GB 4789.3-2016

National Food Safety Standard Food Microbiological Examination: Enumeration of Coliforms

食品安全国家标准 食品微生物学检验 大肠菌群计数

National Health and Family Planning Commission of the People's Republic of China
China Food and Drug Administration

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National Standard of the People's Republic of China

GB 4789.3-2016

National Food Safety Standard

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食品微生物学检验 大肠菌群计数

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Preface

This standard replaces GB/T 4789.3-2010 "National Food Safety Standard Food Microbiological Examination: Enumeration of Coliforms", GB/T 4789.32-2002 "Microbiological Examination of Food Hygiene-Examination: Rapid Detection of Coliform" and content concerning enumeration of coliforms in SN/T 0169-2010 "Detection Method of Coliform, Fecal Coliform and Colibacillus in Import and Export Food".

Compared with GB/T 4789.3-2010, major changes of this standard are as follows:

——Examination principle has been added;

——The application scope has been revised;

——Morphological description of typical bacterial colony has been revised;

——Number of colony chosen on the plate has been revised in Method II

——Confirmatory test of method II has been revised

——The report of plate count in method II has been revised.

National Food Safety Standard

Food Microbiological Examination: Enumeration of Coliforms

1. Scope

This standard specifies the method for the enumeration of coliforms in foods.

Method I in this standard is applicable to the enumeration of coliforms in foods which contain lower level of coliforms; Method II is applicable to the enumeration of coliforms in foods which contain higher level of coliforms

2. Terms and definitions

2.1 Coliforms

A cluster of concurrently aerobic and anaerobic gram negative sporeless bacilli which can ferment lactose and generate acid and gas under a certain culture condition.

2.2 Most probable number, MPN

A Poisson distribution-based indirect count method.

3. Examination principle

3.1 MPN method

MPN method is a quantitative detection method combining statistics and microbiology. After the samples were diluted and cultured, the maximum possible number of coliforms in samples could be calculated through probability theory based on minimum dilution degree that colony does not grow and maximum dilution degree that colony grows.

3.2 Plate count

Coliforms can make acid while fermenting the lactose in solid culture medium. With help of indicator, countable red or purple bacterial colonies could be

formed with or without precipitation ring.

4. Apparatus and materials

In addition to the conventional apparatus for sterilization and incubation in microbiological laboratory, other apparatus and materials are as follows:

4.1 Constant temperature incubator: 36℃±1℃.

4.2 Refrigerator: 2℃~5℃.

4.3 Constant temperature water bath: 46℃±1℃.

4.4 Balance: with sensitivity of 0.1g.

4.5 Homogenizer.

4.6 Shaker.

4.7 Sterile pipette: with nominal capacities of 1mL (graduated in 0.01mL division) and 10mL (graduated in 0.1mL division), or micropipette and pipette tips.

4.8 Sterile conical flask: with nominal capacity of 500mL.

4.9 Sterile culture dish: with diameter of 90mm.

4.10 pH meter or pH colorimetric tube or precise pH test paper.

