NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB 4789.4—2010

National food safety standard

Food microbiological examination: Salmonella

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Foreword

This standard instead of GB/T 4789.4-2008 (Food microbiological examination: Salmonella)

Compared to this standards and GB/T 4789.4-2008, The main changes are as follows:

Modified the name of standard in English and Chinese

Modify the scope of the standard

Modified media and reagents

Modify the equipment and materials

Revised Appendix A

Appendix A and Appendix B are normative appendix

Instead of the standard conditions for the successive release: GB 4789.4-84、GB 4789.4-1994、GB/T 4789.4-2003、GB/T 4789.4-2008.

Food microbiological examination: Salmonella

1. Scope

The standard specifies a method for the detection of Salmonella in food.
The standard applies a method for the detection for Salmonella in food.

2. Equipment and Material

Usual microbiological laboratory equipment and, in particular the following

2.1 Refrigerator: 2□-5□.
2.2 Incubator: 36□±1□,42□±1□
2.3 Homogeneous machine
2.4 Oscillator
2.5 Electric Balance: Sensibility 0.1g
2.6 Asepsis conical flask: Capacities 500ml and 250ml.
2.7 Asepsis pipettes: of capacities 10ml and 1ml, graduated respectively in 0.1ml and 0.01ml divisions or micro-pipette and the suction head.
2.8 Petri dishes: of diameter 90mm.
2.9 Asepsis culture tubes, 3mm*50mm,10mm*75mm
2.10 Asepsis Capillary
2.11 PH-meter or pH color comparison tube or precision pH test strips.
2.12 VITEK Machine.

3. Culture media and reagent

3.1 Buffered peptone water: See appendix A.1
3.2 Tetrathionate Broth: See appendix A.2
3.3 Selenite Cystinol Broth: See appendix A.3
3.4 Bismuth Sulfite Agar: See appendix A.4
3.5 Hekton Enteric Agar: See appendix A.5
3.6 Xylose Lysine Desoxycholate Agar: See appendix A.6
3.7 Salmonella Chromogentic Agar
3.8 Triple sugar/iron agar: See appendix A.7
3.9 Peptone water, Indole reagent: See appendix A.8
3.10 Urea Agar: See appendix A.9
3.11 KCN Agar: See appendix A.10
3.12 Lysine decarboxylation test broth: See appendix A.11
3.13 Bromcresol Fermentation tube: See appendix A.12
3.14 Ortho-nitrophenol β -D-galactosidase: See appendix A.13
3.15 Semisolid Medium: See appendix A.14
3.16 Sodium malonate medium: See appendix A.15
3.17 Salmonella O and H phase Antisera

