National Standard of the People's Republic of China

GB 4789.4-2016

National Standards For Food Safety

Food Microbiological Analysis Salmonella Test

2016-12-23 Issue 2017-06-23 Implementation

Forward

This standard replaces GB4789.4-2010 "National Food Safety Standards for Microbiological Examination of Salmonella in Food", SN0170-1992 "Methods for the Examination of Salmonella in Exported Food (Including Arizona Bacteria)", and SN/T2552.5-2010 "Methods for Microbiological Examination of Milk and Dairy Products - Part 5: Salmonella Examination"

The main changes in the integrated standard compared to GB4789.4-2010 are as follows:

——Revised testing procedures and serological testing procedures;

——Revised Appendix A and Appendix B.

National Standards For Food Safety

Food Microbiological Analysis Salmonella Test

1 Scope

This standard specifies the method of detecting salmonella in foods.

This standard applies to determination of salmonella in foods.

2 Equipment and materials

In addition to routine sterilization and culture equipment in microbiology laboratory, other equipment and materials are as follows:

2.1 Refrigerator: 2~5℃.

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

2.3 Homogenizer .

2.4 Oscillator.

2.5 Balance: 0.1g of sensibility.

2.6 Sterile conical flask: 500mL and 250mL.

2.7 Sterile pipette: 1mL(with 0.01 scale) 10mL(with 0.1 scale).

2.8 Sterile Petri dish: 90mm, 60mm in diameter.

2.9 Sterile test tube: 3mm ×50mm, 10mm ×75mm.

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

2.11 Automatic microbial biochemical identification system.

2.12 Sterile capillary.

3 Medium and Reagents

3.1 Buffer Petone Water (BPW): see A.1.

3.2 Tetrathionate Broth (TTB): see A.2.

3.3 Selenite Cystine (SC) Broth: see A.3.

3.4 Bismuth Sulfite (BS) Agar: see A.4.

3.5 HE agar: see A.5.

3.6 Xylose lysine Desoxycholate (XLD) Agar: see A.6.

3.7 Salmonella chromogenic medium.

3.8 Triple Sugar Iron (TSI) Agar: see A.7.

3.9 Petone water, indole reagent: see A.8.

3.10 Urea agar (pH7.2): see A.9.

3.11 Potassium cyanide (KCN) medium: see A.10.

3.12 Lysine decarboxylase test medium: see A.11.

3.13 Sugar fermentation tube: see A.12.

3.14 O-Nitrophenyl β-D galactopyranoside (ONPG) medium: see A.13.

3.15 Semi-solid agar: see A.14.

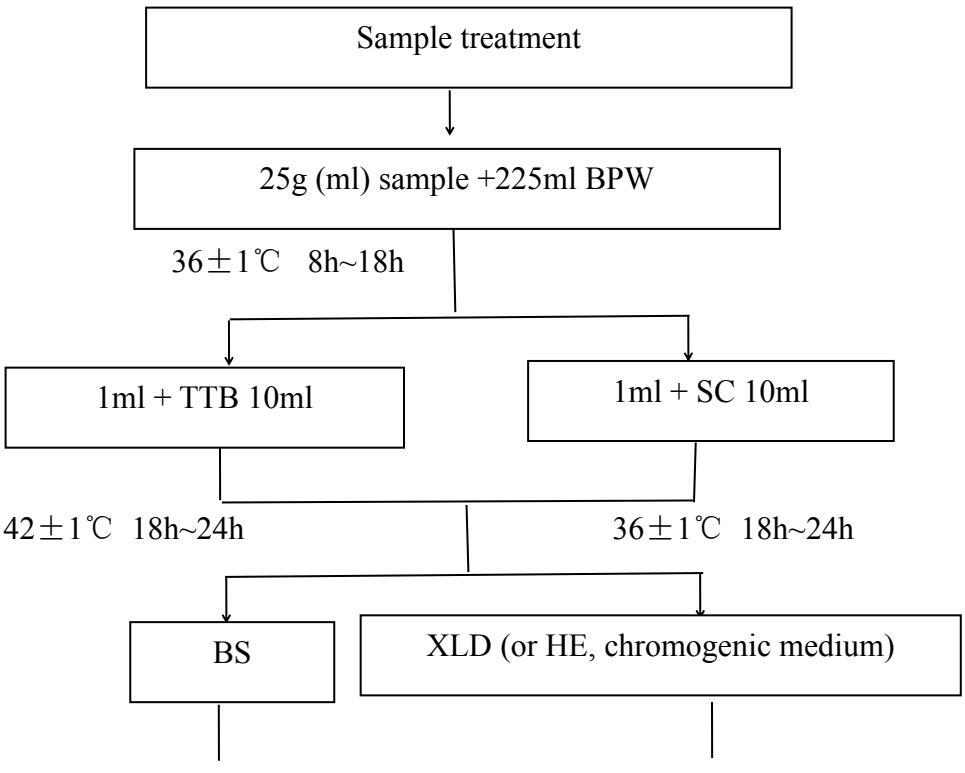
3.16 Sodium malonate medium: see A.15.

