SN

中华人民共和国进出口商品检验行业标准

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

Method for the determination of gentamycin residues in meats and meat products for export —Cylinder plate method

1997-08-15发布　　1998-01-01实施

中华人民共和国国家进出口商品检验局　发布

前　言

本标准是根据 GB/T 1.1—1993《标准化工作导则　第 1 单元：标准的起草与表述规则　第 1 部分：标准编写的基本规定》及 SN/T 0001—1995《出口商品中农药、兽药残留量及生物毒素检验方法标准编写的基本规定》的要求而进行编写的。其中测定方法是参考了国内外有关文献，经研究、改进和验证后而制定的。本标准同时制定了抽样和制样方法。

测定低限是根据国际上对肉品中庆大霉素残留量的最高限量和测定方法的灵敏度而制定的。

本标准的附录 A 是标准的附录。

本标准的附录 B 是提示的附录。

本标准由中华人民共和国国家进出口商品检验局提出并归口。

本标准由中华人民共和国山东进出口商品检验局负责起草。

本标准主要起草人：韩继玲、郑连胜、张铁军、张再生、任怀秀。

本标准系首次发布的行业标准。

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1 范围

本标准规定了出口肉及肉制品中庆大霉素残留量检验的抽样、制样和杯碟测定方法。

本标准适用于出口鸡肉中庆大霉素残留量的检验。

2 抽样和制样

2.1 检验批

以不超过 2 500 件为一检验批。

同一检验批的商品应具有相同的特征，如包装、标记、产地、规格和等级等。

2.2 抽样数量

批量，件	最低抽样数，件
1～25	1
26～100	5
101～250	10
251～500	15
501～1 000	17
1 001～2 500	20

2.3 抽样方法

按照 2.2 规定的抽样数量随机抽取，逐件开启。每件至少取一袋，作为原始样品。如每件中无小包装，或有小包装，但每袋重量超过 2 kg 者，则可用灭菌刀具从抽出的包件中割取不少于 100 g，作为原始样品。原始样品总量不少于 2 kg，混合后，置于清洁容器内，加封后标明标记，及时送交实验室。

2.4 试样制备

从所取回的原始样品中取出部分有代表性样品，将可食部分放入组织捣碎机中捣碎，充分混匀。用四分法缩分至不少于 500 g，装入清洁容器内，作为试样。加封后标明标记。

2.5 试样保存

将试样于－18℃以下冷冻保存。

注：在抽样及制样的操作过程中，必须防止样品受到污染或发生残留物含量的变化。

3 测定方法

3.1 方法提要

中华人民共和国国家进出口商品检验局 1997-08-15 批准　　1998-01-01 实施

试样中庆大霉素残留用磷酸盐缓冲液提取，提取液经离心后，取上清液与表皮葡萄球菌作用，用杯碟法进行测定，根据抑菌圈的大小用标准曲线法进行定量。

3.2 试剂和材料

除另有规定外，所用试剂均为分析纯，水为蒸馏水。

3.2.1 硫酸庆大霉素标准品：已知纯度标准品，1 μg 相当于 0.631 μg 庆大霉素，由中国药品生物制品检定所提供，或等效品。

3.2.2 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，溶于水中，用水定容至 1 000 mL。于 121℃高压灭菌 15 min。

3.2.3 庆大霉素标准储备液：精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。

3.2.4 庆大霉素标准工作液：吸取一定量的庆大霉素标准储备液，用磷酸盐缓冲液稀释成庆大霉素浓度为 20 μg/mL 的中间标准液，再以此中间标准液用磷酸缓冲液稀释成浓度为 0.05，0.10，0.20，0.40，0.80，1.60 μg/mL 的庆大霉素标准工作液。以上溶液均需当日配制。