3.18 Biological identity strips

4. Test Procedure

See chart 1: Salmonella test procedure

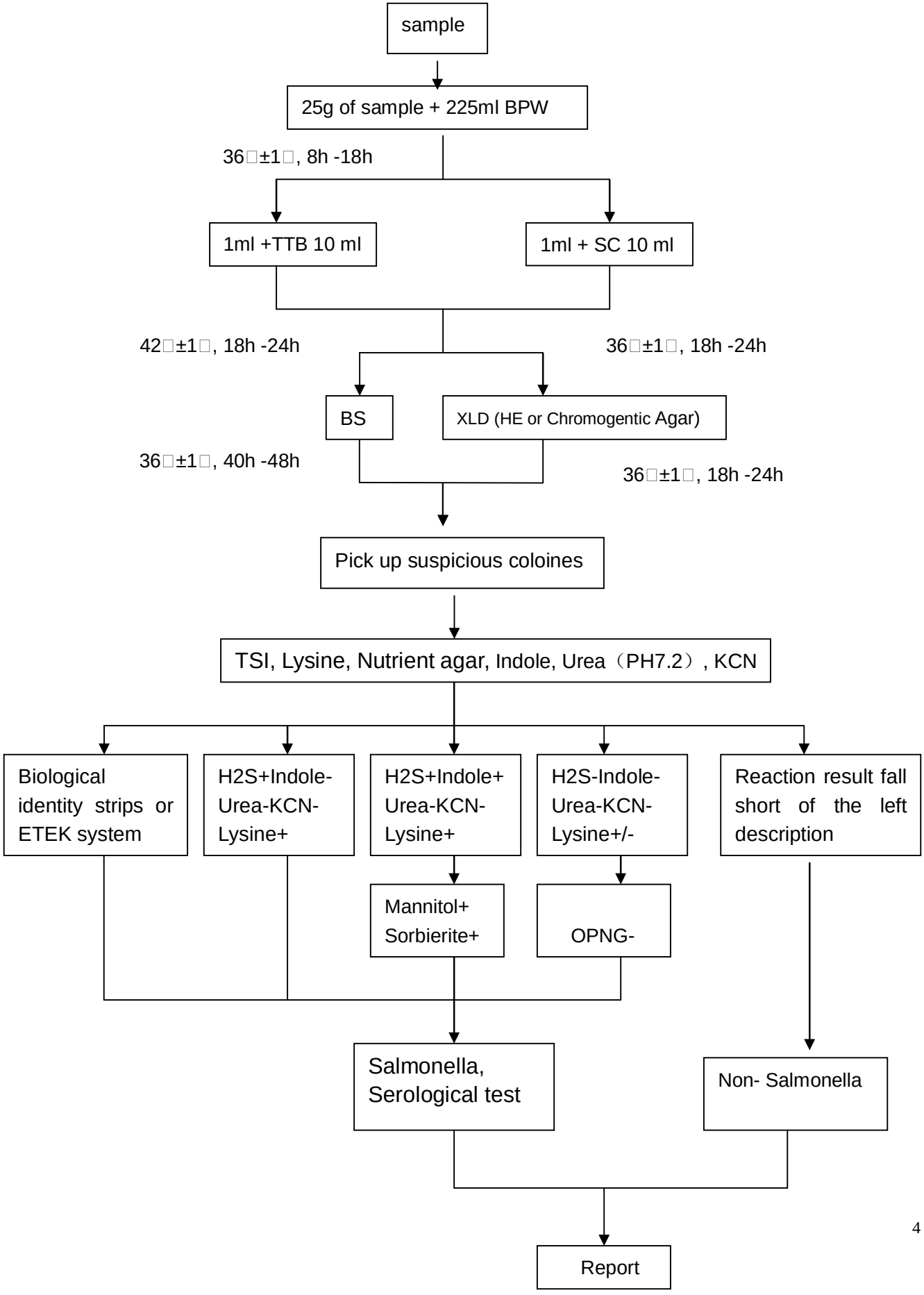

chart 1: Salmonella test procedure

5. Operation steps

5.1 Pre-enrichment

Weigh 25g of sample into a sterlie homogeneous cup containing 225ml of BPW, homogeneous 1min~2min in speed of 8 000 r / min ~ 10 000 r / min, or place into an aseptic homogenizing bag containing 225 mL BPW, use a Slap-type homogenizer beat 1 min ~ 2 min. If the sample is liquid, does not require homogenization, oscillation mixing. For determination of pH, using 1 mol / mL sterile NaOH or HCl pH adjusted to 6.8 ± 0.2. Aseptic sample transfer to 500 mL Erlenmeyer flask, if use a homogeneous bag, 36 ℃ ± 1 ℃ cultured 8 h ~ 18h directly.

If freeze food, should be thawed at below 45℃, less than 15 min, or at 2℃ -5℃, less than 18h.

5.2 Enrichment

Gently shake the incubation suspension, transfer 1ml of the incubation fluid into a tube containing 10ml of TTB, incubate at 42℃±1℃ for 18h-24h. Transfer 1ml of the incubation fluid into a tube containing 10ml of SC, incubate at 36℃±1℃ for 18h-24h.

