SN

中华人民共和国进出口商品检验行业标准

SN 0536—1996

出口肉品中卡那霉素残留量检验方法 杯碟法

Method for the determination of kanamycin residues in meat for export —Cylinder plate method

1996-04-29发布　　1996-10-01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编写的。其中测定方法采用了日本厚生省 1990 年编集的《畜、水产食品中的残留物质检验方法》中卡那霉素检验方法。技术内容与原方法相同，经验证后，按规定格式要求作了编辑性修改。在标准中同时制定了抽样和制样方法。

本标准的附录 A 是标准的附录。

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。

本标准起草单位：中华人民共和国浙江进出口商品检验局。

本标准主要起草人：施伟良、张蓉。

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品中卡那霉素残留量检验的抽样、制样和杯碟测定法。

本标准适用于出口禽肉中卡那霉素残留量的检验。

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2.2规定的抽样件数随机抽取，逐件开启。每件至少取一袋作为原始样品，抽取的原始样品总量不少于2 kg，放入清洁容器内，加封后，标明标记及时送交实验室。

如每件中无小包装或有小包装但每袋重量超过2 kg者，则可用灭菌刀具在抽出的包件中，每件割取不少于100 g样品，混合后置清洁容器内，作为混合原始样。混合原始样的总量不少于2 kg。加封后，标明标记，及时送交实验室。

2.4 试样制备

从取回的原始样品中取出部分有代表性样品，将可食部分放入绞碎机中绞碎，充分混匀。用四分法缩分出不少于500 g作为试样。装入清洁容器内，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。

中华人民共和国国家进出口商品检验局1996-04-29批准　　1996-10-01实施

3 测定方法

3.1 方法提要

用磷酸盐缓冲液抽提试样中残留的卡那霉素，经均质、离心，取上清液进行杯碟法测定。利用被测定样液中的残留卡那霉素与枯草杆菌的作用，产生抑菌圈，从抑菌圈大小用标准曲线法进行定量测定。

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 卡那霉素标准品：775 μg/mg，由中国药品生物制品检定所提供。

3.2.2 缓冲液Ⅰ(pH6.0±0.1)：称取 3.5 g 无水磷酸二氢钾及 16.73 g 无水磷酸氢二钾溶于水中，定容 1 000 mL，121℃高压灭菌 15 min。

3.2.3 缓冲液Ⅱ(pH8.0±0.1)：称取 13.3 g 无水磷酸二氢钾及 6.2 g 氢氧化钾溶于水中，定容 1 000 mL，121℃高压灭菌 15 min。

3.2.4 缓冲液Ⅲ(pH7.9±0.1)：称取 0.523 g 无水磷酸二氢钾及 16.73 g 无水磷酸氢二钾溶于水中，定容 1 000 mL，121℃高压灭菌 15 min。

3.2.5 试验菌种：枯草芽孢杆菌(*Bacillus subtilis*)，由中国药品生物制品检定所提供，菌株号 63501。

3.2.6 生理盐水：称取 8.5 g 氯化钠，溶解于 1 000 mL 水中，121℃高压灭菌 15 min。

3.2.7 卡那霉素标准储备液：准确称取适量的卡那霉素标准品，精确到±0.1 mg，用缓冲液Ⅰ(3.2.2)溶解并定容至 1 000 μg/mL，置 4℃冰箱保存，可使用一个月。

3.2.8 卡那霉素标准工作液：吸取一定量卡那霉素标准储备液，用缓冲液Ⅱ(3.2.3)稀释成 0.25、0.5、1.0、2.0 μg/mL 的标准工作液。以上溶液均须当日配制和使用。

3.2.9 培养基

保存及增菌用培养基(见附录 A 中 A1)。

基层及菌种用培养基(见附录 A 中 A2)。

3.3 仪器和设备

3.3.1 培养皿：内径 90 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：不锈钢管，外径(8.0±0.1)mm，内径(6.0±0.1)mm，高度(10.0±0.1)mm。

