National Standard of the People's Republic of China

GB 4789.4-2016

National Food Safety Standard

Food Microbiological Examination: *Salmonella*

食品安全国家标准

食品微生物学检验 沙门氏菌检验

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Foreword

This standard replaces GB 4789.4-2010 “Food Hygiene Microbiological Examination: *Salmonella*”, SN 0170-1992 “Examination method of *Salmonella* (including *Arizona*) in Exported Food”, and SN/T 2552.5-2010 “Microbiological examination of milk and dairy products: Part 5 Examination of *Salmonella*”.

Compared to GB 4789.4-2010, major changes of this standard are as follows:

— Examination procedure and serology test procedure has been revised;
— Annex A and Annex B have been revised.

National Food Safety Standard

Food Microbiological Examination: *Salmonella*

1. Scope

This standard specifies the testing method for *Salmonella* in foods.
This standard applies to the examination of *Salmonella* in foods.

2. Apparatus and materials

In addition to the conventional apparatus for sterilization and incubation in microbiological laboratory, other apparatus and materials are as follows:

2.1 Refrigerator: 2 ℃~5 ℃.

2.2 Constant temperature incubator: 36 ℃±1 ℃, 42 ℃±1℃.

2.3 Homogenizer.

2.4 Shaker.

2.5 Electronic balance: with sensitivity of 0.1g.

2.6 Sterile conical flask: with nominal capacities of 500 mL and 250mL.

2.7 Sterile pipette: with nominal capacities of 1 mL (graduated in 0.01 mL division) and 10 mL (graduated in 0.1 mL division), or micropipette and pipette tips.

2.8 Sterile culture dish: with diameter of 60mm and 90 mm.

2.9 Sterile test tube: 3mm×50mm, 1 0 mm×75mm.

2.10 pH meter or pH colorimetric tube or precision pH test paper.

2.11 Automatic microorganism biochemical identificationsystem.

2.12 Sterile capillary.

3. Culture mediums and reagents

3.1 Buffered peptone water (BPW): See Section A.1 in Annex A.

3.2 Tetrathionate brilliant green (TTB) enrichment broth: See Section A.2 in Annex A.

3.3 Selenite cystinol (SC) enrichment broth: See Section A.3 in Annex A.

3.4 Bismuth sulfite (BS) agar: See Section A.4 in Annex A.

3.5 HE agar: See Section A.5 in Annex A.

3.6 Xylose lysine deoxycholate (XLD) agar: See Section A.6 in Annex A.

3.7 *Salmonella* chromogenic medium.

3.8 Triple sugar iron (TSI) agar: See Section A.7 in Annex A.

3.9 Peptone water, Indole reagent: See Section A.8 in Annex A.

3.10 Urea agar (pH 7.2): See Section A.9 in Annex A.

3.11 Potassium cyanide (KCN) medium: See Section A.10 in Annex A.

3.12 Lysine decarboxylation test broth: See Section A.11 in Annex A.

3.13 Sugar fermentation tube: See Section A.12 in Annex A.

3.14 Ortho-nitrophenol-β-D-galactopyranoside (ONPG) medium: See Section A.13 in Annex A.

3.15 Semisolid medium: See Section A.14 in Annex A.

3.16 Sodium malonate medium: See Section A.15 in Annex A.

3.17 *Salmonella* O, H and Vi antisera.

3.18 Biochemical identification kit.

4. Examination procedures

The examination procedures of Salmonella are shown in Figure 1.

