Salmonella Loop-Mediated Isothermal Amplification (LAMP) Protocol

LAMP is an isothermal nucleic acid amplification test that runs at a constant temperature (1) and exhibits high tolerance to matrix inhibitors (2-4). Many LAMP assays have been developed and used for *Salmonella* detection in human food and/or animal food (5). The *Salmonella* LAMP assay described in this protocol specifically:

- Targets the *Salmonella* invasion gene (*invA*; GenBank accession number M90846) (6).
- Is rapid, reliable, and robust in a variety of human and animal food matrices (3, 7-12).
- The limits of detection for different *Salmonella* serovars in pure culture range from 1.3 to 28 cells per rection (8, 9, 11). In food matrices, the assay can detect 1 CFU in 25 g test portions with overnight enrichment (8, 9, 11).
- Is versatile and can be run on multiple platforms and with various reagent options (13).
- Has been validated in a variety of food categories in single-laboratory validation studies (11, 13) and in dry dog food through a multi-laboratory validation study (14).
- Has been adopted by the National Antimicrobial Resistance Monitoring System (NARMS) as the uniform *Salmonella* screening method in retail meat testing (15).

The LAMP assembly and data analysis protocol described below is for three instrument platforms: Genie II, Genie III, and 7500 Fast (13). A video article (16) demonstrating the entire protocol from sample preparation to result interpretation on Genie II has been published, which can be accessed through this text link and video link. Use of other platforms and protocols shall be first validated per the FDA's guidelines (17), AOAC's Appendix J (18) or ISO 16140 (19, 20), and approved by the FDA's Microbiology Methods Validation Subcommittee (MMVS).

A. Equipment and Materials

Items 1-3 are needed to run the LAMP assay on Genie II or Genie III, whereas items 4-8 are needed to run the assay on 7500 Fast. Items 9-12 are common equipment and materials.

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. 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. Trypticase soy agar (TSA) or other nonselective agars
2. Trypticase soy broth (TSB) or brain heart infusion (BHI) broth
3. Peptone diluent (0.1%)
4. PrepMan Ultra Sample Preparation Reagent (Thermo Fisher Scientific #4318930)
5. Isopropanol (70%)
6. DNA AWAY (Thermo Fisher Scientific #7010PK) or equivalent
7. LAMP master mix: three options; GspSSD Isothermal Mastermix (OptiGene Ltd. #ISO-001 for 400 reactions or #ISO-002 for 2,000 reactions), GspSSD2.0 Isothermal Mastermix (**RECOMMENDED**; 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), among which GspSSD2.0 Isothermal Mastermix had the best performance (13)

8. Molecular grade water

9. *Salmonella* LAMP primers and primer mix (10×)

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

Table 1. *Salmonella* LAMP primers

Primer name	Sequence (5′-3′)	Length (bp)
Sal4-F3	GAACGTGTCGCGGAAGTC	18
Sal4-B3	CGGCAATAGCGTCACCTT	18
Sal4-FIP	GCGCGGCATCCGCATCAATA-TCTGGATGGTATGCCCGG	38
Sal4-BIP	GCGAACGGCGAAGCGTACTG-TCGCACCGTCAAAGGAAC	38
Sal4-LF	TCAAATCGGCATCAATACTCATCTG	25
Sal4-LB	AAAGGGAAAGCCAGCTTTACG	21

   b. Prepare the LAMP primer mix (10×) according to the worksheet (**Table 2**). Add appropriate volumes of stock solutions for each primer and 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 (up to two years).

Table 2. Worksheet for preparing the LAMP primer mix (10×)

Component	Volume (µl)
Sal4-F3 primer (100 µM)	10
Sal4-B3 primer (100 µM)	10
Sal4-FIP primer (100 µM)	180
Sal4-BIP primer (100 µM)	180
Sal4-LF primer (100 µM)	100
Sal4-LB primer (100 µM)	100
Molecular grade water	420
Total	1,000

10. LAMP controls: always include a positive control (PC) and a no template control (NTC) in every LAMP run.

   a. Use DNA extracts from any *Salmonella* reference strain, e.g., *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 19585 (LT2), as the PC. Inoculate the bacterial strain on a nonselective agar plate (e.g., trypticase soy agar [TSA]) and incubate 24 ± 2 h at 35 ± 2°C. Transfer 3-5 isolated 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.* 10^9 CFU/ml. Serially dilute the overnight culture in peptone diluent to obtain *ca.* 10^7 CFU/ml. Transfer 500 µl of this dilution to a microcentrifuge tube and heat 10 min at 100 ± 1°C in a heat block. Cool to room temperature, centrifuge again 2 min at 12,000 × *g*, and transfer supernatant to a new microcentrifuge tube. Store all tubes containing control DNA extracts at -20°C.

   Alternatively, a positive culture control may be used as the PC. Inoculate appropriate preenrichment broth at the time of sample preenrichment (see **BAM Chapter 5 Section C**)

with a *Salmonella* reference strain, e.g., *Salmonella enterica* subsp. *enterica* serovar Gaminara Sal57 (a Green Fluorescent Protein [GFP] strain derived from FDA SAL5695; Microbiologics, St. Cloud, MN; #01278UV-V), incubate the broth and extract DNA concurrently with the food samples consistent with laboratory QA/QC procedures.

b. Use molecular grade water as the NTC.

