SN

中华人民共和国进出口商品检验行业标准

SN 0537—1996

出口肉品中红霉素残留量检验方法 杯碟法

Method for the determination of erythromycin 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进行编写的。其中测定方法采用《美国公职分析化学家协会公定分析方法》第 15 版中抗生素红霉素残留量分析方法。技术内容与原方法相同，经验证后，按规定格式要求仅作了编辑性修改。在标准中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红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 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。

本标准起草单位：中华人民共和国上海进出口商品检验局。

本标准主要起草人：李慧珠、朱治平、吴仲梁。

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品中红霉素残留量的检验抽样、制样和杯碟测定方法。

本标准适用于出口禽肉中红霉素残留量的检验。

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，抽取原始样品的总量不少于 2 kg，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，可用灭菌刀具从抽出的包件中，每件割取不少于 100 g，混合后，置于清洁容器内，作为原始试样，混合的试样总量不少于 2 kg，加封后，标明标记，及时送交实验室。

2.4 试样的制备

从取回的原始样品中取出部分样品，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

利用被测定样液中的残留红霉素与藤黄微球菌作用，产生抑菌圈。试样用20%的甲醇-二甲氧基甲烷溶液提取。提取液经离心浓缩后，溶于缓冲溶液(pH8.0)中并定容，用杯碟法测定，对照红霉素标准曲线定量。

3.2 试剂和材料

3.2.1 红霉素标准品：由卫生部药品生物制品检定所提供。

3.2.2 二甲氧基甲烷[$CH_2(COCH)_2$]：化学纯。

3.2.3 磷酸盐缓冲液(pH8.0)：0.1 mol/L，称取16.73 g无水磷酸氢二钾及0.53 g无水磷酸二氢钾，溶于水，定容至1 000 mL。

3.2.4 试验菌种：藤黄微球菌(*Micrococcus Luteus*)，菌株号28001。

3.2.5 红霉素标准储备液：准确称取一定量红霉素标准品(精确至0.10 mg)，用二甲氧基甲烷溶液，使红霉素液的浓度为1 000 μg/mL，再用磷酸盐缓冲液(pH8.0)稀释，使最终浓度为100 μg/mL，保存于4℃冰箱备用(不宜超过一周)。

3.2.6 红霉素标准工作液：取上述储备液用磷酸盐缓冲液稀释，配制成0.025，0.05，0.10，0.20，0.40，0.80 μg/mL的标准工作液，作为制备标准曲线的标准工作液。需当天配制，当天使用。

3.2.7 肉汤培养基(见附录A中A1)。

3.2.8 菌种培养基(见附录A中A2)。

3.2.9 检定培养基(见附录A中A3)。

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：外径(7.8±0.1)mm，内径(6.0±0.1)mm，高(10±0.1)mm的不锈钢管。

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，带均质杯(300 mL)。

3.3.6 离心机：不低于4 000 r/min。

3.3.7 水浴锅：0～60℃±1℃。

3.3.8 恒温培养箱：(35±1)℃。

3.3.9 高压灭菌器。

3.3.10 其他：玻璃器皿。

3.4 测定步骤

3.4.1 试液制备：准确称取20.0 g样品于均质杯内，加入50 mL20%甲醇-二甲氧基甲烷溶液，9 000 r/min，均质2 min，移入离心管4 000 r/min，离心10 min，将上清液移入100 mL容量瓶中(视情况过滤)。试样再分别用20%甲醇-二甲氧基甲烷如上法提取两次，将上清液合并，用甲醇-二甲氧基甲烷定容至100 mL。用移液管吸取10 mL提取液至试管中，置于40℃水浴，通以氮气吹干，取出试管，加磷酸盐缓冲液，溶解残渣并定容至2 mL，作为样液。

3.4.2 菌液的制备：将菌种接种于肉汤培养基(3.2.7)内，置(35±1)℃培养(24±2)h。再转种到菌种培养基(3.2.8)斜面上，置(35±1)℃培养16～18 h后，加入3 mL肉汤培养基(3.2.7)，洗下菌苔，吸取1 mL此菌液转种至克氏瓶中菌种培养基(3.2.8)的表面，置(35±1)℃培养(24±2)h。用15 mL灭菌生理盐水洗下琼脂表面的菌苔，制成菌悬液，置4℃冰箱保存(可保存二周)。