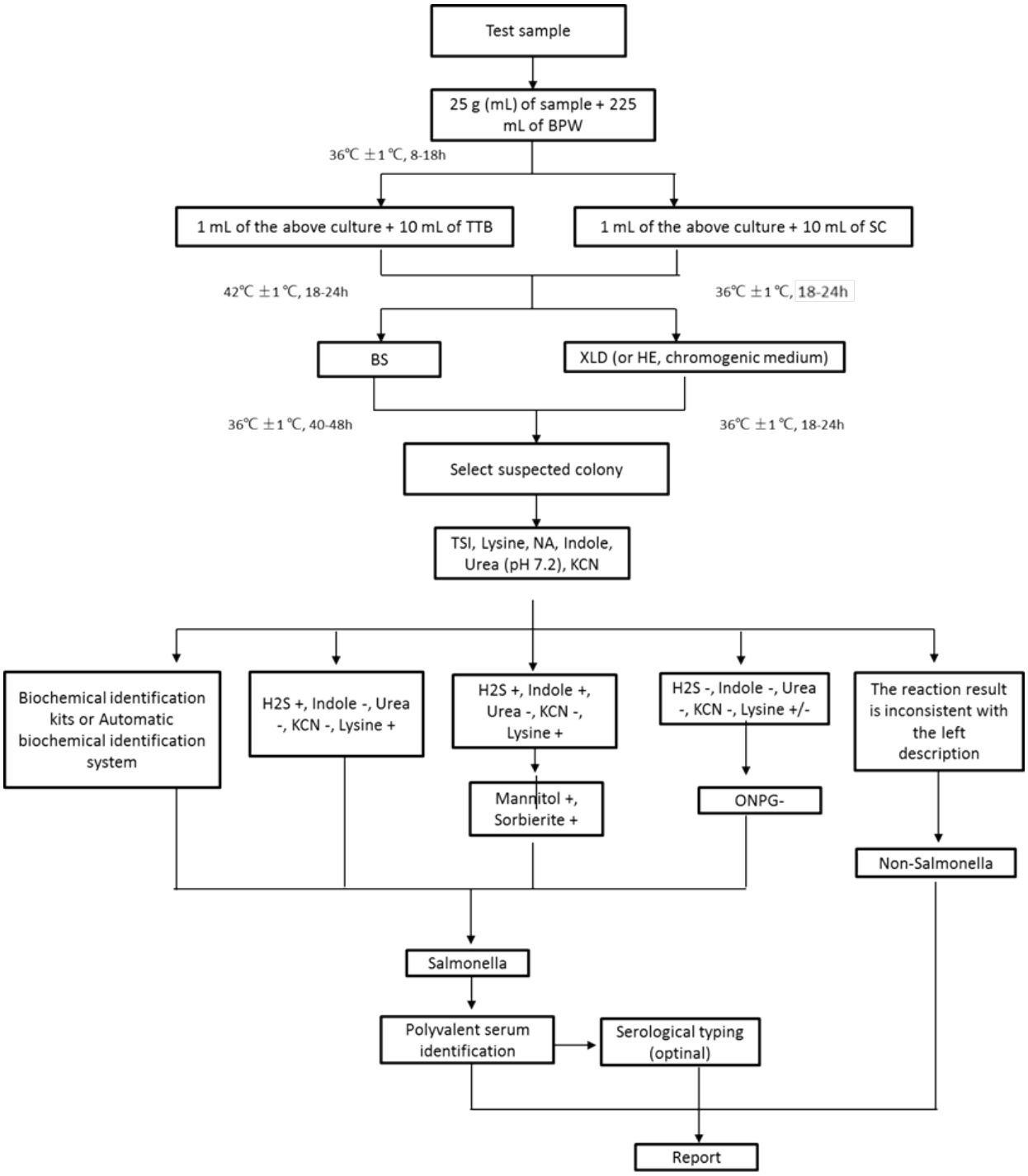

Figure 1 Examination procedures of *Salmonella*

5. Operating steps

5.1 Pre-enrichment

Aseptic technique: Weigh 25 g (mL) of sample and place into a sterile homogenizing cup which contains 225 mL of BPW, homogenize for 1 min~2 min in the speed of 8000 r/min~10000 r/min, or place into a sterile homogenizing bag which contains 225 mL of BPW, and homogenize it for 1 min~2 min with a slapping homogenizer. If the sample is liquid, shake and mix well, with no need for homogenization. For determination of pH, adjust the pH to 6.8±0.2 with 1 mol/mL sterile NaOH or HCl. Aseptically transfer the sample into a 500 mL conical flask or other vessels (if the homogenizing cup does not have hole cover, there is no need to transfer the sample). Directly

incubate in case of using a homogenizing bag. Incubate at 36 ℃±1 ℃for 8 h~18 h.

And the frozen product should be unfrozen at below 45℃ for less than 15 min, or at2 ℃~5 ℃ for less than 18h.

5.2 Enrichment

Lightly shake the cultured sample mixture, take 1 mL of the culture and transfer incubate into 10 mL of TTB, and then incubate at 42 ℃±1 ℃ for 18 h~24 h. Meanwhile, take 1 mL of the culture and transfer incubate into 10 mL of SC, and then incubate at 36 ℃±1 ℃ for 18 h~24 h.

5.3 Isolation

Take one loop of the enriched culture, streak inoculate on a BS agar plate and a XLD agar plate (or HE agar plate or *Salmonella* chromogenic medium plate). Incubate at 36 ℃±1 ℃ for 18 h~24 h (XLD agar plate, HE agar plate and *Salmonella* chromogenic medium plate) or for 40 h~48 h (BS agar plate). Observe the colonies growing on each plate, and the colony characteristics on each plate can be seen in Table 1.

Table 1 Colony characteristics of *Salmonella* on different selective mediums

Selective agar plate	*Salmonella*
BS agar	The colonies show black with metallic luster, tan or grey, and the mediums surrounding the colony show black or brown; there are some greyish-green colonies surround by an unchanged medium.
HE agar	The colonies show blue-green or green, with a black center or almost a black whole colony; some strains show yellow, with a black center or almost a black whole colony.
XLD agar	The colonies show pink, with or without a black center, some strains have a large lustrous black center or a black whole colony; while some strains show yellow, with or without a black center.
Salmonella chromogenic medium	It should be judged in accordance with the instruction of the chromogenic medium.

5.4 Biochemical test

5.4.1 Pick over 2 of the typical or suspected colonies from each selective agar plate, inoculate on TSI agar by streaking on the slant firstly and then stabbing in the bottom; without sterilizing the incubation needle, directly inoculate on the lysine decarboxylase test medium and nutrient agar plate, incubate at 36 ℃±1 ℃ for 18 h~24 h, up to 48 h if necessary. Table 2 can be referred to for the reaction results of *Salmonella* on TSI agar and lysine and lysine decarboxylase test medium.

Table 2 Reaction results of *Salmonella* on TSI agar and lysine and lysine decarboxylase test medium

TSI agar				Lysine decarboxylase test medium	Preliminary judgment
Slant	Bottom	Gas production	H_2S		
K	A	+(-)	+(-)	+	Suspected *Salmonella*
K	A	+(-)	+(-)	-	Suspected *Salmonella*
A	A	+(-)	+(-)	+	Suspected *Salmonella*
A	A	+/-	+/-	-	non-*Salmonella*
K	K	+/-	+/-	+/-	non-*Salmonella*
Note: K: alkali production, A: acid production; +: positive, -: negative; + (-): majority is positive, minority is negative;+/-: positive or negative.					

5.4.2 The colony can be inoculated in peptone water (for indole test), urea agar (pH 7.2) and KCN media while inoculating on the TSI agar and lysine and lysine decarboxylase test medium. Alternatively, the suspected colonies on the nutrient agar plate can be used for inoculation after the preliminary judgment. Incubate at 36 ℃±1 ℃ for 18 h~24 h, up to 48h if necessary. Judge the result according to Table 3. The picked plates should be kept at 2 ℃ ~5 ℃ or room temperature for at least 24 h, for reexamination when necessary.

Table 3 Preliminary identification of the biochemical reaction of *Salmonella*

Reaction no.	H_2S	Indole	pH7.2 Urea	KCN	Lysine decarboxylase
A1	+	-	-	-	+
A2	+	+	-	-	+
A3	-	-	-	-	+/-
Note: +: positive; -: negative; +/-: positive or negative.					

5.4.2.1 Reaction no. A1: The strains with typical reaction can be judged as *Salmonella*. The strains can be judged as *Salmonella* according to table 4 when there is an abnormal item among Urea, KCN and Lysine decarboxylase. The strains can be judged as non-*Salmonella* when there are two abnormal items.

Table 4 Preliminary identification of the biochemical reaction of *Salmonella*

pH7.2 Urea	KCN	Lysine decarboxylase	Judgment results
-	-	-	Salmonella paratyphi A (require serological identification results)
-	+	+	Salmonella IV or V (conform to the biochemical characteristics of the group thereof)
+	-	+	Salmonella variation (require serological identification results)
Notes: + refers to positive; - refers to negative.			

5.4.2.2 Reaction no. A2: Perform mannitol and sorbierite test for supplement, and the above two test results of *Salmonella* indole positive variation are both positive, but the eventual judgment should be made with the help of the serological identification results.

5.4.2.3 Reaction no. A3: Perform ONPG test for supplement. While the strains of lysine decarboxylase negative are *Salmonella* paratyphi A, the strains of ONPG negative and lysine decarboxylase positive are *Salmonella*.

