



***Guidelines for Assuring Quality***  
***of***  
***Food and Water Microbiological***  
***Culture Media***

*Culture Media Special Interest Group  
for the  
Australian Society for Microbiology, Inc.*

**2<sup>nd</sup> edition**  
**2014**

The Australian Society  
**for Microbiology**  
bringing Microbiologists together



## FOREWORD to the First Edition

The Culture Media Special Interest Group (SIG) of the Australian Society for Microbiology was formed in 1991 by a group of interested individuals after an upsurge in interest in the issue of media quality and the appearance that no common standards or consensus existed in this area in Australia.

Increased interest, especially amongst medical microbiologists, in what was being done, or should be done, by way of assuring the quality of microbiological media made the issue contentious.

The National Association of Testing Authorities (NATA), Australia, were amongst those seeking guidance in the area of Media Quality Control, being in the position of accrediting microbiology laboratories in the fields of biological testing and medical testing. They found little in the way of consistency and knew of no locally applicable guidelines on which to base their assessments and recommendations.

A working party of the Culture Media SIG developed a set of guidelines "Guidelines for Assuring Quality of Medical Microbiological Culture Media" which were approved in September 1996. This document has been widely used over the past six years and is acknowledged as a valuable resource by microbiologists in medical as well as food, water and pharmaceutical laboratories.

It is now opportune to build from the guidelines for medical microbiological media, to provide, new guidelines of immediate relevance to food and water laboratories.

Many laboratories are now working to the new technical requirements for the competence of testing and calibration laboratories ISO17025. As part of this technical standard the requirements for media quality control are embedded in Section 4.6 "Purchasing services and supplies." NATA has within the ISO17025 standard, specific requirements for Biological Testing, which include requirements for media quality control. However this NATA document does not elaborate in detail about how to perform Quality Control on the media. One of the purposes of this document is to provide more details on how to perform some of the recommended Quality Control procedures.

This document is intended to offer guidance to food and water microbiology laboratories of any size, whether they prepare media in-house, purchase it commercially, or obtain it from a central facility within their greater organisation. To this end, some compromises have been necessary.

The document seeks to give specific direction in key areas, however it is recognised that considerable variability exists in the resources to which different laboratories have access, and hence options and alternatives are offered. It is intended that selections be made from alternatives, with every consideration given to the practice of good science, and that alternative approaches not covered specifically by these guidelines, must be subjected to studies in the laboratory applying them in order to validate their effectiveness and consistency in reaching the desired outcome.

The over-riding aim of generating guidelines such as these is to promote a consistently high standard of quality in the performance of microbiology in Australia.

These guidelines have been produced, revised and reviewed by the Victorian branch of the Culture Media SIG and various interested people and parties throughout Australia and overseas.

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## FOREWORD to the Second Edition

The Culture Media SIG published the first edition of the guidelines for assuring the quality of food and water microbiological culture media in 2004. This document set out to guide laboratories on how to assure the quality of control culture media, regardless of whether the media was produced in-house or sourced from outside the laboratory. It brought together information from disparate sources and was an important resource for laboratories seeking to meet the requirements of ISO/IEC 17025: *General requirements for the competence of testing and calibration laboratories* and for The National Association of Testing Authorities (NATA) that assessed laboratories for compliance to this standard. These guidelines, combining food and water microbiological culture media, preceded the conversion of the International Standards Organisation (ISO) technical specifications for quality control of culture media used in food microbiology (1), to a full ISO Standard that also incorporates media used in water microbiology (2).

This edition of the Guidelines aims to capture and reflect changes that have occurred since the first edition, to re-invigorate the document's relevance in quality control and quality assurance of microbiological culture media. There is also a harmonization of the style and format of the Guidelines to that of the medical versions (3,4,5).

The document complements ISO11133, as Australian water microbiology standards, and some food microbiology standards, are not currently harmonized with ISO standards. It is anticipated that ISO11133 will be adopted as an Australian Standard soon, with the addition of an Annex to cover Australian specific requirements. Until that time, these ASM Guidelines help to fill the gap.

In circumstances not covered by these Guidelines, well-documented in-house procedures that deal with assuring quality (in those circumstances) should be applied.

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Any suggestions for amendments or changes, questions arising, should be directed to the National Convenor of the SIG via email.

Please send to [admin@theasm.com.au](mailto:admin@theasm.com.au)

Please include as the Subject Line:  
*Food and Water Microbiological Media - QC Guidelines 2<sup>nd</sup> edition – Attention: Culture Media SIG Convenor*

*Please include as much detail as you can in the body of the email. Acknowledgement of receipt of your email will be made. Any amendments agreed to by the Special Interest Group will be carried forward to be included in the next edition/revision.*



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Recommended control strains & acceptance criteria for growth performance testing of water microbiological culture media.

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## 1.0 Introduction

ISO/IEC 17025 *General requirements for the competence of testing and calibration laboratories* requires laboratories to “ensure that purchased supplies and reagents and consumable materials that affect the quality of tests and/or calibrations are not used until they have been inspected or otherwise verified as complying with standard specifications or requirements... Records of actions taken to check compliance shall be maintained”. This is interpreted by the National Association of Testing Authorities Australia (NATA), to mean that each testing laboratory is responsible for ensuring that an appropriate level of quality assurance (QA) is performed on the media it uses, whether derived from in-house or commercial sources; and that this procedure is fully documented (6).

## 1.1 Application

These guidelines are applicable to suppliers, producers and users of microbiological culture media for food and water testing. They should be used in conjunction with other relevant accreditation documents to implement a comprehensive QA program (2, 6, 7, 8). These guidelines may also be beneficial to laboratories other than those involved in food and water microbiology.

For testing media prepared from basic individual ingredients, quantitative testing is recommended.

For testing commercially available dehydrated media, quantitative testing is recommended for enumeration media. Qualitative testing may be sufficient for other types of media; quantitative batch testing will give greater assurance of media quality.

For finished media (other than enumeration media), qualitative testing is recommended.

For commercially supplied, ready-to-use finished media, and which have been quality tested by the manufacturer in accordance with NATA, further testing may not be required; performance monitoring is recommended.

## 1.2 Scope

These guidelines pertain primarily to food and water microbiological culture media used for cultivation, isolation and identification of food-borne and/or water-borne microorganisms. Most of the media and microorganisms referred to in this document are those described by Australian Standards AS5013 series, AS4276 series and AS3896. Cultures recommended by ISO 11133 are also included.



### 1.3 Definitions

**Culture Media:** Formulations 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. (*Note: This is taken to include diluents and other suspending fluids.*)

**Ready-To-Use-Media:** Culture media supplied in containers in ready-to-use form (e.g. Petri dishes, tubes, bottles or other containers).

**Manufacturer:** Manufacturers of food and water microbiological culture media are those facilities where ingredients are weighed, mixed, sterilised, dispensed and final products are labelled and packaged. This includes facilities who prepare media for sale outside their organisation or for distribution within their organisation, or for their own use.

**User:** Consumers of microbiological culture media including those who purchase or receive it from a physically separate location within or outside their organisation.

**Quality Assurance:** Planned and systematic activities implemented within the quality system encompassing processes before, during and after the manufacture of microbiological culture media that verify the adequacy of the media for its intended purpose.

**Quality Control:** The final inspection and testing of the finished product to ensure its compliance with predetermined performance criteria.

**Lot of Culture Media:** Fully traceable unit of a raw material (e.g. dehydrated culture media, antibiotics, supplements, blood etc.), referring to a defined amount which is consistent in type and quality and having been assigned the same lot number.

**Batch of Culture Media:** Fully traceable unit of a medium referring to a defined amount of semi-finished 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 period, having been assigned the same batch number.

**Performance of Culture Media:** The response of a culture media to challenge by test organisms under defined conditions.

**Validation/Validated:** The collection of data that demonstrates the reproducibility of a specific property of a medium or process. Data should be comprehensively documented and verify that, under usual conditions, the medium or process is reliable in producing the expected outcome.

**Reference Media:** Control media used for comparative evaluation of performance, independent of the medium under test and demonstrated to be suitable for control use with regard to preparation and consistency of performance.



**Test Organisms:** These are microorganisms generally used for quality control and performance testing of culture media.

**Reference Strain (Master):** A microorganism defined to at least the genus and species level, catalogued and described according to its characteristics and stating its origin.

**Reference Stocks:** A set of separate cultures obtained in the laboratory by a single sub-culture from the reference strain.

**Working Culture:** A primary sub-culture from a reference stock.

Other definitions pertaining to preparation, quality control and quality assurance of microbiological media can be found in ISO11133.



## **2.0 Media Manufacturer Quality Assurance Practices**

### **2.1 Requirements**

Process quality assurance is integral to the manufacture of food and water microbiological culture media, just as HACCP or its equivalent is an integral part of all good food manufacturing practices. Quality assurance practices should include tests to verify that the steps taken (to ensure freedom from contamination, freedom from significant physical or chemical imperfections (e.g. pH, gel strength), the correct performance of the media when used appropriately) are robust and reproducible .

Performance of media listed in the Appendices 2 and 3 should comply with expected results shown when tested according to methods described in these Guidelines.

Media not listed in the Appendices should also be tested to demonstrate satisfactory performance and a low failure rate; at a minimum, the quality control guidelines provided by the manufacturers of dehydrated culture media in their technical manuals, or other appropriate reference texts (9) should be followed.

### **2.2 Contamination and Significant Physical Imperfections**

Testing for contamination should include sampling, incubation and inspection of individual units of each batch produced. The sampling procedures recommended are summarised in Appendix 1 including notes on interpretation.

Incubation of all samples should be for a *minimum* of 48 hours at a suitable temperature ( $30 \pm 2^\circ\text{C}$  is recommended) before inspection. Sterility testing should always be undertaken when media is aseptically dispensed. However, where media is terminally sterilized a protocol may be established for release on the basis of a validated sterilization process. Such a validated process eliminates conventional sterility testing as a release criterion.

