

SN

中华人民共和国出入境检验检疫行业标准

SN/T 1538.2—2007

培养基制备指南 第2部分：培养基性能测试实用指南

Guidelines on preparation and production of culture media—
Part 2: Practical guidelines on performance testing of culture media

(ISO/TS 11133-2:2003, Microbiology of food and animal feeding stuffs—
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前 言

SN/T 1538《培养基制备指南》分为两个部分：

——第 1 部分：实验室培养基制备质量保证通则；

——第 2 部分：培养基性能测试实用指南。

本部分为 SN/T 1538 的第 2 部分，对应于 ISO/TS 11133-2:2003《食品和动物饲料——培养基制备指南——培养基性能测试实用指南》，与其的一致性程度为修改采用，主要差异如下：

——按照 GB/T 1.1 标准要求 and 汉语习惯对一些编排格式进行了修改；

——将一些国际标准的表述方式改为适用于我国标准的表述方式；

——对“前言”和“引言”进行了修改；

——对“规范性引用文件”的描述按照国家标准的要求进行了修改；

——将 ISO 标准中的部分“注”(4.2.2, 4.2.3.2, 4.2.3.3, 5.2.1, 5.3.1.2, 5.3.3, 5.4, 5.4.2.1)按中文标准习惯改为正文；

——将标准中的国际标准用相应的国家标准或行业标准替代如：用 GB/T 2828.1 替代了 ISO 2589-1:1999；用 SN/T 1538.1—2005 替代了 ISO/TS 11133-1:2000；

——将 4.1.2“国际标准中提到的培养基”改为“国际/国家标准中提到的培养基”；

——对 4.2.2 中对培养基微生物污染的要求进行了限定；

——对 4.2.3.1 和 5.4.2.1 的部分段落顺序进行了调整；

在 5.3 和 5.4 对采用其他方法的培养基性能测试作了说明；

——将标准中提及的用接种环接种的量(5.3.2.2, 5.3.3.2, 5.4.3.1, 5.4.4.2)全部统一为用“直径 3 mm 的接种环接种一环”；

——在附录 B 中增加了注解“可采用能溯源到以上菌株的其他菌株进行培养基的性能测试”。

本部分的附录 B 为规范性附录，附录 A 为资料性附录。

本部分由国家认证认可监督管理委员会提出并归口。

本部分由中华人民共和国山西出入境检验检疫局，中国检验检疫科学研究院，中华人民共和国内蒙古出入境检验检疫局，中华人民共和国广西出入境检验检疫局，中华人民共和国上海出入境检验检疫局，中华人民共和国吉林出入境检验检疫局，中华人民共和国山东出入境检验检疫局负责起草。

本部分主要起草人：李卫华、赵贵明、刘中学、刘军义、李小虹、王振国、雷质文。

本部分系首次发布的出入境检验检疫行业标准。

引 言

微生物学实验室的主要工作是对各种微生物进行保存、复苏、培养、检测和(或)计数,所用的培养基应对样品和被测微生物都具有特异性。微生物学分析过程则要求培养基符合标准或满足最低性能要求,并保证能够获得重现性结果,这样才能使分析结果准确、可靠。

培养基制备指南是微生物学实验室质量控制程序的一个必要组成部分,该标准能对培养基进行有效的监控,从而保证结果的可信和有效性。

本部分是 SN/T 1538 系列标准的第 2 部分,提出了培养基控制要求和常用培养基的性能测试方法,为培养基的性能测定提供了依据,从根本上保证了培养基的质量。

培养基制备指南

第 2 部分:培养基性能测试实用指南

1 范围

SN/T 1538 的本部分规定了培养基的通用质量控制要求并列举了固定和液体培养基的性能测试方法。

本部分适用于市售和自制培养基的性能测试。

2 规范性引用文件

下列文件中的条款通过 SN/T 1538 的本部分的引用而成为本部分的条款。凡是注日期的引用文件,其随后所有的修改(不包括勘误的内容)或修订版均不适用于本部分,然而,鼓励根据本部分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部分。

GB/T 2828.1 计数抽样检验程序 第 1 部分:按接收质量限(AQL)检索的逐批检验抽样计划

SN/T 1538.1 培养基制备指南 第 1 部分:实验室培养基制备质量保证通则

3 术语和定义

SN/T 1538.1 确立的术语和定义适用于 SN/T 1538 的本部分。

4 常规质量控制要求

4.1 基本要求

4.1.1 培养基

培养基的质量由基础成分的质量、培养基的配方、制备过程的控制、微生物污染的消除及包装和储存条件等因素所决定(参见附录 A)。

供应商或制备者应确保培养基的理化特性满足相关标准的要求,以下特性的质量评价结果应符合相应的规定:

- 分装数量;
- 外观、色泽和均一性;
- 琼脂凝胶的硬度;
- 水分含量;
- pH;
- 缓冲能力;
- 微生物污染。

培养基的各种成分、营养添加剂或选择剂应进行适当的质量评价。

4.1.2 基础成分

国际和(或)国家标准中提到的培养基通常可以直接使用。但因其中一些基础生物成分质量不稳定,可允许对其用量进行适当的调整,如:

- 根据营养需要改变蛋白胨、牛肉膏、酵母浸膏的用量;
- 根据所需凝胶作用的效果改变琼脂的用量;

- 根据缓冲要求决定缓冲物质用量；
- 根据选择性要求决定胆盐、胆抽提物和脱氧胆盐、抗菌染料的用量；
- 根据抗生素的效价决定其用量。

4.2 微生物学要求

4.2.1 概述

应选择能代表整批产品的样品进行微生物学性能测试。

4.2.2 微生物污染

按批次的不同选择适量的培养基在适当条件下培养,测定其微生物污染。生产商应根据每种固体或液体培养基成分、制备要求和包装类型的不同,规定或建立其污染限值。

分别从初始和最终制备的培养基中抽取或制备至少一个(或1%)的平板或试管,置于37℃或按特定标准中规定的温度培养18 h。

培养基的统计学抽样参见 GB/T 2828.1。

注:该条款只适用于即用型培养基。

4.2.3 生长特性

4.2.3.1 概述

选择下列方法对每批成品培养基、营养成分或添加剂进行评价:

- a) 定量方法;
- b) 半定量方法;
- c) 定性方法。

按本部分或其他等同技术对培养基进行定量、半定量或定性评价。采用定量方法时,应使用参考培养基(见特定标准或附录 B)进行对照;采用半定量和定性方法时,使用参考培养基(见特定标准或附录 B)或能得到“阳性”结果的培养基进行对照有助于结果的解释。参考培养基应是从近期批次中选出的已知质量良好的培养基。培养基的稳定性测试应使用来自不同供应商的培养基或即用型培养基。

选择性培养基上目标菌菌落的外观、大小和形态都应十分典型,非目标菌应部分或全部被抑制。

4.2.3.2 生长率

按规定用适当器具将适量测试菌株的工作培养物(5.2.2.1)接种至固体、半固体和液体培养基中。

每种培养基上菌株的生长率应达到所规定的最低限值(见相关标准或附录 B)。

定量方法中生长率 P_R 的计算见式(1)

$$P_R = \frac{N_s}{N_o} \dots\dots\dots(1)$$

式中:

N_s ——从一个或多个待测培养基平板上得到的菌落总数;

N_o ——从一个或多个规定的参考培养基平板上获得的菌落总数(该菌落总数应 ≥ 100 CFU)。

非选择性培养基上目标菌的生长率最低应为0.7,该类培养基应易于目标菌生长;选择性培养基上目标菌的生长率最低应为0.1。通常应达到这个要求,特殊情况时可以放宽判定标准(见相关标准或附录 B)。

半定量方法采用生态测量技术在平板上划线,平板连续划线区域得分的总和即为生长指标 G ,这个值随培养基的不同而变化。将该值与已知测定值和(或)参考培养基的 G 值相比较,确保变化范围不超过标准要求。条件允许时,可建立每种培养基的允许范围参数。

定性方法通常采用划线观察法。

4.2.3.3 选择性

为定量评价培养基的选择性,应按规定以适当器具将适量测试菌株的工作培养物(5.2.2.2)接种至选择性培养基和参考培养基中,培养基的选择性应达到规定值(见相关标准或附录 B)。

用式(2)计算选择因子 S_F :

$$S_F = D_o - D_s \quad \dots\dots\dots(2)$$

式中:

D_o ——能在参考培养基上生长至少 10 个菌落的最高稀释度;

D_s ——能在测试培养基上显示生长的最高稀释度。

S_F 、 D_o 和 D_s 用 \log_{10} 表示,例: $D_o 10^{-4} = \log_{10} 4.0$, $D_s 10^{-3} = \log_{10} 3.0$ 选择因子 $S_F = 1.0$ 。

非目标菌在选择性培养基上的 S_F 至少为 2。通常应达到这个要求,特殊情况时可以放宽判定标准(见相关标准或附录 B)。在半定量和定量法中,非目标菌的生长应该部分或全部被抑制。

4.2.4 生理生化特性(选择性和特异性)

确定培养基的菌落形态学、鉴别特性和选择性,以获得培养基的整体性能。

规定培养基的基本特性,使用一套适当的测试菌株对培养基上目标菌的生理生化特性和非目标菌的被抑制程度进行测试。

4.2.5 抗菌试验特性

抗生素的抗菌作用取决于抗生素在培养基琼脂中的扩散程度和与琼脂中其他成分的拮抗作用。测定食品样品中抗菌物质的培养基应符合相关标准的要求。

4.3 性能评价和结果解释

若按照规定得到的所有测试菌株的性能测试结果均符合规定,则该批培养基品质优良;若只有基本要求和微生物学要求符合规定,则该批培养基可被接受。

5 培养基性能测试方法

5.1 概述

本部分列举了液体、固体培养基的定量、半定量和定性测试方法。通常使用半定量和定性测试方法就能满足实验室对培养基性能测试的要求。

评价新的培养基或新的供应商的产品时,应采用定量测试方法。

附录 B 或 SN/T 1538.1 中列出了相应的测试菌株。

在制、修订微生物学标准时,应在标准中对相关测试菌株和所使用的培养基做出规定。

为成功分离目标菌(如:沙门氏菌),应使用适当的样品、培养基和标准菌株建立对照实验,证明整个方法的生长率和灵敏度,同时证明培养基成份符合要求。

5.2 测试菌株

5.2.1 概述

附录 B 针对不同的培养基列出了所推荐使用的目标菌株(生长率)和非目标菌株(选择性)。测试菌株应符合 SN/T 1538.1—2005 中 5.2.2 的要求。如根据测试需要选用强阳性菌株、微弱生长的阳性菌株,非特异性菌株或受损的菌株等。

SN/T 1538.1—2005 的附录 B 提供了标准菌株的保存方法。

5.2.2 工作菌株的制备

工作菌株是将标准储备菌株接种到非选择性肉汤中所制备的处于稳定生长期的纯菌株,也可以采用其他制备方法,但要保证接种菌株的纯度和操作的规范性,以确保工作菌株的可靠性。

如经过冷冻的菌种复苏后能在选择性培养基上存活,则该菌株可以使用。

5.2.2.1 生长率测试用工作菌株

目标菌的定量、半定量和生长率试验常用每平板(或试管)的接种水平为 10 CFU~100 CFU。

5.2.2.2 选择性测试用工作菌株

培养基选择性试验是将浓度为 10^4 CFU/mL~ 10^6 CFU/mL 的非目标菌工作菌株悬浮液接种到平

板上或试管中。

5.2.2.3 培养条件

按相关标准和附录 B 中的条件对接种后的培养基进行培养。

5.3 固体培养基测试方法

可采用与下列方法效果等同的方法进行固体培养基的测试。如定量方法只能用定量方法所替代。

5.3.1 定量测试方法

5.3.1.1 概述

该方法适用于大多数固体培养基,但不适用于部分霉菌培养基的测试。

5.3.1.2 测试步骤

——按 5.2.2 的方法使用工作菌株;

