May 02, 2024

Protocol for Identifying Highly Pathogenic Salmonella Using the HPS Multiplex PCR Assay

DOI
dx.doi.org/10.17504/protocols.io.6qpvr3on3vmk/v1

Dayna Harhay[1], Kerry D. Brader[1], Jim Bono[1], Gregory P Harhay[1], Mick Bosilevac[1], Tatum S Katz[1]

[1]US Meat Animal Research Center, Agricultural Research Service, US Department of Agriculture, Clay Center, NE, USA

USDA-ARS Salmonella G…

Tatum S Katz
US Meat Animal Research Center, Agricultural Research Servic...

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.6qpvr3on3vmk/v1

Protocol Citation: Dayna Harhay, Kerry D. Brader, Jim Bono, Gregory P Harhay, Mick Bosilevac, Tatum S Katz 2024. Protocol for Identifying Highly Pathogenic Salmonella Using the HPS Multiplex PCR Assay. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.6qpvr3on3vmk/v1

Protocol status: Working
We use this protocol and it's working

Created: July 05, 2023

Last Modified: May 02, 2024

Protocol Integer ID: 84558

Keywords: Non-typhoidal Salmonella enterica, NTSE, Highly Pathogenic Salmonella, HPS, Polymerase Chain Reaction, PCR, multiplex PCR assay, virulence genes, pathogenicity, NP108, CRIS 3040-42000-020-00D, Meat Safety and Quality Research Unit

Funders Acknowledgement:
CRIS
Grant ID: 3040-42000-020-00D

Disclaimer

The use of product and company names is necessary to accurately report the methods and results; however, the United States Department of Agriculture (USDA) neither guarantees nor warrants the standard of the products, and the use of names by the USDA implies no approval of the product to the exclusion of others that may also be suitable. The USDA is an equal opportunity provider and employer.

Abstract

This protocol describes how to perform a multiplex PCR assay for the identification of Highly Pathogenic *Salmonella* (HPS). The assay targets eight virulence genes including: *sseK2* (HPS-6), *sseK3* (HPS-1), *avrA* (HPS-3), *lpfB* (HPS-5), *spvD* (HPS-4), *sspH2* (HPS-7), *gtgA* (HPS-2) and *invA* (i). Comparative genomic research using 23 complete closed *Salmonella* genome sequences (1-23) revealed these genes to be shared among *Salmonella* serotypes noted for being invasive and/or causing most cases of salmonellosis (i.e. consistently on the CDC's list of top 20 serotypes attributed to human illness) but to varying degrees are absent among serotypes that are less frequently associated with human illness. Validation of this assay with 1303 *Salmonella* of 69 different serotypes confirmed the utility of this assay for identifying HPS (Harhay *et. al.*, in preparation). *Salmonella* samples that result in the amplification of five or more targets are identified as HPS, and the markers amplified are reported as an HPS index (HPSi) (Figure 1). The assay described is intended for use with DNA lysates of *Salmonella* isolates, not lysates of enrichment cultures, where more than one *Salmonella* serotype may be present. Notably, the HPS assay is not serotype specific (i.e. the results do not indicate the presence of a particular serotype) rather it provides an indication of the potential pathogenicity of the *Salmonella* being assayed. This protocol includes information on generation of template DNA, primer sequences needed (Table 1), construction of the PCR master mix (Table 2), thermal cycler program used for amplification (Table 3), parameters for DNA gel electrophoresis, and amplicon visualization and scoring (Figure 1). Further evidence of the utility of the HPS gene targets is their recent addition to the AMRFinderPlus Reference Gene Catalog.

