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Title: Loop-Mediated Isothermal Amplification for Screening *Salmonella* in Animal Food and Confirming *Salmonella* from Culture Isolation

Authors and Affiliations:

Kelly J. Domesle[1], Shenia R. Young[1], Qianru Yang[1], Beilei Ge[1]

[1]Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, MD, USA

Corresponding Authors:

Beilei Ge (beilei.ge@fda.hhs.gov)

Email Addresses for Co-authors:

kelly.domesle@fda.hhs.gov
shenia.young@fda.hhs.gov
qianru.yang@fda.hhs.gov
beilei.ge@fda.hhs.gov

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all set**

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps.

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Dr. Beilei Ge:** This *Salmonella* LAMP method has been incorporated within the FDA's Bacteriological Analytical Manual, both as a screening method in animal food and a confirmation method for any presumptive *Salmonella* isolates.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Ms. Kelly Domesle:** The method is rapid, reliable and robust. Compared to PCR, its main advantages include rapid testing, simple instrumentation, low false-negative rates, and a flexible workflow.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Ms. Shenia Young:** Rapid screening for pathogens such as *Salmonella* plays an important role in quickly detecting a potentially contaminated product and can prevent illnesses or outbreaks in humans and animals.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Preparation of DNA Samples

2.1. Begin by preparing DNA templates for the LAMP *(pronounce as one word, 'lamp')* assay **[1]**. There are two types of samples used to make DNA templates. To prepare templates from animal food samples, aseptically weigh 25 grams of food sample into a sterile filter bag **[2]**.

2.1.1. WIDE: Establishing shot of talent at the lab bench with the food samples.

2.1.2. Talent weighing the food sample into a sterile filter bag.

~~2.1.3.~~

2.2. Add 225 milliliters of sterile buffered peptone water to the bag **[1]** and incubate it at 35 degrees Celsius for 24 hours **[2]**.

2.2.1. Talent adding BPW to the bag.

2.2.2. Talent putting the bag in an incubator and closing the door.

2.3. After the incubation, transfer 1 milliliter from the filtered side of the bag to a microcentrifuge tube **[1]**. Centrifuge the tube at 900 *x g* for 1 minute **[2]**, then transfer the supernatant to a new microcentrifuge tube **[3]**. *Videographer: This step is important!*

Author NOTE: the centrifuge steps in 2.3 and 2.4 have different speed/time settings. The shots taken are reflective of the actual speed/time needed for step 2.4.1. Please obscure the speed/time in the shots

2.3.1. Talent transferring liquid from the bag to a microcentrifuge tube.

2.3.2. Talent putting the tube in the centrifuge and closing the door. *Videographer: Obtain multiple usable takes because this shot will be reused in 2.4.1.*

2.3.3. Talent transferring the supernatant to a new tube.

2.4. Centrifuge the sample at 16,000 x *g* for 2 minutes **[1]** and discard the supernatant **[2]**. Resuspend the pellet in 100 microliters of the sample preparation reagent **[3]** and heat it at 100 degrees Celsius for 10 minutes in a dry heat block **[4]**. Then, cool the sample to room temperature and store it at -20 degrees Celsius **[5]**.

2.4.1. *Use 2.3.2.*

2.4.2. Talent discarding the supernatant.

2.4.3. Talent resuspending the pellet.

2.4.4. Talent placing the sample on the heat block. NOTE: Use the shot that shows the 100 degree temperature. Videographer NOTE: Mis-slated 2nd 2.4.3.

Videographer: Obtain multiple usable takes because this shot will be reused in 2.5.2.

2.4.5. Talent putting the sample in the freezer. NOTE: the freezer used in the shot was set at -25 degrees Celsius, so please blur the temperature in the shot *Videographer: Obtain multiple usable takes because this shot will be reused in 2.6.1.*

2.5. The second type of sample for this LAMP assay comes directly from presumptive *Salmonella* cultures. To prepare DNA templates, transfer 500 microliters of an overnight *Salmonella* culture to a microcentrifuge tube **[1]** and heat it in a 100-degree Celsius heat block for 10 minutes **[2]**.