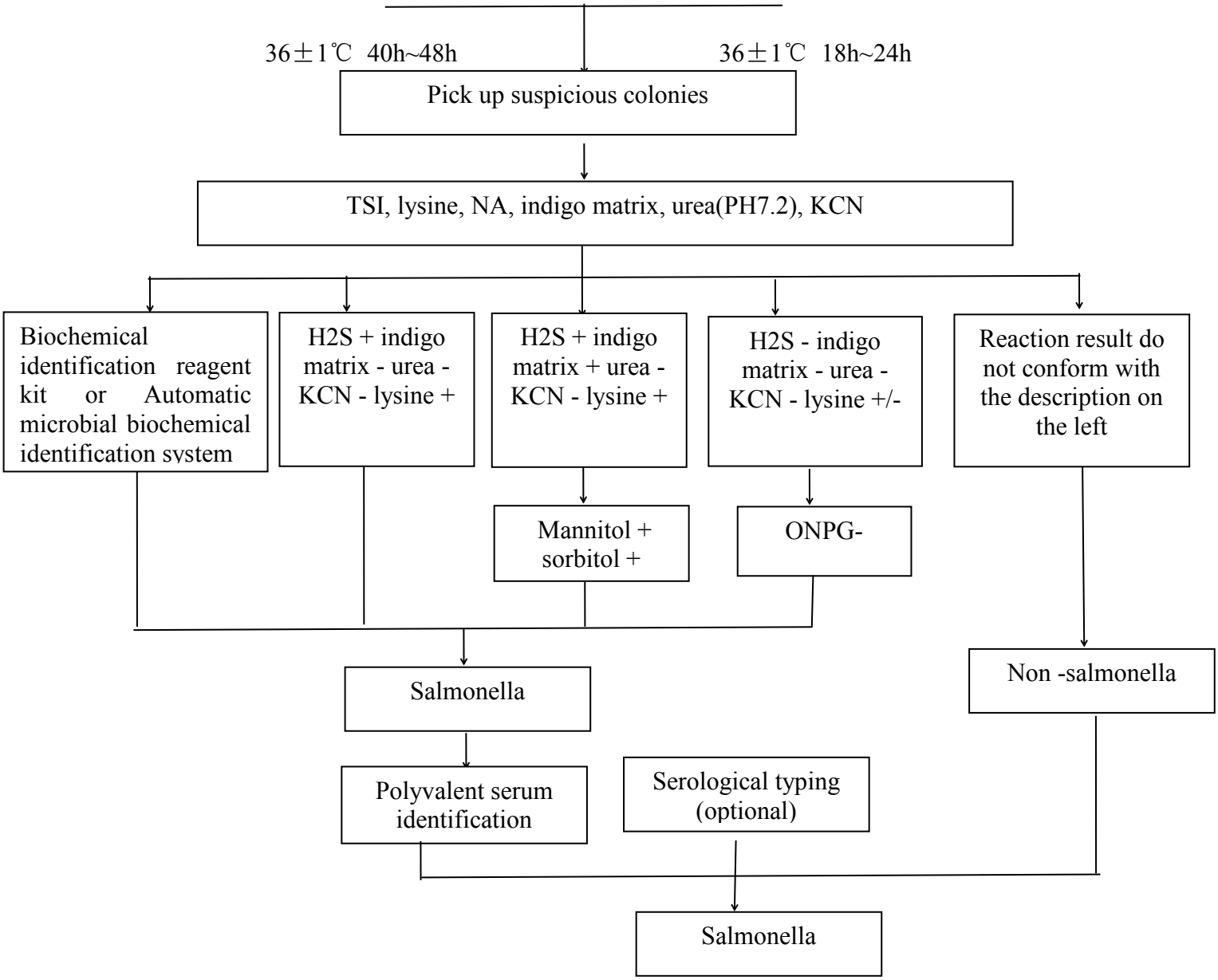
3.17 Salmonella O, H and Vi diagnosed serum.

3.18 Biochemical identification reagent kit.

4 Test procedure

The test procedure of salmonella is shown in Figure 1





5 Operation steps

5.1 Pre-enrichment

Aseptically weigh 25g(ml) sample and place it in a sterile homogenous cup or suitable container containing 225ml BPW. Homogenize at 8000r/min~10000r/min for 1~2min, or place in a sterile homogenization bag containing 225ml BPW, and tap with a tapping homogenizer 1~2min. If the sample is liquid, it does not need to be homogenized. Use 1mol/l sterile NaOH or HCL adjusted the PH to 6.8±0.2. Aseptically transfer the sample to a 500ml flask or other suitable container(if the homogenization cup has a non-porous cover does not transfer the sample) if use

homogenization bag, it can be directly cultured and cultured at 36±1℃ for 8h~18h.

