MMS Salmonella qPCR Genotyping 2.0 Assay

Product Manual

Version 1.0

Catalog Number

PD781 (100 reactions)

PD781-25 (25 reactions)

This product is intended for food and veterinary diagnostics.

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A. Introduction and Principles of the Test

Salmonella is generally classified as follows:

- Salmonella enterica
  - Salmonella enterica arizonae
  - Salmonella enterica diarizonae
  - Salmonella enterica enterica
  - Salmonella enterica houtenae
  - Salmonella enterica indica
  - Salmonella enterica salamae
- Salmonella bongori

Each of these subspecies is further divided into multiple serovars. Of these, Salmonella enterica subsp. enterica serovar Enteritidis and Salmonella enterica subsp. enterica serovar Typhimurium are some of the most prevalent serovars that can also infect humans. Our fourplex, probe-based real-time qPCR assay is intended for detection of all known serovars of Salmonella, with the ability of distinguishing the Enteritidis and Typhimurium serovars, in less than1 hour (with extracted DNA as the starting material).

The assay targets a 130bp-long segment of the bacterial spaQ gene, a highly conserved gene that is present only in Salmonella. It also targets a 155bp and 92bp segments of serovar Enteritidis and serovar Typhimurium, respectively. These two segments are highly conserved and are present only in their respective serovars.

Target	**spaQ gene (Pan Salmonella)**	**Typhimurium (S. typhimurium)**	**Enteritidis (S. enteritidis)**	**IC (Internal Control)**
Detection Channel	FAM	ROX	Cy5	HEX

Due to the variety of possible input samples (food pre-enrichment, animal tissue, animal fluids, etc.), a synthetic DNA spike-in is included in the kit to serve as the extraction internal control. This approach ensures that the extraction and amplification steps are accounted for, thus eliminating the possibility of false negatives due to erroneous extraction and/or amplification.

Starting from the crude sample, the whole process takes approximately 21-23 hours for food samples (pre-enrichment + DNA extraction + qPCR) and around 3 hours for veterinary samples (DNA extraction + qPCR).

B. Intended Use

This kit is intended for detection of all known serovars of Salmonella in food or veterinary samples, with the ability of distinguishing the Typhimurium and the Enteritidis serovars. The kit does *NOT* include reagents for pre-enrichment and DNA extraction, so buffered peptone water (BPW) and one of our DNA extraction kits are needed alongside this kit. See Section D of this manual (Required Materials That Are Not Included in the Kit) for a full list of needed reagents, labware, and consumables.

The protocol in this kit has been validated on the PCR machines listed in Section E (Compatible PCR Instruments).

C. Kit Contents and Storage

The kit comes in a package of five tubes, as indicated in Table1. All components must be stored at -20°C. The MMS Oligo Mix should not be exposed to light for extended periods of time.

Table1: *Components of the MMS Salmonella qPCR Genotyping Assay*

Component	PD781 Volume (µL)	PD781-25 Volume (µL)
qPCR Mix	1,000	250
MMS Oligo Mix	200	50
Negative Control	100	50
Positive Control	100	50
Extraction Internal Control (IC)	1,000	250