3.4.3 菌液用量测定：测试前应先用0.05 μg/mL红霉素标准工作液对该菌液浓度的平板进行预测试，经培养后，其标准工作液所呈现的抑菌圈直径在10 mm以上时，则该菌液浓度即可使用，否则应调

整菌液至合适的浓度。

3.4.4 检定平板的制备：于无菌平皿中加入 10 mL 融化的测试培养基(3.2.9)，保持水平，待其凝固。再吸取加有菌液的该培养基 4 mL 注入上述基层培养基上，保持水平，待其凝固后，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，所用平板需当天制备。

3.4.5 标准曲线的制备

以 0.10 μg/mL 浓度的标准工作液作为参考浓度。每个标准工作液浓度取 3 个检定平板为一组，在每个检定平板上的其中 3 个牛津杯内加满参考浓度溶液，另 3 个牛津杯内加满标准工作液，参考浓度溶液与标准工作液要间隔放置，5 个标准浓度共 15 个检定平板，含量最低的标准浓度 0.025 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。这样参考浓度 0.10 μg/mL的工作液将得出 45 个抑菌圈直径的数值，而其他的标准浓度将得出 9 个抑菌圈的数值。

将陶瓦盖盖好，置 35℃培养(18±1)h，翻转平板，除去牛津杯，精确地测量产生的抑菌圈直径(精确到 0.1 mm)，求出每组 3 个检定平板上 0.10 μg/mL 浓度的抑菌圈直径读数与其他标准浓度的抑菌圈直径读数的平均值，再求出参考浓度 0.10 μg/mL 的所有 45 个抑菌圈直径数值的平均值，用 45 个参考浓度抑菌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标准浓度的抑菌圈读数的平均值。

将这些校正后的值，包括 0.10 μg/mL 浓度的平均值在半对数坐标纸上，以抑菌圈直径为纵坐标(算术级)，以 μg/mL 计的浓度为横坐标(对数级)，通过式(1)和式(2)的 L 和 H 点绘出最佳直线。

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

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

式中：L ——标准曲线上的最低浓度(0.05 μg/mL)抑菌圈直径，mm；

H ——标准曲线上的最高浓度(0.8 μg/mL)抑菌圈直径，mm；

c ——参考浓度 0.1 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标准浓度(0.05、0.20、0.40、0.80 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 3 个间隔的牛津杯内加满红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 35℃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小于 10 mm，即报告为未检出。如抑菌圈的直径大于等于 10 mm，经校正后，从标准曲线上查出相应的红霉素的含量，得出结果以 μg/mL 计。

如果样品中红霉素含量的单位用 mg/kg 表示时，可将 μg/mL 按照式(3)进行换算：

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

式中：X ——样品中红霉素的含量，mg/kg；

c ——从标准曲线上查出的样液红霉素浓度，μg/mL；

m ——最终试液所代表的试样量，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 时，必要时，尚需进行确准试验(可用薄层层析法)。

4 测定低限和回收率

4.1 测定低限

本方法的测定低限为 0.05 mg/kg。

4.2 回收率

本方法的回收率数据：

红霉素添加浓度为 0.05 μg/g，回收率为 78.27%；

红霉素添加浓度为 0.10 μg/g，回收率为 80.28%；

红霉素添加浓度为 0.20 μg/g，回收率为 86.14%。

附 录 A
（标准的附录）
培 养 基

A1 肉汤培养基

胰酶消化的明胶	5.0 g
酵母浸膏	1.5 g
牛肉膏	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
蒸馏水	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 菌种培养基

胰酶消化的明胶	6.0 g
胰蛋白胨的酶消化物	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
蒸馏水	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 检定培养基

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 the 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 on the general rules for drafting and presentation of the standards and SN/T 0001—1995 General rules for drafting the standard methods for the determination of pesticide, veterinary drug residues and biotoxin in commodities for export. In this standard, the testing method for erythromycin residues in A. O. C., 15th Edition, 1990 is adopted as the method of determination, which is thus technically identical with that of original; only minor editorial modification required for the stipulated format was made after going through verification. In addition, methods of sampling and sample preparation are also specified in this standard.

The limit of determination is defined in the standard on the basis of the current international maximum limit for erythromycin residues in cereals and sensitivity of the method. Annex A is the nomative annex.

This standard was propose by and 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 drafter of the standard is Li Huizhu, Zhu zhiping, Wu Zhongliang.