5.3 Isolation

Streak a loopful of incubated TTB and SC onto BS and XLD (or HE or Chrom Agar). Make fraction streaks to obtain well isolated colonies, incubate the plates of XLD (or HE or Chrom Agar) at 36℃±1℃ for 18h-24h or 40h-48h (BS dishes). Observe the typical colonies on the each plate. (See table 1)

Table 1: Salmonella on isolation Media

Isolation agar plate	Salmonella
BS	Black with metallic shine, tan or grey colonies surround by a black or brown medium; Some celadon colonies surround by a original medium.
HE	Blue-green colonies or some yellow colonies with a black centre.
XLD	Pink to red colonies or yellow colonies with a black centre or none a black centre.
Chrom Agar	Purple-red colonies.

5.4 Biochemical confirmation

5.4.1 Pick up 2 of the typical colonies of presumptive Salmonella from each selective agar plate, streak the slant of a TSI tube and stab in the bottom. Don' t sterilize the vaccination needle, straight stab two times into the butt of one LIA tube and streak the Nutrient agar plate, incubate at 36℃

±1℃ for 18h-24h, delay to 48h if necessary. See table 2 about growth characteristic for Salmonella on TSI and Lysine.

Table 2: Growth characteristic for Salmonella on TSI and Lysine

TSI				Lysine	Prejudgement
Slant	Bottom	Gas formation	H2S		
K	**A**	**+(-)**	**+(-)**	**+**	presumptive Salmonella
K	**A**	**+(-)**	**+(-)**	**-**	presumptive Salmonella
A	**A**	**+(-)**	**+(-)**	**+**	presumptive Salmonella
A	**A**	**+/-**	**+/-**	**-**	Non- Salmonella
K	**K**	**+/-**	**+/-**	**+/-**	Non- Salmonella
Mark: K: Alcaligenes, A: Acid; + positive,-Negative;+(-) majority positive, minority negative;+/- Pos or Neg					

5.4.2 At the same time, the colony can be inoculated in peptone water(for indole reaction), Urea, KCN media, also can inoculate suspicious colonies from nutrient agar, incubate at 36℃±1℃ for 18h-24h, delay to 48h if necessary. See table 3 to judge the result. The picked plate should be kept at 2℃-5℃ or room temperature for 24h, reexamine if necessary.

Table 3: Biochemical reaction for Salmonella interpretation

Reaction ID	H2S	Indole	PH7.2 Urea	KCN	Lysine
A1	+	-	-	-	+
A2	+	+	-	-	+
A3	-	-	-	-	+/-
Mark: +Positive; -Negative; +/-Pos or Neg					

5.4.2.1 Reaction ID A1: Salmonella typical reaction. If there is a abnormal item in 3 of Urea, KCN and Lysine reaction, follow table 4. Non Salmonella show 2 of abnormal items.

Table 4: Biochemical reaction for Salmonella interpretation

PH7.2 Urea	KCN	Lysine	Judgement
-	-	-	S.paratyphi A (Serological Confirmation)
-	+	+	Salmonella IV or V (Up to home group characteristics
+	-	+	Salmonella variation (Serological Confirmation)
Mark: +Positive; -Negative;			

5.4.2.2 Reaction ID A2: Additional Mannitol and Sorbierite testing,

Salmonella indole positive variation testing be showed positive, and need be complied with serological confirmation.

5.4.2.3 Reaction ID A3: Additional ONPG testing. ONPG negative and Lysine positive Salmonella, Lysine negative S.paratyphi A.

5.4.2.4 Follow table 5 to identify Salmonella biochemical group.

Table 5: Biochemical identity for Salmonella group

Item	I	II	III	IV	V	VI
Galactitol	+	+	-	-	+	-
Sorbierite	+	+	+	+	+	-
Salicin	-	-	-	+	-	-
ONPG	-	-	+	-	+	-
Malonate salt	-	+	+	-	-	-
KCN	-	-	-	+	+	-
Mark: +Positive; -Negative;						

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5.4.3 If use Biological identity strips or VETEK identity system, the primary Salmonella result can follow table 2. Pick up the suspicious colonies from Nutrient plate, made the appropriate turbidity suspension, then use Biological identity strips or VETEK system to identify.

