Assessing Robustness of *Salmonella* and *Listeria* detection by Loop-mediated AMPlification (LAMP)

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Abstract

Validation demonstrates that methods perform “as advertised.” For major pathogen platforms “official” validations typically cover one enrichment temperature and a narrow range of incubation times. Unfortunately, many issues, from equipment failure to human error, can disrupt the validation envelope causing delays or loss of results.

Our objective was to determine the robustness of LAMP pathogen assays by testing wide variations in temperature, incubation time and other conditions, to facilitate efficient laboratory operation in “real world” conditions.

Thirty-two samples of cheese were inoculated with ca 1.5 CFU/25g of *Salmonella abaetetuba*. Samples were enriched 1/10 in Buffered Peptone Water, incubated at 30, 35, 37 or 42°C, and tested using 3M *Salmonella* MDA2 after 18, 24, 30 and 48 hours.

Thirty-four samples of cheese were inoculated with *Listeria monocytogenes* at 10-15 CFU/25g. Samples were enriched 1/10 in Demi-Fraser broth incubated at 30, 37 or 42°C; 17 with Ferric Ammonium Citrate (FAC) and 17 without, and tested using 3M *Listeria* species and *Listeria monocytogenes* MDA2 kits after 24, 30, 35 and 48 hours.

For *Salmonella*, the probability of detection (POD), calculated following AOAC guidelines, was similar between all incubation times and temperatures, with POD in the range 0.6 – 0.8. For *Listeria* spp., all samples were positive for all incubation conditions (POD 1.00). The *Listeria monocytogenes* assay was positive for all conditions, except for one sample enriched with no-FAC and incubated at 30°C for 48 hours. Within the range tested, these variables did not affect performance of the methods.

Data suggest that LAMP detection of *Listeria* (spp. and *monocytogenes*) and *Salmonella* following enrichment at 35°C instead of the AOAC validated 37°C entails no loss of performance.

Detection of *Salmonella, Listeria* spp. and *L. monocytogenes* by LAMP using appropriate 3M MDA2 kits appears to be very robust to significant incubation perturbations, showing that all three methods should remain reliable following a range of equipment failure or human error.

Introduction

- Rapid pathogen detection methods are officially validated for a narrow set of conditions
- Many reasons, from operational efficiency to human error, make understanding the robustness of methods to a wide range of perturbations valuable
- We set out to investigate the robustness of *Listeria* spp., *L. monocytogenes*, and *Salmonella* spp. assays using a commercially available LAMP platform

What is Loop-Mediated Isothermal Amplification (LAMP)?

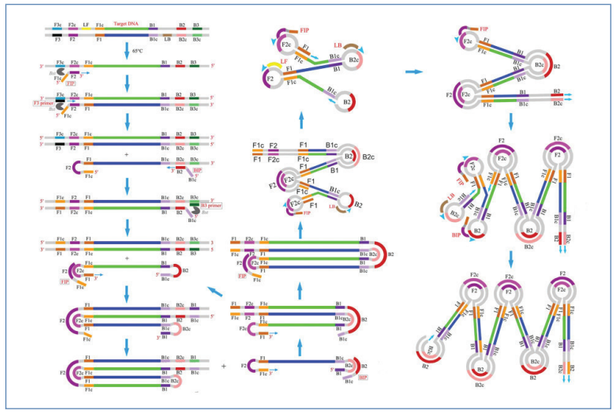


Image from Li, et al., 2016, Frontiers in Plant Science

A different DNA amplification technology:

Primer Design:
- recognize distinct target regions of DNA
- create stem loops through duplication of target regions

Bst DNA Polymerase:
- 5' to 3' polymerase with strand displacement
- Continuous amplification at a constant temperature – no temperature cycling

Amplification:
- Continuous amplification of multiple copies of the region of interest
- Loop structure physically excludes many potential inhibitors

How does detection work?

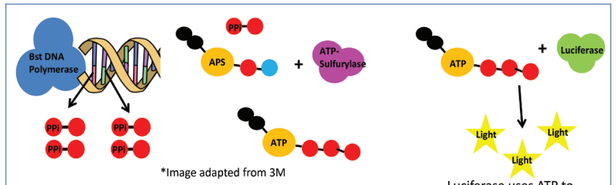


*Image adapted from 3M

Amplification creates multiple copies of the region of interest, releasing PPi (pyrophosphate) as a byproduct.