——选择能够代表一批培养基的适当数量的平板进行测试,并保证平板表面足够干燥。用同样的方法制备参考培养基平板(见 SN/T 1538.1—2005 中 4.4.4);

——用工作菌株的稀释液均匀涂布测试平板和参考平板,并按 5.2.2 的要求,选择菌落数在一定范围的平板进行计数。可使用改进的 Miles-Misra 表面滴落法、其他滴落计数法或螺旋平板法或倾注平板技术进行菌落计数,并按标准规定的培养条件培养平板;

——计算每一平板或每一滴液体中的菌落数,评价菌落的大小和表面特征。

5.3.1.3 计算

根据平板上菌液的体积和稀释倍数,计算出培养基上菌落的平均值。滴落计数法需要确定液滴的数量和体积。

5.3.1.4 结果解释

计算生长率 P_R (4.2.3.2) 和选择因子 S_F (4.2.3.3, 必要时),解释结果。

5.3.2 半定量测试方法

5.3.2.1 概述

该方法适用于固体和液体培养基的性能测试,但仅能作为固体培养基的补充测试。

用于测试的培养基应干燥到相同的程度,整个操作步骤应规范化。

5.3.2.2 测试步骤

——按常规的方法用大约 15 mL 琼脂培养基制备平板;

——按 5.2.1 制备和使用工作菌株;

——用 3 mm 接种环按图 1 划线平板。A 部分用接种环按 0.5 cm 的间隔划 4 条平行线,按同样的方法在 B 区和 C 区域划线,最后在 D 区内划一条连续的曲线。划线时最好将模板图放在平板下面;

——按标准中规定的培养时间和温度对接种后的平板进行培养。

注:操作时用接种环而不用接种针,接种环应完全浸入培养基中。取一满环接种物,将接种环接触容器边缘 3 次可去除多余的液体。划线时接种环与琼脂表面的角度应为 $20^\circ \sim 30^\circ$ 。接种环压在琼脂表面的压力和划线速度前后一致,整个划线应快速连续,移取液体培养物时应将接种环伸入培养液下部以防止环上产生气泡或泡沫。

通常用同一个接种环对 A~D 部分进行划线,操作过程不需要对接种环灭菌。但为了得到低生长指数 G ,在接种不同部分时应更换接种环或对其灭菌。

5.3.2.3 计算

培养后,评价菌落的形状、大小和密度,并计算生长指数 G 。每条有菌落生长的划线记作 1 分,每个培养皿上最多为 16 分。如果仅一半的线有菌落生长,记作 0.5 分。如果划线上没有菌落生长或生长量少于划线的一半,则记作 0。记录每个平板的得分总和便得到 G 。如,菌落在 A 区和 B 区全部生长,而在 C 区有一半线生长则 G 为 10。

5.3.2.4 结果解释

目标菌的生长指标 G 大于 6 时,培养基可接受。非选择性培养基的 G 值通常较高。

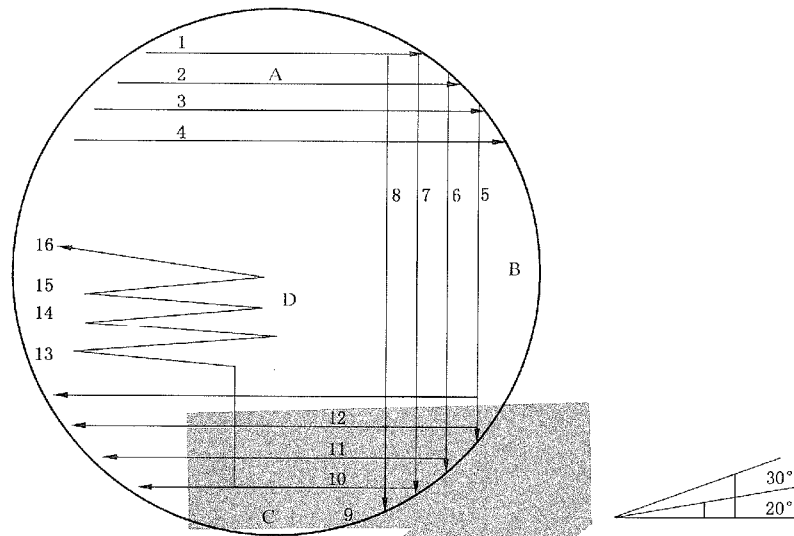


图1 改进的划线法接种模式图和接种角度

目标菌在培养基上应呈现典型的生长,而非目标菌的生长应部分或完全被抑制。

5.3.3 定性测试方法

5.3.3.1 概述

该方法可用作固体培养基性能测试的补充。

5.3.3.2 测试步骤

- 按常规的方法用大约 15 mL 琼脂培养基制备平板;
- 按 5.2.2 的方法制备和使用工作菌株;
- 用 3mm 接种环取测试菌培养物,在测试培养基表面划平行直线。可同时在一个平板上接种多个菌株,但划线不能交叉;
- 按标准中规定的培养时间和温度对接种后的试管进行培养。

注:也可使用其他划线技术。

5.3.3.3 结果解释

培养后按以下方法对培养基计分:

- 0 表示无生长;
- 1 表示微弱生长;
- 2 表示生长良好。

目标菌得分应为 2,并且有典型的菌落外观、大小和形态。非目标菌的生长应该部分或全部被抑制。

5.4 液体培养基测试方法

可采用与下列方法效果等同的方法进行液体培养基的测试。如,定量方法只能用定量方法所替代。

5.4.1 概述

测定液体培养基的生长率应采用适当的接种方法。本条介绍用于评价液体培养基生长率和选择性的定量、半定量和定性方法。但液体培养基的细菌总数或得分应通过将培养物倾注或划线平板后才能得到。液体培养基特性反应的定性评价通过肉眼观察来实现。

5.4.2 定量测试方法

该方法还适用于对新制备培养基、肉汤或稀释剂的评价。

5.4.2.1 测试步骤

- 从每批液体培养基中选择适当数量的试管或每份 10 mL 的培养基进行测试；
- 接种目标菌：用少量目标菌株培养物（每管 10 CFU~100 CFU，接种物制备见 5.2.2）接种测试肉汤和参考肉汤，混匀；
- 接种非目标菌：用大量非目标菌培养物（每管大于 1 000 CFU，接种物制备见 5.2.2）接种测试肉汤和参考肉汤，混匀；
- 接种目标菌和非目标菌混合培养物：用少量（每管 10 CFU~100 CFU，接种物制备见 5.2.2）目标菌接种测试肉汤和参考肉汤，同时每个试管中接种大量非目标菌（每管大于 1 000 CFU，接种物制备见 5.2.2），混匀；
- 按标准中规定的培养时间和温度对接种后的试管进行培养；
- 培养后，从每一种肉汤中移取一定量的菌液，必要时可取原菌液的稀释液按 5.3.1 的方法涂布于非抑制性琼脂平板上；
- 稀释剂和运输培养基：按每管 100 CFU~1 000 CFU 接种测试菌（接种物制备见 5.2.2），混匀。稀释剂在室温下放置 45 min 后进行平板计数；运输培养基按常规培养时间和温度对接种后的试管进行培养，然后进行平板计数。

改进的 Miles-Misra 方法、其他的涂布方法或螺旋平板涂布法等都可以用于平板菌落计数。

注：检测混合菌时，应在能够鉴别这些菌株的非选择性琼脂平板上涂布接种（例：平板计数琼脂中加入 MUG 可用于大肠杆菌和沙门氏菌的计数）。当非选择性琼脂平板不能鉴别出混合菌时，应使用经性能测试合格的选择性琼脂培养基。

5.4.2.2 结果读取、计算和解释

培养后，对目标菌和非目标菌进行计数，以区分不同类型的培养基。并根据不同的测试目的，进行计算和解释。

- a) 按 P_R （见 4.2.3.2）和 S_F 值（见 4.2.3.3）对参考肉汤和测试肉汤进行比较和解释：
 - 目标菌的 P_R 值不应小于 0.1（测试培养基与参考培养基菌落总数的差别不超过一个数量级）；
 - 非目标菌的 S_F 值至少应达到 2；
 - 混合菌中，目标菌株的生长不应受到非目标菌生长的抑制，即目标菌始终应为优势菌。
- b) 在混合菌中目标菌数量的最低值及非目标菌数量的最高值应符合以下要求：
 - 目标菌的最低浓度应达到 10^6 CFU/mL~ 10^8 CFU/mL；
 - 在选择性肉汤培养物中非目标菌的最高浓度不应超过 10^4 CFU/mL。
- c) 稀释剂和运输培养基不应引起目标菌和非目标菌数量太大的变化。微生物在这些培养基中培养后的数量变化应在最初计数的 $\pm 50\%$ 的范围内。

注：液体培养基与菌种生长特性相关的质量在菌种生长早期表现最为明显。如测试菌在参考肉汤和测试肉汤中生长率和选择性的很多信息要在对数生长期，尤其是前对数生长期获得。因此，要观察培养基质量的微小变化，应在接种测试菌后的短时间内（如 6 h 或 12 h）从液体培养基中挑取培养物划线接种平板。

5.4.3 半定量测试方法

5.4.3.1 测试步骤

- 挑选一定数量试管或在一批培养基中吸取每份 10 mL 的样品进行测试；
- 目标菌、非目标菌以及混合菌的接种：在装有测试肉汤的试管中接种 10 CFU~100 CFU 的目标菌，同时接种更多数量（每管大于 1 000 CFU）的非目标菌，混匀；
- 非目标菌的接种：在测试肉汤试管中接种较多数量（大于 1 000 CFU）的非目标菌，混匀培养；
- 按标准方法中规定的培养时间和温度进行培养；
- 用 3 mm 接种环取一环混合菌培养液划线接种到特定目标菌选择性平板上；
- 用 3 mm 接种环取一环非目标菌培养液划线接种到非选择性平板（如 TSA）上；

——将两个平板按标准要求培养(见特定标准)。

5.4.3.2 计算和结果解释

选择性平板上有至少 10 个目标菌菌落生长,则表示液体测试肉汤的生长率为良好。

非选择性平板上没有菌落生长(或者少于 10 CFU),则表示液体测试肉汤的选择性为良好。

5.4.4 定性测试方法

5.4.4.1 概述

单管定性法适用于液体培养基的性能测试。这项测试不能用于四硫磺酸盐肉汤等混浊培养基。

5.4.4.2 测试步骤

——用 3 mm 接种环取一环工作菌株培养物直接接种到用于性能测试的液体培养基中;

——按标准方法中规定的培养时间和温度进行培养。

5.4.4.3 结果解释

用目测的浊度值(如 0~2)评价培养基(使用试管或瓶子培养):

——0 表示无混浊;

——1 表示很轻微的混浊;

——2 表示严重的混浊。

目标菌的浊度值应为 2。

注 1:有时可以观察到微生物生长后聚集成细胞团,沉积在试管或瓶子的底部。发生这种情况时,小心振荡试管后进行观察。

注 2:该方法也可用于对液体培养基产气和颜色变化等反应特性的评价。

6 测试结果的记录

6.1 制备商信息

培养基制造商或供应商应按客户的要求提供培养基常规信息(见 SN/T 1538.1—2005 中 4.1.1)和相关测试菌株生长特性信息。

6.2 溯源性

按照质量体系的要求,对所有培养基性能测试的数据归档,并在有效期内进行适当的保存。建议使用内部质量控制单(参见附录 A)进行文件记录并评价测试结果。

附录 A

(资料性附录)

