

SN

中华人民共和国进出口商品检验行业标准

SN/T 0475—95

出口商品中粪链球菌群 检 验 方 法

**Examination methods of the fecal streptococcus
group in commodities for export**

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出口商品中粪链球菌群检验方法

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1 主题内容与适用范围

本标准规定了出口商品中粪链球菌群的抽样、制样和多管法、滤膜法及平板计数检验方法。
本标准适用于食品中粪链球菌群的检验,也适用于羽绒制品填充料中粪链球菌群的检验。

2 设备与材料

- 2.1 取样工具:镊子、手术剪、刮勺、乳胶手套等。
- 2.2 具盖样品瓶、金属样品桶或其他容器。
- 2.3 高压蒸汽灭菌器。
- 2.4 天平:称量 1 000 g,感量 0.01 g。
- 2.5 均质器及 1 000 mL 具盖均质杯。
- 2.6 移液管:容量 1 mL,10 mL(大口径)。
- 2.7 培养皿:60 mm×15 mm,90 mm×15 mm 或 50 mm×12 mm 玻璃皿或塑料皿。
- 2.8 各种规格的培养基容器。
- 2.9 玻璃、耐高温塑料、陶瓷或不锈钢过滤器。
- 2.10 滤膜:直径 47 mm,微孔直径 $0.45 \pm 0.4 \mu\text{m}$,亦可用性能相同的其他滤膜替代。
- 2.11 振荡器。
- 2.12 恒温培养箱: $36 \pm 1^\circ\text{C}$, $44.5 \pm 0.5^\circ\text{C}$ 。
- 2.13 恒温水浴箱: $44.5 \pm 0.5^\circ\text{C}$ 。

3 培养基

- 3.1 叠氮化钠葡萄糖肉汤[参见附录 A(补充件)中 A1]。
- 3.2 Pfizer 肠球菌选择性琼脂(PSE 琼脂)[参见附录 A(补充件)中 A2]。
- 3.3 KF 链球菌琼脂[参见附录 A(补充件)中 A3]。
- 3.4 脑心浸液[参见附录 A(补充件)中 A4]。
- 3.5 脑心浸液琼脂[参见附录 A(补充件)中 A5]。

4 抽样与制样

4.1 检验批

以不超过 2 500 件(以商品包装件数计,如包、箱、袋等)为一检验批。如另有规定,则按不同商品所规定的报验批为检验批。

同一批商品应具有相同的特征,如包装、标记、产地、规格和等级等。

4.2 抽样数量

在无特殊规定时,按照公式 $\sqrt{N}/2$ (式中 N 为该批商品的总件数),遵循四舍五入规则计算抽样件数;如另有规定,则按不同商品所规定的抽样数进行抽样。

4.3 抽样方法与样品保存

按4.2规定的抽样件数随机抽取,以无菌操作逐件开启。

4.3.1 食品:对于有内包装(如袋、瓶或罐装者)的食品,每件至少取一完整未开封的小包装单位作为原始样品;如无内包装或包装较大者,则使用灭菌工具取样。原始样品总量不少于1 kg(1 L),装入清洁灭菌容器内,加封后标明标记。如不能及时检验,冷冻食品样品应保持冷冻状态;非冷冻样品需在2~5℃保存。

4.3.2 羽绒制品填充料:通常情况下是在填充前临时包内取样,必要时从制品中取样。使用灭菌镊子或戴经消毒的乳胶手套从每件的不同部位抽取100 g作原始样品,放入灭菌样品桶内,加封后标明标记。如不能及时检验,样品需在2~5℃保存。

