JVDI: Supplementary material

Naberhaus SA, et al. Emergence of *Salmonella enterica* serovar 4,[5],12:i:- as the primary serovar identified from swine clinical samples and development of a multiplex real-time PCR for improved *Salmonella* serovar-level identification

Supplementary Table 1. Primer and probe sequences utilized for the multiplex real-time PCR for differentiation of *Salmonella* Typhimurium and 4,[5],12:i:- serovars.

Component	Gene and sequence	PCR product (bp)	Reference
	invA		
Forward primer	CATTTCTATGTTCGTCATTCCATTACC	133	[2]
Reverse primer	AGGAAACGTTGAAAAACTGAGGATTCT		
Probe	56-FAM-TCTGGTTGA-ZEN-TTTCCTGATCGCACTGAATATC-3IABkFQ		
	fliA		
Forward primer	CATTACACCTTCAGCGGTAT	254	[1]
Reverse primer	CTGGTAAGAGAGCCTTATAGG		
Probe	5Cy55-CGGCATGATTATCCGTTTCTACAGGG-3IAbRQSp		
	hin-iroB		
Forward primer	GTGTGGCATAAATAAACCGA	274	[1]
Reverse primer	AGGCTTACCTGTGTCATCCA		
Probe	5Hex-TAACGCGCT-ZEN-CACGATAAGGC-3IABkFQ		
	fljB		
Forward primer	TGGTGCTGTTAGCAGAC	297	[1]
Reverse primer	TCAACACTAACAGTCTGTCG		
Probe	5TexRd-XN-AACCGCCAGTTCACGCAC-3IAbRQSp		
	IAC (XIPC695)		
Forward primer	TTCGGCGTGTTATGCTAACTTC	NA	ISU-VDL internal control
Reverse primer	GGGCTCCCGCTTGACAATA		
Probe	5Cy5-CTCCGCAGA-TAO-AATCCAGGGTCACG-3IAbRQSp		

IAC = internal amplification control; ISU-VDL = Iowa State University, Veterinary Diagnostic Laboratory (Ames, IA); NA = not applicable.

References

1. Maurischat S, et al. Rapid detection and specific differentiation of *Salmonella enterica* subsp. *enterica* Enteritidis, Typhimurium and its monophasic variant 4,[5],12:i:- by real-time multiplex PCR. Int J Food Microbiol 2015;193:8–14.
2. Pusterla N, et al. Use of quantitative real-time PCR for the detection of *Salmonella* spp. in fecal samples from horses at a veterinary teaching hospital. Vet J 2010;186:252–255.

Supplementary Table 2. Results of real-time PCR exclusivity testing to ensure no cross-reactivity of non-Typhimurium, non-4,[5],12:i:- serovars of *Salmonella* or other non-*Salmonella* organisms commonly found in feces.

Salmonella serovar	*n*	*invA*	*fliA*	*hin-iroB*	*fljB*
Agona	2	+	–	–	–
Anatum*	2	+	–	+	+
Derby	2	+	–	–	–
Heidelberg	2	+	–	+	+
Infantis	2	+	–	+	+
Johannesburg	2	+	–	–	+
Mbandaka*	2	+	–	+	+
Ohio*	2	+	–	+	+
Senftenberg	2	+	–	–	–
Worthington*	2	+	–	+	+
Bovismorbificans	1	+	–	–	+
Brandenburg	1	+	–	–	+
Cerro	1	+	–	–	–
Choleraesuis var kunzendorf	1	+	–	+	+
Give	1	+	–	–	+
Havana	1	+	–	–	–
Kiambu	1	+	–	+	+
Krefeld*	1	+	–	+	+
Litchfield	1	+	–	+	+
Liverpool	1	+	–	+	+
London	1	+	–	+	+
Muenchen*	1	+	–	+	+
Newport*	1	+	–	+	+
6,7:Nonmotile	1	+	–	+	+
Rissen	1	+	–	–	–
Saintpaul	1	+	–	+	+
Schwarzengrund	1	+	–	–	+
Uganda*	1	+	–	+	+