4.11 Colony counter.

5. Culture mediums and reagents

5.1 Lauryl sulfate tryptose (LST) broth: See A.1

5.2 Brilliant green lactose bile (BGLB) broth: See A.2.

5.3 Violet red bile agar (VRBA): See A.3 .

5.4 Sterile phosphate buffer solution: See A.4.

5.5 Sterile physiological saline solution: See A.5.

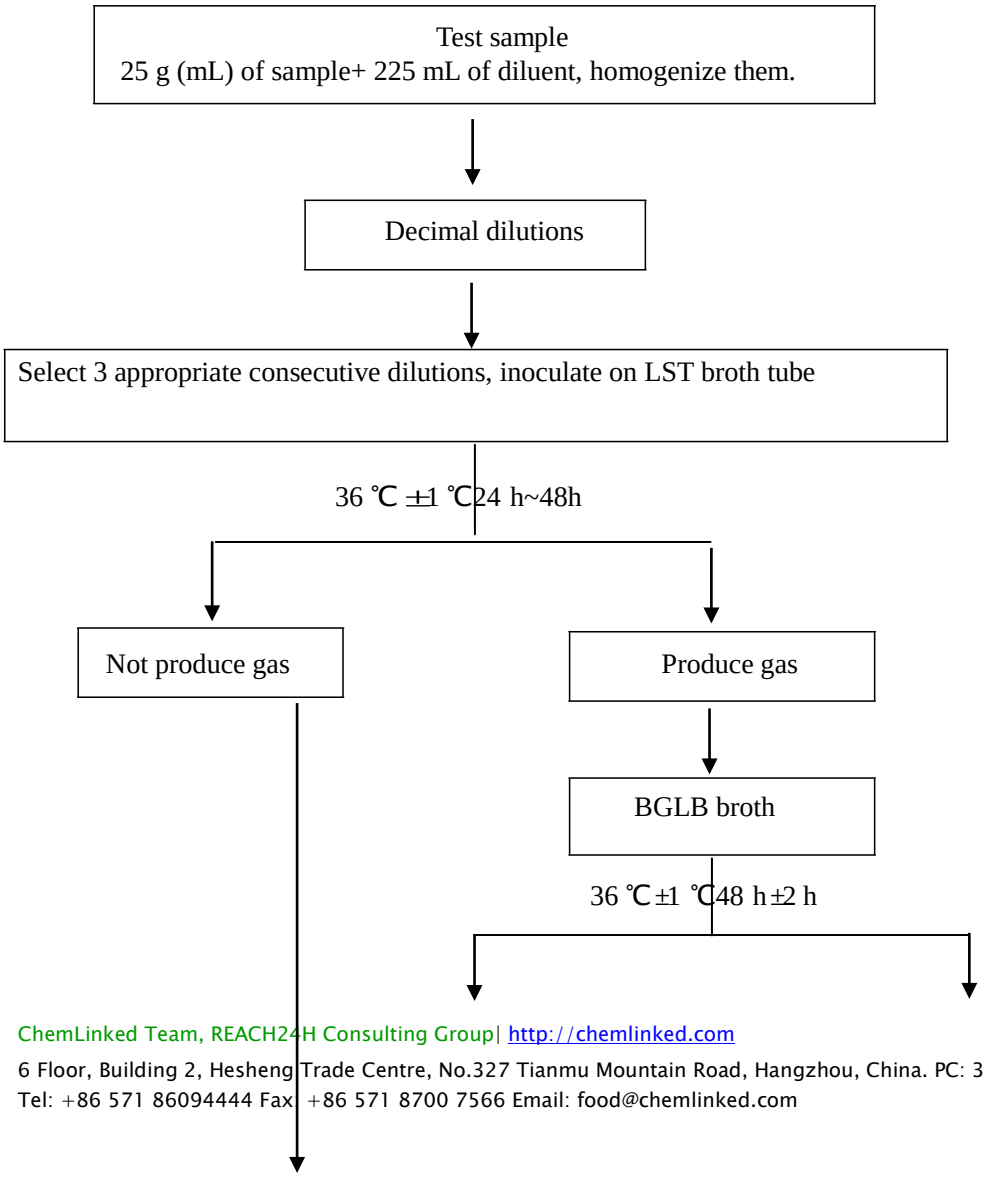
5.6 1mol/L NaOH: See A.6 .

5.7 1mol/L HCL: See Section A.7.

Method I Coliforms MPN count

6. Examination procedures

See Figure 1 for the examination procedures of Coliforms MPN count.



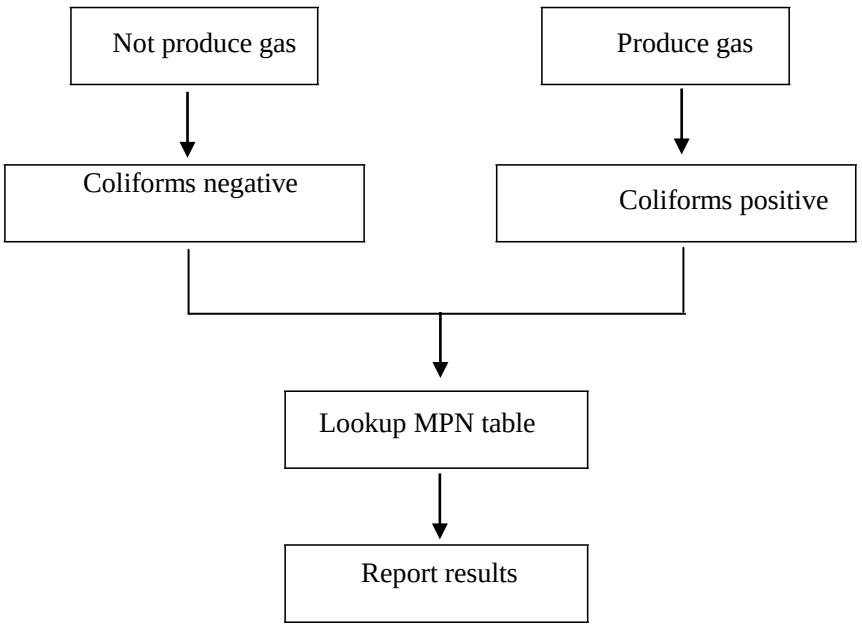


Figure 1 Examination procedures of Coliforms MPN count

7. Operation procedures

7.1 Dilution of sample

7.1.1 Solid and semi-solid sample: Weigh 25g of sample and put it into a sterile homogenizing cup containing 225mL of phosphate buffer solution or physiological saline solution, and homogenize at 8000r/min~10000r/min for 1min~2min, or put it into a sterile homogenizing bag containing 225mL of phosphate buffer solution or physiological saline solution and homogenize it for 1min~2min in a slapping homogenizer to get a 1:10 homogeneous sample solution.

7.1.2 Liquid sample: Pipette 25mL of sample with a sterile pipette, put it into a sterile conical flask (an appropriate amount of sterile glass beads should be put into the flask in advance) containing 225mL of phosphate buffer solution or physiological saline solution, or in other sterile container for adequate shaking or put it into mechanical oscillator for shaking, and mix well to get a 1:10 homogeneous sample solution.