D. Required Materials That Are Not Included in the Kit

- For food homogenization (food samples only)
  - Buffered Peptone Water (BPW)
  - Autoclave
  - Balance with at least 0.1g increments and a minimum of 3kg capacity
  - Stomacher blender
  - Food mixing bags
  - Incubator capable of reaching 37°C for 24 hours straight
- For DNA extraction
  - Choose one of our DNA extraction kits depending on your sample type:
    - Food: *DNAbler - Cells and Tissue 2.0 Kit (Cat. No. DE25050)*
      OR
      Mag Bacterial DNAbler (Ref DE92050)
      + our MagEx32 automated extraction system (NE032)
      OR
      DNAbler - Gram Negative Bacteria Direct Lysis Kit (Ref DE90100)
    - Animal tissue: *DNAbler – Cells and Tissue 2.0 Kit (Cat. No. DE25050)*
    - Animal fluids: *DNAbler – Blood 2.0 Kit (Cat. No. DE26050)*
  - 1mL serological pipette and sterile tips to withdraw food homogenate (5 or 10mL would work as well)
  - Pipettes and filtered pipette tips in the range of 1–1,000µL
  - Dry bath capable of reaching up to 100°C
  - 1.5mL or 2.0mL microcentrifuge tubes and screw cap tubes
  - Microcentrifuge with 1.5mL/2.0mL rotor
  - Centrifuge with rotor for 1.5mL tubes capable of producing at least 10,000g
  - Pipettes and filtered pipette tips in the range of 100–1,000µL
  - Vortex
  - 96-100% ethanol
- For qPCR
  - Real-time PCR thermal cycler (see next section: Compatible Instruments)
  - PCR plates compatible with your real-time PCR thermal cycler
  - Optical adhesive seal and an applicator to seal off the PCR plate
  - PCR plate microcentrifuge
  - Pipettes and filtered pipette tips in the range of 1–1,000µL
  - 1.5mL or 2.0mL microcentrifuge tubes
  - Microcentrifuge with 1.5mL/2.0mL rotor
  - (highly recommended) Ice or a cooled PCR stand/rack

E. Compatible PCR Instruments

The MMS Salmonella qPCR Genotyping Assay has been thoroughly validated on the following real-time PCR systems:

- QuantStudio™ 5 Real-Time PCR System (ABI, A28574)
    - The "Design and Analysis 2, Version 2.7.0" is needed
- CFX Opus 96 Real-Time PCR System (Bio-Rad, 12011319)

It is also, in theory, compatible with any open-system real-time PCR system which has the FAM, HEX/VIC, ROX, and Cy5 detection channels. If you have a different real-time PCR system, please contact our technical team to help validate your instrument.

support@havensci.com

F. Good PCR Practices

Contamination is the worst enemy of PCR. Although the positive and negative controls in our kit can help in detecting contamination, dealing with contamination could prove troublesome and costly. Here, we list a few measures that could help you avoid contamination:

- Have physically separate workstation for PCR preparation, sample loading, and nucleic acid extraction. Each workstation should have its own dedicated pipettes and other small instruments, and should preferably be in a separate room.
- Always work under a cabinet (laminar flow for PCR preparation, and safety cabinet for nucleic acid extraction and sample loading).
- Use filtered tips. They are more expensive, but the small extra cost may save you valuable time, effort, and money down the line.
- If you need to analyze the PCR product after PCR cycling has finished, never unseal the plate near your PCR preparation or sample loading workstations. Even the smallest of aerosols could contain tens of millions of PCR templates.

G. Performance Evaluation

Inclusivity

As of December 31st, 2022, there were 1,207 full sequences for all Salmonella enterica and bongori species. The Pan Salmonella oligos in our assay have perfect complementarity to 99.23% of these sequences. Furthermore, the Enteritidis-specific and Typhimurium-specific oligos have perfect complementarity to all published Enteritidis and Typhimurium full genomic sequences (146 and 193 sequences, respectively).

We also verified the assay on 7 salmonella cultures from reference samples, as well as more than 20 environmental samples from either cultures or pre-enriched media in multiple sites (Table2). All samples were detected successfully by our assay.

Table2: *Samples used to test the inclusivity of the MMS Salmonella qPCR Genotyping Assay*

Pathogen Name (strain)		
Salmonella typhimurium (ATCC 14028)	Salmonella typhimurium (ATCC 13311)	Salmonella enteritidis (ATCC 13076)
Salmonella arizonae (ATCC 13314)	Salmonella Stanly (ATCC 7308)	Salmonella Agona (ATCC 51957)
Salmonella Idikan (Environmental)	Salmonella spp. (>20 environmental samples from multiple sites)	

Exclusivity

Wet-lab testing of reference bacterial cultures (ATCC) or intact bacterial cells (Zeptometrix) shows that our assay has no false positives in any of the organisms listed in Table3. Furthermore, in-silico analysis shows that our assay has no cross-reactivity with any of the organisms listed in Table4.