5.5 Serological confirmation

5.5.1 Antigen preparation

Generally use 1.2%-1.5% of incubated agar to slide agglutination. If Salmonella O-antigens don't be agglutinated, the culture should be inoculated in 2%-3% of agar; If the agglutination of Salmonella O-antigens shall be checked by Salmonella V-antigens, then pick up the colonies into 1ml of saline solution, boiling by spirit lamp. If the agenesis of Salmonella H-antigen, then inoculate the culture on the centre of 0.55%-0.65% semisolid plate, check the fringe part of far-flung colonies. Or the strain by 0.3% ~ 0.4% with semi-solid agar in a small glass tube 1 to 2 times, since bacterial culture and then check the remote access

5.5.2 Examination of multivalent thalli antigens(O)

Respective place half of a colony onto the top of each area of slide, add one drop of multivalent thalli 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.

5.5.3 Examination of multivalent flagellum antigens (H)
Same as 5.5.2

5.5.4 Serological type confirmation (Selective item)

5.5.4.1 Examination for O-antigens

Using A-F of multivalent anti-O serum to test agglutination. Use the saline solution to do comparison. If the bacteria have clumped into more or less distinct units, the strain is considered auto-agglutinable.

If be agglutinated by A-Fof multivalent anti-O serum, then in turn use O4; O3, O10; O7; O8; O9;O2 and O11 of factorial serum to test agglutination. Salmonella judged Group O. If the bacteria have agglutinated by O3, O10 of serum, the culture should be tested by O10, O15, O34, O19 of single factorial serum, Salmonella judged E1, E2, E3, E4 of each subgroup.

If don't be agglutinated by A-F of multivalent anti-O serum, use the follow 9 of multivalent anti-O serum. If be agglutinated by any serum, then be checked by O group serum of the serum. Each multivalent anti-O serum includes the below O gene.

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

5.5.4.2 Examination for H-antigens

O group familiar bacteria type belong to A-F, in turn be checked with H-antigens in table 6.

Table 6 : A-F familiar bacteria type H-antigens

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)	d	No
D (gas)	g, m, p, q	No
E1	h, v	6, w, x
E4	g, s, t	No

E4	i	No

Be checked with 8 of multivalent H serum for singular bacteria type. If be agglutinated by one or two of serum, then be checked by each H gene of the serum, make sure the phase 1 and 2 of H-antigen. 8 of multivalent anti-H serum includes the below H gene.

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

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

H multivalent 3 k, r, y, z, z$_{10}$, 1v, 1w, 1z$_{13}$, 1z$_{28}$, 1z$_{40}$

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

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

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

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

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

H antigen component of each should be the final determining factor under the H serum test results alone, no single factor H serum to use two composite factor H serum check.

1-phase H antigen were detected but have not detected H antigen phase 2 or phase 2 H antigens were detected without the detection phase 1 H antigens can be. Transplanted in the agar slant and then check on behalf of 1 or 2. If still only one phase of the H antigen detection, phase variation of the method to use check another phase. Single-phase bacteria do not check the phase variation.

Phase variation of test methods are as follows:

Small glass tube: The semi solid tube (each tube is about 1 mL ~ 2 mL) dissolved in the alcohol lamp and cooling to 50 ℃, taking the known phase of the H factor serum 0.05 mL ~ 0.1 mL, added in melted semi-solid, the mix after using capillary-packing straw draw for the phase variation in test. Small glass test tube, once solidified, with the inoculation needles picked to be tested, inoculation at one end. The small glass tube flat on the plate inside, and placed next to a group in their wet cotton to prevent water evaporation and shrinkage of agar in a day, test results, when bacterial dissociation phase after another, picked from the other side of the bacteriological examination. Serum concentration of the medium should be appropriate in the proportion of bacteria can not grow too high, too low power can not be the same with bacteria inhibition. 1:200 ~ 1:800 normal serum according to the original amount added.