7.1.3 The pH of the homogenous sample solution should be between 6.5 and 7.5. Adjust the pH with 1mol/L sodium hydroxide (NaOH) or 1mol/L

hydrochloric acid (HCL), when necessary.

7.1.4 Pipette 1mL of the above 1:10 homogenous sample solution with a 1mL sterile pipette or micropipette, slowly pour it into a sterile tube containing 9mL of phosphate buffer solution or physiological saline solution along the tube wall (Make sure that the pipette or pipette tip do not touch the diluent), shake or repeatedly blow and beat with another 1mL sterile pipette to mix well and get a 1:100 homogeneous sample solution.

7.1.5 Based on the estimate of sample contamination status and according to the operation procedures above, prepare decimal ascending dilutions. Use a new 1mL sterile pipette or pipette tip for each dilution. From preparation of homogenous sample solution to completion of inoculation, the whole process should be within 15min.

7.2 Primary fermentation test

For each sample, select three appropriate consecutive dilutions (test sample may be selected in case of liquid sample). Inoculate on 3 tubes of lauryl sulfate tryptone (LST) broth for each dilution, 1mL for each tube (if the inoculation amount exceed 1 mL, double-strength LST broth should be used). Incubate at 36℃±1℃ for 24h±2h and observe whether bubbles are generated in the inverse tubes. If there are bubbles, perform secondary fermentation test. If there are no bubbles, continue incubating to 48h±2h. Tubes without bubbles are coliforms negative and tubes with bubbles go through secondary fermentation test.

7.3 Secondary fermentation test

Take 1 loop of culture from each of the gas-producing LST broth tubes with inoculation loops, transfer inoculate them to brilliant green lactose bile (BGLB) broth tubes. Incubate at 36℃±1℃ for 48h±2h and observe bubble-generation. Gas-producing tubes are recorded as coliforms positive.

7.4 Report of most probable number (MPN) of coliforms

Based on the number of tubes which are coliforms BGLB positive verified

according to the procedures in Section 7.3, search the MPN Table (see Annex B) and report MPN of coliforms in each gram (or mL) of sample.

Method II Coliforms plate count

8. Examination procedures

See Figure 2 for the examination procedures of coliforms plate count.