C. DNA Extraction from Food Sample Preenrichment Broths

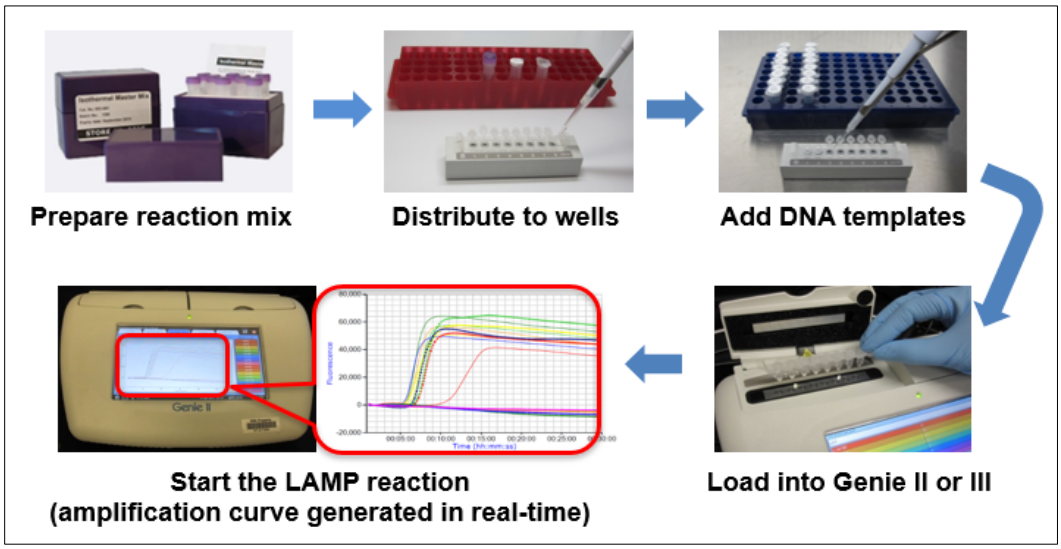
Go to this link (Preparation of DNA Templates; 0:49 to 1:59) for a quick demonstration.

1. Mix well by swirling the sample filter bag containing food preenrichment 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 × *g* to remove large particles and transfer supernatant to a new microcentrifuge tube. Centrifuge 2 min at 16,000 × *g* and discard supernatant. Suspend the pellet in 100 µl PrepMan Ultra Sample Preparation Reagent and heat 10 min at 100 ± 1°C in a heat block. Cool to room temperature, centrifuge again 2 min at 12,000 × *g* and transfer supernatant to a new microcentrifuge tube. Store sample DNA extracts at -20°C.

D. LAMP Reaction Assembly

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



IMPORTANT: Before starting to assemble a LAMP reaction on the bench, first set up a new LAMP run and enter sample information on Genie instruments or 7500 Fast (see **Section E.1 or E.2, respectively, through step c**).

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.

Follow these steps to assemble a LAMP reaction:

1. 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.

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

3. Prepare the LAMP reaction mix according to the worksheet (**Tables 3a or 3b**). After all components have been added to a microcentrifuge tube, vortex gently then flick wrist to ensure all reagents are pooled at the bottom of the tube.

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 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 the GspSSD Isothermal Mastermix or GspSSD2.0 Isothermal Mastermix

Component	Volume (µl) for 1 reaction	Volume (µl) for 9 reactions	Volume (µl) for 18 reactions	Volume (µl) for 100 reactions
GspSSD or GspSSD2.0 Isothermal Mastermix	15	135	270	1,500
Primer mix (10×)	2.5	22.5	45	250
Molecular grade water	5.5	49.5	99	550
Total	23	207	414	2,300

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

Component	Volume (µl) for 1 reaction	Volume (µl) for 9 reactions	Volume (µl) for 18 reactions	Volume (µl) for 100 reactions
WarmStart LAMP Master Mix (2×)	12.5	112.5	225	1,250
LAMP Fluorescent Dye	0.5	4.5	9	50
Primer mix (10×)	2.5	22.5	45	250
Molecular grade water	7.5	67.5	135	750
Total	23	207	414	2,300

4. Place the Genie Strip or MicroAmp Fast Reaction Plate/Strip in the holder and distribute 23 µl of the LAMP reaction mix to each well.

5. Vortex all DNA templates and centrifuge briefly to pool reagents. In a separate clean area, 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.

6. Remove the Genie Strip or MicroAmp Fast Reaction Plate/Strip from the holder and flick wrist to ensure all reagents have pooled at the bottom of the tube. Centrifuge the MicroAmp Fast Reaction Plate briefly in a mini plate spinner as needed.

7. Load the Genie Strip into Genie II or Genie III block(s) or load the MicroAmp Fast Reaction Plate/Strip into the 7500 Fast plate holder, ensuring caps are secure and completely sealed before closing the instrument's lid or tray.

E. LAMP Run

1. Run LAMP on either one of the Genie instruments or 7500 Fast (alternative platform).Run on Genie

   a. Turn on the Genie II or Genie III instrument and tap once to access the home screen.

   b. Tap [LAMP & Anneal] and select [Edit] to access the run profile screen.

Isothermal amplification phase	Anneal phase
65°C, 30 min	98°C to 80°C with 0.05°C decrement per sec

   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 saves automatically when complete.

2. Run on 7500 Fast (alternative platform)

   a. Turn on 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.

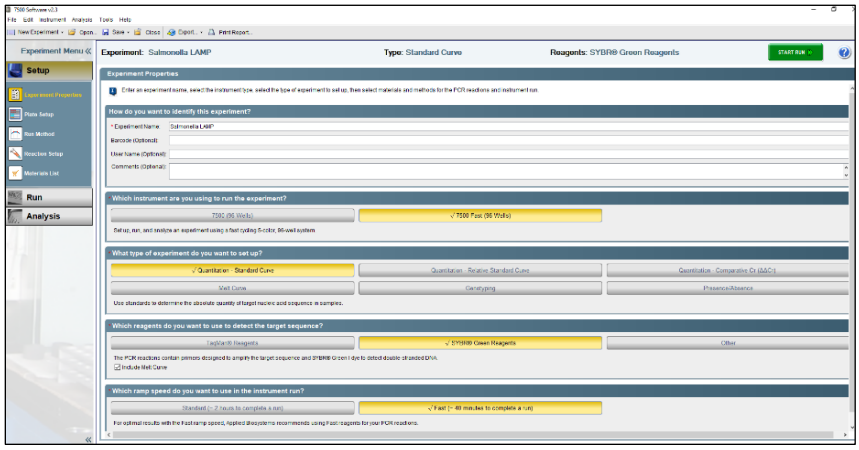
      i. In the [Experimental Properties] tab, select the following parameters:

         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)

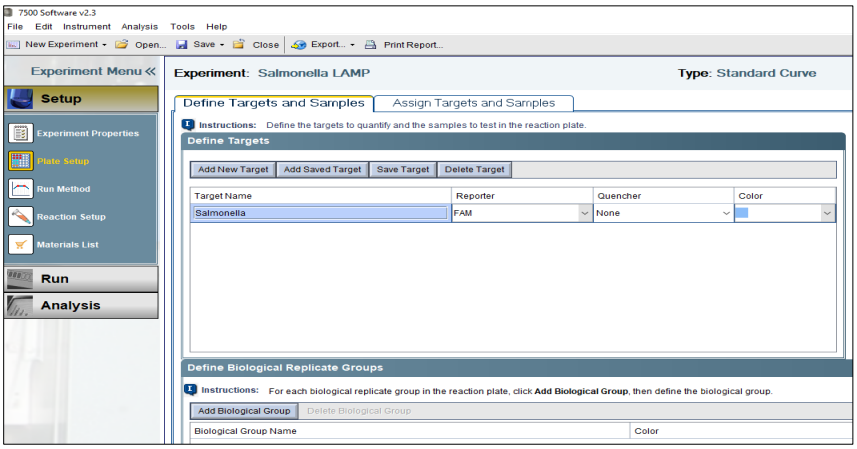


      ii. In the [Plate Setup] tab, under [Define Targets], enter/select these parameters:

a) Target Name: Salmonella

b) Reporter: FAM

c) Quencher: None



iii. In the [Run Method] tab, click either [Graphical View] or [Tabular View] and set up the following parameters:

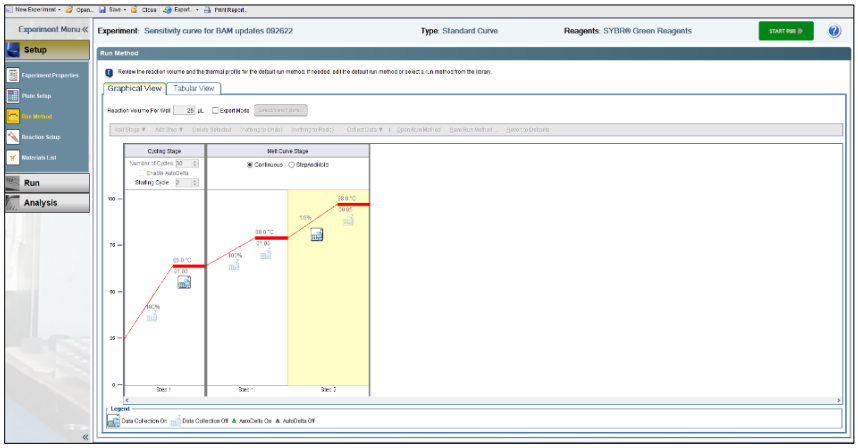
a) Reaction Volume Per Well: “25” µl

b) Delete [Holding Stage], Step 2 of the [Cycling Stage], and Steps 2 and 4 of the [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,” select “Collect Data on Hold.” This enables a LAMP reaction of 30 min at 65°C with fluorescence readings collected every min.

d) Set up [Melt Curve Stage] (2 steps, “Continuous”): Step 1 - Ramp Rate “100%,” Temperature “80°C,” Time “1:00;” Step 2 - Ramp Rate “1.5%,” Temperature “98°C,” Time “0:05,” “Collect Data on Ramp.”

Isothermal amplification	Melt curve analysis
65°C, 30 min	80°C to 98°C with 0.05°C increment per sec



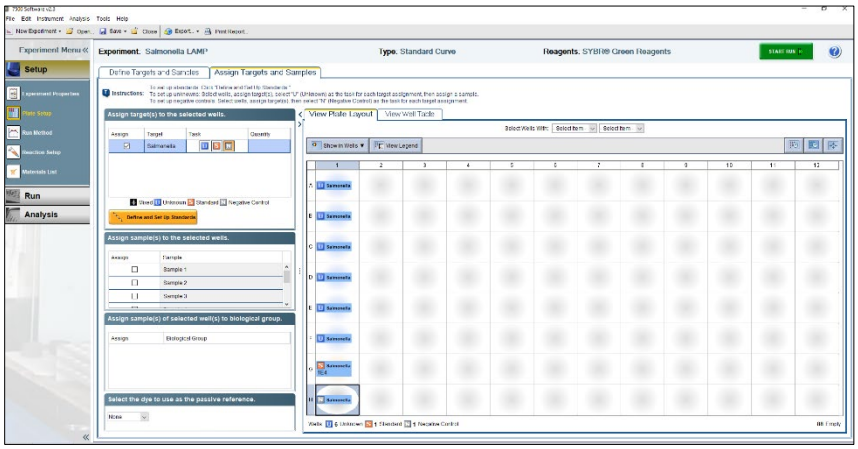
c. Set up a new run and enter experiment name and sample information.

i. Open the “Salmonella LAMP.edt” template from the home screen.

ii. In the [Experimental Properties] tab, type in an experiment name.

iii. In the [Plate Setup] tab, under [Define Samples], click [Add New Sample] and type in sample names. View tab [Assign Targets and Samples] and assign targets and samples to the plate layout. **IMPORTANT**: Make sure that under [select the dye as the passive reference] on the bottom left, change from "ROX" to "None."

iv. Save experiment (.eds) file, using the same experiment name used in step ii.



d. Click the [Start Run] button from the top right corner of the screen.

e. 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).

f. The run saves automatically when complete.