5 样品制备

5.1 液体食品

用无菌吸管吸取25 mL样品,放入装有225 mL灭菌生理盐水的500 mL稀释瓶中,迅速振摇,充分混匀,制成1:10样品匀液。

5.2 水产品、禽类制品

以无菌操作取25 g样品放入装有225 mL灭菌生理盐水的均质杯内,以8 000 r/min均质1~2 min,制成1:10样品匀液。

5.3 羽绒制品填充料

取10 g样品放入适当大小的锥形烧瓶内,加入400 mL无菌水于锥形烧瓶中,用灭菌胶塞塞紧瓶口,置振荡器振荡10 min。

对上述各样品稀释液可根据需要进一步按10倍递增稀释。

6 接种培养和计数

6.1 多管法

6.1.1 用适当稀释的样液接种一套叠氮化钠葡萄糖肉汤管。接种量为1 mL或1 mL以下时,使用10 mL单料管;接种量为10 mL,使用10 mL双料管。接种后的试管置 $36\pm 1^\circ\text{C}$ 培养 24 ± 2 h,检查各试管的混浊情况。若混浊不明显,继续培养至 48 ± 2 h后记录结果。

6.1.2 培养 24 ± 2 h或 48 ± 2 h之后,对所有呈现混浊的叠氮化钠葡萄糖肉汤管进行确证试验。用接种环将各管的培养物划线于PSE琼脂平板上,倒置平皿于 $36\pm 1^\circ\text{C}$ 培养 24 ± 2 h。琼脂平板上出现的带棕色环的棕黑色菌落,确证为粪链球菌群细菌。

6.1.3 MPN值的记录和计算

根据接种的样品量和确证为粪链球菌群细菌的管数,查MPN表[参见附录B(补充件)],报告每克(毫升)样品中粪链球菌群MPN值。

6.2 滤膜法

6.2.1 平板培养基制备:倾注或吸取4~5 mL融化的KF琼脂于平皿(60 mm×15 mm)中,若平板表面有气泡,可用火焰灼除。若使用盖合紧密的塑料培养皿(50 mm×12 mm),可预先制成储备平板,于4~10℃冰箱内保存4周效果不减。

6.2.2 样品量的选择与样品过滤:根据样品的污染程度,使用样液的量为100,10,1,0.1或0.01 mL。使用灭菌滤膜过滤样液,以能在滤膜上生长出20~100个菌落为宜。

6.2.3 接种与培养:将已滤过样液的滤膜紧贴于KF琼脂培养基表面上,膜下避免出现气泡。倒置平皿于 $36\pm 1^\circ\text{C}$ 培养 48 ± 2 h。

6.2.4 粪链球菌群的计数与计算:粪链球菌群细菌在 KF 琼脂平板上的滤膜上形成暗红色至粉红色菌落,使用低倍大视野双目解剖镜或效能相当的其他光学仪器帮助计数。选择生长 20~100 个菌落的滤膜,根据所使用的样品量,算出该样品每克(毫升)中的粪链球菌群数。

6.2.5 确证试验:由于 KF 培养基有良好的选择性能,在 KF 琼脂平板上的滤膜上生长的红色或粉红色菌落,实际上都是粪链球菌群细菌。按下列程序作进一步确证试验:将滤膜上的典型菌落接种于脑心浸液琼脂斜面,置 $36 \pm 1^\circ\text{C}$ 培养 48 ± 2 h;用其培养物做过氧化氢酶试验,过氧化氢的浓度为 3%。阳性反应者为非链球菌群细菌。阴性反应者接种于脑心浸液肉汤,置 $45.5 \pm 0.5^\circ\text{C}$ 培养 48 ± 2 h;同样方式接种一管胆盐肉汤(无菌 10%牛胆液 40 mL 加入 60 mL 无菌脑心浸液配成),置 $36 \pm 1^\circ\text{C}$ 培养 3 d。在上述两种情况下生长者为粪链球菌群细菌。

6.3 平板计数法

6.3.1 平板制备:按附录 A(补充件)中 A2 或 A4 制备琼脂培养基,倾注平板前将融化的培养基于 $45 \sim 50^\circ\text{C}$ 保温,保温时间不要超过 4 h。