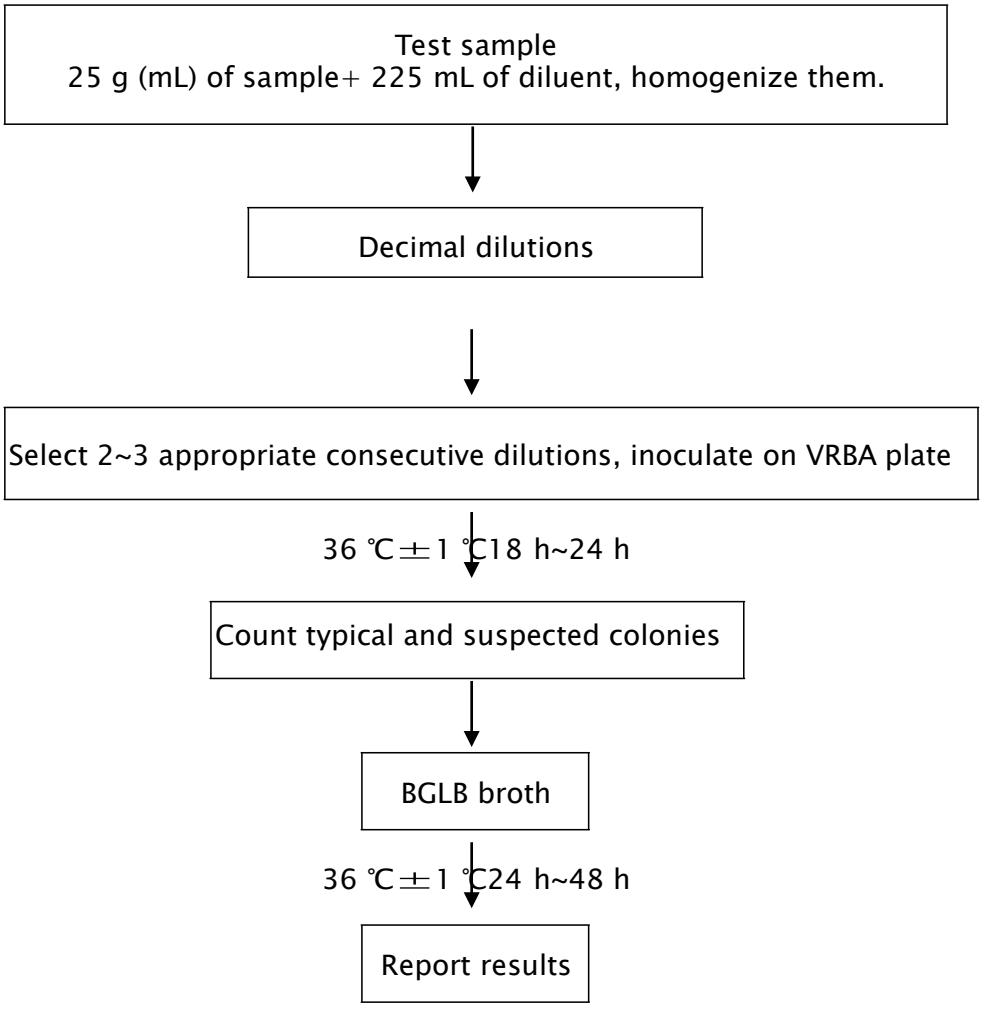


Figure 2 Examination procedures of coliforms plate count

9. Operation procedures

9.1 Dilution of sample

Perform in accordance with Section 7.1.

9.2 Plate count

9.2.1 Select 2~3 appropriate consecutive dilutions, inoculate on 2 sterile plates for each dilution, 1mL for each plate. At the same time, add 1mL of physiological saline solution into a sterile plate, as blank control.

9.2.2 Pour 15mL~20mL of violet red bile agar (VRBA) which has been melted and kept temperature of 46℃ into each plate in time. Rotate the plates carefully to make the culture medium and the sample solution mix well. After the agar is coagulated, add 3mL~4mL of VRBA more to cover the plate surface. Inverse the plate and incubate at 36℃±1℃ for 18h~24h.

9.3 Selection of colony plate count

Select the plates with colony counts between 15CFU and 150CFU and respectively count the typical and suspected coliforms colonies (for example, its colony diameter is smaller than typical coliforms colony) appearing on plates. Typical colonies are purple red, surrounded by red bile salt precipitation ring, with diameter of 0.5mm or bigger. For the plates whose minimum dilution degree is lower than 15CFU, specific number of coliforms colonies should be recorded.

9.4 Verification test

Pick 10 typical and suspected colonies of different types from the VRBA plates, and pick all typical and suspected colonies for those plates with less than 10 coliforms colonies, transfer inoculate them into the BGLB broth tubes respectively. Incubate at 36 ℃ ±1 ℃ for 24h~48h and observe bubble generation. All gas-producing BGLB broth tubes can be reported as coliforms positive.

9.5 Report of coliforms plate count

The percentage of the test tubes finally verified to be coliforms positive multiplies by the number of the plate colonies counted in Section 9.3 and the dilution ratio, that's the number of coliforms in each gram (ml) of the sample. For example, 1mL of 10^{-4} diluted sample solution can produce 100 typical and suspected colonies on VRBA plates, and 10 of them are selected to inoculate into

the BGLB broth tubes, and 6 tubes are verified to be positive. Thus the number of coliforms in this sample is: $100\times6/10\times10^4$/g (mL) = 6.0×10^5 CFU/g (mL). If no coliforms colony is cultured on the plate of all the dilution degree (including the sample liquid), then multiplies the minimum dilution degree by a coefficient less than 1.

Annex A
Culture mediums and reagents

A.1 Lauryl sulfate tryptose (LST) broth

A.1.1 Composition

Typtone or Trypticase	20.0g
Sodium chloride	5.0g
Lactose	5.0g
Potassium hydrogen phosphate	2.75g
Potassium dihydrogen phosphate	2.75g
Lauryl sodium sulfate	0.1g
Distilled water	1000mL

A.1.2 Preparation method

Dissolve all the above components in distilled water and adjust the pH to 6.8±0.2. Dispense 10mL of the solution into each test tube having small glass backward tubes, and sterilize for 15min in the autoclave set at 121℃.

A.2 Brilliant green lactose bile (BGLB) broth

A.2.1 Composition

Peptone	10.0g
Lactose	10.0g
Oxgall or oxbile solution	200.0mL
0.1% brilliant green water solution	13.3mL
Distilled water	1000mL

A.2.2 Preparation method

Dissolve peptone and lactose in about 500mL of distilled water, add 200mL of oxgall solution (dissolve 20.0g of dehydrated oxgall powder in 200mL of

distilled water, and adjust the pH to 7.0~7.5), dilute to 975mL with distilled water, adjust the pH to 7.2±0.1, and then add 13.3mL of 0.1% brilliant green water solution, dilute to 1000mL with distilled water, filter the solution through cotton, and then dispense 10mL of the solution into each test tube having small glass backward tubes. Sterilize for 15min in the autoclave set at 121℃.

A.3 Violet red bile agar (VRBA)

A.3.1 Composition

Peptone	7.0g
Yeast extract	3.0g
Lactose	10.0g
Sodium chloride	5.0g
Bile salt or No.3 bile salt	1.5g
Neutral red	0.03g
Crystal violet	0.002g
Agar	15g~18g
Distilled water	1000mL

A.3.2 Preparation method

Dissolve all the above components in distilled water, stand for a few minutes, stir thoroughly, and adjust the pH to 7.4±0.1. Boil for 2 min, melt and keep the temperature of the culture medium between 45℃~50℃ and pour plate. Prepare it immediately before use, with no more than 3 hours.

