GB

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GB 4789.4-2016

National Food Safety Standard – Food Microbiological Examination – Salmonella Test

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Foreword

This Standard replaced GB 4789.4-2010 *National Food Safety Standard – Food Microbiological Examination – Salmonella Test*, SN 0170-1992 *Method for Detection of Salmonella (Including Arizona) in Food for Export*, and SN/T 2552.5-2010 *Microbiological Examination Method for Milk and Milk Products Hygiene – Part 5: Detection of Salmonella*.

Compared with GB 4789.4-2010, the combined standard has the major changes as follows:

--- Modify the detection process and serological detection operation procedures;

--- Modify the Appendix A and Appendix B.

National Food Safety Standard – Food Microbiological Examination – Salmonella Test

1 Scope

This Standard specifies the detection method of Salmonella in food.

This Standard is applicable to the test of salmonella in food.

2 Devices and Materials

In addition to the microbial laboratory routine sterilization and cultivation device, other devices and materials are as follows:

2.1 Refrigerator: 2°C~5°C.

2.2 Constant temperature incubator: 36°C±1°C, 42°C±1°C.

2.3 Homogenizer.

2.4 Oscillator.

2.5 Electronic balance: sensitivity 0.1g.

2.6 Sterile conical flask: capacity of 500mL and 250mL.

2.7 Sterile pipettes: 1mL (with 0.01mL scale), 10ml (with 0.1ml scale) or micro pipette and sucker.

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

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 microbe biochemical identification system.

2.12 Sterile capillary tube.

3 Medium and Reagent

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

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3.17 Salmonella O, H and Vi diagnosed serum.

3.18 Biochemical identification reagent kit.

4 Test Procedures

Salmonella test procedure is shown in Figure 1.

Generally, use 1.2%~1.5% agar culture as the antigens for the slide agglutination test. Firstly, remove the self-agglutination reaction; drop one drop of saline on the clean slide; mix the to-be-tested culture into the saline drops; so that it becomes uniform turbid suspension; shake the slide gently for 30s~60s; observe the reaction under the black background (if necessary, use magnifier to observe); if there is visible O agglutination, then it is considered to have self-agglutination; otherwise it is considered to have no self-agglutination. The culture without self-agglutination shall take the serological identification as per the following method.

5.5.2 Identification of polyvalent bacterial antigen (O)

Draw 2 zones with size of 1cm×2cm; pick up 1-ring of to-be-tested bacteria; separately place 1/2-ring on the upper part of each zone on the slide; thereof, the lower part of one zone is added 1 drop of polyvalent bacterial (O) antiserum; the lower part of the other zone is added 1 drop of saline to control. Then use sterile inoculation ring or needle to separately grind the bacteria moss on two zones into emulsion. Tilt the slide to shake and mix for 1min; observe against the black background; any degree of agglutination was positive reaction. When O serum is not agglutinated, inoculate the strains onto the medium with higher agar content (e.g. 2%~3%) to re-check; if the O agglutination reaction is prevented due to the presence of Vi antigen, pick up bacteria moss to make concentrated bacteria liquid in 1mL of saline; boiling on the alcohol lamp flame then check.

5.5.3 Identification of polyvalent flagellum antigen (H)

The operation is the same as 5.5.2. When H antigen was poorly developed, inoculate the strain into the center of 0.55%~0.65% semi-solid agar plate; when colonies were growing, take bacteria from the edge to check; or inoculate the strain with the small glass tube containing 0.3%~0.4% semi-solid agar for once or twice, take bacteria from the far end, culture and then check.

5.6 Serological classification (optional)

5.6.1 Identification of O antigen

Use A~F polyvalent O serum to do the slide agglutination test; meanwhile use saline to control. The self-agglutination substances in the saline is rough strain, which can't be classified.

The substance that is agglutinated by A~F polyvalent O serum shall successively use O4; O3, O10; O7; O8; O9; O2 and O11 factor serum to do the agglutination test. Judge the O groups according to the test results. The strains that are agglutinated by O3, O10 serum shall use O10, O15, O34, O19 single factor serum to do the agglutination test; judge the subgroups of E1, E4; the final determination of each O antigen composition shall be based on the test results of O single factor serum; If there is no O single factor serum, use two O complex factor serum to check.