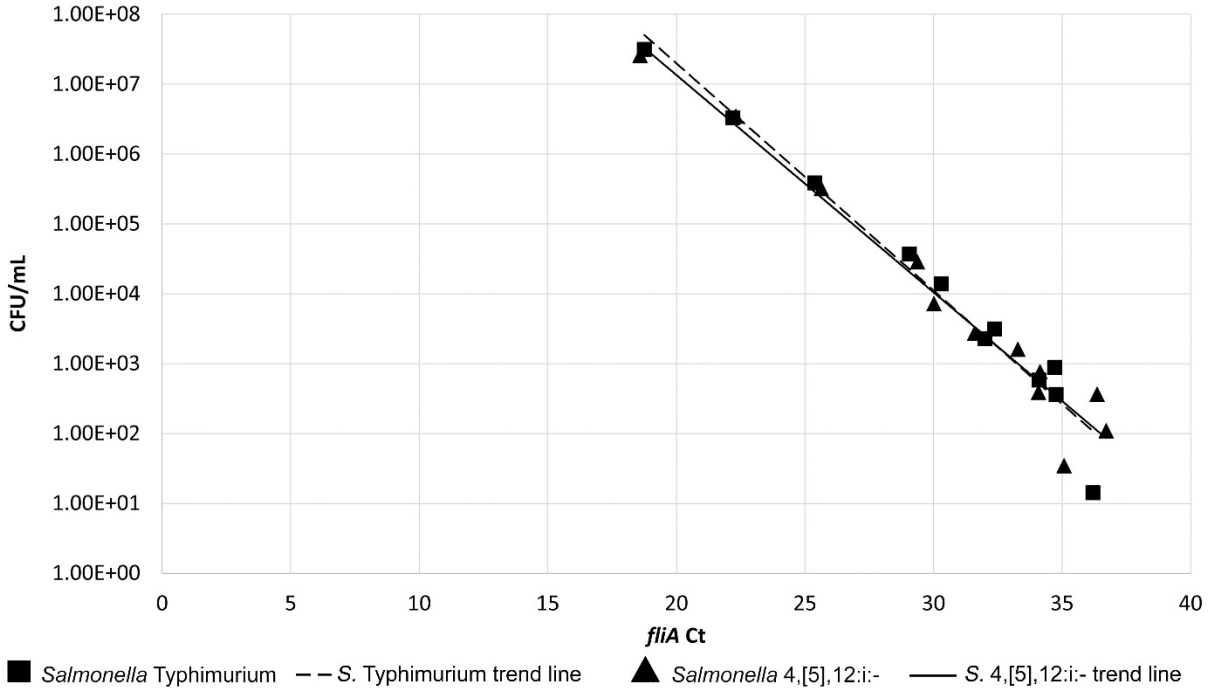
One isolate each of *Citrobacter freundii*, *Enterobacter* spp., *Enterococcus faecium*, *Enterococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Streptococcus suis* were tested and found negative in all 4 assays.

* Cycle threshold (Ct) value was >8 more than Ct value of *fliA* and *invA*, similar to the findings with *S*. 4,[5],12:i:-.

Supplementary Table 3. Results of real-time PCR inclusivity testing of *Salmonella* Typhimurium and 4,[5],12:i:- isolates to ensure correct identification of serovar compared to traditional serotyping.

Salmonella serovar	*n*	*invA*	*fliA*	*hin-iroB*	*fljB*
4,[5],12:i:-	61	+	+	-	+*
Typhimurium	45	+	+	+	+
Isolate A (4,[5],12:i:-)	1	+	+	+	+*
Isolate B (Typhimurium)	1	+	+	-	+

* Denotes that cycle threshold (Ct) value was >8 more than Ct value of *fliA* and *invA*.



Supplementary Figure 1. Determination of the limit of detection of the *fliA* probe of the multiplex real-time PCR assay compared to standard plate counts to identify *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- following inoculation into *Salmonella*-negative feces.