SN

中华人民共和国进出口商品检验行业标准

SN 0671—1997

出口肉及肉制品中林可霉素残留量检验方法　杯碟法

Method for the determination of lincomycin 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。其中测定方法采用了日本厚生省1990年编写的《畜、水产食品中残留物质检验方法》中大环内酯类抗生素残留量分析方法。但在技术内容上稍作改变，经验证后，按规定格式要求作了编辑性修改。同时在标准中制定了抽样和制样方法。

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

本标准附录A为标准的附录。

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。

本标准由中华人民共和国上海进出口商品检验局负责起草。

本标准主要起草人：李慧珠、王培梁。

本标准系首次发布的行业标准。

中华人民共和国进出口商品检验行业标准

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1 范围

本标准规定了出口肉及肉制品中林可霉素残留量检验的抽样、制样和杯碟测定方法。

本标准适用于出口猪肉中林可霉素残留量的检验。

2 抽样和制样

2.1 检验批

以不超过2 500件为一检验批。

同一检验批的产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。如每件中无小包装或有小包装，但每袋小包装重量超过2 kg者，可用灭菌刀具从包件中割取不少于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，加封并标明标记，及时送交实验室。

2.4 试样制备

从混合原始样品中取出部分代表性样品，将可食部分放入绞碎机内绞碎，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。

2.5 试样保存

将试样于－18℃以下冷冻保存。

注：在抽样和制样的操作过程中，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

试样中林可霉素残留用缓冲溶液(pH8.0)提取。提取液经离心后取上清液与藤黄微球菌作用,根据产生抑菌圈的大小用标准曲线法定量。必要时,用纸色谱法进行确证。

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 林可霉素标准品:已知纯度≥85.4%,由卫生部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):称取 33.46 g 无水磷酸氢二钾及 1.046 g 无水磷酸二氢钾,溶于水中并定容至 1 000 mL。

3.2.3 试验菌种:藤黄微球菌(*Micrococcus luteus*),由卫生部药品生物制品检定所提供,菌号 CMCC 28001。

3.2.4 林可霉素标准贮备液:称取一定量林可霉素标准品(精确至 0.1 mg),用磷酸盐缓冲液溶解并稀释,配成浓度为 100 μg/mL 的林可霉素标准贮备液,保存于 4℃冰箱中(不宜超过一个月)。

3.2.5 林可霉素标准工作液:取上述贮备液用磷酸盐缓冲液稀释,分别配制成浓度为 0.15,0.30,0.60,1.20,2.40 μg/mL 的标准工作液,作为绘制标准曲线用。需当天配制,当天使用。

3.2.6 培养基 1(附录 A 中 A1)。

3.2.7 培养基 2(附录 A 中 A2)。

3.2.8 培养基 3(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,于 121℃,高压灭菌 15 min。

3.2.10 展开剂:磷酸盐缓冲液(3.2.2)-甲醇(1+1)。

3.3 仪器与设备

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 绞碎机:电动。

3.3.6 均质器:10 000 r/min,带均质杯(300 mL)。

3.3.7 离心机:8 000 r/min。

3.3.8 水浴锅:恒温±1℃。

3.3.9 恒温培养箱:(30±1)℃,搁板应保持水平。

3.3.10 高压灭菌器。

3.3.11 长方形培养皿:1.5 cm×8 cm×23 cm。

3.3.12 色谱缸:20 cm×15 cm×30 cm。

3.3.13 色谱纸:6 cm×20 cm 滤纸。

3.3.14 微量注射器:10 μL 或 50 μL。

3.4 测定步骤

3.4.1 样液制备

称取 30 g 试样(精确至 0.1 g)于均质杯内,加入 5 mL 磷酸盐缓冲液,以 8 000 r/min 均质 2 min。移入离心管中,以 8 000 r/min 离心 10 min。按上述各步骤再重复两次,合并三次上清液。用 1 mol/L 氢氧化钠溶液将其调至 pH8.0,以磷酸盐缓冲液(3.2.2)定容至 20 mL,再离心,取上清液作为样液。

3.4.2 菌悬液的制备

将菌种(3.2.3)接种于培养基 1(3.2.6)中,混匀,置(30±1)℃培养(24±2)h。再转种到培养基 2(3.2.7)斜面上,置(30±1)℃培养 16～18 h 后,加入 3 mL 培养基 1(3.2.6),洗下菌苔。吸取 1 mL 此菌液,转种至克氏瓶中培养基2(3.2.7)的表面,置(30±1)℃培养(24±2)h。用 15 mL 生理盐水(3.2.9)洗下琼脂表面上的菌苔,制成菌悬液,置 4℃冰箱保存(可保存 2 周)。

