Application Note

Utilization of Medicinal Genomics PathoSEEK® Detection Assays and MaGiC Lysis for Detection of Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, and Salmonella in Nicotine Salt Pouches

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Introduction

Rapid microbiological methods (RMMs) including qPCR-based assays have been increasingly adopted in the nicotine testing industry to improve testing efficiency, reduce turnaround time, and enable greater automation within quality control laboratories. While compendial methods described in United States Pharmacopeia (USP) chapters remain the established standard, these culture-based approaches can be time and resource intensive, requiring selective media, extended incubation periods, and multiple handling steps. In contrast, qPCR-based methods offer a rapid and sensitive alternative for the detection of specified microorganisms.

To support their implementation, alternative methods must demonstrate equivalency to compendial procedures. This application note evaluates the equivalency of multiplex qPCR assays for the qualitative detection of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella spp*., and *E. coli spp*. in nicotine salt pouch matrices against the USP compendial method.

These microorganisms are commonly included in microbiological testing specifications for nicotine products and the availability of reliable rapid detection methods is of significant relevance to industry testing needs. The alternative method evaluated in this study is designed to support efficient, automated workflows while maintaining performance comparable to compendial approaches.

Materials and Methods

For the live culture inoculations, cultures of *E. coli* ATCC 8739, *S. typhimurium* ATCC 13311, *P. aeruginosa* ATCC 9027 & *S. aureus* ATCC 6538 were grown and enumerated to prepare concentrated stocks with known CFU/mL. Dilution series were prepared to achieve the desired contamination levels for spiking.

Twenty one-gram nicotine salt pouch test portions were inoculated with live culture near the limit of detection (1-2 CFU), at a mid level (~50 CFU), and at a high level (~100 CFU). The method was assessed for each organism individually, resulting in 240 total test portions.

The Medicinal Genomics PathoSEEK® Salmonella & E. Coli Multiplex Detection Assay v3 w/ MaGiC Lysis Kit and PathoSEEK® P. Aeruginosa & S. Aureus Multiplex Detection Assay v2 With MaGiC Lysis Kit User Guides were followed in addition to the MaGiC Lysis Automation on BMS Myra Liquid Handler User Guide.

One gram of sample matrix and 9 mL of Buffered Peptone Water (BPW) were added to a 15 mL or 50 mL conical tube. Appropriately diluted live culture was added to each tube containing sample matrix and BPW to achieve the desired spike level. Each tube was vortexed to mix. Prepared sample tubes were enriched at 37°C for 20-24 hours. After enrichment, samples were removed from the incubator and vortexed to mix thoroughly.

DNA Extraction was performed using MaGiC Lysis™ reagents on the Biomolecular Systems Myra Liquid Handling Robot. Real-Time PCR Setup (qPCR) was performed using the BMS Myra Liquid Handling Robot. Samples were analyzed using the Agilent AriaMX Real-Time qPCR System with the PathoSEEK® P. aeruginosa & S. aureus Multiplex Detection Assay v2 and the PathoSEEK® E. coli and Salmonella Detection Assay v3.

For data analysis, samples were considered qPCR positive for *Salmonella* spp., *Staphylococcus aureus*, and *Escherichia coli* if a Cq value of less than 40 was obtained on the relevant detection channels. Samples were considered qPCR positive for *Pseudomonas aeruginosa* if a Cq value of less than 35 was obtained on the appropriate detection channel. This allowance exists because trace levels of environmental *Pseudomonas aeruginosa* DNA are commonly present in laboratory water systems and may result in late signal, Cq > 35, in No Template Control Samples.

Results

Table I: Low-level live organisms spiked into nicotine salt pouch matrix 1-2 CFU

Pouch Flavor	*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Salmonella spp.*		*E. Coli spp.*	
	PathoSEEK	Compendial Method	PathoSEEK	Compendial Method	PathoSEEK	Compendial Method	PathoSEEK	Compendial Method
Groovy Grape	Y	Y	Y	Y	Y	Y	Y	N
Breezy Mango	Y	Y	N	N	N	N	Y	N
Cinnamon	Y	Y	Y	Y	N	N	N	N
Mint	Y	Y	Y	Y	Y	N	Y	N
Icy Berries	Y	Y	Y	Y	N	N	Y	Y
Mighty	Y	Y	N	N	N	N	N	N
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Strawberry	Y	Y	Y	Y	N	N	N	N
Punchy Lime	Y	Y	Y	Y	N	N	N	N
Peppermint	Y	Y	N	N	N	N	N	N
Mint	Y	Y	Y	Y	N	N	Y	Y
Peppermint	Y	Y	Y	Y	N	N	N	N
Peppermint	Y	Y	Y	Y	N	N	N	N
Mint	Y	Y	Y	Y	N	N	Y	N
Mint	Y	Y	Y	Y	N	N	Y	Y
Peppermint	Y	Y	Y	Y	N	N	Y	Y
Mint	Y	Y	N	N	N	N	Y	Y
Peppermint	Y	Y	Y	Y	N	N	N	N
Mint	Y	Y	N	N	N	N	N	N
Citrus	Y	Y	N	N	N	N	N	N

