A clinical evaluation of a fully-Integrated, Low-Cost, Ultra-Rapid PCR Device with Point-of-Care Applications for Chlamydia trachomatis and Neisseria gonorrhoeae

Claire Ferrao1, Anna Dixon1, Justin Hardick2, Daniel Adlerstein1 and Charlotte Gaydos2

1 Atlas Genetics Ltd., Trowbridge (UK), BA14 0XG 2 John Hopkins University, Baltimore (USA), MD 21205

Introduction

Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) represent significant global public health problems. Point of care (POC) testing can drastically reduce disease burden of these organisms by providing rapid results which reduce time to treatment. Additionally, POC testing can be deployed in resource poor settings where complex diagnostic technology is not often available, further reducing disease burden.

The iO™ Platform

The iO™ system comprises a disposable test-specific cartridge and small-footprint instrument which is capable of performing multiple targets and tests (NAT) with equivalent sensitivity and accuracy to central laboratory analyzers. All steps take place automatically on the test cartridge and are complete in less than 30 minutes. The cartridge contains all the necessary assay reagents, in ambient-stable form, either dried or liquid. Microfluidic technology reconstitutes the dried reagents and moves the sample and all other liquid components through the cartridge. No user interaction is required following the initial addition of raw sample. All functions of fluidic movement, temperature control, thermal cycling and electrochemical detection are controlled by the iO™ instrument.

Multiplexing is achieved on the same cartridge in two ways. First, the cartridge contains multiple discrete channels, where a different test can be performed in each. Second, multiplexing within the same channel (a single reaction) is achieved through the use of multiple electrochemical labels, with different Redox potentials, for each target.

In this study, we sought to adapt this cartridge for detection of two distinct NG targets as well as an internal control (IC) which represent individual processes of the prototype iO™ cartridge; extraction, amplification and detection. Reagents required for each step were deposited on the sub-circuits and dried in a humidity controlled oven. Individual samples were added to the extraction sub-circuit followed by DNA extraction and purification. The eluted DNA was used to reconstitute dried amplification reagents followed by ultra-rapid amplification on a flat-bed thermocycler. Amplified samples were transferred to sub-circuits containing dried detection reagents. The formulation consisted of target-specific electrochemically-labelled probes and a double-strand DNA-specific exonuclease which allows the cleavage of the electrochemical labels only when in duplex with any amplified target. Cleaved labels were detected by applying a voltage to a screen-printed carbon electrode. Each electrochemical label has a discrete oxidation potential which allows a measurable current indicating the presence of each target in the sample to be detected.

De-identified, waste, clinical samples (vaginal swabs expressed in 1X TE buffer, N=60) were tested using sub-circuits which represent individual processes of the prototype iO™ cartridge; extraction, amplification and detection. Reagents required for each step were deposited on the sub-circuits and dried in a humidity controlled oven. Individual samples were added to the extraction sub-circuit followed by DNA extraction and purification. The eluted DNA was used to reconstitute dried amplification reagents followed by ultra-rapid amplification on a flat-bed thermocycler. Amplified samples were transferred to sub-circuits containing dried detection reagents. The formulation consisted of target-specific electrochemically-labelled probes and a double-strand DNA-specific exonuclease which allows the cleavage of the electrochemical labels only when in duplex with any amplified target. Cleaved labels were detected by applying a voltage to a screen-printed carbon electrode. Each electrochemical label has a discrete oxidation potential which allows a measurable current indicating the presence of each target in the sample to be detected.

Following the detection step, a cut-off threshold for negative and positive samples was established. There were 5 negative and 2 positive samples that were removed from the analysis due to mechanical failures.

Of the samples that were included in the analysis sensitivity and specificity for NG detection were 100% (28/28) and 96% (24/25), respectively.

For a positive sample to be identified both NG targets were required to be detected and for negative samples, the internal control was required to be detected. The test is developed to be combined in a cartridge with a previously developed test for CT which was shown to have 98.3% sensitivity and 58% specificity.

Results

The iO™ reader and test cartridge

An example of an electrochemical trace obtained for one of the two NG targets and the internal control (IC) detected in a duplex reaction. This is accomplished using two target-specific probes each with an electrochemical label that oxidizes at a specific potential when differential pulse voltammetry is applied.

Sensitivity and specificity of the iO™ NG assay on clinical samples

<table>
<thead>
<tr>
<th>Laboratory Sample status</th>
<th>Atlas end to end sub-circuit results</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>28</td>
<td>Sensitivity 100%</td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>Specificity 96%</td>
</tr>
</tbody>
</table>

Conclusions

• The results show sensitive and specific electrochemical detection of NG targets in clinical samples using multiplex PCR and detection.

• The method demonstrates the application of ultra-rapid amplification and multiplex electrochemical detection using elements of a fully-integrated point-of-care system.

• This development allows the combining of an existing CT assay and the two target NG assay onto the same integrated cartridge

• This sensitive, low-cost, rapid PCR POC device that could impact management and treatment of CT and NG infections by reducing time to treatment.

Atlas Genetics Ltd.
Derby Court, Epsom Square, White Horse Business Park, Trowbridge, Wiltshire, BA14 0XG, United Kingdom.

T: +44 (0)1225 717930   |   E:  daniel.adlerstein@atlasgenetics.com   |   www.atlasgenetics.com