3.4.3 菌悬液最佳用量测定

测试前应先用 0.60 μg/mL 林可霉素标准工作液对上述菌悬液的用量进行预测试。以不同量的菌悬液加入经熔化并冷却至 45～50℃的培养基 3(3.2.8)中，经充分混合并经(30±1)℃培养(18±1)h 后，选择能使该标准工作液产生直径为 18.50 mm(±10%)清晰、完整的抑菌圈的菌悬液，加入量为菌悬液的最佳用量。

3.4.4 检定平板的制备

将培养基 3(3.2.8)熔化，冷却至 40～45℃，加入最佳用量(从 3.4.3 中求得)菌悬液使其充分混合后，取 7.5 mL 注入灭菌培养皿中，保持水平，待凝固制成检定平板。所用平板需当天制备。

3.4.5 标准曲线的制备

以 0.60 μg/mL 浓度的标准工作液作为参考浓度。标准曲线上的每个标准工作液浓度各取 3 个检定平板为一组。在每个平板的表面放置 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 个牛津杯内加满参考浓度溶液，另 3 个牛津杯内加满其他一种浓度的标准工作液。参考浓度溶液与标准工作液要间隔放置。5 种浓度的标准工作液共用 15 个检定平板。含量最低的标准工作液(0.15 μg/mL)的 3 个检定平板作为判断阴性结果的对照，其他 12 个检定平板用来绘制标准曲线。这样，0.60 μg/mL 参考浓度溶液将得出 45 个抑菌圈直径的数值，而其他浓度的标准工作液将得出各 9 个抑菌圈的数值。

将陶瓦盖盖好，置(30±1)℃培养(18±1)h。翻转平板，除去牛津杯。精确地测量所产生的抑菌圈的直径(精确到 0.1 mm)。求出每组 3 个检定平板上 0.60 μg/mL 浓度的抑菌圈直径读数与其他浓度标准工作液的抑菌圈直径读数的平均值。再求出参考浓度(0.60 μg/mL)的所有 45 个抑菌圈直径数值的平均值。用 45 个参考浓度抑制圈直径的平均值与每组中 9 个参考浓度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，用式(1)、(2)算出 L 和 H。在半对数坐标纸上，以抑菌圈直径(mm)为纵坐标(算术级)，以浓度 μg/mL 为横坐标(对数级)，标出 L 和 H 点。通过 L 和 H 点连一直线即为标准曲线。

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

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

式中：L——标准曲线上的最低浓度(0.15 μg/mL)抑菌圈直径，mm；

H——标准曲线上的最高浓度(2.40 μg/mL)抑菌圈直径，mm；

c——参考浓度 0.60 μg/mL 的 45 个抑菌圈直径的平均值，mm；

a、b、d、e——分别表示标准曲线中其他浓度标准工作液(0.15，0.30，1.20，2.40 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，在每个检定平板上放置 6 个已灭菌的牛津杯(按制备标准曲线方法放置)。3 个间隔的牛津杯内加满林可霉素参考浓度标准溶液，另外 3 个牛津杯内加满被检样液。将陶瓦盖盖好，置(30±1)℃培养(18±1)h。翻转平板，除去牛津杯。如有抑菌圈产生，精确测量其直径。

3.5 结果计算和表述

如样液抑菌圈的直径<10 mm，即报告为“阴性”。

抑菌圈的直径≥10 mm，经校正后，从标准曲线上查出相应的林可霉素的浓度。再用式(3)计算试样中林可霉素的含量。

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

式中：X——试样中林可霉素残留的含量，mg/kg；

c——从标准曲线上查出的样液中林可霉素浓度，μg/mL；

m——每毫升最终样液所代表的试样量，g/mL。

3.6 确证试验

如测定结果呈阳性，即所显抑菌圈的平均直径≥10 mm 时，必要时，尚需进行确证试验，可用纸层色谱法以证明抑菌物确系林可霉素。

用磷酸盐缓冲液(3.2.2)喷涂于色谱用纸(6 cm×20 cm)上，自然干燥后备用。

取上述样液(3.4.1)6.6 mL(相当 10 g 试样)，用氢氧化钠(1 mol/L)调节 pH 至 8.0。置于 40℃水浴中，通以氮气蒸发近干。加 1 mL 磷酸盐缓冲液(3.2.2)，作待测液。

