

IAFP, July 18-21 2021

INTRODUCTION

Rapid methods such as PCR have been making in-roads into the routine nutraceutical and dietary supplements testing, the potential of multiplex PCR for routine detection of multiple pathogens has yet to be ascertained. The diversity and ever-growing list of matrices in these industries further exacerbates the challenges for such methods and thereby the method compatibility should be reviewed with the adoption of new technology.

PURPOSE

To evaluate the performance of GENE-UP® NutraPlexPro™ (NP) a triplex real-time PCR assay for detection of *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* simultaneously in various nutraceutical and dietary supplement matrices.

METHODS

For inclusivity, *E. coli* (14), *S. aureus* (7), *Salmonella* spp. (3) were grown in Nutraceutical Universal Enrichment (NUB) broth at 35°C±2 for 24-28h, while for the exclusivity non-target strains were grown for 24h in non-selective broth and tested on NP. For analytical sensitivity, N=3 target strains were individually grown in NUB for 24h at 35°C ±2° then evaluated with NP. For matrix verification samples, eight diverse categories: eggs (5), vitamins (1), fruit drinks (5), organics (5), plant extracts (4), probiotics (5), and protein supplements (10) involving total 35 matrices @ 10g size were tested to determine the compatibility with NP. Each matrix was spiked with ≤800CFU per test portion of each organism and enriched in NUB at 1:10 at 35°C ±2° for 24-28h and tested on NP. All presumptive results were confirmed by culture based methods.

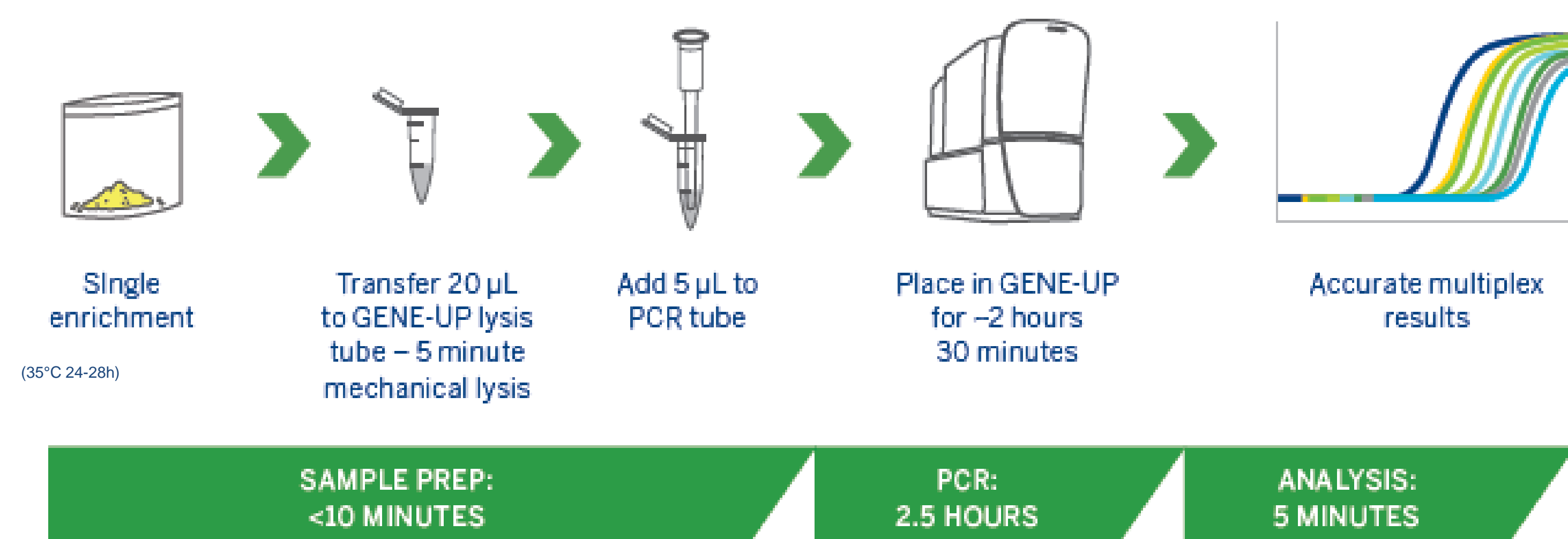


Figure 1. Schematic of GENE-UP® NutraPlexPro™ workflow.