The use of inspected sterility samples to determine significant physical imperfections is acceptable.

Inspection for significant physical imperfections should include: uneven distribution of media; variable amounts of medium in Petri dishes/tubes/bottles; colour; gross deformation of the surface of the media.



### **2.3 Control Strains of Bacteria**

The control strains specified in these guidelines (see Appendices 2 and 3) should be used. The cultures listed in the Appendices reflect the minimal cultures that should be used to QC media. Control strains should be cultures that exhibit typical microscopic, macroscopic and biochemical characteristics of the species, and are traceable to a recognised reference culture collection. Records of identity verification and lineage should be recorded (see NATA requirements (6, 8)). For those media used to select or isolate a specific pathogen from other background microflora, additional culture(s) that verify that the pathogen can be effectively discriminated can be used. It is in such situations where the microbiology laboratory may wish to add well characterized wild strains to supplement its culture collection. Use of cultures for which no lineage history is available is unacceptable.

### **2.4 Maintenance of Cultures used for Quality Control Testing**

The cultures used for Quality Control Testing of media have been selected because of growth attributes or biochemical characteristics. Over an extended period, it is expected that these cultures will be consistent in their phenotypic properties. It is desirable to minimise the number of transfers between the master culture and the working culture such that there is limited population or genetic change. The most effective system for managing the culture collection is the hierarchical or tiered system that includes Master, Stock and Working cultures (see Figure 1).

When a culture is first received by a laboratory it should be activated and tested for purity and identity. If pure, growth from this plate is used to prepare freeze dried ampoules, frozen glycerol broths or beads, or some equivalent system which minimises change but allows long term viability of the micro-organism. In addition to the purity check, and at the same time of preservation, the identity of the culture should be verified including the particular characteristics utilised for media growth performance checks. The preserved culture generated by this process is termed MASTER culture and should not be accessed frequently.



Concurrently with establishing the MASTER culture, the STOCK cultures should also be prepared. The STOCK cultures are usually glycerol broths or beads that are stored frozen. Sufficient vials should be prepared to last 3-12 months. The number of vials will be determined by the laboratory's usage rate. These "STOCK" cultures may be accessed to prepare WORKING cultures which are used for media growth performance checks or test method controls.

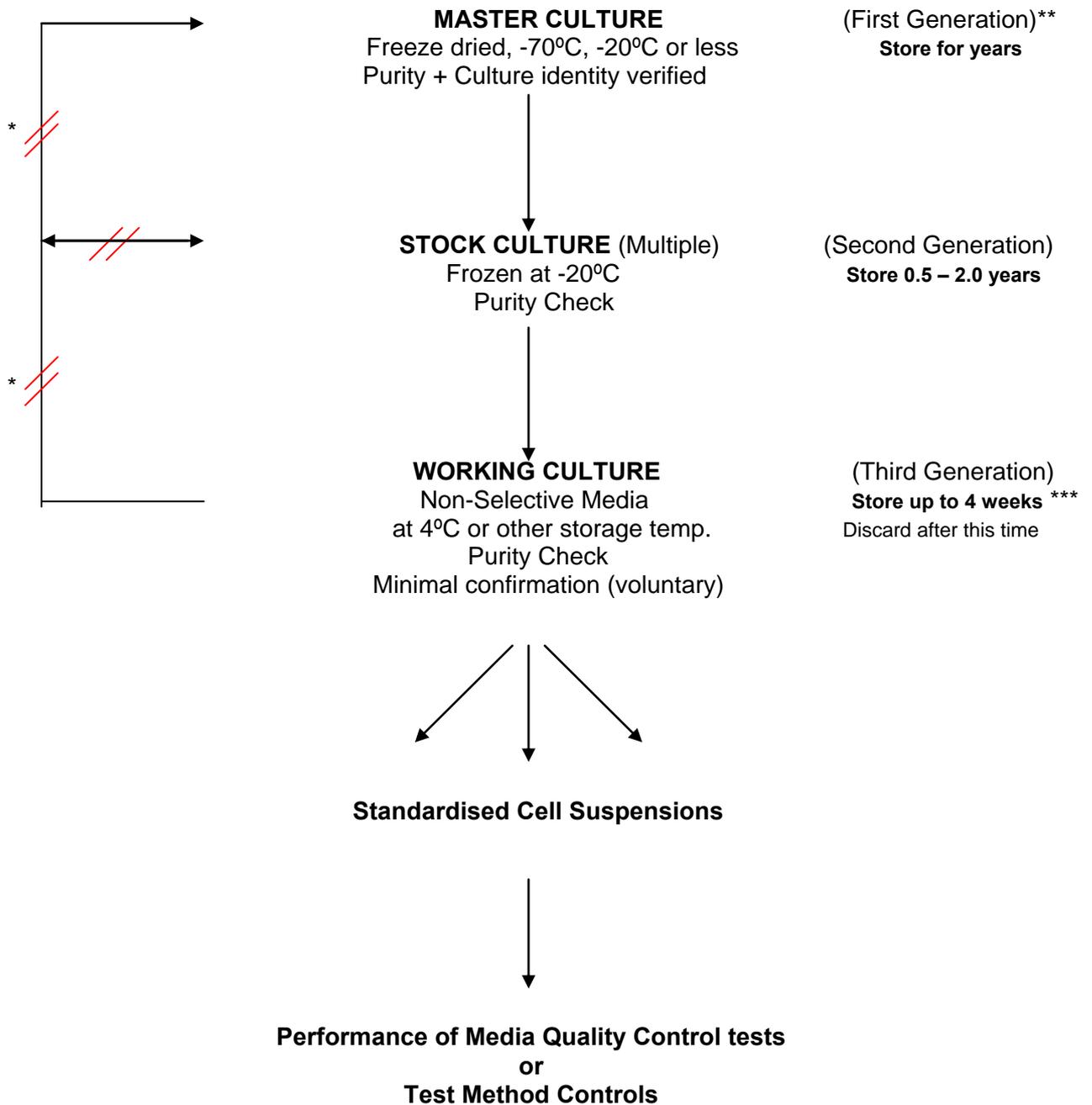
WORKING cultures may be a slope, broth or plate of a non – selective medium such as Tryptone Soy or Nutrient broth/agar. The Working cultures are generated from the Master and Stock cultures as outlined in Figure 1. This procedure produces a Working culture within 5 subcultures of the original culture. Each working culture must be checked for purity and if needed with simplified confirmatory tests to verify the identity of the organism.

If the received culture is viable and pure, the master culture prepared should be only one passage removed from the received culture, the stock culture is therefore two passages removed. The working culture will have had little opportunity to undergo genetic variation and should therefore be typical of the original reference culture. The purpose of establishing this hierarchical system is to minimise the risk of genetic change.

Ideally, MASTER cultures should be stored at  $-70^{\circ}\text{C}$  or freeze dried. However if these resources are not available, the MASTER should be stored in a dedicated freezer which is infrequently opened, and operating at, or as close as possible, to  $-20^{\circ}\text{C}$  **or lower**. By contrast the "STOCK" culture may be stored in the freezer section of a laboratory fridge/freezer and accessed many times throughout the year to prepare the working cultures.



**Figure 1: Maintaining a Culture Collection\***



\* The hierarchical system is not reversible and working cultures must not be used to replace master cultures.  
\*\* A maximum of five subcultures (generations) only allowed.  
\*\*\* Informative – guide only



## 2.5 Test Procedures for Culture Media

To perform the test procedure for culture media the following is recommended:

- a. Suspend three to five isolated colonies in a small volume of suitable medium and use growth from an 18-24 hour culture of the quality control organism. Adjust the turbidity to approximate a McFarland 0.5 turbidity standard. This basic suspension should contain approximately  $10^7$ - $10^8$  cfu/mL. Alternatively, use a thawed frozen culture suspension initially adjusted to give this count, or other internally validated methodology.
- b. For testing the nutritive capacity of a medium, inoculate each test plate with a calibrated or disposable loop loaded with diluted suspension to provide  $10^2$  - $10^3$  cfu/plate. A standardised methodology should be used to distribute CFUs over the plate to generate isolated colonies. If isolated colonies are not achieved, use a ten-fold lighter inoculum. Methods should be supported by validation data, generated by the laboratory.
- c. For testing the inhibitory capacity of a selective medium inoculate each test plate with a calibrated or disposable loop to provide  $10^4$  -  $10^5$  cfu/plate.
- d. For testing the performance of liquid medium for its nutritive capacity a cell suspension should be prepared so that the chosen aliquot will deliver approximately  $10^1$ - $10^2$  cfu per unit of test medium.
- e. For testing the performance of liquid medium for its inhibitory capacity, heavier inocula of the order of  $10^4$ - $10^5$  cfu will normally be used. Broths should be subsequently sub-cultured to check correct inoculum.
- f. Incubate the inoculated test media under conditions specified in the relevant standard/test method. Refer to Appendices 2 and 3 for specific conditions.

## 2.6 Parameters to be Measured in Test Procedures

For the interpretation of the results of the tested media, it is necessary to have tools which enable the comparison of the amount of growth obtained. The use of a reference medium is therefore mandatory for quantitative methods; for qualitative methods, the use of a reference medium helps to interpret results.



### 2.6.1 Productivity

Where it is necessary to demonstrate the growth of a microorganism in a medium, the productivity should be measured.

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

$$P_R = N_S / N_O \quad \text{where}$$

$N_S$  is the total colony count obtained on the tested culture medium

$N_O$  is the total colony count obtained on the defined reference culture medium. It should be  $\geq 100$ cfu.

For qualitative evaluations, visual checks are carried out and growth scores allocated (eg, '0' corresponds to no growth, '1' corresponds to weak growth (either reduction in amount of growth or colony size), '2' corresponds to good growth).

