SN

中华人民共和国出入境检验检疫行业标准

SN/T 0669—2011
代替 SN 0669—1997

出口肉及肉制品中庆大霉素残留检测方法 杯碟法

Determination of gentamycin residues in meats and meat products for export—Cylinder plate method

2011-02-25 发布　　2011-07-01 实施

中华人民共和国
国家质量监督检验检疫总局　发布

前　言

本标准按照 GB/T 1.1—2009 给出的规则起草。

本标准代替 SN 0669—1997《出口肉及肉制品中庆大霉素残留量检验方法　杯碟法》。

本标准与 SN 0669—1997 相比，主要技术变化如下：

——将原标准由定量检测方法变更为定性检测方法；

——将原标准名称《出口肉及肉制品中庆大霉素残留量检验方法　杯碟法》修订为《出口肉及肉制品中庆大霉素残留检测方法　杯碟法》；

——重新核实了原标准的检测低限，由 0.1 mg/kg 修订为 0.05 mg/kg。

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国山东出入境检验检疫局、中国海洋大学。

本标准主要起草人：刘云国、林洪、刘帅帅、贾俊涛、雷质文、林黎明、李正义、马维兴、唐静、张健、房保海、祝素珍、赵丽青、姜英辉、马云、韩青、李明哲、颜显辉。

本标准所代替标准的历次版本发布情况为：

——SN 0669—1997。

出口肉及肉制品中庆大霉素残留检测方法 杯碟法

1 范围

本标准规定了出口肉及肉制品中庆大霉素残留检测的杯碟方法。

本标准适用于出口鸡肉中庆大霉素残留的筛选检测，阳性结果应用其他方法进行确认。

2 规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件，仅注日期的版本适用于本文件。凡是不注日期的引用文件，其最新版本(包括所有的修改单)适用于本文件。

GB/T 6682 分析实验室用水规格和试验方法

3 试样制备与保存

3.1 试样制备

将代表性样品中的可食部分放入绞碎机中绞碎。充分混匀，用四分法缩分至不少于 500 g，作为试样。装入清洁容器内，加封后，标明标记。在制样的操作过程中，应防止样品受到污染或发生残留物含量的变化。

3.2 试样保存

将试样于－18 ℃以下冷冻保存。

4 原理

用磷酸盐缓冲液(pH8.0±0.1)提取试样中的庆大霉素残留物，离心后取上清液作为样液，以表皮葡萄球菌(*Staphylococcus epidermidis*)作为指示菌，用杯碟法进行检测。

5 仪器和设备

5.1 培养皿：内径 90 mm、底部平整光滑的玻璃皿或塑料皿，配陶瓦盖，或玻璃盖内嵌入干燥的滤纸。

5.2 牛津杯：不锈钢圆筒，外径 8.0 mm±0.1 mm，内径 6.0 mm±0.1 mm，高 10.0 mm±0.1 mm。

5.3 克氏瓶：800 mL。

5.4 游标卡尺：测量范围 0 mm～200 mm，精度 0.02 mm。

5.5 均质器：转速不低于 8 000 r/min。

5.6 离心机：转速不低于 4 000 r/min。

5.7 恒温水浴锅：0 ℃～100 ℃，精度±1 ℃。

5.8 恒温培养箱：36 ℃±1 ℃，搁板应保持水平。

5.9 高压灭菌锅。
5.10 半对数坐标纸。
5.11 旋转蒸发器。
5.12 可调移动器:10 μL～100 μL,100 μL～1 000 μL。

6 试剂和材料

除另有规定外,所用试剂均为分析纯,试验用水为蒸馏水、去离子水,试验用水应符合 GB/T 6682 的规定。

6.1 试验菌种

表皮葡萄球菌(*Staphylococcus epidermidis*),菌号 CMCC 12228。

6.2 培养基

6.2.1 增菌用液体培养及见 A.1。
6.2.2 保存及传代用液体培养及见 A.2。
6.2.3 检定用液体培养及见 A.3。

6.3 硫酸庆大霉素标准品

已知纯度标准品,密封避光,防潮,4 ℃保存。

6.4 磷酸盐缓冲液(pH8.0±0.1)