Precision

A positive and a negative samples for Salmonella arizona, Salmonella, serovar Typhimurium, and Salmonella serovar Enteritidis were run at least 5 different times using our assay. The samples were called correctly 100% of the time.

Table3: *Reference samples used for the exclusivity test for the MMS Salmonella qPCR Genotyping Assay. None of the samples showed cross reactivity with our assay.*

Pathogen Name (strain)		
A. baumannii (Zeptometrix 307-0294)	K. pneumoniae (ATCC 27736)	P. mirabilis (Zeptometrix Z050)
B. cereus (ATCC 10876)	K. pneumoniae (Zeptometrix Z460; CTX-M/NDM)	P. paraeruginosa (ATCC 9027)
B. fragilis (Zeptometrix Z029)	L. monocytogenes (ATCC 13932)	P. shigelloides (Zeptometrix N/A)
B. parapertussis (Zeptometrix A747)	M. avium (Zeptometrix Serotype 2)	Providencia ssp. (Zeptometrix NDM)
B. pertussis (Zeptometrix A639)	M. bovis (Zeptometrix Z321)	S. aureus (Zeptometrix MRSA; COL)
B. spizizenii (ATCC 6633)	M. catarrhalis (Zeptometrix Ne 11)	S. aureus (ATCC 25923)
C. difficile (Zeptometrix NAP1)	M. intracellular (Zeptometrix Z310)	S. epidermidis (Zeptometrix HER 1292)
C. jejuni (Zeptometrix N/A)	M. pneumoniae (Zeptometrix M129)	S. epidermidis (ATCC 12228)
C. pneumoniae (Zeptometrix IOL-207)	M. tuberculosis (Zeptometrix H37Ra-1)	S. lungdunesis (Zeptometrix Z097)
E. cloacae (Zeptometrix Z101)	M. tuberculosis (Zeptometrix Rif Resistant)	S. maltophilia (Zeptometrix Z074)
E. coli (Zeptometrix ETEC; ST+, LT+)	MRSA (Zeptometrix MRSA Community Strain)	S. pneumoniae (Zeptometrix Z053)
E. faecalis (ATCC 51299)	MRSA (Zeptometrix MRSA Hospital Strain)	S. pyogenes (Zeptometrix Z018)
E. faecalis (Zeptometrix VRE, Z345, vanB)	MRSA (Zeptometrix MRSA mecC)	S. sonnei (Zeptometrix N/A)
E. faecium (Zeptometrix VRE, Z347, vanA)	MSSA (Zeptometrix MSSA – empty cassette)	S.marcescens (Zeptometrix Z053)
E.coli O157:H7 (Zeptometrix EDL933)	N. meningitidis (Zeptometrix serogroup A)	V. cholerae (non-toxigenic) (Zeptometrix N/A)
H. influenzae (Zeptometrix MinnA)	P. aeruginosa (Zeptometrix IMP-1)	V. parahaemolyticus (Zeptometrix N/A)
K. aerogenes (Zeptometrix Z052)	P. aeruginosa (Zeptometrix VIM)	V. vulnificus (Zeptometrix N/A)
K. oxytoca (Zeptometrix Z115)	P. aeruginosa (Zeptometrix Z139, VIM-1)	Y. enterocolitica (Zeptometrix Clinical Isolate)

Table4: *Species used for the in-silico exclusivity analysis. None of the listed species have any potential for cross reactivity.*

