SN

中华人民共和国出入境检验检疫行业标准

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进出口食品中大肠杆菌检验方法 谷氨酸脱羧酶法

Inspection of *Escherichia coli* in food for import and export—Glutamate decarboxylase assay

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前　言

本标准的附录A和附录C是规范性附录，附录B是资料性附录。

本标准由国家认证认可管理委员会提出并归口。

本标准起草单位：中华人民共和国山西出入境检验检疫局。

本标准主要起草人：李卫华、付英文、廉慧锋、张建军、巩红霞、宋洁。

本标准系首次发布的行业出入境检验检疫标准。

进出口食品中大肠杆菌检验方法 谷氨酸脱羧酶法

1 范围

本标准规定了进出口食品中大肠杆菌的抽样和检测方法。

本标准适用于进出口冻肉产品、奶及奶制品、速冻蔬菜、脱水蔬菜、水、食醋、非酒精饮料、核桃仁、保健茶等食品中大肠杆菌的检验。

2 规范性引用文件

下列文件中的条款通过本标准的引用而成为本标准的条款。凡是注日期的引用文件，其随后所有的修改单(不包括勘误的内容)或修订版均不适用于本标准，然而，鼓励根据本标准达成协议的各方研究是否可使用这些文件的最新版本。凡是不注日期的引用文件，其最新版本适用于本标准。

SN 0330 出口食品中微生物学检验通则

3 术语和定义

下列术语和定义适用于本标准。

3.1

大肠杆菌 *Escherichia coli*

又称大肠埃希氏菌，发酵乳糖产酸、产气。吲哚及甲基红(MR)试验阳性，V-P 试验阴性，柠檬酸盐利用试验阴性，谷氨酸脱羧酶(GAD)试验阳性。

3.2

谷氨酸脱羧酶 glutamate decarboxylase (GAD)

促进谷氨酸脱羧的酶。大肠杆菌能特异性产生这种酶，促进谷氨酸脱羧，产生 γ-氨基丁酸和 CO_2。

3.3

最近似值 most possible number(MPN)

一种统计学方法，表示 95%可信限下的推测值，可用于计量群体中的细菌数目。

4 抽样

按 SN 0330 规定执行。

5 测定方法

5.1 方法原理

大肠杆菌在酸性环境下产生谷氨酸脱羧酶，这种酶分解 GAD 反应液中的谷氨酸，产生 γ-氨基丁酸和二氧化碳(区别于肠杆菌科的其他细菌)，使反应液 pH 值发生变化，从而引起反应液中指示剂颜色变化。

5.2 试剂和材料

5.2.1 标准菌株

阳性菌株：大肠杆菌(*Escherichia coli*)；

阴性菌株：肺炎克雷伯氏菌(*Klebsiella pneumoniae*)。

5.2.2 **培养基和试剂**

培养基和试剂见附录 A。

5.3 **仪器设备**

5.3.1 培养箱：35℃±1℃。

5.3.2 高压灭菌锅：121℃±1℃。

5.3.3 离心机：500 g(转速计算方法参见附录 B)。

5.3.4 pH 计：精度±0.1。

5.3.5 均质器：8 000 r/min。

5.3.6 冰箱：0℃～4℃；－18℃以下。

5.3.7 移液管：1 mL、5 mL、10 mL。

5.3.8 试管：20 mm×200 mm、15 mm×150 mm。

5.3.9 螺帽离心试管：10 mL。

5.3.10 发酵管：根据不同样品选择不同型号。

5.4 **样品制备与保存**

5.4.1 **样品制备**

固体或半固体样品：无菌操作称取 25 g 样品，放入装有 225 mL 磷酸缓冲液(PBS)稀释液的灭菌均质杯内，8 000 r/min 均质 1 min～2 min(也可用灭菌研钵研磨代替)，制成 1∶10 的样品匀液。

干粉样品：无菌操作称取 25.0 g 样品，放入装有 225 mL PBS 稀释液和适量玻璃珠的 500 mL 广口瓶(或三角瓶)中，以 30 cm 振幅，7 s 振荡 25 次(也可机械振荡 30 s)，制成 1∶10 的样品匀液。