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_4z_{23}, z_4z_{24}, z_4z_{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 composition shall be based on the test results of H single factor serum; if there is no H single factor serum, then use two H complex factor serum to verify.

When detecting H antigen in Phase-1 and failing to detect H antigen in Phase-2, or when detecting H antigen in Phase-2 and failing to detect H antigen in Phase-1, 1 generation ~ 2 generations can be inoculated on the agar slope then check. If there is still one-phase H antigen is found out, then use phase variation method to check another phase. Single-phase bacteria don't have to do phase variation test.

The phase variation test method is as follows:

Simple plate method: dry the surface moisture on the 0.35%~0.4% semi-solid agar plate; pick up 1-ring of factor serum to drop onto the semi-solid plate surface; stand for a moment; when serum is absorbed into agar, dibble the to-be-tested strains in the center of serum; after culturing, take bacteria from the edge of growing bacteria moss to test.

Small glass tube method: melt the semi-solid tube (each tube about 1mL~2mL) onto the alcohol lamp; and cool off to 50°C; take 0.05mL ~ 0.1mL of known phase H factor serum, add it into the molten semi-solid substance; after mixing evenly; use capillary pipette to absorb and place into small glass tube for phase variation test; after coagulating, use inoculation needle to pick up to-be-tested bacteria; inoculate onto one end. Place the small glass tube horizontally onto the plate; put wet cotton beside it, so that prevent the moisture is vaporized and dry shrink; check the result every day; after the other phase bacteria dissociation, the bacteria can be picked up from the other end to check. The concentration of serum in the medium shall have appropriate proportion, when it is too high, the bacteria can't grow; when it is too low, the same phase bacteria power can't be suppressed. Generally, it is added with serum amount of 1:200~1:800.

Small inverted tube method: place the small glass tube (the lower-end opening shall remain a gap rather than flush) with two ends open into the semi-solid tube; the upper end of small glass tube shall be higher than the medium surface; backup after sterilization. Heating and melting on the temporarily-used alcohol lamp; cool off to 50°C; pick up 1-ring of factor serum; add into the semi-solid substance of the small casing;

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

30mL of distilled water; add agar into 600mL of distilled water. Then separately mix evenly; boiled to dissolve. Cool off to about 80°C; firstly, mix the ferrous sulfate and disodium hydrogen phosphate evenly; pour into the base fluid; mix evenly. Mix bismuth and ammonium citrate and sodium sulfite evenly; pour into base fluid, and mix evenly again. Adjust the pH to be 7.5±0.2; immediately pour into the agar solution; mix evenly; cool off to 50°C~55°C. Add brilliant green solution; after fully mixing, immediately pour onto the flat plate.

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; when it exceeds 48h, it shall reduce its selectivity; this medium shall be prepared at the current day, and used in the following 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

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

In addition to potassium cyanide, add other compositions into the distilled water; boiled to dissolve; after separately-packing, perform autoclave sterilization at 121°C for 15min. Place it into the refrigerator to make it fully cool off. Add 2.0mL of 0.5% potassium cyanide solution (final concentration is 1:10000) into every 100mL of medium; separately-pack into sterile test tubes; each tube contains 4mL; immediately use the sterile rubber stopper to cover; place into 4°C refrigerator; it can store at least 2 months. Meanwhile, take the medium adding no potassium cyanide as control medium; separately-pack into test tubes for backup.

A.10.3 Test method

Inoculate the agar medium into the peptone water and become the diluted broth; pick up 1-ring to inoculate into the potassium cyanide (KCN) medium. Pick up another 1-ring to inoculate into the control medium. Culture at 36°C±1°C for 1d~2d; observe the results. If the bacteria grow, it is positive (not inhibiting); the bacteria don't grow into negative (inhibiting) for 2d.

NOTE: potassium cyanide is highly toxic; be careful when using; never contaminate, so as to avoid poison. In Summer, the separately-packed medium shall be performed in the refrigerator. The main cause for the test failure is due to sealing not tight; potassium cyanide gradually decomposes; hydrocyanic acid gas escape; so that the drug concentration is reduced; the bacteria grow, and cause the false positive reaction. Each link of the test must be pay special attention to.

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 purple-ethanol solution	1.0mL
L-lysine or DL-lysine	0.5g/100mL or 1.0g/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.

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 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

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