Screening of *Salmonella* in Animal Food by Loop-Mediated Isothermal Amplification (LAMP)

The LAMP method can be used to screen animal food samples for the presence of *Salmonella* (1). The LAMP assay runs at a constant temperature (2) and exhibits high tolerance to matrix inhibitors (3-5). Samples are enriched in buffered peptone water (BPW) prior to DNA extraction for LAMP. The LAMP assay specifically targets the *Salmonella* invasion gene *invA* (GenBank accession number M90846) (6) and is rapid, reliable, and robust in multiple food matrices (1, 4, 7-11). The limits of detection of the LAMP assay when tested using different *Salmonella* serovars in pure culture ranged from 1.3 to 28 cells per reaction (1). In animal food, the method was capable of detecting 1 CFU in 25 g test portions (1). The LAMP method has been validated in six animal food matrices (dry cat food, dry dog food, cattle feed, horse feed, poultry feed, and swine feed) in a pre-collaborative study (1) and in dry dog food in a multi-laboratory collaborative study (12). A recent method extension study adds dairy feed to the list of validated animal food matrices.

LAMP assembly and data analysis protocols described below are for three instrument platforms: Genie II, Genie III, and AB 7500 Fast. A video article (13) demonstrating the entire protocol on Genie II (from sample preparation to result interpretation) was published, which can be accessed through this [link](#) for the text and another [link](#) for the video. Contact: Beilei Ge, FDA-CVM ([Beilei.Ge@fda.hhs.gov](mailto:Beilei.Ge@fda.hhs.gov)). Use of other platforms and protocols must first be validated per FDA’s Microbiological Methods Validation Guidelines (14), AOAC’s Appendix J (15) or ISO 16140-2 (16), and approved for use by FDA’s Microbiological Methods Validation Subcommittee.

**A. Equipment and Materials**

Items 1-3 are needed for assay runs on Genie II or Genie III, whereas items 4-8 are needed for assay runs on AB 7500 Fast. Items 9-12 are common equipment.

1. Genie II or Genie III (OptiGene Ltd., West Sussex, United Kingdom; also available through select U.S. distributors) capable of temperature control up to 100°C with ± 0.1°C accuracy and simultaneous fluorescence detection via the FAM channel
2. Genie 8-well strips (OptiGene Ltd. #OP-0008)
3. Genie strip holder (OptiGene Ltd. #GBLOCK)
4. AB 7500 Fast (Thermo Fisher Scientific, Waltham, MA) capable of temperature control/cycling and simultaneous fluorescence detection via the FAM channel
5. MicroAmp Fast optical 96-well reaction plates (Thermo Fisher Scientific #4346906) or MicroAmp Fast 8-tube strips (Thermo Fisher Scientific #4358293)
6. MicroAmp optical adhesive film (Thermo Fisher Scientific #4311971) or MicroAmp optical 8-cap strips (Thermo Fisher Scientific #4323032)
7. MicroAmp 96-well support base (Thermo Fisher Scientific #4379590) or equivalent
8. Mini plate spinner centrifuge
9. Pipettes (0.5-10 µl, 2-20 µl, 20-200 µl, and 200-1000 µl) and tips (aerosol-resistant)
10. Vortex mixer
11. Microcentrifuge (capable of spinning at 16,000 × g) and tubes (0.5 to 2 ml)
12. Heat block capable of maintaining 100 ± 1°C

B. Media and Reagents
1. PrepMan Ultra sample preparation reagent (Thermo Fisher Scientific #4318930)
2. Peptone water (0.1%)
3. Isopropanol (70%)
4. DNA AWAY (Thermo Fisher Scientific #7010PK) or equivalent
5. LAMP master mix: three choices, which are GspSSD isothermal master mix (OptiGene Ltd. #ISO-001 for 400 reactions or #ISO-002 for 2,000 reactions), GspSSD2.0 isothermal master mix (OptiGene Ltd. #ISO-004 for 300 reactions or ISO-005 for 1,500 reactions), and WarmStart LAMP kit (New England Biolabs, Ipswich, MA; #E1700S for 100 reactions or #E1700L for 500 reactions)
6. Molecular grade water
7. *Salmonella* LAMP primers and worksheet for preparing the LAMP primer mix (10×)

LAMP primers (Table 1) are synthesized by Integrated DNA Technologies (Coralville, IA) or equivalent with standard desalting purification. Prepare stock solutions of each primer (100 µM) by rehydrating it with appropriate amount of sterile molecular grade water. Mix well by vortexing for 10 s and store at -20°C (-80°C for long-term storage).