液体样品：用灭菌移液管吸取 25 mL 样品(吸取样品时，移液管插入液面下不超过 2.5 cm，排出液体时不要将移液管插入稀释液中)，放入盛有 225 mL PBS 稀释液的 500 mL 广口瓶(或三角瓶)中，按上述方法充分振荡，制成 1∶10 的样品匀液。

用 1 mL 移液管准确吸取 1∶10 的样品匀液 1 mL，放入装有 9 mL 磷酸缓冲液的试管中，按上述方法充分振荡，制成 1∶100 的样品稀释液(操作时注意移液管不要与装有稀释液的容器接触)。

按照上述同样的方法依次制成 10 倍系列稀释液(估计样品的污染程度并决定样品的最高稀释度，应保证接种后最高稀释度所有试管为阴性)，整个操作过程不应超过 15 min。

5.4.2 **样品保存**

若样品不能及时测定，冷冻样品应置于－18℃以下冰箱保存；非冷冻易腐食品置于 0℃～4℃冰箱保存。测定前，冷冻样品可于 0℃～4℃融化，时间不超过 18 h，也可在温度不超过 45℃的环境中融化，时间不超过 15 min。

5.5 **测定步骤**

每个样品至少选择三个适当的连续稀释度，每个稀释度接种三管 LST 肉汤，每管接种 1 mL(必要时用双料 LST 肉汤接种 10 mL 液体样品或 10 mL 固体样品匀液)。分别接种一管大肠杆菌、肺炎克雷伯氏菌和 PBS 稀释液作为阳性、阴性和空白对照。置于 35℃±1℃培养 20 h±2 h。取产气试管中的培养液 5 mL，转移到 10 mL 螺帽锥形离心管中，置于离心机中 500 g 离心 10 min，弃去上清液。用 5 mL 磷酸盐缓冲液将沉淀物重新悬浮(机械振荡 1 min～2 min 或手摇振荡至沉淀溶解)，500 g 重复离心一次，弃去上清液。在沉淀物中加入 1.0 mL GAD 反应液，充分振荡混合后置于 35℃±1℃培养，4 h 内观察反应结果。

注：将上述 LST 试管的培养时间延长 24 h 可提高某些被测定样品(如经高温处理的样品)的灵敏度。

5.6 **结果判定和报告**

5.6.1 **结果判定**

GAD 反应液在 4 h 内变为蓝色为判定为阳性反应，其他颜色均判定为阴性反应。

5.6.2 **结果报告**

记录 4 h 内的阳性管数，并对照记录结果查 MPN 表(见附录 C)，报告每克(或毫升)样品中大肠杆菌的 MPN 值。当 MPN 小于 0.3/g(mL)时，可报告为："1 mL(或 1 g)样品中未检出大肠杆菌"。

附 录 A
（规范性附录）
培养基和试剂

A.1 月桂基硫酸盐蛋白胨(LST)肉汤

胰蛋白胨	20 g
氯化钠	5.0 g
乳糖	5.0 g
磷酸氢二钾(K_2HPO_4)	2.75 g
磷酸二氢钾(KH_2KO_4)	2.75 g
月桂基硫酸钠	0.1 g
蒸馏水	1 000 mL

将以上各成分溶解于蒸馏水中(必要时加热)，分装到加有倒立发酵管的 15 mm×150 mm 试管中，每管 10 mL。

双料 LST 肉汤的配制：

除蒸馏水以外的其他成分的量加倍，并分别溶解于蒸馏水中(必要时加热)，分装到加有倒立发酵管的 20 mm×200 mm 试管中，每管 10 mL。

分装好的试管放入高压锅，121℃高压灭菌 15 min。最终 pH 值为 6.8±0.2。

注：月桂基硫酸盐口服有害。使用时应避免吸入本品的粉尘，避免与眼睛及皮肤接触。大量使用应穿适当防护服。与眼睛接触后，应立即用大量水冲洗后请医生诊治。密闭保存于干燥处。