取 1.0 μg/mL 林可霉素标准工作液，以及待测液各 10～20 μL，分别用微量注射器吸取并点在上述备用纸上。将色谱纸移放在盛有展开液(3.2.10)的色谱缸中，展开 1～2 h 后取出。待溶剂挥发后，将色谱纸轻轻贴在加有 60 mL 含有最佳用量菌悬液的培养基 3(3.2.8)的长方形培养皿中，移至 4℃的冰箱中放置 1 h。取出培养皿，移去色谱纸，将培养皿置于(30±1)℃进行培养(20±1) h。根据培养皿上出现的抑菌圈的位置，测得抑菌圈的 R_f 值。对照标准，以确定被检测试样中抗生素是否是林可霉素。

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

猪肉中林可霉素添加浓度及其回收率的实验数据：

在 0.10 mg/kg 时，回收率为 82.7%；

在 0.20 mg/kg 时，回收率为 94.5%；

在 0.40 mg/kg 时，回收率为 90.7%。

附 录 A
（标准的附录）
培 养 基

A1 培养基 1

明胶的胰酶消化物	5.0 g
酵母浸膏	1.5 g
牛肉浸膏	1.5 g
氯化钠	3.5 g
葡萄糖	1.0 g
无水磷酸氢二钾	3.68 g
无水磷酸二氢钾	1.32 g
水	1 000 mL

调节溶液 pH 到 7.0±0.1，分装试管，于 121℃高压灭菌 15 min。

A2 培养基 2

胰酪胨	4.0 g
酵母浸膏	3.0 g
牛肉浸膏	1.5 g
葡萄糖	1.0 g
琼脂	15.0 g
水	1 000 mL

调节溶液 pH 到 6.5±0.1，分装试管，于 121℃高压灭菌 15 min。

A3 培养基 3

培养基的成分同 A2，调节溶液 pH 到 8.0±0.1，分装试管，于 121℃高压灭菌 15 min。

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". In this standard,the testing method for residues of mocrolide antibiotics in "Methods for the determination of residues in animal and aquatic foods"approved by the Public Health Department of Japan in 1990 is adopted as the method of determination, which is thus technically equivalent to that of the original, but with slight modification. After going through verification this standard was drafted according to the editorial requirements for the stipulated format. In addition,the methods of sampling and sample preparation are also specified in this standard.

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

Annex A of this standard is an normative 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 Shanghai Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafters of this standard are Li Huizhu and Wang Peiliang .

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 lincomycin residues in meats and meat products for export —Cylinder plate method

SN 0671—1997

1 Scope

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

This standard is applicable to the determination of lincomycin residues in pork for export.

2 Sampling and sample preparation

2.1 Inspection lot

The quantity of an inspection lot should not exceed 2 500 packages.

The characteristics 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 at least one bag shall be taken as a primary sample. In case there are no small bags inside each 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 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

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on Aug. 15, 1997

Implemented from Jan. 1, 1998

be sealed and labeled.

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 lincomycin residues in the test sample are extracted with buffer solution (pH 8.0). After centrifugalizing, the supernatant is reacted with *Micrococcus luteus* with formation of inhibition zone. Determination is made by measuring the size of inhibition zone, using standard curve method. If necessary, carry out the confirmation test by paper chromatography.

3.2 Reagents and materials

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

3.2.1 Lincomycin standard: Known purity ≥ 85.4%, provided by Drug and Biological Product Inspection Institute of Public Health Ministry, or equivalent.

3.2.2 Phosphate buffer solution (pH 8.0): Dissolve 33.46 g of anhydrous dipotassium hydrogen phosphate and 1.046 g anhydrous potassium hydrogen phosphate in water and dilute to 1 000 mL with water.

3.2.3 Test strain: *Micrococcus luteus*, No. 28001 (CMCC), provided by Drug and Biological Product Inspection Institute of Public Health Ministry.

3.2.4 Lincomycin standard stock solution: Accurately weigh a proper quantity of lincomycin standard and dissolve in phosphate buffer solution to prepare a lincomycin standard stock solution of 100 μg/mL in concentration. Store in a 4℃ refrigerator, which can be used within a month.

3.2.5 Lincomycin standard working solution: Pipet a series of lincomycin standard stock solution, and dilute separately with sufficient phosphate buffer solution (pH 8) to prepare a series of standard working solutions of 0.15, 0.30, 0.60, 1.20 and 2.40 μg/mL in concentrations, which should be used on the same day of preparation.