F. LAMP Result Interpretation

For LAMP assays run on Genie instruments, the results can be interpreted on Genie instrument panel directly (**RECOMMENDED**) or using the Genie Explorer software. For LAMP runs on 7500 Fast, the LAMP results shall be interpreted using the 7500 software.

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. Result interpretation on the instrument panel of Genie II (firmware v2.34.15)

   a. Tap the [🗀] icon in the bottom left corner of the home screen and select [log] to navigate to the run file (.gen) for the LAMP run of interest.

   b. Observe the five tabs associated with the 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 annealing temperatures (°C) for any amplified product in that well. **RECOMMENDED abbreviations for "Peak Ratio" and "Anneal Peak" are T_p and T_a, respectively**.

d. Examine the control wells first. For the NTC well, the "Peak Ratio (T_p)" should be blank while "Anneal Peak (T_a)" can be either blank (both Genie II and Genie III) or < 83°C (Genie II only). The PC well should have "Peak Ratio (T_p)" less than 10 min and "Anneal Peak (T_a)" around 89 ± 2°C. Runs with either or both controls not in these ranges are considered invalid and should be repeated.

e. Examine the sample wells. **All samples with "Peak Ratio (T_p)" ≤ 20 min and the correct "Anneal Peak (T_a)" (89 ± 2°C) are considered LAMP-positive for *Salmonella*.** For samples with the correct "Anneal Peak (T_a)" (89 ± 2°C) but "Peak Ratio (T_p)" > 20 min or blank, manually observe amplification curves and repeat LAMP on sample DNA extracts having valid LAMP amplifications (typical sigmoid amplification curves as shown in **Figures 1c, 2c, and 3a**). If the same results are obtained for the repeat run, the samples are considered LAMP-positive for *Salmonella*.

f. Tap the [Results] tab, export data to a single page pdf report or a CSV file or print the results directly. These outputs can also be transferred to a computer through the USB drive or with a USB memory stick.

2. Result interpretation on the Genie Explorer software (v2.0.7.11) (alternative way)

   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 run file (.gen) for the LAMP run of interest.

   b. Observe the seven tabs associated with the run: [Profile], [Temperature], [Amplification], [Amplification Rate], [Anneal], [Anneal Derivative], and [Result] (**Figure 2**).

   c. Click the [Amplification Rate] tab to view a graphic display of fluorescence ratio over time (**Figure 2d**). Click the [Graph Settings] icon at the top right of the screen and adjust the "Peak Detection Threshold Ratio" from 0.020 to 0.010.

   d. Click the [Result] tab for a tabular view of the results (**Figure 2g**). There are four columns (Graph Name, Well Number, Well Name, Peak Value) with "Amp Time" shown in the top block and "Anneal Derivative" shown in the bottom block for each well. The "Amp Time" (min:sec) is equivalent to the "Peak Ratio" on the Genie II instrument while the "Anneal Derivative" (°C) is equivalent to the "Anneal Peak" on Genie II. **RECOMMENDED abbreviations for "Peak Value" for "Amp Time" and "Peak Value" for "Anneal Peak" are T_p and T_a, respectively.**

   e. Interpret the LAMP results (evaluation of controls, positive/negative sample calls, and suggested repeats for inconclusive results) and report final LAMP results following similar steps as when using the Genie II instrument panel (see **Section F.1**) with one exception that the NTC well and negative samples should have blank "Peak Value" for "Anneal Derivative" (T_a) as the LAMP software settings eliminate those with < 83°C results.

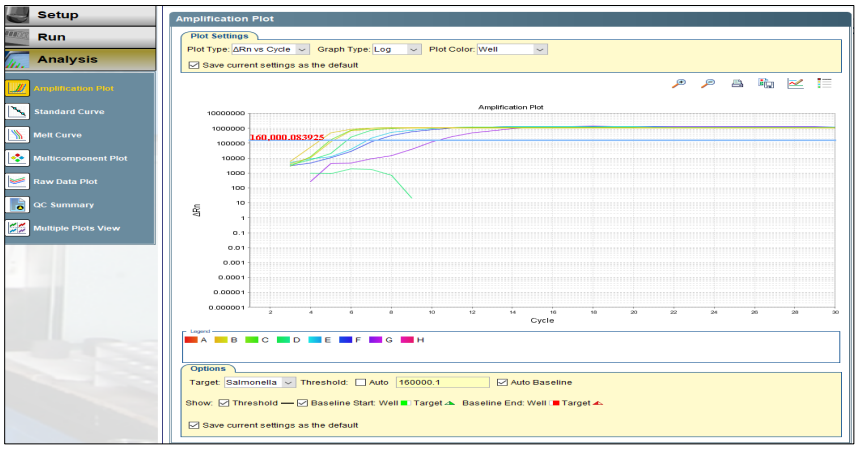
   f. Generate a Genie experiment report from the run by clicking the [] icon in the bottom right of the software screen. Alternatively, export data as a text file by clicking the [] icon or as an image file by clicking the [] icon or print directly.

3. Result interpretation on the 7500 software (v2.3) (alternative platform)

The LAMP runs on 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.

b. Click the [Amplification Plot] tab, choose “Log” as the [Graph Type] to view a graphic display of ΔRn over cycle in log scale. Observe the curve to see if the auto threshold is placed within the exponential phase of the amplification curve. If not, uncheck “Auto” next to [Threshold] and adjust it to be within the exponential phase of the amplification curve according to the instrument manual.



After setting the threshold, choose “Linear” as the [Graph Type] to view a graphic display of ΔRn over cycle in linear scale (**Figure 3a**).