A.2 GAD 反应液

L-谷氨酸	1 g
溴甲酚绿	0.05 g
氯化钠	90 g
曲拉通 X-100	3 mL
无菌水	1 000 mL

上述成分充分溶解后过滤除菌，反应液最终 pH 为 3.4。保存于 4℃冰箱备用。

注：曲拉通 X-100 口服有害，对眼睛、呼吸系统、皮肤有刺激性。大量使用时应穿适当防护服。万一接触到眼睛，应立即用大量水冲洗后请医生诊治。于 0℃～4℃避光干燥保存。

A.3 磷酸盐缓冲液

储备液

磷酸二氢钾(KH_2PO_4)	34.0 g
蒸馏水	500 mL

将磷酸二氢钾(KH_2PO_4)溶于蒸馏水中，用 1 mol/L 氢氧化钠约 175 mL 调至 pH7.2。加蒸馏水至 1 L 贮存于冰箱。

稀释液

取贮存液 1.25 mL，加蒸馏水稀释至 1 000 mL，分装合适的容器后，121℃高压灭菌 15 min。

附 录 B
（资料性附录）
相对离心力的计算

B.1 相对离心力计算见式(B.1)：

$$RCF = 1.118 \times 10^{-5} N^2 r \quad \cdots\cdots (B.1)$$

式中：

N——转头转速，单位为转每分钟(r/min)；

r——转头半径，离心管中轴底部内壁到离心机转轴中心的距离，单位为厘米(cm)；

RCF——相对离心力，单位为重力加速度(g)。

B.2 转速的计算见式(B.2)：

$$N = \sqrt{RCF \times 10^5 / 1.118r} \quad \cdots\cdots (B.2)$$

示例：转头半径 r=9.0 cm，相对离心力 RCF=500 g，通过式(B.2)求出转速：

$$N = \sqrt{500 \times 10^5 / 1.118 \times 9.0} = \sqrt{4\ 969\ 191} = 2\ 230(\text{r/min})$$

附 录 C
（规范性附录）
1 g(mL)样品中最近似值(MPN)表

使用三管法，接种量分别是 0.1 g(mL)、0.01 g(mL)、0.001 g(mL)。

阳性管数			MPN	阳性管数			MPN
0.1	0.01	0.001		0.1	0.01	0.001	
0	0	0	<3	2	0	0	9.1
0	0	1	3	2	0	1	14
0	0	2	6	2	0	2	20
0	0	3	9	2	0	3	26
0	1	0	3	2	1	0	15
0	1	1	6.1	2	1	1	20
0	1	2	9.2	2	1	2	27
0	1	3	12	2	1	3	34
0	2	0	6.2	2	2	0	21
0	2	1	9.3	2	2	1	28
0	2	2	12	2	2	2	35
0	2	3	16	2	2	3	42
0	3	0	9.4	2	3	0	29
0	3	1	13	2	3	1	36
0	3	2	16	2	3	2	44
0	3	3	19	2	3	3	53
1	0	0	3.6	3	0	0	23
1	0	1	7.2	3	0	1	39
1	0	2	11	3	0	2	64
1	0	3	15	3	0	3	95
1	1	0	7.3	3	1	0	43
1	1	1	11	3	1	1	75
1	1	2	15	3	1	2	120
1	1	3	19	3	1	3	160
1	2	0	11	3	2	0	93
1	2	1	15	3	2	1	150
1	2	2	20	3	2	2	210
1	2	3	24	3	2	3	290
1	3	0	16	3	3	0	240
1	3	1	20	3	3	1	460
1	3	2	24	3	3	2	1 100
1	3	3	29	3	3	3	>1 100

注：表内所列检样量如改用 1 g(mL)、0.1 g(mL)和 0.001 g(mL)时，表内数字应相应降低 10 倍；如改用 0.01 g(mL)、0.001 g(mL)和 0.000 1 g(mL)时，则表内的数字应相应增加 10 倍，其余类推。

CONTENTS

Preface

Annex A and Annex C are normative, Annex B are informative.

