GB

NATIONAL STANDARD OF THE

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National food safety standard - Food microbiological examination -Examination of clostridium perfringens

食品安全国家标准 食品微生物学检验 产气荚膜梭菌检验

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1 Scope

This standard specifies the inspection methods for Clostridium perfringens in food.

This standard applies to the inspection of Clostridium perfringens in food.

2 Equipment and materials

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

a) Constant temperature incubator: 36 °C ± 1 °C;

b) Refrigerator: 2 °C ~ 5 °C;

c) Constant temperature water bath: 50 °C ± 1 °C, 46 °C ± 0.5 °C;

d) Balance: The sensitivity is 0.1 g;

e) Homogenizer;

f) Microscope: 10X ~ 100X;

g) Sterile pipettes: 1 mL (with 0.01 mL scale), 10 mL (with 0.1 mL scale) or micropipettes and tips;

h) Sterile test tube: 18 mm × 180 mm;

i) Sterile Petri dish: 90 mm in diameter;

j) pH meter or pH colorimetric tube or precision pH test paper;

k) Anaerobic culture device.

3 Media and reagents

3.1 Tryptone-sulfite-cycloserine (TSC) agar: See A.1 in Appendix A.

5 Operation steps

5.1 Preparation of sample

5.1.1 The samples shall be tested as soon as possible, after collection. If they cannot be tested in time, they can be stored at 2 °C ~ 5 °C. If the test cannot be performed within 8 hours, weigh aseptically 25 g (mL) of sample. Add it into an equal amount of buffered glycerol-sodium chloride solution (add double volume for liquid sample). Store it as soon as possible, in a -60 °C low temperature refrigerator or with dry ice.

5.1.2 Aseptically weigh 25 g (mL) of the sample, into a homogenizer bag, which contains 225 mL of 0.1% peptone water (if it is the frozen-preserved sample in 5.1.1, after thawing at room temperature, add 200 mL of 0.1% peptone water). Continuously homogenize on a slap-type homogenizer, for 1 min ~ 2 min. OR otherwise place it in a homogenizer cup, which contains 225 mL of 0.1% peptone water, to homogenize it at 8000 r/min ~ 10000 r/min, for 1 min ~ 2 min, as a 1:10 dilution.

5.1.3 Use the above 1:10 dilution solution, to prepare a serial dilutions of 10^{-2} ~ 10^{-6}, based on the proportion of 1 mL of dilution solution: 9 mL of 0.1% peptone water.

5.2 Cultivation

5.2.1 Pipette 1 mL of each dilution into a sterile petri dish. Make two parallels for each dilution. Pour 15 mL of TSC agar, which is cooled to 50 °C (can be kept such temperature, in a constant temperature water bath at 50 °C ± 1 °C) to each plate. Slowly rotate the plate, to mix the dilution and agar well.

5.2.2 After the above agar plates are solidified, add 10 mL of TSC agar, which is cooled to 50 °C (which can be kept in a constant temperature water bath at 50 °C ± 1 °C), to cover the surface of the plate evenly.

5.2.3 After the agar is solidified, it is placed in an anaerobic culture device, for incubation at 36 °C ± 1 °C, for 20 h ~ 24 h.

5.2.4 Typical Clostridium perfringens are black colonies on TSC agar plates.

5.3 Confirmatory test

5.3.1 Randomly select 5 (if less than 5, select 5) black colonies from a single plate. Inoculate them into FTG medium, respectively, to incubate them at 36 °C ± 1 °C for 18 h ~ 24 h.

5.3.2 Smear with the above-mentioned culture medium. Observe the purity by Gram staining. Clostridium perfringens is a gram-positive stubby bacillus, which has sometimes visible spores. If the culture medium is not pure, it shall be streaked and inoculated on TSC agar plates for purification. Perform anaerobic culture at 36 °C ±

1 °C, for 20 h ~ 24 h. Pick a single typical black colony. Inoculate it into FTG medium, to culture it at 36 °C ± 1 °C, for 18 h ~ 24 h. It is used for subsequent confirmatory tests.

5.3.3 Take 1 mL of vigorously growing FTG culture medium. Inoculate it into the iron-containing milk medium. After culturing it in a water bath at 46 °C ± 0.5 °C for 2 h, observe whether there is a phenomenon of "violent fermentation" every hour. The characteristics of this phenomenon are: The milk coagulum quickly forms a sponge-like mass, after breaking up AND usually rises to the surface of the medium. Those, that do not ferment within 5 h, are negative. Clostridium perfringens ferments lactose, coagulates casein, produces a large amount of gas, showing the phenomenon of "violent fermentation", BUT the medium does not turn black.