称取 13.3 g 磷酸二氢钾及 6.2 g 氢氧化钾,溶解于 1 000 mL 水中,于 121 ℃灭菌 15 min。

6.5 庆大霉素标准储备液

准确称取适量的硫酸庆大霉素标准品,用磷酸盐缓冲液配制成浓度为 1 000 μg/mL 的标准储备液。保存于 4 ℃冰箱中,可使用 1 个月。

6.6 庆大霉素标准工作液

取一定量庆大霉素标准储备液,用磷酸盐缓冲液稀释成浓度为 0.05 μg/mL 的庆大霉素标准工作液,应当日配制。

6.7 生理盐水

称取 8.5 g 氯化钠,溶解于 1 000 mL 水中,分装后,于 121 ℃灭菌 15 min。

7 检测方法

7.1 样液制备

称取 10 g 试样,置于均质杯中,加入 10 mL 磷酸盐缓冲液,8 000 r/min 均质 3 min 后,室温放置 60 min,移入离心管中 4 000 r/min 离心 15 min,取上清液于作为待测样液。

7.2 菌悬液制备

将试验菌种接种于装有培养基Ⅰ的试管中,于 36 ℃±1 ℃培养 24 h±1 h。移 1 mL 菌液于盛有适

量培养基Ⅱ的克氏瓶中，使菌液均匀覆盖在琼脂表面，于 36 ℃±1 ℃培养 24 h±1 h。然后用适量生理盐水将菌苔洗下，备用。置 4 ℃冰箱中(不宜超过 1 周)。

7.3 检定平板的制备

在测定前，需进行预测试，以求得菌悬液最佳用量。用 0.05 μg/mL 庆大霉素标准工作液对加有不同量的菌悬液的平板进行测试，经培养后能使得 0.05 μg/mL 标准工作液在平板上产生直径在 10 mm 以上的清晰、完整的抑菌圈的菌悬液用量为最佳用量。

将培养基Ⅲ融化，冷却至 50 ℃左右，加入最佳用量的菌悬液，使其充分混匀后，取 10 mL，注入灭菌培养皿中，保持水平，凝固后即为检定平板。制备好的平板置 2 ℃～8 ℃冰箱可保存 2 d～3 d。

7.4 样液的测定

取制备好的检定用平板两个，在平板底部作好标记，把牛津杯适当间隔置于平板上，每个平板最多不超过 6 个，在其中一个牛津杯中加入 280 μL 0.05 μg/mL 庆大霉素标准工作液作为阳性对照，其余牛津杯中加入 280 μL 样液，冷藏放置 30 min 后，36 ℃±1 ℃培养 8 h 后开始观察，如果 0.05 μg/mL 庆大霉素标准工作液产生清晰、完整的抑菌圈时终止培养，如果无明显抑菌圈，继续观察至 24 h。用游标卡尺测量标准工作液和样液的抑菌圈直径大小。每份样品做两个平板上的平行试验。

8 结果判断和报告

如样液在平板上无抑菌圈，0.05 μg/mL 庆大霉素标准工作液的抑菌圈直径大于 10 mm，即报告“阴性”。

如样液在平板上呈现抑菌圈，抑菌圈直径在 10 mm 以上，即报告“初筛阳性”。

如样液在平板上呈现抑菌圈，抑菌圈直径小于 10 mm，大于 8 cm，或者两个平行结果不一致时，视为可疑，应重新测试后判定。

阳性样品需要用确证方法进行定性及定量分析。

9 检测限

本方法的检测限为 0.05 mg/kg。

附 录 A
（规范性附录）
培 养 基

A.1 培养基Ⅰ

胰蛋白胨	10.0 g
牛肉浸膏	5.0 g
氯化钠	2.5 g
水	1 000 mL

将上述成分于水中加热溶解，调节 pH 为 6.5～6.6，分装于试管或克氏瓶中，于 121 ℃灭菌 15 min。

A.2 培养基Ⅱ

胰蛋白胨	10.0 g
牛肉浸膏	5.0 g
氯化钠	2.5 g
琼脂	14.0 g～16.0 g
水	1 000 mL

将上述成分于水中加热溶解，调节 pH 为 6.5～6.6，分装于试管或克氏瓶中，于 121 ℃灭菌 15 min，灭菌后可制成斜面。

A.3 培养基Ⅲ

蛋白胨	6.0 g
酵母浸膏	4.0 g
牛肉浸膏	1.5 g
葡萄糖	1.0 g
琼脂	15 g
水	1 000 mL

将各成分加热并溶解于水中，调节 pH 为 6.5，于 121 ℃灭菌 15 min，临用前用 1 mol/L 氢氧化钠溶液，调节 pH 至 8.0。

Foreword

This standard was drafted in accordance with the GB/T 1.1—2009.

This standard was an amendment to the SN 0669—1997《Determination of gentamycin residues in meat and meat produces for export-Cylinder plate method》.

General technique rules and testing methods in SN 0669—1997 were not amended expect text format and some language expression. The main changes between this standard and SN 0669—1997 are as follows:

—This standard was revised to a qualitative testing method from a quantitative one.