A.4 Phosphate buffer solution

A.4.1 Composition

Potassium dihydrogen phosphate (KH_2PO_4)	34.0g
Distilled water	500mL

A.4.2 Preparation method

Stock solution: Weigh 34.0g of potassium dihydrogen phosphate and dissolve in

500mLof distilled water. Adjust the pH to 7.2±0.2 with about 175mL of 1mol/L sodium hydrochloride solution, dilute to 1000mL with distilled water, and store in a refrigerator. Dilute solution: Take 1.25mL of the stock solution, dilute to 1000mL with distilled water, dispense the solution into suitable containers, and sterilize for 15min in the autoclave set at 121℃.

A.5 Sterile physiological saline

A.5.1 Composition

Sodium chloride	8.5g
Distilled water	1000mL

A.5.2 Preparation method

Weigh 8.5g of sodium chloride, dissolve in 1000mL of distilled water, and sterilize for 15 min in the autoclave set at 121℃.

A.6 1mol/L NaOH

A.6.1 Composition

NaOH	40.0g
Distilled water	1000mL

A.6.2 Preparation method

Weigh 40g of sodium hydroxide, dissolve in 1000mL of sterile distilled water.

A.7 1mol/L HCl

A.7.1 Composition

HCl	90mL
Distilled water	1000mL

A.7.2 Preparation method

Take 90mL of concentrated hydrochloric acid, dilute to 1000mL with sterile distilled water.

Annex B
Coliforms most probable number (MPN) index table

B.1 Coliforms most probable number (MPN) index table

See Table B.1 for the most probable number (MPN) of coliforms per gram (or millimeter) of the test sample.

Table B.1 Coliforms most probable number (MPN) index table

mber of positive tubes			MPN	% confidence limit		mber of positive tubes			MPN	% confidence limit	
0.10	0.01	0.001		Lower limit	Upper limit	0.10	0.01	0.001		Lower limit	Upper limit
0	0	0	<3.0	--	9.5	2	2	0	21	4.5	42
0	0	1	3.0	0.15	9.6	2	2	1	28	8.7	94
0	1	0	3.0	0.15	11	2	2	2	35	8.7	94
0	1	1	6.1	1.2	18	2	3	0	29	8.7	94
0	2	0	6.2	1.2	18	2	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	8.7	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1,000
2	0	2	20	4.5	42	3	3	0	240	42	1,000
2	1	0	15	3.7	42	3	3	1	460	90	2,000
2	1	1	20	4.5	42	3	3	2	1100	180	4,100
2	1	2	27	8.7	94	3	3	3	>1100	420	--

Note 1: This table adopts three dilutions [0.1g (or 0.1mL), 0.01g (or 0.01mL) and 0.001g (or 0.001mL), for which, three tubes are inoculated.

Note 2: If the tested amounts as shown in this table are changed to 1g (or 1mL), 0.1g (or 0.1mL) and 0.01g (or 0.01mL), figures in this table should be decreased by 10 times accordingly; if the tested amounts are changed to 0.01g (or 0.01mL), 0.001g (or 0.001mL) and 0.0001g (or 0.0001mL), figures in this table should be increased by 10 times accordingly, so on and so forth.

中华人民共和国国家标准

GB 4789.3—2016

食品安全国家标准
食品微生物学检验　大肠菌群计数

2016-12-23 发布　　2017-06-23 实施

中华人民共和国国家卫生和计划生育委员会
国家食品药品监督管理总局　发布

前　言

本标准代替 GB 4789.3—2010《食品安全国家标准　食品微生物学检验　大肠菌群计数》、GB/T 4789.32—2002《食品卫生微生物学检验　大肠菌群的快速检测》和 SN/T 0169—2010《进出口食品中大肠菌群、粪大肠菌群和大肠杆菌检测方法》大肠菌群计数部分。

本标准与 GB 4789.3—2010 相比，主要变化如下：

——增加了检验原理；

——修改了适用范围；

——修改了典型菌落的形态描述；

——修改了第二法平板菌落数的选择；

——修改了第二法证实试验；

——修改了第二法平板计数的报告。

食品安全国家标准

食品微生物学检验　大肠菌群计数

1　范围

本标准规定了食品中大肠菌群(Coliforms)计数的方法。

本标准第一法适用于大肠菌群含量较低的食品中大肠菌群的计数;第二法适用于大肠菌群含量较高的食品中大肠菌群的计数。

2　术语和定义

2.1

大肠菌群　Coliforms

在一定培养条件下能发酵乳糖、产酸产气的需氧和兼性厌氧革兰氏阴性无芽胞杆菌。

2.2

最可能数　Most probable number;MPN

基于泊松分布的一种间接计数方法。

3　检验原理

3.1　MPN 法

MPN 法是统计学和微生物学结合的一种定量检测法。待测样品经系列稀释并培养后,根据其未生长的最低稀释度与生长的最高稀释度,应用统计学概率论推算出待测样品中大肠菌群的最大可能数。