OPTIONAL: On rare occasions, the auto baseline may need to be adjusted when the amplification curve begins before the maximum baseline which manifests as ΔRn below 0 examined with ‘Linear’ as the [Graph Type]. Uncheck “Auto Baseline” and manually adjust the baseline under “Analysis Settings” toward the left side of the graph according to the instrument manual.

c. Click the [Melt Curve] tab, select “Derivative Reporter” as the [Plot] type to view a graphic display of “Derivative Reporter (−Rn′)” over temperature (**Figure 3b**).

d. Click the [View Plate Layout] tab, select wells to see C_T values (**Figure 3c**) and for each well, check corresponding [Amplification Plot] and [Melt Curve] tabs.

e. Click the [View Well Table] tab, which shows a tabular view of the results for each well, including C_T and T_m (melting temperature, °C) (**Figure 3d**).

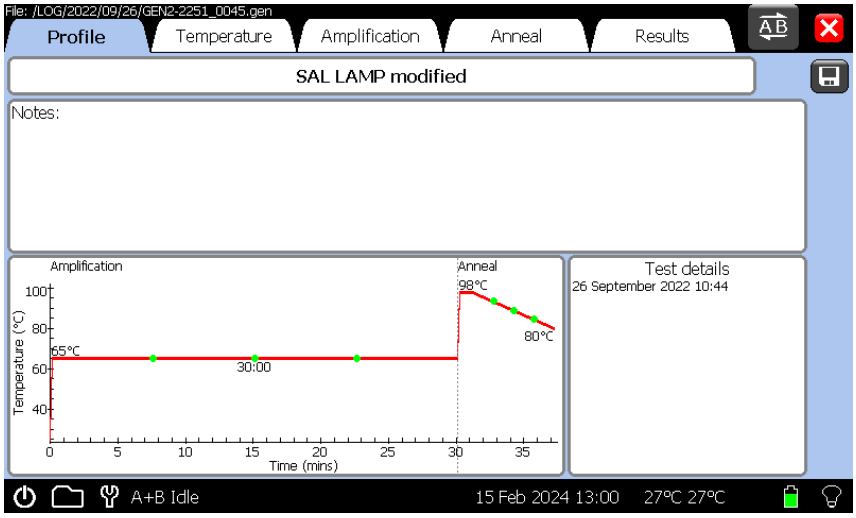
f. Examine the control wells first. The NTC well should report “Undetermined” for C_T while T_m can be < 83°C. The PC well should have a C_T less than 10 cycles (one min per cycle) and T_m around 89 ± 2°C.

g. Examine the sample wells. **All samples with $C_T \leq 20$ cycles and the correct T_m (89 ± 2°C) are considered LAMP-positive for *Salmonella*.** For samples with the correct T_m (89 ± 2°C) but C_T > 20 cycles or “undetermined,” manually observe amplification curves and repeat LAMP on sample DNA extracts having valid LAMP amplifications (typical sigmoid amplification curve as shown in **Figures 1c, 2c, and 3a**). If the same results are obtained for the repeat run, the samples are considered LAMP-positive for *Salmonella*.

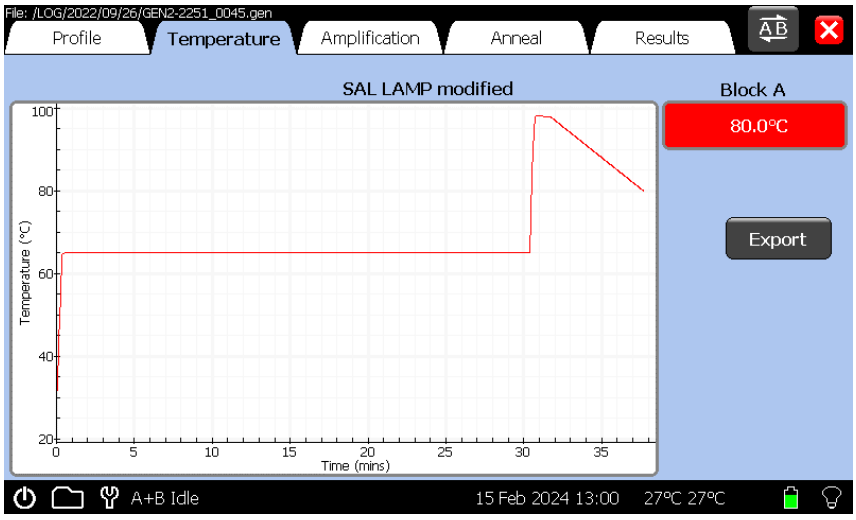
h. Export data to a .txt file or an .xls file or PowerPoint slides or print the results in pdf format.

Figure 1. Representative LAMP results displayed on the Genie II instrument panel (firmware v2.34.15). 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 × 10^5 cells to 2.6 cells per reaction. PC is *S.* Typhimurium ATCC 19585 (LT2) at 1.7 × 10^4 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.