Small inverted tube method: Will be open at both ends of the small glass tube (to leave a gap open lower end, do not flush) on the semi-solid tube, a small glass tube should be high for the upper surface of the medium, after sterilization reserve. Temporary lights used on the heating when dissolved in alcohol, cooling to 50 ℃, picked factor serum 1 part, by adding a small tube inside the semi-solid, slightly stirred, to mix, once solidified, will be tested isolates were inoculated in a small tube in the semi-solid surface, the daily test results, when bacterial dissociation phase after another, from the outside casing to take semi-solid surface bacteria examination, or switching to 1% soft agar slant, in 37 ℃ training before making

agglutination test.

Easy plates: Dry the 0.7%-0.8% of semisolid medium plate, drop a loopful of gene serum on the surface of plate, wait a moment. Stab the centre of serum, pick up the bacteria from far-flung lawn to test after incubation.

5.5.4.3 Examination for V-antigens

Using the anti-V-antigens to check the bacteria type. For example:

5.5.4.4 Judgement for bacteria type

In accordance with results of serological confirmation, indicate the type of salmonella follow appendix B.

6. Expression of results

The above biochemical and serological identification results, indicate the presence or absence of salmonella in 25g of samples.

Appendix A
(normative appendix)
Culture Media and Reagent

A.1 Buffered Peptone Water

A.1.1 Composition

Peptone	10.0g
Sodium chloride	5.0g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	9.0g
Potassium dihydrogen phosphate (KH_2PO_4)	1.5g
Distilled Water	1000.0ml

pH 7.2±0.2

A.1.2 Preparation

Dissolve the components in the distilled water, equably mix and place for 10min, by heating to completely solve, adjust the PH to 7.2±0.1, autoclave 15min at 121℃.

A.2 Tetrathionate Broth

A.2.1 Base broth

Peptone	10.0g
Peptone from casein	5.0g
Sodium chloride	3.0g
Calcium carbonate	45.0g
Distilled water	1000.0ml

pH 7.0±0.2

Dissolve the components except for Calcium carbonate in the distilled water, equably mix and place for 10min, add Calcium carbonate. Adjust the PH to 7.0±0.2, autoclave 20 min at 121℃.

A.2.2 Sodium Hyposulfite solution

Sodium Hyposulfite	50.0g
Distilled water	100.0ml

Autoclave 20min at 121℃.

A.2.3 Iodine solution

Iodine piece	20.0g
Potassium iodide	25.0g
Distilled water	add water to 100.0ml

Dissolve the Potassium iodide in a little water, completely solve, add iodine piece, also add water to standard quantity, should be kept in brown bottles, tightly fill in cover.

A.2.4 Brilliant green water

Brilliant green	0.5g
Distilled water	100.0ml

Dissolve the Brilliant green in the water, can be stored in dark more than 1day.

A.2.5 OX Bile salt solution

OX bile salt	10.0g
Distilled water	100.0ml

By heating to boiling, autoclave 20min at 121℃.

A.2.6 Preparation

Base broth	900.0ml
Sodium Hyposulfite solution	100.0ml
Iodine solution	20.0ml
Brilliant green water	2.0ml
OX Bile salt solution	50.0ml

According to the above order in the base broth using sterilized operation before using, equably mix every adding one component.

A.3 Selenite Cystinol Broth

A.3.1 Composition

Peptone	5.0g
Lactose	4.0g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	10.0g
Sodium selenite	4.0g
L- Cystine	0.01g
Distilled water	1000.0ml

pH 7.0±0.2

A.3.2 Preparation

Dissolve the components except for Sodium selenite and L- Cystine in the distilled water, equably mix and place for 10min, by heating to boiling for 5min.Cool to below 55℃, add Sodium selenite and 10ml of 1g/L of L- Cystine solution (Weigh 0.1g L- Cystine, add 15ml of 1mol/L NaOH solution, also add aseptic distilled water to 100ml), mix and adjust the PH to 7.0±0.2

