Platform and Reagent Extensions of a *Salmonella* Loop-Mediated Isothermal Amplification (LAMP) Method in Animal Food

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Abstract

The *Salmonella* LAMP method was recently added to the FDA's Bacteriological Analytical Manual (BAM) to screen animal food for *Salmonella* contamination and to confirm presumptive *Salmonella* isolated from any food source. In this study, we extended the *Salmonella* LAMP method to two platforms (Genie II and AB 7500 Fast) and three master mixes (ISO-001, ISO-004, and NEB WarmStart) for a total of six platform-master mix combinations. Sensitivity was evaluated with a serially diluted *Salmonella* Typhimurium LT2 culture, inclusivity with 100 different *Salmonella* serovars, and exclusivity with 30 non-*Salmonella* strains. Seven animal food matrices were evaluated including dry cat food, dry dog food, cattle feed, dairy feed, horse feed, poultry feed, and swine feed. All six platform-master mix combinations showed comparable sensitivity, consistently detecting down to the 1 cell/reaction level, except for the AB 7500 Fast-NEB WarmStart kit which was 10-fold less sensitive. All six combinations were comparable in inclusivity and exclusivity. In animal food matrices, all six combinations had comparable performance in probability of detection among fractional samples as compared to the FDA's BAM reference culture method for *Salmonella*. There was no difference between the Genie II and the AB 7500 FAST platforms, which are available in almost all FDA field regulatory laboratories. Regardless of platform, master mix ISO-004 was the fastest, frequently detecting positives within 5 min of the real-time assay starting. In conclusion, LAMP was demonstrated to be a versatile and robust molecular method, highly amenable to different laboratory workflows. This improvement in *Salmonella* testing methods will greatly enhance the efficiency of FDA's human and animal food testing programs for FSMA compliance, ensuring the safety of these FDA-regulated products.

Introduction

- **LAMP for *Salmonella* detection**
  - Rapid, reliable, and robust isothermal amplification assay successfully applied in human food and animal food[1]
  - Completed SLV in six animal food matrices (dry cat food, dry dog food, cattle feed, horse feed, poultry feed, and swine feed)[2]
  - Completed MLV in dry dog food[3]
  - Incorporated into BAM Chapter 5 *Salmonella* as a screening method in animal food and confirmation method for presumptive *Salmonella* isolates from all food categories[4]
- **LAMP is a versatile molecular method**
  - Amenable to multiple assay platforms and various reagent choices
  - Evaluation on these platforms and new reagents has not been done
- **Objective**

To conduct platform and reagent extensions on the *Salmonella* LAMP assay in a variety of animal food matrices.

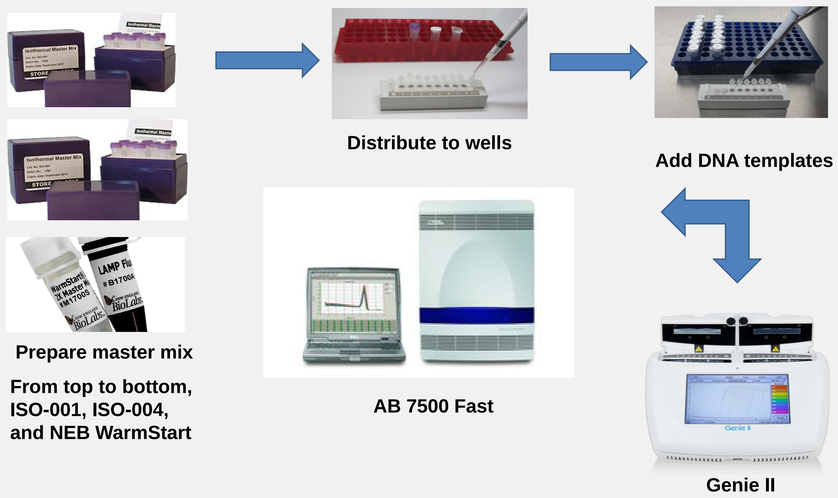
Materials and Methods

- **Study design followed "FDA's Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, 3rd edition – Section 5.0. Method Modification and Method Extension Criteria for Existing Validated Microbiology Methods"**

Table 1. Samples, platforms, and reagents tested in this study

Sample DNA extract	LAMP reagent	Platform	
		Genie II	AB 7500 Fast
Limit of detection	ISO-001	SLV	This study
	ISO-004	This study	This study
	NEB WarmStart	This study	This study
Inclusivity	ISO-001	SLV	This study
	ISO-004	This study	This study
	NEB WarmStart	This study	This study
Exclusivity	ISO-001	SLV	This study
	ISO-004	This study	This study
	NEB WarmStart	This study	This study
Seven animal food matrices (dry cat food, dry dog food, cattle feed, dairy feed, horse feed, poultry feed, and swine feed)	ISO-001	SLV	This study
	ISO-004	This study	This study
	NEB WarmStart	This study	This study

Fig. 1. LAMP flowchart



Results

- All six platform-master mix combinations showed comparable sensitivity, consistently detecting down to the 10^0 cell/reaction level, except for AB 7500-NEB WarmStart kit which was 10-fold less sensitive. All six combinations were comparable in inclusivity and exclusivity

Fig. 2. LAMP amplification graphs on AB 7500 Fast and Genie II

- Regardless of platform, master mix ISO-004 was the fastest (< 5 min), followed by ISO-001 and NEB WarmStart.
- In animal food matrices, all six combinations had comparable performance in probability of detection among fractional samples and compared to the BAM reference culture method.

Table 2. LAMP positive detection in animal food matrices

Platform/ Reagent	Dry cat food	Dry dog food	Cattle feed	Dairy feed	Horse feed	Poultry feed	Swine feed
BAM	11 (55)	6 (30)	4 (20)	18 (90)	11 (55)	8 (40)	2 (10)
Genie-001	10 (50)	6 (30)	8 (40)	17 (85)	12 (60)	10 (50)	2 (10)
Genie-004	10 (50)	6 (30)	8 (40)	17 (85)	14 (70)	12 (60)	2 (10)
Genie-NEB	10 (50)	6 (30)	8 (40)	16 (80)	11 (55)	9 (45)	2 (10)
AB-001	10 (50)	6 (30)	8 (40)	17 (85)	15 (75)	7 (35)	2 (10)
AB-004	10 (50)	6 (30)	8 (40)	18 (90)	12 (60)	14 (70)	2 (10)
AB-NEB	12 (60)	5 (25)	4 (20)	5 (25)	11 (55)	6 (30)	2 (10)

Conclusions

- LAMP was amenable to any of these six platform-master mix combinations. From a user-friendly perspective, Genie II-ISO-004 will be chosen for future matrix extension work in food matrices.

FDA Missions Relevance: Improvement in *Salmonella* testing methods will greatly enhance the efficiency of FDA's food and feed testing programs for FSMA compliance.

References & Disclaimer

References

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3. Ge, B. et al. 2019. Front Microbiol. 10:562.
4. Andrews, W. H. 2020. *Bacteriological Analytical Manual* Chapter 5: *Salmonella*. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella

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