5.4.2.4 Identify *Salmonella* biochemical groups according to Table 5 if necessary.

Table 5 Identification of *Salmonella* biochemical groups

Items	I	II	III	IV	V	VI
Galactitol	+	+	-	-	+	-
Sorbierite	+	+	+	+	+	-
Salicin	-	-	-	+	-	-
ONPG	-	-	+	-	+	-
Malonate	-	+	+	-	-	-
KCN	-	-	-	+	+	-
Notes: + refers to positive; - refers to negative.						

5.4.3 If biochemical identification kit or automatic microorganism biochemical identification system is used. Pick the suspected colonies on the nutrient agar plate, and prepare a bacterial suspension with appropriate turbidity, then identify it by the biochemical identification kit or automatic microorganism biochemical identification system according to the preliminary judgment results.

5.5 Serological identification

5.5.1 Identify the self-agglutination of targeted testingmaterials

Generally use 1.2%-1.5% of incubated agar to slide agglutination. Firstly, self- agglutination should be excluded. Add a drop saline on a clean slide and mix the testing material with the saline to even turbid suspension. Shake the slide lightly for 30s-60s, and observe the reaction under black background (the magnifying glass comes to help when necessary), if that is agglutinate, then it is determined as self-agglutination, otherwise viewed as non-self-agglutination.

5.5.2 Identification of multivalent somatic antigens(O)

Respective place half of a colony onto the top of each area of slide, add one drop of multivalent somatic O- serum on the bottom of one area, add other one drop of saline solution on the bottom of other area as comparison. Disperse two areas as to obtain a homogeneous and turbid suspension. Rock the slide gently for 60s. Observe the result against a dark background. All of agglutination phenomena are considered positive reaction. Place the colony on high concentration agar culture (2%-3%) and observe again when there is no agglutination phenomena. If Vi antigens prevent the agglutination of O antigens, then pick the colony and mix with 1 mL saline and heat to boil on the alcohol burner and then check again.

5.5.3 Identification of multivalent flagellar antigens(H)

The same as that specified in Section 5.5.2. When the H antigens does not grow well, place the colony onto the center of 0.55%-0.65% semisolid agar culture, pick the colony around the margin when colony is growing and

check; or place the colony into the 0.3%- 0.4% semisolid agar glass tube for once or twice, and pick the far-end colony andcheck.

5.6 Serological typing (optional)

5.6.1 Identification of O-antigens

A~F multivalent anti-O serum is used for slide agglutination test, while the normal saline is used as control. If the auto-agglutinable strains in the normal saline are rough strains, they cannot be serotyped.

For the strains agglutinated by A~F multivalent anti-O serum, perform agglutination test using O4; O3, O10; O7; O8; O9;O2 and O11 factor serum in turn. For the strains agglutinated by O3 and O10 serum, perform agglutination test using O10, O15, O34, O19 single-factor serum, and judge the E1, E2, E3, E4 subgroup. The final judgment of each O-antigen component should be in accordance with the test result of O single-factor serum. For those have no O single-factor serum, they should be verified by two O combined-factor serums.

For the strains not agglutinated by A~F multivalent anti-O serum, firstly examine it with 9 multivalent anti- O serums. For the strains agglutinated by any serum, examine it with the O-group serums included in the above multivalent serum to determine the O-group. The O-factor serums included in each multivalent anti- O serum are as follows:

O multivalent 1 A, B, C, D, E and F group (also include 6 and 14 group)
O multivalent 2 13, 16, 17, 18 and 21 group
O multivalent 3 28, 30, 35, 38 and 39 group
O multivalent 4 40, 41, 42 and 43 group
O multivalent 5 44, 45, 47 and 48 group
O multivalent 6 50, 51, 52 and 53 group
O multivalent 7 55, 56, 57 and 58 group
O multivalent 8 59, 60, 61 and 62 group
O multivalent 9 63, 65, 66 and 67 group

5.6.2 Identification of H-antigens

For the common bacteria types in A~F group, examine the H-antigens in Phase 1 and Phase 2 with H factors listed in Table 6.

Table 6 H-antigens for common bacteria types in A~F group

O group	Phase 1	Phase 2
A	a	No
B	g, f, s	No
B	i, b, d	2
C1	k, v, r, c	5, z15
C2	b, d, r	2, 5
D(Non gas-producing)	d	No
D (Gas-producing)	g, m, p, q	No
E1	h, v	6, w, x
E4	g, s, t	No
E4	i	

For the uncommon bacteria types, firstly examine it with 8 multivalent anti-H serums. For the strains agglutinated by one or two serum(s), examine it with the H-group serums contained in the above one or two multivalent serum(s) to determine H-antigens in Phase 1 and Phase 2. The H-factor serums contained in the above 8

multivalent anti-O serums are as follows:

H multivalent 1	a, b, c, d, i
H multivalent 2	eh, enx, enz15, fg, gms, gpu, gp, gq, mt, gz51
H multivalent 3	k, r, y, z, z10, 1v, 1w, 1z13, 1z28, 1z40
H multivalent 4	1, 2; 1, 5; 1, 6; 1, 7; z6
H multivalent 5	z4z23, z4z24, z4z32, z29, z35, z36, z38
H multivalent 6	z39, z41, z42, z44
H multivalent 7	z52, z53, z54, z55
H multivalent 8	z56, z57, z60, z61, z62

The final judgment of each H-antigen component should be made in accordance with the test result of H single-factor serum. If there are some with no H single-factor serum, they should be verified by two H combined-factor serums.

As to the case that H antigens in Phase 1 were detected but H antigens in Phase 2 were not detected or that H antigens in Phase 2 were detected but H antigens in Phase 1 were not detected, retest can be performed after inoculating on the agar slant for 1~2 generations. If still only one phase of H antigens is detected, it is allowed to employ phase variation method to check the other phase. Single-phase bacteria do not need phase variation examination.

Phase variation test methods are as follows:

Easy plate method: Dry the water on the surface of 0.35%~0.4% semisolid agar plate, pick one loop of factor serum and drop on the surface of flat plate, stand for a moment. Inoculate the bacteria to be tested on the center point of serum, incubate it and then pick the bacteria from the edge of the spreading lawn to test after the serum is absorbed into the agar.