### 2.6.2 Selectivity

Where it is necessary to demonstrate that a medium suppresses the growth of a microorganism, the selectivity should be measured.

For quantitative methods, the Selectivity Factor  $S_F$  is calculated quantitatively as follows:

$$S_F = D_O - D_S \quad (S_F, D_O \text{ and } D_S \text{ are expressed in } \log_{10} \text{ units})$$

$D_O$  is the the highest dilution showing growth of at least 10 colonies on the non-selective reference medium.

$D_S$  is the highest dilution showing comparable growth on the test medium.

e.g: if  $D_O 10^{-4} = \log_{10} 4.0$  and  $D_S 10^{-3} = \log_{10} 3.0$  then the selectivity factor  $S_F = 1.0$

NOTE The  $S_F$  of non-target microorganisms on most selective media should be at least 2.

For qualitative methods the unwanted strain(s) should be inhibited partly or completely.

### 2.6.3 Specificity

The specificity is given by essential characteristics that differentiate related organisms - by the presence, absence and/or grade of expression of biochemical responses and colony sizes and morphology.



## 2.7 Growth Recovery of Control Microorganisms

For lot/batch control of culture media and nutritive ingredients for culture media, growth should be assessed by quantitative or qualitative methods.

Verification of each new lot/batch of medium is made by comparison to previous batches of the same medium or a current batch of a reference medium. **However, comparison with a previous batch of medium is discouraged because of the possibility of insidious decline of performance standards.**

*For Example: Lot/batch A when first tested only recovered 75% of the pathogen. This is later used as the control for lot/batch B. Lot/Batch B only recovers 75% of the pathogen as compared to A. Combining the two batches shows only a 56% recovery of the test organism. This decline in recovery would be further compounded with lot/batch C.*

Each laboratory needs to set its own acceptance/rejection criteria, with reference to Appendices 2 and 3 and the recommendations below.

### 2.7.1 Quantitative recovery (typically used for raw material testing)

#### 2.7.1.1 Non-selective solid media

- Perform viable counts on both the test and reference medium;
- compare the results as described in 2.6.1. The  $P_R$  should be calculated using the counts from both media. An acceptance criteria of at least 70% recovery is recommended;
- the medium also needs to be assessed for typical morphology and colony size to complete the performance evaluation on the medium.

#### 2.7.1.2 Selective solid media

- Perform viable counts on both the test and reference medium;
- compare the results as described in 2.6.1. The  $P_R$  should be calculated using the counts from both media. An acceptance criteria of at least 50% recovery is recommended;
- the medium also needs to be assessed for typical morphology and colony size to complete the performance evaluation on the medium.

It is also relevant to demonstrate the capacity of the test medium to suppress the negative control organism. The  $S_F$  of negative control microorganisms on most selective media should be at least 2.

**2.7.1.3 Non-selective liquid media**

Between  $10^1$ - $10^2$  cfu of the test organism is inoculated into the test broth, incubated and then a standard aliquot is removed to enumerate by quantitative methods, to demonstrate the recovery of an adequate number of test organisms.

**2.7.1.4 Selective liquid media**

- Test organism:  $10^1$ - $10^2$  cfu is inoculated into the test broth, reference broth (TSB or BHI) and solid reference medium. The solid reference medium is used to confirm the cfu in the inoculum;
- negative control organism:  $10^4$ - $10^5$  cfu is inoculated into a second set of the same media.
- Test organism and negative control organism as a mixed culture:  $10^1$ - $10^2$  cfu of the test organism, and  $10^4$ - $10^5$  cfu of the negative control organism is inoculated into a third set of media. The solid reference medium here should (where possible) be a non-selective agar that allows differentiation of test organism and negative control organism;
- after incubation, remove a measured volume from each broth and spread on solid non-selective media;
- after incubation of solid media, determine the percentage recovery for the test organism and the degree of inhibition for the negative control organism. For the mixed culture, the percentage recovery of the positive organism should not be compromised.

*Selective Liquid Medium Testing*

Test Medium			Non Selective Reference Medium		
+ve control org. $10^1$ - $10^2$ cfu	-ve control org. $10^4$ - $10^5$	Mix(+ve &-ve)	+ve control org. $10^1$ - $10^2$ cfu	-ve control org. $10^4$ - $10^5$	Mix(+ve &-ve)
↓ subculture	↓ subculture	↓ subculture	↓ subculture	↓ subculture	↓ subculture
Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)
Count	Count	Count Both Org.	Count	Count	Count Both Org.
Determine: % Recovery of the +ve organism % Inhibition of -ve organism % Recovery of the +ve organism from mix should equal or exceed from +ve organism alone.					



### 2.7.2 Qualitative recovery (typically used for batch testing)

The use of the term 'semi-quantitative' has been discontinued in international standards for quality control of culture media (2).

A standardised methodology must be used to distribute CFUs over the plate. Different streak plate techniques may be used; eg, a 5 zone streak plate (Figure 2) or an ecometric method (Figure 3). Each laboratory needs to standardise its method and all of the operators trained to follow their procedure.

Fig.2

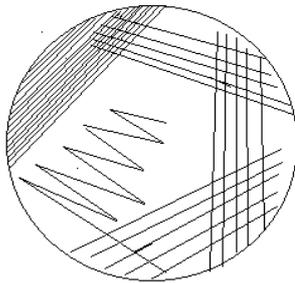
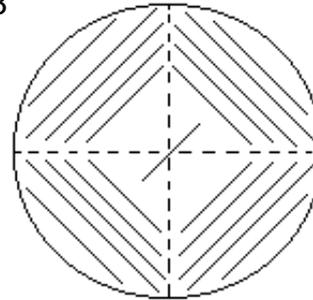


Fig.3



The growth (e.g. number of streak lines or quadrants grown) for both test and reference media should be compared and the growth index  $G_i$  calculated or determined. For example: If a 21 streak line plate is prepared, then the number of streak lines on the reference medium is recorded as the Absolute Growth Index ( $AGI$ ), whilst the number of streak lines on the test medium is recorded as the Relative Growth Index ( $RGI$ ).

The % Relative Growth Index is calculated as follows:

$$\%RGI = \frac{RGI}{AGI} \times 100$$

A simplified qualitative method involves using standardized streaking technique and inocula, with test organisms streaked onto both test and reference media. The growth on the plates after incubation is assessed and recorded as follows: no growth, weak growth and good growth, or could be scored (only indicative) as 0,1,2. The score of wanted microorganisms should be good growth (or 2) and display typical appearance, size, morphology and (if appropriate) biochemical response of colonies.



### **2.7.2.1 Non-selective solid media**

- Perform viable counts on both the test and reference medium;
- compare the results as described in 2.7.2. Calculate the %RGI using the counts from both media. An acceptance criteria of a %RGI of at least 70% is recommended;
- the medium also needs to be assessed for typical morphology and colony size to complete the performance evaluation on the medium.

### **2.7.2.2 Selective solid media**

- Perform viable counts on both the test and reference medium;
- compare the results as described in 2.7.2. Calculate the %RGI using the counts from both media. An acceptance criteria of a %RGI of at least 50% is recommended for the test organism;
- the medium also needs to be assessed for typical morphology and colony size to complete the performance evaluation on the medium.

It is also relevant to demonstrate the capacity of the test medium to suppress the negative control organism. The recommended acceptance criteria for negative control microorganisms on most selective media is less than 25%.

### **2.7.2.3 Non-selective liquid media**

Between  $10^1$ - $10^2$  cfu of the test organism is inoculated into the test broth, incubated and then a standard aliquot is removed to enumerate by quantitative methods, to demonstrate the recovery of an adequate number of test organisms.

A simplified qualitative method involves using standard inocula of working cultures that are directly inoculated into the medium being tested and a reference broth. The qualitative evaluation should be carried out visually by allocating growth scores as follows: zero turbidity or 0, very light turbidity or 1, good turbidity or 2. Score for the wanted microorganisms should be good turbidity or 2. Note that liquid media can be carefully shaken before interpreting turbidity, but that media with turbid ingredients cannot be tested by this method.

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



#### **2.7.2.4 Selective liquid media**

Inoculate a test broth with positive control bacterium, another test broth with negative control bacterium, and a third test broth with a mixture of positive and negative control bacteria. After incubation, a standard loop (10 $\mu$ l) from the test broths for the positive bacterium, and the mixture, are plated out onto a selective medium for growth of the positive bacterium; a standard loop (10 $\mu$ l) from the test broth for the negative control bacterium is plated onto a non-selective medium. The test medium is considered to have passed if at least 10 colonies of the positive control develop on the selective medium and no growth or less than 10 colonies of the negative control develop on the non-selective medium.

### **2.8 Interpretation of Results**

A medium's performance is regarded as satisfactory if all test strains grow or are inhibited as is appropriate for the medium being tested, and colonial morphology and reactions produced in the medium are typical for the organism on that particular type of medium. However, to be able to accept all batches of "satisfactory" medium, it is essential to have documented the acceptance and rejection criteria or what the laboratory might call its media specifications. In addition, there needs to be a general procedure of how to proceed if a batch of medium is rejected – does the laboratory retest, throw out or what protocol needs to be followed.

#### **2.8.1 Recommended Results for Quantitative Recovery**

Productivity:  $\geq 70\%$  (wanted organism) nonselective medium

$\geq 50\%$  (wanted organism) selective medium

$\leq 25\%$  (unwanted organism)

Selectivity:  $>2$  (Log)

Specificity: Reject if fails to produce typical colonial morphology, size or biochemical response

Reject if fails to suppress background flora.



