Clear Safety™ *Salmonella*: Automated Targeted NGS Detection and Serotyping from Sample Enrichment

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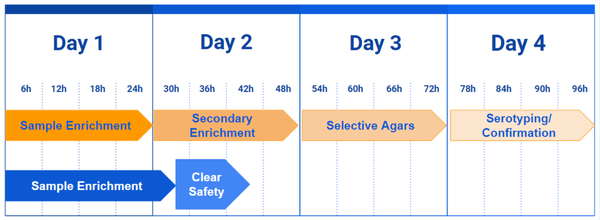
BACKGROUND

Salmonella serotyping is an important tool to help identify strains of concern for implementation of risk-based mitigation strategies. Current methods (traditional anti-sera, DNA arrays, bead-based arrays, and whole genome sequencing) require that analysts culture and isolate *Salmonella* colonies prior to performing the serotyping methods.

Clear Safety™ *Salmonella* uses an automated NGS-based platform to simultaneously detect *Salmonella* and identify the most common serotypes from sample enrichments avoiding the need for isolating *Salmonella* colonies and reducing the time to results from >72h to 40h.



Highlighted Features of Clear Safety *Salmonella*

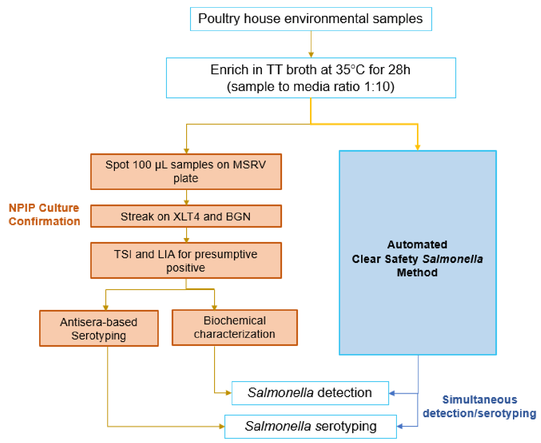
- Targeted NGS generates millions of sequencing reads to detect multiple genes for each target pathogen that allows for built-in redundancy resulting in less false negatives and false positives.
- Automated platform simplifies the workflow minimizing user intervention in performing the assay and result analysis.
- NGS technology allows for high throughput (192 samples) detection of *Salmonella* and serotyping of the 63 most common *Salmonella* serotypes
- Clear Safety *Salmonella* targets DNA from live cells, reducing false positives from dead cell DNA
- Clear Safety *Salmonella* is AOAC and NPIP-approved for detection in food and environmental samples

Figure 1: Clear Safety *Salmonella*: An Automated, Targeted NGS Platform

Integrated, end-to-end automation platform for direct detection and serotyping of *Salmonella* from sample enrichment. Steps include sample lysis, live and dead sample treatment, PCR, library preparation, sequencing and analytics

METHODS

Figure 2: Paired Study Design of NPIP Program Standard and Clear Safety *Salmonella*



Sampling

A total of 363 poultry environmental samples (boot swabs, dust swabs and hatchery waste) sampled from primary production houses were collected following the National Poultry Improvement Plan (NPIP) program standards. Swabs were either pre-moistened or had 30mL of double strength skim milk added to them. Samples were analyzed by three independent laboratories following NPIP recommendations. Samples were stored at refrigerator temperatures (2-4°C) for no more than 5 days.

Enrichment

Poultry environmental samples were enriched in 150mL (1:10 sample to media ratio) of pre-warmed tetrathionate broth (TT) and incubated at 37 ± 2°C for 28-48h.

Clear Safety *Salmonella*

1. 50µL of TT enrichment was added to 450µL CL Prep Solution in a 1.2 mL sample tube in a sample rack.
2. Sample rack and reagents were loaded onto the Clear Safety Platform. Bacteria lysis, PCR, library preparation and sequencing were performed by the automated workflow.

NPIP Reference Method

1. 100µL of TT enrichment were spotted on MSRV incubated at 42 ± 2°C for 24h
2. Outer edge of MSRV growth were streaked on BGN and XLT4 agar, incubated at 35 ± 2°C for 24h
3. Suspect colonies were streaked on TSI and LIA slants incubated at 35 ± 2°C for 24h.
4. *Salmonella* isolates were serotyped with anti-sera.

