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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 13091-2018

Replacing GB/T 13091-2002

Determination of Salmonella in feeds

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Table of Contents

Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009. This standard replaces GB/T 13091-2002 "Determination of Salmonella in feeds". Compared with GB/T 13091-2002, except for editorial modifications, the main changes of this standard are as follows: - DELETE the terms and definitions (see Chapter 3 of the 2002 edition); - DELETE the principle (see Chapter 4 of the 2002 edition); - ADD the test procedure diagram (see Figure A.1 in Appendix A); - MODIFY the enrichment equipment; ADD the homogenization bag and homogenizer options (see 6.1;

9.1.2 of the 2002 edition); - CHANGE the separation medium from "phenol red brilliant green agar and DHL agar" to "BS agar, DHL agar or Salmonella chromogenic medium" (see 6.3;

9.4 of the 2002 edition); - MODIFY the identification process and identification table (see 6.4;

9.5 of the 2002 edition); - ADD the step of "preliminary judgment using trisaccharide iron test and lysine decarboxylase test" (see 6.4.1); - ADD the option of "Biochemical identification kit or automatic microbial biochemical identification system" for Salmonella identification (see 6.4.3). This standard was proposed by AND shall be under the jurisdiction of the National Feed Industry Standardization Technical Committee (SAC/TC 76). Drafting organizations of this standard. Institute of Agricultural Quality Standards and Testing Technology, Chinese Academy of Agricultural Sciences [National Feed Quality Supervision and Inspection Center (Beijing)]. The main drafters of this standard. Rao

Zhenghua, Liu Xiaolu, Fan Xia. This standard replaces the standard previously issued as follows: - GB/T 13091-1991, GB/T 13091-2002. Determination of Salmonella in feeds

1 Scope

GB/T 13091-2018 is the Chinese national standard on determination of salmonella in feeds, in the field of agriculture. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 17 September 2018 by the State Administration for Market Regulation; Standardization Administration of China, and has been in force since 1 April 2019. Classification: ICS 65.120, CCS B46. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This standard specifies the inspection methods for Salmonella in feed. This standard applies to the inspection of Salmonella in feed and feed additives.

2 Normative references

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) is applicable to this standard.

GB/T 6682 Water for analytical laboratory use - Specification and test methods

3 Medium or material

Unless otherwise stated, only reagents confirmed to be of analytical grade are used in the analysis.

3.1 Water. It shall meet the requirements of grade-3 water in GB/T 6682.

3.2 Buffered peptone water (BPW). See B.1 in Appendix B.

3.3 Magnesium chloride malachite green (RV) enrichment solution. See B.2 in Appendix B.

3.4 Cystine selenite (SC) enrichment solution. See B.3 in Appendix B.

3.5 Bismuth sulfite (BS) agar. See B.4 in Appendix B.

3.6 Deoxycholate-Hydrogen-Sulfide-Lactose (DHL) agar. See B.5 in Appendix B.

3.7 Salmonella chromogenic medium.

4 Instruments and equipment

4.1 Refrigerator. 2 °C ~ 5 °C.

4.2 Constant temperature incubator. 36 °C ± 1 °C, 42 °C ± 1 °C.

4.3 Homogenizer.

4.8 Sterile petri dishes. Diameter 60 mm, 90 mm.

4.9 Sterile test tubes. 3 mm x 50 mm, 10 mm x 75 mm.

4.10 pH meter or pH colorimetric tube or precision pH test paper.

4.11 Automatic microbial biochemical identification system.

5 Samples

5.1 Sampling principles The collection of samples shall follow the principles of randomness and representativeness. The sampling process shall follow aseptic procedures, to prevent all possible external contamination.

5.2 Sampling method

5.2.4 For solid products with individual packages greater than 500 g, use a sterile sampler to take appropriate samples from different parts of the same package; put them into the same sterile sampling container, as one sample.

5.3 Storage and transport of collected samples

5.3.1 The samples shall be sent to the laboratory for testing, as soon as possible.

6 Test method (see Appendix A for the test procedure diagram)

6.1 Pre-enrichment Weigh 25g (mL) of sample under sterile conditions. Add it into a 500 mL sterile conical flask, which was filled with 225 mL of sterile BPW. Place it in an oscillator. Oscillate at 8000 r/min ~ 10000 r/min for 2 min ~ 3 min. If the sample is in liquid form, shake and mix well. Incubate it at 36 °C ± 1 °C for 18 h ± 2 h.

6.2 Selective enrichment After the pre-enrichment culture is shaken, take 1 mL to inoculate it into a test tube, which contains 10 mL of RV. Incubate it at 42 °C ± 1 °C for 18 h ~ 24 h. Take another 1 mL of pre-enrichment culture. Inoculate it into a test tube, which contains 10 mL of SC. Incubate it at 36 °C ± 1 °C for 18 h ~ 24 h.

6.3 Isolation and culture Use an inoculation loop, to take 1 loop of selective enrichment solution. Streak and inoculate it on a BS agar plate. Incubate it at 36 °C ± 1 °C for 40 h ~ 48 h. Take another loop of selective enrichment solution. Streak and inoculate it on a DHL agar

plate or a Salmonella chromogenic medium plate.

6.4 Biochemical test

6.4.1 Pick 2 or more typical or suspicious colonies from the selective agar plate. Inoculate on trisaccharide iron agar. First streak the slope. Then puncture the bottom layer. Do not sterilize the inoculation needle. Directly inoculate lysine decarboxylase test medium and nutrient agar plate. Culture at 36 °C ± 1 °C for 18 h ~ 24 h. If necessary, it can be extended to 48 h. The changes in the characteristics of the trisaccharide iron agar medium are as shown in Table

2.Table 3 shows the reaction results of Salmonella in the trisaccharide iron agar and lysine decarboxylase test medium.

6.5 Serological identification

6.5.1 Checking the culture for autoagglutination Use 1.2% ~ 1.5% agar culture as the antigen, for slide agglutination test. Firstly, to rule out self-agglutination reaction, add a drop of normal saline to a clean glass slide; mix the culture to be tested in the normal saline, to make a homogeneous turbid suspension; shake the slide gently for 30 s ~ 60 s; observe the reaction in dark (observe with a magnifying glass if necessary). If there is visible bacterial agglutination, it is considered to have self-agglutination; otherwise, there is no self-agglutination. For the non-self- agglutinating culture, refer to the following method for serological identification.

6.5.2 Identification of the O antigen Draw 2 areas of about 1 cm x 2 cm on the slide. Pick 1 loop of bacteria to be tested. Put 1/2 loop on the upper part of each area on the slide. Add 1 drop of multivalent bacteria O serum, to the lower part of one of the areas.

6.5.3 Identification of H antigen The operation is the same as 6.5.2.When the H antigen is underdeveloped, inoculate the strain in the center of a 0.55% ~ 0.65% semi-solid agar plate. When the colony spreads and grows, take bacteria from the edge for inspection. OR inoculate the strain 1 ~ 2 times in small glass tubes, which contain 0.3% ~ 0.4% semi-solid agar plate. Take the bacteria from the far end for culture and re-inspection.

6.5.4 Identification of Vi antigen The operation is the same as 6.5.2.Check with Vi factor serum.