Table II: Percent detection in twenty test portions: Low-level live organisms spiked into nicotine salt pouch

	PathoSEEK Results	**Compendial Method Results**
Pseudomonas aeruginosa	100%	100%
Salmonella typhimurium	15%	10%
Staphylococcus aureus	70%	70%
Escherichia coli	50%	30%

Table III: Mid-level live organisms spiked into nicotine salt pouch matrix - 50 CFU

Pouch Flavor	*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Salmonella spp.*		*E. Coli spp.*	
	PathoSEEK	Compendial Method	PathoSEEK	Compendial Method	PathoSEEK	Compendial Method	PathoSEEK	Compendial Method
Groovy Grape	Y	Y	Y	Y	Y	Y	Y	Y
Breezy Mango	Y	Y	Y	Y	Y	Y	Y	Y
Cinnamon	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Icy Berries	Y	Y	Y	Y	Y	Y	Y	Y
Mighty	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Strawberry	Y	Y	Y	Y	Y	Y	Y	Y
Punchy Lime	Y	Y	Y	Y	Y	Y	N	N
Peppermint	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Peppermint	Y	Y	Y	Y	Y	Y	Y	Y
Peppermint	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Peppermint	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Peppermint	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Citrus	Y	Y	N	N	Y	Y	Y	Y

Table IV: Percent detection in twenty test portions: Mid-level live organisms spiked into nicotine salt pouch

	PathoSEEK Results	**Compendial Method Results**
Pseudomonas aeruginosa	100%	100%
Salmonella typhimurium	100%	100%
Staphylococcus aureus	95%	95%
Escherichia coli	95%	95%

Table V: High-level live organisms spiked into nicotine salt pouch matrix - 100 CFU

Pouch Flavor	*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Salmonella spp.*		*E. Coli spp.*	
	PathoSEEK	Compendial Method	PathoSEEK	Compendial Method	PathoSEEK	Compendial Method	PathoSEEK	Compendial Method
Groovy Grape	Y	Y	Y	Y	Y	Y	Y	Y
Breezy Mango	Y	Y	Y	N	Y	Y	Y	Y
Cinnamon	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Icy Berries	Y	Y	Y	Y	Y	Y	Y	Y
Mighty	Y	Y	Y	N	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Strawberry	Y	Y	Y	Y	Y	Y	Y	Y
Punchy Lime	Y	Y	Y	Y	Y	Y	Y	Y
Peppermint	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Peppermint	Y	Y	Y	Y	Y	Y	Y	Y
Peppermint	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Peppermint	Y	Y	Y	Y	Y	Y	N	Y
Mint	Y	Y	Y	Y	Y	Y	Y	N
Peppermint	Y	Y	Y	Y	Y	Y	Y	Y
Mint	Y	Y	Y	Y	Y	Y	Y	Y
Citrus	Y	Y	Y	N	Y	Y	Y	Y

Table VI: Percent detection in twenty test portions: High-level live organisms spiked into nicotine salt pouch

	PathoSEEK Results	**Compendial Method Results**
Pseudomonas aeruginosa	100%	100%
Salmonella typhimurium	100%	100%
Staphylococcus aureus	100%	85%
Escherichia coli	95%	95%

Discussion

The results were concordant between the two methods in 96% of the samples tested. Of the discordant results, nine samples were positive by qPCR but negative by the USP method: five at the low level (1–2 CFU) level and four at the high level (100 CFU). In one instance, a sample was positive on the USP method but negative by qPCR at the high level (100 CFU).

These results indicate that the PathoSEEK qPCR Method is more sensitive than the USP method.

Conclusions

Medicinal Genomics' PathoSEEK® Pseudomonas aeruginosa & Staphylococcus aureus Multiplex Detection Assay v2 and PathoSEEK® E. coli and Salmonella Multiplex Detection Assay v3 with MaGiC Lysis are suitable for the rapid molecular detection of *Staphylococcus aureus*, P*seudomonas aeruginosa*, *Salmonella* spp., and *E. coli* spp. in nicotine salt pouch matrices and produced results that exceeded the capabilities of the compendial method.

Ordering information

Product	Part Number
PathoSEEK® Salmonella & E. coli Multiplex Detection Assay v3 with MaGiC Lysis Kit	P/N 420525
PathoSEEK ® P.aeruginosa & S. aureus Multiplex Detection Assay v2 with MaGiC Lysis Kit	P/N 420526
Internal Control	P/N 420337
PathoSEEK ® P. aeruginosa & S. aureus Positive Control	P/N 420338
PathoSEEK Salmonella & E. coli Positive Control	P/N 420313

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