RESULTS

Organism	# strains	<i>E. coli</i> Channel	<i>Staphylococcus</i> Channel	<i>Salmonella</i> Channel	Internal Control Channel
Inclusivity					
<i>Escherichia coli</i>	14	(+)	(-)	(-)	(+)
<i>Salmonella</i> spp	3	(-)	(-)	(+)	(+)
<i>Staphylococcus aureus</i>	7	(-)	(+)	(-)	(+)
Exclusivity*					
	23	(-)	(-)	(-)	(+)
Challenge study					
<i>K. pneumoniae</i> , <i>S. epidermidis</i> , <i>E. faecalis</i>	NA	(-)	(-)	(-)	(+)
<i>E. coli</i> , <i>S. epidermidis</i> , <i>E. faecalis</i>	NA	(+)	(-)	(-)	(+)
<i>S. aureus</i> , <i>K. pneumoniae</i> , <i>E. faecalis</i>	NA	(-)	(+)	(-)	(+)
<i>Salmonella</i> , <i>K. pneumoniae</i> , <i>S. epidermidis</i>	NA	(-)	(-)	(+)	(+)

*Exclusivity strains included: *S. chromogenes*, *S. urealyticus*, *S. epidermidis*, *S. galinarum*, ATCC 49148, *S. saprophyticus*, *S. hyicus*, *S. scheiferi*, *S. sciuri*, *S. xylosus*, *S. warneri*, *L. brevis*, *E. tarda*, *E. cloacae*, *E. aerogenes*, *K. pneumoniae*, *L. monocytogenes*, *H. alvei*, *B. cereus*, *E. faecalis*, *C. freundii*, *P. vulgaris*, *C. albicans*, *S. cerevisiae*. NA: not applicable. **Samples were enriched in were grown in Nutraceutical Universal Enrichment (NUB) broth at 35°C±2° for 24-28h.

Table 1. NutraPlexPro inclusivity, exclusivity, and challenge data.

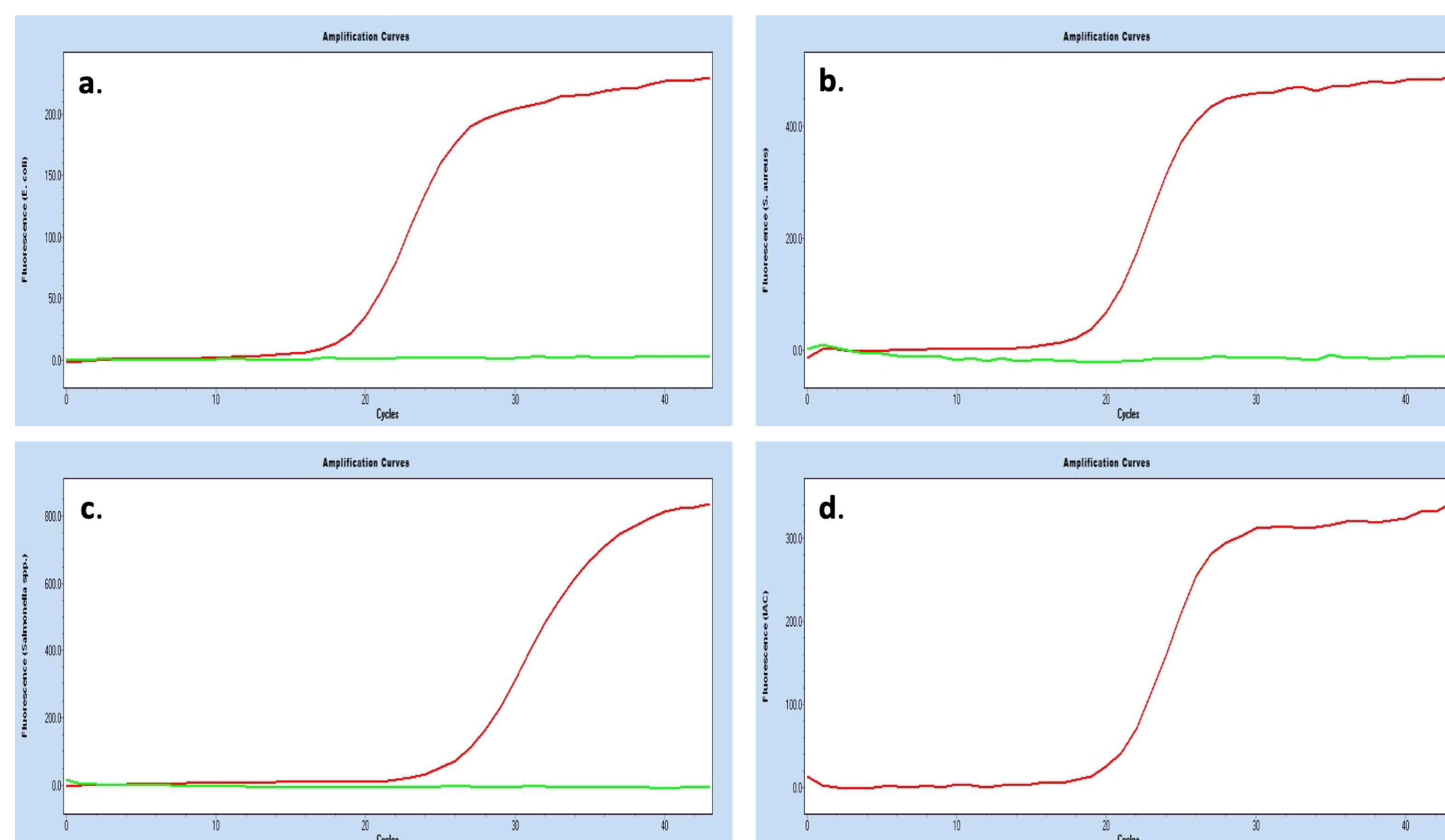


Figure 2. NutraPlexPro representative graphs from matrix trials a) *E. coli*, b) *Staphylococcus*, c) *Salmonella* channels, d) Internal Amplification Control

Note: *each matrix spiked at ≤800CFU/test portion. Samples were enriched in were grown in 1:10 Nutraceutical Universal Enrichment (NUB) broth at 35°C±2° for 24-28h. All presumptive results were confirmed by culture based methods. These graphs are representative from the following list of samples: eggs (5), vitamins (1), fruit drinks (5), organics (5), plant extracts (4), probiotics (5), and protein supplements (10).

