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中华人民共和国出入境检验检疫行业标准

SN/T 1538.1—2005

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

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

(ISO/TS 11133-1:2000, Microbiology of food and animal feeding stuffs—Guidelines on preparation and production of culture media—Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory, MOD)

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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1部分，对应于ISO/TS 11133-1：《食品和动物饲料微生物学——培养基制备指南——实验室培养基制备质量保证通则》(2000年英文版)。本部分与ISO/TS 11133-1的一致性程度为修改采用，主要差异如下：

——按照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；

——删除了原标准第3章“术语和定义”中的引用标准；

——在“规范性引用文件”部分用ISO 19000取代了ISO 8402，加入了标准GB/T 6682；

——将部分中提到的“特定国际标准”改为“特定标准”；

——在4.2.4条款中加入了特定的温度值，如“4℃”、“20℃”等；

——在4.3.3中增加了对称量脱水培养基的描述；

——在4.3.7.1中增加了对不同培养基灭菌的描述；

——在4.3.7.3中对过滤器的组装条件进行了修改；

——在4.5中增加了培养基弃置的注解；

——在5.2.5条款中加入了菌落计数技术；

——删除了原标准中的注解“1)”。

本部分的附录A、附录B和附录C均为资料性附录。

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负责起草。

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本部分系首次发布的检验检疫行业标准。

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。

SN/T 1538《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1部分，规范了培养基相关术语，规定了培养基贮存、制备、使用、弃置等方面的通用要求，并提出了成品培养基的质控方法。

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

1　范围

本SN/T 1538的部分规定了与培养基制备质量保证相关的通用术语和食品或饲料微生物学分析用培养基的最低要求。

本部分适用于以下三种培养基：

——商品化即用型培养基；

——商品化脱水合成培养基(包括完全培养基和需添加补充物的基础培养基)；

——各别成分制备的培养基。

2　规范性引用文件

下列文件中的条款通过SN/T 1538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，其随后所有的修改单(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 6682　分析实验室用水规格和试验方法(eqv ISO 3696:1987)

GB/T 19000　质量管理体系　基础和术语(idt ISO 9000:2000)

EN 1659　体外诊断方法——微生物培养基——术语和定义

EN 12322　体外诊断医疗设备——微生物培养基——培养基性能标准

3　术语和定义

下列术语和定义适用于SN/T 1538的本部分。

3.1　概述

本条款规定了与质量保证相关的通用定义以及与培养基和质控菌株相关的术语。

3.2　质量保证术语

3.2.1

质量保证　quality assurance

为使人们能确信某个产品或服务能满足规定的质量要求所必须的在质量体系中执行的全部有计划和系统的活动。

3.2.2

质量控制　quality control

为达到质量要求所采取的操作技术和活动。

3.2.3

内部质量控制　internal quality control

与控制分析和必要的纠偏活动相关的实验室制备工作的连续控制计划。

3.2.4

培养基批量　batch of culture media；lot of culture media

是培养基完整的可追溯单位。是指满足产品要求(内部控制)和质量保证测试，产品型号和质量稳定的一定量的半成品或成品。这些产品在特定的生产周期生产，而且编号相同。

3.2.5

培养基的性能　performance of culture media

在特定条件下培养基对测试菌株的反应。

3.3　**培养基术语**

3.3.1

培养基　culture medium

液体、半固体或固体形式的，含天然或合成成分，用于保证微生物繁殖或保持其活力的物质。

注：当与其他词组成词组时，这个术语常被缩写为“medium”。(例如：“enrichment medium”为增菌培养基)。

3.3.2　**按化学成分分类**

3.3.2.1

纯化学培养基　chemically defined culture medium

只含有化学成分的培养基(即分子结构和纯度已知)。

3.3.2.2

非纯化学培养基　chemically incomplete culture medium

全部或部分由天然物质，加工过的物质或其他不纯的化学物质构成的培养基。

注：ISO/TC 34/SC 9 中对培养基中使用的各种不纯的化学物质进行了详细的描述和命名，参见附录 A。

3.3.3　**按状态分类**

3.3.3.1

液体培养基　liquid culture medium

一种或多种成分组成的水溶液(如：蛋白胨水、营养肉汤等)。

注 1：通常要将固体颗粒加到液体培养基中。

注 2：试管、三角瓶或瓶子中的液体培养基一般称作“肉汤”。

3.3.3.2

固体培养基和半固体培养基　solid culture medium and semi-solid culture medium

含有不同浓度固化物(如：琼脂、明胶等)的液体培养基。

注 1：琼脂作为固化物的培养基在各国应用广泛，目前通常用缩略语“琼脂”作为这种固体培养基的同义词。它可以和其他名词连接使用。如“平板计数琼脂”。

注 2：倾注到平皿内的固体培养基一般称之为“平板”；倒入试管并摆放成斜面的固体培养基，当培养基凝固后通常称作“斜面”。

3.3.4　**按用途分类**

3.3.4.1

运输培养基　transport medium

在取样后和实验室样品处理前保护和维持微生物活性的培养基。

注：运输培养基中通常不允许包含使微生物的增殖的物质，但是培养基应能保护菌株，确保它们不变质(如：Stuart 运输培养基或 Amies 运输培养基)。

3.3.4.2

保藏培养基　preservation medium

用于在一定期限内保护和维持微生物活力，防止长期保存对微生物的不利影响，或使微生物在长期保存后容易复苏(如：Dorset 卵黄培养基)的培养基。

3.3.4.3

复苏培养基　resuscitation medium

能够使受损或应激的微生物修复，使微生物恢复正常生长能力，但不一定促进微生物繁殖的培养基。

3.3.4.4

增菌培养基　enrichment medium

大多为液体培养基，能够给微生物的繁殖提供特定的生长环境。

3.3.4.4.1

选择性增菌培养基　selective enrichment medium

能够保证特定的微生物在其中繁殖，而部分或全部抑制其他微生物生长的培养基（如：Rappaport-Vassiliads 培养基）。

3.3.4.4.2

非选择性增菌培养基　non-selective enrichment medium

能够保证大多数微生物生长（如：营养肉汤）的培养基。

3.3.4.5

分离培养基　isolation medium

支持微生物生长的固体或半固体培养基。

3.3.4.5.1

选择性分离培养基　selective isolation medium

支持特定微生物的生长而抑制其他微生物生长的分离培养基（如：PALCAM 琼脂、MarConkey 琼脂）。

3.3.4.5.2

非选择性分离培养基　non-selective isolation medium

对微生物没有选择性抑制的分离培养基（如：营养琼脂）。

3.3.4.6

鉴别培养基　differential medium

能够进行一项或多项微生物生理和（或）生化特性鉴定的培养基（如尿素培养基、Kligler 琼脂）。

注：能够用于分离培养的鉴别培养基被称作为分离和（或）鉴别培养基[例如木糖赖氨酸脱氧胆盐（XLD）琼脂]。

3.3.4.7

鉴定培养基　identification medium

能够产生一个特定的鉴定反应而不需要做进一步确认实验的培养基。

注：用于分离的鉴定培养基被称为分离和（或）鉴定培养基。

3.3.4.8

多种用途培养基　media having multiple uses

同时可归为几类不同用途的特定培养基。例如，按 3.3.4.3 分类，血琼脂是一种复苏培养基；按 3.3.4.5分类为分离培养基；按 3.3.4.6 分类为鉴别培养基，用于溶血的检测。