3.3.3 游标卡尺：测量范围 0～200 mm，精度 0.02 mm。

3.3.4 绞碎机：电动。

3.3.5 均质器：不低于 10 000 r/min。

3.3.6 离心机：不低于 4 000 r/min。

3.3.7 恒温培养箱：(36±1)℃，搁板应保持水平。

3.3.8 高压灭菌器。

3.3.9 恒温水浴锅。

3.3.10 其他：实验室常用玻璃器皿。

3.4 测定步骤

3.4.1 样液制备

称取 20.0 g 试样于均质杯内，加入 20 mL 缓冲液Ⅲ(3.2.4)，搅匀，放置 60 min，均质 2 min(10 000 r/min)后，移入离心管中离心 30 min(4 000 r/min)。取上清液检查 pH，必要时用 1 mol/L 氢氧化钠溶液调节 pH 至 7.9±0.1，此溶液作为样液。

3.4.2 芽胞悬液的制备

取适量生理盐水(3.2.6)加入已培养好的枯草杆菌 63501 试管斜面中，制成悬液。取 1 mL 悬液涂布于装有增菌用培养基(见附录 A 中 A1)的克氏瓶中，置培养箱(36±1)℃培养 7 天。镜检芽孢数达 85%以上，用适量生理盐水洗下菌苔，置于(65±1)℃恒温水浴中加热 30 min，经离心(3 000 r/min)

20 min，弃去上清液。再加生理盐水洗涤，经离心后弃去上清液，如此重复洗涤芽孢悬液三次。置常温24 h后再置于(65±1)℃恒温水浴中加热30 min，然后用适量生理盐水制成芽孢悬液。置4℃冰箱中可保存数月。

3.4.3 芽胞悬液用量的确定

在实际测定前，应先用0.25 μg/mL卡那霉素标准工作液对加有不同量的芽胞悬液的平板进行预测试，经培养后能使0.25 μg/mL标准工作液产生直径≥10 mm清晰、完整的抑菌圈的最佳芽胞悬液用量。

3.4.4 检定用平板

注入10 mL基层培养基(见附录A中A2)于灭菌培养皿内，水平静置，使其凝固。将基层培养基(见附录A中A2)熔化，冷却至50℃左右，加入适量(从3.4.3测得)的芽胞悬液使之充分混合后，取约7 mL注入已铺有基层培养基的平板中，保持水平，使其凝固。所用平板须当天制备。

3.4.5 标准曲线的制备

用卡那霉素标准工作液(3.2.8)制备标准曲线，以0.5 μg/mL浓度的标准工作液为参考浓度。每个标准工作液浓度取三个检定平板作为一组，每个平板上置4个牛津杯，使牛津杯在半径为2.8 cm的圆面成90°角间距，对角两个杯加0.5 μg/mL参考浓度标准工作液，另两个对角杯加其他浓度的一种标准工作液。4个浓度标准工作液共12个检定平板，用于制备标准曲线。其中0.5 μg/mL参考浓度的标准工作液将得到24个数值，而其他标准工作液分别得到6个数值。

将瓦盖盖好，置(36±1)℃培养(17±1)h。经培养后，翻转平板，除去牛津杯，精确地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0.5 μg/mL参考浓度24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减去每组平板参考浓度的抑菌圈直径平均值的差，即为各该组平板的校正值。将校正后的值用式(1)和式(2)算出L和H点的直径，在半对数坐标纸上，以抑菌圈直径为横坐标(算术级)，卡那霉素浓度为纵坐标(对数级)，通过L和H点连一直线即为标准曲线。

$$L = (7a + 4b + c - 2d)/10 \quad \cdots\cdots(1)$$

$$H = (7d + 4c + b - 2a)/10 \quad \cdots\cdots(2)$$

式中：L——标准曲线上最低浓度抑菌圈的直径；

H——标准曲线上最高浓度抑菌圈的直径；

b——参考浓度0.5 μg/mL的24个抑菌圈直径的平均值，mm；

a,c,d——分别表示标准曲线中其他标准浓度(0.25、1.0、2.0 μg/mL)的抑菌圈直径经校正后的平均值，mm。

3.4.6 样液的测定

每份样液取3个检定平板，在每个平板上置4个已灭菌的牛津杯(按制备标准曲线方法放置)，在对角的2只牛津杯里注满试验样液，在剩余的2只牛津杯里分别注满卡那霉素标准工作液(高稀释度0.25 μg/mL及参考浓度0.5 μg/mL)。于(36±1)℃下培养(17±1)h，然后翻转平板，除去牛津杯。如有抑菌圈产生，准确量取抑菌圈直径(精确到0.1 mm)。