5.3.4 Use an inoculating loop (needle), to take the FTG culture medium, for puncture and inoculate the buffered kinetic-nitrate medium. Incubate it at 36 °C ± 1 °C, for 24 h. The growth of bacteria along the puncture line is examined under transmitted light, to determine the presence or absence of motility. Motile strains grow diffusely along the puncture line, whilst non-motile strains grow only along the puncture line. Then add dropwise 0.5 mL of reagent A and 0.2 mL of reagent B, to check for the presence of nitrite. If red appears within 15 minutes, it indicates that nitrate has been reduced to nitrite; if there is no color change, add a little zinc powder and leave it for 10 minutes. If red appears, it indicates that the strain cannot reduce nitrate. Clostridium perfringens has no power AND can reduce nitrate to nitrite.

5.3.5 Use an inoculating loop (needle), to puncture the FTG culture medium and inoculate the lactose-gelatin medium. Incubate it at 36 °C ± 1 °C, for 24 h. Observe the results. If gas production is found and the medium turns from red to yellow, it indicates that lactose is fermented and acid is produced. The test tube is placed at about 5 °C for 1 h, to check the liquefaction of gelatin. If the medium is solid, incubate it for another 24 h at 36 °C ± 1 °C. Repeat the check for gelatin liquefaction. Clostridium perfringens can ferment lactose and liquefy gelatin.

6 Results and reports

6.1 Typical colony count

Select a plate, which has a typical number of colonies, between 20 CFU and 200 CFU, to count the number of typical colonies. if:

a) The number of typical colonies, on only one dilution plate, is between 20 CFU and 200 CFU, THEN, count the typical colonies on the plate of this dilution;

b) The number of typical colonies, on the lowest dilution plate, is less than 20 CFU, THEN, count the typical colonies on the plate of this dilution;

c) The number of typical colonies, on a plate of a certain dilution, is greater than 200

Appendix A

Medium and reagents

A.1 Tryptone-sulfite-cycloserine (TSC) agar

A.1.1 Basic ingredients

Tryptone: 15.0 g

Soy peptone: 5.0 g

Yeast powder: 5.0 g

Sodium metabisulfite: 1.0 g

Ferric ammonium citrate: 1.0 g

Agar: 15.0 g

Distilled water: 900.0 mL

pH: 7.6 ± 0.2

A.1.2 D-cycloserine solution

Dissolve 1 g of D-cycloserine, in 200 mL of distilled water. Sterilize it by membrane filtration. Store it at 4 °C for later use.

A.1.3 Preparation method

Heat and boil the basic ingredients, until they are completely dissolved. Adjust the pH. Divide it into 500 mL flasks, 250 mL per flask. Sterilize it by autoclaving, at 121 °C for 15 min. Keep it at 50 °C ± 1 °C, for later use. Before use, add 20 mL of D-cycloserine solution to every 250 mL of base solution. Mix well. Pour it on a plate.

A.2 Liquid thioglycolate medium (FTG)

A.2.1 Composition

Tryptone: 15.0 g

L-cystine: 0.5g

Yeast powder: 5.0 g

Glucose: 5.0 g

Sodium chloride: 2.5 g

Sodium thioglycolate: 0.5 g

Resazurin: 0.001 g

Agar: 0.75 g

Distilled water: 1000.0 mL

pH: 7.1 ± 0.2

A.2.2 Preparation method

Heat and boil the above ingredients, until they are completely dissolved. Adjust the pH after cooling. Divide it into test tubes, 10 mL per tube. Sterilize it, by autoclaving at 121 °C for 15 min. Before use, boil or heat with flowing steam for 15 min. Quickly cool it to the inoculation temperature.

A.3 Buffer kinetic-nitrate medium

A.3.1 Composition

Peptone: 5.0 g

Beef powder: 3.0 g

Potassium nitrate: 5.0 g

Disodium hydrogen phosphate: 2.5 g

Galactose: 5.0 g

Glycerin: 5.0 mL

Agar: 3.0 g

Distilled water: 1000.0 mL

pH 7.3 ± 0.2

A.3.2 Preparation method

Heat and boil the above ingredients, until they are completely dissolved. Adjust the pH. Divide it into test tubes, 10 mL per tube. Sterilize it, by autoclaving at 121 °C for 15 min. If not in use on the same day, refrigerate it at about 4 °C. Before use, boil or heat with flowing steam for 15 min. Quickly cool to the inoculation temperature.

A.4 Lactose-gelatin medium

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