3.3.5　按制备方法分类

3.3.5.1

即用型培养基　ready-to-use medium

以即用形式置于容器中（例如：平皿、试管或其他容器）供应的培养基。

3.3.5.2

商品化脱水合成培养基　culture medium prepared from commercially dehydrated formulations

不立即使用的干粉形式的（如：粉末、小颗粒、冻干等形式）培养基。

这类培养基溶于水后能制成下列培养基：

——完全即用型培养基；

——不完全即用型培养基，使用时需加入不稳定成分。

3.3.5.3

实验室制备各别成分培养基　culture medium prepared from individual components in the laboratory

3.4 测试菌株术语

3.4.1 概述

测试菌株通常指用于培养基质量控制和性能测定的微生物。根据用途不同定义如下：

3.4.2

标准菌株 reference strain

至少定义到属或种水平的菌株。按菌株特性进行分类和描述，最好有明确的来源。

3.4.3

标准储备菌株 reference stocks

将实验室分离到的或供应商提供的标准菌株转接一代后得到的一套完全相同的独立菌株。

3.4.4

工作菌株 working culture

由标准储备菌株转接一代获得的菌株。

4 培养基质量控制[1)]

4.1 证明文件

4.1.1 生产企业提供的文件

生产企业应提供下列材料：

——培养基、独立成分、添加成分名称及产品编号；

——批号；

——培养基使用前的 pH；

——储藏信息和有效期；

——性能评价和所用的测试菌株；

——技术数据清单；

——质控证书；

——必要的安全/危害数据。

4.1.2 实验室检查记录单

实验室收到培养基后，应检查：

——培养基的名称和批号；

——接收日期；

——有效期；

——包装及其完整性。

4.2 贮存

4.2.1 概述

应严格按照供应商提供的贮存条件、有效期和使用方法进行培养基的保存和使用。

4.2.2 脱水培养基及其添加成分的质量管理和质量控制

新购买的培养基一般为脱水的粉状或颗粒状，保存在密闭的容器中。用于菌种选择或鉴定的添加成分通常为冻干物或液体。培养基的购买应有计划，以利于存货的周转（即掌握先购先用的原则）。实验室应保存有效的培养基目录清单，清单应包括以下内容：

——容器密闭性复查；

——首次开封日期；

——内容物的感官检查。

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1) 培养基质量常见问题，参见附录 C。

对于新开封的脱水培养基，应对其质量进行检查。通过粉末的流动性、均匀性、结块情况和色泽变化等判断脱水培养基的质量的变化。若发现培养基受潮或物理性状发生明显改变则不应再使用。

4.2.3 商品化即用型培养基

应严格按照供应商提供的贮存条件、有效期和使用方法进行保存和使用。

4.2.4 商品化的脱水合成培养基和各别成分制备的培养基

这类培养基的贮存有效期各不相同，一般无法做统一规定。可参照不同标准中的规定执行。

培养基灭菌后分装到平皿、试管或测试瓶中，不能立即使用的培养基应避光、干燥保存。

除特殊说明和标准规定，通常情况下基础培养基（如使用前加入添加成分的培养基）应在 4℃冰箱中保存不超过 3 个月，或在室温（20℃）下保存不超过 1 个月，以保证其成分不会改变；不稳定的选择性物质和其他添加成分应即配即用；对发生化学反应或含有不稳定成分的固体培养基也应即配即用，不可二次融化。

观察培养基颜色变化，是否有蒸发/脱水情况，是否有微生物生长。当培养基发生这类变化时，应禁止使用。

使用和进一步加热前，应事先将培养基放置到室温。

4.3 培养基的实验室制备

4.3.1 概述

使用脱水培养基和其他含有有害物质（如胆盐或其他选择剂）的成分时，应遵守良好实验室规范和生产厂商提供的使用说明。

使用商品化脱水合成培养基制备培养基时，应严格按照厂商提供的使用说明配制。如质量/体积、pH、制备条件、灭菌条件和操作步骤等。

使用各别成分制备培养基时，应按配方准确配制，记录 4.1.2 列出的内容，并记录所使用成分的特性（如代号和批号等）。

4.3.2 水

配制培养基应使用蒸馏水或相同质量的水，以排除测试条件下抑制或影响微生物生长的物质。如果蒸馏水是用氯消毒的水制备的，在蒸馏前应先对氯进行中和（见 GB/T 6682）。

盛放蒸馏水的容器最好是由中性材料制成的（如中性玻璃、聚乙烯等），在初次使用前要确认容器中不含有任何抑制因子。

注：有时需使用新制备的蒸馏水，避免水中溶解的二氧化碳。

为保证蒸馏水的质量，蒸馏水的电阻率最少应达到 300 000 Ωcm。

警告：采用离子交换器（去离子）生产的去离子水，微生物含量较高，这种水在过滤灭菌后仍可能带有细菌生长的抑制因子。所以配置培养基时最好不要使用这种方法生产的去离子水，而应使用蒸馏水。

4.3.3 称量和复水

称量所需量的脱水培养基（注意缓慢操作，必要时佩带口罩或在通风柜中操作，以防吸入含有有毒物质的培养基粉末），先加入少量的水，充分混合（注意避免培养基结块），然后再加水至所需的量。

4.3.4 溶解和分装

脱水培养基加水后适当加热，并不停搅拌使其快速溶解，必要时，重新溶解。含琼脂的培养基在加热前应先浸泡几分钟。用各别成分制备的培养基应将不同成分分别加入适量的水中，并充分溶解，然后再加水至所需的量。

4.3.5 pH 的测定和调整

用 pH 计测 pH，必要时进行调整。在实验室用各别成分制备的培养基，除特殊说明外，培养基灭菌后冷却到 25℃时，pH 的变化不应超过 0.2 个单位。一般使用浓度约为 40 g/L（约 1 mol/L）的氢氧化钠溶液或浓度约为 36.5 g/L（约 1 mol/L）的盐酸溶液调整培养基的 pH。

注：商品化的培养基高压灭菌前后 pH 值可能变化很大。但采用优质蒸馏水或去离子水配制时，灭菌前无需调节

pH 值。

4.3.6 分装

将配制好的培养基分装到适当的容器中，根据不同用途，容器的体积可为培养基的 1 倍、2 倍或 3 倍。

4.3.7 灭菌

4.3.7.1 概述

培养基和试剂应采用湿热灭菌(4.3.7.2)或过滤灭菌(4.3.7.3)。

亮绿培养基等特定的培养基中含有对光和热敏感的物质，只能煮沸灭菌。煮沸后应迅速冷却，避光保存；明胶、血清、糖类等不耐高温的物质，应采用高压锅低温灭菌法/间歇灭菌法灭菌；有些试剂则不需灭菌，可直接使用(参见相关标准或供应商使用说明)。

4.3.7.2 湿热灭菌

湿热灭菌在高压锅或制备培养基的容器中进行，高压灭菌一般采用 121℃灭菌 15 min。当培养基体积超过 1 000 mL 时，要对灭菌条件进行适当的调整，但应按照标准或使用说明的规定进行。高压灭菌过程要通过放置到特定位置的热电耦或测试条进行监测，以保证灭菌的效果。

注：大容量(>1 000 mL)的培养基灭菌时可能会造成过度加热。

灭菌效果的控制是关键。加热后采用适当的方法冷却，以防加热过度。这对于肠道菌培养基和大容器中的培养基等的灭菌十分重要。

4.3.7.3 过滤灭菌

过滤灭菌可在真空负压或正压的条件下进行。使用孔径为 0.22 μm 的滤膜和过滤垫。过滤前先将滤膜和滤垫灭菌。过滤器于 121℃灭菌 15 min(可以整体灭菌也可以拆卸后灭菌)，灭菌后在无菌条件下组装。