6.3.2 接种与培养:取 1 mL 适当稀释的样液加入 90 mm×15 mm 的培养皿内,倾入 12~15 mL 已融化约 45°C 的 KF 或 PSE 琼脂培养基,轻轻转动培养皿,使样液与培养基充分混合,让细菌均匀分散在培养基内。从样品稀释液制备至倾注平板时间不要超过 20 min。待平板凝固后倒置平皿进行培养;KF 平板置 $36 \pm 1^\circ\text{C}$ 培养 48 ± 2 h;PSE 平板置 $36 \pm 1^\circ\text{C}$ 培养 24 ± 2 h。

6.3.3 菌落计数及报告结果:粪链球菌群细菌在 KF 平板上形成暗红至粉红色菌落,边缘整齐。表面以下菌落常呈椭圆或晶体状;在 PSE 琼脂平板上形成带棕色环的棕黑色菌落。使用有适当光源的低倍大视野双目解剖镜或效能相当的其他光学仪器,对有 30~300 个菌落的平板进行菌落计数。按粪链球菌群数/g(mL)报告结果。

若需进一步鉴定分离的粪链球菌,可参照附录 C(参考件)进行。

附录 A
培养基
(补充件)

A1 叠氮化钠葡萄糖肉汤

牛肉浸膏	4.5 g
胰蛋白胨或多肽	15.0 g
葡萄糖	7.5 g
氯化钠	7.5 g
叠氮化钠(NaN_3)	0.2 g
蒸馏水	1 000 mL

以上各成分混匀,不断搅拌加热溶解。用适当大小的试管分装,每管 10 mL,121℃ 高压灭菌 15 min,灭菌后的培养基 pH 约为 7.2。若制备双料浓度的叠氮化钠葡萄糖肉汤,可将上述配方中蒸馏水改为 500 mL。

A2 Pfizer 肠球菌选择性琼脂(PSE 琼脂)

蛋白胨	20.0 g
酵母浸膏	5.0 g
细菌学用胆汁	10.0 g
氯化钠	5.0 g
柠檬酸钠	1.0 g
七叶甙(Esculin)	1.0 g
柠檬酸铁铵	0.5 g
叠氮化钠(NaN_3)	0.25 g
琼脂	15.0 g
蒸馏水	1 000 mL

灭菌后的 pH 为 7.1。于 45~50℃ 保温培养基,从保温开始至倾注平板的时间不要超过 4 h。

A3 KF 链球菌琼脂

3号胨或多肽	10.0 g
酵母浸膏	10.0 g
氯化钠	5.0 g
甘油磷酸钠	10.0 g
麦芽糖	20.0 g
乳糖	1.0 g
叠氮化钠	0.4 g
琼脂	20.0 g
蒸馏水	1 000 mL

将各成分加热溶解,用 10% 的碳酸钠调整 pH 值至 7.2,121℃ 灭菌 15 min,冷却至 50~60℃ 时加入

无菌 1% 氯化三苯四氮唑(2,3,5-triphenyltetrazolium chloride)水溶液 10 mL。培养基于 45~50℃ 保温不要超过 4 h。倾注或移取 4~5 mLKF 琼脂至 60 mm×15 mm 的皿中,若出现气泡可用火焰灼除。若使用盖合紧密的塑料皿(50 mm×12 mm),可预先制成平板,置 4~10℃ 储存,4 周内可使用。

A4 脑心浸液

小牛脑浸液(固体)	12.5 g
牛心浸液(固体)	5.0 g
胨	10.0 g
葡萄糖	2.0 g
氯化钠	5.0 g
磷酸氢二钠	2.5 g
蒸馏水	1 000 mL

灭菌后的 pH 值为 7.4。

A5 脑心浸液琼脂

在脑心浸液中加入 15.0 g 琼脂,灭菌后的 pH 为 7.4。制成试管斜面备用。

附录 B

1 g 样品中最可能数(MPN)表
(补充件)

