Detection and Identification of Microorganisms using PathoKey® SQ FLEX Pathogen ID Assay

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INTRODUCTION

The PathoKey® SQ Pathogen ID Assay is an automated Next Generation Sequencing (NGS) based In-Vitro Diagnostic test intended for the detection and identification of microorganisms simultaneously.

The PathoKey® Assay allows for the selection of up to four different pathogen targets per sample via the use of specific primer pools for target amplification. Pathogen targets include bacteria, fungi, parasites, viruses and antimicrobial resistant genes. The assay has a Limit of Detection (LoD) of 10^3 – 10^4 CFU/mL or copies/mL and has been shown to work with clinical samples like whole blood, plasma, serum, nasopharyngeal swabs, saliva, urine, stool, FFPE, CSF, BAL and skin swabs. Additionally, the assay has a built-in positive control that acts as an external control to monitor workflow operation, a negative control to detect workflow contamination and an extraction control to confirm validity of the extraction process.

The PathoKey® Assay workflow utilizes an automated robotic liquid handling system (Sentosa® SX 101) for total nucleic acid extraction, PCR setup and NGS library preparation for up to 24 samples. Template preparation is done via emulsion PCR using Ion PGM Hi-Q View OT2 Kit on the Sentosa® ST401 system and sequencing is performed on the Ion Torrent NGS platform. Data interpretation is subsequently performed by the Sentosa® SQ Reporter software and a report containing the list of pathogens detected in each sample is generated. This minimizes hands-on time and human error while enabling high throughput.

WORKFLOW

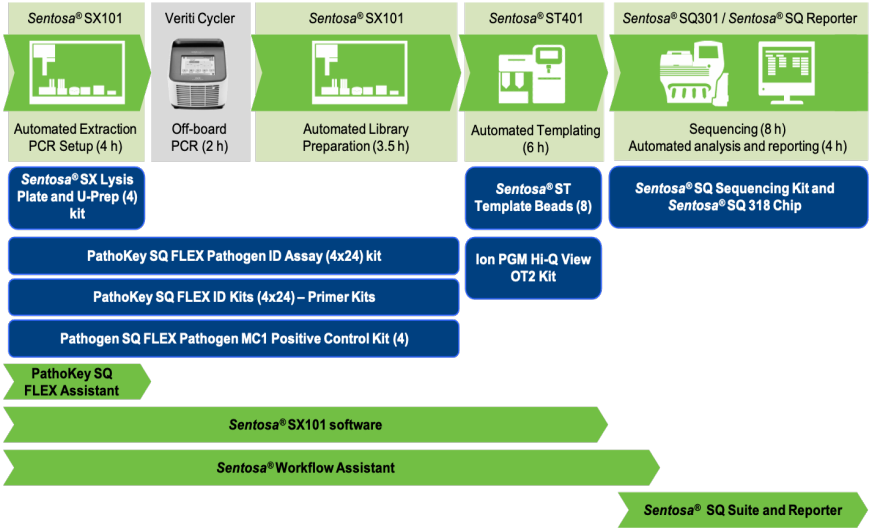

Figure 1. PathoKey® SQ Pathogen ID Assay Workflow

RESULTS

Analytical Sensitivity

The Limit of Detection (LoD) of the assay workflow for the bacteria, fungi, parasites, viruses and AMR bacteria strains tested was determined as 10^3 CFU/mL or copies/mL in biological matrices like nasopharyngeal and skin swabs, saliva, urine, whole blood, plasma, FFPE, BAL and CSF (Tables 1, 2 and 3).

Table 1. LoD for bacteria in nasopharyngeal swabs and plasma

Bacteria	Matrix	LoD (CFU/mL)
Steptococcus pneumoniae	Nasopharyngeal	10^3
Stenotrophomonas maltophila	Nasopharyngeal	10^3
Acinetobacter baumannii	Nasopharyngeal	10^3
Citrobacter freundii	Nasopharyngeal	10^3
Neisseria gonorrhoea	Nasopharyngeal	10^3
Vibrio parahaemolyticus	Plasma	10^3
Clostridium difficile	Plasma	10^3
Prevotella melaninogenica	Nasopharyngeal	10^3
Bacillus cereus	Plasma	10^3
Salmonella enterica	Plasma	10^3
Helicobacter pylori	Nasopharyngeal	10^3

Table 2. LoD for fungi and parasites in various biological matrices

Fungi	Matrix	LoD (CFU/mL)
Aspergillus sydowii	Nasopharyngeal	10^3
Pleurostoma richardsiae	Skin	10^3
Setosphaeria rostrata	Nasopharyngeal	10^3
Rhizopus microsporous	Nasopharyngeal	10^3
Rhodotprula mucilaginosa	Skin	10^3
Candida krusei	Skin	10^3
Cryptococcus neoformans	CSF	10^3
Candida albicans	Nasopharyngeal	10^3
Parasites	**Matrix**	**LoD (CFU/mL)**
Plasmodium falciparum	Whole blood/ Plasma/FFPE	10^3
Trypanosoma cruzi	Whole blood/ Plasma/FFPE	10^3
Acanthomoeba polyphaga	Whole blood/ Plasma/FFPE	10^3