3.2.5 试验菌种：表皮葡萄球菌(*Staphylococcus epidermidis*)，菌号 CMCC 12228，由中国药品生物制品检定所提供。

3.2.6 培养基Ⅰ(见附录 A 中 A1)。

3.2.7 培养基Ⅱ(见附录 A 中 A2)。

3.2.8 培养基Ⅲ(见附录 A 中 A3)。

3.2.9 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℃高压灭菌 15 min。

3.2.10 展开剂：三氯甲烷-甲醇-浓氨水(1+1+1)。

3.2.11 碘片。

3.3 仪器和设备

3.3.1 培养皿：内径为 90 mm，高度为 20 mm，底部平整、光滑的玻璃皿或塑料皿，具陶瓦盖。

3.3.2 牛津杯：不锈钢圆筒，外径(8.0±0.1) mm，内径(6.0±0.1) mm，高(10±0.1) mm。

3.3.3 克氏瓶：800 mL。

3.3.4 游标卡尺：测量范围 0～200 mm，精度 0.02 mm 或抑菌圈测量仪。

3.3.5 均质器：转速不低于 10 000 r/min。

3.3.6 离心机：转速不低于 4 000 r/min。

3.3.7 恒温水浴锅：0～100℃，精度±1℃。

3.3.8 恒温培养箱：(36±1.0)℃，搁板必须保持水平。

3.3.9 高压灭菌器。

3.3.10 半对数坐标纸。

3.3.11 旋转蒸发器。

3.3.12 色谱缸：20 cm×15 cm×30 cm。

3.3.13 薄层硅胶 G 板：5 cm×10 cm 或 10 cm×20 cm。

3.3.14 微量注射器：10 μL 或 20 μL。

3.4 测定步骤

3.4.1 样液制备

称取 10 g 试样(精确至 0.1 g)于均质杯内，加入 10 mL 磷酸盐缓冲液(3.2.2)，均质 3 min(8 000 r/min)后，室温放置 60 min，移入离心管中离心 30 min(4 000 r/min)，取上清液于大试管中，再加入 10 mL 磷酸盐缓冲液，使与残渣混匀，进行二次离心，合并两次上清液于茄形瓶中，用 1 mol/L 氢氧化钠溶液调 pH 至 8.0±0.1，并用缓冲液定容至 20 mL，作为待测样液。

3.4.2 菌悬液的制备

将试验菌种(3.2.5)接种于培养基Ⅱ(3.2.7)的试管中,经(36±1)℃培养(24±1) h。移1 mL菌液于盛有适量培养基Ⅰ(3.2.6)的克氏瓶中,使菌液均匀覆盖在琼脂表面,于(36±1)℃培养(24±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个月)。

3.4.3 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0.10 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0.10 μg/mL标准工作液在平板上产生直径大于等于10 mm清晰、完整的抑菌圈的菌悬液用量为最佳用量。

将培养基Ⅲ(3.2.8)熔化,冷却至50℃左右,加入最佳用量菌悬液,使其充分混合后,取10 mL,注入灭菌培养皿中,保持水平,待其凝固制成检定平板。所用平板需当天制备。

3.4.4 标准曲线的制备

用庆大霉素标准工作液(3.2.4)绘制标准曲线,以0.2 μg/mL浓度的标准工作液为参考浓度,以0.05 μg/mL浓度的标准工作液作阴性对照。每个标准工作液浓度取3个检定平板作为一组。在每个平板上置6个牛津杯,使牛津杯在半径为2.8 cm的圆面上成60°角间距,其中3个杯中加满参考浓度的标准工作液,另三个杯中加满其他浓度的一种标准工作液。参考浓度溶液与标准工作液要间隔放置。5个浓度的标准工作液共计15个检定平板,用于绘制标准曲线。这样0.2 μg/mL参考浓度标准工作液将得到45个抑菌圈直径的数值,而其他标准工作液分别得到9个抑菌圈直径的数值。

盖好皿盖,置(36±1)℃培养(17±1) h后,翻转平板,除去牛津杯。精确测量各个浓度的抑菌圈直径(精确到0.1 mm),求得各自的平均值。再求出0.2 μg/mL参考浓度的45个抑菌圈直径的平均值。用参考浓度的抑菌圈直径的总平均值,与每组平板参考浓度的抑菌圈直径平均值之差,来校正其他各浓度标准工作液的抑菌圈读数的平均值。

将校正后的值,按式(1)、式(2)求出L点和H点。在半对数坐标纸上,以抑菌圈直径(mm)为纵坐标(算术级),庆大霉素浓度为横坐标(对数级),标出L和H点。通过L点、H点连一直线,即为标准曲线。

$$L = \frac{3a + 2b + c - e}{5} \qquad \cdots\cdots(1)$$

$$H = \frac{3e + 2d + c - a}{5} \qquad \cdots\cdots(2)$$

式中:L——标准曲线上的最低浓度(0.10 μg/mL)抑菌圈直径,mm;