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 erythromycin residues in meat for export —Cylinder plate method

SN 0537—1996

1 Scope

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

This standard is applicable to the determination of erythromycin residues in poultry meat 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 and 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 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 there are not small bags inside each package, or if there are small bags inside the package, but the content of a bag exceed 2 kg, cut out a part from the meat in each package of not less than 100 g with asterile knife. Mix the parts of the meat as the mixed primary sample, which shall not exceed 2 kg. Place in aclean container, seal, label and send to laboratory in time.

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

2.4 Preparation of test sample

Part of representative sample is taken from the 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 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 factor which may cause the change of residue content.

3 Method of determination

3.1 Principle

The erythromycin residue in the test sample solution are made reacted with Micrococcus luteus, and inhibition zone is formed. It is extracted from the sample with 20% methanol-2-methoxy methane. After centrifugalizing and concentrating, the residues are dissolved in pH 8.0 buffer, which should be defermined by microbiological cylinder-plate method and determined quantitatively by erythromycin standard curve method.

3.2 Reagents and materials

3.2.1 Erythromycin standard: provided by Drug and Biological product Inspection Institute of Public health Branch.

3.2.2 2-methoxy methane: Chemical pure.

3.2.3 Phosphate buffer (pH 8.0): 0.1 mol/L, dissolve 16.73 g K_2HPO_4 and 0.53 g anhydrous KH_2PO_4 in water and dilute to 1 000 mL.

3.2.4 Test strain: Micrococcus lutens, No. 28001.

3.2.5 Erythromycin standard solution: Accurately weigh a proper quantity of erythromycin standard and dissolve in sufficient 2-methoxy methane to make 1 000 μg/mL, and dilute with pH 8.0 buffer to make 100 μg/mL standard stock solution. Store in a refrigerator (which can be used within a week).

3.2.6 Erythromycin standard working solution:

Pippet a certain amount of erythromycin standard stock solution dilute with sufficient buffer to make 0.025, 0.05, 0.10, 0.20, 0.40, 0.80 μg/mL, erythromycin standard working solution.

3.2.7 Nutrient broth (see appendix A1).

3.2.8 Media for strain (see appendix A2).

3.2.9 Media for testing (see appendix A3).

3.3 Apparatus and equipment

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

3.3.2 Stainless steel cylinders: (7.8±0.1)mm(od), (6.0±0.1)mm(id), (10±0.1)mm long.

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

3.3.4 Grinder: electric.

3.3.5 Homogenizer: not less than 10 000 r/min, with homogenizing cylinder (300 mL).

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

3.3.7 Water-bath: 0—60℃±1℃.

3.3.8 Incubator: (35±1)℃.

3.3.9 Autoclave.

3.3.10 Other: pipe, dropper, test tube, centrifugal tube, Roux bottle, conical flask, etc.

3.4 Procedure of determination

3.4.1 Preparation of test solution

Weigh 20.0 g of the sample (accurate to 0.1 g) in homogenouse cup, add 50 mL 20% methanol-2-methoxy methane, homogenize at 9 000—10 000 r/min for 2 min and centrifuge at 4 000 r/min for 10 min, move the supernatant (filter if necessary) into 100 mL volumetric flask. Extract the sample twice again as above, each 20% 2-methanol-2-methoxy methane. Combine the supernatants into the volumetric flask mentioned above and dilute to 100 mL with methanol. Pipet 10 mL extraction into a test tube, then dry with a gentle stream of nitrogen in 40℃ water bath. Remove the tube, add 2 mL pH 8.0 buffer to dissolve the residues and close the tube to prepare for microbiogical determination.

3.4.2 Preparation of test organism

Inoculate strain into nutrient broth (3.2.7), mix well and incubate at (35±1)℃ for (24±2)h. Transfer the incubated culture to a test tube, incubate at (35±1)℃ for 16—18 h. Pipet 3 mL nutrient broth, wash down the growth colony, incubated 1 mL organism suspension to the surface of medium (3.2.8) in the Roux bottle, incubate at (35±1)℃ for (24±2)h. Wash growth colony from the agar surface with 15 mL sterile washing solution. Store this stock suspension in the refrigtrator (4℃) no longer than 2 weeks.