注：一些滤膜上附着有蛋白质(如抗生素)。为达到有效过滤，应事先将滤膜用无菌水润湿。

4.3.7.4 监测

应对经湿热或过滤灭菌的培养基进行监测，尤其要对 pH、色泽、灭菌效果和均匀度等指标进行监测。

4.3.8 添加成分的制备

制备含有有毒物质的添加成分(尤其是抗生素)时应小心操作(必要时在通风柜中操作)，避免因粉末的扩散造成实验人员过敏或发生其他不良反应；制备溶液时应小心按产品使用说明操作；不要使用过期的试剂；抗生素工作溶液应现用现配；批量配制的抗生素溶液分装后冷冻贮存，但解冻后的贮存溶液不能再次冷冻；厂商应提供冷冻对抗生素活性影响的有关资料，也可由使用者自行测定。

4.4 培养基的使用

4.4.1 琼脂培养基的融化

将培养基放到沸水浴中或采用有相同效果的方法(如高压锅中的蒸汽)使之融化。经过高压的培养基应尽量减少重加热时间，避免过度加热。培养基融化后放入 47℃±2℃的恒温水浴锅中保温，直至使用。融化后的培养基应尽快使用，放置时间一般不应超过 4 h 。

4.4.2 培养基的脱气

必要时，将培养基在使用前放到沸水浴或蒸汽浴中加热 15 min，加热时松开容器的盖子；加热后盖紧，并迅速冷却至使用温度。

4.4.3 添加成分的加入

对热不稳定的添加成分应在培养基冷却至 47℃±2℃时再加入。灭菌的添加成分在加入之前，应先放置到室温，避免冷的液体造成琼脂凝结或形成片状物。将加入添加成分的培养基缓慢充分混匀，尽快分装到待用的容器中。

4.4.4 平板的制备和储存

倾注融化的培养基到平皿中，使之在平皿中形成一个至少 2 mm 厚的琼脂层(直径 90 mm 的平皿

通常要加入 15 mL 琼脂培养基)。将平皿盖好皿盖后放到水平平面使琼脂冷却凝固。

注：在培养过程中，培养基会损失水分。当水分损失的量大于培养基总量的 15%时，就会影响微生物的生长。造成培养基水分损失的因素很多，如培养基成分，平皿中培养基总量和培养箱的类型等(如使用带风扇的培养箱，培养箱中的湿度偏低，平板在培养箱中放置的位置靠近加热管，培养温度过高等)，操作时应注意避免。

凝固后的培养基应立即使用或存放于暗处和(或)4℃～12℃冰箱的密封袋中，最多存放一周或按厂商提供的标准执行。在平板底部做好标记，标记的内容包括名称、制备日期和(或)有效期。也可以使用适宜的培养基编码系统进行标记。

将倒好的平板放在密封的袋子中冷藏保存可延长贮存期限。为了避免产生冷凝水，平板应冷却后再装入袋中。贮存前不要对培养基表面进行干燥处理。

对于采用表面接种形式培养的固体培养基，应先对琼脂表面进行干燥：揭开平皿盖，将平板倒扣于烘箱/培养箱中(温度设为 25℃～50℃)；或放在有对流风的无菌净化台中，直到培养基表面的水滴消失为止。注意不要过度干燥。商业化的平板琼脂培养基应按照厂商提供的说明使用。

4.4.5 培养

培养时每垛最多堆放六个平板，平板间要留有空隙以保证空气流通，使培养物的温度尽快与培养箱温度达到一致；液体培养基温度与培养箱达到一致取决于很多因素，如体积、内容物量、容器类型、培养类型等；使用厌氧罐时，堆放的平板数可以超过六个。

4.5 培养基的弃置

所有污染和未使用的培养基的弃置应采用安全的方式，并且要符合相关法律法规的规定。

注：应根据相关法律法规和标准的要求以及各实验室的实际情况，在所制定的药品试剂安全管理规范当中加入有关培养基弧置的具体要求，以确保所弃置培养基的安全性。

5 成品的质量控制

5.1 物理指标控制

培养基的实验室测试至少应包括：

——20℃～25℃的 pH 值；

并应观察以下内容：

——加入培养基的量，琼脂层的厚度；

——色泽；

——透明度和(或)是否存在肉眼可见的杂质；

——凝胶稳定性、黏稠度和湿度。

5.2 微生物指标控制

5.2.1 污染的控制

从每批制备好的培养基中选取部分样品进行污染测试。

5.2.2 测试菌株

测试菌株是具有其代表种的稳定特性并能有效证明实验室特定培养基最佳性能的一套菌株。测试菌株主要购置于标准菌种保藏中心，也可以是实验室自己分离的具有良好特性的菌株。实验室应检测和记录标准储备菌株的特性；新复苏的菌株可能会有非特异性反应，使用时应引起注意；最好使用从食品中分离的菌株。

每种培养基的测试菌株应包括：

——具典型反应特性的强阳性菌株；

——微弱生长的阳性菌株(对培养基中选择剂等试剂敏感性强的菌株)；

——非特异性菌株。如：产生不同发酵反应和荧光反应的菌株；

——阴性菌株。

注：国际食品微生物委员会(ICFM)和培养基菌株卫生工作组(WPCM)介绍了培养基评估用测试菌株的有效收集方法。

5.2.3 即用型培养基和试剂

商品化即用型培养基的生产厂如果经过 ISO 9001 体系认证或满足相应质量要求，应向使用方提供相应的资质证明。这样，使用者就不必再进行大量的培养基测试工作，但应保证培养基的贮存条件。

5.2.4 商品化合成脱水培养基制备的培养基

对每批制备好的培养基除用标准菌株进行测试，还应用实际样品进行检测，以更好地保证培养基的质量。不含指示剂或选择剂的培养基，只需用阳性菌株进行检测；含有指示剂或选择剂的培养基，应使用能证明其指示或选择作用的菌株进行试验；复合培养基(即需要加入添加成分的培养基)需要用具备 5.2.2 性能的菌株逐批进行验证；实验室制备的需加入添加成分的即用型培养基本条款同样适用。

5.2.5 各别成分制备的培养基

除了按照 5.2.4 条款进行培养基菌株测试外，还应对各别成分制备的培养基用 Miles & Misra 技术、螺旋平板技术或菌落总数技术进行测试，以监控基础材料的质量，培养基的性能和实验室内部的配制规范。