H——标准曲线上的最高浓度(1.60 μg/mL)抑菌圈直径,mm;

c——参考浓度(0.20 μg/mL)的45个抑菌直径的平均值,mm;

a、b、d、e——分别表示标准曲线中其他浓度标准工作液(0.10、0.40、0.80、1.60 μg/mL)的抑菌圈直径经校正后的平均值,mm。

3.4.5 样液的测定

每份样品采用三个检定平板,在每个检定平板上置6个已灭菌的牛津杯。在每个检定平板上3个间隔的牛津杯内加满庆大霉素标准参考浓度溶液,另外3个牛津杯内加满被检样液。将陶瓦盖盖好,置(36±1)℃培养(17±1) h。翻转平板,除去牛津杯。如果有抑菌圈产生,精确测量其直径。

3.5 结果计算和表述

如样液抑菌圈的平均直径小于10 mm,即报告试样中庆大霉素残留为"阴性"。

如样液抑菌圈的平均直径大于等于10 mm,经校正后,从标准曲线上查出相应的庆大霉素浓度。按式(3)计算试样中庆大霉素含量。

$$X = \frac{c}{m} \qquad \cdots\cdots(3)$$

式中:X——试样中庆大霉素残留的含量,mg/kg;

c——由标准曲线上查出的样液中庆大霉素浓度,μg/mL;

m——最终试液所代表的试样的浓度，g/mL。

注：如测定结果呈阳性，即所显抑菌圈的平均直径大于等于 10 mm，必要时，尚需进行确证试验（可用薄层色谱法，参见附录 B），以证明抑菌物质确为庆大霉素。

4 测定低限和回收率

4.1 测定低限

本方法的测定低限为 0.10 mg/kg。

4.2 回收率

鸡肉中庆大霉素的添加浓度及其回收率的试验数据：

0.10 mg/kg，回收率为 97.7%；

0.20 mg/kg，回收率为 92.5%；

0.40 mg/kg，回收率为 98.0%。

附 录 A
（标准的附录）
培 养 基

A1 培养基 I

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

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

A2 培养基 II

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

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

A3 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	15.0 g
蒸馏水	1 000 mL

将各成分加热并溶解于蒸馏水中，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。

附 录 B
（提示的附录）
确 证 试 验

采用薄层色谱法进行确证试验。

B1 将普通硅胶 G 薄层板（5 cm×10 cm）于 105℃活化 2 h，冷却后备用。

B2 取样液（3.4.1）20.0 mL（相当于 10.0 g 样品），用氢氧化钠溶液（1 mol/L）调节 pH 至 8.0，于 65～70℃减压浓缩至干。用 1.0 mL 磷酸盐缓冲液（3.2.2）溶解残渣，溶液作待测样液。

B3 用微量注射器分别吸取浓度为 20 μg/mL 的庆大霉素标准工作液及待测样液各 2.0 μL，分别点在上述薄层板上（间距 2 cm）。将薄层板移放至盛有展开剂（3.2.10）的展开缸中展开，至溶剂展延至薄层

板前沿(约 20 min)后取出。待溶剂挥发后,将薄层板置于另一充满饱和碘蒸气之展开缸内,观察显色情况。根据薄层板上待测液斑点的颜色及位置,测得 R_f 值,对照标准液斑点,以确定样液中的抑菌物质是否为庆大霉素。

Foreword

This standard was drafted in accordance with the requirements of GB/T 1.1—1993,"Directives for the work of standardization—Unit 1: Drafting and presentation of standards—Part 1: General rules for drafting standards"and SN/T 0001—1995"General rules for drafting the standard methods for the determination of pesticide, veterinary drug residues and biotoxins in commodities for export". The method of determination of this standard was drafted by referring to relevant domestic and foreign literatures through research, modification and verification. In addition, the methods of sampling and sample preparation are also specified in this standard.

The limit of determination in this standard is defined on the basis of the current international maximum limit for gentamycin residues in meats and the sensitivity of the method.

Annex A of this standard is a normative annex.

Annex B of this standard is an informative annex.

This standard was proposed by and is under the charge of the State Administration of Import and Export Commodity Inspection of the People's Republic of China.

This standard was drafted by Shangdong Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafter of this standard are Han Jiling, Zheng Liansheng, Zhang Tiejun, Zhang Zaisheng and Ren Huaixiu.