Small glass tube: Melt the semi-solid tube (each tube is about 1 mL~2 mL) on the alcohol lamp and cool down to 50 ℃, add 0.05 mL~0.1 mL of the H-factor serum of known phase into the melted semi-solid, mix well and then pipette it with a capillary into the small glass tubes for phase variation test. Pick the bacteria to be tested with an inoculation needle and inoculate on one end after solidification. Place the small glass tube into a flat plate, and place a ball of wet cotton beside the small glass tube to prevent water evaporation and agar shrinkage, check the results every day, and pick the culture on the other end for bacteriological examination after dissociation of bacteria in the other phase. Serum concentration of the medium should be in appropriate proportion, because the bacteria cannot grow under a too high concentration, and the motility of the bacteria in the same phase cannot be inhibited. It should be added in the proportion of 1:200~1:800 to the original serum amount.

Small inverted tube method: Place a small glass tube with both ends opened (leaving a gap at the lower opened end, and do not make the end flat) in the semi-solid tube, the upper end of the small glass tube should be higher that the upper surface of the medium, and store for use after sterilization. Before use, heat and melt it on the alcohol lamp and cool down to 50 ℃, pick one loop of factor serum, add into the small inverted tube inside the semi-solid, slightly stir to mix well. After solidification, inoculate the bacteria to be tested onto the semi-solid surface inside the small inverted tube, check the results every day, and pick the culture for bacteriological examination the semi-solid surface inside the small inverted tube after dissociation of bacteria in the other phase, or transfer and inoculate on a 1% soft agar slant, incubate at 36 ℃ and then perform agglutination test.

5.6.3 Identification of Vi-antigens

Check the bacteria type with Vi factor serum. The known bacteria types with Vi- antigens are *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella dublin*.

5.6.4 Judgment of bacterial type

In accordance with the results of serological confirmation, the judgement of the bacterial type should be made based on Annex B or *Salmonella* antigen table.

6. Results and reports

Based on the above results of biochemical tests and serological identification, report whether *Salmonella* is detected or not detected in 25 g (mL) of the sample.

Annex A

Culture Mediums and Reagents

A.1 Buffered peptone water(BPW)

A.1.1 Composition

Peptone	10.0 g
Sodium chloride	5.0 g
Sodium hydrogen phosphate (containing 12 crystal water)	9.0 g
Potassium dihydrogen phosphate	1.5 g
Distilled Water	1000 mL

A.1.2 Preparation

Dissolve the components in the distilled water, stir well and place for 10min, by heating to completely solve, adjust the PH to 7.2±0.2, autoclave 15min at 121℃.

A.2 Tetrathionate brilliant green (TTB) enrichment broth

A.2.1 Base solution

Peptone	10.0 g
Beef extract	5.0 g
Sodium chloride	3.0 g
Calcium carbonate	45.0 g
Distilled water	1000 mL

Place all the above components excluding calcium carbonate in the distilled water, boil to dissolve, add calcium carbonate. Adjust the pH to 7.0±0.2, and sterilize for 20 min in the autoclave set at 121℃.

A.2.2 Sodium hyposulfite solution

Sodium hyposulfite (containing 5 crystal water)	50.0 g
Distilled water	add to 100 mL

Sterilize for 20 min in the autoclave set at 121℃.

A.2.3 Iodine solution

Iodine plate	20.0 g
Potassium iodide	25.0 g
Distilled water	add to 100 mL

Dissolve the potassium iodide in a little distilled water completely, add iodine plate, shake the glass bottle until the iodine plate is completely dissolved, and then add distilled water to the specified volume, store in brown bottles, and plug the bottle stopper. Then the obtained solution is ready to use.

A.2.4 0.5% brilliant green aqueous solution

Brilliant green	0.5 g
Distilled water	100 mL

After completely dissolved, store in dark place for not less than 1 d, and sterilize it naturally.

A.2.5 OX bile salt solution

OX bile salt	10.0 g
Distilled water	100 mL

Heat and boil to completely dissolved, and sterilize for 20 min in the autoclave set at 121℃.

A.2.6 Preparation

Base solution	900 mL
Sodium hyposulfite solution	100 mL
Iodine solution	20.0 mL
Brilliant green aqueous solution	2.0 mL
OX bile salt solution	50.0 mL

Before use, aseptically add all the above components into the base solution according to the above order, shake well after each adding.

A.3 Selenite cystinol (SC) enrichment broth

A.3.1 Composition

Peptone	5.0 g
Lactose	4.0 g
Sodium hydrogen phosphate	10.0 g
Sodium hydrogen selenite	4.0 g
L-cystine	0.01 g
Distilled water	1000 mL

A.3.2 Preparation

Place all the above components except sodium hydrogen selenite and L-cystine in the distilled water, boil to dissolve, cool down to below 55 ℃, aseptically add sodium hydrogen selenite and 10 mL of 1 g/L L-cystine solution (weigh 0.1 g of L-cystine, add 15 mL of 1 mol/L NaOH solution to dissolve it, and then add sterile distilled water to 100 mL. The amount should be doubled in case of using DL- cystine). Shake well and adjust its pH to 7.0±0.2.

A.4 Bismuth sulfite (BS) agar

A.4.1 Composition

Peptone	10.0 g
Beef extract	5.0 g
Glucose	5.0 g

Iron sulfate	0.3 g
Sodium hydrogen phosphate	4.0 g
Brilliant green	0.025 g or 5.0 mL of 5.0 g/L aqueous solution
Ammonium bismuth citrate	2.0 g
Sodium sulfite	6.0 g
Agar	18.0 g~20.0 g
Distilled water	1000 mL

A.4.2 Preparation

Place the first three components in 300 mL of distilled water (for base solution preparation). Place the ferrous sulfate and disodium hydrogen phosphate in 20 mL and 30 mL of distilled water, respectively. Place ammonium bismuth citrate and sodium sulfite in another 20 mL and 30 mL of distilled water, respectively. Place the agar in 600 mL of distilled water. Then shake well and boil to dissolve it. Cool down to 80 ℃, firstly mix the ferrous sulfate and disodium hydrogen phosphate thoroughly, and pour into the base solution, and then mix well. Mix the ammonium bismuth citrate and sodium sulfite thoroughly, and pour into the base solution, and then mix well. Adjust the pH to 7.4±0.2, immediately pour into the agar solution, mix well again, and then cool down to 50 ℃ ~55 ℃. Add brilliant green solution, mix well and immediately pour plates.

Note: Pressure sterilization is not needed by the medium. Overheating is not advisable during preparation, because it can lead to the reduction of its selectivity. Store it in dark place at the room temperature. There would be a decline of its selectivity if it exceeds 48h, and the medium should be prepared on that very day and be put into use on the next day.