A.4 Bismuth Sulfite Agar

A.4.1 Composition

Peptone	10.0g
Peptone from casein	5.0g
Glucose	5.0g
Iron sulfate	0.3g
Disodium hydrogen phosphate dodecahydrate	4.0g

($Na_2HPO_4.12H_2O$)	
Brilliant green	0.025g
Ammonium iron citrate	2.0g
Sodium sulfite	6.0g
Agar	18.0g
Distilled water	1000.0ml
pH 7.5±0.2	

A.4.2 Preparation

Dissolve the first three basic component in 300ml of distilled water, add Iron sulfate in 20ml of distilled water, add $Na_2HPO_4.12H_2O$ in 30ml of distilled water, add Ammonium iron citrate in 20ml of distilled water, add Sodium sulfite in 30ml of distilled water, add agar in 600ml of distilled water, equably mix and place for 30min, by heating to completely solve, cool to 80℃, mix Iron sulfate and Sodium sulfite, dumping to base broth, mix it , adjust the PH to 7.5±0.2, dumping to agar, mix it , cooling to 50℃-55℃, and add Brilliant green solution ,mix and pour plates to give thick layers.

Note: Do not autoclave. The freshly prepared medium is strongly inhibitory and is thus especially suitable for heavily contamination samples.

A.5 Hekton Enteric Agar

A.5.1 Composition

Peptone from casein	3.0g
Lactose	12.0g
Sucrose	12.0g
Salicin	2.0g
Bile salt mixture	20.0g
Sodium chloride	5.0g
Agar	18.0-20.0g
Distilled water	1000.0ml
0.4%bromothymol blue	16.0ml
Andrade indicator	20.0ml
Solution A	20.0ml
Solution B	20.0ml
pH 7.5±0.2	

A.5.2 Preparation

Dissolve the first seven components in 400ml of distilled water as base broth, add agar in 600ml of distilled water, by heating to solve. Add solution A and B in base broth, adjust the PH to 7.5±0.2,also add indicator complete mixture. Cooling to 50℃-55℃, pour plates.

Note:

1. Do not autoclave and fulsome heating.
2. Solution A:

Sodium Hyposulfite	34.0g
Ammonium iron citrate	4.0g

Distilled water	100.0ml

3. Solution B

Deoxy sodium cholate	10.0g
Distilled water	100.0ml

4. Andrade indicator:

Acid fuchsin	0.5g
1 mol/L NaOH solution	16.0ml
Distilled water	100.0ml

Dissolve fuchsin in distilled water, add NaOH solution. After unchanged, also add 1-2ml of NaOH.

A.6 Xylose Lysine Desoxycholate Agar

A.6.1 Composition

Yeast Extract	3.0g
L-lysine	5.0g
Xylose	3.75g
Sucrose	7.5g
Lactose	7.5g
Deoxy sodium cholate	2.5g
Ammonium iron citrate	0.8g
Sodium thiosulfate	6.8g
Sodium chloride	5.0g
Agar	15.0g
Phenol red	0.08g
Distilled water	1000.0ml
pH 7.4±0.2	

A.6.2 Preparation

Dissolve the components except for phenol red in 1000ml of distilled water, by heating, adjust the PH to 7.4±0.2. Add the indicator, cooling to 50℃-55℃, pour plates.

Note: Do not autoclave and fulsome heating. The plates should be freshly prepared and kept in dark.

A.7 Triple sugar/iron Agar

A.7.1 Composition

Peptone	20.0g
Peptone from casein	3.0g
Sucrose	10.0g
Lactose	10.0g
Glucose	1.0g
Iron sulfate	0.5g
Phenol red	0.025g
Sodium chloride	5.0g
Sodium thiosulfate	0.5g

Agar	12.0g
Distilled water	1000.0ml
pH 7.4±0.2	

A.7.2 Preparation

Dissolve the components except for phenol red and agar in 400ml of distilled water, equably mix and place for 10min, by heating to complete solove, adjust the PH to 7.4±0.2. Dissolve the agar in 600ml of distilled water, equably mix an place for 10min, by heating to solve.