3.2　平板计数法

大肠菌群在固体培养基中发酵乳糖产酸,在指示剂的作用下形成可计数的红色或紫色,带有或不带有沉淀环的菌落。

4　设备和材料

除微生物实验室常规灭菌及培养设备外,其他设备和材料如下:

4.1　恒温培养箱:36 ℃ ±1 ℃。

4.2　冰箱:2 ℃～5 ℃。

4.3　恒温水浴箱:46℃±1℃。

4.4　天平:感量 0.1 g。

4.5　均质器。

4.6　振荡器。

4.7　无菌吸管:1 mL(具 0.01 mL 刻度)、10 mL(具 0.1 mL 刻度)或微量移液器及吸头。

4.8　无菌锥形瓶:容量 500 mL。

4.9　无菌培养皿:直径 90 mm。

4.10 pH 计或 pH 比色管或精密 pH 试纸。

4.11 菌落计数器。

5 培养基和试剂

5.1 月桂基硫酸盐胰蛋白胨(lauryl sulfate tryptose，LST)肉汤：见 A.1。

5.2 煌绿乳糖胆盐(brilliant green lactose bile，BGLB)肉汤：见 A.2。

5.3 结晶紫中性红胆盐琼脂(violet red bile agar，VRBA)：见 A.3。

5.4 无菌磷酸盐缓冲液：见 A.4。

5.5 无菌生理盐水：见 A.5。

5.6 1 mol/L NaOH 溶液：见 A.6。

5.7 1 mol/L HCl 溶液：见 A.7。

第一法 大肠菌群 MPN 计数法

6 检验程序

大肠菌群 MPN 计数的检验程序见图 1。

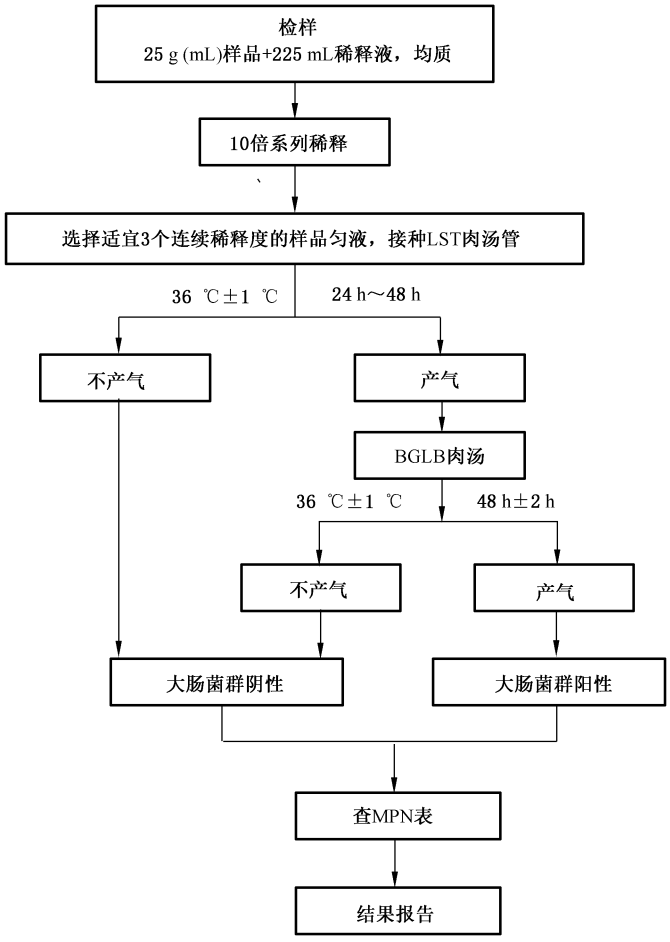


图 1 大肠菌群 MPN 计数法检验程序

7 操作步骤

7.1 样品的稀释

7.1.1 固体和半固体样品：称取 25 g 样品，放入盛有 225 mL 磷酸盐缓冲液或生理盐水的无菌均质杯内，8 000 r/min～10 000 r/min 均质 1 min～2 min，或放入盛有 225 mL 磷酸盐缓冲液或生理盐水的无菌均质袋中，用拍击式均质器拍打 1 min～2 min，制成 1∶10 的样品匀液。

7.1.2 液体样品：以无菌吸管吸取 25 mL 样品置盛有 225 mL 磷酸盐缓冲液或生理盐水的无菌锥形瓶（瓶内预置适当数量的无菌玻璃珠）或其他无菌容器中充分振摇或置于机械振荡器中振摇，充分混匀，制成 1∶10 的样品匀液。

7.1.3 样品匀液的 pH 应在 6.5～7.5 之间，必要时分别用 1 mol/L NaOH 或 1 mol/L HCl 调节。

7.1.4 用 1 mL 无菌吸管或微量移液器吸取 1∶10 样品匀液 1 mL，沿管壁缓缓注入 9 mL 磷酸盐缓冲液或生理盐水的无菌试管中（注意吸管或吸头尖端不要触及稀释液面），振摇试管或换用 1 支 1 mL 无菌吸管反复吹打，使其混合均匀，制成 1∶100 的样品匀液。