A.5 HE agar (Hektoen Enteric Agar)

A.5.1 Composition

Peptone	12.0 g
Beef extract	3.0 g
Lactose	12.0 g
Sucrose	12.0 g
Salicin	2.0 g
Bile salt	20.0 g
Sodium chloride	5.0 g
Agar	18.0 g~20.0 g
Distilled water	1000 mL
0.4% bromothymol blue solution	16.0 mL
Andrade indicator	20.0 mL
Solution A	20.0 mL
Solution B	20.0 mL

A.5.2 Preparation

Dissolve the first seven components in 400 mL of distilled water, as base solution; place the agar in 600 mL of distilled water. Then stir well respectively and boil to dissolve them. Add solution A and B into the base solution, and adjust the pH to 7.4±0.2. Then add the indicator, and mix with agar solution. After cooling to 50 ℃~55 ℃, pour plates.

Notes:
1. The medium does not need pressure sterilization, do not heat it excessively during preparation to avoid reducing its selectivity.

2. Preparation of Solution A

Sodium hyposulfite	34.0 g
Ammonium iron citrate	4.0 g
Distilled water	100 mL

3. Preparation of Solution B

Sodium deoxycholate	10.0 g
Distilled water	100 mL

4. Andrade indicator

Acid fuchsin	0.5 g
1 mol/L sodium hydroxide solution	16.0 mL
Distilled water	100 mL

Dissolve the acid fuchsin in distilled water, add sodium hydroxide solution. Add another 1 mL~2 mL of sodium hydroxide solution if the color fading of the acid fuchsin is not complete after several hours.

A.6 Xylose lysine desoxycholate (XLD) agar

A.6.1 Composition

Yeast extract	3.0 g
L-lysine	5.0 g
Xylose	3.75 g
Lactose	7.5 g
Sucrose	7.5 g
Sodium deoxycholate	2.5 g
Ammonium iron citrate	0.8 g
Sodium thiosulfate	6.8 g
Sodium chloride	5.0 g
Agar	15.0 g
Phenol red	0.08 g
Distilled water	1000 mL

A.6.2 Preparation

Place all the above components except phenol red and agar in 400 mL of distilled water, boil to dissolve them, and adjust the pH to 7.4±0.2. Place the agar into another 600 mL of distilled water and boil to dissolve it.

Mix the above two solutions thoroughly, add the indicator, cool down to 50 ℃~55 ℃, and pour plates.

Notes: Pressure sterilization is not needed by the medium. Overheating is not advisable during preparation, because it can lead to the reduction of its selectivity. Store it in dark place at the room temperature. And the medium should be prepared on that very day and be put into use on the next day.

A.7 Triple sugar iron (TSI) agar

A.7.1 Composition

Peptone	20.0 g
Beef extract	5.0 g
Sucrose	10.0 g
Lactose	10.0 g
Glucose	1.0 g
Ammonium ferrous sulfate (containing 6 crystal water)	0.2 g
Phenol red	0.025 g or 5.0 mL of 5.0 g/L solution
Sodium chloride	5.0 g
Sodium thiosulfate	0.2 g
Agar	12.0 g
Distilled water	1000 mL

A.7.2 Preparation

Place all the above components except phenol red and agar in 400 mL of distilled water, boil to dissolve them, and adjust the pH to 7.4±0.2. Place the agar in another 600 mL of distilled water, and boil to dissolve it.
Mix the above two solutions fully, add the indicator, mix well and dispense into test tubes in quantities of 2 mL~4 mL, sterilize for 10 min in the autoclave set at 121 ℃ or 15 min at 115 ℃. Setting in a sloping position showing orange red color is allowable.

A.8 Peptone water, indole reagent

A.8.1 Peptone water

Peptone (or tryptone)	20.0 g
Sodium chloride	5.0 g
Distilled water	1000 mL

Add all the above components into distilled water, boil to dissolve them, and adjust the pH to 7.4±0.2. Dispense into small test tubes, and sterilize for 15 min in the autoclave set at 121 ℃.

A.8.2 Indole reagent

A.8.2.1 Kovacs reagent: Dissolve 5 g of p-Dimethylaminobenzaldehyde in 75 mL of pentanol, and then slowly add 25 mL of concentrated hydrochloric acid.

A.8.2.2 O-Bohr reagent: Dissolve 1 g of p-Dimethylaminobenzaldehyde in 95 mL of 95% ethanol, and then slowly add 20 mL of hydrochloric acid.

A.8.3 Test method

Pick a small amount of the culture and inoculate, and then incubate at 36 ℃±1 ℃ for 1 d~2 d, up to 4 d~5 d if necessary. Add approximately 0.5 mL of the Kovacs reagent and lightly shake the tube, and the formation of a crimson color indicates a positive reaction. Or add 0.5 mL of O-Bohr reagent, flow down along the tube wall and

cover the culture surface, and the formation of a rose color at the connecting surface indicates a positive reaction.

A.9 Urea agar (pH 7.2)

A.9.1 Composition

Peptone	1.0 g
Sodium chloride	5.0 g
Glucose	1.0 g
Potassium dihydrogen phosphate	2.0 g
0.4% phenol red	3.0 mL
Agar	20.0 g
Distilled water	1000 mL
20% urea solution	100 mL

A.9.2 Preparation

Place all the above components excluding urea, agar and phenol red into 400 mL of distilled water, boil to dissolve them and adjust the pH to 7.2±0.2. Place the agar into another 600 mL of distilled water, and boil to dissolve it. Mix the above two solutions fully, add the indicator, mix well, dispense and sterilize for 15 min in the autoclave set at 121 ℃. Cool down to 50 ℃~55 ℃ and add the sterile filtered urea solution. The final concentration of urea is 2%. Dispense the medium into sterile test tubes, and allow it to set in a sloping position. The obtained medium is ready to use.

A.9.3 Test method

Pick the agar culture and inoculate, and then incubate at 36 ℃±1 ℃ for 24 h, and observe the results. For the urease positive strains, the medium turns into red because of the alkali-production.

A.10 Potassium cyanide (KCN) medium

A.10.1 Composition

Peptone	10.0 g
Sodium chloride	5.0 g
Potassium dihydrogen phosphate	0.225 g
Sodium hydrogen phosphate	5.64 g
Distilled water	1000 mL
0.5% Potassium cyanide	20.0 mL

A.10.2 Preparation

Place all the above components excluding potassium cyanide into distilled water and boil to dissolve them, dispense and sterilize for 15 min in the autoclave set at 121 ℃. Cool the medium fully in the refrigerator. Add 2.0 mL of 0.5% potassium cyanide solution into each 100 mL of the medium (the final concentration is 1:10000), dispense into sterile test tubes in the quantities of 4 mL. Stuff the tubes with the sterile rubber stoppers immediately, and then store in a 4 ℃ refrigerator, at least stable for 2 months. Meanwhile, take the medium without potassium cyanide as control medium, dispense into test tubes for later use.