## 2.8.2 Recommended Results for Qualitative Recovery

%RGI            $\geq 70\%$  (wanted organism) nonselective medium  
                   $\geq 50\%$  (wanted organism) selective medium  
                   $\leq 25\%$  (unwanted organism)

Growth observed: good growth (wanted organism) or zero/weak growth (unwanted organism)

Turbidity observed: good turbidity (wanted organism) or zero/very light turbidity (unwanted organism)

Specificity:    Reject if fails to produce typical colonial morphology, size or biochemical response  
                  Reject if fails to suppress background flora.

## 2.9 Reporting Quality Assurance Data to Users

Manufacturers testing food and water microbiological culture media according to these Guidelines may affix compliance labels to, or issue certification with, batches of products that have been found to comply. Such labels or certification need only declare that testing of that specific batch has complied with the requirements of these Guidelines.

If compliance labels are used, customers should be supplied with a Product Specification.

The specification should detail intended use and storage conditions, strains tested, testing method, incubation temperature, period and atmosphere, the final pH of the medium and the procedure used for testing for contamination.

If compliance certificates are issued, such certifications should also include the strains tested and their performance, incubation temperature, period and atmosphere, the final pH of the medium and the procedure used for testing for contamination.



### **3.0 Packaging, Transport and Storage**

Prepared media should be packaged in such a way as to minimise moisture loss and provide protection against physical and microbial contamination. Such packaging should consider the ways in which the media is stored, handled and transported.

Where transportation of media occurs, appropriate packaging and modes of transportation should be used to ensure against exposure to potentially detrimental conditions.

Prepared media should be stored in such a way as to minimise moisture loss and provide protection against physical and microbial contamination, as well as against light-induced damage and thermal damage. Prepared media should be stored in unopened or resealed packages at 2-8°C unless documented validation has been conducted on samples of each medium type to demonstrate that storage under alternative conditions is not detrimental to its performance when tested according to these Guidelines.

#### **3.1 Shelf Life of Prepared Media**

All prepared media should be marked with an expiry date. This should be validated under the conditions of packaging, transportation and storage that will prevail under normal circumstances. The date of manufacture should be provided (this may be on the product, or on the packaging, or on the conformity certificate).

Validations of expiry dates should be based on evaluations of the performance of samples of each type of medium according to these guidelines. Where media is prepared commercially or for distribution outside the manufacturing laboratory, such validations should include simulated transportation phase(s) in the storage/testing protocol. Such simulated phases should reflect the least favourable conditions likely to be encountered during transportation. Conditions to which the media are exposed during transport should be evaluated using suitable measuring devices i.e. temperature indicator or electronic monitor.

Revalidation of expiry date should be done whenever significant changes are made to usual conditions of packaging, storage and transportation or to the formulation of the medium.

**Validation of Shelf Life Example: Method 1**

Prepare a batch of the medium to be shelf life validated. This should be of a size that will allow testing with a number of different microorganisms per x number of weeks. (10 organisms for 10 weeks = 110 plates / broths. Package and store the batch of medium as is the normal protocol of the laboratory, e.g. plates wrapped in cellophane or plastic, store at 2-8°C in dark; broths caps tightened, packaged in cardboard or plastic/cellophane, stored as appropriate in low light or dark. Label packages week 0 to 10.

In this example the batch of medium is constant but there may be week to week variation in the operator and the conduct of the test.

Using quantitative or qualitative recovery testing procedures, inoculate test microorganisms onto media to be validated and a freshly made control/ reference batch each week. Record all results: Growth, colony size, colonial morphology, biochemical responses, volume (can be determined by weight), gel strength, gas, turbidity, clarity, haemolysis etc. The test medium will progressively get older but a fresh batch of the reference medium is used each time. Continue until test medium displays noticeable character changes such as reduction in colony size, reduction in amount of growth, media colour changes, drying of medium (cracking, loss of volume) etc.

Determine at which week the last acceptable results were recorded. This then represents the upper limit of the shelf life of that batch of medium. The laboratory may decide that an acceptable safety margin can be included in the shelf-life. This is usually a reduction in the shelf life expectancy. If the medium tested is acceptable at 10 weeks, the laboratory may decide to place an 8 week expiry date on the medium.

Where media is to be transported, a simulated or real transport phase should be included in the shelf life testing protocol. This could be done either during the x number of weeks testing period or after determining the shelf life under ideal conditions.

The procedures used, the results obtained, and the conclusions drawn, should be fully documented.

**Validation of Shelf Life Example: Method 2**

If a type of medium is made regularly i.e. weekly, collect a number of plates each week from the batch (if 10 organisms to be tested, collect 10 plates/broths) for the predicted shelf life number of weeks i.e. 10 weeks. Ensure that test media is packaged and stored correctly as per laboratory protocol. When enough media has been collected, the testing protocol can begin. During this collecting phase, test media could be transported and returned to laboratory to be included in test. Oldest collected media could be 10 weeks and the youngest is fresh. Label all packages with week number.

In this example, the test batch of medium changes, but the operator, inoculation techniques, incubation conditions, control/reference batch and recording of results are constant.

Using quantitative or qualitative recovery testing procedures, inoculate test microorganisms onto every week's media to be validated and fresh control/reference batch. In this example all testing is completed in 1-2 days rather than progressively over weeks as in Example 1. Record all results: Growth, colony size, colonial morphology, biochemical responses, volume (can be determined by weight), gel strength, gas, turbidity, clarity, haemolysis etc. It is important to note all changes and at which week they occurred.

Determine at which week the last acceptable results were recorded. This then represents the upper limit of the shelf life of that batch of medium. The laboratory may decide that an acceptable safety margin can be included in the shelf-life. This is usually a reduction in the shelf life expectancy. If the medium tested is acceptable at 10 weeks, the laboratory may decide to place an 8 weeks expiry date on the medium.



## **4.0 Quality Assurance Practices for media prepared off-site**

### **4.1 General Requirements**

Laboratories who receive prepared media accompanied by a media quality control certificate should retain these certificates in an appropriate file for a minimum of 4 years (8).

Laboratories who obtain prepared culture media either from a commercial source or a central facility, that carries a compliance label should record the following data in a log book or similar.

- Date received
- Product
- Batch number
- Expiry date
- Date manufactured
- Condition upon delivery
- Size of delivery

If performance testing is undertaken upon receipt the results should also be recorded.

### **4.2 Physical Inspection of Plates/Tubes, Bottles**

Users of commercially prepared media, or media supplied from a central accredited facility to satellite laboratories on a non-commercial basis (i.e. within one organisation), should undertake a brief inspection of the media on receipt in their laboratory.

Examination should include:

- Integrity of packaging
- Broken or cracked petri dishes/bottles/tubes
- Quality and accuracy of labelling
- Expiry date
- Dehydration
- Discolouration
- Sloped or uneven filling of petri dishes
- Contamination
- Crystalline pattern on surface of medium (indicative of freezing)
- Large bubbles
- Presence of leakage



### 4.3 Remedial Action for Deficiencies Observed

Where significant defects are found, the users should notify the manufacturers providing all of the following details:

- Products affected (catalogue number or identification code, and product name)
- Quantity affected and quantity received
- Batch number and expiry date (and timestamp where present)
- Date received by user
- Detailed description of problem or deficiency

Whenever possible, samples of the defective media should be retained by the user and provided to the manufacturer at their request. Any corrective action or response made by the manufacturer should be fully documented in the User's Laboratory Manual in accordance with accreditation requirements (7).

### 4.4 Performance Monitoring

It is recommended that users of commercially prepared media monitor performance of the media they purchase. Testing should include nutrient and inhibitory performance, but not contamination.

Once the laboratory has been able to demonstrate the reliability of the products, they may reduce the frequency of testing. Upon any failure of the media - either on quality control performance tests or in-use monitoring - a return to the monitoring of each batch should be undertaken until reliability is re-established.

Laboratories are also strongly encouraged to confirm the ability to support growth for *all* media used for recovery of *fastidious* organisms or those organisms with unique growth requirements (8).

Where media provided has been tested in a manner that may not include your particular end-use needs, a monitoring program should be implemented to include these needs (eg, blood agar plates, *Listeria* spp. and CAMP test).



## 5.0 References

1. ISO/TS11133-2:2003. *Microbiology of food and animal feeding stuffs –Guidelines on preparation and production of culture media –Part 2: Practical guidelines on performance testing of culture media*. International Standards Organisation, Geneva.
2. ISO 11133. *Microbiology of food, animal feed and water –Preparation, production, storage and performance testing of culture media*. International Standards Organisation, Geneva.
3. *Guidelines for Assuring Quality of Medical Mycology Culture Media*. 2012. Culture Media & Mycology Special Interest Groups, Australian Society for Microbiology.
4. *Guidelines for Assuring Quality of solid media used in Australia for the Cultivation of Medically Important Mycobacteria*. 2012. Culture Media & Mycobacteria Special Interest Groups, Australian Society for Microbiology.
5. *Guidelines for Assuring Quality of Medical Microbiological Culture media*. 2012. Culture Media Special Interest Group, Australian Society for Microbiology.
6. *Biological Testing ISO/IEC17025 Application Document. Annex G: Media Preparation and Quality Control. Current edition*. National Association of Testing Authorities (NATA), Sydney, Australia.
7. *ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories*. International Standards Organisation, Geneva.
8. *Biological Testing ISO/IEC17025 Application Document*. 2015. National Association of Testing Authorities (NATA), Sydney, Australia
9. *Handbook of Culture Media for Food and Water Microbiology : Edition 3*. Edited by JEL Corry, Gordon DW Curtis and RM Baird 2011. Royal Society for Chemistry, [www.rsc.org](http://www.rsc.org)
10. AS1199.1-2003 (ISO2859-1:1999). *Sampling Procedures for Inspection by Attributes. Part 1: sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection*. 2003. Standards Australia, Sydney.
11. *Biological Testing ISO/IEC17025 Application Document. Annex H. Maintenance of Microbiological Reference Culture Collections (MRCC)*. 2011. National Association of Testing Authorities, Sydney, Australia.