Organism Name (NCBI TaxID)		
Gallus gallus domesticus (taxid:9031)	Cronobacter (taxid:413496)	Campylobacter (taxid:194)
Bacillus (taxid:1386)	Clostridium (taxid:1485)	Bacillus/Clostridium group (taxid:1239)
Staphylococcus aureus (taxid:1280)	Escherichia coli (taxid:562)	Escherichia coli O157:H7 (taxid:83334)
Legionella (taxid:445)	Yersinia (taxid:629)	Yersinia enterocolitica (taxid:630)
Legionella pneumophila (taxid:446)	Listeria (taxid:1637)	Shigella (taxid:620)

Limit of detection (LoD)

Salmonella samples were diluted to a final concentration of 1,000 copies/µL (Salmonella serovar enteritidis, Salmonella serovar typhimurium, and Salmonella enterica arizonae). From these, further serial dilutions (with equal amounts of the IC spiked in) showed that our assay has the following limit of detection for each target:

Target **(sample used)**	**Pan-Salmonella** **(Salmonella enterica arizonae)**	**Enteritidis-specific** **(Salmonella serovar enteritidis)**	**Typhimurium-Specific** **(Salmonella serovar typhimurium)**
Limit of Detection	8 Copies/reaction	40 Copies/reaction	8 Copies/reaction

H. Sample Preparation and DNA Extraction (Food Samples)

Food sample preparation

The food sample must first be homogenized in buffered peptone water (BPW) then incubated at 37°C for 18-24 hours, according to the ISO 6579-1:2017 standard (Detection of Salmonella spp). The BPW must be autoclaved then set aside to reach room temperature before use.

1. Weigh 25g of food sample and place it in a laboratory-grade food homogenization bag
2. Add 225mL of autoclaved buffered peptone water (BPW) onto the food bag
3. Homogenize the food sample immersed in BPW for 1 minute in a stomacher homogenizer
4. Place the food bag in an incubator at 37°C for 18-24 hours
5. Take out the food homogenization bag from the incubator and let it sit at room temperature without mixing for at least 5 minutes
6. Proceed to DNA extraction depending on your choice of kit (see the following subsections)

DNA extraction from pre-enriched food

After sample preparation, proceed to DNA extraction according to your choice of kit. Haven Scientific offers 3 DNA extraction kits that are compatible with the MMS Salmonella qPCR Genotyping Assay:

- DNAbler - Cells and Tissue 2.0 Kit (Cat. No. DE25050)

Spin column-based, versatile option that produces highly pure, highly concentrated, high molecular weight DNA that is suitable for all genomic applications. Compatible with all types of food matrices.

- Mag Bacterial DNAbler (Ref DE92050)

Automated magnetic bead-based high-throughput option that produces highly pure, highly concentrated, high molecular weight DNA that is suitable for all genomic applications. Compatible with all types of food matrices. Works with our MagEx32 (NE032) automated nucleic acid extraction system.

- DNAbler - Gram Negative Bacteria Direct Lysis Kit (Ref DE90100)

Direct extraction method, very quick and reliable, yields crude DNA suitable for pathogen detection by qPCR at an affordable price. Compatible with many types of food matrices.

Using the DNAbler – Cells & Tissue 2.0 kit (spin column)

The MMS Salmonella qPCR Genotyping Assay is equipped with an Internal Extraction Control (IC). It should be added to the lysate during extraction to ensure proper control of all steps of the process. Refer to the full manual of our (DNAbler – Cells & Tissue 2.0) kit for the detailed protocol.

