

# 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 *Enterococci* (VRE) and *Klebsiella pneumoniae* carbapenemase (KPC) colonization 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 proper patient isolation and management. Savyon Diagnostics has recently finalized the development of a novel molecular-based diagnostic screening test for simultaneous detection of MRSA, VRE and KPC directly from a variety of swab sample types. The test utilizes Savyon's proprietary NanoChip®XL molecular electronic microarray system. The aim of this work is to demonstrate the compatibility of different swabs (amies agar and amies liquid swabs, Copan Italia, Spa, Brescia., Italy) with our PCR - NanoCHIP® technology for screening large number of samples for simultaneous detection of MRSA, VRE and KPC in variety of clinical samples.

**Methods.** After routine diagnostic procedure positive Amies 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 order to allow fast and easy DNA extraction from clinical samples. Pathogen and antibiotic resistance specific genes were amplified through multiplex PCR and subjected to the NanoCHIP® system. The generated amplicons were electronically addressed to discrete loci on the NanoCHIP® cartridge, pre-activated with specific capture oligonucleotides. Detection was achieved through specific fluorescent reporter oligonucleotides. The output analysis of each sample was compared to the characterization of the respective original swab sample, as characterized by real-time PCR in various laboratories.

**Results.** The novel extraction protocol demonstrated a full compatibility with the downstream PCR-NanoCHIP® technology. The results were in complete accordance with the characterizations of the tested samples in terms of clinical sensitivity and specificity.

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

## Background

Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Enterococci* (VRE) and *Klebsiella pneumoniae* carbapenemase containing *K. pneumoniae* (KP-KPC) colonization 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 proper patient isolation and management. Savyon Diagnostics has recently finalized the development of a novel molecular-based diagnostic screening test for simultaneous detection of MRSA, VRE and KP-KPC directly from a variety of clinical samples.

The test utilizes Savyon's proprietary NanoCHIP® molecular electronic microarray system. This system enables medium-high throughput screening from sample to results at the same working day and with minimal hands-on time.

The NanoCHIP® offers the following features:

- ❖ Automated testing process from sample to result, using primary samples
- ❖ Use dermal, nasopharyngeal, or perineum swab samples, culture enriched isolates, positive blood cultures and wound, pus or surgical swabs
- ❖ Vast reduction of general microbiology process in the laboratory; only validation of positive samples
- ❖ DNA extraction solution is provided. Alternatively user may use a variety of automated and manual validated DNA extraction techniques
- ❖ Up to 96 samples per run
- ❖ Short turnaround time: Less than 8 hours from sample to result
- ❖ CE-IVD

Copan Eswabs™ and Amies Gel swabs are commonly used for collecting, transporting and maintaining ICP samples. As the first step in each molecular diagnostic system would be extraction of DNA from the clinical specimen, efficient extraction from the different types of swabs is challenging on the way to achieve a reliable diagnostics. As part of the development the ICP test the need was raised to develop a method which will efficiently process the various swabs that the kit was defined to comply with. As mentioned above the ICP kit provides convenient solution for DNA extraction from different swab types as well as swab samples. This procedure is essential for simplifying the whole process and reducing costs.

## Methods

After routine diagnostic procedure positive Amies Gel as well as liquid (eSwab™) medium transport swabs were kept at 4° C as well as at -20° C.

- The FLOQSwab of the Eswab™ was carefully pulled out of the tube with liquid amies transport media
- Both the Rayon swab of the Amies Gel and the FLOQSwab of Eswab™ devices were eluted in 200 µl of Savyon Lysis buffer
- Specimens were extracted with heat-lysis protocol: 100° C for 10 minutes followed with 14,000 rpm centrifuge for 5 minutes

The extracted DNA was used as template to amplify pathogen and antibiotic resistance specific genes through multiplex PCR and subjected to the NanoCHIP® system.

The NanoCHIP® System is an **automated multiplex** platform capable of detecting and analyzing **multiple targets** together with **multiple samples** on the same run utilizing the **electronic micro-array technology**. More information on the system and its function can be found at Booth #81 and at: [www.savyondx.com](http://www.savyondx.com) or [www.nanochipxl.com](http://www.nanochipxl.com)

## Objective

The aim of this work is to demonstrate the compatibility of different swabs contained in the Amies Gel and Eswab™ collection devices, (Copan Italia Spa, Brescia, Italy) with our PCR - NanoCHIP® technology for screening large number of samples for simultaneous detection of MRSA, VRE and KPC in variety of clinical samples, and to present the newly developed protocol for processing the swabs efficiently