## Appendix 1 Sampling Plan for Microbiological Culture Media

**Small Batches ( $\leq 100$  units):** 1% or 1 unit from beginning and 1% or 1 unit from end of batch (1).

**Double Sampling Plan ( $> 100$  units)** NORMAL SAMPLING PLAN, AQL - 2.5, GENERAL INSPECTION LEVEL = 1 (10)

Batch Size (units made)	Sample Number		1 <sup>st</sup> Sample		2 <sup>nd</sup> Sample	
	1 <sup>st</sup> sample	2 <sup>nd</sup> sample	Accept	Reject	Accept	Reject
101 – 150	5	5	0	2	1	2
151 - 280	8	8	0	2	1	2
281 - 500	13	13	0	2	1	2
501 - 1200	20	20	0	3	3	4
1201 - 3200	32	32	1	3	4	5
3201 – 10000	50	50	2	5	6	7
10000 +	80	80	3	6	9	10

### Interpretation:

**Small Batches ( $< 100$  units):** A 2% sample plan is recommended as being the most cost effective option for sampling small batches of media. The samples to be tested should be taken from the beginning and the end of the manufacturing process. When sterility testing small batches, it is more economical to reject a batch, and prepare a new one, than devote time and resources to repeat testing. If the number of contaminated/defective items in the sample is zero, the batch may be accepted. If the number of contaminated/defective items in the sample is equal to or greater than one, the batch is to be rejected.

**Large Batches ( $> 100$  units):** A double normal sampling plan provides for a second set of samples to be taken where larger lots are prepared, and fail to be accepted after the first sample is examined. If, after inspection of the initial sample, the number of contaminated items lies between the 'Accept' and 'Reject' levels, a second sample may be taken and tested. **If the cumulative total** of contaminated items, i.e. first sample plus second sample, is equal to or less than the second sample level of acceptance, the batch may be accepted. If however, the cumulative total of contaminated items, i.e. first sample plus second sample, is equal to or greater than the second sample level of rejection, the batch is to be rejected.

APPENDIX 2 Batch Quality Control for Growth and Performance Testing of Media for Food Microbiology									
Media	Microorganisms	Standard# (# = current issue)	Function	Incubation	Aust.Std QC strains*	ISO11133 QC strains^	Method of Control	Criteria	Characteristic reactions
			see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Appendix to the ISO Standard; OR, suggested strains where no equivalents listed in Australian Standards)	^where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7		
DILUENTS 0.1% peptone 0.1% peptone salt solution (PSS) PSS+BCP  Ringer's solution 1/4 strength		AS5013.11.x (ISO6887-x)	D	20-25°C/ 45min-1hr		E.coli WDCM00013 or 00012 S.aureus WDCM00034	Quantitative	+/- 30% colonies at time 0	n/a
		AS5013.20							
0.1% peptone water + 3% NaCl	Vibrio parahaemolyticus	AS5013.18	D	20-25°C/ 45 min-1hr	V.parahaemolyticus NCTC10884		Quantitative	+/- 50% colonies at time 0	-
Agar Listeria according to Ottaviani and Agosti	Listeria monocytogenes	AS5013.24.1 (ISO11290-1 MOD) AS5013.24.2 (ISO11290-2 MOD)	P	36-38°C/ 40-48h		L.monocytogenes WDCM00021 or 00109	Qualitative	growth	Blue-green colonies with opaque halo
			SE			E.coli WDCM00013 or 00012		no growth	-
			SP			Enterococcus faecalis WDCM00087 or 00009		growth	Blue-green colonies withOUT opaque halo
						Listeria innocua WDCM00017			
Alkaline peptone water	Vibrio parahaemolyticus	AS5013.18	P	34-38°C/ 6-8h	V.parahaemolyticus NCTC10884		Qualitative	growth on subculture to TCBS	-
Baird-Parker medium (B-P)	coagulase-positive staphylococci	AS5013.12.1 (ISO6888-1)	P	36-38°C/22-50h		S.aureus WDCM00034 or 00032	Quantitative	P <sub>R</sub> ≥ 0.5	Black/grey colonies, with opacity halo
			SE			S.epidermidis WDCM00036	Qualitative	Black/grey colonies, without opacity halo	
						S.saprophyticus WDCM00159		Inhibition	
Baird-Parker medium (B-P) containing rabbit plasma fibrinogen (RPF)	coagulase-positive staphylococci	AS5013.12.2 (ISO6888-2)	P	36-38°C/22-50h		S.aureus WDCM00034 or 00032	Quantitative	P <sub>R</sub> ≥ 0.5	Black/grey colonies, with opacity halo
			SE			S.epidermidis WDCM00036	Qualitative	Black/grey colonies, without opacity halo	
						S.saprophyticus WDCM00159		Inhibition	
Bolton Broth	Campylobacter	ISO10272	SE	36-38°C/4-6hr, then ~41.5°C/40-48h		C.coli WDCM00004 OR C.jejuni WDCM00005 or 00156 AND E.coli WDCM00013 or 00012 AND P.mirabilis WDCM00023	Qualitative	>10 cols on mCCDA	Grey, flat, moist, sometimes with metallic sheen
			SE			E.coli WDCM00013 or 00012 P.mirabilis WDCM00023		no growth on TSA	n/a
Brain Heart Infusion Broth (BHIB)	coagulase-positive staphylococci	AS5013.12.1 (ISO6888-1)	NS	36-38°C/ 22-26h		S.aureus WDCM00034	Qualitative	turbidity	-
Brilliant Green Lactose Bile Broth	coliforms	AS5013.3 (ISO 4831) AS5013.4 (ISO4832)	P	29-31°C/ 22-50h		E.coli WDCM00013 or 00012 Citrobacter freundii WDCM00006	Qualitative	turbidity & gas in Durham tube	Gas production and turbidity
			SE			Enterococcus faecalis WDCM00087 or 00009		inhibition, no gas production	n/a

Function  
D=dilution  
SE=selectivity  
NS=nonselective  
P=productivity  
SP=specificity

All incubation conditions are aerobic unless otherwise indicated.

italics not used for ease of reading

ASM Guidelines for Assuring Quality of Food and Water Microbiological Culture Media 2nd edition

APPENDIX 2 Batch Quality Control for Growth and Performance Testing of Media for Food Microbiology									
Media	Microorganisms	Standard# (# = current issue)	Function see footnotes	Incubation as recommended/listed in Std or in ISO11133	Aust.Std QC strains* (*as listed in the Australian Appendix to the ISO Standard; OR, suggested strains where no equivalents listed in Australian Standards)	ISO11133 QC strains^ ^where listed for these media; not all strains required as a minimum	Method of Control see Guidelines Section 2.7	Criteria	Characteristic reactions
Bromocresol purple cellobiose broth +3% NaCl	Vibrio parahaemolyticus	AS5013.18	SP	34-38°C/ 4d	V.parahaemolyticus NCTC10884 E. aerogenes WDCM00175 (not listed in this specific Std but suitable for intended task described here)		Qualitative	growth growth	no fermentation Fermentation of cellobiose
Brucella Broth	Campylobacter	ISO10272	P	~41.5°C/ 2-5d		C.coli WDCM00004 OR C.jejuni WDCM00005 or 00156	Qualitative	turbidity	-
Buffered glucose broth +3% NaCl	Vibrio parahaemolyticus	AS5013.18	SP	34-38°C/ 18-24h	V.parahaemolyticus NCTC10884 S.aureus WDCM00034(not listed in this specific Std but suitable for intended task described here)		Qualitative	growth growth	Voges-Proskauer -ve Voges-Proskauer +ve
Buffered Peptone Water (BPW)	salmonellae	AS5013.10 (ISO6579)	NS	36-38°C/ 16-20h	Salmonella Hofit IMVS 1799		Qualitative	Good growth	turbidity
	diluent	AS5013.11.1 (ISO6887-1)	D	20-25°C/ 45min-1hr		E.coli WDCM00013 or 00012 S.aureus WDCM00034		+/- 30% colonies at time 0	na
	Listeria monocytogenes	AS5013.24.2 (ISO11290-2 MOD)		18-22°C/ 55-65min		L.monocytogenes WDCM00019			
CFC agar	Pseudomonas spp	AS5013.21 (ISO13720)	P	24-26°C/40-48h		Pseudomonas fluorescens WDCM00115 Pseudomonas fragi WDCM00116	Quantitative	P <sub>R</sub> ≥ 0.5	-
			SE			E.coli WDCM00013 or 00012	Qualitative	no growth	-
CT-SMAC agar	E.coli O157	AS5013.26 (ISO16654)	P	36-38°C/18-24h		E.coli WDCM00014	Qualitative	growth	translucent sorbitol-negative colonies
			SP			E.coli WDCM00013 or 00012		growth	opaque sorbitol-positive colonies
Dichloran 18% Glycerol agar (DG18)	yeasts & moulds	AS5013-29				see ISO Std			
		ISO21527-2	P	24-26°C/ 5d		S.cerevisiae WDCM00058	Qualitative	growth	Characteristic colonies
			SE			E.coli WDCM00013 B.subtilis WDCM00003		inhibition	n/a
Dichloran Rose Bengal Chloramphenicol agar (DRBC)	yeasts & moulds	AS5013-29				see ISO Std			
		ISO21527-2	P	24-26°C/ 5d		Candida albicans WDCM00054 Aspergillus brasiliensis WDCM00053 S.cerevisiae WDCM00058	Qualitative	growth	Characteristic colonies according to each species
			SE			E.coli WDCM00013 B.subtilis WDCM00003		inhibition	n/a
EC broth	coliforms/ E.coli	AS5013.9 AS5013.15 (ISO7251)	P	43-45°C/22-50h		E.coli WDCM00013 or 00012	Qualitative	growth	Gas production and turbidity
			SE			Pseudomonas aeruginosa WDCM00025		no growth	-
EMB agar (Eosin Methylene Blue agar)	coliforms/ E.coli	AS5013.9	P	29-31°C/ 18-24h OR 36-38°C/ 18-24h	E.coli WDCM00013 or 00012		Qualitative	growth	Green metallic sheen, and/or dark purple centres, and/or opaque, ununclated, mucoid and pink coloration

