Text S1. Considerations for interpreting serotype in *Salmonella* based on the conventions of the White-Kauffmann-Le Minor Scheme

The Kauffmann-White-Le Minor Scheme is the international standard for designation of *Salmonella* serotypes. An electronic copy is available at http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089. The Scheme is based primarily on O group and H antigens; however, additional O epitopes have been described for some O groups, referred to here as ancillary O antigens. Also, additional characteristics unrelated to serotype antigens need to be considered for some serotypes.

1) The genes responsible for serotype are subject to horizontal genetic transfer which can result in the same complement of serotype antigens existing in different genetic backgrounds. Identification of strains to the subspecies level should accompany serotype determination; the same antigenic profile in different subspecies is considered different serotypes. Some examples are listed in Table 1

Table 1. Examples serotypes with the same antigenic profiles but belonging to different subspecies

# isolates reported to US National Surveillance, 2007 to 2011	Serotype	Sub-species	O Group	H Phase 1	H Phase 2
72	IIIa 41:z4,z23:-	IIIa	41	z4,z23	-
13	Waycross	I	41	z4,z23	[e,n,z15]
0	IV 41:z4,z23:-	IV	41	z4,z23	-
152	Cerro	I	18	z4,z23	[1,5]
143	IIIa 18:z4,z23:-	IIIa	18	z4,z23	-
11423	Javiana	I	9	l,z28	1,5
0	II 9,12:l,z28:1,5	II	9	l,z28	1,5
89	IV 48:g,z51:-	IV	48	g,z51	-
63	IIIa 48:g,z51:-	IIIa	48	g,z51	-

2) For some serotypes, additional markers must be considered in assigning serotype. This includes primarily "biotypes", serotypes that have unique phenotypic profiles. Table 2 lists serotypes that have same antigenic profile and are differentiated by phenotypic properties. Serotype Gallinarum is also identified by phenotypic profile and is discussed in item 4 below. See Kauffmann-White-Le Minor Scheme for details. The SeqSero output for strains belonging to these serotypes includes the antigenic profile only with a notation that additional characterization must be done in order to assign to a specific serotype.

Table 2. Serotypes that are also defined by phenotypic profile.

Serotype	Sub-species	O Antigen	O Group	H Phase 1	H Phase 2	Phenotypic profile
Fulica	I	4,[5],12	4	a	[1,5]	rhamnose -, gas from glucose -, dulcitol -, trehalose -, Simmons citrate -, L(+) tartrate -, mucate -, H2S -, tetrathionate-reductase -
Hessarek	I	4,12,[27]	4	a	1,5	typical for subspecies I, i.e., rhamnose +, gas from glucose +, dulcitol +, trehalose +, Simmons citrate +, L(+) tartrate +, mucate +, H2S +, tetrathionate-reductase +
Schleissheim[1]	I	4,12,[27]	4	b	-	dulcitol-, gelatinase +
Paratyphi B[2]	I	[1],4,[5],12	4	b	1,2	L(+) tartrate -, dulcitol+, gelatinase -
Paratyphi B var. L(+) tartrate+[2]	I	[1],4,[5],12	4	b	1,2	L(+) tartrate +, dulcitol+, gelatinase -
Choleraesuis	I	6,7	7	c	1,5	dulcitol -, mucate -, H2S -, L(+) tartrate +
Choleraesuis var. Decatur	I	6,7	7	c	1,5	typical for subspecies I, i.e., dulcitol +, mucate +, H2S +, L(+) tartrate +
Choleraesuis var. Kunzendorf	I	6,7	7	c	1,5	dulcitol -, mucate -, H2S +, L(+) tartrate +
Paratyphi C	I	6,7,[Vi]	7	c	1,5	dulcitol +, mucate -, H2S +, L(+) tartrate +; may also be Vi +
Typhisuis	I	6,7	7	c	1,5	dulcitol -, mucate -, H2S -, L(+) tartrate -
Miami	I	[1],9,12	9	a	1,5	H2S +, citrate +, L(+) tartrate +
Sendai	I	[1],9,12	9	a	1,5	H2S -, citrate -, L(+) tartrate -

[1] A monophasic variant of serotype Paratyphi B var. L(+) tartrate+ , serotype I 4,5,12:b:- , is fairly common in the United States and is often confused with serotype Schleissheim.

[2] Serotypes Paratyphi B and Paratyphi B var. L(+) tartrate+ also represent pathovars; serotype Paratyphi B is associated with paratyphoid fever and Paratyphi B var. L(+) tartrate+ is associated with uncomplicated gastrointestinal infections. They can also be differentiated by virulence markers.

3) The core of the O antigen is the O group; most genes involved in O group biosynthesis are found in the *rfb* cluster, which is targeted in the SeqSero pipeline. For some O groups, additional O epitopes have been described, referred to here as ancillary O antigens. Ancillary O antigens are typically encoded outside the rfb region; for example, O5 in serogroup O4 has been shown to be encoded by a bacteriophage. In many instances, ancillary O antigens are not the basis for distinguishing two serotypes and are not required for serotype assignment. There are three exceptions to this:

i. For serogroup O13, determination of ancillary O antigens O22 versus O23 is required for serotype assignment. The genetic basis for this difference is currently unknown.
ii. For serogroup O8, ancillary O antigen O6 has been shown to be variably expressed (Mikoleit 2012). Serotypes within group O8 that differ only O6, e.g., Hadar and Istanbul, Newport and Bardo, should be considered the same serotype.
iii. For serogroup O6,14, ancillary O antigens O24 and O:25 are variably expressed (unpublished data). Characterization of 6,14,24 and 6,14,25 serotypes that share the same H antigens (e.g., Carrau and Madelia) suggest that these are likely unique lineages; however, since O24 and O25 are variably expressed, it is impossible to distinguish O24+/O24- and O25+/O25- lineages based on serotyping alone. They should be considered the same serotype.

4) Serotype Gallinarum (antigenic formula I 9,12:nonmotile) has a non-expressed H g,m *fliC* allele; so, it looks like serotype Enteritidis when serotype is determined based on genetic markers. It also has a unique phenotypic profile, a unique pathotype, and has been shown to be a distinct lineage by genetic criteria (reference). Using phenotypic serotyping methods, nonmotile variants of Group O9 serotypes have an antigenic profile indistinguishable from serotype Gallinarum, I 9,12:nonmotile but can be differentiated from serotype Gallinarum by phenotypic profile and other methods. While serotype Gallinarum is rare, strains identified with antigenic profile 9:g,m:- using genetic methods should be further characterized to rule out the possibility of a serotype Gallinarum strain being mis-identified as serotype Enteritidis. The gene *sdf* has been shown to pre present in commonly circulating serotype Enteritidis strains but not in serotype Gallinarum strains.

5) Although not related to the conventions of the Kauffmann-White-Le Minor Scheme, rough, nonmotile and monophasic strains as determined by phenotypic methods may possess non-expressed serotype determinants that can be detected by genetic methods. In particular, some monophasic variants, e.g. some isolates of serotype I 4,5,12:b:- and serotype IIIb 61:-:1,5,7, may appear diphasic using genetic methods to determine serotype.