Attachments

HPS_protocol_outline...
283KB

Table 1.xlsx
10KB

Table 2.xlsx
11KB

Table 3.xlsx
10KB

Figure 1.pdf
94KB

Materials

Bacterial Lysis (BAX lysis) Procedure to prepare DNA template

Equipment:
Thermal cycler (BIO-RAD T100)
Lysis Program (see below)
PCR plates & Microseal B (BIO-RAD; #MSB-1001)
Multi-Channel Pipettors

Buffers and reagents:
BAX Lysis Buffer (Hygiena; item #ASY2011; ref. #D14403062)
BAX Protease Vial (Hygiena; item #ASY2012; ref. #D11134081)
Control strain (S. Typhimurium ATCC - 14028S (7))
Overnight Culture of samples in question

HPS Multiplex PCR Reaction

Equipment:
Thermal cycler (BIO-RAD T100)
Program (Table 3)
PCR plates & Microseal B (BIO-RAD; #MSB-1001)
Multi-Channel Pipettors (10ul and 20ul 12 channel preferrable)

Buffers and reagents:
Bullseye HS Taq Pol (MidSci; BE225108)
Deoxynucleotide (dNTP) Solution Mix (New England Biolabs; N0447L)
Primer list: Table 1.
Master Mix: Table 2.

Agarose gel electrophoresis and staining, to visualize DNA amplicons (see Figure 1)

Equipment:
4x26-Well Combs, Fixed Height, 1.5 mm Thickness, Multichannel Pipet Compatible (BIO-RAD; #1704457)
Sub-Cell GT Horizontal Electrophoresis System, 15 x 25 cm tray, with gel caster (BIO-RAD; #1704484)
0.5 L Erlenmeyer flask
Ethidium Bromide Extractor (GVS; item #10448031). Use as per manufacturers instruction.

Buffers and reagents:
HPS Marker generated using Control strain (S. Typhimurium ATCC - 14028S (7))
10x TBE (Fisher Scientific; #BP1333) - NOTE: it is important to use TBE; buffers such as SB result in poor band separation.
Bullseye General Purpose Agarose GP2 (MidSci; #BE-A500)
6x loading dye (purchased or homemade)

Tables and Figures

Table 1. HPS primer list

Primer name	position	primer size	Tm	sequence 5'-3'	product size
HPS-6-F	4 F	21	55.3	GCACGTTTTAATGCCGCTTTT	844
HPS-1-F	71 F	21	56.5	TGCCTTCATCAGGTAGTGCAA	741
HPS-6-R	847 R/811 R	19	56.8	GATGCTCGCTGCGGTTAAC	
HPS-3-F	74 F	20	57.0	CGAGTTTATCGCCTCAGCCT	662
HPS-3-R	735 R	20	55.2	TCGTTGTRCGCCTTGAGTAT	
HPS-5-F	39 F	20	56.8	GCTGGCGTTGATTGCTCAAA	557
HPS-5-R	595 R	20	57.4	TCAATGAGCCTTTTGCCGGA	
HPS-4-F	12 F	20	57.1	TGGTAGTGCGTCATCCCAAG	452
HPS-4-R	463 R	22	56.4	GAGCTATGTAGTTCTCTGCGCT	
HPS-7-1397-F	1397 F	22	58.9	ACTGTCTGAACGTACTTTGCAG	381
HPS-7-1777-R	1777 R	18	60.5	CGGTCCTCGCAGCTTGAG	
HPS-2-F	124 F	20	57.4	CGCCTCAACATGATGGTCT	338
HPS-2-R	461 R	20	57.4	TCCAGGTTGAGGGCAATCAC	
INVA-F	294 F	19	59.7	TATCGCCACGTTCGGGCAA	275
INVA-R	568 R	19	58.1	TCGCACCGTCAAAGGAACC	