注：以上是最低要求。如果食品中含有受损的微生物细胞，还应考虑培养基在受损微生物恢复方面的适用性。菌株保存、传代技术和标准菌株的收集参见附录 B。

附 录 A
（资料性附录）
食品和动物源性产品微生物分析标准中指定的培养基成分

A.1 概述

为了统一对不同微生物标准中培养基成分的描述，ISO/TC 34/SC 9“食品和农产品-微生物”技术委员会对不同的培养基成分进行了分类，详见 A.2～A.5。

A.2 蛋白胨

——酶解酪蛋白[1)]；
——酶解大豆粉；
——酶解动物组织[2)]；
——心酶解物；
——酶解明胶；
——酶解动、植物组织。

A.3 浸膏

——肉浸膏；
——脑心浸膏；
——酵母浸膏；
——细菌学牛胆盐；
——胆盐；
——3 号胆盐。

A.4 琼脂

细菌学琼脂。

A.5 其他

——卵黄乳液；
——脱脂奶粉；
——酸解酪蛋白。

1）包括胃蛋白酶消化的酪蛋白和胰蛋白酶消化的酪蛋白和胰蛋白胨。
2）包括肉胨，胃蛋白酶消化的肉，胰腺消化的肉。

附 录 B
（资料性附录）
质控菌株的保藏和使用

B.1 概述

冻干法、−70℃玻璃珠保存法、液氮保存法等是食品微生物中常用的菌种保藏方法，但这些方法并不可能适用于所有菌株。

参见图 B.1

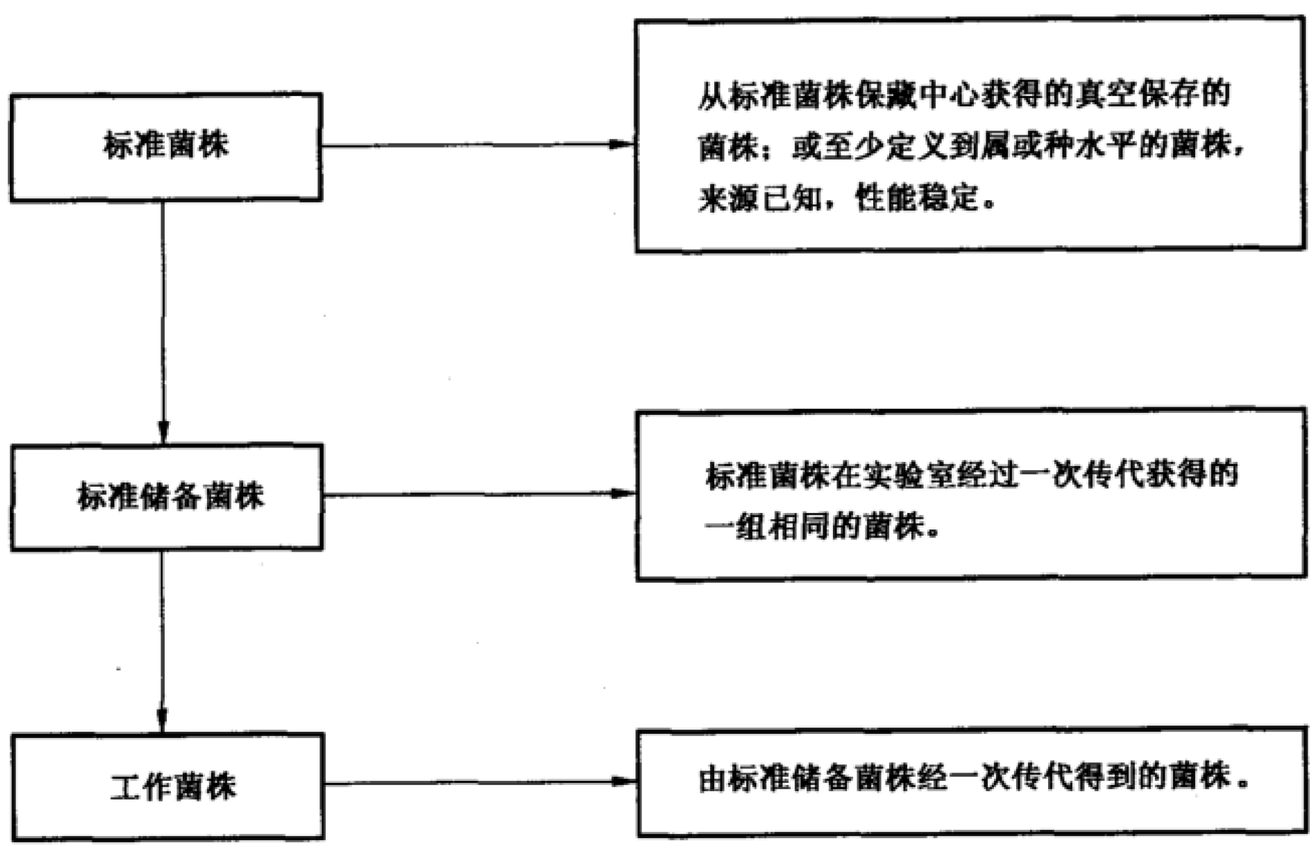

图 B.1

B.2 商业途径获得的标准菌株

对于从菌种保藏中心或经 ISO 9001 认证的商业机构购买的标准菌株，要以原始的包装形式进行保藏，复苏和使用应按照厂商提供的使用说明进行。

B.3 实验室制备的标准菌株的保藏

用于质量控制或性能测定的标准储备菌株在保存和使用时应注意避免交叉污染，减少菌株突变或发生典型的性能变化；标准储备菌株应制备多份，并采用超低温（−70℃）或冻干的形式保存；测试菌株在每种培养基上的生长特性应全部文件化。

B.4 工作菌株

工作菌株由冻干或超低温保存的标准菌株制备（B.3）。转接操作时要注意避免标准菌株可能发生的交叉污染和菌株的退化。制备工作菌株时，应将参考原株转接到多份非选择培养基中培养，得到稳定期的菌株。

对于商品化的保藏菌株必须严格按照厂商的说明执行。

注：工作菌株不能再传代。但工作菌株如果处理或贮存得当[即不存在交叉污染和（或）在一周内不退化]，则可以多次使用。

附 录 C
（资料性附录）
培养基的质量保证——常见问题解答

异常现象	可能原因
培养基不能凝固	制备过程中过度加热 低 pH 值造成培养基酸解 称量不正确 琼脂未完全溶解 培养基成分未充分混匀
pH 值不正确	制备过程中过度加热 水质不佳 外部化学物质污染 测定 pH 值时温度不正确 pH 计未正确校准 脱水培养基质量差
颜色异常	制备过程中过度加热 水质不佳 脱水培养基质量差 pH 不正确 外来污染
产生沉淀	制备过程中过度加热 水质不佳 脱水培养基质量差 pH 未正确控制
培养基出现抑制/重复性差	制备过程中过度加热 培养基脱水质量差 水质不佳 使用成分不正确，如，成分称量不准，添加物浓度不正确
选择性差	制备过程中过度加热 脱水培养基质量差 配方使用不对 添加成分不正确。如加入添加成分时培养基过热或添加浓度错误

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Foreword

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 Practical general guidelines on performance testing of culture media.
This standard is the first part of SN/T 1538, Corresponding to ISO /TS 11133-1 Microbiology of food and animal feeding stuffs—Guidelines on preparation and production of culture media—General guidelines on quality assurance for the preparation of culture media in the laboratory (English version, 2000). 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, Introduction of original standard;
—Delete the quotation standard number in the Chapter 3 of the original standard;
—Substitute ISO 8402 for ISO 19000 and Add GB/T 6682 in normative references;
—Replace special international standard with special standard;
—Add the special degree of temperature in 4.2.4, e.g. 4℃, 20℃;
—Add description of weighing dehydrated medium in 4.3.3;
—Add description of sterilization of different media in 4.3.7.1;
—Modify the assembly condition of the filter in 4.3.7.3;
—Add note of disposal of media in 4.5;
—Add total plate counting in 5.2.5;
—Delete note 1) of the original standard.

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

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

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

The main drafters of this standard are Li weihua, Zhao guiming, Zhang jianjun, Lian huifeng, Liu pei and Gong hongxia.

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

Introduction

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.