1. Take out the food homogenization bag from the incubator and let it sit without mixing for at least 5 minutes, insert the sterile tip of a serological pipette ~2-3 cm below the surface of the BPW solution, withdraw 1mL of the pre-enriched BPW into a clean, RNase-free, 1.5mL microcentrifuge tube

    - ***Make sure to avoid any food clumps and withdraw only from the clear portion of the solution; if the food homogenate has a visible lipid layer at the top, insert the pipette tip deeper to ensure you withdraw only from the liquid layer beneath the lipid layer***

2. Centrifuge the homogenate-containing tube at ≥14,000g for 2 minutes then discard the supernatant; a pellet should be visible at the bottom of the tube

3. Continue the extraction according to the (Bacteria) protocol in the (DNAbler – Cells & Tissue 2.0) kit manual; however, make sure to add 10µL of the Internal Extraction Control (IC) to the sample AFTER adding 200µL of the Lysis/Binding Buffer

4. After extraction, the DNA-containing eluate is ready for use in qPCR; use it immediately or place it at -20°C for up to a week for later use

Using the Mag Bacterial DNAbler kit (automated, magnetic bead-based)

The MMS Salmonella qPCR Genotyping Assay is equipped with an Internal Extraction Control (IC). It should be added to the lysate during extraction to ensure proper control of all steps of the process. Refer to the full manual of our (Mag Bacterial DNAbler) kit for the detailed protocol. Our MagEx32 automated nucleic acid extraction system is needed for this protocol.

1. Take out the food homogenization bag from the incubator and let it sit without mixing for at least 5 minutes, insert the sterile tip of a serological pipette ~2-3 cm below the surface of the BPW solution, withdraw 0.25mL of the pre-enriched BPW into its designated well on the deep-well extraction plate

    - ***Make sure to avoid any food clumps and withdraw only from the clear portion of the solution; if the food homogenate has a visible lipid layer at the top, insert the pipette tip deeper to ensure you withdraw only from the liquid layer beneath the lipid layer***

2. Add 10µL of the Internal Extraction Control (IC) to the sample and continue the extraction according to the (Mag Bacterial DNAbler) kit manual

3. After extraction, the DNA-containing eluate is ready for use in qPCR, use it immediately or place it at -20°C for up to a week for later use

Using the DNAbler - Gram Negative Bacteria Direct Lysis kit (direct method)

The Internal Extraction Control (IC) is not compatible with our (DNAbler - Gram Negative Bacteria Direct Lysis) Kit. It should NOT be added to the lysate during extraction. Rather, it should be added to the qPCR reaction master mix during qPCR preparation. Refer to the full manual of our (Gram Negative Bacteria Direct Lysis) kit for the detailed protocol.

1. Take out the food homogenization bag from the incubator and let it sit without mixing for at least 5 minutes, insert the sterile tip of a serological pipette ~2-3 cm below the surface of the BPW solution, withdraw 1mL of the pre-enriched BPW into a clean, RNase-free, 1.5mL microcentrifuge tube

    - ***Make sure to avoid any food clumps and withdraw only from the clear portion of the solution; if the food homogenate has a visible lipid layer at the top, insert the pipette tip deeper to ensure you withdraw only from the liquid layer beneath the lipid layer***

2. Centrifuge the homogenate-containing tube at ≥14,000g for 2 minutes then discard the supernatant; a pellet should be visible at the bottom of the tube

3. Continue the extraction according to the (DNAbler - Gram Negative Bacteria Direct Lysis) kit manual

I. Sample Preparation and DNA Extraction (Animal Fluids or Tissue)

Veterinary sample preparation and DNA extraction is straightforward. Use one of our DNAbler kits according to your sample type following the kit's detailed manual:

- DNAbler - Cells and Tissue 2.0 Kit (Cat. No. DE25050)

Spin column-based, versatile option that produces highly pure, highly concentrated, high molecular weight DNA that is suitable for all genomic applications. Compatible with mammalian cells and tissue.

- DNAbler – Blood 2.0 Kit (Cat. No. DE26050)

Spin column-based, versatile option that produces highly pure, highly concentrated, high molecular weight DNA that is suitable for all genomic applications. Compatible with animal body fluids.

NOTE: Do not forget to add 10µL of IC to the samples during extraction AFTER addition of the Lysis Buffer.

J. Running qPCR Reactions

qPCR reactions are prepared using the MMS Salmonella qPCR Genotyping Assay. During extraction, the Extraction Internal control (IC) provided in this kit should have been added to each sample, which enables monitoring of the extraction process, and whether PCR inhibitors are present in the sample.