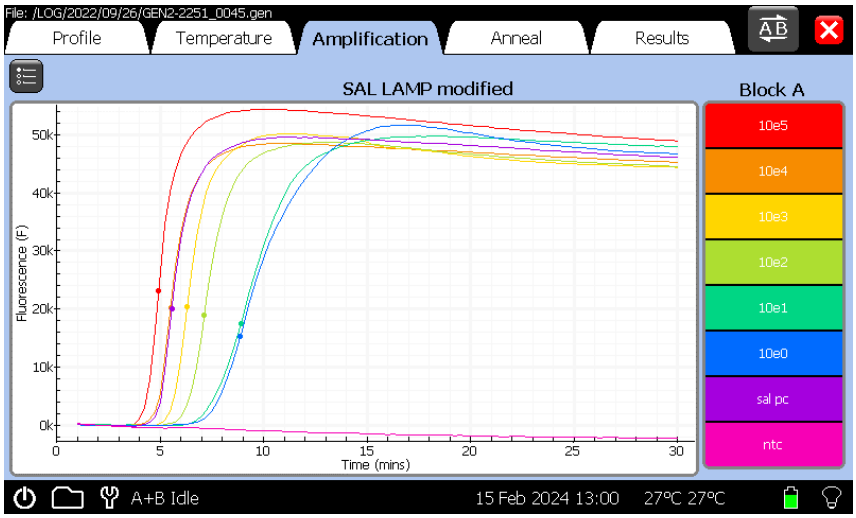
a. The [Profile] tab



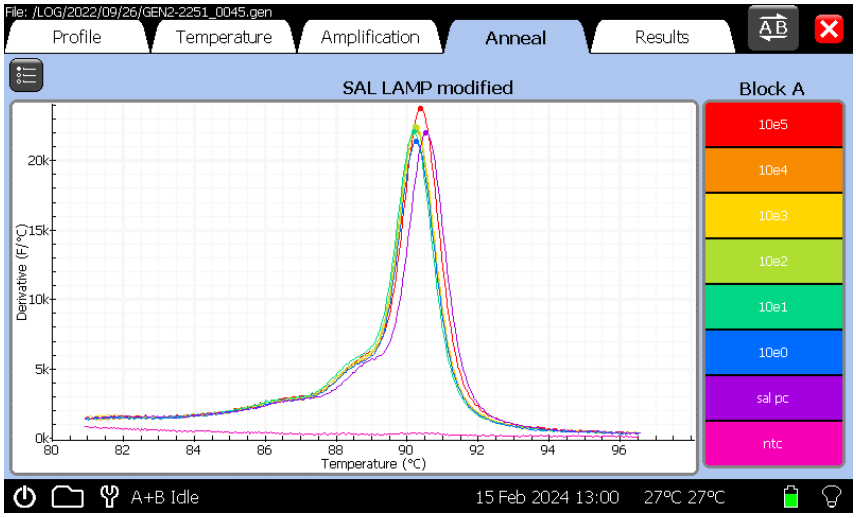
b. The [Temperature] tab



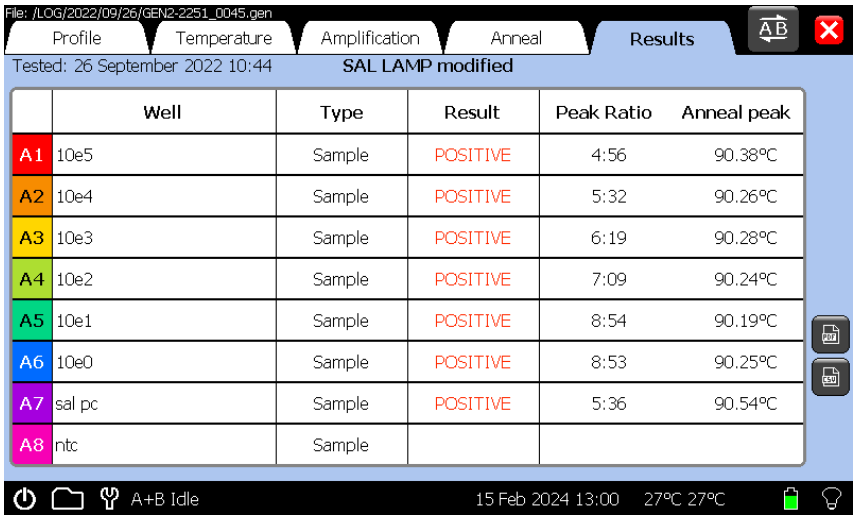
c. The [Amplification] tab



d. The [Anneal] tab



e. The [Results] tab

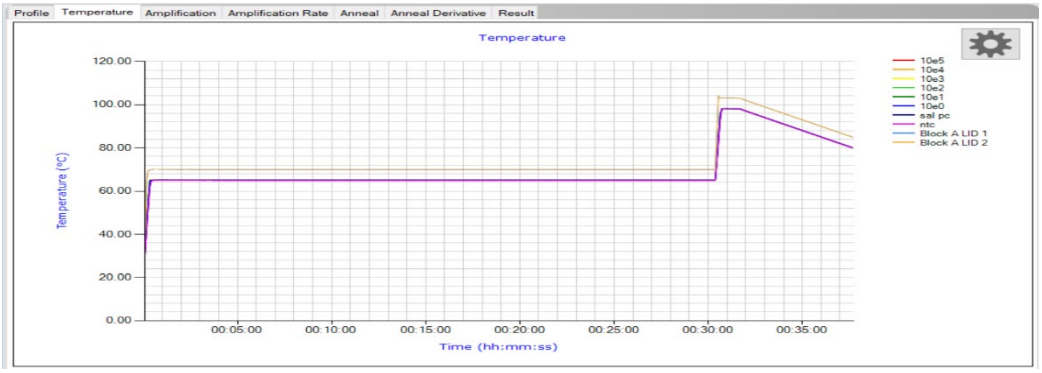


	Well	Type	Result	Peak Ratio	Anneal peak
A1	10e5	Sample	POSITIVE	4:56	90.38°C
A2	10e4	Sample	POSITIVE	5:32	90.26°C
A3	10e3	Sample	POSITIVE	6:19	90.28°C
A4	10e2	Sample	POSITIVE	7:09	90.24°C
A5	10e1	Sample	POSITIVE	8:54	90.19°C
A6	10e0	Sample	POSITIVE	8:53	90.25°C
A7	sal pc	Sample	POSITIVE	5:36	90.54°C
A8	ntc	Sample			