Sample Description*	Analysis	Analysis Results	
		NutraPlexPro	Culture-based
Spike with >1000 CFU/mL	<i>S. aureus</i>	(+)	(+)
<i>Salmonella enterica</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> **	<i>S. aureus</i>	(+)	(+)
	<i>Escherichia coli</i>	(+)	(+)
	<i>Salmonella</i>	(+)	(+)
200mL NutraPlexPro:	<i>S. aureus</i>	(+)	(+)
<i>Salmonella enterica</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> (low)	<i>Salmonella</i>	(+)	(+)
	<i>E. coli</i>	(+)	(+)
Spike with 25 CFU/mL	<i>S. aureus</i>	(+)	(+)
<i>Salmonella enterica</i> , <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> **	<i>E. coli</i>	(+)	(+)
	<i>Salmonella</i>	(+)	(+)
	<i>Staphylococcus aureus</i> **	(+)	(+)
Spike with >1000 CFU/mL	<i>S. aureus</i>	(+)	(-)
<i>Escherichia coli</i> , and 25 CFU/mL <i>Staphylococcus aureus</i> **	<i>E. coli</i>	(+)	(+)
	<i>Salmonella</i>	NA	N/A
Spike with >1000 CFU/mL	<i>S. aureus</i>	(-)	(-)
<i>Bacillus subtilis</i>	<i>E. coli</i>	(-)	(-)
	<i>Salmonella</i>	(-)	(-)
	<i>Staphylococcus aureus</i>	(-)	(-)
200mL NutraPlexPro:	<i>S. aureus</i>	(+)	(+)
<i>Staphylococcus aureus</i> spike (high)	<i>E. coli</i>	(-)	(-)
	<i>Salmonella</i>	(-)	(-)
200mL NutraPlexPro:	<i>S. aureus</i>	(+)	(+)
<i>Staphylococcus aureus</i> spike (low)	<i>Salmonella</i>	(-)	(-)
	<i>E. coli</i>	(-)	(-)
200mL NutraPlexPro:	<i>S. aureus</i>	(-)	(-)
<i>Salmonella enterica</i> spike (high)	<i>Salmonella</i>	(+)	(+)
	<i>E. coli</i>	(-)	(-)
200mL NutraPlexPro:	<i>S. aureus</i>	(-)	(-)
<i>Salmonella enterica</i> spike (low)	<i>Salmonella</i>	(+)	(+)
	<i>E. coli</i>	(-)	(-)
200mL NutraPlexPro:	<i>S. aureus</i>	(-)	(-)
<i>Escherichia coli</i> spike (high)	<i>Salmonella</i>	(-)	(-)
	<i>E. coli</i>	(+)	(+)
200mL NutraPlexPro:	<i>S. aureus</i>	(-)	(-)
<i>Escherichia coli</i> spike (low)	<i>Salmonella</i>	(-)	(-)
	<i>E. coli</i>	(+)	(+)
200mL of NUB (Negative Control)**	<i>S. aureus</i>	(-)	(-)
	<i>E. coli</i>	(-)	(-)
	<i>Salmonella</i>	(-)	(-)

*Samples were enriched in were grown in Nutraceutical Universal Enrichment (NUB) broth at 35°C±2° for 24-28h. High spike refers to >1000CFU/ml, while low spike refers to 200-800 CFU/ml.

** Data were collected in triplicate. Culture plate-based testing, MacConkey Agar was used for *E. coli*, Mannitol Salt Agar was used for *S. aureus*, and for *Salmonella* spp., XLD Agar was used with incubation at 30-35°C for 18-24 hours.

Table 2. NutraPlexPro comparative performance data against the culture methods as a reference.

CONCLUSIONS

- The NutraPlexPro kit has displayed:
 - Inclusivity was 100%, and no exclusivity strains were detected.
 - The analytical sensitivity of target strains was 10³- 10⁴ CFU/ml (data not shown).
 - Target organisms detection in a myriad of matrices, even in some of the more difficult matrices. In certain other cases, mitigation steps would allow for target organisms detection (data not shown).
 - Additionally, the NutraPlexPro was compared to traditional plating methods and was determined to show equivalent, if not better, sensitivity than the traditional plating methods.
- Overall, the GENE-UP NutraPlexPro provides a reliable microbiology testing option in food, nutraceutical, and dietary supplement matrices compared to the traditional methodologies for the detection of select pathogens i.e. *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*.