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National food safety standard - Food microbiological examination - Examination of Shigella

食品安全国家标准 食品微生物学检验 志贺氏菌检验

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1 Scope

The document lays down the method of examination for Shigella in food.

It applies to the examination of Shigella in food.

2 Equipment and materials

Besides the sterilising and culturing equipment usual in a microbiological laboratory, the following are listed: a) constant-temperature incubator, 36 °C +/- 1 °C; b) refrigerator, 2 °C to 5 °C; c) membrane filtration system; d) anaerobic culture apparatus, 41.5 °C +/- 1 °C; e) electronic balance, readability 0.1 g; f) microscope, 10 times to 100 times.

g) homogeniser; h) shaker; i) sterile pipettes, 1 mL graduated in 0.01 mL and 10 mL graduated in 0.1 mL, or micropipettes and tips; j) sterile homogenising cup or sterile homogenising bag, capacity 500 mL; k) sterile Petri dishes, diameter 90 mm; l) pH meter or pH colorimetric tube or precision pH paper; m) automated microbiological biochemical identification system.

3 Culture media and reagents

3.1 Shigella enrichment broth with novobiocin: see A.1 of Annex A. 3.2 MacConkey (MAC) agar: see A.2. 3.3 xylose lysine deoxycholate (XLD) agar: see A.3. 3.4 Shigella chromogenic medium. 3.5 triple sugar iron (TSI) agar: see A.4. 3.6 nutrient agar slant: see A.5. 3.7 semi-solid agar: see A.6. 3.8 glucose ammonium salt medium: see A.7. 3.9 urea agar: see A.8.

3.10 beta-galactosidase medium: see A.9. 3.11 amino acid decarboxylase test medium: see A.10. 3.12 sugar fermentation tubes: see A.11. 3.13 Simmons citrate medium: see A.12. 3.14 mucate medium: see A.13. 3.15 peptone water and indole reagent: see A.14. 3.16 Shigella diagnostic sera. 3.17 biochemical identification kit.

4 Examination procedure

The examination procedure for Shigella is given as Figure 1. The flow chart runs from the test sample, 25 g (or 25 mL) of sample plus 225 mL of Shigella enrichment broth, through anaerobic culture at 41.5 °C +/- 1 °C for 16 h to 20 h, to plating on XLD and on MAC or Shigella chromogenic medium at 36 °C +/- 1 °C for 20 h to 48 h; suspect colonies are picked, carried to TSI, semi-solid and nutrient agar slant, and then split between serological typing and biochemical identification, which converge on the grouping and typing result for the genus Shigella and finally the report.

5 Operating steps

5.1 Enrichment. Under aseptic conditions 25 g (mL) of the test sample is taken and added to a homogenising cup holding 225 mL of sterilised Shigella enrichment broth and homogenised with a rotary-blade homogeniser at 8 000 r/min to 10 000 r/min; or it is added to a homogenising bag holding 225 mL of the same broth and homogenised for 1 min to 2 min with a paddle homogeniser. A liquid sample need only be shaken to mix. It is then cultured anaerobically at 41.5 °C +/- 1 °C for 16 h to 20 h.

5.2 Isolation. The enriched Shigella broth is streaked separately onto an XLD agar plate and onto a MAC agar plate or a Shigella chromogenic medium plate and cultured at 36 °C +/- 1 °C for 20 h to 24 h; the colony morphology on each plate is observed. Single colonies of Shigella sonnei are larger in diameter than those of the other shigellae. Where the colonies are atypical or too small to observe easily, culture is continued to 48 h before observing again. Table 1 gives the colony characteristics on each selective agar plate: on MAC agar, colourless to pale pink, semi-transparent, smooth, moist, round, with an even or uneven edge; on XLD agar, pink to colourless, semi-transparent, smooth, moist, round, with an even or uneven edge; on Shigella chromogenic medium, judgement follows the instructions supplied with the medium.

5.3.1 Preliminary biochemical test. Two or more typical or suspect colonies are picked from the selective agar plates and inoculated one each into TSI, semi-solid agar and nutrient agar slant, held at 36 °C +/- 1 °C for 20 h to 24 h, and the results read separately.

5.3.2 Any strain that on triple sugar iron agar shows an alkaline slant and an acid butt (glucose fermented, lactose and sucrose not fermented), no gas (Shigella flexneri type 6 may produce a little gas) and no hydrogen sulfide, and that is non-motile in the semi-solid tube, has the growth on its nutrient agar slant from 5.3.1 taken for biochemical tests and serological typing.

5.4.1 Biochemical test. The growth on the nutrient agar slant cultured under 5.3.1 is used for the biochemical tests: beta-galactosidase, urea, lysine decarboxylase, ornithine decarboxylase, and the decomposition of salicin and aesculin. Apart from ornithine being

positive for Shigella sonnei and for Shigella boydii type 13, and beta-galactosidase being positive for Shigella sonnei, Shigella dysenteriae type 1 and Shigella boydii type 13, cultures of the genus Shigella give negative results in these biochemical tests. Because the biochemical characteristics of Shigella flexneri type 6 resemble those of Shigella dysenteriae or Shigella boydii, indole, mannitol, raffinose and glycerol tests may also be added where needed, and Gram staining and the oxidase test may be done; the organism is to be a Gram-negative rod, oxidase negative. A strain whose biochemical reactions do not fit may not be judged to belong to the genus Shigella even if it agglutinates with some Shigella typing serum. Table 2 sets out the biochemical characteristics of the four groups of the genus Shigella (group A Shigella dysenteriae, group B Shigella flexneri, group C Shigella boydii, group D Shigella sonnei) against ten reactions; its cells carry positive, negative, mostly negative, mostly positive, delayed positive and variable-biotype marks with footnotes, and the pairing of the marks with the rows and columns is not reported here.

5.4.2 Additional biochemical test. Because some anaerogenic Escherichia coli and some A-D (Alkalescens-Dispar) organisms share part of their biochemical characteristics with Shigella and can agglutinate with some Shigella typing serum, a culture that has matched the biochemical characteristics of the genus in the previous tests is put through the glucose ammonium salt, Simmons citrate and mucate tests as well, cultured at 36 °C for 24 h to 48 h. Table 3 sets out the biochemical distinction between the genus Shigella, anaerogenic Escherichia coli and A-D organisms over those three reactions; its notes state that Shigella is generally negative in all three, while anaerogenic Escherichia coli and A-D (alkaline-atypical) organisms are positive in at least one.

5.4.3 Where a biochemical identification kit or an automated microbiological biochemical identification system is chosen, the identification may be run on the growth of the nutrient agar slant cultured under 5.3.1, on the basis of the preliminary judgement of 5.3.2.