使用三管法,接种量为 0.1,0.01,0.001 g(mL)。

表 B1

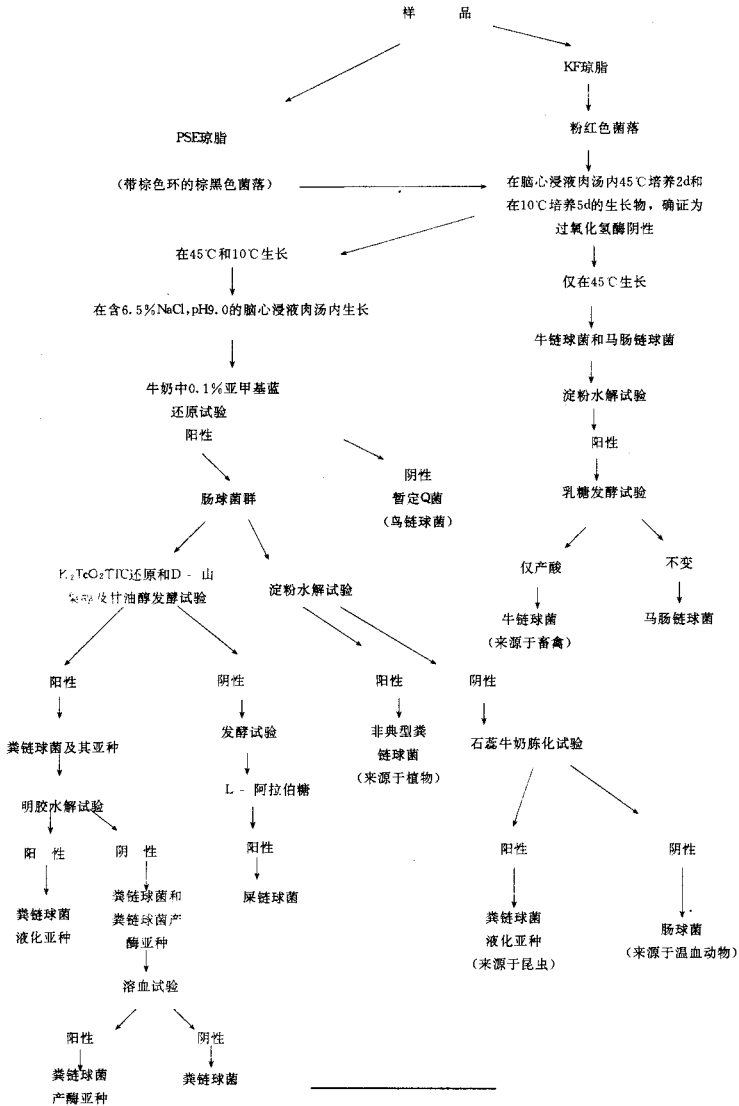
阳性管数				阳性管数			
0.1	0.01	0.001	MPN	0.1	0.01	0.001	MPN
0	0	0	<3	0	3	1	13
0	0	1	3	0	3	2	16
0	0	2	6	0	3	3	19
0	0	3	9	1	0	0	3.6
0	1	0	3	1	0	1	7.2
0	1	1	6.1	1	0	2	11
0	1	2	9.2	1	0	3	15
0	1	3	12	1	1	0	7.3
0	2	0	6.2	1	1	1	11
0	2	1	9.3	1	1	2	15
0	2	2	12	1	1	3	19
0	2	3	16	1	2	0	11
0	3	0	9.4	1	2	1	15

续表 B1

阳性管数				阳性管数			
0.1	0.01	0.001	MPN	0.1	0.01	0.001	MPN
1	2	2	20	2	3	1	36
1	2	3	24	2	3	2	44
1	3	0	16	2	3	3	53
1	3	1	20	3	0	0	23
1	3	2	24	3	0	1	39
1	3	3	29	3	0	2	64
2	0	0	9.1	3	0	3	95
2	0	1	14	3	1	0	43
2	0	2	20	3	1	1	75
2	0	3	26	3	1	2	120
2	1	0	15	3	1	3	160
2	1	1	20	3	2	0	93
2	1	2	27	3	2	1	150
2	1	3	34	3	2	2	210
2	2	0	21	3	2	3	290
2	2	1	28	3	3	0	240
2	2	2	35	3	3	1	460
2	2	3	42	3	3	2	1 100
2	3	0	29	3	3	3	>1 100