RESULTS, cont'd

Table 3. LoD for AMR bacteria and viruses in urine and saliva

AMR bacteria (genes)	Matrix	LoD (CFU/mL)
Klebsiella pneumoniae, K6 (SHV, SUL1)	Urine	10^3
MRSA (ErmA, mecA)	Urine	10^3
Enterococcus faecium (vanA)	Urine	10^3

Viruses	Matrix	LoD (copies/mL)
Adenovirus	Saliva	10^3
BK virus	Saliva	10^3
Cytomegalovirus	Urine	10^3
Herpes simplex virus I	Saliva	10^3
Herpes simplex virus 2	Urine	10^3
Human herpesvirus 6	Saliva	10^3
Human herpesvirus 7	Saliva	10^3
Varicella-Zoster virus	Saliva	10^3
Enterovirus	Urine	10^3
JC virus	Saliva	10^3

Clinical Evaluation

The assay was clinically evaluated with 41 patient urine samples, 43 urine DNA samples and 22 urine samples spiked with CDC AR Bank Isolates from POPLAR healthcare. The PathoKey® SQ Pathogen ID Assay was able to detect 91.6% of bacteria and 90.5% of AMR genes when compared to MicroPath ID qPCR technology platform (Tables 4 and 5). An example of the lab test report for one of POPLAR's sample is shown in Figure 2.

Table 4. Bacteria detection in POPLAR healthcare clinical samples

Bacteria detected with MicroID qPCR	Detected with PathoKey® Assay?
Enterobacter cloacae	Yes
Enterococcus avium	Yes
Enterococcus faecalis	Yes
Enterococcus faecium	Yes
Escherichia coli	Yes
Klebsiella pneumoniae	Yes
Proteus mirabilis	Yes
Pseudomonas aeruginosa	Yes
Salmonella enterica subsp. enterica serovar Concord	Yes
Shigella flexneri	Yes
Streptococcus agalactiae	Yes
Ureaplasma urealyticum	No

Table 5. AMR detection in POPLAR healthcare clinical samples

AMR genes detected with MicroID qPCR	Detected with PathoKey® Assay?
AAC	Yes
AADA	Yes
APH	Yes
CTX	Yes
dhfr	No
ErmA	Yes
ErmB	Yes
ErmC	Yes
gyrA	Yes
KPC	Yes
mecA	Yes
NDM	Yes
OXA	Yes
QnrA	No
QnrS	Yes
SHV	Yes
SUL1	Yes
TEM	Yes
TetM	Yes
vanA	Yes
VIM	Yes

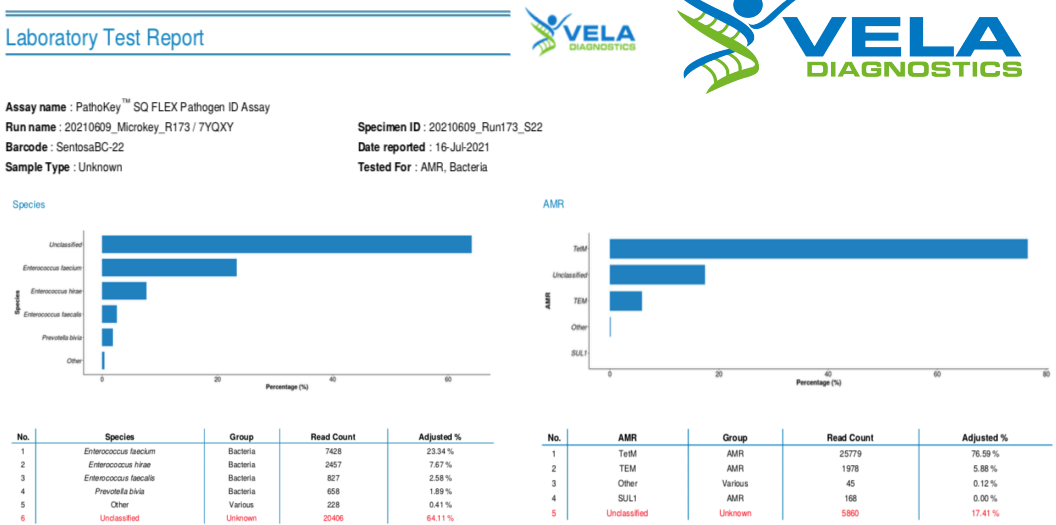

Figure 2. PathoKey® SQ Pathogen ID Assay Laboratory Test Report

CONCLUSION

We have developed a rapid and automated pathogen genotyping assay to enable the identification of various group of pathogens (bacteria, fungi, parasites, viruses and antimicrobial resistant genes) in a single sample which will contribute significantly to the study of host-microbiome research and greatly reduce the need to purchase multiple assay kits from different suppliers.

CONTACT

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