## The NanoCHIP® Workflow

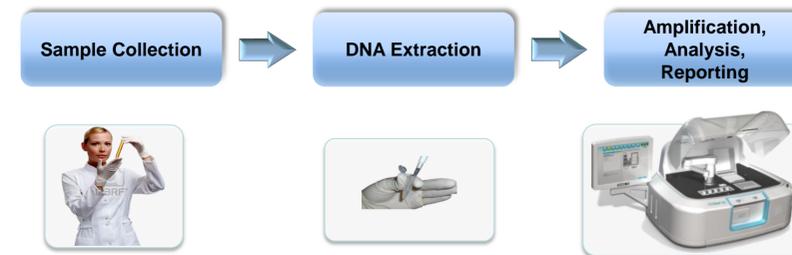
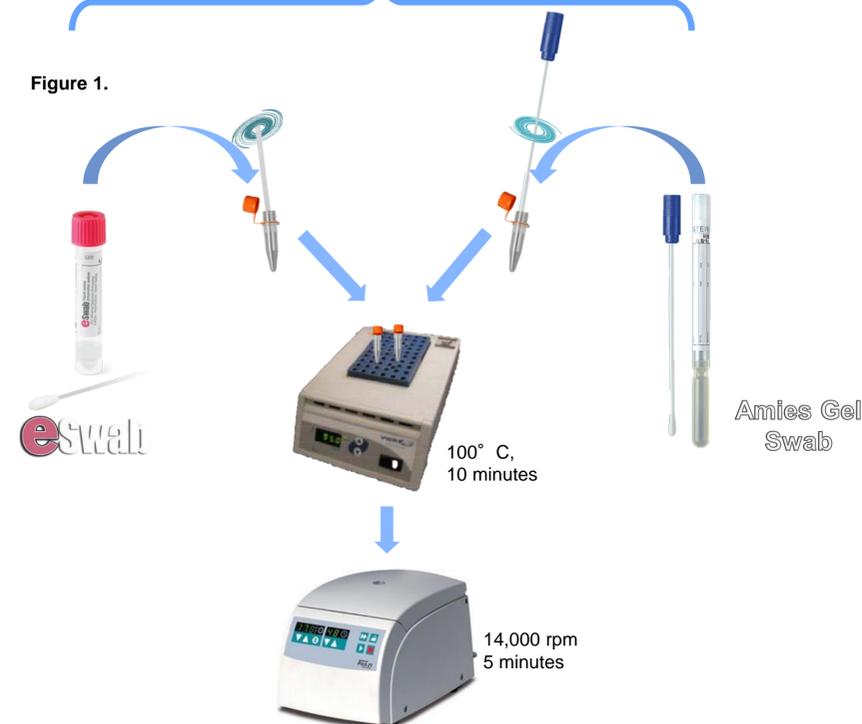


Figure 1.



## Results

**Table 1.** Efficient DNA extraction and detection of MRSA, VRE and KPC characterized samples by ICP kit

		TP	FN*	TN	FP	Sensitivity	Specificity
MRSA	Eswab	85	2	153		97.7%	100%
	Gel swab	29	2	56		93.5%	100%
	Total	114	4	209		96.6%	100%

VRE	Eswab	51		159		100%	100%
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KPC	Eswab	52		153		100%	100%
	Gel swab	7		50		100%	100%
	Total	59		203		100%	100%

\* Results are in agreement with QR-PCR

Both types of swab samples were analyzed by the routine microbiological protocol for CRE, MRSA and VRE screening. Following the routine analysis and quantitative culturing, samples were kept either at 4° C or at -20° C. The DNA was extracted from selected samples following the protocol demonstrated in Figure1. The extracted DNA was kept at -20° C up to one month and was analyzed by the NanoCHIP® ICP test. Direct QR-PCR analysis of KPC-CRE and MRSA/MSSA using the same extracted DNA submitted to the NanoCHIP® ICP analysis was performed, in order to validate discrepant results.

The data represents summary of results from clinical studies in Israel and abroad

## Conclusions & Summary

- ❖ The results shown in 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 colonization, offering reliable diagnosis in various types of swab samples
- ❖ The NanoCHIP® performance protocol is compatible with both Copan Eswab™ and Amies Gel swab collection devices
- ❖ The new protocol in this work is user friendly, easy to perform and fast
- ❖ 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

## Materials



**COPAN**  
Eswab™ - a FLOQSwab™ and 1mL of modified Liquid Amies



Amies gel transport swabs without charcoal