This standards was proposed and administrated by National Regulatory Commission for Certification and Accreditation.

This standards was drafted by Shanxi Entry-Exit Inspection and Quarantine of the Peoples Republic of China.

The main drafters of this standard are Li weihua, Fu yingwen, Lian huifeng, Zhang jianjun, Gong hongxia, Song jie.

This standard is a professional standard of entry-exit inspection and quarantine promulgated for the first time.

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

Inspection of *Escherichia coli* in food for import and export—Glutamate decarboxylase assay

1 Scope

This standard has stipulated the sample and determination method of *Escherichia coli* in food for import and export.

This standard apply to the *Escherichia coli* inspection of frozen meat product, milk and milk products, quick-freeze vegetable, dehydration vegetable, water, vinegar, non alcoholic beverage, walnut-meat and health care tea etc..

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of this standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below.

SN0330 General guidance for microbiological examination of export foods

3 Terms and Definitions

For the purpose of this standard, the terms and definition given in the following.

3.1

Escherichia coli

Ferment lactose, produce acid and gas. Result of indole and methyl red (MR) test are positive, V-P test is negative and the citrate test is negative, Decarboxylase (GAD) test is positive.

3.2

Glutamate Decarboxylase (GAD)

A kind of enzyme which can promote the decarboxylation action of glutamic acid. The Decarboxylase be produced by *Escherichia coli*, promote the decarboxylation action of glutamic acid, produce γ-propalanine (aminobutyric acid) and CO_2.

3.3

Most Possible Number(MPN)

A kind of statistics method, represent the presumed number under the credible limit of 95%, the

number of active germs can be measured by it.

4 Sampling

Conduct to the related section of Standard SN0330.

5 Determination

5.1 General description of the method

In the acid environment, *Escherichia coli* produce Decarboxylase which can degradation the glutamic acid in GAD reaction liquid and produce γ-propalanine and CO_2 (different from the other bacteria of Enterobacteriaceae), cause the change of pH in medium, and cause the change of color in GAD reaction liquid.

5.2 Reagent and material

5.2.1 Reference culture

Positive reference culture: *Escherichia coli*;

Negative reference culture: *Klebsiella pneumoniae*.

5.2.2 Medium and reagent (refer to Annex A)

5.3 Apparatus and glassware

5.3.1 incubator: 35℃ ± 1℃.

5.3.2 autoclave: 121℃ ± 1℃.

5.3.3 centrifuges: 500 g (refer to annex B).

5.3.4 pH meter: accuracy to ± 0.1.

5.3.5 homegenizor: 8 000 r/min.

5.3.6 refrigerator: 0℃ ~ 4℃; below − 18℃.

5.3.7 pipette: 1 mL, 5 mL, 10 mL.

5.3.8 test tube: 20 mm × 200 mm, 15 mm × 150 mm.

5.3.9 screw cap centrifuge tube:10 mL.

5.3.10 fermentation tube:use different types according to different samples.

5.4 Preparation and preservation of test sample

5.4.1 Preparation of the sample

Solid or half solid sample:take 25 g sample by aseptic manipulation,put into to sterilized homegenizor with 225 mL phosphate buffer solution(PBS),homogenate with homegenizor at 8 000 r/min for 1 min~2 min (or grind with sterilization mortar),and 1 : 10 initial suspension (primary dilution) was prepared.

Dry-powder sample:weighing 25 g sample by aseptic manipulation,put into to 500 mL sterile wide neck flask (or triangle bottle) which have 225 mL phosphate buffer solution and some glass pearl,vibrate quickly with amplitude of 30 cm,for twenty-five times in 7 s. (or replace with mechanical vibration for 30 s). and 1 : 10 initial suspension was prepared.

Liquid sample:Absorb 25 mL liquid sample with fresh sterile pipette (when absorb sample,the tip of pipette which insert under the liquid surface do not exceed 2.5 cm. And the pipette should not touch the dilute liquid or container when discharging liquid),put into to 500 mL sterilized wide neck flask (or triangle bottle) which have 225 mL phosphate buffer solution,vibrate quickly according to the method described above,and 1 : 10 initial suspension was prepared.