**Table 1. LAMP primers for screening *Salmonella* in animal food**

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5’-3’)</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sal4-F3</td>
<td>GAACGTGTCGCGGAAGTC</td>
<td>18</td>
</tr>
<tr>
<td>Sal4-B3</td>
<td>CGGCAATAGCGTCACCTT</td>
<td>18</td>
</tr>
<tr>
<td>Sal4-FIP</td>
<td>GCGCIGCCATCCGCAATCAATA-TCTGGATGGATGATGCCGG</td>
<td>38</td>
</tr>
<tr>
<td>Sal4-BIP</td>
<td>GCGAACGGCGAAGCGTACTG-TCGCCACCCTCAAGGGAAC</td>
<td>38</td>
</tr>
<tr>
<td>Sal4-LF</td>
<td>TCAATCGGCATCAATACTCATCTG</td>
<td>25</td>
</tr>
<tr>
<td>Sal4-LB</td>
<td>AAAGGGAAGGCCAGCTTTACG</td>
<td>21</td>
</tr>
</tbody>
</table>

The primers are designed based on the *Salmonella invA* sequence (GenBank accession number M90846).

Prepare the LAMP primer mix (10×) according to a worksheet (Table 2). Add appropriate volumes of primer stock solutions and sterile molecular grade water into a microcentrifuge tube. Mix all reagents well by vortexing for 10 s. Aliquot to 500 µl per microcentrifuge tube and store at -20°C.
Table 2. Worksheet for preparing the LAMP primer mix (10×)

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sal4-F3 primer (100 µM)</td>
<td>10</td>
</tr>
<tr>
<td>Sal4-B3 primer (100 µM)</td>
<td>10</td>
</tr>
<tr>
<td>Sal4-FIP primer (100 µM)</td>
<td>180</td>
</tr>
<tr>
<td>Sal4-BIP primer (100 µM)</td>
<td>180</td>
</tr>
<tr>
<td>Sal4-LF primer (100 µM)</td>
<td>100</td>
</tr>
<tr>
<td>Sal4-LB primer (100 µM)</td>
<td>100</td>
</tr>
<tr>
<td>Molecular grade water</td>
<td>420</td>
</tr>
<tr>
<td>Total</td>
<td>1,000</td>
</tr>
</tbody>
</table>

8. LAMP controls. Always include a positive control (PC) and a no template control (NTC) in every LAMP run.

a. DNA extracted from a Salmonella reference strain, e.g., Salmonella enterica serovar Typhimurium ATCC 19585 (LT2), may be used as the PC. Inoculate the bacterium on a nonselective agar plate and incubate 24 ± 2 h at 35 ± 2°C. Transfer several single colonies to 5 ml of trypticase soy broth (TSB) or brain heart infusion (BHI) broth and incubate 16 ± 2 h at 35 ± 2°C to reach ca. 10⁸ to 10⁹ CFU/ml. Serially dilute the overnight culture in 0.1% peptone water to obtain ca. 10⁶ to 10⁷ CFU/ml. Transfer 500 µl of this dilution to a microcentrifuge tube and heat 10 min at 100 ± 1°C in a dry heat block. Cool to room temperature and store extracted DNA at -20°C.

Alternatively, a positive culture control may be used as the PC. Inoculate BPW at the time of sample enrichment with a Salmonella reference strain, e.g., Salmonella enterica serovar Gaminara Sal57 (a Green Fluorescent Protein [GFP] strain derived from FDA SAL5695; Microbiologics, St. Cloud, MD; #01278UVV), incubate the broth and extract DNA concurrently with the animal food samples (see Section C) consistent with local QA/QC procedures.

b. Sterile molecular grade water is used as the NTC.