Mix the above both solution, add Phenol red, dispense the mixed mdium in quantities of 2ml-4ml, autoclave 10min at 121℃ or 15min at 115℃.Allow to set in a sloping position to give hyacinth color.

A.8 Peptone water, indole reagent.

A.8.1 Peptone water

Peptone	20.0g
Sodium chloride	5.0g
Distilled water	1000.0ml
pH 7.4±0.2	

A.8.2 Indole reagent

A.8.2.1 Kovacs reagent: Dissolve 5g of 4-Dimethylaminobenzaldehyde in 75ml of 2-Methybutan-2-ol, and tardily add 25ml of Hydrochloric acid.

A.8.2.2 O-bohr reagent: Dissolve 1g of 4-Dimethylaminobenzaldehyde in 95ml of 95% Ethanol, and tardily add 20ml of Hydrochloric acid.

A.8.3 Test Method

Inoculate a tube containing 5ml of peptone medium with the suspected colony, incubate at 36℃±1℃ for 1d-2d, delay to 4d-5d if necessary. Add 0.5ml of the Kovacs reagent, the formation of a red ring indicates a positive reaction. Or add 0.5ml of O-bohr reagent, the formation a rose color indicates a positive reaction.

A.9 Urea Agar (PH7.2)

A.9.1 Composition

Peptone	1.0g
Sodium chloride	5.0g
Glucose	1.0g
Potassium dihydrogen phosphate	2.0g
0.4%Phenol red	3.0ml
Lactose	1.0g
Agar	20.0g
Distilled water	100.0ml
pH 7.2±0.2	

A.9.2 Preparation

Prepare the components except for Urea and agar, adjust PH, add agar, by heating to melt, dispense the medium, autoclave 15min at 121℃. Cooling to 50℃

-55℃, add, under aseptic conditions the urea solution The last concentration of Urea medium is 2%, the last PH is 7.2±0.2. Dispense the medium in the aseptic tubes, allow to set in a sloping position.

A.9.3 Test Method

Streak the agar slope surface, incubate at 36℃±1℃ for 24hr, and examine. If the reaction is positive, splitting of urea liberates ammonia, which changes the color of phenol red to rose-pink.

A.10 KCN Agar(PH7.2)

A.10.1 Composition

Peptone	10.0g
Sodium chloride	5.0g
Potassium dihydrogen phosphate	0.225g
Sodium dihydrogen phosphate	5.64g
0.5%KCN	20.0ml
Distilled water	1000.0ml

A.10.2 Preparation

Prepare the components except for KCN, autoclave 15min at 121℃. Cool the medium in the refrigeratory. Add 2ml of 0.5% KCN in per 100ml of medium, dispense in the quantities 4ml of aseptic tubes. Stuff the tube with the aseptic rubber cover, be kept in the 4℃ for 2 months. At the same time, dispense the non KCN medium to compare the test result.

A.10.3 Test Method

Inoculate a loopful of incubated peptone medium with the suspected colony in KCN medium, the other loopful inoculated in non KCN medium. Incubate at 36℃±1℃ for 1d-2d, and examine. The growth of bacteria indicate positive, the without growth of bacteria after 2d incubation indicate negative.

Note: KCN is poison, be carefully use it, do not contaminate. Dispense the medium in the refrigeratory in summer. KCN medium can decompound gas and lead to the debasement of concentration, thus maybe give false positive reaction

A.11 L-Lysine decarboxylation medium

A.11.1 Composition

Peptone	5.0g
Yeast Extract	3.0g
Glucose	1.0g
Distilled water	1000.0ml
L-Lysine monohydrochloride	0.5g/100ml
1.6%Bromocresol Purple	1.0ml
pH 6.8±0.2	

A.11.2 Preparation

Dissolve the components except for Lysine, dispense the medium in the quantities 100ml of bottles, respectively add Lysine. (L-lysine: 0.5%, DL-Lysine:1%), adjust PH to 6.8±0.2, don't add Lysine in comparison medium. Dispense the medium in quantities 5ml of aseptic tube, seal a layer of olefin oil.