SN/T 1538 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 first part of these series standard, it standard the terms and definitions of media; regulate the general guideline of the storage, preparation, using and disposal of media; propose the quality control of finished product. Part 2 of these standard will draft whereafter.

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

1 Scope

This standard provides the general terminology related to quality assurance of the preparation of culture media and specifies the minimum requirements to be used for the microbiological analysis of products intended for human consumption or animal feeding.

These requirements are applicable to three categories of culture media used in laboratories that prepare and/or use culture media for performing microbiological analyses:

—commercially manufactured ready-to-use media;

—media prepared form commercially available dehydrated formulations (either complete e. g. plate count agar or basal media to which supplements are added e. g. Baird-Parker agar);

—media prepared from its individual components.

2 Normative references

This standard 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 standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

GB/T 19000 Quality management systems—Fundamentals and vocabulary (idt ISO 9000:2000)

GB/T 6682 Specification and test method of water in analysis laboratory (eqv ISO 3696:1987)

EN 1659 Invitro diagnostic systems—Culture media for microbiology—Terms and definitions

EN 12322 Invitro diagnostic medical devices—Culture media for microbiology —Performance criteria for culture media

3 Terminology

3.1 General

This clause gives the general definitions related to quality assurance and provides different types of terminology related to culture media and to control cultures.

3.2 Terminology of quality assurance

3.2.1

Quality assurance

All the planned and systematic activities implemented within the quality system and demonstrated as needed, to provide adequate confidence that an entity will fulfill the requirements for quality.

3.2.2

Quality control

Operational techniques and activities that are used to fulfill the requirements for quality.

3.2.3

Internal quality control

A continuous control program of the laboratory' s work prepared by or for them, and based on control analysis together with follow-up and if necessary, corrective actions.

3.2.4

Batch of culture media; lot of culture media

Fully traceable unit of a medium referring to a defined amount of bulk, semi-finished product or end product, which is consistent in type and quality and which has passed the requirements of production(in-process control)and quality assurance testing, and which has been produced within one defined production period, having been assigned the same lot number.

3.2.5

Performance of culture media

The response of a culture medium to challenge by test organisms under defined conditions.

3.3 Terminology of culture media

3.3.1

Culture medium

Formulation of substances, in liquid, semi-solid or in solid form, which contain natural and/or synthetic constituents intended to support the multiplication, or to preserve the viability, of microorganisms.

When used in connection with compound words, this term is often shortened into "medium" (e.g. enrichment medium).

3.3.2 Culture media classified by composition

3.3.2.1

Chemically defined culture medium

Culture medium consisting only of chemically defined constituents(i.e. of known molecular structure and degree of purity).

3.3.2.2

Chemically incomplete culture medium

Culture medium consisting entirely or partly of natural materials, processed or otherwise, the chemical composition of which is not completely defined.

NOTE For the various chemically undefined components used in culture media ISO/TC34/SCghas specified harmonized designations-see Annex A.

3.3.3 Culture media classified by consistency

3.3.3.1

Liquid culture medium

consisting of an aqueous solution of one or more constituents(e.g. peptone water, nutrient broth)

NOTE 1 In some cases, solid particles are added to the liquid culture medium.

NOTE 2 Liquid media in tubes, flasks or bottles are commonly called "broth".

3.3.3.2

Solid culture medium and semi-solid culture medium

Liquid culture medium containing solidifying materials(e.g. agar-agar, gelatine, etc.) in different concentrations.

NOTE 1 Due to the world-wide use of culture media solidified with agar-agar, the shortened term "agar" is often used synonymously for solid culture media and therefore in connection with nouns, e.g. " Plate count agar".

NOTE 2 Solid culture media poured into Petri dishes are commonly called "plates" . Solid culture media poured into tubes that are kept in slanted positions while the media are solidifying are often called "slants".

3.3.4 Culture media classified by intent of use

3.3.4.1

Transport medium

Culture medium designed to preserve and maintain the viability of microorganisms for the time period between sample collection and laboratory processing of the sample.

NOTE Transport media usually contain substances that do not permit multiplication of microorgani but ensure their preservation(e.g. Stuart' s or Amies Transport medium).

3.3.4.2

Preservation medium

Culture medium designed t preserve and maintain the viability of microorganisms over an extended period, to protect them against the adverse influences which may occur during long-term storage and to allow recover after this period (e.g. Dorset egg medium).

3.3.4.3

Resuscitation medium

Culture medium enabling stressed and damaged microorganisms to repair and recover their capacity

for normal growth without necessarily promoting their multiplication.

3.3.4.4
Enrichment medium
Predominantly liquid culture medium which, due to its composition, provides particularly favorable conditions for multiplication of microorganisms.

3.3.4.4.1
Selective enrichment medium
Enrichment medium which supports the multiplication of specific microorganisms whilst partially or totally inhibiting the growth of other microorganisms(e.g. Rappaport-Vassiliadis medium).

3.3.4.4.2
Non-selective enrichment medium
Enrichment medium which supports the growth of most microorganisms(e.g. nutrient broth).

3.3.4.5
Isolation medium
Solid or semi-solid culture medium which supports the growth of microorganisms.

3.3.4.5.1
Selective isolation medium
Isolation medium which growth of specific microorganims, while inhibiting other microorganisms (e.g. PALCAM agar, MacConkey agar).

3.3.4.5.2
Non-selective isolation medium
Isolation medium which is not devised to selectively inhibit microorganisms (e.g. nutrient agar).

3.3.4.6
Differential medium
Which permits the testing of one or more physiological/biochemical characteristics of the microorganisms for their identification(e.g. Urea medium, Kligler agar).
NOTE differential media which can be used as isolation media are referred to as isolation/differential media [e.g. xylose lysine desoxycholate(XLD)agar].

3.3.4.7
Identification medium
Culture medium designed to produce a specific identification reaction which dose not require any further confirmatory test.
NOTE identification media which can be used as isolation media are referred to as isolation/identification media.

3.3.4.8

Media having multiple uses

Certain culture media may be assigned to several categories, e.g. Blood agar is a resuscitation medium according to 3.3.4.3 an isolation medium according to 3.3.4.5 and a differential medium according to 3.3.4.6 used for detection of haemolysis.

3.3.5 Culture media classified according to preparation method

3.3.5.1

Ready-to-use medium

Culture medium which is supplied in containers in ready-to-use form (e.g. Petri dishes or tubes or other containers).

3.3.5.2

Culture medium prepared from commercially dehydrated formulations

Culture medium in dry form which is not ready for immediate use (e.g. powders, granules, lyophilized products).

Rehydration will make one of two kinds of medium:

—a complete ready-to-use medium.

—an incomplete medium to which labile components are added at the time of use .

3.3.5.3

Culture medium prepared from individual components in the laboratory

3.4 Terminology for test organisms

3.4.1

General

These are microorganisms generally used for quality control and performance testing of culture media. They are defined according to their source as follows.

3.4.2

Reference strain

Microorganisms defined to at least the genus and species level, catalogued and described according to its characteristics and preferably stating its origin.

3.4.3

Reference stocks

A set of separate identical cultures obtained in laboratory by a single sub-culture from the reference strain either in the laboratory or from a supplier.

3.4.4

Working culture

A primary sub-culture from a reference stock.

4 Practices for quality control of culture media[1)]

4.1 Documentation

4.1.1 Documentation required from the manufacturer

The following details should be available from the manufacturer:

—Name of the medium, individual components and any supplements, and their product codes.

—Batch code.

—pH value of the medium before use.