Absorb 1 mL 1 : 10 initial suspension with fresh sterile pipette to test tube which have 9 mL phosphate buffer solution,vibrate quickly according to the method described above,and a further decimal dilution (1 : 100) was prepared (notice that the pipette should not touch the dilute liquid or container when discharging liquid).

The further decimal dilutions will be prepared according to the same method described above(estimate the pollution level of sample,prepare a sufficient number of dilutions to ensure that all the tubes for the final dilution will yield a negative result). The entire operation should not exceed 15 min.

5.4.2 Preservation of the test sample

If sample can not be determinated in time,refrigerant food sample should preserved below −18℃; Perishable food should preserved at 0℃~4℃. Before determination,the refrigerant.

Sample should be melt at 0℃~4℃ for 18 h or 45℃ for less than 15 min.

5.5 Determination procedure

For each sample determinated, select at least three consecutive dilutions (all the tubes for the final dilution will yield a negative result), for each of them, using fresh sterile pipette, transfer 1 mL of dilutions respectively to three tube of lauryl sulfate tryptose broth(LST) (inoculate 10 mL liquid sample or initial suspension of solid sample to double-strength LST broth when necessary). and inoculate *Escherichia coli*, *Klebsiella pneumoniae* and Phosphate buffer solution as positive, negative and blank control respectively. incubate all inoculated tube in the incubator set at 35℃ ±1℃ for 20 h±2 h. For each tube incubated showing gas formatin, transfer 5 mL inoculated solution into 10 mL conical screw-cap test tubes, the bacteria are concentrated by centrifugal at 500 g for 10 min, discard the supernatant. Re-suspending the sediments with 5 mL phosphate buffer solution (vibrating 2 min~3 min on vortex agitator or vibration by hand until the sediment dissolve), then centrifugal at 500 g for another 10 mins, discard the supernatant. Transfer 1.0 mL GAD reaction liquid into test tube, vibrating by vortex agitator, incubate in the incubator set at 35℃ ±1℃ for 4 h. Observing reaction result.

note: The sensitivity of the sample (for example the sample handling by high temperature) will be improve when lasting the culture time of LST pipe for another 24 h.

5.6 Judgement and report of result

5.6.1 Judgement of result

The color of GAD reaction liquid change into blue in 4 h indicates a positive reaction, other colors indicate negative reaction.

5.6.2 Report of result

The numbers of positive pipe cultured in 4 h is recorded, check the MPN table (see appendix C) according to the record number. Reports the MPN of *Escherichia coli* in the sample of 1 g(or 1 mL). If the MPN is lower than 0.3 presumptive *Escherichia coli* was used, express the result in the following way: *Escherichia coli* is absent from 1 mL or 1 g sample.

Annex A
(Normative)
Culture medium and reagent

A.1 Lauryl sulfate tryptose broth

Typtose	20.0 g
Sodium chloride	5.0 g
Lactose	5 g
Dipotassium hydrogen phosphate	2.75 g
Potassium dihydrogen phosphate	2.75 g
Sodium lauryl sulfate	0.1 g
Distilled water	1 000 mL

Dissolve the components in the distilled water (by heating if necessary), dispense the medium in quantities of 10 mL into tubes of 15 mm × 150 mm with fermentation tubes in.

Double-strength LST:

Double the quantity of all components except Distilled water, Dissolve the components in the distilled water (by heating if necessary), dispense the medium in quantities of 10 mL into tubes of 20 mm × 200 mm with fermentation tubes in.

Sterilize for 15 min in the autoclave set at 121℃. The pH is 6.8±0.2 after sterilization.

Note: Sodium lauryl sulfate is harmful take orally, avoiding sucks in the dust article contact with eye and skin. Wearing properly protection clothes if use in large quantities. Wash with plenty of waters immediately when contact eye and then see a doctor. Preserve in dry place pressure-tightly.