If the IC was not added during DNA extraction, or if the DNAbler – Gram Negative Bacteria Direct Lysis Buffer was used for extraction, the IC **MUST** be added to the qPCR reaction master mix as in step3 of this protocol. This does not enable monitoring of the extraction process, but does determine whether PCR inhibitors are present in the sample

1. Thaw all kit components at room temperature then place them on ice. Mix each tube by gentle flicking followed by a quick spin down to collect the tube's component to the bottom of the tube. ***Do NOT exceed 5 freeze-thaw cycles!***

2. Prepare a reaction master mix by mixing the following components (5% overage should be included to account for pipetting errors):

    a. *10 µL of qPCR Mix / reaction*
    b. *2 µL of the MMS Oligo Mix / reaction*

Example

Component	Per 1 reaction	Per 10 reactions (+5% overage)
qPCR Mix	*10 µL*	*105 µL*
MMS Oligo Mix	*2 µL*	*21 µL*

NOTE: Don't forget to figure in the positive and negative controls in your calculations!

3. (Only if IC was not added to the lysate during extraction. If it was added, proceed to step4) Dilute the IC 100X fold by mixing 1 µL of it with 99 µL of RNase-free water, then add 1 µL of this 100X diluted IC to the reaction master mix prepared in step2 so that the total volume per sample is 13 µL instead of 12 µL. In the example of step2, for 10X reactions +5% overage, an additional 10.5 µL of 100X diluted IC should be added.

4. Transfer 12 µL of the reaction master mix (prepared in step2) into each designated well on a 96-well optical qPCR plate.
5. Add 8 µL of each sample's extracted nucleic acid to its designated well; add 8 µL of the positive and negative controls to their designated wells.

NOTE: If you wish to add a smaller sample volume, add RNase-free water up to 20 µL

6. Seal the plate with optical cap strips or an optical adhesive seal. Spin down the plate to collect all components to the bottom of the plate.

7. On the Real-Time PCR thermal cycle software, define the detection filters as follows:

Target	Pan Salmonella	Typhimurium	Enteritidis	IC
Detection Channel	FAM	ROX	Cy5	HEX

8. On the software, define your Positive and Negative Controls.

9. Run the Real-Time PCR thermal cycler for 40 cycles as follows:

Step1: *2 minutes - 95°C (initial denaturation)*

Step2: *10 seconds - 95°C (denaturation)*

Step3: *30 seconds - 60°C (annealing/extension)* → ***DATA COLLECTION***

Step4: *go to step2 (X39 times)*

10. Use the next section (Data Interpretation) to call the samples positive or negative

K. Data Interpretation

Follow the 3 steps below sequentially to interpret the qPCR results:

1. Adjustment of software settings
2. Checking qPCR run validity
3. Sample calling

Adjustment of software settings

Adjust the thermal cycler's analysis software settings as follows:

- Quantstudio5:
  - Open the run file using the "Design and Analysis 2" software
  - Go to the "Quality Check" tab on the top of the screen, press on the "Actions" button, select "Primary Analysis Settings…"
  - Stay on the first tab named "General", and, on the "Default Settings" row, uncheck the box under the "Auto Threshold" column, a number should appear under the "Threshold" column, set it at 30,000
  - On the "Default Settings" row, uncheck the box under the "Auto Baseline" column, a number should appear under the "Baseline start" and "Baseline end" columns, set them at 3 and 12, respectively
  - Press the "Save" button on the bottom right, the "Quality Check" tab will reappear, press the "Analyze" button on the top right to apply the new settings
  - Look for qPCR false amplification curves (artifacts) and omit them from analysis; qPCR amplification curves follow a sigmoidal shape (exponential fluorescence increase, figure1) whereas qPCR artifacts have a linear amplification shape (figure2)