—The name of original standard "Method for the determination of gentamycin residues in meat and meat produces for export-Cylinder plate method" was revised to "Determination of gentamycin residues in meat and meat produces for import and export-Cylinder plate method".

—Testing threshold of the primary standard was re-verified,0.1 mg/kg was revised to 0.05 mg/kg.

Since the implementation of the standard date,SN 0669—1997 was abolished at the same time.

This standard was proposed by and is under the charge of China National Regulatory Commission for Certification and Accreditation.

This standard was drafted by the Shandong Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China and Ocean University of China.

This standard was mainly drafted by Liu Yunguo,Lin Hong,Liu Shaishuai,Jia Juntao,Lei Zhiwen,Lin Liming,Li Zhengyi,Ma Weixing,Tang Jing,Zhang Jian,Fang Baohai,Zhu Suzhen,Zhao Liqing,Jiang Yinghui,Ma Yun,Han Qing,Li Mingzhe,Yan Xianhui.

This standard replaced the previous version of the release of the standard as follows:

—SN 0669—1997.

Determination of gentamycin residues in meats and meat products for export—Cylinder plate method

1 Scope

This standard specifies the gentamycin residues in meat and meat products for export by cylinder plate method.

This standard is applicable to the determination of gentamycin residues in chicken for export. Any positive result should be confirmed by other method.

2 Normative Reference

The following normative documents contain provision which, through reference in this text, constitute provisions of this standard for dated reference, subsequent amendments to (excluding editing corrections), or revisions of, any of these publications do not apply. However, parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies.

GB/T 6682 Specifications and test method of water—Used in assay laboratory

3 Preparation and Storage of test sample

3.1 Preparation of test sample

Part of representative sample is taken from the primary sample, and the edible portions are homogenized by grinding in a grinder. The homogenized sample is thoroughly mixed and reduced to at least 500 g by quartering as the test sample. The test sample is placed in a clean container which shall be sealed and labeled. In the course of sampling and sample preparation, precaution shall be taken to avoid contamination or any factor which may cause the change of residue content.

3.2 Storage of test sample

The test sample should be stored below −18 ℃.

4 Principle of method

The gentamycin residues in the test sample are extracted with phosphate buffer (pH8.0±0.1). The supernatant is used for test sample liquid after centrifugation. Cylinder plate method is used to determine the gentamycin in the supernatant. *Staphylococcus epidermidis* used as indicator bacteria.

5 Apparatus and equipment

5.1 Culture dishes, 90 mm(i.d.) glass or plastic dish with smooth and flat bottom, and with clay cover, or glass cover was embedded with dry filter paper.

5.2 Cylinder, stainless steel, 8 mm±0.1 mm(od), 6 mm±0.1 mm(id), 10 mm±0.1 mm height.

5.3 Roux bottle, 800 mL.

5.4 Vernier caliper, measuring range: 0 mm～200 mm, precision: 0.02 mm.

5.5 Homogenizer, Speed ≥8 000 r/min.

5.6 Centrifuge, Speed ≥4 000 r/min.

5.7 Constant temperature water bath pot, 0 ℃～100 ℃, precision, ±1 ℃.

5.8 Incubator, 36 ℃±1 ℃, its shelf kept horizontal.

5.9 Autoclave.

5.10 Semi-logarithm coordinate paper.

5.11 Rotary evaporator.

5.12 Finnpepette, 10 μL～100 μL, 100 μL～1 000 μL.

6 Reagents and Materials

All chemical reagents used in this method are of analytically pure. Test water should be distilled water or deionized water in conformity with GB/T 6682.

6.1 Bacterium

Staphylococcus epidermidis CMCC 12228.

6.2 Media

6.2.1 Medium for preservation and transportation of the bacterium, see A.1.

6.2.2 Liquid medium for reproduction of the bacterium, see A.2.

6.2.3 Medium for bioassay, see A.3.

6.3 Gentamicin sulfate standard

Known purity, Sealed and dark, damped, preserved at 4 ℃.

6.4 Phosphate buffer (pH8.0±0.1)

Dissolve 13.3 g of KH_2PO_4 and 6.2 g of NaOH in 1 000 mL of water, autoclave at 121 ℃ for 15 min.

6.5 Gentamicin standard stock solution

Accurately weigh a proper amount of gentamicin sulfate standard, dissolve with phosphate buffer to prepare a gentamicin standard stock solution of 1 000 μg/mL in concentration, store at 4 ℃, which can be used within a month.

6.6 Gentamicin standard working solution

Dilute the gentamicin standard stock solution with phosphate buffer solution to obtain the middle standard solution, and then further dilute the middle standard solution with phosphate buffer to obtain a series of gentamicin standard working solution of 0.05 μg/mL, which should be prepared and used on the same day.