7.1.5 根据对样品污染状况的估计，按上述操作，依次制成十倍递增系列稀释样品匀液。每递增稀释 1 次，换用 1 支 1 mL 无菌吸管或吸头。从制备样品匀液至样品接种完毕，全过程不得超过 15 min。

7.2 初发酵试验

每个样品，选择 3 个适宜的连续稀释度的样品匀液（液体样品可以选择原液），每个稀释度接种 3 管月桂基硫酸盐胰蛋白胨（LST）肉汤，每管接种 1 mL（如接种量超过 1 mL，则用双料 LST 肉汤），36 ℃±1 ℃ 培养 24 h±2 h，观察倒管内是否有气泡产生，24 h±2 h 产气者进行复发酵试验（证实试验），如未产气则继续培养至 48 h±2 h，产气者进行复发酵试验。未产气者为大肠菌群阴性。

7.3 复发酵试验（证实试验）

用接种环从产气的 LST 肉汤管中分别取培养物 1 环，移种于煌绿乳糖胆盐肉汤（BGLB）管中，36 ℃±1 ℃培养 48 h±2 h，观察产气情况。产气者，计为大肠菌群阳性管。

7.4 大肠菌群最可能数（MPN）的报告

按 7.3 确证的大肠菌群 BGLB 阳性管数，检索 MPN 表（见附录 B），报告每 g（mL）样品中大肠菌群的 MPN 值。

第二法 大肠菌群平板计数法

8 检验程序

大肠菌群平板计数法的检验程序见图 2。

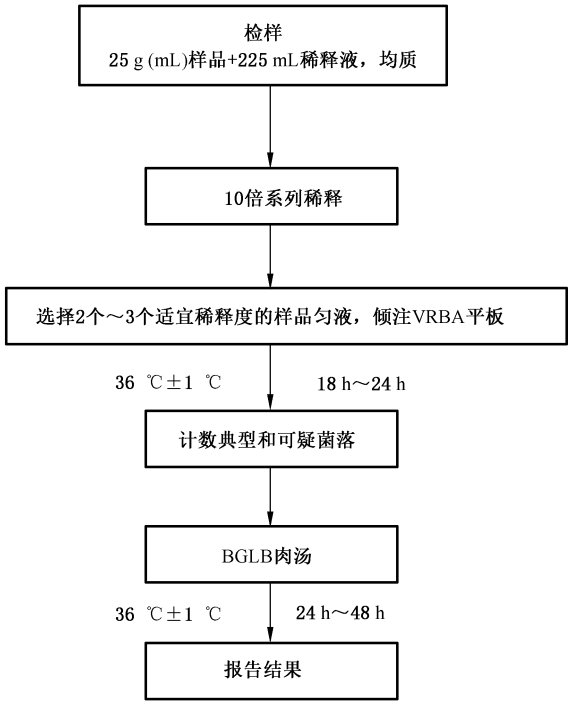


图 2　大肠菌群平板计数法检验程序

9　操作步骤

9.1　样品的稀释

按 7.1 进行。

9.2　平板计数

9.2.1　选取 2 个～3 个适宜的连续稀释度，每个稀释度接种 2 个无菌平皿，每皿 1 mL。同时取 1 mL 生理盐水加入无菌平皿作空白对照。

9.2.2　及时将 15 mL～20 mL 融化并恒温至 46 ℃的结晶紫中性红胆盐琼脂(VRBA)约倾注于每个平皿中。小心旋转平皿，将培养基与样液充分混匀，待琼脂凝固后，再加 3 mL～4 mLVRBA 覆盖平板表层。翻转平板，置于 36 ℃±1 ℃培养 18 h～24 h。

9.3　平板菌落数的选择

选取菌落数在 15 CFU～150 CFU 之间的平板，分别计数平板上出现的典型和可疑大肠菌群菌落(如菌落直径较典型菌落小)。典型菌落为紫红色，菌落周围有红色的胆盐沉淀环，菌落直径为 0.5 mm 或更大，最低稀释度平板低于 15 CFU 的记录具体菌落数。

9.4　证实试验

从 VRBA 平板上挑取 10 个不同类型的典型和可疑菌落，少于 10 个菌落的挑取全部典型和可疑菌落。分别移种于 BGLB 肉汤管内，36 ℃±1 ℃培养 24 h～48 h，观察产气情况。凡 BGLB 肉汤管产气，即可报告为大肠菌群阳性。

9.5 大肠菌群平板计数的报告

经最后证实为大肠菌群阳性的试管比例乘以 9.3 中计数的平板菌落数，再乘以稀释倍数，即为每 g(mL) 样品中大肠菌群数。例：10^{-4}样品稀释液 1 mL，在 VRBA 平板上有 100 个典型和可疑菌落，挑取其中 10 个接种 BGLB 肉汤管，证实有 6 个阳性管，则该样品的大肠菌群数为：$100\times6/10\times10^{4}$/g(mL)＝$6.0\times10^{5}$CFU/g(mL)。若所有稀释度(包括液体样品原液)平板均无菌落生长，则以小于 1 乘以最低稀释倍数计算。

