Clear Labs

An automated next-generation sequencing method for simultaneous detection and serotyping of *Salmonella* directly from enrichments

Atul K. Singh, Andrew Lin, Anay Campos, James Maloney, Adam Allred, Justin Ng, Prasanna Thwar, and Ramin Khaksar

BACKGROUND

From 2017 to 2021, the number of chicken samples that tested positive for *Salmonella* by USDA-FSIS decreased by 50% *(1)*. Despite significant progress in reducing the prevalence of *Salmonella* in poultry, the number of *Salmonella* illnesses has remained consistent at ~15 illnesses/100,000 people in the U.S. *(2)* Numerous studies have indicated that prevalence alone is not an adequate measure of the risk associated with poultry, and that other variables, such as the *Salmonella* serotype should be considered. *(1, 3, 4, 5)*. Over 2,600 *Salmonella* serotypes have been identified to date, and different *Salmonella* serotypes exhibit varying levels of virulence and pathogenicity *(6)*. CDC estimates that most illnesses are caused by fewer than 100 of these serotypes, with the 20 most prevalent serotypes being responsible for approximately 70% of all *Salmonella* infections in the U.S. *(1)*. USDA has considered focusing on the *Salmonella* serotypes that are more likely to cause illness *(1)* similar to the strategy of designating STEC O serogroups as adulterants in raw beef products *(1)*. One impediment to this shift in strategy is the misperception that there are no affordable, rapid serotyping tests available. Indeed, most current serotyping methods are only capable of analyzing an isolated *Salmonella* colony, which can take approximately 6 days to obtain. However, automated targeted NGS, such as Clear Safety™ *Salmonella,* can provide rapid, affordable, high-throughput serotyping results.

Here we evaluate Clear Safety™ *Salmonella* for detection and serotyping accuracy. Clear Safety™ *Salmonella* is able to identify the 63 serotypes listed in Table 1. A total of 251 *Salmonella* and 40 non-*Salmonella* pure reference cultures were analyzed with Clear Safety™ *Salmonella* to evaluate serotyping accuracy. Additionally, chicken carcass rinses were artificially contaminated with 20 different serotypes and pre-enriched in Clear *Salmonella* Media and analyzed with Clear Safety™ *Salmonella* to evaluate its ability to accurately serotype from sample enrichments.

Table 1: 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	

METHODS

Sample Preparation

1) Pure Reference Cultures
   a) *Salmonella* and non-*Salmonella* reference cultures were collected from various sources: ATCC, American Type culture collection; CLCB, Clear Labs Culture Bank; CLSI, Cornell Life Science Institute; NCTC, National Collection of Type Cultures UK; NZRM, New Zealand Reference Material; BEI resources established by National Institute of Allergy and Infectious Diseases (NIAID); DARPA SAFE, Defense Advanced Research Project Agency Systems and Assays for Food Examination.

   a) All reference cultures were grown in 4 mL of tryptic soy broth (TSB) incubated at 35ºC for 16-24 h.

1) Artificially Contaminated Chicken Carcass Rinse
   a) Six whole chicken carcasses were rinsed according to USDA Microbiological Laboratory Guidelines 4.13 *(7)*. Whole carcasses were placed in a sterile bag and rinsed by adding 400 ml of sterile buffered peptone water (BPW; Accumedia, Neogen, Lansing, MI) and shaking the bag for 1 min by hand in a rocking motion. Rinsates were removed to new bag with a sterile pipette.

   a) Thirty mL aliquots of carcass rinsates were artificially inoculated with 4 CFU/sample of representative strains from each 20 *Salmonella* serotypes commonly associated with human-illness (See Table 3)

   a) Inoculated carcass rinsates were mixed with 30 mL of Clear *Salmonella* Media (CSM; Clear Labs, San Carlos, CA) supplemented with 20 mg/L of novobiocin and incubated for 24h at 35ºC).

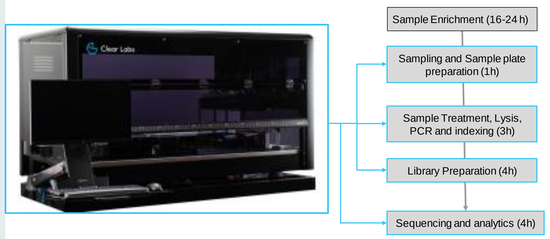
Clear Safety™ *Salmonella* Assay

1) Sample Plate Preparation
   a) Remove 50 µL homogenized enriched sample and combine it with 450 µL CL Prep Solution in the sample rack containing the sample tubes.

   a) Load the sample rack, reagents, plates, tips, and flow cell onto the Clear Safety™ platform according to the manufacturer's instructions.

   a) Start Automated Workflow.

Figure 1: Clear Safety *Salmonella*



An integrated, end-to-end automated platform for direct detection and serotyping of *Salmonella* from sample enrichments. Automated workflow includes sample lysis, live and dead sample treatment, PCR, library preparation, sequencing and analytics

RESULTS

Table 2: Summary of Clear Safety™ *Salmonella* results for Pure Reference Cultures

Strains	Number of strains tested	Number of strains correctly identified		Detection accuracy[a]	Serotyping accuracy[b]
		Salmonella spp.	Serotype		
Inclusive strains					
S. enterica subsp. *Enterica* [c]	206	206	206	100%	100%
Non-claimed serotypes	24	24	N/A	100%	N/A
S. enterica subsp. *non-enterica*	17	17	N/A	100%	N/A
S. bongori	4	4	N/A	100%	N/A
Exclusive strains (non-targets)					
Non-*Salmonella*	40	0	N/A	0%	N/A

[a] Detection accuracy: Refers to positive detection of *Salmonella* genus with Clear Safety™ *Salmonella*.