Table 2. HPS Master Mix

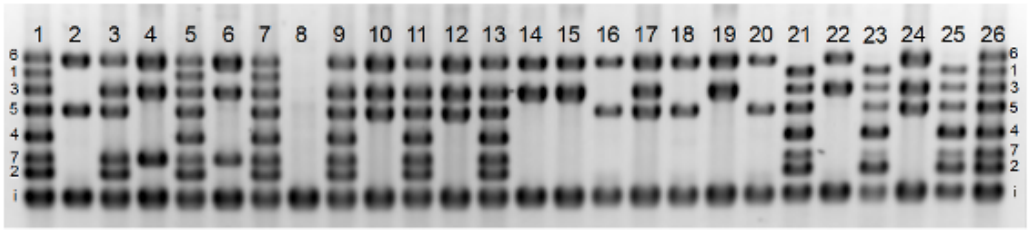
Master Mix Recipe	1 rxn	25 rxn	50 rxn	500 rxn
Reagent	µL	µL	µL	µL
PCR grade H2O	16.011	400.275	800.55	8005.5
10x Combination Buffer w/ MgCl2	2.5	62.5	125	1250
dNTP	0.59	14.75	29.5	295
(200 uM) HPS-1-F	0.031	0.775	1.55	15.5
(200 uM) HPS-6-F	0.031	0.775	1.55	15.5
(200 uM) HPS-6-R	0.031	0.775	1.55	15.5
(200 uM) HPS-2-F	0.02	0.5	1	10
(200 uM) HPS-2-R	0.02	0.5	1	10
(200 uM) HPS-3-F	0.031	0.775	1.55	15.5
(200 uM) HPS-3-R	0.031	0.775	1.55	15.5
(200 uM) HPS-4-F	0.02	0.5	1	10
(200 uM) HPS-4-R	0.02	0.5	1	10
(200 uM) HPS-5-F	0.02	0.5	1	10
(200 uM) HPS-5-R	0.02	0.5	1	10
(200 uM) HPS-7-F	0.031	0.775	1.55	15.5
(200 uM) HPS-7-R	0.031	0.775	1.55	15.5
(200 uM) INVA-F	0.031	0.775	1.55	15.5
(200 uM) INVA-R	0.031	0.775	1.55	15.5
TAQ (Bullseye Taq)	0.5	12.5	25	250
Final Volume µL	20	500	1000	10000

Table 3. Thermal cycler program adapted from (24)

Step	Degrees	Action	Time
1	95		15:00
2	95		0:30
3	65		1:00
4	72		1:00
5	Go to	step 2	8x
6	95		0:30
7	64		0:45
		-1.5°C per cycle	
8	72		1:00
9	Go to	step 6	4x
10	95		0:30
11	56		0:30
12	72		1:00
13	Go to	step 10	12x
14	95		0:30
15	56		0:30
16	72		1:00
		+10 sec/cycle	
17	Go to	step 14	9x
18	4		Hold

Figure 1. Example agarose gel depicting the various HPS banding patterns.

Figure 1.



Well #	Serotype	HPSi✦	Result	Well #	Serotype	HPSi✦	Result
1	Typhimurium (ATCC 14028S)	6135472i	HPS	14	Cerro	63i	non-HPS
2	Lille	65i	non-HPS	15	Cerro	63i	non-HPS
3	Typhimurium	63572i	HPS	16	Lille	65i	non-HPS
4	Anatum	637i	non-HPS	17	Lubbock	635i	non-HPS
5	Typhimurium	6135472i	HPS	18	Lille	65i	non-HPS
6	Anatum	637i	non-HPS	19	Cerro	63i	non-HPS
7	Typhimurium	6135472i	HPS	20	Lille	65i	non-HPS
8	Montevideo	i	non-HPS	21	Dublin	135472i	HPS
9	Typhimurium	635472i	HPS	22	Cerro	63i	non-HPS
10	Lubbock	635i	non-HPS	23	Dublin	135472i	HPS
11	Typhimurium	635472i	HPS	24	Lubbock	635i	non-HPS
12	Lubbock	635i	non-HPS	25	Dublin	135472i	HPS
13	Typhimurium	635472i	HPS	26	Typhimurium (ATCC 14028S)	6135472i	HPS

Figure 1. Example agarose gel depicting the various HPS banding patterns that can be observed using the methods described and template DNA from different *Salmonella* serotypes. Well number designations are indicated at the top of the figure. *Salmonella* Typhimurium strain ATCC 14028S is used as a positive control and marker, since it produces all 8 amplicons (bands) that can be generated by the assay. The bands in marker lanes (1 and 26) are labeled with the corresponding HPS marker numbers. The HPS index (HPSi✦) for each well is indicated in the table, and the interpretation (≥ 5 targets amplified = HPS, or 1 to 4 amplicons = non-HPS) listed in the Result column.