C. DNA Extraction from Animal Food Sample Enrichments

Go to this link (Preparation of DNA Templates; 0:49 to 1:59) for a quick demonstration.

1. Mix well by swirling the Whirl-pak filter bag containing animal food enrichment broths. Transfer 1 ml from the filtered side of the bag to a microcentrifuge tube. Vortex briefly.

2. Extract DNA using the PrepMan Ultra sample preparation reagent as follows: Centrifuge 1 min at 900 x g to remove large particles and transfer supernatant to a new microcentrifuge tube. Centrifuge 2 min at 16,000 x g and discard supernatant. Suspend the pellet in 100 µl of PrepMan Ultra sample preparation reagent and heat 10 min at 100 ± 1°C in a dry heat block. Cool to room temperature and store extracted DNA at -20°C.
D. LAMP Assembly

1. Overview
   
   Go to this link (Assembly of a LAMP Reaction; 2:25 to 3:49) for a quick demonstration.

   ![Diagram of LAMP assembly process]

   - **Prepare reaction mix**
   - **Distribute to wells**
   - **Add DNA templates**
   - **Start the LAMP reaction** (amplification curve generated in real-time)
   - **Load into Genie II or III**

2. LAMP reaction assembly

   **IMPORTANT:** To prevent cross-contamination, it is highly recommended to physically separate the areas used for preparing the LAMP reaction mix and adding DNA templates, either in different areas of the same laboratory or in different laboratories.

   a. Clean bench with isopropanol and a DNA- and DNase-degrading solution such as DNA AWAY and clean pipettes and plate/strip holders with DNA AWAY.

   b. Thaw LAMP isothermal master mix (GspSSD, GspSSD2.0, or WarmStart), 10× primer mix, molecular grade water, PC *Salmonella* DNA, and sample DNA extracts at room temperature.

   c. Prepare the LAMP reaction mix according to a worksheet (Tables 3a or 3b). After all components have been added to a microcentrifuge tube or other appropriate tube, vortex gently then flick contents down.

   Always include a PC and an NTC in every LAMP run. When using only one Genie II block or running LAMP on Genie III (8 samples total), prepare the LAMP reaction mix according to the column for 9 reactions (1 extra for pipetting loss). When using both Genie II blocks (A and B, 16 samples total), prepare the LAMP reaction mix according to the column for 18 reactions (2 extras for pipetting loss). When running LAMP on AB 7500 Fast, prepare the LAMP reaction mix for up to 100 reactions (96 samples plus 4 extras for pipetting loss). For all other sample numbers, adjust the volumes accordingly.
Table 3a. Worksheet for preparing the LAMP reaction mix using GspSSD or GspSSD2.0

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µl) for 1 reaction</th>
<th>Volume (µl) for 9 reactions</th>
<th>Volume (µl) for 18 reactions</th>
<th>Volume (µl) for 100 reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>GspSSD or GspSSD2.0 isothermal master mix</td>
<td>15</td>
<td>135</td>
<td>270</td>
<td>1,500</td>
</tr>
<tr>
<td>Primer mix (10×)</td>
<td>2.5</td>
<td>22.5</td>
<td>45</td>
<td>250</td>
</tr>
<tr>
<td>Molecular grade water</td>
<td>5.5</td>
<td>49.5</td>
<td>99</td>
<td>550</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>207</td>
<td>414</td>
<td>2,300</td>
</tr>
</tbody>
</table>

Table 3b. Worksheet for preparing the LAMP reaction mix using WarmStart

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µl) for 1 reaction</th>
<th>Volume (µl) for 9 reactions</th>
<th>Volume (µl) for 18 reactions</th>
<th>Volume (µl) for 100 reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>WarmStart isothermal master mix</td>
<td>12.5</td>
<td>112.5</td>
<td>225</td>
<td>1,250</td>
</tr>
<tr>
<td>LAMP fluorescent dye</td>
<td>0.5</td>
<td>4.5</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>Primer mix (10×)</td>
<td>2.5</td>
<td>22.5</td>
<td>45</td>
<td>250</td>
</tr>
<tr>
<td>Molecular grade water</td>
<td>7.5</td>
<td>67.5</td>
<td>135</td>
<td>750</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>207</td>
<td>414</td>
<td>2,300</td>
</tr>
</tbody>
</table>

d. Place the Genie strip or MicroAmp reaction plate/strip in the holder and distribute 23 µl of the LAMP reaction mix to each well.