RESULTS

Table 1: Consolidated Data from Coordinating Labs

Clear Safety	NPIP Reference Method		Totals
	Positive	Negative	
Positive	113	3	116
Negative	5	242	247
Total	118	245	363

In this paired study, 363 poultry environmental samples were analyzed by Clear Safety *Salmonella* and the NPIP program standard. With a Cohen's Kappa measure of inter-rater reliability of 0.95, there is **near perfect agreement** of the two methods for detection of *Salmonella*. Clear Safety *Salmonella* proves to be accurate with diagnostic sensitivity, specificity, positive predictive value and negative predictive values all surpassing the USDA MLG acceptance criteria for alternative methods of > than 90%.

Table 2: Statistical Analysis of the Data

Statistical parameter	Consolidated data	Acceptance criteria
Cohen Kappa	0.95	0.81 - 1.00
Diagnostic sensitivity (dSN)	95.8	> 90%
Diagnostic specificity (dSP)	98.8	> 90%
Positive Predictive Value (PPV)	95.8	> 90%
Negative Predictive Value (NPV)	98.0	> 90%

Clear Safety *Salmonella* serotyping results from sample enrichments agreed with traditional anti-sera serotyping from isolates in 86.1% of the samples.

Many factors may have contributed to the lack of agreement in serotyping results for some samples. While classical anti-sera serotyping is included in the NPIP reference method, the accuracy of this method can vary depending on the quality of anti-sera and experience of analysts. In addition, Clear Safety *Salmonella* results from enrichment could differ from classical serotyping of isolates if the sample contained multiple serotypes and the analyst did not pick enough isolates to get a full representation. Bias in secondary selective enrichments favoring growth of certain serotypes over others could also contribute to these discrepancies.

Table 3: Clear Safety Serotyping vs Classical Anti-sera

	No. of Positive Samples	Serotype Matched with Ref Method	Serotyping Accuracy
Laboratory-1	48	40	83.3%
Laboratory-2	35	30	85.7%
Laboratory-3	25	23	92.0%
Total	108	93	86.1%

CONCLUSION

Clear Safety *Salmonella* is an automated sequencing system that accurately detects *Salmonella* and confirms serotypes 12h after enrichment, compared to the culture confirmation methods that takes around 4-7 days. In this independent study, the Clear Safety *Salmonella* assay has shown high agreement with the reference method. Thus, the findings of this study support the claim that the Clear Safety *Salmonella* assay presents a valuable, affordable, and high throughput automated solution that can improve identification of *Salmonella* in poultry production houses. Clear Safety *Salmonella* offers rapid detection and serotyping that helps food producers make informed decisions in implementing risk-based mitigation strategies to minimize Salmonella contamination and outbreaks.

Table 4: List of Identifiable serotypes from Clear Safety *Salmonella*

Enteritidis	Braenderup	Muenchen	Virchow	Gaminara
Typhimurium	Cerro	Oranienburg	Tennessee	Havana
I 4,[5],12:i:-	Derby	Panama	Liverpool	Idikan
Newport	Dublin	Paratyphi B	Uganda	Lille
Kentucky	Give	Poona	Gallinarum/Pullorum	Pomona
Abaetetuba	Hadar	Reading	Minnesota	Putten
Agona	Heidelberg	Rissen	Ohio	Roodepoort
Alachua	Infantis	Saintpaul	Molade	Muenster
Albany	Javiana	Schwarzengrund	Litchfield	Norwich
Anatum	Johannesburg	Senftenberg	Sandiego	Worthington
Bareilly	Mbandaka	Stanley	Meleagridis	Ouakam
Berta	Mississippi	Thompson	Barranquilla	
Blockley	Montevideo	Typhi	Cubana	

The Clear Safety *Salmonella* platform offers molecular sequence-based serotyping of 63 serotypes that is **98.4% of most commonly identified** in the poultry industry.

For more information regarding Clear Safety *Salmonella*, please contact us at inquiries@clearlabs.com

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