注：表内所列样品量如改为 1,0.1,0.01 g(mL)时，表内数字应相应降低 10 倍；如改为 0.01,0.001,0.000 1 g(mL)时，则表内数字相应增加 10 倍，其余可类推。

附录 C
粪链球菌鉴定简图
(参考件)



附加说明：

本标准由中华人民共和国国家进出口商品检验局提出。

本标准由中华人民共和国湖北进出口商品检验局负责起草。

本标准主要起草人张宗显、王丽。

**Professional Standard of the People's Republic
of China for Import and Export Commodity Inspection**

SN/T 0475—95

**Examination methods of the fecal streptococcus
group in commodities for export**

1 Scope and field of application

This standard specifies the methods of sampling, sample preparation, multiple-tube technique, membrane filter technique and plate count of the fecal streptococcus group in commodities for export.

This standard is applicable to the examination of the fecal streptococcus group in food and also in filler of feather products for export.

2 Apparatus and materials

- 2.1 Sampling tools, sampling device suitable for products, such as scissors, forceps, scoop and latex glove etc.
- 2.2 Sampling jars, metallic barrels or containers with suitable covers.
- 2.3 Autoclave.
- 2.4 Balance, with weights, 1 000 g capacity, sensitivity of 0.01 g.
- 2.5 Homogenizer, homogenizing cup of 1 000 mL capacity, with suitable covers.
- 2.6 Pipets (large bore), 1 and 10 mL graduated.
- 2.7 Petri dishes, 60 mm×15 mm, 90mm×15 mm or 50 mm×12 mm, glass or plastic.
- 2.8 Medium containers with different capacity.
- 2.9 Filters, glass, plastic, ceramic or stainless steel.
- 2.10 Filtering membrane, 47 mm in diameter, $0.45 \pm 0.04 \mu\text{m}$ in diameter of millipore or other membrane equivalent.
- 2.11 Shaker.
- 2.12 Incubators maintained at $36 \pm 1^\circ\text{C}$ and $44.5 \pm 0.5^\circ\text{C}$ respectively.
- 2.13 Constant temperature water baths maintained at $44.5 \pm 0.5^\circ\text{C}$.

3 Media

- 3.1 Azide dextrose broth [refer to appendix A(Supplement) A1].
- 3.2 Pfizer selective enterococcus(PSE) agar[refer to appendix A(Supplement) A2].
- 3.3 KF streptococcus agar [refer to appendix A(Supplement) A3].
- 3.4 Brain-heart infusion [refer to appendix A(Supplement) A4].
- 3.5 Brain-heart infusion agar[refer to appendix A(Supplement) A5].

Approved by the State Administration of Import and Export
Commodity Inspection of the People's Republic of China on
Sep. 6, 1995

Implemented from
Jan. 1, 1996

4 Sampling and sample preparation

4.1 Inspection lot

The quantity of an inspection lot should not be more than 2 500 packages. If otherwise specified, regard quantity of package specified by different commodity as an inspection lot.

The characteristics of the cargo within the same inspection lot such as packing, mark, origin, specification and grade etc., should be the same.

4.2 Quantity of the sample taken

In the absence of stipulation, the quantity of sample to be taken is calculated by the formula $\sqrt{N}/2$ (N is the total number of package) and the rule rounding off to the whole number. If otherwise specified, the quantity of sample to be taken is the same that specified by different commodity.

4.3 Sampling procedure and storage of sample

A number of packages specified in 4.2 is taken at random and opened one by one aseptically.

4.3.1 Food: For foods which have internal packing (i. e. bag, bottle, can), from each package at least one small unopened package shall be taken as primary sample; in absence of internal packing or more large packing, cargo is sampled by sterile tool. Total weight of all primary samples should be not less than 1 kg (1 L), the samples shall be placed into a sterile clean container, then sealed and labeled. If test is not implemented in time, the frozen food should be maintained under frozen state. The unfrozen sample shall be stored at 2 to 5°C.