If it is a frozen product, it should be thawed not more than 15min below 45℃, or not more than 18 hours at 2~5℃.

5.2 Enrichment

Shake the cultured sample mixture gently, transfer 1ml, transfer to 10ml TTB, and cultured at 42 ℃±1 ℃ for 18h~24h. at the same time, another 1ml was taken, transferred to 10ml LSC, and cultured at 36 ℃ ± 1 ℃ for 18h ~ 24h.

5.3 Separation

Take one loop of the enrichment solution with a diameter of 3mm inoculation loop, respectively, streak inoculation on a BS agar plate and an XLD agar plate (or HE agar plate or salmonella chromogenic medium plate), cultured at 36℃±1℃ for 40h~48h(BS agar plate) or 18h~24h(XLD agar plate, HE agar plate, salmonella chromogenic medium plate), observe the colonies growing on each plate. The characteristics of the colonies on the table are shown in table 1.

Table 1 Colony characteristics of Salmonella on different selective agar plates

Selective agar plate	Salmonella
BS agar	The colonies are black with metallic luster, brown or gray, and the culture medium around the colonies can be black or brown, some strains form gray-green Colored colonies, the surrounding medium is unchanged.
HE agar	Blue-green or blue, most colonies are black or almost completely black; some strains are yellow, and the center is black or almost completely black.
XLD agar	The colonies are pink, with or without black centers, and some strains can have large shiny black centers, or all black Colonies some strains are yellow colonies with or without black centers.
Salmonella chromogenic culture	Base Judge according to the instructions of the chromogenic medium.

5.4 Biochemical test

5.4.1 Pick two or more typical or suspicious colonies from the selective agar plate respectively, inoculate trisaccharide iron agar, first line the bevel, and the puncture the bottom layer, do not

sterilize the inoculation needle, directly inoculate the lysine decarboxylase test medium and nutrient agar plate, and culture at 36℃±1℃ for 18h~24h, can be extended to 48h if necessary. In the medium of trisaccharide iron agar and lysine decarboxylase, the reaction of salmonella see table 2.

Table 2 The results of the reaction of Salmonella in the test medium of trisaccharide iron agar and lysine decarboxylase

Trisaccharide iron agar				Lysine decarboxylase test medium	Preliminary judgment
Bevel	Bottom layer	Gas production	Hydrogen sulfide		
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, + (-): most positive, few negative, +/- : positive or negative.					

5.4.2 While inoculating trisaccharide iron agar and lysine decarboxylase test medium, peptone water can be directly inoculated (for indigo substrate test). Urea agar (pH7.2) and potassium cyanide (KCN) medium can also be used to pick suspicious colonies from the nutrient agar plate after preliminary judgment of the results . Incubate at 36 ℃±1 ℃ for 18h~24h, if necessary, it can be extended to 48h, according to Table 3 to determine the results. Store plates with picked colonies, keep at 2 ℃ ~ 5 ℃ or room temperature for at least 24h, in case of review if necessary.

Table 3 Preliminary identification table of biochemical reaction of Salmonella

Reaction number	Hydrogen sulfide (H2S)	Indigo matrix	pH7.2 Urea	Potassium cyanide (KCN)	Lysine decarboxylase
A1	+	-	-	-	+
A2	+	+	-	-	+
A3	-	-	-	-	+/-
Note: + positive, - negative, +/- positive or negative.					

5.4.2.1 Reaction number A1: The typical reaction is judged as Salmonella. For example, one of

the three items of urea, KCN and lysine decarboxylase is unusual, according to Table 4 can be determined as Salmonella. If there are 2 abnormalities, it is non-Salmonella.

Table 4 Preliminary identification table of biochemical reaction of Salmonella

pH7.2 Urea	Potassium cyanide (KCN)	Lysine decarboxylase	judgement result
-	-	-	Salmonella paratyphi A (to Seeking serological identification results)
-	+	+	Salmonella Ⅳ or Ⅴ (requirements biochemical characteristics of the co-group)
+	-	+	Individual variants of Salmonella (required Serological identification results)
Note: + means positive, - means negative.			

5.4.2.2 Reaction number A2: supplemented with the mannitol and sorbitol test, the positive results of both the Salmonella indica matrix variants were positive, but it needs to be combined with the results of serological identification.

5.4.2.3 Reaction number A3: Make ONPG. ONPG negative is Salmonella, meanwhile lysine decarboxylase is positive, Paratyphoid A typhimurium was negative for lysine decarboxylase.

