b>BD ProbeTec™</b> ET <i>Chlamydia trachomatis/Neisseria gonorrhoeae</i> (CT/GC) Amplified DNA Assay Collection Kit for Male Urethral Specimens, 100 units.
440450	<b>BD ProbeTec™</b> ET CT/GC/AC Reagent Pack, 384 tests.
440451	<b>BD ProbeTec™</b> ET CT/GC Control Set, 20 positive and 20 negative.
440452	<b>BD ProbeTec™</b> ET CT/GC Diluent Tubes, 2 mL x 400.
440453	<b>BD ProbeTec™</b> ET Diluent (CT/GC), 4 x 225 mL.
440455	<b>BD ProbeTec™</b> ET Sample Tubes and Caps, 4 x 100.
440456	<b>BD ProbeTec™</b> ET Caps, 4 x 100.
440457	<b>BD ProbeTec™</b> ET Accessories (20 Primer covers, Amplification sealers and Disposal bags, 20 each).
440458	<b>BD ProbeTec™</b> ET Pipette Tips, 6 x 120.
440461	<b>BD ProbeTec™</b> ET <i>Chlamydia trachomatis/Neisseria gonorrhoeae</i> (CT/GC) Amplified DNA Assay Male Urethral Specimen Collection and DRY TRANSPORT Kit, 1x100.
440476	<b>BD ProbeTec™</b> ET <i>Chlamydia trachomatis/Neisseria gonorrhoeae</i> (CT/GC) Amplified DNA Assay Endocervical Specimen Collection and DRY TRANSPORT Kit, 100 each.
440454	<b>BD ProbeTec™</b> ET Urine Processing Kit, 4 x 25.
440928	<b>BD ProbeTec™</b> ET Urine Preservative Transport Kit, 100/box
440474	<b>BD ProbeTec™</b> ET CT/AC Reagent Pack, 384 tests.
440477	<b>BD ProbeTec™</b> ET Instrument, Ex U.S.
440478	<b>BD ProbeTec™</b> ET Instrument, U.S. and Canada.
440479	<b>BD ProbeTec™</b> ET Priming and Warming Heater, 220V.
440480	<b>BD ProbeTec™</b> ET Priming and Warming Heater, 120V.
440482	<b>BD ProbeTec™</b> ET Lysing Heater, 220V.
440483	<b>BD ProbeTec™</b> ET Lysing Heater, 120V.
440487	<b>BD ProbeTec™</b> ET Pipettor.
440502	<b>BD ProbeTec™</b> ET Lysing Rack.
440704	<b>BD ProbeTec™</b> ET CT Reagent Pack, 384 tests.
440705	<b>BD ProbeTec™</b> ET CT/GC Reagent Pack, 384 tests.

The following strains are available from:  
American Type Culture Collection (ATCC™)  
10801 University Boulevard  
Manassas, VA 20110-2209, USA.  
ATCC # VR-902B *Chlamydia trachomatis* LGV2  
ATCC Strain # 19424 *Neisseria gonorrhoeae*

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**Approved by:**

*Date Effective:* \_\_\_\_\_

*Supervisor:* \_\_\_\_\_ *Date:* \_\_\_\_\_

*Director:* \_\_\_\_\_ *Date:* \_\_\_\_\_

*Reviewed:*

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