Function  
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NS=nonselective  
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Batch Quality Control for Growth and Performance Testing of Media for Food Microbiology									
Media	Microorganisms	Standard <sup>#</sup>	Function	Incubation	Aust.Std QC strains*	ISO11133 QC strains <sup>A</sup>	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Appendix to the ISO Standard; OR, suggested strains where no equivalents listed in Australian Standards)	<sup>A</sup> where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7		
EE Broth (Enterobacteriaceae Enrichment broth)	Enterobacteriaceae	AS5013.8 (ISO5552)	P	36-38°C/22-26h		E.coli WDCM00013 or 00012	Qualitative	growth on VRBG	Pink to red colonies, ± precipitation halo
			SE					no growth on TSA	na
Fraser Broth	Listeria monocytogenes	AS5013.24.1 (ISO11290-1 MOD)	P	36-38°C/ 46-50h		(L.monocytogenes WDCM00021 OR 00109) AND (E.coli WDCM00013 or 00012) AND (E.faecalis WDCM00087 or 00009)	Qualitative	> 10 colonies on Agar Listeria according to Ottaviani and Agosti	Blue green colonies with opaque halo
			SE					no growth	-
Giolitti-Cantoni Broth	coagulase-positive staphylococci	AS5013.12.3 (ISO6888-3)	P	36-38°C/22-50h (tubes sealed with agar plug)		(S.aureus WDCM00034 or 00032) AND (E.coli WDCM00013 or 00012)	Qualitative	>10colonies on Baird-Parker or RPF	Characteristic colonies according to each medium
			SE	36-38°C/46-50h (tubes sealed with agar plug)				E.coli WDCM00013 or 00012	no growth
Half Fraser Broth	Listeria monocytogenes	AS5013.24.1 (ISO11290-1 MOD) AS5013.24.2 (ISO11290-2 MOD)	P	29-31°C/ 22-26h		(L.monocytogenes WDCM00021 OR 00109) AND (E.coli WDCM00013 or 00012) AND (E.faecalis WDCM00087 or 00009)	Qualitative	> 10 colonies on Agar Listeria according to Ottaviani and Agosti	Blue green colonies with opaque halo
			SE					no growth	-
Hektoen agar	shigellae	AS5013.25 (ISO21567)	P	36-38°C/ 20-24h		S.sonnei WDCM00127	Qualitative	growth	translucent, green moist colonies
			SP					E.coli WDCM00013 or 00012	growth
Lactose gelatin medium	Clostridium perfringens	AS5013.16 (ISO7937)	SP	36-38°C/ 22-26h, anaerobic		C.perfringens WDCM00007 or 00080	Qualitative	growth	gelatin liquefaction
Lactose-sulfite medium	Clostridium perfringens	AS5013.16 (ISO7937)	SP	46°C/ 18-24h, waterbath		C.perfringens WDCM00007 or 00080	Qualitative	growth	growth, gas production, presence of blackening (iron sulphide precipitate)
Lauryl Tryptose Broth (LTB)	coliforms	AS5013.9 AS5013.3 (ISO 4831)	P	29-31°C/ 22-50h		E.coli WDCM00013 or 00012 Citrobacter freundii WDCM00006	Qualitative	turbidity & gas in Durham tube	Gas production and turbidity
			SE					no growth	n/a
	Escherichia coli	AS5013.15 (ISO7251)	P	36-38°C/22-50h		E.coli WDCM00013 or 00012	Qualitative	turbidity & gas in Durham tube	Gas production and turbidity
			SE					no growth	n/a
Lysine Decarboxylase Broth (LDC Broth)	salmonellae	AS5013.10 (ISO6579)	SP	36-38°C/ 21-27h oil overlay in tube	Salmonella Hofit IMVS 1799		Qualitative	Good Growth	turbidity and purple/ pale purple
mCCD agar	Campylobacter	ISO10272	P	~41.5°C/40-48h, microaerobic		C.jejuni WDCM00005 C.coli WDCM00072	Qualitative	growth	smooth, flat, translucent, colourless to grey colonies spreading along the streak line
			SE			E.coli WDCM00013 or 00012 S.aureus WDCM00034		inhibition	-

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Media	Microorganisms	Standard#	Function	Incubation	Aust.Std QC strains*	ISO11133 QC strains^	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Appendix to the ISO Standard; OR, suggested strains where no equivalents listed in Australian Standards)	^where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7		
MacConkey agar	shigellae	AS5013.25 (ISO21567)	P SP	36-38°C/ 20-24h	S.sonnei WDCM00127 E.coli WDCM00013 or 00012		Qualitative	growth growth	translucent, lactose-negative colonies opaque, lactose-positive colonies
Minerals Modified Glutamate agar (MMGA)	β-D-glucuronidase positive Escherichia coli	AS5013-19.1 (ISO16649-1)	P SP	36-38°C/ 22-26h	E.coli WDCM00012 Enterococcus faecalis WDCM00009	E.coli WDCM00013 or 00012 Enterococcus faecalis WDCM00087 or 00009	Qualitative	acid production no growth	colour change to yellow -
Mollers decarboxylase broth base with 1% NaCl	Vibrio parahaemolyticus	AS5013.18	NS	34-38°C/ 4d	V.parahaemolyticus NCTC10884		Qualitative	growth	turbidity and yellow (acid)
Mollers decarboxylase broth base with 1% NaCl and arginine	Vibrio parahaemolyticus	AS5013.18	SP	34-38°C/ 4d	V.parahaemolyticus NCTC10884		Qualitative	growth	turbidity and yellow (acid)
Mollers decarboxylase broth base with 1% NaCl and lysine	Vibrio parahaemolyticus	AS5013.18	SP	34-38°C/ 4d	V.parahaemolyticus NCTC10884		Qualitative	growth	turbidity and purple (alkaline)
Mollers decarboxylase broth base with 1% NaCl and ornithine	Vibrio parahaemolyticus	AS5013.18	SP	34-38°C/ 4d	V.parahaemolyticus NCTC10884		Qualitative	growth	turbidity and purple (alkaline)
Mueller-Kauffmann Tetrathionate Broth with novobiocin (MKTn)	salmonellae	AS5013.10 (ISO6579)	P SE	36-38°C/ 21-27h	Salmonella Hofit IMVS 1799 AND E.coli WDCM00013 or 00012 AND Pseudomonas aeruginosa WDCM00025	E.coli WDCM00013 or 00012 Enterococcus faecalis WDCM00087 or 00009	Qualitative	> 10 colonies on XLD or other medium of choice Partial inhibition ≤ 100 colonies on TSA < 10 colonies on TSA	characteristic salmonellae colonies according to each medium - -
MYP agar (Mannitol egg-Yolk Polymyxin)	Bacillus cereus	AS5013.2 (ISO7932, MOD)	P SE SP	29-31°C/ 21-48h 29-31°C/ 40-48h		Bacillus cereus WDCM00001 E.coli WDCM00013 or 00012 Bacillus subtilis WDCM00003	Quantitative Qualitative	P <sub>R</sub> ≥ 0.5 total inhibition -	pink colonies surrounded by zone (halo) of precipitin - yellow colonies without precipitation halo
Nitrate motility medium	Clostridium perfringens	AS5013.16 (ISO7937)	SP	36-38°C/ 22-26h, anaerobic		C.perfringens WDCM00007 or 00080	Qualitative	growth	nitrate positive
Nutrient agar	salmonellae Enterobacteriaceae Pseudomonas spp	AS5013.10 (ISO6579) AS5013.8 (ISO5552) AS5013.21 (ISO13720)	P	36-38°C/ 22-26h 24-26°C/ 40-48h	Salmonella Hofit IMVS 1799 Pseudomonas fluorescens WDCM00015	E.coli WDCM00013 or 00012	Qualitative	good growth	n/a
Oxford agar	Listeria monocytogenes	AS5013.24.1 (ISO11290-1 MOD) AS5013.24.2 (ISO11290-2 MOD)	P SE	36-38°C/ 40-48h		L.monocytogenes WDCM00021 or 00109 E.coli WDCM00013 or 00012 Enterococcus faecalis WDCM00087 or 00009	Qualitative	growth no growth	Grey-black colonies with sunken centre, surrounded by black halo -