实验室自制培养基测试结果记录单样本

表 A.1 记录卡片样本

培养基内部质量测试控制卡				
培养基:		制备体积:	倾倒日期:	内部批号:
脱水培养基(批号):	供应商:	批:	总量:	日期/签名:
添加剂:	供应商:	批:	总量:	日期/签名:
制备详情:				
物理质量控制				
预期 pH:	测定 pH:	质量确认: 是 <input type="checkbox"/> 否 <input type="checkbox"/>	缺陷:	日期/签名:
预期质量:	观察:	质量确认: 是 <input type="checkbox"/> 否 <input type="checkbox"/>	缺陷:	日期/签名:
预期颜色:	观察:	质量确认: 是 <input type="checkbox"/> 否 <input type="checkbox"/>	缺陷:	日期/签名:
预期透明度/可见杂质	观察:	质量确认: 是 <input type="checkbox"/> 否 <input type="checkbox"/>	缺陷:	日期/签名:
预期凝胶稳定性/粘稠度/ 湿度:	观察:	质量确认: 是 <input type="checkbox"/> 否 <input type="checkbox"/>	缺陷:	日期/签名:
微生物污染				
测试平板或试管编号: 培养:	结果:	质量确认: 是 <input type="checkbox"/> 否 <input type="checkbox"/>	污染平板或试管编号:	日期/签名
微生物生长——生长率		控制方法:	定量 <input type="checkbox"/> 定性 <input type="checkbox"/>	
菌株: 培养: 参考培养基:	判定标准:	结果:	质量确认: 是 <input type="checkbox"/> 否 <input type="checkbox"/>	日期/签名
微生物生长——选择性		控制方法:	定量 <input type="checkbox"/> 定性 <input type="checkbox"/>	
菌株: 培养: 参考培养基:	判定标准:	结果:	质量确认: 是 <input type="checkbox"/> 否 <input type="checkbox"/>	日期/签名
微生物生长——特异性		控制方法:	定量 <input type="checkbox"/> 定性 <input type="checkbox"/>	
菌株: 培养: 参考培养基:	判定标准:	结果:	质量确认: 是 <input type="checkbox"/> 否 <input type="checkbox"/>	日期/签名
本批发放:				
储存详情:		本批发放: 是 <input type="checkbox"/> 否 <input type="checkbox"/>		日期/签名:

附录 B
(规范性附录)
常用培养基推荐测试菌株

表 B.1~B.6 参考了欧洲药典中使用的控制菌株和药典中关于培养基用食品微生物的建议(ICFMH 工作会议),其中包括了即将制定和修订的标准(新标准或其修订稿)的内容。培养基的有效批是指一批性能满意的培养基,允许等效使用其他菌种保藏机构的菌株(如:NCCTC,CIP 等)。表中所有的培养基均出自 EN 和 ISO 标准。

表 B.1 计数用选择性培养基

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应
Baird-Parker	S ^e	凝固酶阳性 葡萄球菌	EN 6888	生长率	24h~ 48h/37°C	金黄色葡萄球菌 ATCC 6538 ^a , 金黄色葡萄球菌 ATCC 25923 ^b	TSA	定量	PR \geq 0.5	黑色/灰色菌落带透明带(蛋黄分解反应)
				选择性	48h/37°C	大肠杆菌 ATCC 25922 或 8739 ^b	—	定性	全部抑制	—
				特异性	24h~ 48h/37°C	表皮葡萄球菌 ATCC 12228 ^a	—	定性	—	黑色/灰色菌落带透明带无蛋黄分解反应
RPFA	S	凝固酶阳性 葡萄球菌	EN 6888-2	生长率	24h~ 48h/37°C	金黄色葡萄球菌 ATCC 6538 或 金黄色葡萄球菌 ATCC 25923 ^b	TSA	定量	PR \geq 0.5	黑/灰色菌落带有混浊的晕环
				选择性	48h/37°C	大肠杆菌 ATCC 25922 或 8739 ^b	—	定性	全部抑制	—
				特异性	24h~ 48h/37°C	表皮葡萄球菌 ATCC 12228 ^a	—	定性	—	黑/灰色菌落带有混浊的晕环
氯霉素培养基 OGA (OGY)	S	酵母菌/霉菌	ISO 7954	生长率	3d~ 5d/37°C	白色念珠菌 ATCC 10231 黑曲霉 ATCC 1640 ^b 圆弧青霉 ATCC 16025 酿酒酵母 ATCC 9763	沙保罗葡萄糖琼脂(SDA)	定量	PR \geq 0.5	不同菌种菌落特征不同
				选择性	3d~ 5d/37°C	大肠杆菌 ATCC 25922 或 8739 ^b 枯草芽孢杆菌 ATCC 6633	—	定性	全部抑制	—
				生长率	72h/30°C	清酒杆菌 ATCC 15521 ^b 有雪片球菌 ATCC 29538 乳酸乳杆菌 ATCC 19435 ^b	MRS	定量	PR \geq 0.5	不同菌种菌落特征不同

表 B.1 (续)

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应	
MRS	S	乳酸菌	ISO 15214	选择性	72h/30℃	大肠杆菌 ATCC 25922 或 8739 ^b 蜡样芽孢杆菌 ATCC 11778	—	定量	全部抑制	—	
MYP	S	枯草芽孢杆菌	EN ISO 7932	生长率	24h~ 48h/30℃	蜡样芽孢杆菌 ATCC 11778 ^b	TSA	定性	PR \geq 0.7	具沉淀带的粉色菌落	
				选择性	48h/37℃	大肠杆菌 ATCC 25922 或 8739 ^b	—	全部抑制	—		
Oxford	S	单核细胞增生 李斯特氏菌	EN ISO 11290	特异性	48h/37℃	枯草芽孢杆菌 ATCC 6633 ^b	—	—	—	—	不具沉淀带黄色菌落
				生长率	48h/37℃	单核细胞增生李斯特氏菌 1/2a ATCC 19111	TSA	定量	PR \geq 0.5	具黑色沉淀带灰黑 菌落	
				选择性	48h/37℃	单核细胞增生李斯特氏菌 4b ATCC 13932 ^b	—	全部抑制	—		
PALCAM	S	单核细胞增生 李斯特氏菌	EN ISO 11290	生长率	48h/37℃	大肠杆菌 ATCC 25922 或 8739 ^b	—	定性	全部抑制	—	—
				选择性	48h/37℃	粪肠球菌 ATCC 29212 或 19433	—	—	—	—	
				生长率	48h/37℃	白色念珠菌 ATCC 10231	TSA	定量	PR \geq 0.5	具黑色沉淀带灰黑 菌落	
TS(C)	S	空肠弯曲杆菌	EN ISO 7937	选择性	72h/30℃	单核细胞增生李斯特氏菌 1/2a ATCC 19111	—	定性	全部抑制	—	—
				生长率	20h/37℃	单核细胞增生李斯特氏菌 4b ATCC 13932 ^b	TSA	定量	PR \geq 0.7	黑色菌落	
				选择性 TS	20h/37℃	大肠杆菌 ATCC 25922 或 8739 ^b	—	全部抑制	—	白色菌落	
VRBG	S	肠杆菌科	ISO 7402 ISO 8907	生长率	24h/37℃	产气荚膜梭菌 ATCC 13124	—	定性	—	—	带有或不带沉淀带的 粉色或红色菌落
				选择性	20h/37℃	产气荚膜梭菌 ATCC 12916	TSA	定量	PR \geq 0.5	全部抑制	—

表 B.1 (续)

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应
VRBL	S	大肠菌群	ISO 4832	生长率	20h/30℃	大肠杆菌 ATCC 25922 或 8739 ^b	TSA	定量	PR \geq 0.5	带有或不带沉淀带的淡紫色菌落
				选择性	24h/30℃	粪肠球菌 ATCC 29212 或 19433 ^b	—	定性	全部抑制	—
				特异性	24h/30℃	铜绿假单胞菌 ATCC 27853	—	定性	—	无色至浅棕色菌落
CT-SMAC	S	大肠杆菌 O157	ISO 16654	生长率	24h/37℃	大肠杆菌 O157 : H7 ATCC 43894 或 43895(无毒菌株)	TSA	定量	PR \geq 0.5	透明菌落,表面淡黄棕色,菌落直径约 1mm
				选择性	24h/37℃	金黄色葡萄球菌 TACC 6538 或 ATCC 25923 ^b	—	定性	全部抑制	—
				特异性	24h/37℃	大肠杆菌 ATCC 11775 或 25922 ^b	—	定性	—	粉色菌落
BGBLB	L ^c	大肠菌群	ISO 4831	生长率	24h~48h/30℃	大肠杆菌 ATCC 25922 或 8739 ^b	—	半定量	浊度 2+ 导管 1/3 产气	产气并混浊
				选择性	24h~48h/30℃	弗氏柠檬酸杆菌 ATCC 43864	—	定性	—	—
				生长率	24h~48h/30℃	粪肠球菌 ATCC 29212 或 19433 ^b	—	定性	—	—
LST	L	大肠菌群	ISO 4831	生长率	24h~48h/30℃	大肠杆菌 ATCC 25922 或 8739 ^b	—	半定量	浊度 2+ 导管 1/3 产气	产气并混浊
				选择性	24h~48h/30℃	弗氏柠檬酸杆菌 ATCC 43864	—	定性	—	—
				生长率	24h~48h/44℃	粪肠球菌 ATCC 29212 或 19433 ^b	—	定性	—	—
EC	L	大肠杆菌	ISO 7251	生长率	24h~48h/44℃	大肠杆菌 ATCC 25922 或 8739 ^b	—	半定量	浊度 2+ 导管 1/3 产气	产气并混浊
				选择性	24h~48h/44℃	铜绿假单胞菌 ATCC 27853 ^b	—	定性	—	—

注 1: 固体培养基也可以使用半定量平板技术。

注 2: 可使用能溯源到以上测试菌株的其他菌株进行培养基的性能测试。

^a S= 固体培养基。^b 实验室使用的菌株(最少使用数量)。^c L= 液体培养基。

表 B.2 计数用非选择性培养基

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应	
PCA	S ^a	全部微生物	ISO 4833	生长率	72h/30℃	大肠杆菌 ATCC 25922 或 8739 ^b 金黄色葡萄球菌 ATCC 6538 或 6538P 枯草芽孢杆菌 ATCC 6633 ^b	TSA	定量	PR≥0.7		
		注：可使用能溯源到以上测试菌株的其他菌株进行培养基的性能测试。									
		a S=固体培养基。 b 实验室使用的质控菌株(最少使用数量)。									

表 B.3 选择性增菌培养基

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应
EE	L ^a	肠杆菌科	ISO 7402	生长率	24h/37℃	大肠杆菌 ATCC 25922 或 8739 ^b 鼠伤寒沙门氏菌 ATCC 14028	—	半定量	VRBG 上 > 10CFU	粉色至红色菌落, 有或没有沉淀带
			ISO 8523	选择性		粪肠球菌 ATCC 29212 或 19433 ^b 单核细胞增生李斯特氏菌 1/2a ATCC 19411 或单核细胞增生李斯特氏菌 4b ATCC 13932 ^b + 大肠杆菌 ATCC 25922 或 8739 ^b	—	半定量	Oxlord 或 PAL-CAM 上 > 10CFU	灰色至黑色菌落具有黑色环状带
一倍稀释的 Fraser	L	单核细胞增生李斯特氏菌	EN ISO 11290-1	生长率	24h/30℃	粪肠球菌 ATCC 29212 或 19433 ^b 大肠杆菌 ATCC 25922 或 8739 ^b + 粪肠球菌 ATCC 29212 或 19433 ^b	—	半定量	TSA 上全抑制 TSA 上 < 100CFU	—
				选择性		单核细胞增生李斯特氏菌 1/2a ATCC 19411 或单核细胞增生李斯特氏菌 4b ATCC 13932 ^b + 大肠杆菌 ATCC 25922 或 8739 ^b	—	半定量	Oxlord 或 PAL-CAM 上 > 10CFU	灰色至黑色菌落具有黑色环状带

表 B.3 (续)