5.4.2.4 Identify the biochemical group of Salmonella according to Table 5 if necessary.

Table 5 Identification of various biochemical groups of Salmonella

project	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ	Ⅵ
dulcitol	+	+	-	-	+	-
sorbitol	+	+	+	+	+	-
salicin	-	-	-	+	-	-
ONPG	-	-	+	-	+	-
malonate	-	+	+	-	-	-
KCN	-	-	-	+	+	-
Note: + indicates positive, - indicates negative.						

5.4.3 If you choose a biochemical identification kit or fully automated microbial biochemical identification system, you can pick up suspicious colonies on the liquid plate,prepare a

suspension of bacteria with appropriate turbidity using physiological saline, use a biochemical identification kit or fully automated microbial biochemical identification system.

5.5 Serological identification

5.5.1 Check the culture for self-coagulation

Generally 1.2% to 1.5% agar culture is used as the antigen for the slide agglutination test. First exclude the self-agglutination reaction. Add a drop of physiological saline on the clean slide, mix the culture to be tested in the drop of physiological saline to make it a homogeneous turbid suspension, and gently slide the slide, shake for 30s~60s, observe the reaction on a black background (observe with a magnifying glass if necessary), if there is visible bacterial agglutination, it is considered to have self-coagulation, otherwise there is no self-coagulation. Serological identification of cultures without self-coagulation was performed according to the following method.

5.5.2 Identification of multivalent bacterial antigen (O)

Make two area of about 1cm×2cm on the slide, pick up one loop of bacteria, and put 1/2 ring on the top of each area on the slide, Add one drop of multivalent bacterial (O) antiserum to the lower part of one area, and add one drop of normal saline to the lower part of the other area as a control. Reuse Sterile inoculation loops or needles grind the moss in the two areas into an emulsion. Shake the slide for 1 min and mix it against the dark back, observes that any agglutination is a positive reaction. When the serum O does not agglutinate, inoculate the strain 2%~3%) Recheck on the medium; if the O agglutination reaction is prevented due to the presence of Vi antigen, you can pick the moss in 1mL normal saline, make a concentrated bacterial solution, boil it on the flame of an alcohol lamp, and then check.

5.5.3 Identification of multivalent flagellar antigen (H)

The operation is the same as 5.5.2. When the H antigen is stunted, inoculate the strain in the center of a 0.55%~0.65% semi-solid agar plate and wait for the colony.When spreading and growing, take a bacteria check at the edge of the edge, or inoculate the strain once through a small glass tube filled with 0.3%~0.4% semi-solid agar 1~2 times, take the culture from the remote and then check again.

5.6 Serological typing (optional)

5.6.1 Identification of O antigen

A~F polyvalent O serum was used for slide agglutination test, and normal saline was used as control. Rough-type bacteria that self-coagulate in normal saline which cannot be typed. Those who were agglomerated by A~F multivalent O sera were sequentially tested with O4; O3, O10; O7; O8; O9; O2 and O11 factor sera. According to the test results, determine the O group. Strains that are agglutinated by O3 and O10 serum, then use O10, O15, O34, O19 single factor serum for agglutination test, to determine the E1 and E4 subgroups, the final determination of each O antigen component should be based on the test results of O single factor serum. If no single factor serum, two O complex factor serums should be used for verification.

Those who are not agglomerated by A~F multivalent O serum, first check with 9 kinds of multivalent O serum, if one of them is agglutination, then use this serum included group O sera were checked one by one to determine the group O. The O factors included in each multivalent O serum are as follows:

O Multivalent 1 A, B, C, D, E, F group (and including 6, 14 groups)

O Multivalent 2 13, 16, 17, 18, 21 group

O Multi-price 3 28,30,35,38,39 group

O Multi-price 4 40,41,42,43 group

O Multi price 5 44,45,47,48 group

O Multi-price 6 50,51,52,53 group

O multi-price 7 55,56,57,58 group

O Multi-price 8 59,60,61,62 group

O multi-price 9 63,65,66,67 group

5.6.2 Identification of H antigen

For the common bacterial types belonging to each O group of A~F, check the H antigens of phase 1 and phase 2 with the H factor serum described in Table 6 in turn.