e. Vortex all DNA templates and centrifuge briefly as needed. Add 2 µl of DNA template to each well and cap tightly. When using the MicroAmp optical adhesive film, apply it after all DNA templates have been added.

f. Remove the Genie strip or MicroAmp reaction plate/strip from the holder and flick wrist to ensure all reagents have pooled at the bottom of the tube. Centrifuge the MicroAmp reaction plate briefly in a mini plate spinner as needed.

g. Load the Genie strip into block(s) of Genie II or Genie III and the MicroAmp plate/strip into the plate holder of AB 7500 Fast, ensuring caps are secure and a good seal before closing the lid.

**E. LAMP Run**

1. Run on Genie
   a. Turn on the Genie II or Genie III instrument and tap once to access the home screen.
   b. Set up a new run by tapping the default [LAMP & Anneal] profile and select [Edit].
c. Enter sample names using the touchscreen keyboard and tap the [ ] icon to save.
d. Click the [ ] icon in the upper right of the screen and select the block(s) containing Genie strip(s) to start the run.
e. OPTIONAL: While the LAMP reaction is in progress, tap the [Temperature], [Amplification], and [Anneal] tabs to view real-time results (~ 40 min).
f. The run will automatically save when complete.

2. Run on AB 7500 Fast
   a. Turn on AB 7500 Fast and launch 7500 software (v2.3) from a connected computer.
   b. Set up a LAMP experiment template by clicking [Advanced Setup] from the home screen and save it as “Salmonella LAMP.edt” for all future LAMP runs.
      i. In the [Experimental Properties] tab, select the following parameters:
         a) Instrument: 7500 Fast (96 Wells)
         b) Type of experiment: Quantitation – Standard Curve
         c) Reagents: SYBR® Green Reagents and check [Include Melt Curve]
         d) Ramp speed: Fast (~ 40 minutes to complete a run)
      ii. In the [Plate Setup] tab, under [Define Targets]:
           a) Add a new target “Salmonella” and select “FAM” as the Reporter and Quencher is “none.”
      iii. In the [Run Method] tab, select [Tabular View] and set up the following:
           a) Reaction Volume Per Well: 25 µl
           b) Delete all default stages but [Cycling Stage] and [Melt Curve Stage].
           c) Set up [Cycling Stage] (1 step): Number of Cycles “30,” Starting Cycle “2,” Ramp Rate “100%,” Temperature “65°C,” Time “1:00,” Collect Data on Hold.
           d) Set up [Melt Curve Stage] (2 steps, “Continuous”): Step 1 - Ramp Rate “100%,” Temperature “80°C,” Time “1:00;” Step 2 - Ramp Rate “3.5%,” Temperature “98°C,” Time “0.05,” Collect Data on Ramp.

<table>
<thead>
<tr>
<th>Isothermal amplification phase</th>
<th>Anneal phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>65°C, 30 min</td>
<td>98°C to 80°C with 0.05°C decrement per sec</td>
</tr>
</tbody>
</table>

   c. Set up a new run and enter experiment name and sample information.
   i. Open the “Salmonella LAMP.edt” template from the home screen.
   ii. In the [Experimental Properties] tab, type in an “Experiment Name.”
In the [Plate Setup] tab, under [Define Samples], click [Add New Sample] and type in sample names. View tab [Assign Targets and Samples] and add samples to the plate layout.

Save experiment (.eds) file, using the experiment name.

Click “Start Run” from the top right corner of the screen.

OPTIONAL: While the LAMP reaction is in progress, click the [Temperature Plot], [Amplification Plot], and [Melt Curve] tabs in the [Run] tab to view real-time results (~ 40 min).

The run will automatically save when complete.

F. LAMP Result Interpretation

Go to this link (LAMP Results Interpretation; 3:50 to 5:48) for a quick demonstration.