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应
Fraser	L	单核细胞增生李斯特氏菌	EN ISO 11290-1	选择性	24h~48h/37℃	+ 粪肠球菌 ATCC 29212 或 19433 ^b	—	—	TSA 上全抑制	—
						大肠杆菌 ATCC 25922 或 8739 ^b	—	—	TSA 上 <100CFU	—
ITC	L	小肠耶尔森氏菌	ISO 10273	生长率	48h/25℃	小肠耶尔森氏菌 ATCC 23715 或 9610 ^b	—	半定量	CIN 或 SSDC 上 >10CFU	不同培养基上菌落特征不同(见标准)
						大肠杆菌 ATCC 25922 或 8739 ^b	—	—	—	—
Park & Sanders	L	弯曲杆菌属	ISO 10272	生长率	见标准	铜绿假单胞菌 ATCC 27853 ^b	—	半定量	TSA 上全抑制	—
						铜绿假单胞菌 ATCC 27853 ^b	—	—	—	—
Park & Sanders	L	弯曲杆菌属	ISO 10272	生长率	见标准	奇异变形菌 ATCC 29906	—	半定量	Karmal 或其他选择性培养基上 >10CFU	不同培养基上菌落特征不同(见标准)
						大肠杆菌 ATCC 43478	—	—	—	—
Preston	L	弯曲杆菌属	ISO 10272	生长率	18h/42℃	或空肠弯曲杆菌 ATCC 33291 或 29428 ^b	—	半定量	TSA 上全抑制	—
						大肠杆菌 ATCC 25922 或 8739 ^b	—	—	—	—
Preston	L	弯曲杆菌属	ISO 10272	选择性	18h/42℃	大肠杆菌 ATCC 25922 或 8739 ^b	—	半定量	Karmal 或其他选择性培养基上 >10CFU	不同培养基上菌落特征不同(见标准)
						奇异变形菌 ATCC 29906	—	—	—	—

表 B.3 (续)

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应
PSB	L	小肠耶尔森氏菌	ISO 10273	生长率	3d~ 5d/25℃	小肠耶尔森氏菌 ATCC 23715 或 9610 ^b	—	半定量	CIN 或 SSDC 上 >10CFU	不同培养基上菌落特征不同(见标准)
						+大肠杆菌 ATCC 25922 或 8739 ^b +铜绿假单胞菌 ATCC 27853 ^b				
				选择性	3d~ 5d/25℃	铜绿假单胞菌 ATCC 27853 ^b 奇异变形菌 ATCC 29906	—	半定量	TSA 上全抑制	—
						鼠伤寒沙门氏菌 ATCC 14028 或肠炎沙门氏菌 ATCC 13076 ^b +大肠杆菌 ATCC 25922 或 8739 ^b +铜绿假单胞菌 ATCC 27853 ^b				
MKTn	L	沙门氏菌	ISO 6579	生长率	24h/37℃	鼠伤寒沙门氏菌 ATCC 14028 或肠炎沙门氏菌 ATCC 13076 ^b +大肠杆菌 ATCC 25922 或 8739 ^b +铜绿假单胞菌 ATCC 27853 ^b	—	半定量	XLD 或其他选择性培养基上 >10CFU	不同培养基上菌落特征不同(见标准)
						大肠杆菌 ATCC 25922 或 8739 ^b 粪肠球菌 ATCC 29212 或 19433				
Pappaport Vassiliadis	L	沙门氏菌	EN 12824	生长率	24h/41.5℃	鼠伤寒沙门氏菌 ATCC 14028 ^b 肠炎沙门氏菌 ATCC 13076 ^b +大肠杆菌 ATCC 25922 或 8739 ^b +铜绿假单胞菌 ATCC 27853 ^b	—	半定量	BGA 或其他选择性培养基上 >10CFU	不同培养基上菌落特征不同(见标准)
						大肠杆菌 ATCC 25922 或 8739 ^b 粪肠球菌 ATCC 29212 或 19433				
RVS	L	沙门氏菌	ISO 6579	生长率	24h/41.5℃	鼠伤寒沙门氏菌 ATCC 14028	—	半定量	XLD 或其他选择性培养基上 >10CFU	不同培养基上菌落特征不同(见标准)

表 B.3 (续)

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应
RVS	L	沙门氏菌	ISO 6579	生长率	24h/41.5℃	肠炎沙门氏菌 ATCC 13076 ^b	—	半定量	XLD 或其他选择性培养基上 >10CFU	不同培养基上菌落特征不同(见标准)
						+ 大肠杆菌 ATCC 25922 或 8739 ^b				
						+ 铜绿假单胞菌 ATCC 27853 ^b				
				选择性	24h/41.5℃	大肠杆菌 ATCC 25922 或 8739 ^b	—	半定量	TSA 上全抑制	—
						粪肠球菌 ATCC 29212 或 19433				
亚硝酸盐 胱氨酸	L	沙门氏菌	EN 12824	生长率	24h/37℃	鼠伤寒沙门氏菌 ATCC 14028 ^b	—	半定量	BGA 或其他选择性培养基上 >10CFU	不同培养基上菌落特征不同(见标准)
						或肠炎沙门氏菌 ATCC 13076 ^b				
						+ 大肠杆菌 ATCC 25922 或 8739 ^b				
				选择性	24h/37℃	铜绿假单胞菌 ATCC 27853 ^b	—	半定量	TSA 上 <<10CFU	—
						大肠杆菌 ATCC 25922 或 8739 ^b				
						粪肠球菌 ATCC 29212 或 19433				

注：可使用能溯源到以上测试菌株的其他菌株进行培养基的性能测试。

^a L=液体培养基。

^b 实验室使用的质控菌株(最少使用数量)。

表 B.4 非选择性增菌培养基

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应
BHI	L ^a	葡萄球菌	ISO 6888	生长率	24h/37℃	金黄色葡萄球菌 ATCC 25923 ^b		定性	浊度 1~2	—
布鲁氏杆菌培养基	L	弯曲杆菌	ISO 10272	生长率	2d~ 5d/25℃	大肠弯曲杆菌 ATCC 43478 空肠弯曲杆菌 ATCC 33291 或 29428 ^b	—	定性	浊度 1~2	—
蛋白胨盐	L	稀释液	ISO 6887	稀释	45min/ 20℃~25℃	大肠杆菌 ATCC 25922 或 8739 ^b	TSA	定量	+/-50% 菌落数 /至(+/-50% 原始菌落数)	—
巯基乙酸 盐培养基	L	梭状芽孢 杆菌	ISO 7937	生长率	24h/37℃	金黄色葡萄球菌 ATCC 25923 产气荚膜梭菌 ATCC 13124 ^b		定性	浊度 1~2	—
TSYEB	L	单核细胞增 生李斯特 氏菌	ISO 11290	生长率	24h/25℃	单核细胞增生李斯特氏菌 1/2a ATCC 13811 单核细胞增生李斯特氏菌 4b ATCC 13932 ^b		定性	浊度 1~2	—
注：可使用能溯源到以上测试菌株的其他菌株进行培养基的性能测试										
^a L=液体培养基。										
^b 实验室使用的质控菌株(最少使用数量)。										

表 B.5 选择性分离培养基

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应
改进的 Butzler	S ^a	弯曲杆菌	ISO 10272	生长率	24h~72h/42℃	大肠弯曲杆菌 ATCC 43478	—	定性	良好生长(2)	不同培养基上菌落特征不同(见标准)
CCDA	S ^a	弯曲杆菌	ISO 10272							
Karmali	S ^a	弯曲杆菌	ISO 10272							
Preston	S ^a	弯曲杆菌	ISO 10272							
Skirrow	S ^a	弯曲杆菌	ISO 10272	选择性	24h~72h/42℃	大肠杆菌 ATCC 25922 或 8739 ^b 金黄色葡萄球菌 ATCC 25923	—	定性	全部或部分抑制(0~1) 全部抑制(0)	无典型菌落 —
CIN	S	小肠耶尔森氏菌	ISO 10273	生长率	24h/30℃	小肠耶尔森氏菌 ATCC 23715 或 9610 ^b	—	定性	良好生长(2)	不同培养基上菌落特征不同(见标准)
SSDC	S	小肠耶尔森氏菌		选择性	24h/30℃	大肠杆菌 ATCC 25922 或 8739 ^b 金黄色葡萄球菌 ATCC 25923	—	定性	全部或部分抑制(0~1) 全部抑制(0)	无典型菌落
亮绿琼脂 (BGA)	S	沙门氏菌	EN 12824/ ISO 6579	生长率	24h~48h/37℃	鼠伤寒沙门氏菌 ATCC 14028 ^b 肠炎沙门氏菌 ATCC 13076	—	定性	良好生长(2)	不同培养基上落特征不同(见标准)
XLD				选择性	24h~48h/37℃	大肠杆菌 ATCC 25922 或 8739 ^b 粪肠球菌 ATCC 29212 或 19433	—	定性	全部或部分抑制(0~1) 全部抑制(0)	无典型菌落 —

注：可使用能溯源到以上测试菌株的其他菌株进行培养基的性能测试。

^a S=固体培养基。^b 实验室使用的质控菌株(最少使用数量)。

表 B.6 非选择性分离培养基

培养基	类型	微生物	标准	用途	培养条件	测试菌株	参考培养基	控制方法	判定标准	特性反应
营养琼脂	S ^a	肠杆菌科	ISO 7402 ISO 8523	生长率	24h/37℃	大肠杆菌 ATCC 25922 或 8739 ^c	---	定性	生长良好(2)	—
		沙门氏菌	EN 12824 ISO 6579		鼠伤寒沙门氏菌 ATCC 14028 ^c					
		小肠耶尔森氏菌	ISO 10273		小肠耶尔森氏菌 ATCC 23715 或 9610 ^c					
TSYEA 琼脂	S	单核细胞增生李斯特氏菌	FN ISO 11290	生长率	24h/37℃	单核细胞增生李斯特氏菌 1/2a ATCC 19111 或单核细胞增生李斯特氏菌 4b ATCC 13932 ^b	定性	生长良好(2)	---	
注：可使用能溯源到以上测试菌株的其他菌株进行培养基的性能测试。										
^a S= 固体培养基。										
^b 实验室使用的质控菌株(最少使用数量)。										
^c 可选质控菌株。										

Preface

SN/T 1538 *Guidelines on preparation and production of culture media* consist of the following parts:

- Part 1: *General guidelines on quality assurance for the preparation of culture media in the laboratory*;
- Part 2: *Practical implementation of the general guidelines on quality assurance for the preparation of culture media in the laboratory*;

This part is the second part of SN/T 1538, corresponding to ISO/TS 11133-2:2003 *Microbiology of food and animal feeding stuffs—Guidelines on preparation and production of culture media*. The consistency degree of this standard and ISO/TS 11133-1 is modified, the main different showing bellows:

- Modify some editing pattern according to the customs of Chinese;
- Change some of the expression of International standard to National standard of China;
- Modify *Foreword*, Delete *Introduction* of original standard;
- Modify “Normative references” according to the standard of China;
- Change some note to text according to the Chinese custom.
- Substitute the International standard with National standard or SN standard. for example: substitute ISO 2589-1:1999 with GB/T 2828. 1, and substitute ISO/TS 11133-1:2000 with SN/T 1538. 1;
- Substitute “Culture media described in the International Standards” with “Culture media described in the International/National Standards” in 4. 1. 2;
- Qualification the “microbial contamination” in 4. 2. 2;
- Adjust order of part of the paragraph in 4. 2. 3. 1;
- Add “note” in 5. 3 and 5. 4: “The methods which similar to the original standard may be used”;
- Unify the inoculation quantity with loop: “inoculate one loop with the inoculator which the diameter is 3mm”;
- Adjust order of part of the paragraph in 5. 4. 2. 1;
- Add note in annex B “The culture which can be traced to the culture metioned above can be used in the performance of culture media”.

Annex B is normative. Annex A is informative.

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

This part was drafted by Shanxi Entry-Exit Buru Inspection, Quarantine of the Peoples Republic of China, China Import and Export Commodity Inspection Technology Institue, Neimenggu, Guangxi, Shanghai, Jilin, Shandong Extry-Exit Bum of CIQ.

SN/T 1538.2—2007

The main drafters of this standard are Li weihua, Zhao guiming, Liu zhongxue, Liu junyi, Li xiaohong, Wang zhenguo, Lei zhiwen.

This part 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.

