Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Urine using the BD ProbeTec™ CT Q× and GC Q× Amplified DNA Assays on the BD Viper™ System in Extracted Mode

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**ABSTRACT**

We have developed two novel assays for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) on the BD Viper™ System in extracted mode. Here we describe the analytical performance of the new BD ProbeTec™ CT Q× and GC Q× Amplified DNA Assays with Neat urine specimens (without preservative) as well as urine specimens stored in the Urine Preservative Transport (UPT) for the BD ProbeTec (CT/GC) Q× Amplified DNA Assays. Both the Neat and UPT urine samples utilized pierceable caps and were extracted on the BD Viper System.

The analytical sensitivities of the CT Q× and GC Q× assays with Neat and UPT-treated urine were determined by spiking pooled CT/GC negative specimens from healthy donors at the following target levels: 0, 1, 3, 7, 15, and 30 Elementary Bodies (EB)/mL for CT and 0, 5, 10, 20, 50, and 100 cells/mL for GC. The 95% limits of detection (LODs) for CT and GC with Neat urine were 14 EB/mL (95% confidence intervals: 8, 21 EB/mL) and 21 cells/mL (12, 30), respectively, while for UPT-treated urine the LODs were 6 EB/mL (4, 9) and 25 cells/mL (13, 38).

Specimen stability studies were conducted using multiple pools of Neat and UPT-treated urine that were spiked with CT and GC organisms at a low level, near the analytical LODs of the Q× assays, and which were then stored under different conditions. CT and GC DNA was successfully detected in Neat urine stored at 30°C for up to 30 hours and at 2-8°C for up to 7 days. With UPT-treated urine, CT and GC DNA was stable at 2-30°C for up to 30 days. Stability of CT/GC DNA was also demonstrated when Neat urine specimens were held at 2-8°C for up to 24 hours and 30°C for up to 8 hours prior to transfer to the UPT.

The BD ProbeTec CT Q× and GC Q× assays, when used in conjunction with extraction on the BD Viper System, were able to detect low numbers of organisms in both Neat and UPT-treated urine. The stability of the CT/GC DNA in Neat and UPT-treated urine specimens in tubes with pierceable caps was also demonstrated over a broad range of temperatures that provide flexible alternatives for transport and storage prior to extraction on the BD Viper System.

**METHODS**

**Preparation of Urine Pools**
- Male and female neat urine samples were obtained from healthy volunteers and pooled in equal volumes.
- Studies were conducted either directly with the pooled neat urine or after treatment of the pooled samples with the UPT.

**Specimen Processing and Analysis**
- After preparation according to the methods outlined below, all samples were dispensed into BD Viper Sample Tubes that were fitted with pierceable caps.
- Specimen processing and testing were conducted on the BD Viper System in extracted mode.

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**Analytical Sensitivity** (Figures 1 & 2):
- Aliquots of neat and UPT-treated urine specimens were spiked with CT at 0, 1, 3, 7, 15, & 30 Elementary Bodies (EB)/mL, and 0, 5, 10, 20, 50, & 100 cells/mL for GC.
- 72 assay replicates were generated at each target level for each specimen type.

**CT/GC DNA Stability in Neat and UPT Urine Specimens** (Figures 3-5):
- CT/GC negative specimens were prepared from pooled neat urine specimens.
- CT/GC positive specimens were created by spiking pooled neat urine at 45 CT EB/mL and 150 GC cells/mL.
- 32 assay replicates were generated at each time-point and for each stability condition. (Table 1)

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**Table 1. CT/GC Q′ Urine Specimen Stability Study Design**

<table>
<thead>
<tr>
<th>Urine Specimen</th>
<th>Condition</th>
<th>Storage Temperature</th>
<th>Test Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>30°C</td>
<td></td>
<td>0, 8, 24, 30h</td>
</tr>
<tr>
<td></td>
<td>2-8°C</td>
<td></td>
<td>0, 1, 3, 7 days</td>
</tr>
<tr>
<td>UPT</td>
<td>8h hold at 30°C prior to UPT</td>
<td>2-8°C</td>
<td>0, 14, 21, 30 days</td>
</tr>
<tr>
<td></td>
<td>24h hold at 2-8°C prior to UPT</td>
<td>30°C</td>
<td></td>
</tr>
</tbody>
</table>

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**Figures 1 & 2. Analytical Sensitivity Data**

**CT Q′ Urine Specimens Limits of Detection**
Error bars show 95% Confidence Intervals of the point estimate

**GC Q′ Urine Specimens Limits of Detection**
Error bars show 95% Confidence Intervals of the point estimate
**METHODS** (continued)

The analytical limits of detection for the BD ProbeTec CT/GC Q<sup>x</sup> Amplified DNA Assays with neat and UPT urine were shown to be as follows:

<table>
<thead>
<tr>
<th>Assay</th>
<th>Specimen Matrix</th>
<th>LOD Point Estimate</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT Q&lt;sup&gt;x&lt;/sup&gt;</td>
<td>Neat</td>
<td>14</td>
<td>8,21</td>
</tr>
<tr>
<td></td>
<td>UPT</td>
<td>6</td>
<td>4,9</td>
</tr>
<tr>
<td>GC Q&lt;sup&gt;x&lt;/sup&gt;</td>
<td>Neat</td>
<td>21</td>
<td>12,30</td>
</tr>
<tr>
<td></td>
<td>UPT</td>
<td>25</td>
<td>13,38</td>
</tr>
</tbody>
</table>

- In neat urine specimen pools, CT and GC DNA was shown to be stable for 7 days at 2-8°C and 30 hours at 30°C.
- In UPT-treated urine specimen pools, CT and GC DNA was shown to be stable for up to 30 days at 2-30°C.