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# Comparison of Commercial Nucleic Acid Amplification Assays for the Detection of Neisseria gonorrhoeae, Chlamydia trachomatis and Trichomonas vaginalis

Interior Health
Every person matters

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## Abstract

**Background:** We compared the Seegene Allplex STI4 assay to the Hologic Aptima Combo 2 and Aptima *Trichomonas vaginalis* (TV) assays, for the detection of *Neisseria gonorrhoeae* (GC), *Chlamydia trachomatis* (CT) and TV.

**Methods:** A total of 418 pre-tested, frozen clinical specimens were supplied for this study, which had been run previously on the Hologic Aptima Combo 2 assay and/or Aptima TV assays. Specimens were frozen at -80°C after testing, and included Aptima vaginal, unisex (urethral and cervical) swabs, and urines. There were 107 CT, 64 GC and 50 TV positive specimens (including 18 co-positives) and 216 negatives tested. Primary specimen containers were de-capped, swabs were removed, then were extracted on the Hamilton STARlet. The BioRad CFX96 Real Time PCR Detection System and Seegene Viewer software were used to obtain and analyze results. Results were then compared to Aptima testing to assess sensitivity and specificity of the Seegene STI4 assay. Repeat Aptima testing was performed in discordant cases, to determine if the specimen was still positive after freeze/thaw cycles. For any remaining discordant CT or GC specimens, the Aptima confirmatory test (using a second primer set) was performed with the specimen called positive if both the Aptima Combo 2 and confirmatory assay was positive for the target. For TV, specimens positive on Allplex only underwent sequencing. Otherwise, the Aptima results were considered the gold standard.

**Results:** The Seegene STI4 assay provided accurate results in 411 of 418 specimens (98.3%) as shown in Table 1. The sensitivity and specificity for GC was 100%. TV had a sensitivity of 96.1% and a specificity of 100.0%. For CT, the sensitivity was 95.3% and the specificity was 100%. In all cases of decreased sensitivity, false negatives were attributed to specimens with lower relative light unit values by Aptima testing.

**Conclusions:** The Seegene STI4 assay performed well in the detection of sexual transmitted infections. A drawback to the Seegene system is the need to de-cap and remove swabs from the sample tubes before running them on the extractor, which hinders work flow and is a potential source of contamination. Seegene is experimenting with pierceable caps to determine if this aspect of workflow can be improved.

## Introduction

- Rapid, accurate diagnosis of sexually transmitted infections (STI) is important clinically and for public health follow up.
- We compared the performance of the Allplex STI4 assay (Seegene) to the Aptima Combo 2 (Hologic) and Trichomonas vaginalis (TV) assays for detection of Neisseria gonorrhoeae (GC), Chlamydia trachomatis (CT) and TV.

## Methods

- 418 frozen clinical specimens were tested, including 59 vaginal, 5 urethral and 125 cervical swabs, and 229 urines.
- These specimens were previously run on the Aptima Combo 2 assay and/or TV assays and frozen at -80°C.
- There were 107 CT, 64 NG and 50 TV positive specimens (including 18 co-positives) and 216 negatives.
- For Allplex, primary specimens were de-capped, had swabs removed and were extracted on the Hamilton STARlet using the STARMag Universal Cartridge Kit.
- For Allplex, the manufacturer suggested pre-treatment process for concentrating urine specimens was omitted.
- Results were obtained and analyzed using the CFX96 Real Time PCR Detection System (Bio-Rad) and Seegene Viewer software and Panther system (Hologic).
- For discordants, repeat Aptima testing was performed to assess specimen viability after freeze/thaw cycles.
- For any remaining discordant CT or GC specimens, the Aptima confirmatory test (using a second primer set) was performed with the specimen called positive if both the Combo 2 and confirmatory assay were positive.
- For TV, specimens positive on Allplex only underwent sequencing. Otherwise, the Aptima results were considered the gold standard.
- Sequencing for TV was performed in the DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad) using the ABI BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), then analyzed by electrophoresis on the ABI 3730xl DNA Analyzer (Applied Biosystems).

## Results

- The Seegene STI4 assay provided accurate results in 411/418 specimens (98.3%) and Aptima in 410/418 (98.1%).
- Both assays had sensitivity and specificity >95% (Table 1).

## Results

- Allplex false negatives occurred in specimens with relative light unit (RLU) values less than 1000 by Aptima Combo 2 or TV testing, while Aptima false positives tended to occur with RLU values <400 for CT and <1100 for GC (Table 2)

Table 1. Summary of Results

| Target | n | Allplex results | | Aptima results | |
|---|---|---|---|---|---|
| | | Sens (%) | Spec (%) | Sens (%) | Spec (%) |
| CT | 107 | 95.3 | 100.0 | 100.0 | 99.2 |
| GC | 64 | 100.0 | 100.0 | 100.0 | 98.2 |
| TV | 50 | 96.0 | 100.0 | 98.3 | 100.0 |

Table 2. Summary of Discordant Specimens

| Specimen type | Expected Result | Allplex Result | Allplex Cycle threshold (Ct) | Aptima Original RLU | Aptima Repeat RLU | Confirmatory Results |
|---|---|---|---|---|---|---|
| Urine | GC | GC/TV | 41.21 (TV) | TV neg | TV neg | TV confirmed sequencing |
| Cervix | CT | Neg | N/A | 537 | 690 | CT 6884 RLU |
| Urine | CT/GC | GC | N/A | 2290 | 1592 | CT 6905 RLU |
| Cervix | CT | Neg | N/A | 268 | 167 | CT 6969 RLU |
| Vagina | CT | Neg | N/A | 820 | 686 | CT 6153 RLU |
| Cervix | CT | Neg | N/A | 356 | 875 | CT 6871 RLU |
| Vagina | TV | Neg | N/A | 208 | Insufficient specimen | N/A |
| Urine | TV | Neg | N/A | 129 | 135 | N/A |
| Cervix | Neg | Neg | N/A | 352 (CT) | N/A | CT negative |
| Urine | Neg | Neg | N/A | 409 (GC) | N/A | GC negative |
| Urine | Neg | Neg | N/A | 194 (CT) | N/A | CT negative |
| Cervix | Neg | Neg | N/A | 1038 (GC) | N/A | GC negative |
| Vagina | Neg | Neg | N/A | 370 (GC) | N/A | GC negative |
| Vagina | Neg | Neg | N/A | 263 (GC) | N/A | GC negative |
| Cervix | Neg | Neg | N/A | 1014 (GC) | N/A | GC negative |

## Conclusion

- The Allplex STI4 and Aptima assays performed well in the detection of STIs
- For Alllplex, the need to de-cap and remove swabs from the specimens before extraction is a hindrance to workflow and a potential source of contamination.

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