Foreword

The main objectives are to maintain, resuscitate, grow, detect and/or enumerate a wide variety of microorganisms in the microbiological laboratory, the requirements for media are specific to both the sample and/or organisms to be detected. The procedure of microbiology tests depend on culture media being consistent of standard and specify the minimum requirements, these can provide accurate and reliable result.

Guidelines on preparation and production of culture media are an essential part of internal quality control procedures of microbiology laboratory. It can monitoring the media effectively, and provide the result reliable and effective.

This standard is the second part of these series standard, it provides criteria for quality control of culture media and performance testing methods for culture media, provide basis for the culture media, assure the quality of culture media.

Guidelines on preparation and production of culture media—Part 2: Practical guidelines on performance testing of culture media

1 Scope

This part of SN/T 1538 sets criteria for routine quality control and give some example methods for the performance testing of solid and liquid culture media.

This part applies to performance testing and evaluating for commercial and produced culture media.

2 Normative references

This part of SN/T 1538 incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this part only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

GB/T 2828.1 (Sampling procedures for inspection by attributes—Part 1: Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection)

SN/T 1538.1—2005 Guidelines on preparation and production of culture media—Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory

3 Terms and definitions

For the purposes of this document, the terms and definitions given in SN/T 1538.1 apply.

4 Criteria for routine quality control

4.1 General quality criteria

4.1.1 Quality of culture media

The quality of culture media depends on the quality of the basic ingredients, correct formulation, quality preparation procedures, elimination of contaminant microbial agents and appropriate packaging and storage conditions(see AnnexA).

The manufacturer or producer in the laboratory shall comply with the physico-chemical characteristics of the culture media as specified in the corresponding standard. Furthermore, quality assessment shall ensure that the culture medium conforms to stated recommendations, including:

- distributed quantity and/or thickness;
- appearance, color and homogeneity;
- gel consistency;
- moisture content;
- pH value;
- buffering capacity;
- microbial contamination.

The individual components and any nutritive or selective supplements shall also undergo suitable quality assessment procedures.

4.1.2 Quality of basic media components

Culture media described in the International/National Standards were judged satisfactory; however, due to the variability of their quality, it may be acceptable for media manufacturers to modify the concentration of some basic biological ingredients, as listed below:

- peptones and meat or yeast extracts variable in their nutritive properties;
- agar variable in its gelling properties;
- buffering substances;
- bile salts, bile extract and desoxycholate, antibacterial dyes, depending on their selective properties;
- antibiotics depending on their activity.

4.2 Microbiological quality criteria

4.2.1 General

The microbiological performance tests shall be carried out on a sample which is representative of a batch of end product.

4.2.2 Microbial contamination

An appropriate quantity, depending on the size of the batch of culture medium, shall be tested for microbial contamination by incubation under appropriate conditions. Target limits for the percentage of contaminated plates or containers of liquid medium should be established for each medium or specified by the manufacture. Manu-

facturers should draw up specifications based on media components, processing limits and type of packaging.

The samples to be tested should be at least 1 plate or tube or 1% of plates or tubes from the beginning and 1 plate or tube or 1 % of plates or tubes from the end of a pouring or dispensing process. The plates or tubes should be incubated for at least 18 h at 37°C or under the incubation conditions which are used routinely for this medium according to the specific standard.

For statistical sampling plans refer to the GB/T 2828. 1.

Note: this item meet for ready for use culture media.

4.2.3 Growth

4.2.3.1 General

To evaluate, each batch of complete culture medium, nutrient components or supplements, growth shall be appropriately assessed by either:

- a) quantitative; or
- b) semi-quantitative; or
- c) qualitative methods.

Quantitative, semi-quantitative or qualitative evaluations shall be performed by the methods described in this part or by another generally accepted technique. The use of a specific reference medium is therefore mandatory for quantitative methods(see the specific standard or Annex B) ,For semi-quantitative or qualitative methods, the use of a specific reference medium(see corresponding specific standard or Annex B) or a culture medium giving a "positive" reaction helps to interpret results. The reference medium must be of known good quality chosen from a recently released batch, or, if comparing long term stability, a batch from another supplier, or a ready-to-use medium, etc.

In addition, growth of the target strains shall be typical in appearance, size and morphology of the colonies growth of the non-target strains shall be partly or completely inhibited.

4.2.3.2 Productivity

Solid, semi-solid or liquid culture media shall be inoculated with an appropriate inoculum(5.2.2.1) of the working culture of each of the defined test microorganisms using an appropriate device.

Productivity shall reach a defined minimum limit (see corresponding specific standard or Annex B).

For quantitative methods the Productivity Ratio $P_R(1)$ is determined as follows:

$$P_R = \frac{N_s}{N_o} \dots\dots\dots (1)$$

Where

N_s — the total colony count obtained on the culture medium under test (obtained from one or more

plates);

N_0 —the total colony count obtained on the defined reference culture medium obtained from one or more plates, and shall be ≥ 100 CFU

NOTE: The Productivity Ratio of a non selective medium is at least 0.7 for microorganisms that can grow easily on that medium. The P_R of the target microorganisms on a selective medium should be at least 0.1. These values are generally achievable, however less rigorous criteria can be accepted for certain combinations of media and test microorganisms (see corresponding specific standard or Annex B)

For semi-quantitative methods, the scores of consecutive sectors of a plate inoculated by the ecometric technical are summed to obtain the growth index G_1 , which varies according to the culture medium. It is therefore important to compare them with previous indices and/or G_1 of a reference medium and to ensure that variation are not excessive. The expected range of variations for each culture medium can also be established once sufficient experience of the method has been gained.

Qualitative evaluations shall be carried out visually by allocating growth scores.

4.2.3.3 Selectivity

To assess selectivity quantitatively, selective culture media and a reference medium are inoculated with an appropriate inoculum (5.2.2.2) of the defined test microorganism using an appropriate device. Selectivity has to reach defined values (see corresponding specific standard or Annex B).

The Selectivity Factor $S_F(2)$, is calculated as follows:

$$S_F = D_0 - D_S \dots\dots\dots (2)$$

Where

D_0 —the highest dilution showing growth of at least 10 colonies on the reference medium;

D_S —the highest dilution showing comparable growth on the test medium.

S_F , D_0 and D_S are expressed in \log_{10} units, e. g. $D_0 10^{-4} = \log_{10} 4,0$ and $D_S 10^{-3} = \log_{10} 3,0$ then the selectivity factor is $S_F = 1,0$.

The S_F of non-target microorganisms on a selective medium should be at least 2. This value is generally achievable. However less rigorous criteria can be accepted for certain combinations of media and test microorganisms (see corresponding specific standard or Annex B).

For semi-quantitative and qualitative methods the growth of the non-target strain (s) shall be inhibited partly or completely.

4.2.4 Biochemical and physiological characteristics (selectivity and specificity)

The colony morphology and the diagnostic features together with the degree of selectivity should be established in order to obtain a complete picture of the performance of a medium. The

essential characteristics of specificity shall be defined and achieved. For differential media the quality of biochemical/physiological characteristics of the target microorganism(s) and the degree of inhibition of non-target microorganisms should be determined with an appropriate set of test strains.

4.2.5 Antimicrobial testing characteristics

The antimicrobial action of antibiotics depends upon their agar diffusion characteristics and any antagonistic effects from the components present. Media for testing the presence or absence of antimicrobial substances in food samples should conform to reference methods.

4.3 Performance evaluation and interpretation of results

A batch of culture medium performs satisfactorily if all the test microorganisms used perform according to the given specifications. It shall be accepted if both general and microbiological quality criteria are met.

5 Methods for use in performance testing of culture media

5.1 General

Examples of quantitative, semi-quantitative and qualitative testing methods for solid culture media and liquid media are described. In most cases in the user's laboratory semi-quantitative and qualitative techniques will meet the performance testing requirements of a batch of culture medium.

For special cases, e.g. evaluation of a new medium or a new manufacturer, etc. quantitative testing method shall be performed by the user's laboratory.

Familiarity with general microbiological techniques is assumed and therefore the methods are not given in exhaustive detail.

Suitable test microorganisms are listed in Annex B also SN/T 1538. 1.

NOTE: It is the intention in the future, that new and revised individual standards for detection or enumeration of specific microorganisms or groups of microorganisms will describe the relevant test microorganisms to be used, together with the acceptance criteria for each culture medium in the standard.

For the successful isolation of targeted microorganisms in a multistage method, for example detection of *Salmonella*, several complex interactions take place at each growth stage. Here a control test using appropriate samples, culture and reference materials should be set up, so that the productivity or the sensitivity, of the whole method is demonstrated. This is in addition to demonstrating that

each component medium is fit for purpose.

5.2 Test microorganisms

5.2.1 General

The appropriate reference strains of target (productivity) and non-target (selectivity) microorganisms for each culture medium are given in Annex B. The test microorganisms should meet the requirements given in 5.2.2 of SN/T 1538.1—2005, e.g. robust, weakly growing, biochemically unreactive or injured strains, as appropriate.

Guidance on the preservation and maintenance of reference strains is given in Annex B of SN/T 1538.1—2005.

5.2.2 Preparation of the working culture

Working cultures shall be prepared as a pure stationary phase culture in a non-selective broth from the reference stock culture.

Different techniques may be used, but shall guarantee the purity of the inoculum, as well as its standardisation which allows it to be used at a later stage.

Frozen inocula may be used if it can be shown that the microorganism can survive for the chosen period.

5.2.2.1 Working culture for productivity testing

For semi-quantitative or qualitative tests and productivity testing of target microorganisms an inoculum level is used to obtain 10 cfu to 100 cfu per plate or tube of medium.

5.2.2.2 Working culture for selectivity testing

For selectivity testing of culture media a suspension of the non-target microorganism containing 10^4 cfu to 10^6 cfu per ml is inoculated onto the plate or into the tube of medium.

5.2.2.3 Incubation conditions

Incubate the inoculated culture media in accordance with the conditions described in the corresponding standard and given in the appropriate tables in Annex B.

5.3 Methods for solid culture media

The equivalent method may be used in performance testing of solid culture media. quantitative meth-

od is only substituted by quantitative method.

5.3.1 Quantitative plating method

5.3.1.1 General

This is a general method suitable for most solid culture media. It may not be suitable for testing some types of moulds.

5.3.1.2 Procedure

- Use working cultures as described in 5.2.2.
- Select an appropriate number of plates representative of each batch to be tested and ensure the surface of each plate is adequately dried. Plates of the reference medium should be similarly prepared (see 4.4.4 of SN/T 1538.1—2005).
- Spread onto the surface of the test and reference plates an inoculum of the diluted working culture to give counts that fall within the recommended limits given in 5.2.2. The modified Miles-Misra surface drop method and other dropping systems or a spiral plater may also be used. The pour plate method may also be used for culture media normally used for enumeration; in this way. Incubate plates under appropriate conditions as defined in the individual standards.
- Count the colonies present on each plate or from each drop as appropriate. Assess the size and appearance of the colonies.

5.3.1.3 Calculation

Based on the volume spread on the plates and the dilution factor, the mean count on the medium can be calculated. In the case of dropping methods the number of drops and their volume must be considered.

5.3.1.4 Interpretation of results

To interpret the results, the Productivity Ratio P_R (4.2.3.2), and where appropriate the Selectivity Factor S_F (4.2.3.3), should be calculated.

5.3.2 Semi-quantitative streaking method based on ecometry

5.3.2.1 General

The streaking method is suitable for performance testing of solid and liquid culture media, it can only be regarded as a supplementary test for solid culture media.

When using this method the culture media tested should be dried to the same degree and the whole procedure shall be standardized so that results of different batches can be compared.

5.3.2.2 Procedure

- Agar plates are prepared in the usual manner with about 15 ml of agar. Media normally used for the pour plate technique, for example Plate Count Agar (PCA), may also be tested by surface plating on solidified media.
- Use working cultures as described in 5.2.1.
- The plates are streaked as shown in Figure 1 using a 3 mm loop. Four parallel lines are drawn with the loop at approximately 0.5 cm intervals over sector A. Streaking is repeated for sectors B and C and terminated in sector D with a single line. A template can be used beneath the plate to facilitate accurate streaking
- The incubation times and temperatures stated in the standard methods are used.