Table 6 A~F group common bacterial type H antigen table

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(no 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	

Uncommon bacteria type, first check with 8 kinds of multivalent H serum, if one or two kinds of serum agglutination, then use one or two kinds of blood which various H-factor sera included in the clearance are checked one by one, with phase 1 and 2 H antigens. The H factor included in 8 kinds of multivalent H serum is as follows:

H polyvalent 1 a, b, c, d, i

H polyvalent 2 eh,enx,enz$_{15}$,fg,gms,gpu,gp,gq,mt,gz$_{51}$

H polyvalent 3 k, r, y, z, z$_{10}$, lv, lw, lz$_{13}$, lz$_{28}$, lz$_{40}$

H polyvalent 4 1,2;1,5;1,6;1,7;z$_6$

H polyvalent 5 z$_4$ z$_{23}$, z$_4$ z$_{24}$, z$_4$ z$_{32}$, z$_{29}$, z$_{35}$, z$_{36}$, z$_{38}$

H polyvalent 6 z$_{39}$, z$_{41}$, z$_{42}$, z$_{44}$

H polyvalent 7 z$_{52}$, z$_{53}$, z$_{54}$, z$_{55}$

H polyvalent 8 z$_{56}$, z$_{57}$, z$_{60}$, z$_{61}$, z$_{62}$

The final determination of each H antigen component should be based on the test results of H single factor serum. If there have no H single factor serum, it should be checked with two H compound factor serum.

If Phase 1 H antigen is detected but Phase 2 H antigen is not detected, or Phase 2 H antigen is detected but Phase 1 H antigen is not detected, it may be inoculate on the slope of agar for 1 to 2 generations and check again. If only one phase of H antigen is still detected, the phase variation

method should be used to check the other antigen. Single-phase bacteria do not have to do the phase variation check.

The phase variation test method is as follows:

Simple plate method: dry the surface moisture of 0.35%~0.4% semi-solid agar plate, pick one ring of factor serum, and drop it on the surface of the semi-solid plate for a while, wait for the serum to be absorbed into the agar, and plant the strain to be tested in the center of the serum. After cultivation, the bacteria will spread and grow. Check for bacteria at the edge of the moss.

Small glass tube method: dissolve the semi-solid tube (about 1mL~2mL per tube) on an alcohol lamp and cool to 50 ℃, take the known factor H blood 0.05mL~0.1mL, add it to the melted semi-solid, mix well, use a capillary pipette to suck and dispense the small glass for phase variation test. After coagulation, use the inoculation needle to pick up the bacteria to be tested and inoculate one end. Place the small glass tube in a flat dish and place a ball of wet cotton next to it. In order to prevent the water in the agar from evaporating and shrinking, check the results every day. After the bacteria in the other phase dissociate, you can pick the bacteria from the other end for inspection. The concentration of serum in the medium should be in an appropriate ratio. When it is too high, bacteria cannot grow, and when it is too low, the motility of bacteria in the same phase cannot be suppressed. General add in the amount of 1:200~1:800 of the original serum.

Small inverted tube method: place the small glass tube with two ends open (a gap should be left at the lower end, not flush) in the semi-solid tube, the end of the small glass tube should be higher than the surface of the medium, and be used after sterilization. Before use, it is heated and dissolved on an alcohol lamp, cooled to 50 ℃, and 1 ring of serum factor is picked, add to the semi-solid in the small sleeve, stir it slightly to make it mix well, and after solidification, inoculate the strain to be tested on the semi-solid surface layer in the small sleeve, check the results every day, after the other phase of bacteria dissociates, you can take the bacteria from the semi-solid surface outside the casing to check, or replant 1% soft agar slope, and incubate at 36 ℃, do the agglutination test.

5.6.3 Identification of Vi antigen

Check with Vi factor serum. The known bacterial types with Vi antigen are: Salmonella typhi, Salmonella paratyphi C, Dublin sand Salmonella.

5.6.4 Determination of bacteria type

According to the results of serological typing, according to Appendix B or the relevant

Salmonella antigen table to determine the bacterial type.

6 Results and reports

Based on the results of the above biochemical tests and serological identification, it was reported that Salmonella was detected or not detected in the 25g (mL) sample.

Appendix A Medium and Reagent

A.1 Buffer Peptone Water (BPW)

A.1.1 Compositions

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

A.1.2 Preparation

Each composition is added to the distilled water; mix evenly; stand for about 10min; boiled to dissolve; adjust the pH to 7.2±0.2; perform autoclave sterilization at 121°C for 15min.

A.2 Tetrathionate Broth (TTB)

A.2.1 Base fluid

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

In addition to the calcium carbonate, add various compositions into the distilled water; add calcium carbonate; adjust pH to 7.0±0.2; perform autoclave sterilization at 121°C for 20min.

A.2.2 Sodium thiosulfate solution

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

perform autoclave sterilization at 121°C for 20min.

A.2.3 Iodine solution

Iodine tablets	20.0g
Potassium iodine	25.0g
Distilled water add to	100mL

Dissolve Potassium iodine fully in a small amount of distilled water, then put Iodine tablets in the flask, shake the Iodine tablets untill all dissolved, then add distilled water to the specified amount, store in the brown bottle, plug the cap for later use.