This standard is a professional standard promulgated for the first time.

Note: This English version, a translation from the Chinese text, is solely for guidance.

Professional Standard of the People's Republic of China for Import and Export Commodity Inspection

Method for the determination of gentamycin residues in meats and meat products for export —Cylinder plate method

SN 0669—1997

1 Scope

This standard specifies the methods of sampling, sample preparation and determination by cylinder plate method of gentamycin residues in meats and meat products for export.

This standard is applicable to the determination of gentamycin residues in chicken meat for export.

2 Sampling and sample preparation

2.1 Inspection lot

The quantity of an inspection lot should not be more than 2,500 packages.

The characteristic of the cargo within the same inspection lot, such as packing, mark, origin, specification, grade etc., should be the same.

2.2 Quantity of sample taken

Number of packages in each inspection lot	Minimum number of packages to be taken
1—25	1
26—100	5
101—250	10
251—500	15
501—1 000	17
1 001—2 500	20

2.3 Sampling procedure

A number of packages specified in 2.2 are taken at random and opened one by one. From each package at least one bag shall be taken as the primary sample. If there are no small bags inside the package or if there are small bags inside the package, but the content of a bag exceeds 2 kg, cut out a part from the meat in each package of not less than 100 g as a primary sample with a sterilized knife.

The total weight of all primary samples should not be less than 2 kg, which shall be mixed, placed in a clean container, sealed, labeled and sent to laboratory in time.

2.4 Preparation of test sample

Part of representative sample is taken from the mixed primary sample, the edible portions are homogenized by grinding in a high-speed grinder, The ground sample is thoroughly mixed and reduced to at least

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Implemented from Jan. 1, 1998

500 g by quartering as the test sample, which is placed in a clean container, sealed and labled.

2.5 Storage of test sample

The test sample should be stored below −18℃.

Note: In the course of sampling and sample preparation, precautions must be taken to avoid contamination or any factors which may cause the change of residue content.

3 Method of determination

3.1 Principle

The gentamycin residues in the test sample are extracted with phosphate buffer solution. After centrifugalizing, the supernate is made to react with staphylococcus epidermidis, and is assayed by cylinder plate method. Quantitation is made by measuring the size of inhibition zone, using standard curve mathod.

3.2 Reagents and materials

Unless otherwise specified, all the reagents used should be analytically pure, "water" is distilled water.

3.2.1 Gentamycin sulfate standard: Standard of known purity, provided by the Drug and Biological Product Inspection Institute of the Ministry of Health, China, or equivalent. 1 μg is equal to 0.631 μg of gentamycin.

3.2.2 Phosphate buffer solution: pH 8.0±0.1. Weigh 13.3 g of potassium dihydrogen phosphate and, 6.2 g of potassium hydroxide, dissolve and dilute to 1 000 mL with water. Sterilized at 121℃ for 15 min.

3.2.3 Gentamycin standard stock solution: Accurately weigh an adequate amount of gentamycin sulfate standard, dissolve and dilute with phosphate buffer solution to prepare a standard stock solution of 1 000 μg/mL in concentration and store at 4℃ in a refrigerator. It should be used within one month.

3.2.4 Gentamycin standard working solution: Pipet certain amount of standard stock solution, and dilute with phosphate buffer solution to prepare an intermediate solution to 20 μg/mL in concentration. Then dilute this intermediate solution with phosphate buffer solution to abtain a series of standard working solutions of 0.05, 0.10, 0.20, 0.40, 0.80, 1.60 μg/mL in concentrations. All the above solutions should be prepared fresh daily.

3.2.5 Test strain: Staphylococcus epidermidis: CMCC No. 12228, provided by the Drug and Biological Product Inspection Institute of the Ministry of Health, China.