NOTE: Only the loop, not the wire, should be dipped in the culture. The loop should be completely filled with the culture. Excess liquid should be removed by pressing the wider part of the loop three times against the edge of the container. When streaking plates the angle between the loop and agar surface should be 20° to 30° . The pressure of the loop on the agar surface and the rapidity of streaking must be consistent throughout. Dipping the loop in the culture whilst foam and/or bubbles are on the surface of the broth should be avoided.

Normally the same loop is used for streaking all sectors from A to D without flaming the loop between streaks. In some cases where a lower growth index G is expected to demonstrate distinct differences, changing or sterilizing the loop between streaking sectors A and B may be appropriate.

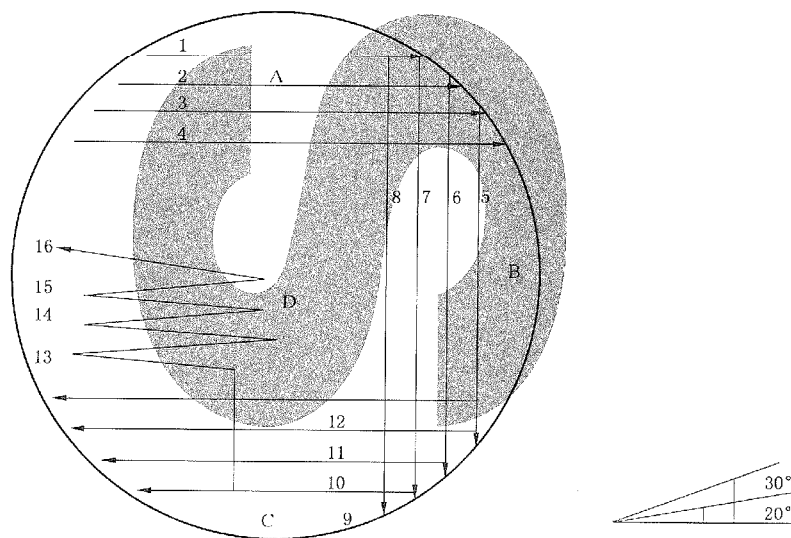


Figure 1—Pattern of inoculation by modified streaking method and angle of loop

5.3.2.3 Calculation

After incubation, the appearance, colony size and intensity of growth are assessed and the growth index G_1 calculated. Each streaking line showing growth is scored with 1. The maximum score per

plate is 16. The streak is scored as 0.5 if growth only occurs along half of its length. A streak without growth or with scanty growth (less than half the length), is scored as 0. The scores are summed to obtain the G_1 . For example, if growth was obtained in sectors A and B and in half of sector C the G_1 would be 10.

5.3.2.4 Interpretation of results

The growth index G_1 given by a target strain should be at least 6 in order to conclude that the medium is acceptable. In the case of non-selective media the G_1 is normally higher. In addition, growth of the target strain shall be typical, and growth of non-target strains shall be partly or completely inhibited.

5.3.3 Qualitative streaking method

5.3.3.1 General

The method is suitable for supplementary performance testing of solid culture media.

5.3.3.2 Procedure

—Agar plates are prepared in the usual manner with about 15 ml of agar. Media normally used for the pour plate technique, for example Plate Count Agar (PCA), may also be tested by surface plating on solidified media

—Use working cultures as described in 5.2.2.

—The test microorganisms are streaked in parallel straight lines with a 3 mm loop on the surface of the test medium. Several test microorganisms can be streaked on the same plate without crossing.

NOTE: Other standardized streaking techniques can be used.

—The incubation times and temperatures stated in the standard methods are used.

5.3.3.3 Interpretation of results

The growth on the plates after incubation is assessed as:

—0 corresponds to zero growth;

—1 corresponds to weak growth;

—2 corresponds to good growth.

Target microorganisms shall score 2 and have typical appearance, size and colony morphology. The growth of non-target microorganisms shall be partly or completely inhibited (0 or 1).

5.4 Methods for liquid culture media

The equivalent method may be used in performance testing of liquid culture media, e. g. the quantitative method is only substituted by quantitative method.

5.4.1 General

To determine the productivity of a liquid medium an appropriate inoculum shall be used. The quantitative, semi-quantitative and qualitative methods described below assess productivity and selectivity. The proposed methods record the quantity of growth after appropriate incubation by plating or streaking from the liquid media onto agar media and enumerating colony forming units (CFU) or calculating scores from the liquid medium. For qualitative methods in liquid media the characteristic reactions are assessed visually.

5.4.2 Quantitative dilution method for target and non-target microorganisms

The method is also appropriate for evaluation of new culture media, broths or diluents.

5.4.2.1 Procedure

- Select an appropriate number of tubes or 10 mL portions of each batch of liquid medium to be tested.
- Inoculation of target microorganisms: Inoculate test broth and reference broth for each test organism with a small number (e. g. 10 CFU to 100 CFU) into each tube; for preparation of the inoculum see 5.2.2) and mix.
- Inoculation of non-target microorganisms: Inoculate test broth and reference broth for each test organism with a higher number (>1 000 CFU into each tube; for preparation of the inoculum see 5.2.2) and mix.
- Inoculation of target and non-target microorganisms as a mixed culture: For testing mixed cultures in selective media inoculate test broth and reference broth with a small number of target microorganisms (e. g 10 CFU to 100 CFU for every tube; for preparation of the inoculum see 5.2.2) and in the same tube with a higher number of non-target microorganisms (>1 000 CFU into each tube; for preparation of the inoculum see 5.2.2) and mix.
- Inoculation of target and non-target microorganisms in diluents and transport media: inoculate diluents with test microorganisms (e. g. 100 CFU to 1 000 CFU into each tube; for preparation of the inoculum see 5.2.2) and mix.
- The incubation times and temperatures stated in the standard methods are used.

Diluents should be incubated for 45 min at room temperature and then plated out. Transport media should be incubated at an appropriate temperature and time according to normal usage and then plated out.

- Remove an aliquot volume or if necessary a dilution from each broth after the incubation step and spread to a non-inhibitory agar plate as described in 5.3.1.

The modified Miles-Misra surface drop method, other dropping systems or a spiral plater can be used to give countable colonies on the plates.

NOTE: To test mixed cultures, spreading should be done when possible on non-selective agar plates which allow

differentiation of the microorganisms in the mixed culture (e. g. Plate Count Agar with MUG for counting *Escherichia coli* and *Salmonella* spp.). When it is not possible to distinguish mixed cultures on non-selective agar, selective agar media should be used providing that their performance has been previously tested.

5.4.2.2 Reading, calculation and interpretation of results

After incubation colonies of target and non-target microorganisms are counted, in the case of mixed cultures distinguishing the different types. Calculation and interpretation shall be done with respect to the aim of examination:

- a) comparative interpretation between reference broth and test broth using P_R and S_F , figures as described in 4.2.3.2 and 4.2.3.3:
 - for target microorganisms P_R should not be < 0.1 (the difference in growth does not exceed one order of magnitude);
 - for non-target microorganisms, S_F should reach at least 2;
 - in mixed cultures the growth of target microorganisms should not be inhibited by the non-target microorganisms, i. e. the target microorganisms should always be the dominant population;
- b) for other cases achieving fixed minimum counts for target microorganisms and maximum counts for non-target microorganisms is more appropriate:
 - target microorganisms should reach 10^6 CFU/mL to 10^8 CFU/mL;
 - non-target microorganisms should not exceed 10^4 CFU/mL in selective broth.
- c) for diluents and transport media neither reduced nor higher numbers of target and/or non-target organism are required. The number of microorganisms after incubation in these media should be within $\pm 50\%$ of the initial count.

NOTE: The quality of a liquid medium with respect to optimal growth properties is indicated most appropriately in the early growth phase. Looking at the length of the log phase and growth in the early log phase gives the most sensitive information on productivity and selectivity of target and non-target microorganisms respectively in the test and reference broths. Therefore if only minor differences in the media quality are being sought, streaking from the liquid media onto the plates should be done after shorter incubation period of e. g. 6 h or 12 h.

5.4.3 Semi-quantitative single tube method for target, non-target and mixed microorganisms

5.4.3.1 Procedure

- Select an appropriate number of tubes or 10 ml portions of each batch to be tested.
- Inoculation of target and non-target organisms as a mixed culture: Inoculate 1 tube of test broth with about 10 CFU to 100 CFU of target microorganism and in the same tube inoculate with a higher number of non-target microorganisms ($> 1\ 000$ CFU for every tube) and mix.
- Inoculation of non-target microorganisms: Inoculate one tube of test broth per microorganism with a higher number ($> 1\ 000$ CFU) and mix.
- The incubation times and temperatures stated in the standard methods are used.

- Remove one loop with 3mm loop from the mixed culture and streak on a plate of the specific selective medium for the target microorganism.
- Remove one loop with 3mm loop from the culture of non-target microorganism and streak on a plate of a non-selective medium (e. g. TSA).
- Incubate both plates under appropriate conditions for a suitable time, as indicated in individual standards.

5.4.3.2 Calculation and interpretation of results

Productivity of the liquid test broth is satisfactory if at least 10 colonies of the target microorganism have grown on the selective agar plate.

Selectivity of the liquid test broth is satisfactory if no growth (or less than 10 CFU) of non-target microorganism occurs on the non-selective agar plate.

5.4.4 Qualitative single tube method

5.4.4.1 General

The method is suitable for performance testing of liquid culture media. The method is only qualitative and scores are therefore only indicative. Turbid media, e. g. tetrathionate broth, cannot be tested by this method.

5.4.4.2 Procedure

- for performance testing of liquid culture media the working cultures are directly inoculated into the medium being tested using a 3 mm loop;
- the incubation times and temperatures stated in the individual standard methods are used.

5.4.4.3 Interpretation of results

Qualitative evaluation shall be carried out visually by allocating growth scores, e. g. from 0 to 2. For tubes and bottles:

- 0 corresponds to zero turbidity;
- 1 corresponds to very light turbidity;
- 2 corresponds to good turbidity.

The score of a target microorganism shall be 2.

NOTE 1: Sometimes the growth of microorganisms can only be observed as a cell aggregation/deposit at the tube or bottle. In this case careful shaking can improve assessment and interpretation.

NOTE 2: Other characteristics such as gas formation, colour change, etc. can also be assessed by this method.

6 Documentation of test results

6.1 Information provided by the manufacturer

The manufacturer or supplier of the culture media shall provide, on request, the specific microbiological growth characteristics and general information relating to the specific batch of culture medium, see 4.1.1 of SN/T 1538.1—2005.

6.2 Traceability

All the data from routine performance testing should be documented in an appropriate way and kept for a sufficient period of time according to the quality system in use. The use of control sheets for documenting and evaluating the results of the tests is recommended (see Annex A).