A.2.4 0.5% bright green water solution

Brilliant green	0.5g
Distilled water	100mL

After dissolving, store it in a dark place for no less than 1d to let it sterilize naturally.

A.2.5 bovine bile salt solution

Bovine bile salt	10.0g
Distilled water	100mL

Heat and boil until completely dissolved, autoclave 121 ℃ for 20min.

A.2.6 Preparation method

Base fluid	900mL
Sodium thiosulfate solution	100mL
Iodine solution	20.0mL
Brilliant green solution	2.0mL
Bovine bile salt solution	50.0mL

Immediately before use, according to the sequence listed above, add aseptic operation to the base solution in sequence, each component should be shaken before adding another ingredient.

A.3 Selenite cystine (SC) enrichment solution

A.3.1 compositions

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

A.3.2 Preparation

In addition to Sodium hydrogen selenite and L-cystine, add the ingredients to distilled water, boil to dissolve, cool to below 55 ℃, and add sodium selenite and 1g/L L-cystine solution 10mL (Weigh 0.1g L-cystine, add 15mL of 1mol/L sodium hydroxide solution to dissolve Solution, and then add sterile distilled water to 100ml, if it is DL-cystine, the amount should be doubled) aseptically. Shake well and adjust the pH to 7.0±0.2.

A.4 Bismuth sulfite (BS) agar

A.4.1 compositions

Peptone	10.0g
Beef paste	5.0g
Glucose	5.0g
Ferrous sulfate	0.3g
Disodium phosphate	4.0g
Brilliant green	0.025g or 5.0g/L aqueous solution 5.0mL
Ammonium bismuth citrate	2.0g
Sodium Sulfite	6.0g
Agar	18.0g~20.0g
Distilled water	1000mL

A.4.2 Preparation

Add the first three ingredients to 300mL of distilled water (making base liquid), ferrous sulfate and disodium hydrogen phosphate are added to 20ml and 30ml distilled water, bismuth ammonium citrate and sodium sulfite were added to another 20mL and 30mL distilled water, and agar was added to 600mL distilled water. Afterwards, stir well and boil to dissolve. When

cooling to about 80℃, mix the ferrous sulfate and disodium hydrogen phosphate first, pour into the base liquid and mix. Mix bismuth ammonium citrate and sodium sulfite, pour into the base liquid, and mix again. Adjust the pH to 7.5±0.2, then pour into the agar liquid and mix Evenly, cool to 50℃~55℃. Add the brilliant green solution, mix thoroughly and immediately pour into the dish.

Note: This medium does not need to be autoclaved, and it should not be heated excessively during the preparation process to avoid reducing its selectivity. Store it in a dark place at room temperature. This medium should be prepared on the same day and used the next day.

A.5 Hektoen Enteric Agar

A.5.1 Compositions

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

A.5.2 Preparation

Dissolve the first seven compositions into 400mL of distilled water as the base fluid;

add agar into 600mL of distilled water. Then separately mix evenly, boiled to dissolve.

Add Solution A and Solution B into base fluid; adjust the pH to 7.5±0.2. Then add the indicator to the solution and combined with AGAR solution and cooled to 50~55℃.

Note :

1. This medium does not need autoclaving and should not be heated excessively during preparation to avoid reducing its selectivity.

2. Preparation of liquid A

Sodium thiosulfate	34.0g
Ammonium ferric citrate	4.0g
Distilled water	100mL

3.Preparation of liquid B

Deoxysodium cholate	10.0g
Distilled water	100mL

4. Andrade indicator

Acide fuchsin	0.5g
1mol/l sodium hydroxide solution	16mL
Distilled water	100mL

Add the Acide fuchsin to distilled water and added the sodium hydroxide solution, then add 1ml~2ml sodium hydroxide solution after a few hours if the color is not faded completely.

A.6 Xylose lysine Desoxycholate (XLD) Agar

A.6.1 Compositions

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

A.6.2 Preparation

In addition to phenolic red and AGAR, the other ingredients were added to 400mL distilled water and dissolved, and adjust the PH to be 7.4±0.2. Add AGAR to 600ml distilled water and boil to dissolve. After the above two solutions are evenly mixed, add the indicator. Cool off to 50~55℃.

NOTE: this medium doesn't need autoclave sterilization, it shall not be heated too much during the preparing process, so that avoid reduce its selectivity, store at the room temperature in the Dark, this medium shall be prepared at the current day, and used in the following day.

A.7 Triple Sugar Iron (TSI) Agar.