3.2.6 Medium 1 (see A1 in annex A).

3.2.7 Medium 2 (see A2 in annex A).

3.2.8 Medium 3 (see A3 in annex A).

3.2.9 Saline: Dissolve 8.5 g of sodium chloride in water and dilute to 1 L with water. Autoclave at 121℃ for 15 min.

3.2.10 Developing agent: Phosphate buffer solution (3.3.2)-Methanol (1+1).

3.3 Apparatus and equipment

3.3.1 Dish: 90 mm (id), glass or plastic dishes with smooth and flat bottom and clay cover.

3.3.2 Oxford cup: Stainless steel cylinder, (8.0±0.1) mm (od), (6.0±0.1) mm (id), (10±0.1) mm height.

3.3.3 Kolle bottle: 800 mL.

3.3.4 Vernier caliper: Measuring range 0—200 mm, precision 0.02 mm, or meter for measuring the inhibition zone.

3.3.5 Grinder: Electric.

3.3.6 Homogenizer: Not less than 10 000 r/min, with homogenizing cup (300 mL).

3.3.7 Centrifuge: Not less than 8 000 r/min.

3.3.8 Water-bath: Thermostatic, ±1℃.

3.3.9 Incubator: (30±1)℃, the shelf must be kept horizontal.

3.3.10 Autoclave.

3.3.11 Rectangular culture dish: 1.5 cm×8 cm×23 cm.

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

3.3.13 Chromatographic paper: 6 cm×20 cm filter paper.

3.3.14 Micro-syringe: 10 μL or 50 μL.

3.4 Procedure

3.4.1 Preparation of sample solution

Weigh ca 30 g of the test sample(accurate to 0.1 g)into a homogenizing cup, add 5 mL of phosphate buffer solution, homogenize at 8 000 r/min for 2 min. Transfer to a centrifuge tube and centrifugalize at 8 000 r/min for 10 min. Extract the residue twice again as above. Combine the three supernatants into a 20 mL volumetric flask. Adjust pH to 8.0 with sodium hydroxide solution(1 mol/L) and dilute to the mark with phosphate buffer solution. The solution, after centrifug alizing, is ready for microbiological determination.

3.4.2 Preparation of organism suspension

Inoculate the test strain (3.2.3) into medium 1(3.2.6), mix well and incubate at (30±1)℃ for (24±2)h. Transfer the incubated culture to the slant surface of medium 2(3.2.7), incubate at (30±1)℃ for 16—18 h. Add 3 mL of medium 1(3.2.6) to wash down the growth colony. Pipet 1 mL of the organism suspension to the surface of medium 2(3.2.7)in the kolle bottle, incubate at (30±1)℃ for (24±2)h. Wash down the growth colony from the agar surface with 15 mL of saline(3.2.9). Store this organism suspension in the refrigerator(4℃), usable within 2 weeks.

3.4.3 Ascertainment of the optimum amount of organism supension used

Before the preparation of the plates, the amount of the organism suspension used should be pretested with 0.60 μg/mL lincomycin standard working solution. Melt the medium 3(3.2.8)and cool to 45—50℃, add different amount of organism suspension and mix thoroughly. Incubate at (30±1)℃ for (18±1) h. When a clear and sharp inhibition zone appears with a diameter of 18.50 mm(±10%) after incubation on the plate, the amount of the organism suspension is considered as optimum.

3.4.4 Preparation of the plates

Melt the medium 3(3.2.8) and cool to 45—50℃. Add optimum amount of organism suspension (ascertained in 3.4.3)and mix thoroughly. Transfer 7.5 mL into a sterilized dish and keep horizontal until congealing as the testing plate. All the plates should be prepared freshly on the day of testing.

3.4.5 Preparation of standard curve

Use the standard working solution of 0.60 μg/mL in concentration as the reference concentration solution. Take three plates as a set for each of the other standard working solution. Place 6 Oxford cup on each plate at 60° intervals on the circle of 2.8 cm radius. Fill three Oxford cup with reference concentration solution (0.60 μg/mL) and three Oxford cup with one of the other concentrations of the standard working solution, alternating so that each 0.60 μg/mL cylinder is followed by a cylinder of the other concentration. Use three plates for each concentration on the curve, for a total of 15 plates. The three plates containing the lowest concentration of standard (0.15 μg/mL)are intended to produce negative results; the other 12 plates are used to construct the standard curve. This will give 45 determinations for 0.60 μg/mL and 9 determinations for each of the other concentrations on the curve.