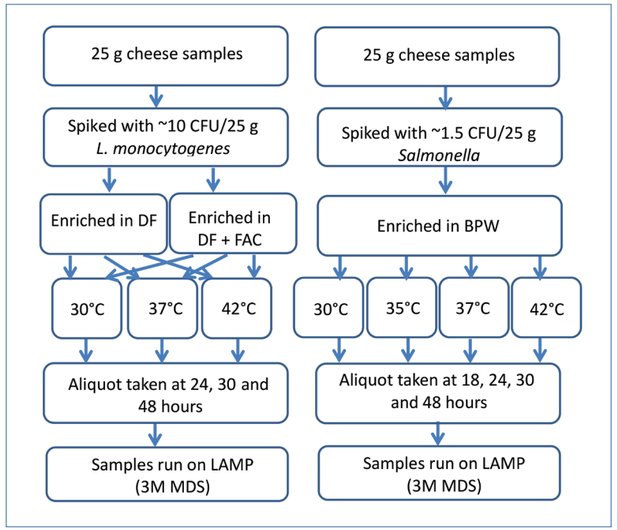
PPi is converted to Adenosine Tri Phosphate (ATP) by ATP-Sulfurylase.

Luciferase uses ATP to generate light. A simple light sensor gives real-time detection in as little as 15 minutes.

Materials and Methods

Materials
- Demi Fraser broth (DF)
- Buffered Peptone Water (BPW)
- Ferric Ammonium Citrate (FAC)
- *Salmonella abaetetuba*, purchased from BioMérieux
- *Listeria monocytogenes*, purchased from Microbiologics®
- LAMP system and kits (3M MDS)

Methods



Results

Listeria spp. and *L. monocytogenes* LAMP assay robustness

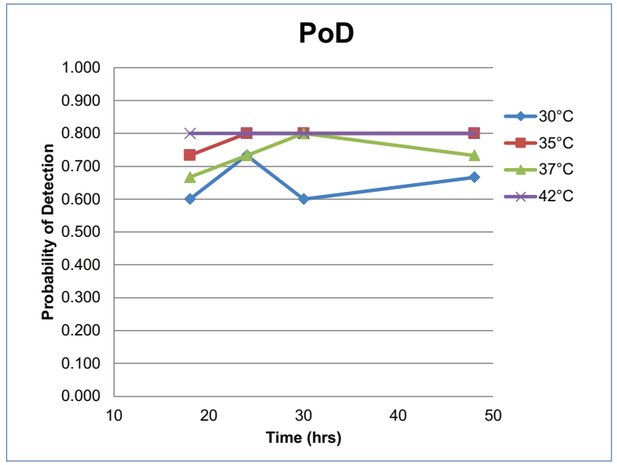
- Seven samples were tested at 30°C and 42°C at 24 and 48 hours
- Three samples were tested at 30°C and 42°C at 30 hours
- Three samples were tested at 37°C for 24, 30 and 48 hours

FAC	Incubation Temp (°C)	Time (hours)	Results (% presumptive) *Listeria* spp.	Results (% presumptive) *L. monocytogenes*
+	30	24	100	100
+	30	30	100	100
+	30	48	100	100
+	37	24	100	100
+	37	30	100	100
+	37	48	100	100
+	42	24	100	100
+	42	30	100	100
+	42	48	100	100
-	30	24	100	100
-	30	30	100	100
-	30	48	100	85.7
-	37	24	100	100
-	37	30	100	100
-	37	48	100	100
-	42	24	100	100
-	42	30	100	100
-	42	48	100	100

While one 30°C, no FAC sample was negative at 48 hours, there is no statistical difference in detection in the presence or absence of FAC in the media, incubation at 30, 37 or 42°C, or incubation time of 24, 30 or 48 hours for either *Listeria* spp. or *L. mono* assays.

Salmonella Assay Robustness

- 15 samples were tested for each incubation temperature at 18, 24 and 48 hours
- 5 samples were tested for each incubation temperature at 30 hours
- Partial recovery due to low level of inoculation allows us to calculate a Probability of Detection (PoD)



A two factor ANOVA with replication showed that there is no statistical difference between incubation temperatures (p-value =0.478), timepoints (p-value =0.700), or any combination of temperatures or timepoints (p-value = 0.999).

Conclusions

- *Listeria* spp. and *L. monocytogenes* LAMP assays are very robust with no differences in detection in the presence or absence of FAC, at temperatures between 30 and 42°C, and at timepoints between 24 and 48 hours
- *Salmonella* spp. LAMP assays are very robust, with no differences in detection at incubation temperatures between 30 and 42°C and at timepoints between 18 and 48 hours

Reference

1. Li, J., C Xiong, Y Liu, J Liang and X Zhou, 2016. “Loop-Mediated Isothermal Amplification (LAMP): “Emergence as an Alternative Technology for Herbal Medicine Identification.” Front. Plant Sci, https://doi.org/10.3389/fpls.2016.01956.

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