A.2 GAD reaction liquid

L-glutamic acid	1.0 g
Bromocresol green	0.05 g
NaCl	90.0 g
Triton X-100	3.0 mL
Sterilized water	1 000 mL

The reagent should mixed thoroughly until all the ingredients to be dissolved, sterilize with filter, store in refrigerator set at 4℃. pH 3.4.

Note: Triton X-100 is harmful when take orally, stimulate eye, respiratory system and skin. Wearing properly protection clothes if use in large quantities. Wash with plenty of waters immediately when contact eye and then see a doctor.

Preserve in dry and dark place in 0℃～4℃.

A.3 Phosphate buffer solution(PBS)

Reserve solution

Dipotassium hydrogen phosphate	34.0 g
Distilled water	500 mL

Dissolving Dipotassium hydrogen phosphate in distilled water, adjust the pH using 175 mL sodium hydrate to 7.2, add sterilized water to 1 000 mL. Store in the refrigerator.

Diluent of PBS

Transfer 1.25 mL reserve solution to 1 000 mL volumetric bottle, add distilled water to 1 000 mL, distribute into suitable vessel, sterilize for 15 min in the autoclave set in 121℃.

Annex B
(informative annex)
Calculation of the relative centrifugal force

B.1 Calculation see formula(B.1):

$$RCF = 1.118 \times 10^{-5} N^2 r \quad \cdots\cdots (B.1)$$

In which:

N—revolving speed, the unit is r/min (revolation per minute);

r—Rotor radius, length from the center of runner shaft to the wall of the centrifugal pipe, the unit is cm;

RCF—Relative centrifugal force, the unit is g.

B.2 The revolving speed see formula(B.2):

$$N = \sqrt{RCF \times 10^5 / 1.118 r} \quad \cdots\cdots (B.2)$$

For example: Rotor radius $r = 9.0$ cm, relative centrifugal force $RCF = 500$ g, Then revolving speed can be from the above formula:

$$N = \sqrt{500 \times 10^5 / 1.118 \times 9.0} = \sqrt{4\,969\,191} = 2\,230 (\text{r/min})$$

Annex C
(Normative)
MPN value in 1 g(mL) test sample

Using 3 tues with 0.1,0.01,0.001 g(mL) portions

Number of positive results			MPN	Number of positive results			MPN
0.1	0.01	0.001		0.1	0.01	0.001	
0	0	0	<3	2	0	0	9.1
0	0	1	3	2	0	1	14
0	0	2	6	2	0	2	20
0	0	3	9	2	0	3	26
0	1	0	3	2	1	0	15
0	1	1	6.1	2	1	1	20
0	1	2	9.2	2	1	2	27
0	1	3	12	2	1	3	34
0	2	0	6.2	2	2	0	21
0	2	1	9.3	2	2	1	28
0	2	2	12	2	2	2	35
0	2	3	16	2	2	3	42
0	3	0	9.4	2	3	0	29
0	3	1	13	2	3	1	36
0	3	2	16	2	3	2	44
0	3	3	19	2	3	3	53
1	0	0	3.6	3	0	0	23
1	0	1	7.2	3	0	1	39
1	0	2	11	3	0	2	64
1	0	3	15	3	0	3	95
1	1	0	7.3	3	1	0	43
1	1	1	11	3	1	1	75
1	1	2	15	3	1	2	120
1	1	3	19	3	1	3	160
1	2	0	11	3	2	0	93
1	2	1	15	3	2	1	150
1	2	2	20	3	2	2	210
1	2	3	24	3	2	3	290
1	3	0	16	3	3	0	240
1	3	1	20	3	3	1	460
1	3	2	24	3	3	2	1 100
1	3	3	29	3	3	3	>1 100

Note: Using 3 tubes with 1,0.1 and 0.01 g(mL) portions numbers in table should be divided by 10; Using 3 tubes with 0.01,0.001 and 0.000 1 g(mL) portions numbers in table should be multiplied by 10. The rest deduced by analogy.

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