3.4.3 Amount of the bulk suspension

Before determination the concentration of the testing strain the medium should be pre-tested by using 0.05 μg/mL Erythromycin standard working solution, after incubatated when the diameter of inhibited zone caused by standard working solution is over iomm, it is suitable for determination, otherwise the concentration of testing strain should be adjusted.

3.4.4 Preparation of plates

Add 10 mL of medium for base to each sterile petri dish. Distribute the medium evenly and allow to harden on a flat, level surface. Melt test medium (3.2.8 Ai), cool to 45℃, and add the optimum amount of culture per 100 mL as determined above. Mix thoroughly. Add 6.0 mL of this inoculated agar to each of the plates prepared above. Distribute the agar evenly by tilting the plates from side to side in a circular motion. Allow to harden. Place the six cylinders, equally spaced, on the inoculated agar surface of the prepared plates. Use the plates the same day they are prepared.

3.4.5 Preparation of standard curve

The reference concentration is 0.10 μg/mL. Fill three cylinders with 0.10 μg/mL standard solution and three cylinders with one of the other concentrations of the standard, alternating so that each 0.10 μg/mL cylinder is followed by a cylinder of the other concentration. Use three plates for each point in the curve, for a total of 15 plates. The three plates containing the lowest concentration of standard (0.025 μg/mL) are intended to produce negative results; the other 12 plates are used to construct the standard response line. This will give 45 determinations for 0.10 μg/mL and 9 determinations for each of the other points on the line.

Replace the covers on the plates and incubate the plates for (18±2)h at 35℃. after incubation, invert the plates to remove the cylinders. Measure the diameter of each zone of inhibition as accurately as possible (at least to the nearest 0.1 mm). Average the readings of the 0.10 μg/mL concentration and the readings of the point tested on each set of three plates. Also average all 45 readings of the 0.10 μg/mL concentration; this average is the correction point for the curve. Correct the average value obtained for each point to the value it would be if the average of the 0.025 IU/mL readings for that set of three plates was the same as the correction point.

Plot these corrected values, including the average of the 0.025 μg/mL concentration, on 2-cycle, semilog graph paper, placing the concentration in μg/mL on the logarithmic scale and the diameter of the zone of inhibition on the arithmetic scale. Construct the best straight line through the point L and H by using the following equations:

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

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

where

L —Calculated zone diameters for the lowest (0.05 μg/mL) concentrations of the standard respone line, mm;

H —Calculated zone diameters for the highest (0.10 μg/mL) concentrations of the standard respone line, mm;

c —Average zone diameter of 45 zone diameters for the reference concentration (0.10 μg/mL), mm;

a, b, d, e —Corrected average zone diameters for each of the other concentrations used for the standard response line, mm.

3.4.6 Determination of sample solution

Use three plates for each sample, place three sterilized cylinders on each plate respectively, fill the interval cylinders with sample solution and 0.10 μg/mL erythromycin standard working solution, incubate at 30℃ for (18±2) h. After incubation, invert the plates to remove the cyclinders. If there are zone of inhibtion on the position of sample cylinders, measure the diameter of each zone as accurately as possibe.

If the diameter of the zone is equal or more than 10 mm, continue the confirmation test.

3.5 Calculation and expression of result

If the value of the inhibition zone is less than 10 mm, report "Negative of erythromycin residues in sample". On the contrary, read the concentration corresponding to this adjusted sample zone size from the curve.

If the residues of erythromycin in sample need to be expressed by mg/kg, the μg/mL can be converted to mg/kg as follows:

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

where

X —Erythromycin content in test sample, mg/kg;

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

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

Note: If the determination of result is positive (the value of the inhibition zone is equal or more than 10 mm, continue the confirmation test if necessary. According to the method of thin layer chromatography.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.025 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of erythromycin and its corresponding recoveries are:

Fortifying concentration	Recovery
0.05 μg/g	78.27%
0.10 μg/g	80.28%
0.20 μg/g	86.14%

Annex A
(Normative Annex)
Medium

A1 Broth medium

Pancreatic digest of gelatin	5.0 g
Yeast extract	1.5 g
Peptone	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 tube and autoclave at 121℃ for 15 min.

A2 Medium for strain culture

Pancreatic digest of gelatin	6.0 g
Peptone	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. Dispense into conical flasks and autoclave at 121℃ for 15 min.

A3 Medium for testing

Ingredients of the medium is the same as A2, adjust the pH to 8.0±0.1, at autoclave 121℃ for 15 min.