A.10.3 Test method

Inoculate the agar medium to the peptone water to get a diluted bacteria solution, and pick a loop of the culture and inoculate on the potassium cyanide (KCN) medium, and take another loop to inoculate on the control medium. Incubate at 36 ℃±1 ℃ for 1 d~2 d, and observe the results. The growth of bacteria indicate positive (not inhibit), and no growth of bacteria after 2 d indicate negative (inhibit).

Note: Because potassium cyanide is highly toxic, it should be used with caution. To avoid poisoning, do not touch it. The medium should be dispensed in a refrigerator in summer. The main cause of the failure to finish the experiment is untight seal. Thus the potassium cyanide decomposes gradually and produces hydrocyanic acid gas. Then the gas escapes, the reagent concentration decreases and bacteria grows. Finally, it causes false positive reactions.

A.11 Lysine decarboxylase medium

A.11.1 Composition

Peptone	5.0 g
Yeast Extract	3.0 g
Glucose	1.0 g
Distilled water	1000 mL
1.6%Bromocresol purple- ethanol solution	1.0 mL
L-lysine or DL-lysine	0.5 g/100 mL or 1.0 g/100 mL

A.11.2 Preparation

Heat to dissolve all the above components except lysine, dispense the medium into bottles in the quantities 100 mL, and then respectively add lysine (L-lysine: 0.5%, DL- lysine: 1%). Adjust the pH to 6.8±0.2. Do not add lysine into the control medium. Dispense the medium into sterile small test tubes in quantities of 5 mL, dropwise add a layer of liquid paraffin, and sterilize for 10 min in the autoclave set at 115℃.

A.11.3 Test method

Pick the culture from the agar slant and inoculate, then incubate at 36 ℃±1 ℃ for 18 h~24 h, and observe the results. Because of alkali production, the medium should be in purple color for the amino acid decarboxylase positive strains. While the medium turns to yellow because of the acid production of glucose, the amino acid decarboxylase positive strains have no basic product. The control tube should be in yellow color.

A.12 Sugar fermentation tube

A.12.1 Composition

Beef extract	5.0 g
Peptone	10.0 g
Sodium chloride	3.0 g
Sodium hydrogen phosphate (containing 12 crystal water)	2.0 g
0.2% Bromthymol phenol blue solution	12.0 mL
Distilled water	1000 mL

A.12.2 Preparation

A.12.2.1 Prepare the glucose fermentation tube with the above components, and adjust the pH to 7.4±0.2. Add 0.5% glucose, dispense the medium into small test tubes containing inverse tubes, and sterilize for 15 min in the autoclave set at 121 ℃.

A.12.2.2 Prepare other glucose fermentation tubes with the above components, dispense the medium into bottles in the quantities of 100 mL, and sterilize for 15 min in the autoclave set at 121 ℃. Prepare the 10% solution of each glucose, and sterilize in the autoclave. Dispense 5 mL of glucose solution into 100 mL of medium, and then aseptically dispense into small testtubes.

Notes: If sucrose is not pure, it can hydrolyze itself after heating. Filtration method should be employed to remove bacteria.

A.12.3 Test method: Pick a small amount of the culture and inoculate, and then incubate at 36 ℃±1 ℃ for 2 d~3 d, and examine. Delayed reaction should be observed for 14 d~30 d.

A.13 ONPG medium

A.13.1 Composition

O-Nitrophenyl-β-D-galactopyranoside (ONPG)	60.0 mg
0.01mol/L sodium phosphate buffer solution (pH 7.5)	10.0 mL
1% peptone water (pH 7.5)	30.0 mL

A.13.2 Preparation

Dissolve OPNG in the buffer solution, add peptone water, sterilize by filtration, dispense the medium into sterile small test tubes in the quantities of 0.5 mL, and stuff the tubes with rubber stoppers.

A.13.3 Test method

Pick a full loop of the culture from the agar slant and inoculate, incubate at 36 ℃±1 ℃ for 1 h~3 h and 24 h, and observe the results. The color will turn to yellow within 1 h~3 h if it produces β-galactosidase; otherwise, there will be no change in the color.

A.14 Semisolid medium

A.14.1 Composition

Beef extract	0.3 g
Peptone	1.0 g
Sodium chloride	0.5 g
Agar	0.35 g~0.4 g
Distilled water	100 mL

A.14.2 Preparation

Prepare the above components, heat to dissolve them, and adjust the pH to 7.4±0.2. Dispense into small test

tubes. Sterilize for 15 min in the autoclave set at 121 ℃. Allow the medium to solidify vertically for later use.

Notes: It is used for motility observation, strain storage, and phase variation of H- antigens.

A.15 Sodium malonate medium

A.15.1 Composition

Yeast extract	1.0 g
Ammonium sulfate	2.0 g
Potassium hydrogen phosphate	0.6 g
Potassium dihydrogen phosphate	0.4 g
Sodium chloride	2.0 g
Sodium malonate	3.0 g
0.2% Bromthymol phenol blue solution	12.0 mL
Distilled water	1000 mL

A.15.2 Preparation

Dissolve all the above components exclude yeast extract in water, adjust the pH to 6.8±0.2, add the indicator, dispense into test tubes, and sterilize for 15 min in the autoclave set at 121 ℃.

A.15.3 Test method

Inoculate the fresh agar culture, incubate at 36 ℃±1 ℃ for 48 h, and observe the results. If the color turns to blue from green, it can be viewed as an indicator of a positive reaction.

Annex B

Common Salmonella Antigen

Refer to Table B.1 for Common Salmonella Antigen.

