Validation of a New DNA Extraction Method from Agar or Liquid Swabs for Molecular Detection of MRSA, VRE and KPC Utilizing the NanoCHIP® Microarray Technology

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Abstract
Objectives. Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-Resistant Enterococcus (VRE) and Klebsiella pneumoniae carbapenemase (KPC) co-infection in hospital admitted patients is the leading cause for Hospital Acquired Infections (HAI). It is now evident that HAI can be widely prevented through screening of patients before or during hospital admission and by immediate treatment of MRSA. An accurate, cost effective and real-time detection method is therefore essential to develop. The aim of this study was to evaluate the performance of a new multiplex PCR using a NanoCHIP® technology for screening large number of samples for simultaneous detection of MRSA, VRE and KPC from various clinical samples.

Methods. After routine diagnostic procedure positive Agar Gel as well as liquid (Eswab®) medium transport swabs were kept either at 4°C or at 20°C. A new protocol was developed in which the transport swabs were transported from the hospital to the laboratory at ambient temperature for an hour, and then placed at 4°C for 8 h. DNA extraction was carried out using the NanoCHIP® system and PCR was run for the respective genes. qPCR was performed with Fluorescence PCR technology using the Eswab® and Gel swabs. The extracted DNA was used as template to amplify pathogenic and antibiotic resistance specific genes through multiple PCR and subjected to the NanoCHIP® system.

Results. The NanoCHIP® system is an automated multiplex platform capable of detecting and analyzing multiple targets in a single run utilizing NanoCHIP® microarray technology. More information on the system and its function can be found at the NanoCHIP® website. The NanoCHIP® system is able to detect and quantify all three targets simultaneously. The extraction procedure is efficient and the sample-to-answer time is less than 2 hours.

Conclusions. The NanoCHIP® system is a rapid and efficient method for the detection of MRSA, VRE and KPC from clinical samples. The system provides a rapid and accurate diagnosis of these pathogens, which can be used in clinical settings to prevent the spread of infections.

Table 1. Efficiency DNA extraction and detection of MRSA, VRE and KPC characterized samples by NanoCHIP®

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>FP</th>
<th>PP</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>100%</td>
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<td>100%</td>
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<tr>
<td>VRE</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
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<tr>
<td>KPC</td>
<td>100%</td>
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</tbody>
</table>

* Results are in agreement with QR-PCR

Materials
Copan® Eswab® / FLOQSwab® test Inc, of liquid Agar Gel
Amersham gel transport swabs without charoral

Methods
After routine diagnostic procedure positive Agar Gel as well as liquid (Eswab®) medium transport swabs were kept either at 4°C or at 20°C. The FLOQSwab of the Eswab® was carefully pulled out of the tube with liquid agar transport medium. Both the Rayon swabs of the Agar Gel and the FLOQSwabs of Eswab® devices were stored in 200μl phosphate buffered saline (PBS) buffer. Specimens were extracted with heat lysis protocol 100°C for 10 minutes followed with 14,000 rpm centrifugation for 5 minutes. The extracted DNA was used as template to amplify pathogenic and antibiotic resistance specific genes through multiple PCR and subjected to the NanoCHIP® system.

The results from this work were achieved in clinical studies in various medical centers in Israel and abroad

The NanoCHIP® has proven to be a useful platform for medium-high throughput screening of MRSA, VRE and KPC co-infection, offering reliable diagnosis in various types of swab samples.

The NanoCHIP® performance protocol is compatible with both Copan Eswab® and Agar Gel swab collection devices.

The new protocol in this work is user-friendly, easy to perform and test.

The newly developed protocol improves the laboratory workflow, minimizes hands-on time and consequently turnaround time, simplifies the pre-PCR DNA extraction procedure, and overall reduces costs.

The NanoCHIP® System is an automated multiplex platform capable of detecting and analyzing multiple targets in a single run utilizing NanoCHIP® microarray technology. More information on the system and its function can be found at the NanoCHIP® website.

Figure 1.