A.7.1 Compositions

Peptone	20.0g
Beef paste	5.0g
Lactose	10.0g
Sucrose	10.0g
Glucose	1.0g
ammonium ferrous sulfate (containing 6 crystal water)	0.2g
phenolic red	0.025g or 0.5g/L solution 5mL
Sodium chloride	5.0g
Sodium thiosulfate	0.2g
AGAR	12.0g
Distilled water	1000mL

A.7.2 Preparation

In addition to phenol red and agar, add other compositions into the 400mL of distilled water; boiled to dissolve; adjust the pH to be 7.4±0.2. Additionally, add agar into 600mL of distilled water, boiled to dissolve.

After mixing the above two solutions, add indicators, mix evenly; separately filling in the tubes, each tube contains 2mL ~ 4mL, perform autoclave sterilization at 121°C for 10min or at 115°C for 15min, after sterilization, make the higher-layer slope, which is in orange red color.

A.8 Petone water, indole reagent

A.8.1 Petone water

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

Add the above compositions into the distilled water, boiled to dissolve, adjust the pH to be 7.4±0.2, separately fill into the small test tubes, perform the autoclave sterilization at 121°C for 15min.

A.8.2 Indole reagent

A.8.2.1 Kovacs reagent: dissolve 5g of paradimethylaminobenzaldehyde into 75mL of Pentanol, then slowly add 25mL of concentrated hydrochloric acid.

A.8.2.2 Europe-wave reagent: dissolve 1g of paradimethylaminobenzaldehyde into 95mL of 95% ethanol. Then slowly add 20mL of concentrated hydrochloric acid.

A.8.3 Test method

Pick up small amount of culture to inoculate, inoculate at 36°C±1°C for 1d~2d, if necessary, can culture for 4d~5d. Add about 0.5mL of Kovacs reagent, shake the test tube gently, if it is positive, it appears dark red in the reagent layer, or add about 0.5mL of Europe-wave reagent, flow down along the tube wall, cover the culture medium Surface, if it is positive, it appears rosy red on the contact place of the liquid.

NOTE: peptone contains rich perchloric acid. After purchasing each batch of peptone, firstly identify by the known bacteria before use.

A.9 Urea agar (pH7.2)

A.9.1 Compositions

Peptone	1.0g
Sodium chloride	5.0g
Glucose	1.0g
Potassium dihydrogen phosphate	2.0g
Phenolic red	3.0mL
AGAR 20.0g	20.0g

Distilled water	1000mL
20% urea solution	100mL

A.9.2 Preparation

In additon to urea, AGAR, phenol infrared, the other ingredients were added to 400ml distilled water, boiled and dissolved, and the PH was adjusted to 7.2 ± 0.2. Add AGAR to 600mL distilled water and boil to dissolve. After mixing the above two solutions, add indicators, mix evenly, separately filling in the tubes. High-pressure sterilization at 121°C for 15min. Cold to 50-55°C, add the sterilized filtered urea slution. The final concentration of urea is 2%. Divide into sterile tubes and bevel them for later use.

A.9.3 Test method

Pick up small amount of culture to inoculate, inoculate at 36°C±1°C for 24h. Urease positive subjects turned the medium red due to alkali production.

A.10 Potassium cyanide (KCN) medium

A.10.1 Compositions

Peptone	1.0g
Sodium chloride	5.0g
Potassium dihydrogen phosphate	0.225g
Disodium hydrogen phosphate	5.64g
Distilled water	1000mL
0.5%Potassium cyanide	20mL

A.10.2 Preparation

The components other than potassium cyanide were added to distilled water, boiled and dissolved, and then autoclaved at 121°C for 15min after separate packing. Leave in the refrigerator to cool thoroughly. Add 0.5% Potassium cyanide solution 2ml to every 100ml medium and packe them into sterile tubes. Each tube is about 4ml and is immediately sealed with a sterile rubber plug and store at 4°C for at least 2 months. At the same time, the medium without potassium cyanide was used as the control medium.

A.10.3 Test method

The AGAR culture is inoculated in peptone to become a diluent. Pick up one ring culture to

inoculated in the potassium cyanide medium. Another ring was selected and inoculated in the control medium. And cultured at 36°C±1°C for 1d~2d, and the results were observed. If there is bacterial growth, it is positive (no inhibition). If there is no baterial growth by 2d ,it is negative (inhibition).

Note : potassium cyanide is a highly toxic drug, use caution, don't contaminated, so as to avoiding poisoning. The metium should be separated in the refrigerator in the summer. The main reason for the failture of the test was that the sealing was not strict and the potassium cyanide was gradually decomposed to produce hydrocyanic acid gas, which led to the decrease of drug concentration and the growth of the bacteria,thus causing false positive reaction. Special attention should be paid to each step in the experiment.