3.5 结果的计算和表述

如样液抑菌圈的直径<10 mm，即报告为"阴性"。

如样液抑菌圈的直径≥10 mm，求出每组三个检定平板上样液和参考浓度标准工作液抑菌圈直径的平均值，经校正后，从标准曲线上查出相应的卡那霉素的浓度，通过式(3)，计算试样中卡那霉素残留量：

$$X = \frac{c}{m} \times 1\,000 \quad \cdots\cdots(3)$$

式中：X——试样中卡那霉素残留的含量，mg/kg；

c——从标准曲线上查出样液中卡那霉素浓度，μg/mL；

m ——每毫升最终样液所代表的试样量，g。

注：如测定结果呈阳性，即所显抑菌圈直径的平均值≥10 mm 时，必要时，尚需进行确证试验（可用薄层色谱法），以证明抑菌物确系卡那霉素。

4 测定低限和回收率

4.1 测定低限

本方法的测定低限为 0.5 mg/kg。

4.2 回收率

回收率实验数据：

卡那霉素添加浓度在 0.5 μg/g 时，回收率为 83.7%；

卡那霉素添加浓度在 1.0 μg/g 时，回收率为 91.5%；

卡那霉素添加浓度在 2.0 μg/g 时，回收率为 97.0%。

附 录 A
（标准的附录）
培养基成分及制备方法

A1 保存及增菌用培养基

蛋白胨	10.0 g
牛肉浸膏	5.0 g
氯化钠	2.5 g
琼脂	13～15 g
蒸馏水	1 000 mL

将上述成分于蒸馏水中加热溶解，调节 pH 使其灭菌后 pH 为 6.5±0.1，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 基层及菌种用培养基

蛋白胨	5.0 g
牛肉浸膏	3.0 g
琼脂	13～15 g
蒸馏水	1 000 mL

将上述成分于蒸馏水中加热溶解，调节 pH 使其灭菌后 pH 为 8.0±0.1，分装于烧瓶中，于 121℃高压灭菌 15 min。

Foreword

This standard was drafted according to 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. Method of determination for kanamycin residues in the "Methods for the determination of residues in animal and aquatic foods" (1990 ed.), a publication of Ministry of Public Health of Japan, is adopted by this standard after verification. Thus the method of determination of this standard is technically identical with that of Japan, with only minor editorial modification. Besides, the methods of sampling and sample preparations are also specified in this standard.

Annex A of this standard is the normative appendix.

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 the Zhejiang Import and Export Commodity Inspection Bureau of the People's Republic of China.

This standard was mainly drafted by Shi Weiliang, Zhang Rong.

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 kanamycin residues in meat for export —Cylinder plate method

SN 0536—1996

1 Scope

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

This standard is applicable to the determination of kanamycin residues in poultry 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 characteristics of the cargo within the same inspection lot, such as packing, mark, origin, specification and grade, 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 at least one bag shall be taken as primary sample. The total weight of all primary samples should not be less than 2 kg, which shall be placed in a clean container, sealed, labeled and sent to laboratory in time.

In case the meat-pieces are not contained in small bags inside each package, or if there are small bags inside the package, but the content of the bag exceeds 2 kg, cut out a part from the meat in each package of not less than 100 g with a sterile knife. Mix the parts of the meat as the primary sample, which shall not less than 2 kg. Place in clean container, seal, label and send to the laboratory in time.

2.4 Preparation of test samples

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on Apr. 29, 1996

Implemented from Oct. 1, 1996

Part of representative sample is taken from the mixed primary sample, the edible portions are homogenized by grinding in a meat 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.

2.5 Storage of test sample

The test sample should be frozen and stored below −18℃.

Note: In the course of sampling and sample preparation, precaution 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 kanamycin in the test sample is extracted with phosphate buffer. After homogenizing and centrifuging, cylinder plate method is used to determine the kanamycin in the supernatant, which is made reacted with *Bacillus subtilis* with formation of inhibition zone, by which kanamycin is determined quantitatively by standard curve method.

3.2 Reagents and materials

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

3.2.1 Kanamycin standard: 775 μg/mg (Provided by the Drug and Biological Product Inspection Institute of China).

3.2.2 Buffer solution Ⅰ (pH 6.0±0.1): Dissolve 3.5 g of anhydrous KH_2PO_4 and 16.73 g of anhydrous K_2HPO_4 and dilute to 1 000 mL with water. Autoclave at 121℃ for 15 min.