A.11.3 Test Method

Inoculate a tube containing 5ml of Lysine medium with the suspected colony Incubate at 36℃±1℃ for 18h-24h, and examine. A purple color after incubation indicates a positive reaction. A yellow color indicates a negative reaction.

A.12 Bromcresol Fermentation tube

A.12.1 Composition

Peptone	10.0g
Peptone from casein	5.0g
Sodium chloride	3.0g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	2.0g
0.2%Bromthymol phenol blue	12.0ml
Distilled water	1000.0ml
pH 7.4±0.2	

A.12.2 Preparation

A.12.2.1 Glucose fermentation tube: Prepare the above components, adjust PH to 7.4±0.2, add 0.5% glucose, dispense the medium in a tube containing inversion canal, autoclave 15min at 121℃.

A.12.2.2 Other glucide fermentation tubes: Prepare the above components, dispense the medium in the quantities 100ml of bottles, autoclave 15min at 121℃. Prepare a kind of 10% of glucide, set in autoclave. Dispense 5 ml of glucide into the 100ml of mediun, dispense, under aseptic condition in the tube.

A.12.3 Test Method

Inoculate a tube containing 5ml of Lysine medium with the suspected colony Incubate at 36℃±1℃ for 18h-24h, and examine. A purple color after incubation indicates a positive reaction. A yellow color indicates a negative reaction.

A.13 ONPG medium

A.13.1 Composition

α-Nitrophenyl β-D-galactopyranoside	60.0mg
0.01mol/L sodium phosphate	10.0ml
1%peptone water	30.0ml

A.13.2 Preparation

Dissolve OPNG in Sodium phosphate, sterilized by filter, dispense the medium in the quantities 5ml of aseptic tube, stuff the tube with the aseptic rubber cover.

A.13.3 Test method

Inoculate a loopful of incubated colony in ONPC medium, incubate at 36℃±1℃ for 18h-24h, and examine. A yellow color after 1h-3h incubation indicates a positive reaction, unchanged color after 24h incubation indicates a negative reaction.

A.14 Semisolid Medium

A.14.1 Composition

Peptone form casein	0.3g
Peptone	1.0g
Sodium chloride	0.5g
Agar	0.35-0.4g
Distilled water	100.0ml

pH 7.4±0.2

A.14.2 Preparation

Dissolve the above components, by heating, adjust PH to 7.4±0.2, dispense in tubes, autoclave 15min at 121℃, allow to set in erective position

Note: Provide motivity observation, culture storage, and variation of H-antigens.

A.15 Sodium malonate medium

A.15.1 Composition

Yeast extract	1.0g
Ammonium sulfate	2.0g
Potassium hydrogen phosphate	0.6g
Potassium dihydrogen phosphate	0.4g
Sodium chloride	2.0g
Sodium malonate	3.0g
0.2%Bromthymol phenol blue	12.0ml
Distilled water	1000.0ml

pH 6.8±0.2

A.15.2 Preparation

Dissolve the components except for yeast extract, adjust PH to 6.8±0.2, add indicator, dispense the tubes , autoclave 15min at 121℃.

A15.3 Test method

Inoculate a tube containing Malonate Broth with freshly colony, incubate at 36℃±1℃ for 48h, and examine. A blue color changed from green color indicates a positive reaction.

Appendix A
(normative appendix)
Common Salmonella antigen

B.1 Common Salmonella antigen

Common Salmonella antigen see Table B.1

Table B.1 Common Salmonella antigen

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. paratyphi A*	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	1,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.kiwa 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_6,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.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]
奥里塔戛林沙门氏菌	*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 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_6,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.bleqdam*	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 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_{63}	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
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_6,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_6,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