- CFX Opus96:
  - Open the run file using the "Bio-Rad CFX Maestro 2.3" software, a screen will appear and the "Quantification" tab on top will be selected
  - Just above the well selector, you will find the amplification channels (FAM, HEX, etc.), put a checkmark only on the FAM channel
  - Press the "Settings" button then select "Baseline Threshold…"
  - Under the "Baseline Cycles" option, select "User Defined", select the entire table, set the "Begin" option to 3 and hit Enter, set the "End" option to 12 and hit Enter
  - Under "Single Threshold", select "User Defined", set the value to 150, press OK
  - Repeat the same steps with all other channels

- Look for qPCR false amplification curves (artifacts) and omit them from analysis; qPCR amplification curves follow a sigmoidal shape (exponential fluorescence increase, figure1) whereas qPCR artifacts have a linear amplification shape (figure2)

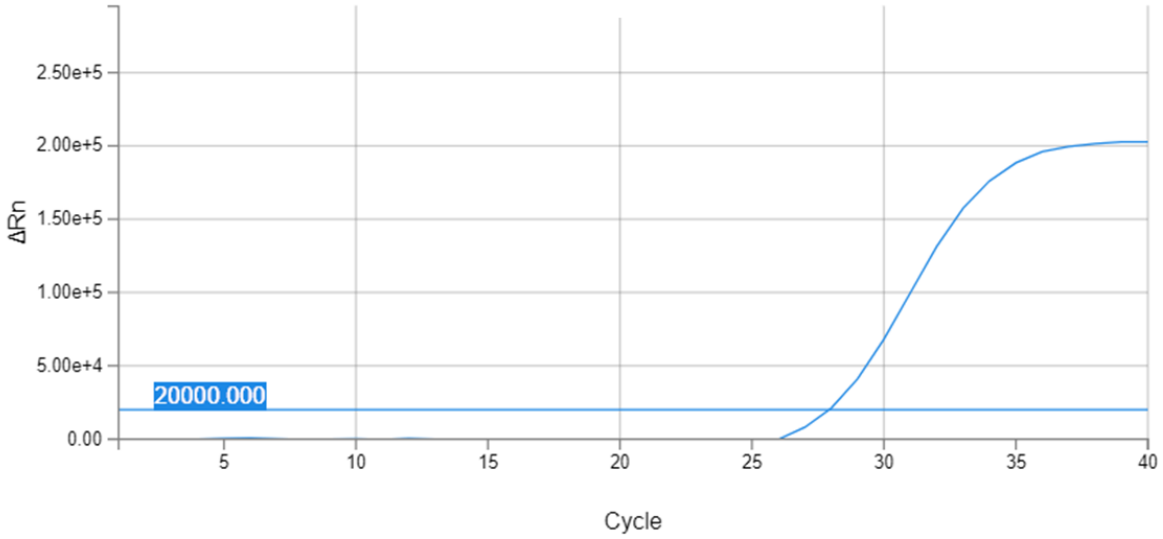

Figure1: *The typical "good" qPCR amplification plot has a sigmoidal shape, in which the fluorescence increases exponentially each subsequent cycle.*