3.2.3 Buffer solution Ⅱ (pH 8.0±0.1): Dissolve 13.3 g of anhydrous KH_2PO_4 and 6.2 g of KOH and dilute to 1 000 mL with water. Autoclave at 121℃ for 15 min.

3.2.4 Buffer solution Ⅲ (pH 7.9±0.1): Dissolve 0.523 g of anhydrous KH_2PO_4 and 16.73 g of anhydrous K_2HPO_4 and dilute to 1 000 mL with water. Autoclave at 121℃ for 15 min.

3.2.5 Test strain: *Bacillus subtilis*, No. 63501 (Provided by the Drug and Biological Product Inspection Institute of China).

3.2.6 Physiological saline: Dissolve 8.5 g of sodium chloride in 1 000 mL of water, autoclave at 121℃ for 15 min.

3.2.7 Kanamycin standard stock solution: Accurately weigh (accurate to 0.1 mg) a proper quantity of kanamycin standard and dissolve in buffer solution Ⅰ (3.2.2) to make a 1 000 μg/mL standard stock solution. Store in a refrigerator at 4℃, which can be used within a month.

3.2.8 Kanamycin standard working solution

Pipet a certain amount of kanamycin standard stock solution, dilute with phosphate buffer solution Ⅱ (3.2.3) to make 0.25, 0.5, 1.0, 2.0 μg/mL standard working solutions, all of the solutions should be prepared and used on the same day.

3.2.9 Media

Medium for stock and enrichment (see A1 of annex A).

Medium for base and strain (see A2 of annex A).

3.3 Apparatus and equipment

3.3.1 Culture dishes: 90 mm(id) glass or plastic dish of flat bottom with clay cover.

3.3.2 Cylinders: Stainless steel, (8.0±0.1)mm(od), (6.0±0.1)mm(id), height(10.0±0.1)mm.

3.3.3 Vernier caliper: Measuring range 0—200 mm, precision 0.02 mm.

3.3.4 Meat grinder; electrodynamic.

3.3.5 Homogenizer; not less than 10 000 r/min.

3.3.6 Centrifuger; not less than 4 000 r/min.

3.3.7 Incubator; (36±1)℃, shelf be kept horizontal.

3.3.8 Autoclave sterilizer.

3.3.9 Water-bath.

3.3.10 Others; Ordinary laboratory glass utensils.

3.4 Procedure of determination

3.4.1 Preparation of sample solution

Weigh 20.0 g of the chopped and mixed sample in a homogenizer, add 20 mL of buffer solution Ⅱ (3.2.4) for extraction. Mix thoroughtly. An hour later, homogenize for 2 min(10 000 r/min) then transfer into a centrifugal tube and centrfuge at 4 000 r/min for 30 min. Check pH value of the supernate and if required, adjust pH to 7.9±0.1 with 1 mol/L NaOH solution. This supernate is used as sample test solution.

3.4.2 Preparation of test spores suspension

Add a proper amount of physiological saline on the surface of cultured *Bacillus subtilis* 63501 slant in the test tube to from suspension. Transfer 1 mL of the suspension to the surface of slant in Mojonner-type flask containing enrichment medium (see annex A1), spread the suspension evenly over the entire surface and incubate at (36±1)℃ for 7 days. If amount of spores is more than 85% by observing with microscope, wash the culture with a proper amount of physiological saline and incubate at (65±1)℃ for 30 min, then discard the supernatant after centrifugalizing at 3 000 r/min for 20 min. The same washing procedure for the culture shall be done for three times, then incubate at (65±1)℃ for 30 min after standing for 24 h at room temperature, finally make spore suspension with a proper amount of physiological saline and store in the refrigerator at about 4℃, which can be used for several months.

3.4.3 Ascertainment of the optimum amount of the spore suspension

Before the actual determination, the plate with different amount of spore suspension in the medium should be pre-tested, using 0.25 μg/mL standard working solution. After incubated, when the diameter of inhibited zone caused by 0.25 μg/mL standard working solution is ≥10 mm, it is the optimum amount of spore suspension used for determination.

3.4.4 Preparation of plates

Pipet 10 mL of the medium (annex A2) into a sterilized dish as the base layer, keep horizonal and make it uniform and solidified for later use. Melt the medium (annex A2) and cool to about 50℃, add the optimum amount (ascertained by 3.4.3) of spore suspension and mix thoroughly. Pipet about 7 mL of above medium into a sterilized dish for the strain layer, keep horizonal and make it uniform and solidified. Use the plates the same day they are prepared.