Table B.1 Common Salmonella Antigen

Bacteria name	Latin bacteria name	O antigen	H antigen	
			Phase 1	Phase 2
Group A				
甲型副伤寒沙门氏菌	*S. paratyphi A*	1,2,12	a	[1,5]
Group B				
基桑加尼沙门氏菌	*S .kisangani*	1,4,[5],12	a	1,2
阿雷查瓦莱塔沙门氏菌	*S. arechavaleta*	4,[5],12	a	1,7
马流产沙门氏菌	*S. abortusequi*	4,12	-	e, n, x
乙型副伤寒沙门氏菌	*S. paratyphi B*	1,4,[5],12	b	1,2
利密特沙门氏菌	*S. limete*	1,4,12,[27]	b	1,5
阿邦尼沙门氏菌	*S. abony*	1,4,[5],12,27	b	e, n, x
维也纳沙门氏菌	*S. wien*	1,4,12,[27]	b	l, w
伯里沙门氏菌	*S .bury*	4,12,[27]	c	z_6
斯坦利沙门氏菌	*S. stanley*	1,4,[5],12,[27]	d	1,2
圣保罗沙门氏菌	*S. saintpaul*	1,4,[5],12	e,h	1,2
里定沙门氏菌	*S .reading*	1,4,[5],12	e,h	1,5
彻斯特沙门氏菌	*S. chester*	1,4,[5],12	e,h	e, n, x
德尔卑沙门氏菌	*S. derby*	1,4,[5],12	f, g	[1,2]
阿贡纳沙门氏菌	*S. agona*	1,4,[5],12	f, g, s	[1,2]
埃森沙门氏菌	*S. essen*	4,12	g, m	-
加利福尼亚沙门氏菌	*S. california*	4,12	g, m, t	[z_{67}]
金斯敦沙门氏菌	*S. kingston*	1,4,[5],12,[27]	g, s, t	[1,2]
布达佩斯沙门氏菌	*S. budapest*	1,4,12,[27]	g, t	-
鼠伤寒沙门氏菌	*S. typhimurium*	1,4,[5],12	i	1,2
拉古什沙门氏菌	*S. Lago*	1,4,[5],12	i	1,5
布雷登尼沙门氏菌	*S. Lago*	1,4,12,[27]	l, v	1,7
基尔瓦沙门氏菌Ⅱ	*S.kilwa II*	4,12	l, w	e, n, x
海德尔堡沙门氏菌	*S.heidelberg*	1,4,[15],12	r	1,2
印地安纳沙门氏菌	*S.indiana*	1,4,12	z	1,7
斯坦利维尔沙门氏菌	*S.stanleyville*	1,4,[5],12,[27]	z_4,z_{23}	[1,2]
伊图里沙门氏菌	*S.ituri*	1,4,12	z_{10}	1,5
C1 group				
奥斯陆沙门氏菌	*S.oslo*	6,7,14	a	e, n, x
爱丁保沙门氏菌	*S.edinburg*	6,7,14	b	1,5
布隆方丹沙门氏菌Ⅱ	*S.bloemfontein* II	6,7	b	[e, n, x]: z_{42}
丙型副伤寒沙门氏菌	*S.paratyphi C*	6,7,[Vi]	c	1,5
猪霍乱沙门氏菌	*S.choleraesuis*	6,7	c	1,5
猪伤寒沙门氏菌	*S.typhisuis*	6,7	c	1,5

罗米他沙门氏菌	*S.lomita*	6,7	e, h	1,5
布伦登卢普沙门氏菌	*S.braenderup*	6,7, 14	e, h	e, n, z_{15}
里森沙门氏菌	*S.rissen*	6,7, 14	f, g	-
蒙得维的亚沙门氏菌	*S.montevideo*	6,7, 14	g, m, [p], s	[1,2,7]
里吉尔沙门氏菌	*S.*riggil	6,7	g,[t]	-
奥雷宁堡沙门氏菌	*S.oranieburg*	6,7, 14	m, t	[2,5,7]
奥里塔蔓林沙门氏菌	*S.oritamerin*	6,7	i	1,5
汤卜逊沙门氏菌	*S.thompson*	6,7, 14	k	1,5
康科德沙门氏菌	*S.concord*	6,7	l, v	1,2
伊鲁木沙门氏菌	*S.irumu*	6,7	l, v	1,5
姆卡巴沙门氏菌	*S.mkamba*	6,7	l, v	1,6
波恩沙门氏菌	*S.bonn*	6,7	l, v	e, n, x
波茨坦沙门氏菌	*S.potsdam*	6,7, 14	l, v	e, n, z_{15}
格但斯克沙门氏菌	*S.gdansk*	6,7, 14	l, v	z_6
维尔肖沙门氏菌	*S.virchow*	6,7, 14	r	1,2
婴儿沙门氏菌	*S.infantis*	6,7, 14	r	1,5
巴布亚沙门氏菌	*S.papuana*	6,7	r	e, n, z_{15}
巴累利沙门氏菌	*S.bareilly*	6,7, 14	y	1,5
哈特福德沙门氏菌	*S.hartford*	6,7	y	e, n, x
三河岛沙门氏菌	*S.mikawasima*	6,7, 14	y	e, n, z_{15}
姆班达卡沙门氏菌	*S.mbandaka*	6,7, 14	z_{10}	e, n, z_{15}
田纳西沙门氏菌	*S.tennessee*	6,7, 14	z_{29}	[1,2,7]
布伦登卢普沙门氏菌	*S.braenderup*	6,7, 14	e, h	e, n, z_{15}
耶路撒冷沙门氏菌	*S.jerusalem*	6,7, 14	z_{10}	l, w
C2 group				
习志野沙门氏菌	*S.narashino*	6.8	a	e, n, x
名古屋沙门氏菌	*S.nagoya*	6,8	b	1,5
加瓦尼沙门氏菌	*S.gatuni*	6,8	b	e, n, x
慕尼黑沙门氏菌	*S.muenchen*	6,8	d	1,2
曼哈顿沙门氏菌	*S.manhattan*	6,8	d	1,5
纽波特沙门氏菌	*S.newport*	6,8,20	e, h	1,2
科特布斯沙门氏菌	*S.kottbus*	6,8	e, h	1,5
茨昂威沙门氏菌	*S.tshiongwe*	6,8	e, h	e, n, z_{15}
林登堡沙门氏菌	*S.lindenburg*	6,8	i	1,2
塔科拉迪沙门氏菌	*S.takoradi*	6,8	i	1,5
波那雷恩沙门氏菌	*S.bonariensis*	6,8	i	e, n, x
利齐菲尔德沙门氏菌	*S.litchfield*	6,8	l, v	1,2
病牛沙门氏菌	*S.bovismorbificans*	6,8,20	r,[i]	1,5
查理沙门氏菌	*S.chailey*	6,8	z_4,z_{23}	e, n, z_{15}
C3 group				
巴尔多沙门氏菌	*S.bardo*	8	e, h	1,2
依麦克沙门氏菌	*S.emek*	8,20	g, m, s	-
肯塔基沙门氏菌	*S.kentucky*	8, 20	i	z6
D group				
仙台沙门氏菌	*S.sendai*	1,9,12	a	1,5
伤寒沙门氏菌	*S.typhi*	9,12,[Vi]	d	-