In the video, the instrument firmware is v2.25.5 and the Genie Explorer software is v2.0.6.3. Check the manufacturer’s site periodically for Genie instrument firmware and software updates.

1. Results on the instrument panel of Genie II (firmware v2.34.13)
   a. Tap the [log] icon in the bottom left corner of the home screen and select [log] to navigate to the LAMP run of interest.
   b. Observe the five tabs associated with each run: [Profile], [Temperature], [Amplification], [Anneal], and [Results] (Figure 1).
   c. Tap the [Results] tab for a tabular view of the results (Figure 1e). There are five columns (Well, Type, Result, Peak Ratio, and Anneal Peak). The “Peak Ratio” column shows the time-to-peak values (min:sec) for each sample (“Well”) and the “Anneal Peak” column shows the melting/annealing temperatures (°C) for any amplified product in that well.
   d. Examine the control wells first. The NTC well should be blank for “Peak Ratio” while “Anneal Peak” can be either blank (both Genie II and Genie III) or < 83°C (Genie II only). The PC well should have a “Peak Ratio” less than 10 min and “Anneal Peak” at 89 ± 2°C. All samples with the correct “Anneal Peak” (89 ± 2°C) and “Peak Ratio” (≤ 20 min) are considered positive for Salmonella. Manually observe the amplification curve for wells with the correct “Anneal Peak” but no “Peak Ratio,” and repeat LAMP on sample DNA extracts having any amplification within 20 min.
   e. In the [Results] tab, save data to a single page pdf report [log], export to a CSV file [csv], or print directly [print]. These outputs can also be copied to a USB stick and transferred to a computer.

2. Results in the Genie Explorer software (v2.0.7.11)

IMPORTANT: The Genie Explorer software uses a default “Peak Detection Threshold Ratio” of 0.020, which is different from the Genie II instrument. To ensure that all valid peaks are identified, and the results match between the instrument panel and software, click the [log] icon in the top right of the “Amplification Rate” tab and adjust the “Peak Detection Threshold Ratio” from 0.020 to 0.010.
The files are transferred from the Genie instrument to the computer via a USB cord that comes with the instrument. Once the files are transferred, the Genie Explorer software can be accessed remotely without the need to be connected to the instrument.

a. Click the [ ] icon on the left panel and navigate to the LAMP run of interest.

b. Besides the five tabs displayed on the instrument panel, observe two additional tabs, [Amplification Rate] and [Anneal Derivative], for a total of 7 tabs (Figure 2).

c. Tap the [Amplification Rate] tab to view a graphic display of the fluorescence ratios by time (Figure 2d) and the [Anneal Derivative] tab to view a graphic display of the fluorescence derivatives by temperature (Figure 2f).

d. Tap the [Result] tab for a tabular view of the results (Figure 2g). There are four columns (Graph Name, Well Number, Well Name, and Peak Value). The “Amp Time” (min:sec) is equivalent to the “Peak Ratio” on the Genie II instrument while the “Anneal Derivative” is equivalent to the “Anneal Peak” (°C) on Genie II.

e. Interpret the LAMP results following the same rules used for the Genie II instrument panel (see section F.1) with the exception that the NTC well and other negative samples should have blank “Anneal Derivative” as the Genie Explorer software settings eliminate those with < 83°C results.

f. Generate a Genie experiment report from the run by clicking the [ ] icon in the bottom right of the software screen. Alternatively, export data as a text file by clicking the [ ] icon or as an image file by clicking the [ ] icon.

3. Results on the AB 7500 software (v2.3)

The LAMP runs on AB 7500 Fast can be analyzed using the 7500 software v2.3, which can be used as a stand-alone software for remote analysis.

a. Open the experiment file (.eds) for the LAMP run of interest and click the [Analysis] tab on the left panel.

b. In the [Amplification Plot] tab, choose “linear” as the graph type to view a graphic display of ΔRn over cycle (Figure 3a). Uncheck “Auto” for [Threshold] and adjust it to be within the exponential phase of the amplification curve according to the instrument manual. OPTIONAL: Type in “160,000” for GspSSD and GspSSD2.0 and “3,000,000” for WarmStart.

