A novel diagnostic approach to detection of respiratory tract infections in immunocompromised patients based on the NanoCHIP® microarray system

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Background

Respiratory diseases are significant causes of morbidity and mortality among immunocompromised patients. The etiologies of these infections include a diverse array of pathogens. Current diagnosis is often made challenging due to the need to detect viral, bacterial as well as fungal pathogens, and therefore involves different laboratories, technologies and diagnostic methodologies. Consequently the process may become time-consuming and laborious, and thus critical in view of the vulnerability of these patients to infections. Savyon Diagnostics has recently developed a diagnostic test for a respiratory tract infections panel (RTIP), based on the NanoCHIP® molecular microarray system. The test is designed for multiplexed detection of bacterial (*Legionella pneumophila*), viral (*Influenza* A and B, *Parainfluenza* (1, 2, and 3), *RSV* A/B, *Adenovirus, CMV, HSV* (1 and 2)), and fungal (*Aspergillus fumigatus*, and *Pneumocystis jirovecii*) pathogens in specimens taken from immunocompromised patients. The test demonstrates a unique feature of simultaneous detection of various types of microorganisms, i.e. RNA and DNA viruses, bacteria, as well as fungi in one PCR reaction tube. By that, the new test offers an efficient tool for patient management and enables proper treatment in a timely manner.

Objective

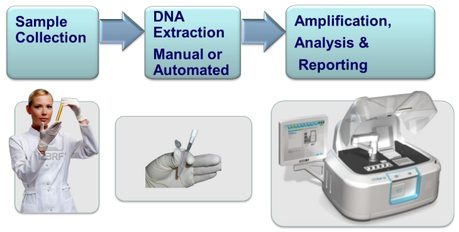
The aim of this work is to demonstrate the concept of the newly developed NanoCHIP® RTIP test and its potential utility for detection of the aforementioned pathogens in immunocompromised patients

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 #91 and at:
www.nanochipxl.com and www.savyondx.com

Methods

- DNA and RNA were extracted from 131 characterized clinical samples, taken from individuals suspected of respiratory tract infections and immunocompromised patients
- The samples were mostly Broncho-alveolar lavage (BAL) and sputum, and the nucleic acids were extracted using either automated system for total nucleic acid extraction NucliSENS® easyMAG® (bioMerieux) or The AllPrep DNA/RNA Mini Kit (Qiagen).
- Target genes were amplified using QIAGEN One-Step RT-PCR Kit
- Resulted amplicons were subjected to the NC400 NanoCHIP® system for detection and analysis:
  - Generated amplicons were electronically addressed to discrete loci in the NanoCHIP® cartridge pre-activated with specific capture oligonucleotides
  - Detection was achieved through specifically designed fluorescent reporter oligonucleotides
- The output analysis of each sample was compared to the original characterization of the sample as determined in the respective clinical laboratory in Rambam Medical Center
- Discrepant analysis was performed using sequencing, Geneproof Real Time PCR (Czech Republic) for CMV ,HSV and ASPERGILLUS, and Real-Time PCR of Fast Track Diagnostics (Malta) for IAV ,IBV ,PIV1-3 and ADV

The NanoCHIP® Workflow



The NanoCHIP® RTI Panel

Type	Pathogen
RNA viruses	*Influenza A virus*
	Influenza B virus
	Parainfluenza virus 1-3
	Respiratory syncytial virus A/B
DNA viruses	Cytomegalovirus
	Herpes Simplex virus 1/2
	Adenovirus
Bacterium	*Legionella pneumophila*
Fungi	*Aspergillus* spp.
	Pneumocystis jirovecii

Results

Table 1. Detection of pathogens in the RTIP test before analysis of discrepancies

Pathogen	TP	FP	TN	FN	Positive Agreement (%)	Negative Agreement (%)	Total Agreement (%)
ADV	13	0	116	2	87	100	98.5
CMV	18	18	94	1	94.7	84	85.5
HSV	14	1	115	1	93.3	99.1	98.5
PIV1	3	2	110	1	NA	98.2	97.4
PIV3	13	2			93	98.2	97.6
RSV	13	0	116	2	87	100	98.5
IVA	14	0	113	4	77.8	100	97
IVB	13	0	117	1	93	100	99.2
Asp	11	9	110	1	92	92.4	92.4
PCP	12	1	117	1	92.3	99.1	98.5
Leg	2	0	129	0	NA	100	100

Results (cont.)

Table 2. Results after discrepant analysis using sequencing to resolve FP and RT-PCR for IVA

Pathogen	TP	FP	TN	FN	Positive Agreement (%)	Negative Agreement (%)	Total Agreement (%)
ADV	13	0	116	2	87	100	98.5
CMV	31	4	95	1	97	96	96.2
HSV	14	1	115	1	93.3	99.1	98.5
PIV1	5	0	110	1	NA	100	99.1
PIV3	15	0			93.8	100	99.2
RSV	15	0	116	0	100	100	100
IVA	14	0	116	1	93.3	100	99.2
IVB	13	0	117	1	93	100	99.2
Asp	17	3	110	1	94.4	97.3	97
PCP	13	0	117	1	93	100	99.2
Leg	2	0	129	0	NA	100	100

Discussion & Summary

- The results were in high accordance with the original characterization of the tested samples
- The new test demonstrates the ability to diagnose simultaneously the most abundant pathogens that present high risk to immunocompromised patients
- The test eliminates the need for separate processes required usually for the various types of pathogens composing the panel
- The test is expected to improve laboratory workflow, to require minimal hands-on time and to be cost-effective
- All in all the test presents a novel and efficient solution to current needs in immunocompromised patient management