塔西沙门氏菌	*S.tarshyne*	9,12	d	1,6
伊斯特本沙门氏菌	*S.eastbourne*	1,9,12	e, h	1,5
以色列沙门氏菌	*S.israel*	9,12	e, h	e, n, z_{15}
肠炎沙门氏菌	*S.enteritidis*	1,9,12	g, m	[1,7]
布利丹沙门氏菌	*S.blegdam*	9,12	g, m, q	-
沙门氏菌 Ⅱ	*Salmonella* II	1,9,12	g, m,[s],t	[1,5,7]
都柏林沙门氏菌	*S.dublin*	1,9,12,[Vi]	g, p	-
芙蓉沙门氏菌	*S.seremban*	9,12	i	1,5
巴拿马沙门氏菌	*S.panama*	1,9,12	l, v	1,5
戈丁根沙门氏菌	*S.goettingen*	9,12	l, v	e, n, z_{15}
爪哇安纳沙门氏菌	*S.javiana*	1,9,12	L, z_{28}	1,5
鸡-雏沙门氏菌	*S.gallinarum-pullorum*	1,9,12	-	-
E1 group				
奥凯福科沙门氏菌	*S.okefoko*	3,10	c	z_6
瓦伊勒沙门氏菌	*S.vejle*	3,{10},{15}	e, h	1,2
明斯特沙门氏菌	*S.muenster*	3,{10}{15}{15,34}	e, h	1,5
鸭沙门氏菌	S.anatum	3,{10}{15}{15,34}	e, h	1,6
纽兰沙门氏菌	*S.newlands*	3,{10},{15,34}	e, h	e, n, x
火鸡沙门氏菌	*S.meleagridis*	3, {10}{15}{15,34}	e, h	l, w
雷根特沙门氏菌	*S.regent*	3,10	f, g, [s]	[1,6]
西翰普顿沙门氏菌	*S.westhampton*	3,{10}{15}{15,34}	g, s, t	-
阿姆德尔尼斯沙门氏菌	*S.amounderness*	3,10	i	1,5
新罗歇尔沙门氏菌	*S.new-rochelle*	3,10	k	l, w
恩昌加沙门氏菌	*S.nchanga*	3,{10}{15}	l, v	l, 2
新斯托夫沙门氏菌	*S.sinstorf*	3,10	l, v	1,5
伦敦沙门氏菌	*S.london*	3,{10}{15}	l, v	1,6
吉韦沙门氏菌	*S.give*	3,{10}{15}{15,34}	l, v	1,7
鲁齐齐沙门氏菌	*S.ruzizi*	3,10	l, v	e, n, z_{15}
乌干达沙门氏菌	*S.uganda*	3,{10}{15}	l, z_{13}	1,5
乌盖利沙门氏菌	*S.ughelli*	3,10	r	1,5
韦太夫雷登沙门氏菌	*S.weltevreden*	3,{10}{15}	r	z_6
克勒肯威尔沙门氏菌	*S.clerkenwell*	3,10	z	l, w
列克星敦沙门氏菌	*S.lexington*	3,{10}{15}{15,34}	z_{10}	1,5
E4 group				
萨奥沙门氏菌	*S.sao*	1,3,19	e, h	e, n, z_{15}
卡拉巴尔沙门氏菌	*S.calabar*	1,3,19	e, h	l, w
山夫登堡沙门氏菌	*S.senftenberg*	1,3,19	g, [s], t	-
斯特拉特福沙门氏菌	*S.stratford*	1,3,19	i	1,2
塔克松尼沙门氏菌	*S.taksony*	1,3,19	i	z_6
索恩保沙门氏菌	*S.schoeneberg*	1,3,19	z	e, n, z_{15}
F group				
昌丹斯沙门氏菌	S.*chandans*	11	d	[e, n, x]
阿柏丁沙门氏菌	*S.aberdeen*	11	i	1,2
布里赫姆沙门氏菌	*S.brijbhumi*	11	i	1,5
威尼斯沙门氏菌	*S.veneziana*	11	i	e, n, x
阿巴特图巴沙门氏菌	*S.abaetetuba*	11	k	1,5

鲁比斯劳沙门氏菌	*S.rubislaw*	11	r	e, n, x
Other groups				
浦那沙门氏菌	*S.poona*	1,13,22	z	1,6
里特沙门氏菌	*S.ried*	1 ,13,22	z_4, z_{23}	[e, n, z_{15}]
密西西比沙门氏菌	*S.mississippi*	1,13,23	b	1,5
古巴沙门氏菌	*S.cubana*	1,13,23	z_{29}	-
苏拉特沙门氏菌	*S.surat*	[1],6,14,[25]	r, [i]	e, n, z_{15}
松兹瓦尔沙门氏菌	*S.sundsvall*	[1],6,14,[25]	z	e, n, x
非丁伏斯沙门氏菌	*S.hvittingfoss*	16	b	e, n, x
威斯敦沙门氏菌	*S.weston*	16	e, h	z_6
上海沙门氏菌	*S.*shanghai	16	l, v	1,6
自贡沙门氏菌	*S.zigong*	16	l, w	1,5
巴圭达沙门氏菌	*S.baguida*	21	z_4,z_{23}	-
迪尤波尔沙门氏菌	*S.dieuoppeul*	28	i	1,7
卢肯瓦尔德沙门氏菌	*S.luckenwalde*	28	z_{10}	e, n, z_{15}
拉马特根沙门氏菌	*S.ramatgan*	30	k	1,5
阿德莱沙门氏菌	*S.adelaide*	35	f, g	-
旺兹沃思沙门氏菌	*S.wandsworth*	39	b	1,2
雷俄格伦德沙门氏菌	*S.riogrande*	40	b	1,5
莱瑟沙门氏菌	*S.lethe* II	41	g, t	-
达莱姆沙门氏菌	*S.dahlem*	48	k	e, n, z_{15}
沙门氏菌IIIb	*Salmonella* IIIb	61	l, v	1,5,7

Note: explanation on marks in the table

{}={} O element means exclusive. The serotype element in the {} cannot coexist with other elements in the {}, take O:3 as an example, when 0:3, 10 colony produces O:15 or O15,34, then it will replace O:10 elements.

[]=O (without underline) or there is no relationship between the existing of H element and transformation of phage, such as [5] element in the O:4 colony. H element in the [] means rare to see in the wild colony, for example, most *S.Paratyphi A* has a (a) phase, but rarely has 2 phase (1,5) colony. So it can be illustrated as 1,2,12:a:[15].

_= underline means the O element is created by lysogenization of phage.