c. In the [Melt Curve] tab, select “Derivative Reporter” as the plot type to view a graphic display of “Derivative Reporter (−Rn)’over temperature (Figure 3b). In the [View Plate Layout] tab, select wells to see corresponding C\textsubscript{T} values (Figure 3c) and [Amplification Plot] and [Melt Curve] for assigned sample(s). The [View Well Table] tab shows a tabular view of this data, including C\textsubscript{T} and T\textsubscript{m} (Figure 3d).

d. Examine the control wells first. The NTC well should report “Undetermined” for C\textsub{T} while T\textsubscript{m} can be < 83°C. The PC well should have a C\textsub{T} less than 10 min and T\textsubscript{m} at 89 ± 2°C. All samples with the correct T\textsubscript{m} (89 ± 2°C) and C\textsub{T} (≤ 20 min) are considered positive for Salmonella. Manually observe the amplification curve for wells with the correct T\textsubscript{m} but no C\textsub{T}, and repeat LAMP on sample DNA extracts having any amplification within 20 min.
Figure 1. Representative LAMP results displayed on the Genie II instrument panel (firmware v2.34.13). In this LAMP run, samples A1 to A6 are 10-fold serial dilutions of *Salmonella enterica* serovar Typhimurium ATCC 19585 (LT2) ranging from $2.6 \times 10^5$ cells to 2.6 cells per reaction. PC is *S. Typhimurium* ATCC 19585 (LT2) at $1.7 \times 10^3$ cells per reaction and NTC is molecular grade water. The master mix is GspSSD2.0. (a) The [Profile] tab shows the programmed temperature profile. (b) The [Temperature] tab shows actual temperatures in the sample wells as LAMP reaction proceeds. (c) The [Amplification] tab shows fluorescence readings during LAMP amplification. (d) The [Anneal] tab shows changes in fluorescence (derivative) during the anneal phase. (e) The [Results] tab shows a tabular view of the LAMP results.

a. The [Profile] tab

![Profile tab image]

b. The [Temperature] tab

![Temperature tab image]
c. The [Amplification] tab

d. The [Anneal] tab

e. The [Results] tab
Figure 2: Representative LAMP results viewed in the Genie Explorer software (v2.0.7.11). This is the same run as in Figure 1. (a) The [Profile] tab shows the programmed temperature profile. (b) The [Temperature] tab shows actual temperatures in the sample wells as LAMP reaction proceeds. (c) The [Amplification] tab shows fluorescence readings during LAMP amplification. (d) The [Amplification Rate] tab shows changes in fluorescence (fluorescence ratio) during LAMP amplification. (e) The [Anneal] tab shows fluorescence readings during the anneal phase. (f) The [Anneal Derivative] tab shows changes in fluorescence (derivative) during the anneal phase. (g) The [Results] tab shows a tabular view of the LAMP results.

a. The [Profile] tab

b. The [Temperature] tab

c. The [Amplification] tab
d. The [Amplification Rate] tab

![Amplification Rate](image)

e. The [Anneal] tab

![Anneal](image)

f. The [Anneal Derivative] tab

![Anneal Derivative](image)

g. The [Result] tab

![Result](image)
Figure 3: Representative LAMP results viewed in the AB 7500 software (v2.3). This is a run using the same master mix and samples as in Figure 1. (a) The [Amplification Plot] tab shows fluorescence readings during LAMP amplification. (b) The [Melt Curve] tab shows fluorescence readings during the melt curve stage. (c) The [View Plate Layout] tab shows C_T values for each well on the plate. (d) The [View Well Table] tab shows a tabular view of the LAMP results.

a. The [Amplification Plot] tab

![Amplification Plot](image)

b. The [Melt Curve] tab

![Melt Curve](image)

a. The “View Plate Layout” tab

![Plate Layout](image)

b. The “View Well Table” tab

<table>
<thead>
<tr>
<th>#</th>
<th>Well</th>
<th>Sample Name</th>
<th>Target Name</th>
<th>C_T</th>
<th>Tm1</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B0</td>
<td>PC</td>
<td>Salmonella</td>
<td>06.789</td>
<td></td>
<td>89.4</td>
</tr>
<tr>
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<td>Salmonella</td>
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H. References