4.3.2 Filler of feather products: The sample should be taken from temporary package before filling. If necessary, sample shall be taken from finished products. 100 g filler shall be taken as primary sample by sterile scissors or hand in sterile latex glove from different position in each temporary package and placed into a sterile barrel, then sealed and labeled.

5 Preparation of test sample

5.1 Liquid food

With sterile pipet transfer 25 mL of sample into flask (capacity in 500 mL) with 225 mL of sterile physiological saline, shake rapidly, mix thoroughly, to bring about uniform sample dilution at one tenth.

5.2 Fishery and poultry products

Aseptically weigh 25 g of sample into homogenizing cup with 225 mL of sterile physiological saline, homogenize at 8 000 r/min for 1—2 min, to bring about uniform sample dilution at one tenth.

5.3 Filler of feather products

Take 10 g of sample into proper flask, to which add 400 mL of sterile water and mouth of flask is plugged with sterile rubber plug, shake on shaker for 10 min.

The above sample dilution shall be further diluted as decimal, if necessary.

6 Inoculation, incubation and count

6.1 Multiple-tube technique

6.1.1 Inoculate a series of tube containing azide dextrose broth with appropriate sample dilution. Use 10 mL single-strength broth for inocula of 1 mL or less and 10 mL double-strength broth for 10 mL inocula. Incubate inoculated tubes at $36 \pm 1^\circ\text{C}$. Examine each tube for turbidity at the end of 24 ± 2 h. If no turbidity is present, reincubate, and read again at the end of 48 ± 2 h.

6.1.2 Subject all azide dextrose broth tubes showing turbidity after 24 ± 2 h or 48 ± 2 h incubation to the confirmed test.

Streak a loopful of growth from each positive azide dextrose broth tube on a petri dish containing PSE agar. Incubate the inverted dish at $36 \pm 1^\circ\text{C}$ for 24 ± 2 h. Brownish black colonies with brown halos on the plate are confirmed as the fecal streptococci.

6.1.3 Recording and computing of Most Probable Number(MPN)

Refer to table of MPN [Appendix B(Supplement)]based on quantities of the inoculated sample and the numbers of tube confirmed the presence of fecal streptococci, report most probable number of fecal streptococci per 1 g(mL) of sample.

6.2 Membrane Filter Technique

6.2.1 Preparation of culture dishes; Pour or pipet 4 to 5 mL liquified KF streptococcus agar into culture dishes (60 mm \times 15 mm). Flame surface if necessary to eliminate bubbles. If tight-fitting plastic dishes (50 mm \times 12 mm) are used, make a stock of prepared dishes in advance and store at 4 to 10°C for use within a 4 week period.

6.2.2 Selection of sample size and filtration; Use volumes of from 100 to 10, 1, 0.1 or 0.01 mL depending on pollution present of sample. Filter dilution of sample through a sterile membrane to give 20 to 100 colonies on membrane surface.

6.2.3 Inoculation and culture

Transfer membrane filtrated the dilution of sample directly to agar medium in petri dish, avoiding air bubbles. Invert culture plates and incubate at $36 \pm 1^\circ\text{C}$ for 48 ± 2 h.

6.2.4 Counting and calculating of fecal streptococci; Colonies produced by fecal streptococci on membrane placed on KF agar plate are dark red to pink. Count with the aid of a low-power binocular wide-field dissecting microscope or equivalent optical device. Compute the numbers per 1g(mL) from sample quantities producing membrane filter counts within the desired 20 to 100 fecal streptococcus colony range.