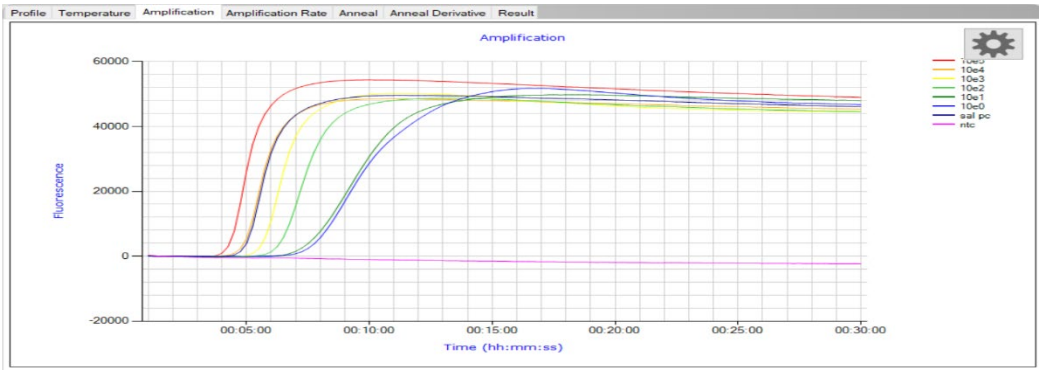
Figure 2: Representative LAMP results viewed in the Genie Explorer software (v2.0.7.11). This is the same run as in **Figure 1**. (a) The [Profile] tab shows the programmed temperature profile. (b) The [Temperature] tab shows actual temperatures in the sample wells as LAMP reaction proceeds. (c) The [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

