Rapid *Salmonella* confirmation method

Using Neogen's ANSR® for *Salmonella* as a rapid confirmation method

Introduction

Identification of presumptive *Salmonella* colonies from selective/differential agar media as *Salmonella* species has historically been achieved using a variety of biochemical and serological procedures. In the case of food and environmental sample analysis, these procedures are specified in reference methods such as those in the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM) and the U.S. Department of Agriculture's Microbiology Laboratory Guidebook (MLG). These methods include conventional biochemical tests, miniaturized biochemical test devices, automated biochemical identification platforms, and serological agglutination tests using *Salmonella*-specific antisera.

The biochemical identification procedures, although accurate and reliable, generally require 4-6 days to obtain results. As an adjunct or alternative to biochemical, nucleic acid-based identification methods hold promise for providing timely and accurate results. This has been acknowledged, for example, by reference in both BAM and MLG to use of nucleic acid-based methods for identification of *Listeria monocytogenes*.

The nucleic acid-based assay is an isothermal nucleic acid amplification procedure, based on the nicking enzyme amplification reaction (NEAR) technology. The method has been evaluated in three AOAC Performance Tested Method℠ validation studies, leading to certification as PTM method 061203, with claims for a wide variety of food and environmental sample types. In these studies, the method exhibited sensitivity comparable to that of the FDA/BAM and USDA/MLG reference culture methods by probability of detection statistical analysis, as well as > 99% inclusivity and 100% exclusivity in testing of target and non-target bacteria.

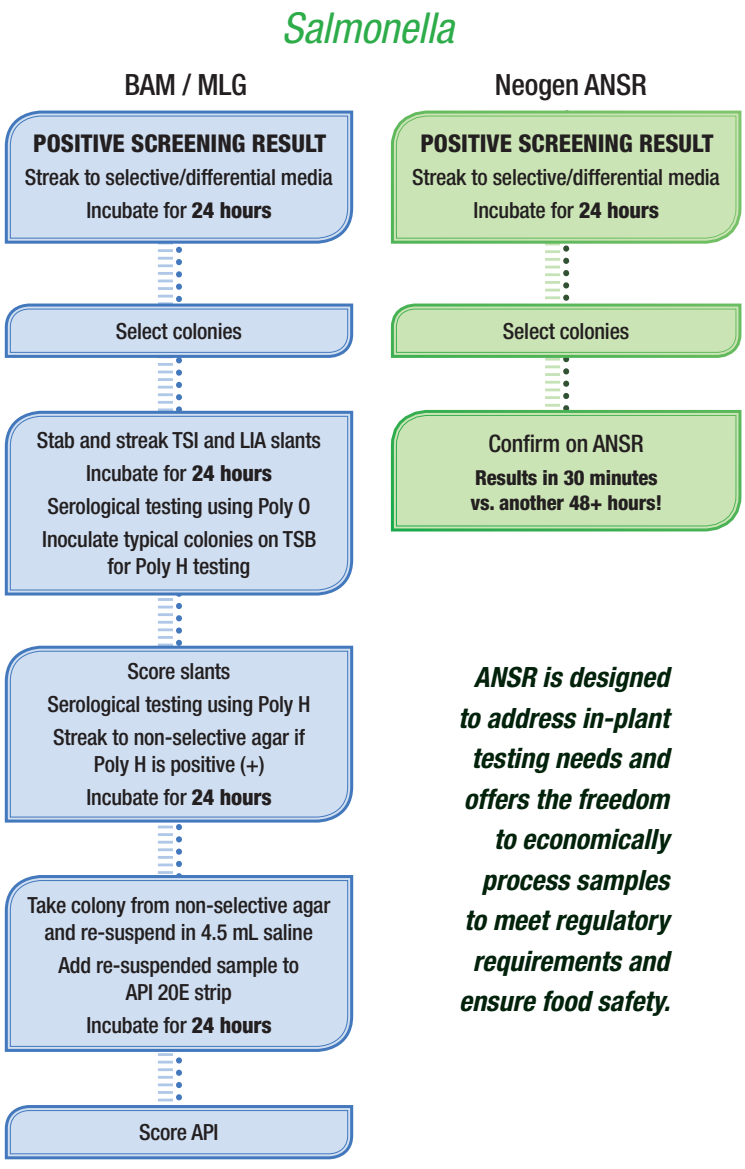
The method's performance in the validation studies, combined with how easy it is perform and its speed at less than 40 minutes, suggested that the method may be used as a confirmation of the identification of presumptive *Salmonella* specie isolates from selective/differential agar plating media. To look into its effectiveness as a confirmatory method, in-house pre-collaborative and interlaboratory collaborative studies were performed in which colonies of *Salmonella* strains and non-*Salmonella* strains were picked from various selective/differential agar media and tested with the assay.