6.2.5 Confirmed test; Because of the good selectivity of KF medium practically all red and pink colonies growing on membrane placed on which are fecal streptococci. To verify use the following procedures; pick selected typical colonies from membrane and inoculate onto a brain-heart infusion agar slant, incubate at $36 \pm 1^\circ\text{C}$ for 48 ± 2 h. When growth is detected on brain-heart infusion agar slant, from which transfer a loopful of growth to a clean glass slide and add a few drops of 3% hydrogen peroxide to the smear. The presence of bubbles constitutes a positive catalase test which indicates the presence of nonstreptococcus species and discontinue confirmation. The absence of bubbles constitutes a negative catalase test. Continue confirmation as indicated below. Transfer a loopful of growth from the brain-heart infusion agar into brain-heart infusion broth and incubate at $44.5 \pm 0.5^\circ\text{C}$ for 48 ± 2 h. Also transfer a loopful of growth into bile broth medium which is prepared by adding 40 ml. sterile 10% oxgall solution to 60 mL sterile brain-heart infusion broth and incubate $36 \pm 1^\circ\text{C}$ for 3 days. Growth in the above both media constitutes a positive test for fecal streptococci.

6.3 Fecal streptococcal plate count

6.3.1 Plating; Prepare agar medium as in appendix A(Supplement) A2 or A4 and hold in a water bath at $45-50^\circ\text{C}$ before making pour plates. Discard any liquid agar medium held over 4 h.

6.3.2 Inoculation and incubation; Place 1 mL of suitable sample or dilution in 90 mm \times 15 mm petri dish, pour 12 to 15 mL of liquefied KF or PSE agar medium about 45°C into each culture dish containing sample or dilution. Thoroughly mix agar and sample together for a uniform dispersion of organisms

and medium over dish bottom by gently rotating the dish on a flat surface. Do not let more than 20 min between making the dilution and pouring the plates. Solidify agar plates and place immediately in inverted position in an incubator. Incubate KF fecal streptococcus pour plates at $36\pm 1^{\circ}\text{C}$ for 48 ± 2 h. Incubate PSE fecal streptococcus pour plates at $36\pm 1^{\circ}\text{C}$ for 24 ± 2 h.

6.3.3 Count colony and report result; Surface and subsurface colonies produced by fecal streptococci on KF agar are dark red to pink with entire edges. Subsurface colonies frequently are ellipsoidal or lens-shaped. Fecal streptococci on PSE agar give brownish-black colonies with brown halos. With a low power binocular wide-field dissecting microscope with suitable light source or equivalent optical device count colonies falling between 30 to 300 CFU/plate. Report results as fecal streptococci per 1 g(mL).

To identify the isolated fecal streptococci refer to appendix C (for reference), if necessary.

Appendix A
Culture media
(Supplement)

A1 Azide dextrose broth

Beef extract	4.5 g
Tryptone or polypeptone	15.0 g
Sodium chloride	7.5 g
Glucose	7.5 g
Sodium azide (NaN ₃)	0.2 g
Distilled water	1 000 mL

Uniformly mix all ingredients, heat with agitation to dissolve dispense 10 mL portion into appropriate tube, autoclave at 121°C for 15 min. pH should be about 7.2 after sterilization.

A2 Pfizer selective enterococcus (PSE) agar

Peptone	20.0 g
Yeast extract	5.0 g
Bacteriological bile	10.0 g
Sodium chloride	5.0 g
Sodium citrate	1.0 g
Esculin	1.0 g
Ferric ammonium citrate	0.5 g
Sodium azide (NaN ₃)	0.25 g
Agar	15.0 g
Distilled water	1 000 mL

pH should be 7.1 after sterilization. Hold medium for not more than 4 h at 45~50°C before plates are poured.

A3 KF Streptococcus agar

Proteose peptone No. 3 or Polypeptone	10.0 g
Yeast extract	10.0 g
Sodium chloride	5.0 g
Sodium glycerophosphate	10.0 g
Maltose	20.0 g
Lactose	1.0 g
Sodium azide (NaN ₃)	0.4 g
Agar	20.0 g
Distilled water	1 000 mL

Heat all ingredients to dissolve. Autoclave at 121°C for 15 min, cool to 50-60°C and add 10 mL sterile 1% aqueous solution of 2,3,5-triphenyltetrazolium chloride. Adjust pH to 7.2 with 10%

Na_2CO_3 , if necessary. Hold medium for not more than 4 h at 45—50°C before plates are poured. Pour or pipet 4 to 5 mL liquefied KF streptococcus agar into culture dishes (60 mm×15 mm). Flame surface if necessary to eliminate bubbles. If tight-fitting plastic dishes (50 mm×12 mm) are used, make a stock of prepared dishes in advance and store at 4—10°C for use within a 4-week period.