3.4.5 Preparation of the standard curve

Prepare the standard curve with kanamycin standard working solution (3.2.8) (using 0.5 μg/mL solution for reference concentration) as follows:

Take 3 test plates as a set for each concentration of standard working solution. Place four Oxford cups on each plate along the circle (2.8 cm radius) at an angle of 90° to each other. Fill 0.5 μg/mL standard working solution into two diagonal cups as reference concentration; into other two diagonal cups, fill the solution of the other concentrations. Thus 4 concentrations totalling 12 test plates are used to prepare the standard curve. Among them, 24 readings are obtained from 0.5 μg/mL reference concentration, while 6

readings obtained from each of the other concentrations.

Place on the clay cover, incubate to (17±1)h at (36±1)℃. Invert the plates and remove the Oxford cups, and measure the inhibition zone diameters of each readings of the point tested on each set of three plates respectively concentration (accurate to 0.1 mm), obtain the average value for each, then calculate the average of 24 zone diameters of the 0.5 μg/mL reference concentration. The difference between the total 24 average diameter and average diameter of inhibition zones of reference concentration in each set is the correction value for each set. After correcting the values of the concentrations of different sets, calculate points L and H by formulas (1) and (2) respectively. On the semilog graph paper, with zone diameters as abscissa (arithmetric) and concentrations as ordinate (logarithmic), draw a straight line between L and H, which is considered a standard curve.

$$L = (7a + 4b + c - 2d)/10 \quad \cdots\cdots (1)$$

$$H = (7d + 4c + b - 2a)/10 \quad \cdots\cdots (2)$$

where

L —Zone diameter for the lowest (0.25 μg/mL)concentration of the standard curve, mm;

H —Zone diameter for the highest (2.0 μg/mL) concentration of the standard curve, mm;

b —Average zone diameter of 24 zone diameters for the reference concentration (0.5 μg/mL), mm;

a, c, d —Corrected average zone diameters for each of the other concentrations (0.25, 1.0, 2.0 μg/mL) used for the standard curve, mm.

3.4.6 Determination of sample solution

Use three plates for each test sample, place four sterilized Oxford cups on each plate respectively at the places as for standard curve preparation. Fill two cups of diagonal position on each plate with sample solution, fill the other two cups with 0.25 and 0.5 μg/mL kanamycin standard working solutions. Incubate at (17±1) h for (36±1)℃. After incubation, invert the plates to remove the cups. If there is zone of inhibition on the position of sample cups, measure the diameter of each zone as accurately as possible (to the nearest 0.1 mm).

3.5 Calculation and expression of result

If the inhibition zone diameter of sample solution is < 10 mm, the result shall be reported as "negative".

If the zone diameter is $\geqslant 10$ mm, find out the average values of the zone diameters of sample solution and reference standard working solutions on the test plate respectively. After correction, obtained from the standard curve the corresponding kanamycin concentration. Then calculate the kanamycin residue content in the test sample according to formula (3):

$$X = \frac{c}{m} \times 1\,000 \quad \cdots\cdots (3)$$

where

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

c —Concentration of kanamycin of sample solution corresponding to the standard from the standard curve, μg/mL;

m —Corresponding mass of test sample in each milliliter of final sample solution, g.

Note: If the result of determination is positive (i.e. the average zone diameters $\geqslant 10$ mm), confirmation test (TLC method may be used) may be carried out, if necessary, to ascertain the inhibiting substance is actually kanamycin.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.5 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of kanamycin and their corresponding recoveries are:

0.5 μg/g, the recovery 83.7%;

1.0 μg/g, the recovery 91.5%;

2.0 μg/g, the recovery 97.0%.

Annex A
(Normative Annex)
Preparation of media and their ingredients

A1 Medium for stock and enrichment

Peptone	10.0 g
Beef extract	5.0 g
sodiun chloride	2.5 g
Agar	13—15 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±0.1. Dispense into separate test tubes or Mojonner-type flasks. Autoclave at 121℃ for 15 min. After autoclaving, make it slanting.

A2 Medium for base and strain

Peptone	5.0 g
Beef extract	3.0 g
Agar	13—15 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 8.0±0.1. Dispense into separate flasks. Autoclave at 121℃ for 15 min. Store for later use.