2.5.1. Talent transferring overnight culture to a microcentrifuge tube.

2.5.2. *Use 2.4.4.*

2.6. Then, cool the sample to room temperature **[2]** and store it at -20 degrees Celsius **[1]**.

2.6.1. *Use 2.4.5.*

2.6.2. Added shot and please insert it before 2.6.1: Sample cooling on the bench.

3. Assembly of a LAMP Reaction

3.1. To prevent cross-contamination, physically separate the areas used for preparing the LAMP master mix and adding the DNA templates. Clean the work surfaces with isopropanol and a DNA- and DNase-degrading solution **[1]**. Then, clean the pipettes and tube strip holders **[2]**.

3.1.1. Talent cleaning the biosafety cabinet surface, with the isopropanol and DNA- and DNase-degrading solutions in the shot and labeled.

3.1.2. Talent cleaning a pipette or tube strip holder, with both in the shot.

3.2. Thaw the isothermal master mix, 10 X primer mix, molecular grade water, positive control DNA, and DNA templates at room temperature **[1]**. Turn on the LAMP instrument and enter the relevant sample information **[2]**. *Videographer: This step is important!*

3.2.1. Isothermal master mix, 10 X primer mix, molecular grade water, positive control DNA, and DNA templates thawing, with the tubes all labeled.

3.2.2. Talent turning on the LAMP instrument and entering sample information. NOTE: Please blur any FDA barcodes on the instruments

3.3. Prepare the LAMP master mix according to manuscript directions **[1]**, vortex and centrifuge it briefly **[2]**, and distribute 23 microliters of the master mix into each well of a tube strip **[3]**. *Videographer: This step is difficult and important!*

3.3.1. Talent preparing the master mix in a microcentrifuge tube.

3.3.2. Talent vortexing the tube, then centrifuging it.

3.3.3. Talent distributing the master mix into the wells of a tube strip.

3.4. Vortex all DNA templates and centrifuge them briefly **[1]**, then add 2 microliters of DNA template to the appropriate well and cap the well tightly **[2]**. Remove the tube strip from the holder and flick it to ensure all reagents have pooled at the bottom of the tube **[3]**. Load the tube strip into the LAMP instrument and start the LAMP run **[4]**. *Videographer: This step is important!*

3.4.1. Talent vortexing and centrifuging the DNA samples.

3.4.2. Talent adding samples to the wells of the strip, then closing the cap right after the sample is added into each well.

3.4.3. Talent removing the strip from the holder and flicking their wrist.

3.4.4. Talent loading the strip, closing the lid, and starting the run.

3.5. While the LAMP reaction is in progress, tap the **Temperature**, **Amplification**, and **Anneal** tabs to see the dynamic changes of various parameters during the LAMP run **[1]**.

3.5.1. Talent tapping the three tabs during the LAMP run (after about 8 min).

4. LAMP Result Interpretation

4.1. The LAMP results can be viewed on the LAMP instrument panel in real-time or using LAMP software **[1]**. To interpret the results on the instrument panel, open the LAMP run of interest **[2]**.

4.1.1. Talent at the LAMP instrument.

4.1.2. Talent opening the LAMP run of interest. NOTE: 4.1.2 – 4.3.2 in one shot

4.2. Observe the five tabs associated with each run **[1]**. The **Profile** and **Temperature** tabs show programmed and actual temperatures in the sample wells as the LAMP reaction proceeds **[2]**.

4.2.1. Talent pointing to the five tabs.

4.2.2. Talent tapping the **Profile** and **Temperature** tabs.

4.3. The **Amplification** and **Anneal** tabs show fluorescence readings and changes in fluorescence during the amplification and anneal phases, respectively **[1]**. The **Results** tab shows a tabular view of the LAMP results that can be interpreted according to manuscript directions **[2]**.

4.3.1. Talent tapping the **Amplification** and **Anneal** tabs.

4.3.2. Talent tapping the **Results** tab.

4.4. The second way to view LAMP results is using the LAMP software. To interpret the results with the software **[1]**, click on the **Computer** icon on the left panel and navigate to the file location to load the LAMP run of interest **[2]**.