This paper presents the results of that studies, and thus, evaluates the ANSR test system's performance as a rapid confirmation method for *Salmonella*.

ANSR assay principles

ANSR is an isothermal nucleic acid amplification assay based on the nicking enzyme amplification reaction (NEAR™) technology. The amplification mechanism involves the binding of an oligonucleotide "template" to a specific sequence of target DNA. The template contains a recognition site for a specific endonuclease. The nicked strand is recognized as damaged and repaired by the action of a thermostable DNA polymerase, displacing the original strand with the newly-synthesized repaired portion. This displaced DNA "product" then binds to a second template and the same reactions lead to formation of a second product. The second product is homologous to the target sequence and is detected using a specific molecular beacon probe. A fluorescent signal is generated in real time, with amplification and detection complete in as little as 10 minutes.

The entire assay is conducted at a constant temperature of 56˚C using a temperature-controlled fluorescence detection instrument. Assay software analyzes the fluorescent signal through time and a data interpretation algorithm interprets results as negative, positive, or invalid based on baseline, rate-of-change, and other criteria. Each tube of ANSR reagents also contains an internal positive control, signaling in a second fluorescence channel irrespective of the presence of target DNA, and indicating proper functioning of the amplification reagents.



ANSR is designed to address in-plant testing needs and offers the freedom to economically process samples to meet regulatory requirements and ensure food safety.

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Using ANSR for *Salmonella* as a rapid confirmation method

Pre-collaborative study

To look into its effectiveness as a confirmatory method, Neogen performed a pre-collaborative study in which colonies of 113 *Salmonella* strains and 37 non-*Salmonella* strains were picked from tryptic soy agar (TSA) and six selective/differential agar media and tested with the assay. Of those tested, 112 *Salmonella* spp. strains produced positive results from all seven media, for inclusivity of 99.1%. One strain of *S.* Weslaco, previously identified as a non-inclusive strain lacking the genetic target for the assay, produced negative results from all seven media. In testing of exclusive strains, 248 of 251 assays produced negative results, for accuracy of 98.8%.

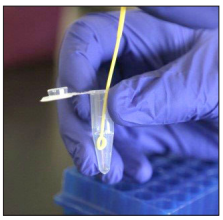
Collaborative study

A collaborative study was conducted to evaluate performance of the assay for identification of *Salmonella* spp. from colony picks taken from selective/differential agar media. A total of 18 laboratories representing industry, government, academic, and commercial testing laboratories participated in the study. Each collaborator tested up to 84 samples, comprised of colony picks of six *Salmonella* spp. and six non-salmonellae taken from six selective/differential agar media as well as tryptic soy agar. A total of 1,441 analyses were performed, of which 1,416 gave the correct identification, for overall accuracy of 98.3%. For identification of *Salmonella* spp., 755 of 756 tests (99.9%) produced the correct result. For identification of non-salmonellae as such, 661 of 685 assays (96.5%) produced the correct result.

See Tables 1, 2 and 3 for inclusive and exclusive isolates used, the results of the exclusive isolates, and results by medium used.

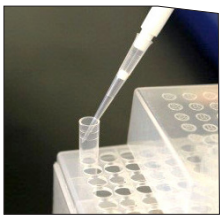
ANSR procedural workflow

1. Pick a colony with an inoculating loop or needle.



2. Resuspend colony in 500 µL of PBS.

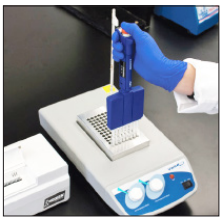
3. Vortex or mix to ensure colony is completely resuspended.