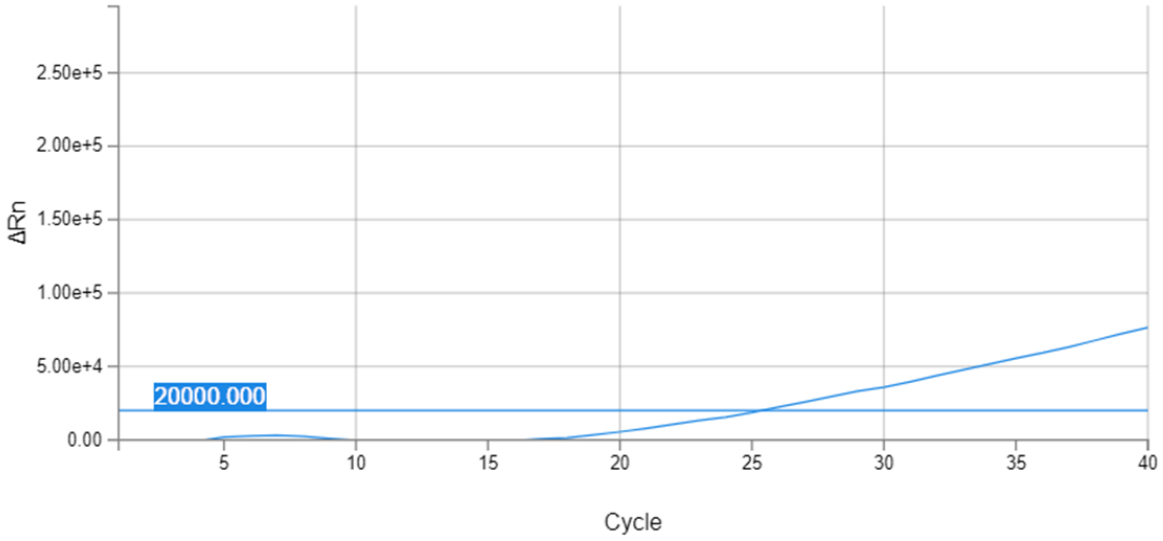

Figure2: *The non-typical "bad" qPCR amplification plot has a linear shape. This is a mere qPCR artifact, not an actual amplification, and it should be omitted from analysis.*

Checking qPCR run validity

Before sample calling, the run must be validated first using the positive and negative controls. For the run to be valid, the Negative Control must return a negative infection result, whereas the Positive Control must return a positive co-infection result (see sample calling below). If not, the whole run is considered invalid.

Sample calling

For the three Salmonella targets, the acceptable cycle threshold of detection is Ct <40. If only the Pan target is detected, Salmonella infection is positive (non-Enteritidis/non-Typhimurium). Enteritidis or Typhimurium infection is positive if the serovar-specific target is detected along with the Pan target. If only the serovar-specific target is detected, serovar-specific infection is presumptive positive, but re-extracting DNA and retesting may be necessary. If all of the Enteritidis-specific, Typhimurium-specific, and Pan targets are detected, there is co-infection of Enteritidis and Typhimurium. If the serovar-specific targets are detected without the Pan target, co-infection is presumptive, but we recommend re-extracting DNA and retesting. If none of the three Salmonella targets is detected, the sample is true negative only if the cycle threshold of the Internal Control was ≤35. Otherwise, there may be a problem in the extraction step, or the sample may be contaminated with PCR inhibitors. In this case, re-extracting DNA and retesting is necessary.

The following table summarizes our sample calling recommendations. A positive (+) sign indicates a Ct < 40 whereas a negative (–) sign indicates no amplification.

	PAN (FAM) CT	TYPHIMUR (ROX) CT	ENTERIT (CY5) CT	IC (HEX) CT	INTERPRETATION
CASE 1	+	–	–	Any	**Salmonella infection**
CASE 2	+	+	–	Any	**Typhimurium infection**
CASE 3	+	–	+	Any	**Enteritidis infection**
CASE 4	+	+	+	Any	**Enteritidis/Typhimurium co-infection**
CASE 5	–	+	–	Any	**Presumptive Typhimurium infection; retest**

CASE 6	–	–	+	Any	**Presumptive Enteritidis infection; retest**
CASE 7	–	+	+	Any	**Presumptive Enteritidis/Typhimurium co-infection; retest**
CASE 8	–	–	–	≤35	**No infection**
CASE 9	–	–	–	>35	**Extraction failed; re-extract the sample**

L. Support

For questions, suggestions, or technical support, feel free to contact us by email on:

support@havensci.com

M. Quality Control

Each lot is tested against predetermined parameters to ensure consistent performance among all lots.