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Media	Microorganisms	Standard <sup>#</sup>	Function	Incubation	Aust.Std QC strains*	ISO11133 QC strains <sup>A</sup>	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Appendix to the ISO Standard; OR, suggested strains where no equivalents listed in Australian Standards)	<sup>A</sup> where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7		
PALCAM agar	Listeria monocytogenes	AS5013.24.1 (ISO11290-1 MOD) AS5013.24.2 (ISO11290-2 MOD)	P	36-38°C/ 40-48h		L.monocytogenes WDCM00021 or 00109 E.coli WDCM00013 or 00012 Enterococcus faecalis WDCM00087 or 00009	Qualitative	growth	Grey-green to black colonies with sunken centre, surrounded by black halo
			SE					no growth	-
Plate Count Agar (PCA)	total aerobic count	AS5013.5 (ISO4833) AS5013.14.3 AS5013.23 (ISO 17410)	P	29-31°C/ 69-75h		E.coli WDCM00013 or 00012 S.aureus WDCM00034 B.subtilis WDCM00003	Quantitative	P <sub>R</sub> ≥ 0.7	-
Preston agar	Campylobacter	AS5013.6	P	41-43°C/ 40-48h, microaerobic	C.jejuni WDCM00005 C.coli WDCM00072 E.coli WDCM00013 or 00012 S.aureus WDCM00034		Qualitative	growth	smooth, flat, translucent, colourless to grey colonies spreading along the streak line
			SE					inhibition	-
Preston Broth with antibiotic supplement	Campylobacter	AS5013.6	P	41-43°C/ 40-48h, microaerobic	C.jejuni WDCM00005 C.coli WDCM00072 E.coli WDCM00013 or 00012 P.mirabilis WDCM00023		Qualitative	growth	growth on subculture on selective medium
			SE					inhibition	inhibited or no growth on subculture on selective medium
Rappaport-Vassiliadis soya peptone (RVS) broth	salmonellae	AS5013.10 (ISO6579)	P	40.5-42.5°C/ 21-27h	Salmonella Hofit IMVS 1799 AND E.coli WDCM00013 or 00012 AND Pseudomonas aeruginosa WDCM00025		Qualitative	> 10 colonies on XLD or other medium of choice	characteristic salmonellae colonies according to each medium
			SE					Partial inhibition ≤ 100 colonies on TSA	-
						E.coli WDCM00013 or 00012 Enterococcus faecalis WDCM00087 or 00009		< 10 colonies on TSA	-
Salt tolerance medium	Vibrio parahaemolyticus	AS5013.18	SP	34-38°C/46-50h	V.parahaemolyticus NCTC10884		Qualitative	NO growth	-
0% NaCl								growth	-
8% NaCl								NO growth	-
11% NaCl									
Sheep blood agar	Listeria monocytogenes	AS5013.24.1 (ISO11290-1 MOD)	SP	35-37°C/ 18-24h	L.monocytogenes WDCM00019 L.innocua WDCM00017 L.ivanovii WDCM00018		Qualitative	growth & β-haemolysis	narrow clear light zone around colonies
								growth & no haemolysis	
	Bacillus cereus	AS5013.2 (ISO7932, MOD)		30°C/ 22-26h	Bacillus cereus WDCM00001		Qualitative	growth & β-haemolysis	wide zone around colonies
Shigella broth	shigellae	AS5013.25 (ISO21567)	P	41-43°C/ 16-20h, anaerobic	S.sonnei WDCM00127 E.coli WDCM00013 or 00012 S.aureus WDCM00034		Qualitative	> 10 colonies on XLD or other medium of choice	characteristic shigellae colonies according to each medium
			SE					inhibition	-

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APPENDIX 2 Batch Quality Control for Growth and Performance Testing of Media for Food Microbiology									
Media	Microorganisms	Standard# (# = current issue)	Function	Incubation	Aust.Std QC strains*	ISO11133 QC strains <sup>A</sup>	Method of Control	Criteria	Characteristic reactions
			see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Appendix to the ISO Standard; OR, suggested strains where no equivalents listed in Australian Standards)	<sup>A</sup> where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7		
Skirrow agar	Campylobacter	AS5013.6	P	41-43°C/ 40-48h, microaerobic	C.jejuni WDCM00005 C.coli WDCM00072		Qualitative	good growth	smooth, flat, translucent, colourless to grey colonies spreading along the streak line
			SE		E.coli WDCM00013 or 00012 S.aureus WDCM00034			inhibition	-
STAA agar	Brocothrix thermosphacta	AS5013.24 (ISO13722)	P	22-25°C/ 44-52h	Brocothrix thermosphacta WDCM00071		Qualitative	growth	-
Sulphite Cycloserine (SC) agar	Clostridium perfringens	AS5013.16 (ISO7937)	P	36-38°C/ 18-22h, anaerobic			Quantitative	$P_R \geq 0.5$	black colonies
			SE					E.coli WDCM00013 or 00012	no growth
Thioglycollate medium	Clostridium perfringens	AS5013.16 (ISO7937)	P	36-38°C/ 18-24h		C.perfringens WDCM00007	Qualitative	good growth (turbidity)	-
Thiosulphate citrate bile salts sucrose (TCBS) agar	Vibrio parahaemolyticus	AS5013.18	P	34-38°C/ 16-20h	V.parahaemolyticus NCTC10884		Qualitative	good growth	blue-green colonies 3-5mm
			SE		E.coli WDCM00013(not listed in this specific Std but suitable for intended task described here)			inhibition	-
Triple Sugar Iron agar +3% NaCl	Vibrio parahaemolyticus	AS5013.18	SP	34-38°C/ 16-20h	V.parahaemolyticus NCTC10884		Qualitative	growth	lac/suc -ve, H2S -ve, no gas
					E.coli WDCM00013(not listed in this specific Std but suitable for intended task described here)				suc +ve, H2S -ve, gas
Tryptone Bile X (TBX) agar	$\beta$ -D-glucuronidase positive Escherichia coli	AS5013-19.1 (ISO16649-1)	P	43-45°C/18-24h	E.coli WDCM00012		Qualitative	growth	blue colonies
			SE		Enterococcus faecalis WDCM00009			no growth	-
			SP		Citrobacter freundii WDCM00006, Pseudomonas aeruginosa WDCM00025			-	white to green-beige colonies
modified Tryptone soya broth (mTSB)	E.coli O157	AS5013.26 (ISO16654)	P	36-38°C/ 18-24h	E.coli WDCM00014		Qualitative	good growth	-
Tryptone soya yeast extract agar (TSYEA)	Listeria monocytogenes	AS5013.24.1 (ISO11290-1 MOD) AS5013.24.2 (ISO11290-2 MOD)	P	36-38°C/ 18-24h		L.monocytogenes WDCM00021 or 00109	Qualitative	good growth	-
Tryptone soya yeast extract broth (TSYEB)	Listeria monocytogenes	AS5013.24.1 (ISO11290-1 MOD) AS5013.24.2 (ISO11290-2 MOD)	P	24-26°C/ 18-24h		L.monocytogenes WDCM00021 or 00109	Qualitative	good growth	turbidity
Tryptone water	E.coli	AS5013.9 AS5013.15 (ISO7251)	SP	44°C/ 46-50h	E.coli WDCM00013 or 00012		Qualitative	Growth	Indole +ve after addition of indole reagent
					Enterobacter aerogenes WDCM00175			Growth	Indole -ve after addition of indole reagent
Tryptone water + 3% NaCl	Vibrio parahaemolyticus	AS5013.18	NS	42°C/ 18-24h	V.parahaemolyticus NCTC10884		Qualitative	growth	-

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Violet Red Bile agar (VRBA)	coliforms	AS5013.4 (ISO4832)	P	29-31°C/22-26h		E.coli WDCM00013 or 00012	Quantitative	P <sub>R</sub> ≥ 0.5	pink to red colonies, with or without precipitation halo
			SE			Enterococcus faecalis WDCM00087 or 00009	Qualitative	inhibition	n/a
			SP			Pseudomonas aeruginosa WDCM00025		n/a	colourless to beige colonies
Violet Red Bile Glucose agar (VRBGA)	Enterobacteriaceae	AS5013.8 (ISO5552)	P	36-38°C/22-26h		E.coli WDCM00013 or 00012	Qualitative	good growth	pink to red colonies, with or without precipitation halo
			SE			Enterococcus faecalis WDCM00087 or 00009		inhibition	n/a
XLD agar	salmonellae	AS5013.10 (ISO6579)	P	36-38°C/ 21-27h	Salmonella Hofit IMVS 1799		Qualitative	good growth	red colonies black centres
			SE		E.coli WDCM00013 or 00012			limited or poor growth	yellow colonies
					Enterococcus faecalis WDCM00087 or 00009			no growth	-

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ASM Guidelines for Assuring Quality of Food Water Microbiological Culture Media 2nd edition

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		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Standard; OR, suggested strains where no equivalents listed in Australian Standards)	<sup>A</sup> where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7		
<b>DILUENTS</b> saline solution, 0.1% peptone, 0.1% peptone salt solution (PSS), phosphate buffer solution, Ringer's solution 1/4 strength		AS4276.1 (ISO8199)	D	20-25°C/ 45min-1hr		E.coli WDCM00013 or 00012 S.aureus WDCM00034	Quantitative	+/- 30% colonies at time 0	n/a
Alkaline peptone water	Vibrio cholerae	AS4276.15	NS	34-38°C/ 18-24h	V.cholerae WDCM00203		Qualitative	Growth	turbidity
Baird-Parker medium (B-P) containing egg yolk	coagulase-positive staphylococci including Staph aureus - membrane filtration method	AS4276.20	P	35-37°C/ 24-48h	S.aureus WDCM00035	S.aureus WDCM00034, WDCM00032	Quantitative	PR ≥ 0.5	Black shiny colonies, opaque zones surrounded by clear zones
			SP		S.epidermidis NCTC6513	S.epidermidis WDCM00036, S.saprophyticus WDCM00159	Qualitative	growth	Black colonies, not shiny, no clearing
			SE	35-37°C/46-50h		E.coli WDCM00013 or 00012	Qualitative	Inhibition	-
BCYE	Legionella	AS3896	P	34-38°C/ 2-5d	(L.pneumophila ATCC <sup>®</sup> 43111 <sup>7</sup> / NCTC11404 OR WDCM00107) AND (L.lingbeachae ATCC <sup>®</sup> 33462 <sup>7</sup> / NCTC11477 OR F.bozemaniae NCTC11368)	L.pneumophila WDCM00107	Qualitative	P <sub>R</sub> ≥ 0.7	colonies 1-2mm diameter, grey-white, circular, smooth, raised with entire edge, ground-glass appearance
BCYE-BMPA	Legionella	AS3896	P	34-38°C/ 2-5d	(L.pneumophila ATCC <sup>®</sup> 43111 <sup>7</sup> / NCTC11404 OR WDCM00107) AND (L.lingbeachae ATCC <sup>®</sup> 33462 <sup>7</sup> / NCTC11477 OR F.bozemaniae NCTC11368)		Qualitative	P <sub>R</sub> ≥ 0.5	colonies 1-2mm diameter, grey-white, circular, smooth, raised with entire edge, ground-glass appearance
			SE	34-38°C/ 72h	P.aeruginosa WDCM00024			inhibition	-
BCYE-GVPC	Legionella	AS3896	P	34-38°C/ 2-5d	(L.pneumophila ATCC <sup>®</sup> 43111 <sup>7</sup> / NCTC11404 OR WDCM00107) AND (L.lingbeachae ATCC <sup>®</sup> 33462 <sup>7</sup> / NCTC11477 OR F.bozemaniae NCTC11368)	L.pneumophila WDCM00107 L.pneumophila WDCM00180	Qualitative	P <sub>R</sub> ≥ 0.5	colonies 1-2mm diameter, grey-white, circular, smooth, raised with entire edge, ground-glass appearance
			SE	34-38°C/ 72h	P.aeruginosa WDCM00024	P.aeruginosa WDCM00025 or WDCM00026		inhibition	-
						E.faecalis WDCM00009 or WDCM00087 E.coli WDCM00012 or WDCM00013		no growth inhibition	- -
BCYE-MWY	Legionella	AS3896	P	34-38°C/ 2-5d	(L.pneumophila ATCC <sup>®</sup> 43111 <sup>7</sup> / NCTC11404 OR WDCM00107) AND (L.lingbeachae ATCC <sup>®</sup> 33462 <sup>7</sup> / NCTC11477 OR F.bozemaniae NCTC11368)		Qualitative	P <sub>R</sub> ≥ 0.5	colonies 1-2mm diameter, grey-white, circular, smooth, raised with entire edge, ground-glass appearance
			SE	34-38°C/ 72h	P.aeruginosa WDCM00024			inhibition	-
Bile aesculin azide agar	Enterococci - membrane filtration method	AS4276.9	SP	34-38°C/ 18-24h	E.faecalis WDCM00009		Qualitative	growth	small colonies producing blackening of the medium
					S.aureus WDCM00032 or WDCM00034				small colonies without blackening of the medium