附 录 A
培养基和试剂

A.1 月桂基硫酸盐胰蛋白胨(LST)肉汤

A.1.1 成分

胰蛋白胨或胰酪胨	20.0 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_2PO_4)	2.75 g
月桂基硫酸钠	0.1 g
蒸馏水	1 000 mL

A.1.2 制法

将上述成分溶解于蒸馏水中,调节 pH 至 6.8±0.2。分装到有玻璃小倒管的试管中,每管 10 mL。121 ℃高压灭菌 15 min。

A.2 煌绿乳糖胆盐(BGLB)肉汤

A.2.1 成分

蛋白胨	10.0 g
乳糖	10.0 g
牛胆粉(oxgall 或 oxbile)溶液	200 mL
0.1%煌绿水溶液	13.3 mL
蒸馏水	800 mL

A.2.2 制法

将蛋白胨、乳糖溶于约 500 mL 蒸馏水中,加入牛胆粉溶液 200 mL(将 20.0 g 脱水牛胆粉溶于 200 mL 蒸馏水中,调节 pH 至 7.0～7.5),用蒸馏水稀释到 975 mL,调节 pH 至 7.2±0.1,再加入 0.1%煌绿水溶液 13.3 mL,用蒸馏水补足到 1 000 mL,用棉花过滤后,分装到有玻璃小倒管的试管中,每管 10 mL。121 ℃高压灭菌 15 min。

A.3 结晶紫中性红胆盐琼脂(VRBA)

A.3.1 成分

蛋白胨	7.0 g
酵母膏	3.0 g
乳糖	10.0 g

氯化钠	5.0 g
胆盐或 3 号胆盐	1.5 g
中性红	0.03 g
结晶紫	0.002 g
琼脂	15 g～18 g
蒸馏水	1 000 mL

A.3.2 制法

将上述成分溶于蒸馏水中，静置几分钟，充分搅拌，调节 pH 至 7.4±0.1。煮沸 2 min，将培养基融化并恒温至 45 ℃～50 ℃倾注平板。使用前临时制备，不得超过 3 h。

A.4 磷酸盐缓冲液

A.4.1 成分

磷酸二氢钾（KH_2PO_4）	34.0 g
蒸馏水	500 mL

A.4.2 制法

贮存液：称取 34.0 g 的磷酸二氢钾溶于 500 mL 蒸馏水中，用大约 175 mL 的 1 mol/L 氢氧化钠溶液调节 pH 至 7.2±0.2，用蒸馏水稀释至 1 000 mL 后贮存于冰箱。稀释液：取贮存液 1.25 mL，用蒸馏水稀释至 1 000 mL，分装于适宜容器中，121 ℃高压灭菌 15 min。

A.5 无菌生理盐水

A.5.1 成分

氯化钠	8.5 g
蒸馏水	1 000 mL

A.5.2 制法

称取 8.5 g 氯化钠溶于 1 000 mL 蒸馏水中，121 ℃高压灭菌 15 min。

A.6 1 mol/L NaOH 溶液

A.6.1 成分

NaOH	40.0 g
蒸馏水	1 000 mL

A.6.2 制法

称取 40 g 氢氧化钠溶于 1 000 mL 无菌蒸馏水中。

A.7 1 mol/L HCl 溶液

A.7.1 成分

HCl	90 mL
蒸馏水	1 000 mL

A.7.2 制法

移取浓盐酸 90 mL,用无菌蒸馏水稀释至 1 000 mL。

附 录 B
大肠菌群最可能数(MPN)检索表

B.1 大肠菌群最可能数(MPN)检索表

每 g(mL)检样中大肠菌群最可能数(MPN)的检索见表 B.1。

表 B.1 大肠菌群最可能数(MPN)检索表

阳性管数			MPN	95%可信限		阳性管数			MPN	95%可信限	
0.10	0.01	0.001		下限	上限	0.10	0.01	0.001		下限	上限
0	0	0	<3.0	—	9.5	2	2	0	21	4.5	42
0	0	1	3.0	0.15	9.6	2	2	1	28	8.7	94
0	1	0	3.0	0.15	11	2	2	2	35	8.7	94
0	1	1	6.1	1.2	18	2	3	0	29	8.7	94
0	2	0	6.2	1.2	18	2	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	8.7	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1 000
2	0	2	20	4.5	42	3	3	0	240	42	1 000
2	1	0	15	3.7	42	3	3	1	460	90	2 000
2	1	1	20	4.5	42	3	3	2	1 100	180	4 100
2	1	2	27	8.7	94	3	3	3	>1 100	420	—

注 1：本表采用 3 个稀释度[0.1 g(mL)、0.01 g(mL)、0.001 g(mL)]，每个稀释度接种 3 管。

注 2：表内所列检样量如改用 1 g(mL)、0.1 g(mL)和 0.01 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，则表内数字应相应增高 10 倍，其余类推。

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