Validation of Copan eNAT, a molecular transport medium, for the collection and preservation of urine specimens for the detection of STI infections with the Seegene Anyplex II STI-7 v1.1 Assay.

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Backgrounds

Urine is used for screening sexual transmitted infections with molecular assays. Copan developed the eNAT, a molecular medium that preserves and stabilizes nucleic acids. eNAT medium can be used to collect, transport and store clinical specimens for the detection of infectious pathogens by molecular amplification assays.

Seegene recommends the use of dry container for urine collection for the detection of urogenital pathogens with the Anyplex II STI-7 v1.1 assay (STI7).

In this study, the Copan eNAT molecular medium is tested for storing urines for the detection of sexual transmitted diseases with the STI7 assay.

Objectives

The objective of this study was to validate the eNAT medium for nucleic acid preservation in urines for STDs detection with the STI7 assays.

Materials and Methods

In this study, 102 urines, collected in dry containers from patients attending a Milan STD clinic were tested as per current method and after adding urine to 1 ml of eNAT molecular medium.

The first 80 urine samples, were used to find the volume of urine to add to 1 ml of eNAT medium, that would give the same sensitivity as urine in dry containers.

To individual 1 ml eNAT medium tubes were added 1, 2, and 3 ml of urine and 3 ml of urine were added to a dry container.

While additional 22 samples were tested in duplicate, 3 ml of urine in dry containers and 3 ml of urine were added to a 1 ml tube of eNAT.

All urine samples, in dry containers and in eNAT, were first vortexed and 350 ul of each sample were used to extract nucleic acids using the Automated Purification Systems (NIMBUS IVD) and eluted in 100 ul of elution buffer.

Purified nucleic acids were tested with the Seegene STI7 assay. (Seegene, distributed by Arrow Diagnostics, Genoa Italy)

Results

<table>
<thead>
<tr>
<th>STI7 Results</th>
<th>3 ml Urine dry Containers</th>
<th>1 ml eNAT+ 1 ml Urine</th>
<th>2 ml Urine</th>
<th>3 ml eNAT+ 3 ml Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEG</td>
<td>43</td>
<td>45</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>POS</td>
<td>37</td>
<td>35</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

Pathogens Detected in each Urine Sample Combination

Pathogens Detected in each Urine Sample Combination

Pathogens Detected in Dry Containers or in 1 ml eNAT

- In the first 80 urine tested, 43 negative and 37 positive were detected from urine collected and transported in dry containers versus 45, 40, 40 negative and 35, 40, 40 positive respectively of 1 ml, 2 ml and 3 ml of urine in 1 ml of eNAT.
- Improved sensitivity and more co-infections were detected when adding 3 ml of urine to 1 ml eNAT medium.
- Loss of sensitivity was noted when adding 1 ml or 2 ml of urine in eNAT medium.
- Inhibition was found in urines transported in dry containers.
- Equivalent results were found in the additional 22 urine samples, (3 ml of urine in dry containers and 3 ml in 1 ml eNAT). 5UU, 12UP, 0 MG, 7 MH, 0 NG, 19 CT, and 0 TV were detected in both samples tested.

Conclusions

Good agreement was found between Copan eNAT 3 ml urine and urine in dry containers for the detection of seven sexually transmitted pathogens with the Seegene STI7 assay.

Copan eNAT medium is suitable for the collection, transport and storage of urine specimens for the detection of STI with molecular assays.

Copan eNAT medium, is available in leak proof tube, is easy to transport and store urines, prevents bacterial overgrowth, stabilizes nucleic acids at room temperature and is compatible with the STI7 assay.

Three ml of urine in a 1 ml tube of eNAT is the optimal urine combination for testing for the detection of STI.

# of Pathogens

0 10 20 30 40

0 5 10 15 20 25 30 35 40

UU UP MG MH NG CT TV

UU UP MG MH NG CT TV