6.7 Physiological saline

Dissolve 8.5 g of sodium chloride in 1 000 mL of distilled water, then dispense into test tubes, autoclave at 121 ℃ for 15 min.

7 Method of detection

7.1 Preparation of sample liquid

Weigh 10 g of sample in a sterilized homogenizing cup, add 10 mL phosphate-buffers, homogenize at 8 000 r/min for 3 min, place at room temperature for 60 min, transfer into a centrifugal tubes and centrifuge at 4 000 r/min for 15 min, transfer the supernatant to a large test tube as sample liquid.

7.2 Preparation of strain suspension

Inoculate the test strain into medium Ⅰ, incubate at 36 ℃ ± 1 ℃ for 24 h ± 1 h. Transfer 1 mL of the culture to the surface of a roux bottle containing optimum amount of medium Ⅱ. Spread the culture evenly over the entire surface. Incubate at 36 ℃ ± 1 ℃ for 24 h ± 1 h, then wash the growth from the agar with a proper amount of physiological saline, store in the refrigerator at 4 ℃ for less than a week.

7.3 Preparation of plate

Before the actual assay, make a pretest to ascertain the optimum amount of the strain suspension. The optimum amount of strain suspension depends on the clear and sharp inhibition zones with the diameters ≥10 mm, caused by 0.05 μg/mL of the standard working solution.

Melt the medium Ⅲ and cool to about 50 ℃, add the optimum amount of strain suspension and mix thoroughly. Pipette 10 mL of the medium into a sterilized dish. Keep horizontal and allow them harden for later use. The plates can be stored in refrigerator at 2 ℃～8 ℃ for 2 d～3 d.

7.4 Determination of sample solution

Take out two prepared plates and mark them on the bottom. Place stainless steel cylinders on the plates with a suitable separation distance. No more than 6 cylinders on one plate. Pipette 280 μL of the 0.05 μg/mL gentamicin standard working solution into one of the cylinders and 280 μL of sample liquid into each of the others. Place the plates in refrigerator for 30 min. Incubate at 36 ℃ ± 1 ℃ for 8 h and start to observe the result. If the 0.05 μg/mL gentamicin standard working solution produces a clear and full inhibiting circle, stop incubation and record the inhibiting circle results produced by sample liquid and the standard working solution. If no clear inhibiting circle exists, continue to incubate and observe until the 24 h have expired. Measure the diameter of inhibiting circles produced by working solution and sample liquid with a vernier caliper. Perform parallel tests for each sample on two plates.

8 Interpreting and reporting the bioassay results

In case of sample liquid does not produce any inhibiting circle while the 0.05 mg/kg gentamicin standard working solution produces a inhibiting circle with a diameter of 10 mm, the bioassay result is negative. In case of the sample liquid produces an inhibiting circle with a diameter of 10 mm or above on the plate, the result is positive. If the inhibiting circle produced by a sample liquid has a diameter less than 10 mm while larger than 8 mm, the bioassay result is suspicious and the bioassay may need to be re-preformed if necessary.

All positive samples should be confirmed by other qualitative and quantitative analysis methods.

9 Limit of determination

The limit of determination of this method is 0.05 mg/kg.

Annex A
（Standard Annex）
Media

A.1 Medium Ⅰ

Peptone	10.0 g
Beef extract	5.0 g
Sodium chloride	2.5 g
Distilled water	1 000 mL

Dissolve the ingredients in distilled water，after heating and stirring，adjust the pH so that the value after autoclaving is 6.5～6.6. Dispense into test tube or Roux bottle，autoclave at 121 ℃ for 15 min.

A.2 Medium Ⅱ

Peptone	10.0 g
Beef extract	5.0 g
Sodium chloride	2.5 g
Agar	14.0 g～16.0 g
Distilled water	1 000 mL

Dissolve the ingredients in distilled water with heating and stirring，adjust the pH so that the value after autoclaving is 6.5～6.6. Dispense into test tube or Roux bottle，autoclave at 121 ℃ for 15 min. Slant could be made after autoclaving.

A.3 Medium Ⅲ

Peptone	6.0 g
Yeast extract	4.0 g
Beef extract	1.5 g
Sodium chloride	2.5 g
Glucose	1.0 g
Agar	15 g
Distilled water	1 000 mL

Dissolve the ingredients in distilled water，after heating and stirring，adjust the pH so that the value after autoclaving is 6.5. Autoclave at 121 ℃ for 15 min. Adjust the pH with sodium hydroxide solution so that the value is pH8.0 before use.