3.2.6 Medium Ⅰ (see A1 in annex A).

3.2.7 Medium Ⅱ (see A2 in annex A).

3.2.8 Medium Ⅲ (see A3 in annex A).

3.2.9 Saline solution: Dissolve 8.5 g of sodium chloride in 1 000 mL of distilled water, autoclave at 121℃ for 15 min.

3.2.10 Developing agent: Chloroform-methanol-concentrated ammonia water(1+1+1).

3.2.11 Iodine.

3.3 Apparatus and equipment

3.3.1 Culture dish: 90 mm(id), 20 mm(ht) glass or plastic dish with flat and smooth bottom and clay cover.

3.3.2 Oxford cylinder: Stainless steel cylinders, (8.0±0.1) mm(od), (6.0±0.1) mm(id), (10.0±0.1) mm(ht).

3.3.3 Kolle bottle: 800 mL.

3.3.4 Vernier caliper: Measuring range 0—200 mm, precision 0.02 mm, or equivalent.

3.3.5 Homogenizer: ≥10 000 r/min.

3.3.6 Centrifuge: ≥4 000 r/m.

3.3.7 Thermostatic water-bath: 0—100℃(±1℃).

3.3.8 Thermostatic culture box: (36±1.0)℃. The shelf must be kept horizontal.

3.3.9 Autoclave.

3.3.10 Semilog graph paper.

3.3.11 Rotatory evaporater.

3.3.12 Chromatographic tank: 20 cm×15 cm×30 cm.

3.3.13 TLC plate: Silica gel G plate 5 cm×10 cm or 10 cm×20 cm.

3.3.14 Micro-syringe: 10 μL,20 μL.

3.4 Procedure

3.4.1 Preparation of the sample solution

Weigh 10 g of the test sample(accurate to 0.1 g)into a homogenizing cup,add 10 mL of phosphate buffer solution,and homogenize for 3 min at 8 000 r/min. Set aside for 60 min at room temperature. Transfer into a centrifuge tube and centrifuge for 30 min at 4 000 r/min. Take out the supernate,and add another 10 mL of phosphate buffer solution into the tube,mix well with the residue and centrifuge as before. Transfer the supernant and combine with that mentioned above into an oval bottle. Adjust the pH with 1 mol/L sodium hydroxide solution to 8.0±0.1,then make up to 20 mL with the buffer solution. The solution is used as sample solution for determination.

3.4.2 Preparation of organism suspension

Inoculate the test strain(3.2.5)into the medium Ⅱ(3.2.7),Incubate for (24±1) h at (36±1)℃. Then pipet 1 mL of organism suspension uniformly onto the surface of the medium Ⅰ(3.2.6) in a Kolle bottle and incubate for (24±1) h at (36±1)℃. Wash down the growth colony with saline solution and store in a refrigerator at 4℃. It shall be used within one month.

3.4.3 Preparation of testing plates

Before the preparation of the plates,the optimum amount of organism suspension used should be pretested with 0.1 μg/mL gentamycin standard working solution,using plates containing different amount of organism suspension. After incubation,when a clear and sharp inhibition zone caused by 0.1 μg/mL standard working solution appears with diameter≥10 mm on the plate,the amount of suspension used is considered as optimum amount.

Melt the medium Ⅲ(3.2.8)and cool to about 50℃,add the optimum amount of organism suspension and mix thoroughly. Pipet 10 mL of above medium into a sterilized culfure dish,keep horizontal and let congeal,thus the testing plate is prepared. All the plates should be prepared on the same day of testing.

3.4.4 Preparation of the standard curve

Prepare the standard curve with gentamycin standard working solutions (3.2.4),using 0.2 μg/mL solution as the reference concentration,and 0.05 μg/mL as the negative concentration. Take three testing plates as a set for each concentration of standard working solution. Place six Oxford cylinders onto each plate along the circle of 2.8 cm radius at an angle of 60°. Fill three Oxford cylinders with reference concentration solution(0.2 μg/mL)and the other three cylinders with one of the other concentration of the standard working solution. The reference solution and the standard solution should be filled alternately with adjacent cylinders. Five standard concentrations totalling 15 testing plates are used to prepare the standard curve. Among them,45 readings are obtained from 0.2 μg/mL reference concentrations,while 9 readings obtained from each of the other concentrations.

Place the clay covers on the plates and incubate at (36±1)℃ for (17±1) h. After incubation,invert

the plates, remove the cylinders. Accurately (to the nearest 0.1 mm) measure the diameters of inhibition zone of each standard concentration and calculate respectively the mean value. Meanwhile, calcutate the mean value of 45 readings of the diameters of inhibition zone of reference concentration (0.2 μg/mL). The difference between the total average of the reference concentration and average of the reference concentration of each set is the correction value for each set. Correct the average value obtained from other concentration of each set.