—Storage information and expiry date.

—Any performance evaluation and test organism used.

—Technical data sheet.

—Quality-control certificate.

—Safety and/or hazard data where needed.

4.1.2 Check list by the laboratory

Laboratory checks upon receipt of the medium

—Name of medium and batch code.

—Date of receipt.

—Expiry date.

—Condition and integrity of packaging.

4.2 Storage

4.2.1 General

In all cases follow the manufacturer's instructions where available regarding storage conditions, expiry date and use.

4.2.2 Quality management and control for dehydrated media and supplements

Media are usually purchased from commercial manufacturers. They are delivered in dehydrated powdered or granulated form in sealed containers and supplements of different selective or diagnostic substances are supplied in either the lyophilized or liquid state. However, purchases should be planned to encourage a regular turnover of stock(i.e. first in-first out). To maintain an effective inventory further checks should include.

—Re-checking of the seal.

—Date of first opened containers.

—visual assessment of contents of opened containers.

Especially after opening a new container, the quality of the medium may depend on the storage environment. Loss of quality of dehydrated media is shown by change in flow characteristics of the powder, homogeneity, caking, colour changes etc.. Any dehydrated medium that has absorbed moisture

1) Culture media-trouble shooting, See Annex C.

or shows obvious changes in physical appearance should be discarded.

4.2.3 Commercially supplied ready-to use media

Follow the manufacturer' s instructions regarding storage conditions, expiry date and use.

4.2.4 Media prepared from commercially available dehydrated formulations and basic individual components

The shelf-life of these types of media varies. It is therefore difficult to state general time limits for storage of prepared media. specific standards may stipulate specific conditions and shelf life.

Sterilized culture media dispensed in plates, tubes or bottles and reagents which are not used immediately shall be protected against light and desiccation.

Unless a validated expiry date has been established or is specified in the International Standard in question, sterile partially complete media, i. e. media to which final components are added immediately before use, shall be kept in a refrigerator (4℃) for not more than 3 months or at room temperature(20℃) for not more than 1 month under conditions which prevent their composition from being modified. However, it is recommended that media to which labile selective supplements have been added should be used on the day of preparation. Solid media containing chemically reactive and/or labile substances should not be stored in bulk for re-melting.

Observe any colour change, sign of evaporation/dehydration or microbial growth. Batches of media showing such changes should not be used.

Prior to use or before further heating, it is recommended that the culture media be equilibrated to ambient temperature.

4.3 Laboratory preparation of media

4.3.1 General

The accurate preparation of culture media is one of the fundamental steps in microbiological examination and it shall be given special care.

Respect good laboratory practice and the manufactuter' s instructions regarding the handing of dehydrated media and other components, particularly those containing hazardous materials i. e. bile salts or other selective agents.

Where media are prepared from dehydrated commercial formulations fellow the manufacturer' s instructions precisely. Document all relevant data, i. e. weights/volume, pH,date of preparation, sterilization conditions, operator.

For media prepared from individual components, follow the recipe precisely and record all details as in(4.1.2)and, in addition, the full identify(i. e. code and batch number)of all the components used.

4.3.2 Water

The water used shall be distilled water or water of equivalent quality i. e. free from substances likely to inhibit or influence the growth of microorganisms under the test conditions. If the distilled water is prepared from chlorinated water, neutralize the chlorine prior to distillation(see GB/T 6682).

The distilled water shall be stored in containers manufactured preferably form inert materials(e. g. neutral glass, polyethylene etc.),which shall be shown to be free of any inhibitory substances prior

to their initial use.

NOTE Insome cases, it may be necessary to use freshly prepared water, free of dissolved carbon dioxide.

In order to be considered as being of good quality, the distilled water shall have a resistivety of at least 300 000 Ωcm.

WARNING Water processed through an ion exchanger(deionised), may have a high microorganism content; it is therefore advisable not to use such water without verifying that the microorganism content of the water is low. Consult the manufacture for the best way to minimize microbial contamination. Heavily contaminated deionised water that has been filter sterilized may still contain substances inhibitory to the growth of some microorganisms.

4.3.3 Weighting and rehydration

Carefully weigh the appropriate amout of dehydrated medium(operating slowly, wearing mask when or operating in fume cupboard when necessary. Prevent to inhale powder especially containing toxic substances)and progressively add the required amount of water avoiding clumping.

4.3.4 Disssolution and dispersion

Dehydrated media needs rapid dispersion by instant and repeated stirring followed by heating, if necessary, to dissolve. Media containing agar should be allowed to soak for several minutes prior to heating with mixing to dissolve. For media prepared from individual components each component should be added separately and allowed to dissolve before finally making up to volume.

4.3.5 Measurement and adjustment of pH

Measure the pH using a PH meter and adjust if necessary i. e. for media prepared from individual components in the laboratory so that after sterilizing and cooling to 25℃ the medium is at the required pH±0.2pH units, unless otherwise stated. The adjustment is normally carried out using a solution of approximately 40g/l(about 1 mol/L)of sodium hydroxide (NaOH)or approximately 36.5g/l(about 1 mol/L)hydrochloric acid(HCl).

NOTE Commercially manufactured media may show significant changes in pH before and after autoclaving. However, provided good quality distilled or de-ionised water is used, pH adjustment prior to autoclaving should not be necessary.

4.3.6 Dispensing

Dispense the medium into appropriate containers having a volume 1,2, to 3 times that of the medium.

4.3.7 Sterilisation

4.3.7.1 General

The sterilization of culture media and of reagents may be carried out by using sterilization by moist heat or sterilization by filtration.

Certain media, such as brilliant green media containing particularly sensitive to heat and light, can be used boiling for autoclaving, and should be rapidly cooled after boiling and protected from strong light. Some material that not be able to bear hyperthermal, such as galatin ,sèrum and saccharide

etc, can be autoclaved by low temperature sterilization/discontinuous sterilization. Also some reagents can be used without sterilization(refer to appropriate international standard or manufacturer' s instructions).

4.3.7.2 Sterilization by moist heat

Sterilization by moist heat is performed in an autoclave or media preparatory. Generally the autoclaving operation 7 takes 15 min at 121℃. For volumes greater than 1 000 mL, adapt the sterilization cycle as necessary. In all cases the autoclave should be monitored by temperature profiling using thermocouples and test strips under typical load conditions to ensure the desired temperatures can be reached.

NOTE Overheating can occur when large volumes of media(>1 000 mL)are processed in an autoclave.

Control of the efficacy of sterilization is essential. After heating it is essential that media be cooled in a manner to prevent boiling over. This is particularly important for sensitive media e. g. Enterobacteriaceae and media in large volumes.

4.3.7.3 Sterilization by filtration

Sterilization by filtration can be performed under vacuum or pressurized conditions. Use membranes and filter elements with a pore diameter of 0. 22 μm. they shall have been sterilized in the autoclave, Refer to the manufacturer's instructions regarding the use of filter elements or membranes that have been purchased in a sterile condition. Sterilize the different parts of the filtration apparatus. assembled or not, in the autoclave for 15 min at 121℃. If necessary, aseptic assembly can be performed in aseptic condition after autoclaving.

Note Some filter membranes may retain proteins(such as antibiotics). In order to obtain the correct concentration the user should pre-wet the filter.

4.3.7.4 Monitoring

After autoclaving, boiling or filtration, all media should be monitored, in particular with respect to pH, colour, sterility and consistency.