Annex A
(informative)

Example of card for recording test results of culture media prepared by the user laboratory

Table A. 1—Example of a card

Control card for internal quality testing of culture media				
culture medium;		volume prepared:	pouring date:	internal batch number
dehydrated medium (&code):	supplier:	batch:	amount:	date/signature:
supplement:	supplier:	batch:	amount:	date/signature:
Process details:				
Physical quality control				
Expected pH-value:	Measured pH:	Quality confirmed: yes <input type="checkbox"/> no <input type="checkbox"/>	defects:	date/signature:
Expected quantity filed and/or layer thickness:	Observed:	Quality confirmed: yes <input type="checkbox"/> no <input type="checkbox"/>	defects:	date/signature:
expected colour:	Observed:	Quality confirmed: yes <input type="checkbox"/> no <input type="checkbox"/>	defects:	date/signature:
Expected clarity/presence of optical artifacts:	Observed:	Quality confirmed: yes <input type="checkbox"/> no <input type="checkbox"/>	defects:	date/signature:
Expected gel stability/consistency/moisture:	Observed:	Quality confirmed: yes <input type="checkbox"/> no <input type="checkbox"/>	defects:	date/signature:
Microbial contamination				
No. of tested plates or tubes Incubation:	result:	Quality confirmed: yes <input type="checkbox"/> no <input type="checkbox"/>	No. of contaminated plates or tubes	date/signature:
Microbiological growth-Productivity		Method of control: quantitative <input type="checkbox"/> qualitative <input type="checkbox"/>		
strains: incubation: reference medium:	criteria:	result:	Quality confirmed: yes <input type="checkbox"/> no <input type="checkbox"/>	date/signature:
Microbiological growth-selectivity		Method of control: quantitative <input type="checkbox"/> qualitative <input type="checkbox"/>		
strains: incubation: reference medium:	criteria:	result:	Quality confirmed: yes <input type="checkbox"/> no <input type="checkbox"/>	date/signature:
Microbiological growth-selectivity		Method of control: quantitative <input type="checkbox"/> qualitative <input type="checkbox"/>		
strains: incubation: reference medium:	criteria:	result:	Quality confirmed: yes <input type="checkbox"/> no <input type="checkbox"/>	date/signature:
Release of the batch				
Details of storage		Release of the batch: yes <input type="checkbox"/> no <input type="checkbox"/>		date/signature:

Annex B
(normative)
Recommended test microorganisms for commonly used culture media

Tables B. 1 to B. 6 have been established taking into account the control strains used in the European Pharmacopoeia and the recommendations from the Pharmacopoeia on food microbiology for culture media (Working Party of ICFMH). These criteria will be included in specific standards when prepared or revised in the future (new standard or revision). A validated batch of media is a batch of media which has shown satisfactory performance. The use of equivalent strains from other reference collections is permitted (e.g. NCTC, CIP...). All cited media are described within EN and ISO standards.

Table B. 1—Selective media for enumeration of microorganisms

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
Baird-Parker	S ^a	Coagulase positive <i>Staphylococci</i>	EN 6888-1	Productivity	24h~ 48h/37°C	<i>S. aureus</i> ATCC 6538; <i>S. aureus</i> ATCC 25923 ^b	TSA	Quantitative	PR ≥ 0.5	Black/grey colonies with clear halo (Egg yolk clearing reaction)
				Selectivity	48h/37°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	—	Qualitative	Total inhibition	—
				Specificity	24h~ 48h/37°C	<i>S. epidermidis</i> ATCC 12228 ^b	—	Qualitative	Black/grey colonies with clear halo Egg yolk clearing reaction	
RPFA	S	Coagulase positive <i>Staphylococci</i>	EN 6888-2	Productivity	24h~ 48h/37°C	<i>S. aureus</i> ATCC 6538 or 6538p <i>S. aureus</i> ATCC 25923 ^b	TSA	Quantitative	PR ≥ 0.5	Black/grey colonies with opacity halo
				Selectivity	48h/37°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	—	Qualitative	Total inhibition	—
				Specificity	24h~ 48h/37°C	<i>S. epidermidis</i> ATCC 12228 ^b	—	Qualitative	Black/grey colonies with opacity halo	

Table B. 1 (continued)

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
Chlorampenicillin or OGA (OGY)	S	Yeasts/Moulds	ISO 7954	Productivity	3d~ 5d/37℃	<i>C. albicans</i> ATCC 10231 <i>A. niger</i> ATCC 16404 ^b <i>P. cyclopium</i> ATCC 16025 <i>S. cerevisiae</i> ATCC 9763	Media batch SDA (Sabouraud Dextros Agar) /ale ady validated	Quantitative	PR≥0.5	Characteristic colonies according to each species
				Selectivity	3d~ 5d/37℃	<i>E. coli</i> ATCC 25922 or 8739 ^b <i>B. subtilis</i> ATCC 6633	—	Quantitative	Total inhibition	
MRS	S	Lactic acid bacteria	ISO 15214	Productivity	72h/30℃	<i>L. sake</i> ATCC 15521 ^b <i>P. damnosus</i> ATCC 29538 <i>L. lactis</i> ATCC 19435 ^b	MRS	Quantitative	PR≥0.5	Characteristic colonies according to each species
				Selectivity	72h/30℃	<i>E. coli</i> ATCC 25922 or 8739 ^b <i>B. cereus</i> ATCC 11778	—	Quantitative	Total inhibition	
MYP	S	<i>Bacillus subtilis</i>	EN ISO 7932	Productivity	24h~ 48h/30℃	<i>B. cereus</i> ATCC 11778 ^b	TSA	Quantitative	PR≥0.7	Pink colonies with precipitation halo
				Selectivity	48h/37℃	<i>E. coli</i> ATCC 25922 or 8739 ^b	—	Qualitative	Total inhibition	
Oxford	S	<i>Listeria monocytogenes</i>	EN ISO 11290	Productivity	48h/37℃	<i>B. subtilis</i> ATCC 6633 ^b <i>L. mono1/2a</i> ATCC 19111 <i>L. mono4b</i> ATCC 13932 ^b	TSA	Quantitative	PR≥0.5	Yellow colonies without precipitation halo Grey to black colonies with black halo
				Selectivity	48h/37℃	<i>E. coli</i> ATCC 25922 or 8739 ^b <i>E. faecalis</i> ATCC 29212 or 19433 <i>C. albicans</i> ATCC 10231	—	Qualitative	Total inhibition	

Table B.1 (continued)

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
PALCAM	S	<i>Listeria monocytogenes</i>	EN ISO 11290	Productivity	48h/37°C	<i>L. mono1/2a</i> ATCC 19111 <i>L. mono4b</i> ATCC 13932 ^b	TSA	Quantitative	PR \geq 0.5	Grey-green to black colonies with black halo
				Selectivity	72h/30°C	<i>E. coli</i> ATCC 25922 or 8739 ^b <i>E. faecalis</i> ATCC 29212 or 19433	—	Qualitative	Total inhibition	—
TS(C)	S	<i>C. jejuni</i>	EN ISO 7937	Productivity	20h/37°C	<i>C. perfringens</i> ATCC 13124 <i>C. perfringens</i> ATCC 12916	TS(C)	Quantitative	PR \geq 0.7	black colonies
				Selectivity TSC	20h/37°C	<i>E. coli</i> ATCC 25922 or 8739	—	Qualitative	Total inhibition	—
				Specificity TS		<i>E. faecalis</i> ATCC 29212 or 19433	—	Qualitative	—	White colonies
				Productivity	24h/37°C	<i>E. coli</i> ATCC 25922 or 8739 ^b <i>S. typhimurium</i> ATCC 14028	TSA	Quantitative	PR \geq 0.5	Pink to red colonies with or without precipitation halo
VRBG	S	<i>Enterobacteriaceae</i>	ISO 7402 ISO 8907	Selectivity	20h/37°C	<i>E. faecalis</i> ATCC 29212 or 19433 ^b	—	Qualitative	Total inhibition	—
				Productivity	24h/37°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	TSA	Quantitative	PR \geq 0.5	Purplish colonies with or without precipitation halo
VRBL	S	<i>coliforms</i>	ISO 4832	Productivity	20h/30°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	TSA	Quantitative	PR \geq 0.5	Colourless to beige colonies
				Selectivity	24h/30°C	<i>E. faecalis</i> ATCC 29212 or 19433 ^b	—	Qualitative	Total inhibition	—
				Specificity	24h/30°C	<i>P. aeruginosa</i> ATCC 27853	—	Qualitative	—	Transparent colonies with a pale yellowish-brown appearance and a diameter of approx. 1mm
CT-SMAC	S	<i>E. coli</i> O157	ISO 16654	Productivity	24h/37°C	<i>E. coli</i> O157:H7 ATCC 43894 or 43895 (non-toxicogenic)	TSA	Quantitative	PR \geq 0.5	—
				Selectivity	24h/37°C	<i>S. aureus</i> ATCC 6538 or ATCC 25923 ^b	—	Qualitative	Total inhibition	—
				Specificity	24h/37°C	<i>E. coli</i> ATCC 11775 or 25922 ^b	—	Qualitative	—	Pink colonies

Table B.1 (continued)

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
BGBLB	L ^c	Coliforms	ISO 4831	Productivity	24h~ 48h/30℃	<i>E. coli</i> ATCC 25922 or 8739 ^b <i>C. freundii</i> ATCC 43864		semi- Quantitative	Turbidity 2+ Gas in 1/3 of Durham tube	Gas production and turbidity
				Selectivity	24~ 48h/30℃	<i>E. faecalis</i> ATCC29212 or 19433 ^b				
LST	L	Coliforms	ISO 4831	Productivity	24~ 48h/30℃	<i>E. coli</i> ATCC 25922 or 8739 ^b <i>C. freundii</i> ATCC 43864		semi- Quantitative	Turbidity 2+ Gas in 1/3 of Durham tube	Gas production and turbidity
				Selectivity		<i>E. faecalis</i> ATCC 29212 or 19433 ^b				
EC	L	<i>Escherichia coli</i>	ISO 7251	Productivity	24~ 48h/44℃	<i>E. coli</i> ATCC 25922 or 8739 ^b		semi- Quantitative	Turbidity 2+ Gas in 1/3 of Durham tube	Gas production and turbidity
				Selectivity	24~ 48h/44℃	<i>P. aeruginosa</i> ATCC 27853 ^b				

Note1: For solid culture media, it is also possible to use a semi-quantitative plating method.
Note2: The culture which can be traced to the culture mentioned above can be used in the performance of culture media.
a S = solid media.
b Strains to be used by the user laboratory (minimum).
c L = liquid medium.

Table B.2—Non selective media for enumeration of microorganisms

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
PCA	S ^a	Total flora	ISO 4833	Productivity	72h/30℃	<i>E. coli</i> ATCC 25922 or 8739 ^b	TSA	Quantitative	RP≥0.7	
						<i>S. aureus</i> ATCC 6538 or 6538 ^b				
						<i>B. subtilis</i> ATCC 6633 ^b				

Note: The culture which can be traced to the culture mentioned above can be used in the performance of culture media
a S = solid media.
b Strains to be used by the user laboratory (minimum).

Table B.3—selective enrichment media

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
EE	L ^a	Enterobacteriaceae	ISO 7402 ISO 8523	Productivity	24h/37°C	E. coli ATCC 25922 or 8739 ^b S. typhimurium ATCC 14028 + E. faecalis ATCC 29212 or 19433 ^b		semi-Quantitative	> 10 col. on VRBG Total inhibition	Pink to red colonies with or without precipitation halo
				Selectivity						
Half-Fraser	L	Listeria monocytogenes	EN ISO 11290-1	Productivity	24h/30°C	L. mono 1/2a ATCC 19111 L. mono 4b ATCC 13932 ^b + E. coli ATCC 25922 or 8739 ^b + E. faecalis ATCC 29212 or 19433 ^b		semi-Quantitative	> 10 on Oxford or PALCAM	Grey to black colonies with black halo
				Selectivity						
Fraser	L	Listeria monocytogenes	EN ISO 11290-1	Productivity	24h~ 48h/30°C	E. coli ATCC 25922 or 8739 ^b E. faecalis ATCC 29212 or 19433 ^b L. mono 1/2a ATCC 19111 L. mono 4b ATCC 13932 ^b + E. coli ATCC 25922 or 8739 ^b + E. faecalis ATCC 29212 or 19433 ^b		semi-Quantitative	Total inhibition on TSA < 100 on TSA	Grey to black colonies with black halo
				Selectivity						

Table B.3 (continued)