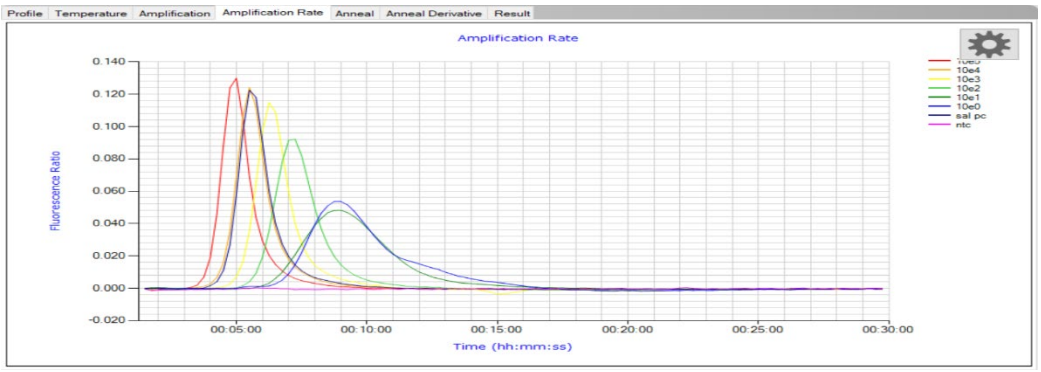
b. The [Temperature] tab



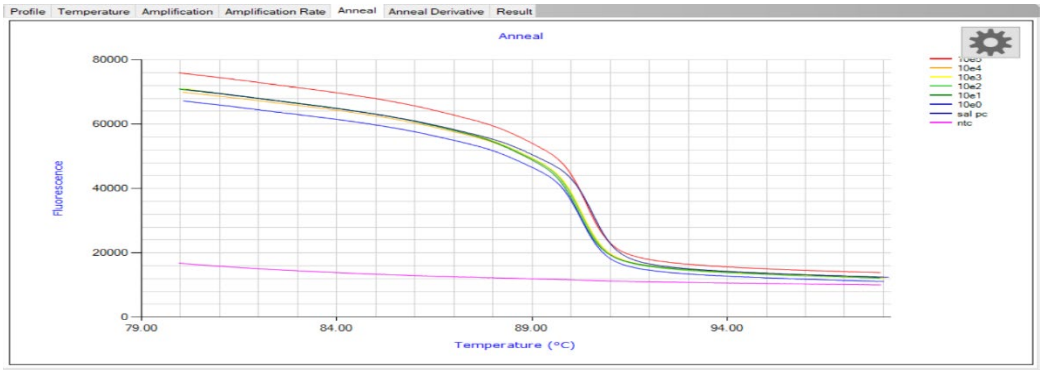
c. The [Amplification] tab



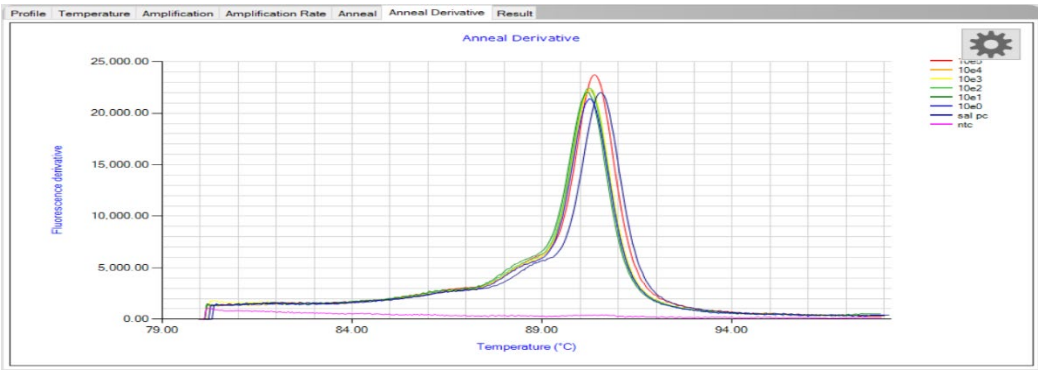
d. The [Amplification Rate] tab



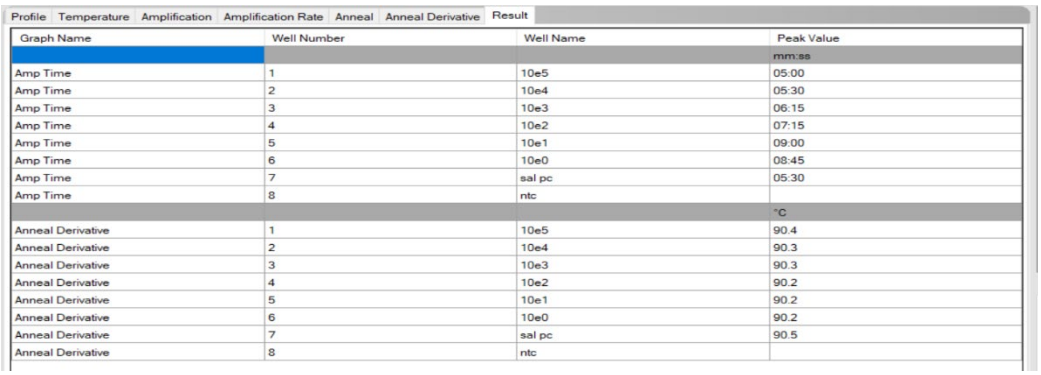
e. The [Anneal] tab



f. The [Anneal Derivative] tab



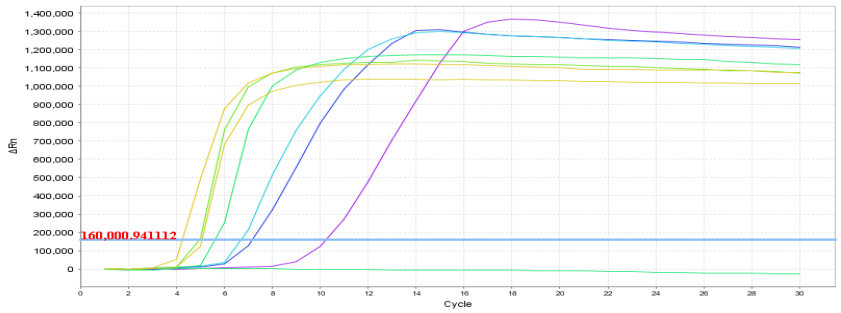
g. The [Result] tab



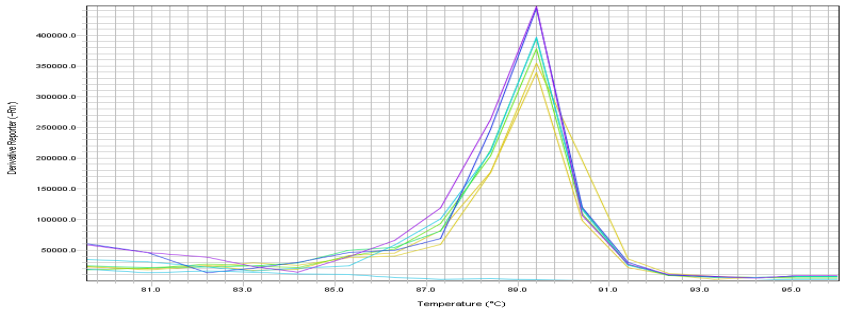
Graph Name	Well Number	Well Name	Peak Value
			mm:ss
Amp Time	1	10e5	05:00
Amp Time	2	10e4	05:30
Amp Time	3	10e3	06:15
Amp Time	4	10e2	07:15
Amp Time	5	10e1	09:00
Amp Time	6	10e0	08:45
Amp Time	7	sal pc	05:30
Amp Time	8	ntc	
			°C
Anneal Derivative	1	10e5	90.4
Anneal Derivative	2	10e4	90.3
Anneal Derivative	3	10e3	90.3
Anneal Derivative	4	10e2	90.2
Anneal Derivative	5	10e1	90.2
Anneal Derivative	6	10e0	90.2
Anneal Derivative	7	sal pc	90.5
Anneal Derivative	8	ntc	

Figure 3: Representative LAMP results viewed in the 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.

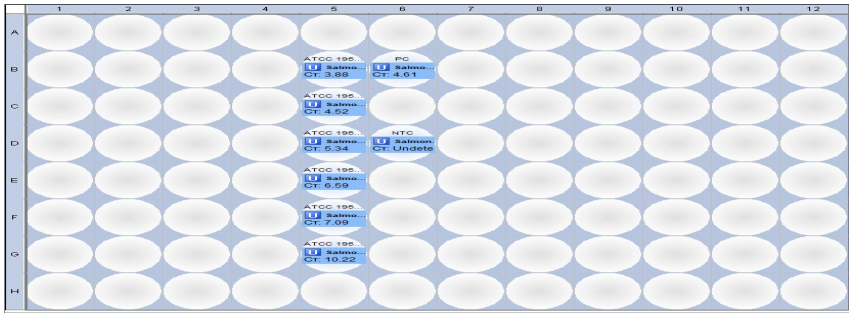
a. The [Amplification Plot] tab



b. The [Melt Curve] tab



c. The “View Plate Layout” tab



d. The “View Well Table” tab

#	Well	Sample Name	Target Name	Dyes	Cт	Tm1
1	B6	PC	Salmonella	FAM-None	04.612	90.8
2	D6	NTC	Salmonella	FAM-None	Undetermined	79.4
3	B5	ATCC 19585 10-5	Salmonella	FAM-None	03.884	90.8
4	C5	ATCC 19585 10-4	Salmonella	FAM-None	04.519	91
5	D5	ATCC 19585 10-3	Salmonella	FAM-None	05.343	91
6	E5	ATCC 19585 10-2	Salmonella	FAM-None	06.594	90.8
7	F5	ATCC 19585 10-1	Salmonella	FAM-None	07.093	90.6
8	G5	ATCC 19585 10-0	Salmonella	FAM-None	10.223	90.6

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