4.4.1. Talent at the computer.

4.4.2. SCREEN: 61239_screenshot.mp4. 0:01 – 0:09.

4.5. Observe the seven tabs associated with each run **[1]**. The **Profile** and **Temperature** tabs show programmed and actual temperatures in the sample wells as the LAMP reaction proceeds **[2]**.

4.5.1. SCREEN: 61239_screenshot.mp4. 0:10 – 0:20. *Video Editor: Emphasize the 7 tabs: Profile, Temperature, Amplification, Amplification Rate, Anneal, Anneal Derivative, and Result.*

4.5.2. SCREEN: 61239_screenshot.mp4. 0:21 – 0:25.

4.6. The **Amplification** and **Amplification Rate** tabs show fluorescence readings and changes in fluorescence during the amplification phase. The **Anneal** and **Anneal Derivative** tabs show fluorescence readings and changes in fluorescence during the anneal phase **[1]**.

4.6.1. SCREEN: 61239_screenshot.mp4. 0:26 – 0:36.

4.7. The **Result** tab shows a tabular view of the LAMP results. There are four columns titled Graph Name, Well Number, Well Name, and Peak Value. The Amp Time and Anneal Derivatives of all eight samples are shown. The results that can be interpreted according to manuscript directions **[1]**.

4.7.1. SCREEN: 61239_screenshot.mp4. 0:37 – 1:02.

Results

5. Representative Results: *Salmonella* Sensitivity Panel

5.1. The LAMP instrument panel and LAMP software can be used to display results of the assay **[1]**. In this LAMP run, samples S1 through S6 are 10-fold serial dilutions of *Salmonella enterica* serovar Infantis ATCC 51741 ranging from 1.1 million to 11 colony forming units per reaction **[2]**.

5.1.1. LAB MEDIA: Figures 2 and 3. *Video Editor: Show 2A next to 3A, 2B next to 3B, 2C next to 3C, 2D next to 3F, and 2E next to 3G.*

5.1.2. LAB MEDIA: Figure 2 E and 3 G. *Video Editor: Zoom in on 2 E and 3 G.*

5.2. The positive control is *Salmonella enterica* serovar Typhimurium LT2 at 17,000 colony forming units per reaction and the no template control, or NTC, is molecular grade water **[1]**.

5.2.1. LAB MEDIA: Figure 2 E and 3 G. *Video Editor: Zoom in on the tables in the photographs.*

5.3. The NTC well has a blank T-max and an anneal temperature of less than 83 degrees Celsius on the LAMP instrument panel, and a blank T-max and blank anneal temperature in the LAMP software **[1]**.

5.3.1. LAB MEDIA: Figure 2 E and 3 G. *Video Editor: Emphasize the NTC row in 2E and 3G.*

5.4. The Positive control well has a T-max of 7 minutes and 45 seconds and annealing temperature of approximately 90 degrees Celsius on both platforms **[1]**.

5.4.1. LAB MEDIA: Figure 2 E and 3 G. *Video Editor: Emphasize the PC rows in both photos (for both Amp time and anneal derivative in 3G).*

5.5. Samples S1 to S6 have T-max between 6 minutes and 30 seconds and 12 minutes and 15 seconds and annealing temperature of approximately 90 degrees Celsius, all indicating a positive detection **[1]**.

5.5.1. LAB MEDIA: Figure 2 E and 3 G. *Video Editor: Emphasize the S1 – S6 rows in 2E and 1 -6 rows in 3G (for both Amp time and anneal derivative in 3G).*

Conclusion

6. Conclusion Interview Statements

6.1. **Ms. Kelly Domesle:** LAMP is very effective and generates a large quantity of DNA, so it is critical that best laboratory practices are used to prevent cross-contamination. Use similar practices when making your DNA templates, as enrichments from contaminated animal food can have high levels of *Salmonella*.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1.*

6.2. **Ms. Shenia Young:** Animal food samples screening positive with LAMP should be confirmed by culture isolation following the procedures in the FDA's Bacteriological Analytical Manual. Negative samples can be reported as such.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

6.3. **Dr. Beilei Ge:** The incorporation of this LAMP method into the FDA's Bacteriological Analytical Manual paves the way for a wider application of this rapid, robust, and user-friendly technology in food safety testing.

6.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.