4.3.8 Preparation of supplements

Manufactured supplements containing toxic agents, particularly antibiotics, must be handled with care(manufactured in fume cupboard when necessary) avoiding dispersion of powder which may give rise to allergic or other reaction in laboratory personnel. Take appropriate precautions and follow the manufacturer's instruction when making solutions. Do not use beyond their stated shelf-life which, for antibiotic working solution, is generally the same day. Under certain circumstances, antibiotic solutions may be stored frozen in suitable aliquots but should not be re-frozen after thawing. The potential loss of activity due to freezing should be discussed with the manufacturer or tested by the user.

4.4 Preparation for use

4.4.1 Melting of agar culture media

Melt a culture medium by placing it in boiling water bath or by any other process which gives identi-

cal results(e.g. a steam flow-through autoclave). Media that have previously been autoclaved should be reheated for a minimum time to maintain media quality. Avoid over-heating and remove when it has melted. Cool the molten medium to 47℃ ±2℃ in a thermostatically controlled water bath until such time as it is to be used. Molten medium should be used as soon as possible but it is recommended that it should not be retained for more than 4 h.

4.4.2 De-aeration of culture media

If necessary, just prior to use, heat the culture medium in boiling water or under a flow of steam for 15 min, with lids or caps loose; after heating, tighten the caps and cool down rapidly to the operating temperature.

4.4.3 Addition of supplements

Heat-labile supplements should be added to the medium after it has been cooled to 47℃ ±2℃. Allow the sterile supplement to come to room temperature before adding it to the agar medium. Cold liquids may cause agar to get or form transparent flakes. Mix all supplements into the medium gently and thoroughly, then distribute into the final containers as quickly as possible.

4.4.4 Preparation and storage of media in Petri dishes.

Pour the molten agar culture medium into Petri dishes so as to obtain a thickness of at least 2 mm(e.g. for 90 mm diameter dishes, 15 mL of agar are normally required). Allow the agar to cool and solidify by placing the Petri dishes with lids in place on a cool, horizontal surface.

Note: During incubation, a loss of moisture of the agar media will occur. A loss of more than 15% of the water content can adversely affect the growth of microorganisms in some circumstances. Factors influencing water loss are medium composition, amount of medium in the plates. the type of incubator i.e. fan-assisted or otherwise, low humidity of the atmosphere in the incubator, the position of the plates near the heating tube, and the high incubation temperature.

Use the solidified medium immediately or store under conditions which prevent its composition from being modified, ie. in the dark and/or in the refrigerator at 4℃ to 12℃ in sealed bags for a maximum period of one week or as directed by the manufacturer or specific standard. Label the dishes on the base with date of preparation and/or expiry date and identity. Alternative coding systems meeting these requirements may be used.

The shelf-life of poured plates will increase if they are stored in sealed plastic bags. In order to avoid the occurrence of condensate, the plates must be cool before being placed into bags. Do not dry the surface of agar plates prior to chill storage.

In general, for the surface inoculation of a solid culture medium, dry the dishes, preferably with the lids removed and with the agar surface facing downwards, in an oven set at a temperature between 25℃ and 50℃ or in a laminar-flow cabinet, until the droplets have disappeared from the surface of the medium. Do not over-dry them. Commercially prepared ready-to use agar plates should be stored and used according to the manufacturer's instructions.

4.4.5 Incubation

Do not stack dishes in piles more than 6 high and leave space for air circulation to allow medium to equilibrate to incubation temperature as rapidly as possible. For liquid media, time to reach incubation temperature is dependant upon a number of factors e.g. volume, loading, container, incubator

type. in the case of anaerobic jars it may be necessary to stack plates in excess of 6 high.

4.5 Disposal of media

Both contaminated and unused media must be disposed of in a manner that is safe and meets any relative law and regulations.

Note: It should be added requirements of media disposal in regulations according to relative law, legislation and specific condition of the laboratory.

5 Quality control of finished product

5.1 Physical quality control

Laboratory testing should include as a minimum:

—pH-value measured at between 20℃ and 25℃;

And by observation:

—quantity filled and/or layer thickness;

—colour;

—clarity/presence of optical artifacts;

—gel stability/consistency/moistness.

5.2 Microbiological quality control

5.2.1 Contamination

An appropriate amount of each batch should tested for contamination.

5.2.2 Test organisms

A set of test organisms should only contain microorganisms with stable characteristics respresentative of their species and that have been shown to be reliable for the demonstration of optimal performance of a particular laboratory prepared medium. The test organisms should primarily comprise strains that are widely available in reference culture collections, but well-characterised strains isolated by the laboratory may also be included. The relevant cultural characteristics of the stock culture should be examined and recorded by the laboratory and the strain renewed should atypical characteristics occur. It is preferable to use strains that have originated from foods although not all culture collections provide such data on their origin.

The test organisms for each medium may include:

—robust positive stains with typical characteristics;

—weakly growing positive stains(i. e. of a more sensitive nature);

—biochemically un-reactive stains e. g. those showing different fermentation or fluorescence reactions;

—completely inhibited strains.

NOTE The International Committee for Food Microbiology and Hygiene' s Working Party on Culture Media(WPCM) have prescribed a validated collection of test strains for media evaluation[1].

5. 2. 3 Ready-to-use media and reagents

Manufacturers of commercially available ready-to-use media especially if approved to ISO 9001 or ISO 9002 standards, will have a quality programme in place and may issue a quality certificate with the media they supply. Under those conditions the user may not need to carry out extensive testing on such media but should ensure that storage conditions are maintained.

5. 2. 4 Media prepared form commercially available dehydrated formulations

Qualitative tests for each batch of prepared medium are the minimum requirements but, where quantitative examinations on samples are to be carried out, quantitative tests on each batch will give greater assurance of media quality. For those media which do contain indicators or selective agents, strains which demonstrate the function of the Indiacator(s) or selective agents, strains which demonstrate the function of the indicator(s) and selectivity must be utilized. For selective agents, strains which demonstrate the function of the indicator(s)and selectivity must be utilized. For complex media, i. e. with added supplements, each batch should be verified with strains with characteristics listed in complex media i. e. with added supplements, each batch should be verified with strains with characteristics listed in complex media. i. e. with added supplements, each batch should be verified with strains with characteristics listed in complex media. i. e. with added supplements, each batch should be verified with characteristics listed in 5. 22. In the case of ready-to-use media to which laboratory-prepared supplements have been added the same applies.

5. 2. 5 Media prepared from basic individual components

It is recommended that in addition to the qualitative tests described in 5. 2. 4 , that some quantitative testing is carried out using techniques such as the modified Miles & Misra technique, spiral plating or total plate counting in order to monitor trends in quality of basic materials, productivity of the medium and in-house laboratory production protocols.

Note: These are minimal guidelines, in practice, foods may contain stressed microorganisms. The suitability of the medium with respect to the recovery of stressed cells should be taken into account. For information on preservation, Maintenance Techniques and reference culture collections see Annex B.

Annex A
(informative)
Designation of the components of the culture media in standards on microbiological analysis of food and animal feeding stuffs products

A.1 General

In order to harmonise the description of the various components in the composition of culture mmedia in microbiological stanfard methods ,ISO/TC 34/SC 9 "food and agricultural products—Microbiology" decided the designations for the categories of components designated in A.2 to A.5.

A.2 Peptones

—Enzymatic digest of casein;[1)]
—Enzymatic digest of soybean meal;
—Enzymatic digest of animal tissues;[2)]
—Enzymatic digest of heart;
—Enzymatic digest of gelatin;
—Enzymatic digest of animal and plant tissues.