After correcting the values, calculate the values of point L and point H by the formula (1) and (2) respectively, and plot the point L and point H on the semilog graph paper, using logarithmic scale for concentration (μg/mL) as abscissa and arithmatic scale for average zone dimeters (mm) as ordinate. Construct a straight line through the point L and H as the standard curve.

$$L = \frac{3a + 2b + c - e}{5} \quad \cdots\cdots (1)$$

$$H = \frac{3e + 2d + c - a}{5} \quad \cdots\cdots (2)$$

where

L—the zone diameter of the lowest concentration (0.1 μg/mL) of the standard curve, mm;

H—the zone diameter of the highest concentration (1.6 μg/mL) of the standard curve, mm;

c—the average of 45 zone diameters for the reference concentration (0.2 μg/mL), mm;

a, b, d, e—the respective corrected average zone diameter for each of the other concentrations (0.10, 0.40, 0.80, 1.60 μg/mL), mm.

3.4.5 Dtermination of sample solution

For each sample solution, use three testing plates. Place 6 cylinders on each plate, and fill three cup with sample solution and alternately to the other three cylinders with standard solution of reference concentration.

Cover the plate and culture at (36±1)℃ for (17±1) h. After incubation, invert the plates to remove the cylinders. If there are zones of inhibition on the positions of sample cylinders, measure the diameter of each zone as accurately as possible.

3.5 Calculation and expression of result

If the average value of the inhibition zone is less than 10 mm, report gentamycin residues in test sample as "negative".

If the average value of the inhibition zone≥10 mm, after correction, obtain the corresponding concentration from the standard curve. Calculate the gentamycin residue content in the test sample by the formula (3):

$$X = \frac{c}{m} \quad \cdots\cdots (3)$$

where

X—Content of gentamycin residues in the test sample, mg/kg;

c—Concentration of gentamycin in the sample solution obtained from the standard curve, μg/mL;

m—Corresponding concentration of the test sample in the final sample solution, g/mL.

Note: If the result of determination is positive (i.e. the average zone diameter≥10 mm), confirmation test (TLC method may be used, cf. annex B) may be carried out, if necessary, to ascertain the inhibiting substance is actually gentamycin.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.1 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of gentamycin in chicken meat and its corresponding recoveries are:

0.10 mg/kg, the recovery 97.7%;

0.20 mg/kg, the recovery 92.5%;

0.40 mg/kg, the recovery 98.0%.

Annex A
(normative)
Media

A1 **Medium I**

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

Dissolve the ingredients in distilled water with heating and stirring, adjust the pH to 6.5—6.6 and dispense into tubes or Kolle bottles. Autoclave at 121℃ for 15 min. Then make a slant for each.

A2 **Medium I**

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

Dissolve the ingredients in distilled water with heating and stirring, adjust the pH to 6.5—6.6 and dispense into tubes or Kolle bottles. Autoclave at 121℃ for 15 min.

A3 **Medium I**

Tryptone	6.0 g
Yeast extract	4.0 g
Beef extract	1.5 g
Glucose	1.0 g
Agar	15.0 g
Distilled water	1 000 mL

Dissolve the ingredients in distilled water with heating and stirring, adjust the pH to 6.5 and autoclave at 121℃ for 15 min. Before using, adjust the pH to 8.0 with sodium hydroxide solution (1 mol/L).

Annex B
(informative)
Confirmation test

Thin-layer chromatograpy is used for the confirmation test.

B1 Activate the silica gel G TLC plate(5 cm×10 cm)at 105℃ for 2 h. Cool down for use.

B2 Pipet 20.0 mL(equivalent to 10.0 g of test sample)of the sample solution(3.4.1), adjust the pH to 8.0 with sodium hydroxide solution(1 mol/L), Concentrate to dryness under reduced pressure of 65—70℃. Add 10 mL of phosphate buffer solution(3.2.2)to dissolve the residue. Use it as the sample solution.

B3 Accurately take 2.0 μL of the gentamycin standard working solution(20 μg/mL)and sample solution

with micro-syringe, spot on the above TLC plate separately (about 2 cm apart from each other). Place the TLC plate in a developing jar containing the developing agent and let develop until the solvent reach the front edge (ca 20 min). Take out and let the solvent volatilize off. Place the TLC plate in another developing jar saturated with iodine vapor and observe the color change. According to the color and its position of sample solution spot on the plate, measure the R_f value. Compare with the standard solution's spot to ascertain whether the antibiotic substance in the sample solution is gentamycin or not.