[b] Serotyping accuracy: Refers to identification of correct serotype with Clear Safety™ *Salmonella*.

[c] 62 of the 63 serotypes within the scope of Clear Safety™ were tested

N/A: Not applicable

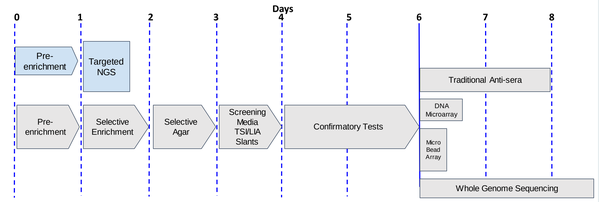
Table 3: Detection and Serotyping of *Salmonella* in poultry carcass rinse enrichments [a] using Clear Safety™ *Salmonella*

S. enterica subsp. *enterica* serotypes	Strain ID	Clear Safety™ *Salmonella* Results	
		Detection [b]	Serotyping
Kentucky	ATCC 9263	Positive	Kentucky
Enteritidis	DARPA SAFE 79	Positive	Enteritidis
Newport	DARPA SAFE 1	Positive	Newport
Typhimurium	ATCC 14028	Positive	Typhimurium
Javiana	ATCC 10721	Positive	Javiana
I 4,[5],12:i:-	DARPA SAFE 74	Positive	I 4,[5],12:i:-
Infantis	CLCB 510	Positive	Infantis
Muenchen	CLCB 236	Positive	Muenchen
Montevideo	ATCC 8387	Positive	Montevideo
Braenderup	CLCB 7	Positive	Braenderup
Thompson	CLCB 9	Positive	Thompson
Saintpaul	CLCB 1038	Positive	Saintpaul
Heidelberg	DARPA SAFE 3	Positive	Heidelberg
Oranienburg	CLCB 18	Positive	Oranienburg
Mississippi	CLCB 251	Positive	Mississippi
Typhi	CLCB 268	Positive	Typhi
Bareilly	ATCC 9115	Positive	Bareilly
Berta	ATCC 8392	Positive	Berta
Agona	CLCB 219	Positive	Agona
Paratyphi B	ATCC 51962	Positive	Paratyphi B
Anatum	ATCC 9270	Positive	Anatum
No Spike	Not applicable	Negative	Not applicable

[a] Carcass rinse samples were artificially inoculated with 4 CFU of each serotype and enriched in CSM at 35°C for 24h.

[b] Clear Safety™ *Salmonella* assay was used to detect serotypes of *Salmonella enterica* subsp. *enterica*

Figure 2: Serotyping Timelines



After enriching samples for 16-28h, automated targeted NGS provided serotyping results in 12h for a total time from sample-to-result of 28-40h. All other serotyping methods require at least 6 days to produce confirmed *Salmonella* isolates in addition to the time to perform the serotyping analysis *(8)*. Times listed here are minimum as some strains may need longer incubation times and some steps may need to be repeated.

CONCLUSIONS

- Clear Safety™ *Salmonella* provided 100% accurate detection of *Salmonella* genus (251/251) and 100% accurate serotyping results (206/206) for serotypes within the scope of Clear Safety, with no false positives among 40 non-*Salmonella* strains
- Clear Safety™ *Salmonella* provided 100% accurate serotyping results from artificially contaminated chicken carcass rinse sample enrichments
- Targeted NGS can identify serotypes from bacterial communities, such as sample enrichments, eliminating the need for time consuming and labor-intensive steps of isolating *Salmonella* colonies
- With the Clear Safety™ platform, users only need to prepare samples and load the reagents and consumables. Sample lysis, PCR, library preparation and sequencing are done automatically without any user intervention

References

(1) USDA-Food Safety Inspection Services: Proposed Regulatory Framework to Reduce Salmonella Illnesses Attributable to Poultry 2022. https://www.fsis.usda.gov/sites/default/files/media_file/documents/FINAL-Salmonella-Framework-10112022-508-edited.pdf
(2) Pew Charitable Trust 2021 https://www.pewtrusts.org/en/research-and-analysis/data-visualizations/2021/12-charts-explore-americas-salmonella-problem-and-steps-to-solve-it
(3) Oscar T. *Salmonella* Prevalence Alone Is Not a Good Indicator of Poultry Food Safety. Risk Anal. 2021 Jan;41(1):110-130.
(4) Oscar TP. Process risk model for *Salmonella* and ground chicken. J Appl Microbiol. 2019 Oct;127(4):1236-1245.
(5) U.S. Government Accountability Office (GAO) GAO-14-744, 2014. Food Safety:USDA Needs to Strengthen Its Approach to Protecting Human Health from Pathogens in Poultry Products. https://www.gao.gov/products/gao-14-744
(6) CDC *Salmonella*. https://www.cdc.gov/salmonella/reportspubs/salmonella-atlas/serotyping-importance.html
(7) USDA Microbiology Laboratory Guidebook, 2023. https://www.fsis.usda.gov/sites/default/files/media_file/documents/MLG-4.13.pdf
(8) Yoshida C, Gurnik S, Ahmad A, Blimkie T, Murphy SA, Kropinski AM, Nash JH. Evaluation of Molecular Methods for Identification of Salmonella Serovars. J Clin Microbiol. 2016 Aug;54(8):1992-8.

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

1559 Industrial Road
San Carlos, CA 94070

Disclaimer:
Clear Safety™ *Salmonella* is for Research Use Only (RUO) and is not intended for diagnostic purposes.

Presented at: IAFP 2023 Toronto · Ontario Canada July 16-19