Bacterial Lysis (BAX lysis) Procedure to prepare DNA template

1 Prepare the BAX Lysis Buffer: Add 150ul Protease solution into 12mL Lysis Buffer.

NOTE: this solution is stable at 4C for two weeks

2 In a PCR plate, dispense 100 µL of the BAX lysis buffer per well, add 2.5 µL of overnight culture, seal with Microseal B, and place in thermal cycler. Run Lysis Protocol listed below (35 minutes).

2.1 Reaction:
Add 100 µL Protease/Lysis Buffer per well in the PCR plate
Add 2.5 µL Sample (overnight culture) per well

2.2 Lysis Thermal Cycler Program:
37 C – 20 min
95 C – 10 min
Cool 4 C – 5 min

NOTE: make sure lid of thermal cycler is set to 96C

3 Move on to PCR or freeze lysates

HPS Multiplex PCR Reaction

4 Make up HPS master mix following Table 2. Store at -20 °C.

5 Thaw master mix, vortex, aliquot 20 µL per well in PCR plate.

6 Add 5 µL of lysate (if frozen, thaw and gently pipette to resuspend) per well. Final reaction volume is 25 µL.

7 Seal plate with Microseal B and place in thermocycler.

8 Run program according to Table 3.

9 Once complete, begin gel or store PCR products at -20 degrees C.

Agarose gel electrophoresis and staining, to visualize DNA amplicons

10 Make a 2% agarose gel (in 0.5L flask, dissolve with heat 5g agarose in 250mL of 1X TBE) and cast in 15x25cm array.

11 After gel is setup, transfer to buffer tank containing enough 1X TBE to cover gel and remove combs.

12 To completed HPS PCR reaction plate, gently remove the Microseal B cover tape, add 6 µL of 6X loading dye to the reaction. Pipette up and down to mix, then with same tip, transfer 6 µL of mixture to the gel and dispense in an appropriate well.

13 Flank with control strain *S. Typhimurium* ATCC – 14028S (7) on either side of reaction wells.

14 Run gel at 170 volts for approximately 60 minutes.

15 Staining

15.1 After electrophoresis, stain the gel with Ethidium Bromide (EtBr) (~1 µg/mL: from a 1% stock solution, add 10 µL EtBr per 100 mL dH_2O) for 30 min. Use a BioRad staining tray with a lid and place this on a rocker to gently agitate the stain with the gel in the tray.

NOTE: use caution and appropriate PPE with EtBr (Toxic if inhaled; Suspected of causing genetic defects)

15.2 Carefully pour off the stain (use gloves and appropriate PPE) into an Ethidium Bromide Extractor (see Materials) or use similar means of safely disposing of the staining solution.

15.3 Add 100ml of water to the tray to de-stain the gel for 15-20 minutes. Again, use a rocker on a low setting. Dispose of the de-stain water appropriately.

16 Image with a UV imager (Figure 1).

Protocol references

1. *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. SL1344 genome sequencing project
Organism: *Salmonella enterica* subsp. enterica serovar Typhimurium str. SL1344 (Taxonomy ID 216597) BioProject Accession: PRJNA50407; ID: 50407; Genbank Accession Number: **NC_016810**

2. *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2 RefSeq Genome
Organism: *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2 (Taxonomy ID 99287) BioProject Accession: PRJNA57799; ID: 57799; Genbank Accession Number: **NC_003197**

3. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. USDA-ARS-USMARC-1808 (Taxonomy ID 1454630) BioProject Accession: PRJNA236699; ID: 236699; Genbank Accession Number: **CP014970**

4. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. CDC H2662 (Taxonomy ID 1454640) BioProject Accession: PRJNA236709; ID: 236709; Genbank Accession Number: **CP014979**

5. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. CDC 2009K-1640 (Taxonomy ID 1454644) BioProject Accession: PRJNA236713; ID: 236713; Genbank Accession Number: **CP014975**

6. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. USDA-ARS-USMARC-1896 (Taxonomy ID 1454636) BioProject Accession: PRJNA236705; ID: 236705; Genbank Accession Number: **CP014977**

7. *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. 14028S
Organism: *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. 14028S (Taxonomy ID 588858) BioProject Accession: PRJNA33067; ID: 33067; Genbank Accession Number: **CP001363**

8. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. USDA-ARS-USMARC-1880 (Taxonomy ID 1454634) BioProject Accession: PRJNA236703; ID: 236703; Genbank Accession Number: **CP014981**

9. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Newport str. CDC 2010K-2159 (Taxonomy ID 1454627); BioProject Accession: PRJNA236696; ID: 236696; Genbank Accession Number: **CP007559**

10. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Newport str. USDA-ARS-USMARC-1925 (Taxonomy ID 1454618) BioProject Accession: PRJNA236687; ID: 236687; Genbank Accession Number: **CP025232**

11. USMARC NAMA *Salmonella* Sequencing Project

Organism: *Salmonella enterica* subsp. *enterica* serovar Newport str. USDA-ARS-USMARC-1928 (Taxonomy ID 1454621) BioProject Accession: PRJNA236690; ID: 236690; Genbank Accession Number: **CP025237**

12. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Newport str. USDA-ARS-USMARC-1929 (Taxonomy ID 1454622) BioProject Accession: PRJNA236691; ID: 236691; Genbank Accession Number: **CP025241**

13. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Newport str. CDC 2009K-1331 (Taxonomy ID 1454625) BioProject Accession: PRJNA236694; ID: 236694; Genbank Accession Number: **CP025248**

14. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Newport str. USDA-ARS-USMARC-1927 (Taxonomy ID 1454620) BioProject Accession: PRJNA236689; ID: 236689; Genbank Accession Number: **CP007216**

15. *Salmonella enterica* subsp. *enterica* serovar Dublin strain:USMARC-69807 Genome sequencing and assembly
Organism: *Salmonella enterica* subsp. *enterica* serovar Dublin (Taxonomy ID 98360) BioProject Accession: PRJNA485466; ID: 485466; Genbank Accession Number: **CP032379**

16. *Salmonella enterica* subsp. *enterica* serovar Dublin strain:USMARC-69840 Genome sequencing and assembly
Organism: *Salmonella enterica* subsp. *enterica* serovar Dublin (Taxonomy ID 98360) BioProject Accession: PRJNA485470; ID: 485470; Genbank Accession Number: **CP032446**

17. *Salmonella enterica* subsp. *enterica* serovar Dublin strain:USMARC-69838 Genome sequencing and assembly
Organism: *Salmonella enterica* subsp. *enterica* serovar Dublin (Taxonomy ID 98360); BioProject Accession: PRJNA485469; ID: 485469; Genbank Accession Number: **CP032449**

18. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Anatum str. USDA-ARS-USMARC-1676 (Taxonomy ID 1454587)BioProject Accession: PRJNA236658; ID: 236658; Genbank Accession Number: **CP014620**

19. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Anatum str. USDA-ARS-USMARC-1735 (Taxonomy ID 1454585) BioProject Accession: PRJNA236656; ID: 236656; Genbank Accession Number: **CP014707**

20. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Montevideo str. CDC 2012K-1544 (Taxonomy ID 1454611); BioProject Accession: PRJNA236681; ID: 236681; Genbank Accession Number: **CP017977**

21. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Montevideo str. CDC 2013K-0218 (Taxonomy ID 1454612) BioProject Accession: PRJNA236682; ID: 236682; Genbank Accession Number: **CP017978**

22. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Montevideo str. CDC 07-0954 (Taxonomy ID 1454607) BioProject Accession: PRJNA236677; ID: 236677; Genbank Accession Number: **CP017974**

23. USMARC NAMA *Salmonella* Sequencing Project
Organism: *Salmonella enterica* subsp. *enterica* serovar Montevideo str. CDC 86-0391 (Taxonomy ID 1454606) BioProject Accession: PRJNA236676; ID: 236676; Genbank Accession Number: **CP007540**

24. Paton AW, Paton JC. 1998. Detection and Characterization of Shiga Toxigenic *Escherichia coli* by Using Multiplex PCR Assays for stx_1, stx_2, *eaeA*, Enterohemorrhagic *E. coli hlyA*, rfb_{O111}, and rfb_{O157}. J Clin Microbiol 36(2):598-602. https://doi.org/10.1128/jcm.36.2.598-602.1998