A.11 Lysine decarboxylase test medium

A.11.1 compositions

Peptone	5.0g
Yeast extract	3.0g
Glucose	1.0g
Distilled water	1000mL
1.6% bromocresol violet ethanol solution	1.0mL
L-lysine or DL-lysine	0.5g/100ml or1.0g/100ml

A.11.2 Preparation

Heating and dissolving the compositions except lysine, separately pack into the bottles for 100mL each one, separately add lysine. Add 0.5% L-lysine, and 1% DL-lysine. Adjust pH to be 6.8±0.2. The control medium doesn't add lysine. Separately pack into the sterile small test tubes, each tube for 0.5mL, drop a layer of liquid paraffin onto it, perform autoclave sterilization at 115°C for 10min.

A.11.3 Test method

Pick up small amount of culture from medium to inoculation, cultured at 36°C±1°C for 18~24h, and observe the result. Amino acid decarboxylase positive because of alkaline product, the medium should be purple. The negative had no alkaline product, but the medium was yellow because of the acid produced by glucose. The control shall be yellow.

A.12 Sugar fermentation tube

A.12.1 Compositions

Beef extract	5.0g
Peptone	10.0g
Sodium chloride	3.0g
Disodium phosphate (including 12 crystal water)	2.0g
Bromothymol blue solution	12mL
Distilled water	1000ml

A.12.2 preparation

A.12.2.1 Accroding to the above composition to preparate the glucose fermentation tubes, adust PH to be 7.4±0.2. Then add glucose with 0.5% and divided into a small test tube with an inverted tube, perform autoclave sterilization at 121°C for 15min.

A.12.2.2 other glucose fermentation tubes can be divided into 100ml per bottles and autoclaving at 121°C for 15min. In addition, 10% solution was prepared for each sugar, and autoclave sterilization was conducted. Add 5ml sugar solution into 100ml medium, and divided into small tubes with sterile preparation.

Note : sucrose is not pure which can decomposed when heating, and should adopt filtration to remove the bacteria.

A.12.3 test method

Pick up small amount of medium to inoculation, and cultured at 36°C±1°C for 2~3d. The delayed reaction should be observed for 14~30d.

A.13 O-Nitrophenyl β-D galactopyranoside (ONPG) medium

A.13.1 composition

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

A.13.2 Preparation

Dissolve ONPG into buffer solution, add peptone water, so that filtrate and sterilize, separately pack into the sterile small test tubes, 0.5mL each tube, cover with rubber stopper tightly.

A.13.3 Test method

Pick up medium from agar slope for 1-ring to inoculate, then culture at 36°C±1°C for 1h~3h and 24h to observe the results. If β-galactosidase generates, then it turns to yellow for 1h~3h, if there is no such enzyme, then it doesn't change color for 24h.

A.14 Semi-solid agar

A.14.1 Compositions

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

A.14.2 Preparation

Prepare as per the above compositions, boiled to dissolve, adjust pH to be 7.4±0.2. Separately pack into small test tubes. Perform the autoclave sterilization at 121°C for 15min. Upright solidification for backup.

NOTE: for the power observation, bacteria preservation, and H antigen phase mutation test.

A.15 Sodium malonate medium

A.15.1 Compositions

Yeast extract	1.0g
Ammonium sulfate	2.0g
Dipotassium phosphate	0.6g

Appendix B
Common Salmonella Antigens

Table B.1 Common Salmonella Antigens

菌名	拉丁菌名	O抗原	H抗原	
			第1相	第2相
A 群				
甲型副伤寒沙门氏菌	S .ParatyphiA	1,2,12	a	[1,5]
B 群				
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	z6
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.Lagos	1,4,[5],12	i	1,5
布雷登尼沙门氏菌	S.Bredeney	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

菌名	拉丁菌名	O抗原	H抗原	
			第1相	第2相
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 群				
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.BloemfonteinⅡ	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

表 B.1（续）

菌名	拉丁菌名	O 抗原	H 抗原	
			第 1 相	第 2 相
C2 群				
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 群				
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	z_6
D 群				
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}

鸡-雏沙门氏菌	S.Gallinarum-Pullorum	1,9,12	—	—
E1 群				
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	1,2
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_{15}	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 群				
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 群				
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

菌名	拉丁菌名	O抗原	H抗原	
			第1相	第2相
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
其他群				
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

注：关于表内符号的说明：

{}={}内O因子具有排他性。在血清型中{}内的因子不能与其他{}内的因子同时存在，例如在O：3,10群中当菌株产生O：15或O：15,34因子时它替代了O：10因子。

[]= O(无下划线)或H因子的存在或不存在与噬菌体转化无关，例如O：4群中的[5]因子。H因子在[]内时表示在野生菌株中罕见，例如极大多数S.Paratyphi A具有一个位相(a)，罕有第2相(1,5)菌株。因此，用1,2,12：a：[1,5]表示。

_=下划线时表示该O因子是由噬菌体溶原化产生的。