A.3 Extracts

—Meat extract;
—Brain-heart extract;
—yeast extract;
—ox bile for bacteriology;
—bile salts;
—bile salts No.3.

A.4 Agar

—Bacteriological agar.

A.5 Other

—Egg yolk emulsion;
—Skim milk powder;
—Acid hydrolysate.

1) This includes peptic digest of casein, tryptic digest of casein and tryptone.
2) This includes meat peptone, peptic digest of meat ,pancreatic digest of meat.

Annex B
(informative)
Guidance on preservation and maintenance of control strains

B.1 General

There are several methods available, i.e. lyophilisation, storage on beads at −70℃, or using liquid nitrogen, for the successful preservation and maintenance of all microorganisms relevant to food microbiology. One method may not be appropriate for all strains.
These guidelines are presented in figure B.1.

B.2 reference strains from commercial sources

If reference strains from reference collections or ISO 9001[2] or ISO 9002[3] approved commercial suppliers are purchased and maintained in their original containers, the manufacturers directions for their cultivation and use must be followed.

B.3 Laboratory-prepared reference stocks

Stock cultures of reference strains (B.2) for quality control and performance testing purposes must be maintained and handled in manner which minimizes the opportunity for cross-contamination, mutation or alteration of typical characteristics, reference stocks should be stored in multiple aliquots either deep-frozen (−70℃) or lyophilized. Their growth characteristics should be fully documented for each medium on/in which they will be utilized as test organisms.

B.4 Working cultures

Working cultures are prepared from lyophilized or deep-frozen reference stocks(B.3). Aliquots must be handled in a manner, which avoids possible cross-contamination of reference stock and/or its deterioration, Working cultures should be prepared by re-suspending an aliquot of the reference stock in a non-selective growth medium and incubating to yield a stationary phase culture.
For commercially available preservation systems the manufacturer's instructions shall be rigorously followed.
NOTE Sib-cultures form working cultures should not be made, However, working cultures may be used more than once provided they are handled and stired appropriately i.e. to avoid cross-contamination and. or deterioration for no longer than one week.

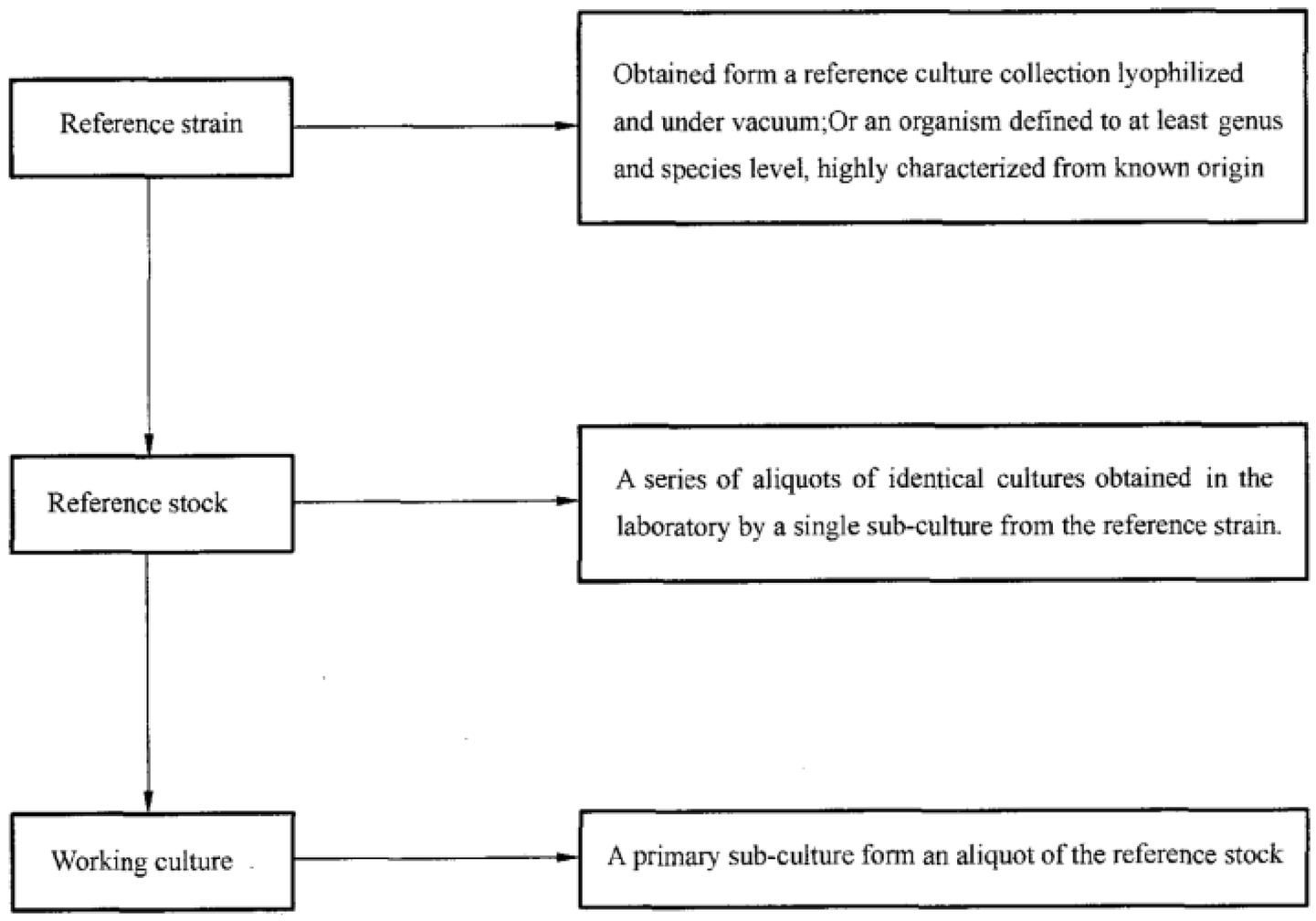
Reference strain
Obtained form a reference culture collection lyophilized and under vacuum;Or an organism defined to at least genus and species level, highly characterized from known origin
Reference stock
A series of aliquots of identical cultures obtained in the laboratory by a single sub-culture from the reference strain.
Working culture
A primary sub-culture form an aliquot of the reference stock

Figure B.1

Annex C
(informative)
Quality assurance of culture media-trouble shooting

Abnormality	Possible reason
Agar medium fails to solidify	Overheating of medium during preparation Low pH causing acid hydrolysis to occur Incorrect weight of agar used Agar not thoroughly dissolved Poor mixing of ingredients
Incorrect pH	Overheating of medium during preparation Poor water quality Extraneous chemical contamination pH measured at incorrect temperature pH meter incorrectly calibrated Poor quality dehydrated medium
Abnormal color	Overheating of medium during preparation Poor water quality Poor quality dehydrated medium Incorrect pH Extraneous contamination
Formation of precipitates	Overheating of medium during preparation Poor water quality Poor quality dehydrated medium Poor pH control
Medium inhibitory/Low productivity	Overheating of medium during preparation Poor quality dehydrated medium Poor water quality Incorrect formulation used, e. g. ingredients not weighed out correctly, supplements added at wrong concentration
Poor selectivity	Overheating of medium during preparation Poor quality dehydrated medium Poor water quality Incorrect formulation used Supplements added incorrectly e. g. when medium too hot or at wrong concentration

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培养基制备指南 第1部分：
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