Place the clay covers on the plates and incubate the plates for(18±1) h at (30±1)℃. After incubation,invert the plates to remove the Oxford cup. Measure the diameter of each zone of inhibition as accurately as possible (to the nearest 0. 1 mm). Average the readings of the 0. 60 μg/mL concentration and the readings of the other concentration on each set of three plates. Also average all 45 readings of the 0. 60 μg/mL concentration as the correction point for the curve. The difference between the total (45 readings)average of 0. 60 μg/mL 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 to the value it would be,if the average of the 0. 60 μg/mL readings for that set of three plates was not the same as the correction point.

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

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

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

where

L—the zone diameter of the lowest concentration(0. 15 μg/mL)of the standard curve,mm;

H—the zone diameter of the highest concentration (2. 40 μg/mL) of the standard curve, mm;

c—the average of 45 zone diameters for the reference concentration (0. 60 μg/mL), mm;

a,b,d,e—the corrected average zone diameter for each of the other concentrations (0. 15, 0. 30, 1. 20 and 2. 40 μg/mL) used for the standard curve, mm.

3.4.6 Determination of sample solution

Use three plates for each sample solution,place 6 Oxford cup on each plate at 60° intervals on the circle of 2. 8 cm radius. Fill three Oxford cup with sample solution alternately with three 0. 60 μg/mL lincomycin standard working solution,incubate at (30±1)℃ for (18±1) h. After incubation,invert the plates to remove the Oxford cup. If there are zones of inhibtion on the position of sample cylinders,measure the diameter of each zone as accurately as possible.

3.5 Calculation and expression of result

If the value of the inhibition zone is less than 10 mm,report lincomycin residues in the test sample as "negative". If the diameter of inhibition zone ≥10 mm, after correction,obtain the corresponding concentration of lincomycin from the standard curve.

Calculate the lincomycin residue content in the test sample by formula(3):

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

where

X—the lincomycin residue content in the test sample,mg/kg;

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

m—the corresponding mass of test sample in each milliliter of final sample solution,g/mL.

3.6 Confirmation test

If the result of determination is positive,i. e. the average of zone diameters≥10 mm,carry out the confirmation test by paper chromatography,if necessary,in order to ascertain the actual presence of lincomycin.

To the chromatographic paper (6 cm×20 cm), spray with phosphate buffer solution(3. 2. 2), let dry for use.

Pipet 6. 6 mL (corresponding 10 g of test sample)of the sample solution(3. 4. 1)and adjust the pH to 8. 0 with sodium hydroxide solution(1 mol/L). Evaporate nearly to dryness under a gentle stream of nitrogen in a 40℃ water-bath. Add 1 mL of phosphate buffer solution(3. 2. 2), the solution is ready for confirmation test.

Spot respectively the linconycin standard working solution(1 μg/mL)and the above sample solution, 10—20 μL for each, onto the above chromatographic paper, using micro-syringe. Place the filer paper in the chromatographic tank containing the developing solution (3. 2. 10). After developing for 1—2 h, take out. Let the solvent evaporate, lay the chromatographic paper lightly on the surface of 60 mL of medium 3 which contains the optimum amount of organism suspension in a rectangular culture dish. Transfer the dish into a refrigerater(4℃)and let stand for 1 h. Remove the chromatographic paper, incubate the culture dish for(20±1) h at (30±1)℃. According the position of the zone of inhibition shown on the medium in the rectangular culture dish, measure the R_f value of the inhibition zone. Compare with the standard to confirm the presence of lincomycin in the test sample.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0. 10 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of lincomycin in pork and their corresponding recoveries are:

0. 10 mg/kg, the recovery 82. 7%;

0. 20 mg/kg, the recovery 94. 5%;

0. 40 mg/kg, the recovery 90. 7%.

Annex A
(normative)
Media

A1 Medium 1

Pancreatic digest of gelatin	5.0 g
Yeast extract	1.5 g
Beef extract	1.5 g
Sodium chloride	3.5 g
Glucose	1.0 g
K_2HPO_4(anhydrous)	3.68 g
KH_2PO_4(anhydrous)	1.32 g
Distilled water	1 000 mL

Adjust the pH to 7.0±0.1. Dispense into test-tubes and autoclave at 121℃ for 15 min.

A2 Medium 2

Casein tryptone	4.0 g
Yeast extract	3.0 g
Beef extract	1.5 g
Glucose	1.0 g
Agar	15.0 g
Distilled water	1 000 mL

Adjust the pH to 6.5±0.1. Dispense into test-tubes and autoclave at 121℃ for 15 min.

A3 Medium 3

Ingredients of the medium are the same as A2, adjust the pH to 8.0±0.1. Dispense into test-tubes and autoclave at 121℃ for 15 min.