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
ITC	L	<i>Yersinia enterocolitica</i>	ISO 10273	Productivity	48h/25°C	<i>Y. enterocolitica</i> ATCC 23715 or 9610 ^b	—	semi-Quantitative	> 10 on CIN or SSDC	Characteristic colonies according to each medium (see standard)
						<i>E. coli</i> ATCC 25922 or 8739 ^b				
						+ <i>P. aeruginosa</i> ATCC 27853 ^b				
Park & Sanders	L	<i>Campylobacter</i>	ISO 10272	Productivity	48h/25°C	<i>P. aeruginosa</i> ATCC 27853 ^b	—	semi-Quantitative	Total inhibition on TSA	—
						<i>P. mirabilis</i> ATCC 29906				
						<i>C. coli</i> ATCC 43478				
						or <i>C. jejuni</i> ATCC 33291 or 29428 ^b				
						+ <i>E. coli</i> ATCC 25922 or 8739 ^b				
						+ <i>P. mirabilis</i> ATCC 29906				
Preston	L	<i>Campylobacter</i>	ISO 10272	Productivity	18h/42°C	<i>C. coli</i> ATCC 43478	—	semi-Quantitative	> 10 on Karmali or other medium of choice	Characteristic colonies according to each medium (see standard)
						or <i>C. jejuni</i> ATCC 33291 or 29428 ^b				
						+ <i>E. coli</i> ATCC 25922 or 8739 ^b				
						+ <i>P. mirabilis</i> ATCC 29906 ^b				
						<i>E. coli</i> ATCC 25922 or 8739 ^b				
						<i>P. mirabilis</i> ATCC 29906				
Preston	L	<i>Campylobacter</i>	ISO 10272	Selectivity	18h/42°C	<i>C. coli</i> ATCC 43478	—	semi-Quantitative	> 10 on Karmali or other medium of choice	Characteristic colonies according to each medium (see standard)
						or <i>C. jejuni</i> ATCC 33291 or 29428 ^b				
						+ <i>E. coli</i> ATCC 25922 or 8739 ^b				
Preston	L	<i>Campylobacter</i>	ISO 10272	Selectivity	18h/42°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	—	semi-Quantitative	Total inhibition on TSA	—
						+ <i>P. mirabilis</i> ATCC 29906 ^b				
						<i>E. coli</i> ATCC 25922 or 8739 ^b				
Preston	L	<i>Campylobacter</i>	ISO 10272	Selectivity	18h/42°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	—	semi-Quantitative	Total inhibition on TSA	—
						+ <i>P. mirabilis</i> ATCC 29906 ^b				
						<i>E. coli</i> ATCC 25922 or 8739 ^b				

Table B.3 (continued)

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
PSB	L	<i>Yersinia enterocolitica</i>	ISO 10273	Productivity	3~ 5d/25℃	<i>Y. enterocolitica</i> ATCC 23715 or 9610 ^b	—	semi- Quantitative	> 10 on CIN or SSDC	Characteristic colonies according to each medium (see standard)
						+ <i>E. coli</i> ATCC 25922 or 8739 ^b				
						+ <i>P. aeruginosa</i> ATCC 27853 ^b				
MKTTh	L	<i>Salmonella</i>	ISO 6579	Productivity	24h/37℃	<i>P. aeruginosa</i> ATCC 27853 ^b	—	semi- Quantitative	Total inhibition on TSA	Characteristic colonies according to each medium (see standard)
						<i>P. mirabilis</i> ATCC 29906				
						<i>S. typhimurium</i> ATCC 14028				
Rappaport Vassiliadis	L	<i>Salmonella</i>	EN 12824	Productivity	24h/41.5℃	<i>S. enteritidis</i> ATCC 13076 ^b	—	semi- Quantitative	> 10 col. on XLD or other medium of choice	Characteristic colonies according to each medium (see standard)
						+ <i>E. coli</i> ATCC 25922 or 8739 ^b				
						+ <i>P. aeruginosa</i> ATCC 27853 ^b				
Rappaport Vassiliadis	L	<i>Salmonella</i>	EN 12824	Selectivity	24h/37℃	<i>E. coli</i> ATCC 25922 or 8739 ^b	—	semi- Quantitative	Total inhibition on TSA	Characteristic colonies according to each medium (see standard)
						<i>E. faecalis</i> ATCC 29212 or 19433				
						<i>S. typhimurium</i> ATCC 14028				
Rappaport Vassiliadis	L	<i>Salmonella</i>	EN 12824	Productivity	24h/41.5℃	<i>S. enteritidis</i> ATCC 13076 ^b	—	semi- Quantitative	> 10 col. on BGA or other medium of choice	Characteristic colonies according to each medium (see standard)
						+ <i>E. coli</i> ATCC 25922 or 8739 ^b				
						+ <i>P. aeruginosa</i> ATCC 27853 ^b				

Table B.3 (continued)

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
Reppaport Vassiliadis	L	<i>Salmonella</i>	EN 12824	Selectivity	24h/41.5°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	—	semi-Quantitative	Total inhibition on TSA <10 on TSA	
						<i>E. faecalis</i> ATCC 29212 or 19433				
RVS	L	<i>Salmonella</i>	ISO 6579	Productivity	24h/41.5°C	<i>S. typhimurium</i> ATCC 14028	—	semi-Quantitative	> 10col. on XLD or other medium of choice	Characteristic colonies according to each medium (see standard)
						<i>S. enteritidis</i> ATCC 13076 ^b				
						<i>E. coli</i> ATCC 25922 or 8739 ^b				
						+ <i>P. aeruginosa</i> ATCC 27853 ^b				
Selenite-cystine	L	<i>Salmonella</i>	EN 12824	Productivity	24h/37°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	—	semi-Quantitative	Total inhibition on TSA <10 on TSA	
						<i>E. faecalis</i> ATCC 29212 or 19433				
						<i>S. typhimurium</i> ATCC 14028 ^b				
						or <i>S. enteritidis</i> ATCC 13076 ^b				
Selenite-cystine	L	<i>Salmonella</i>	EN 12824	Productivity	24h/37°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	—	semi-Quantitative	> 10col. on BGA or other medium of choice	Characteristic colonies according to each medium (see standard)
						+ <i>P. aeruginosa</i> ATCC 27853 ^b				
						<i>E. coli</i> ATCC 25922 or 8739 ^b				
						<i>E. faecalis</i> ATCC 29212 or 19433				
Note: The culture which can be traced to the culture mentioned above can be used in the performance of culture media.										
a L = liquid medium.										
b Strains to be used by the user laboratory (minimum).										

Table B. 4—Non selective enrichment media

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
BHI	L ^a	<i>Staphylococcus</i>	ISO 6888	Productivity	24h/37°C	<i>S. aureus</i> ATCC 25923 ^b		Qualitative	Turbidity 1 to 2	—
Brucella	L	<i>Campylobacter</i>	ISO 10272	Productivity	2d~ 5d/25°C	<i>C. coli</i> ATCC 43478	—	Qualitative	Turbidity 1 to 2	—
						<i>C. jejuni</i> ATCC 33291 or 29428 ^b				
Peptone salt	L	Dilution liquids	ISO 6887	Diluent	45min/20~25°C	<i>E. coli</i> ATCC 25922 or 8739 ^b	TSA	Quantitative	+/- 50% col./ to (+/- 50% of original count)	—
						<i>S. aureus</i> ATCC 25923				
Thioglycollate	L	<i>Clostridium perfringens</i>	ISO 7937	Productivity	24h/37°C	<i>C. perfringens</i> ATCC 13124 ^a		Qualitative	Turbidity 1 to 2	—
TSYEB	L	<i>Listeria monocytogenes</i>	ISO 11290	Productivity	24h/25°C	<i>L. mono</i> 1/2a ATCC 19111		Qualitative	Turbidity 1 to 2	—
						<i>L. mono</i> 4b ATCC 13932 ^b				
Note: The culture which can be traced to the culture mentioned above can be used in the performance of culture media.										
a L = liquid medium.										
b Strains to be used by the user laboratory (minimum).										

Table B.5—Selective isolation media

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
Modified Butzler	S ^a	<i>Campylobacter</i>	ISO 10272	Productivity	24h~ 72h/42°C	<i>C. coli</i> ATCC 43478		Qualitative	Good growth (2)	Characteristic colonies according to each medium (see Standard)
CCDA	S ^a	<i>Campylobacter</i>	ISO 10272	Productivity	24h~ 72h/42°C					
Karmali	S ^a	<i>Campylobacter</i>	ISO 10272	Productivity	24h~ 72h/42°C			Qualitative		
Preston	S ^a	<i>Campylobacter</i>	ISO 10272	Productivity	24h~ 72h/42°C	<i>C. jejuni</i> ATCC 33291 or 29428 ^b				
Skirrow	S ^a	<i>Campylobacter</i>	ISO 10272	Productivity	24h~ 72h/42°C	<i>E. coli</i> ATCC 25922 or 8739 ^b		Qualitative	Total or partial inhibition (0~1)	No characteristic colonies
		<i>Campylobacter</i>	ISO 10272	Selectivity	24h~ 72h/42°C	<i>S. aureus</i> ATCC 25923			Total inhibition(0)	—
CIN	S	<i>Yersinia enterocolitica</i>	ISO 10273	Productivity	24h/30°C	<i>Y. enterocolitica</i> ATCC 23715 or 9610 ^b		Qualitative	Good growth (2)	Characteristic colonies according to each medium (see Standard)
		<i>Yersinia enterocolitica</i>		Productivity	24h/30°C	<i>Y. enterocolitica</i> ATCC 23715 or 9610 ^b			Good growth (2)	Characteristic colonies according to each medium (see Standard)
SSDC	S	<i>Yersinia enterocolitica</i>	ISO 10273	Selectivity	24h/30°C	<i>E. coli</i> ATCC 25922 or 8739 ^b		Qualitative	Total or partial inhibition (0~1)	No characteristic colonies
		<i>Yersinia enterocolitica</i>		Productivity	24h/30°C					

Table B. 5 (continued)

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
SSDC	S	<i>Yersinia enterocolitica</i>	ISO 10273			<i>S. aureus</i> ATCC 25923			Total inhibition(0)	
Brilliant green (BGA)	S	<i>Salmonella</i>	EN 12824/ ISO 6579	Productivity	24h~ 48h/37°C	<i>S. typhimurium</i> ATCC 14028 ^b <i>S. enteritidis</i> ATCC 13076	—	Qualitative	Good growth (2)	Characteristic colonies according to each medium (see Standard)
XLD	S	<i>Salmonella</i>	EN 12824/ ISO 6579	Selectivity	24h~ 48h/37°C	<i>E. coli</i> ATCC 25922 or 8739 ^b <i>E. faecalis</i> ATCC 29212 or 19433	—	Qualitative	Total or partial inhibition (0~1) Total inhibition (0)	No characteristic colonies
Note: The culture which can be traced to the culture mentioned above can be used in the performance of culture media. a S = solid media. b Strains to be used by the user laboratory (minimum).										

Table B. 6—Non selective isolation media

Media	Type	Microorganisms	Standard	Function	Incubation	Control strains	Reference media	Method of control	Criteria	Characteristic reactions
Nutrient agar	S ^a	<i>Enterobacteriaceae</i>	ISO 7402	Productivity	24h/37°C	<i>E. coli</i> ATCC 25922 or 8739 ^c	—	Qualitative	Good growth (2)	—
			ISO 8523							
		<i>Salmonella</i>	EN 12824 ISO 6579	24h/37°C	<i>S. typhimurium</i> ATCC 14028 ^c					
TSYEA agar	S	<i>Yersinia enterocolitica</i>	ISO 10273	Productivity	24h/30°C	<i>Y. enterocolitica</i> ATCC 23715 or 9610 ^c	—	Qualitative	Good growth (2)	—
			EN							
		<i>Listeria monocytogenes</i>	ISO 11290	24h/37°C	<i>L. mono 1/2a</i> ATCC 19111 or <i>L. mono 4b</i> ATCC 13932 ^b					
Note: The culture which can be traced to the culture mentioned above can be used in the performance of culture media. a S = solid media. b Strains to be used by the user laboratory (minimum). c Strains free of choice according to the methods used.										

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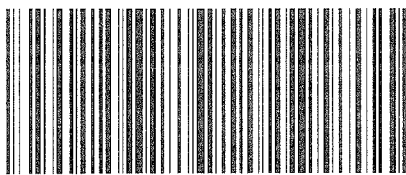
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