A4 Brain-heart infusion

Calf brains infusion solids	12.5 g
Beef heart infusion solids	5.0 g
Proteose peptone	10.0 g
Glucose	2.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate	2.5 g
Distilled water	1 000 mL

pH should be 7.4 after sterilization.

A5 Brain-heart infusion agar

Add 15.0 g of agar to the brain-heart infusion, the pH should be 7.4 after sterilization. Make a brain-heart infusion agar slant for use.

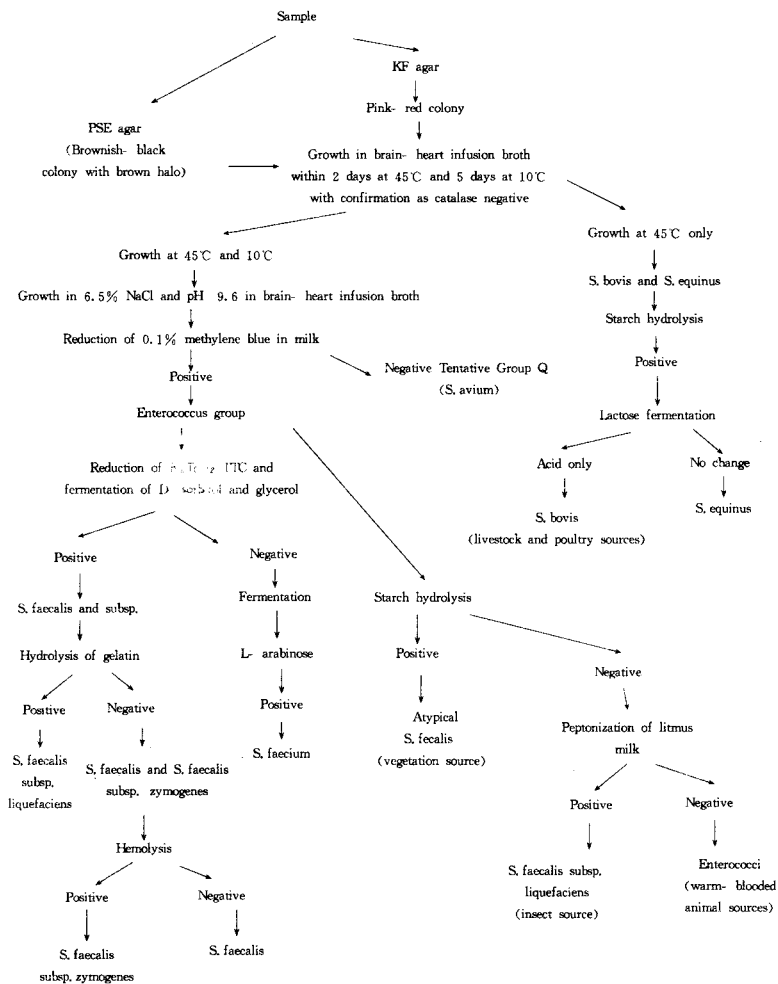
Appendix B
Table of most probable number per 1 g (mL) of sample
(Supplement)

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

number of positive tubes				number of positive tubes			
0.1	0.01	0.001	MPN	0.1	0.01	0.001	MPN
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.

Appendix C
Schematic outline for identification of fecal streptococci
 (For reference)



Additional explanations:

This standard was proposed by the State Administration of Import and Export Commodity Inspection of the People's Republic of China.

This standard was drafted by the Hubei Import and Export Commodity Inspection Bureau of the People's Republic of China.

This standard was mainly drafted by Zhang Zongxian and Wang Li.

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