4. Add 50 µL of colony resuspension to the cluster tube.

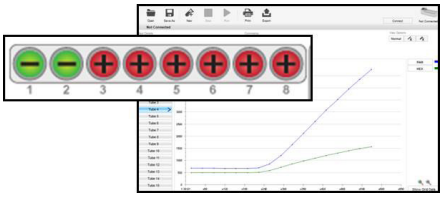
5. Add 450 µL of lysis buffer to the sample.

6. Incubate tubes at 80°C for 20 minutes.



7. Transfer 50 µL of the lysed sample to the lyophilized reagents in the reader.

8. Mix the sample, cap the tubes, close the lid and press START in the ANSR software.



9. Results will be reported in 10 minutes.

Table 1. Inclusive and exclusive isolates used in the ANSR for *Salmonella* collaborative study

Organism	ID No.	Source	Origin (if known)
Salmonella enterica subsp. *arizonae*	700156	ATCC[a]	poult
Salmonella enterica subsp. *enterica* Ser. Typhimurium	23566	ATCC	unknown
Salmonella enterica subsp. *enterica* Ser. Cubana	12007	ATCC	unknown
Salmonella bongori	43975	ATCC	unknown
Salmonella enterica subsp. *enterica* Ser. Cerro	10723	ATCC	unknown
Salmonella enterica subsp. *enterica* Ser. Enteritidis	4931	ATCC	human GI tract
Enterobacter cloacae	13047	ATCC	human CSF
Escherichia coli	25922	ATCC	human
Proteus vulgaris	29905	ATCC	unknown
Providencia alcalifaciens	27970	ATCC	feces
Citrobacter freundii	8090	ATCC	unknown
Klebsiella pneumoniae	13883	ATCC	unknown

[a]*American Type Culture Collection, Manassas, VA*

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Table 2. Interlaboratory study results for the ANSR for *Salmonella* test: Exclusive isolates

Organism	Correct	Misidentified	Total
Salmonella enterica subsp. *arizonae*	126	0	126
Salmonella enterica subsp. *enterica* Ser. Typhimurium	126	0	126
Salmonella enterica subsp. *enterica* Ser. Cubana	126	0	125
Salmonella bongori	126	0	126
Salmonella enterica subsp. *enterica* Ser. Cerro	126	0	126
Salmonella enterica subsp. *enterica* Ser. Enteritidis	125	1	126
Total isolates	**755**	**1**	**756**

Table 3. Results by agar medium

Medium[a]		Correct	Misidentified	Total
BGS	Inclusive	108	0	108
	Exclusive	98	5	103
BS	Inclusive	108	0	108
	Exclusive	99	4	103
DMLIA	Inclusive	108	0	108
	Exclusive	93	3	96
HE	Inclusive	107	1	108
	Exclusive	100	4	104
TSA	Inclusive	108	0	108
	Exclusive	101	3	104
XLD	Inclusive	108	0	108
	Exclusive	101	3	104
XLT-4	Inclusive	108	0	108
	Exclusive	69	2	71
Total	**Inclusive**	**755**	**1**	**756**
	Exclusive	**661**	**24**	**685**

[a] BGS – brilliant green sulfa agar; BS – bismuth sulfite agar; DMLIA – double-modified lysine iron agar; HE – Hektoen enteric agar; TSA – tryptic soy agar; XLD – xylose lysine deoxycholate agar; XLT-4 – xylose lysine tergitol agar.

Conclusion

In the multi-laboratory collaborative evaluation of the assay as a confirmatory method for identification of presumptive *Salmonella* spp. isolates from agar media, it exhibited exceptional accuracy with inclusive strains and also a high degree of exclusivity with non-salmonellae. Of the 18 laboratories participating in the study, 15 reported results with overall accuracy of 99 to 100%.

Based on these results, the ANSR for *Salmonella* was adopted as Official First Action for use as a rapid, accurate adjunct or alternative to biochemical testing for identification of presumptive *Salmonella* spp. isolates.

About Neogen

Neogen's Food Safety Division develops and markets one-stop solutions to simply, rapidly and accurately detect bacterial pathogens, mycotoxins, food allergens, genetic modifications, drug and pesticide residues, ruminant by-products and sanitation concerns in food and animal feed.

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