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APPENDIX 3 Batch Quality Control for Growth and Performance Testing of Media for Water Microbiology									
Media	Microorganisms	Standard <sup>#</sup>	Function	Incubation	Aust.Std QC strains*	ISO11133 QC strains <sup>^</sup>	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Standard; OR, suggested strains where no equivalents listed in Australian Standards)	<sup>^</sup> where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7		
Blood agar + neomycin	Clostridium perfringens	AS4276.17.1 AS4276.17.2	P	34-38°C/ 21-27h	C.perfringensWDCM00007		Qualitative	Growth	large spreading colonies
			SE		E.coli WDCM00090			inhibition	-
Bolton Broth	thermophilic campylobacters	AS 4276.19	SE	41-42°C/ 40-48h, microaerobic (5-6%O <sub>2</sub> , 10% CO <sub>2</sub> )	C.jejuni WDCM00005 or 00156		Qualitative	growth	>10 colonies growth on subculture on mCCDA
					C.coli WDCM00004 or 00072			inhibition	inhibited/ no growth on subculture on mCCDA
			SP		E.coli WDCM00090				
Brain Heart Infusion Broth (BHIB)	coagulase-positive staphylococci including Staph aureus - membrane filtration method	AS4276.20	NS	35-37°C/ 18-24h	S.aureus WDCM00035		Qualitative	Growth	tube coagulase positive
Campylobacter agar mCCDA	thermophilic campylobacters	AS 4276.19	SE	41-42°C/ 40-48h, microaerobic (5-6%O <sub>2</sub> , 10% CO <sub>2</sub> )	C.jejuni WDCM00005 or 00156		Qualitative	growth	smooth, flat, translucent, colourless to grey colonies spreading along the streak line
					C.coli WDCM00004 or 00072			inhibition	-
			SP		E.coli WDCM00090				
Buffered peptone water	salmonellae	AS4276.14 (ISO19250)	NS	34-38°C/ 16-20h	Salmonella Hofit IMVS1799		Qualitative	Good growth	turbidity
Differential reinforced clostridial medium (DRCM)	Clostridium perfringens MPN	AS4276.17.2	P	34-38°C/ 44-52h AnO <sub>2</sub>	C.perfringensWDCM00007		Qualitative	growth	blackening of the medium
EC Broth + MUG	coliforms, E.coli & thermotolerant coliforms MPN	AS4276.6	P	43.5-44.5°C/ 40-48h	E.coli WDCM00090	E.coli WDCM00179	Qualitative	growth	turbidity and gas production and fluorescence
					E.aerogenes WDCM00175			growth	turbidity, no gas production or fluorescence
	E.coli & thermotolerant coliforms -membrane filtration method	AS4276.7	SP		P.aeruginosa WDCM00024	no growth		-	
EHS medium with ONPG and MUG	coliforms and E.coli MPN using enzyme hydrolysable substrates	AS4276.21	SP	34-38°C/ 24-28h	E.coli WDCM00090	E.coli WDCM00090 , WDCM00013 Klebsiella pneumoniae WDCM00206 P.aeruginosa WDCM00025	Qualitative	growth	ONPG +ve, MUG +ve
					E.aerogenes WDCM00175			ONPG +ve, MUG -ve	
					A.hydrophila WDCM00063			ONPG -ve, MUG -ve	
Exeter Broth (Modified)	thermophilic campylobacters	AS 4276.19	SE	41-42°C/ 40-48h, microaerobic (5-6%O <sub>2</sub> , 10% CO <sub>2</sub> )	C.jejuni WDCM00005 or 00156		Qualitative	growth	>10 colonies growth on subculture on mCCDA
					C.coli WDCM00004 or 00072			inhibition	inhibited/ no growth on subculture on mCCDA
			SP		E.coli WDCM00090				

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		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Standard; OR, suggested strains where no equivalents listed in Australian Standards)	<sup>^</sup> where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7		
Improved Formate Lactose Glutamate medium (IFLG) (also known as Minerals Modified Glutamate Medium)	coliforms, Ecoli & thermotolerant coliforms MPN	AS4276.6	P	34-38°C/ 18-24h	E.coli WDCM00090		Qualitative	growth	acid and gas production
			SP		E.aerogenes WDCM00175				acid production
					P.aeruginosa WDCM00024				no acid production
Lactose-gelatin medium	Clostridium perfringens - membrane filtration method	AS4276.17.1	SP	34-38°C/ 21-27h	C.perfringensWDCM00007		Qualitative	Growth	gelatin liquefaction, acid & gas production
					E.coli WDCM00090				NO gelatin liquefaction, acid production
Lactose TTC agar	coliforms, Ecoli & packaged water - membrane filtration method	AS4276.22 (ISO9308-1)	P	34-38°C/ 18-24h	E.coli WDCM00090	E.coli WDCM00179, WDCM00012, WDCM00013 E.aerogenes WDCM00175 P.aeruginosa WDCM00025 or WDCM00026 E.faecalis WDCM00009 or WDCM00087	Qualitative	growth	lactose +ve colonies
			SP		E.aerogenes WDCM00175				lactose +ve colonies
					P.aeruginosa WDCM00024				lactose -ve colonies
			SE					inhibition	-
Lysine decarboxylase broth	salmonellae	AS4276.14 (ISO19250)	SP	34-38°C/ 16-20h	Salmonella Hofit IMVS1799		Qualitative	Growth	turbidity, purple to pale purple
					Citrobacter freundii WDCM00006				turbidity, yellow
MacConkey agar	coliforms, Ecoli & thermotolerant coliforms MPN	AS4276.6	P	34-38°C/ 18-24h	E.coli WDCM00090		Qualitative	growth	red/pink colonies
			SP		E.aerogenes WDCM00175				red/pink colonies
					P.aeruginosa WDCM00024				pale/colourless colonies
Membrane faecal coliform agar (mFC agar)	Ecoli & thermotolerant coliforms -membrane filtration method	AS4276.7	SP	34-38°C/ 18-24h	P.aeruginosa WDCM00024		Qualitative	inhibited	-
			P		E.coli WDCM00090				Quantitative
Membrane faecal coliform broth (mFC broth)	Ecoli & thermotolerant coliforms -membrane filtration method	AS4276.7	SP	34-38°C/ 18-24h	P.aeruginosa WDCM00024		Qualitative	inhibited	-
			P		E.coli WDCM00090				Quantitative
Membrane lauryl sulfate agar (mLSA)	Coliforms- membrane filtration method	AS4276.5	P	34-38°C/ 18-24h	E.aerogenes WDCM00175		Quantitative	P <sub>R</sub> ≥ 0.5	yellow colonies
			SP		P.aeruginosa WDCM00024				Qualitative
	Ecoli & thermotolerant coliforms -membrane filtration method	AS4276.7	P		E.coli WDCM00090		Quantitative	P <sub>R</sub> ≥ 0.5	yellow colonies
Membrane lauryl sulfate broth (mLSB)	Coliforms- membrane filtration method	AS4276.5	P	34-38°C/ 18-24h	E.aerogenes WDCM00175		Qualitative	growth	turbidity and acid production (yellow)
			SP		P.aeruginosa WDCM00024				-
	Ecoli & thermotolerant coliforms -membrane filtration method	AS4276.7	P		E.coli WDCM00090				growth
M-Endo agar	Coliforms- membrane filtration method	AS4276.5	P	34-38°C/ 18-24h	E.aerogenes WDCM00175		Quantitative	P <sub>R</sub> ≥ 0.5	red colonies, possibly metallic sheen
			SP		P.aeruginosa WDCM00024				Qualitative

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		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Standard; OR, suggested strains where no equivalents listed in Australian Standards)	<sup>A</sup> where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7		
M-Endo broth	Coliforms- membrane filtration method	AS4276.5	P	34-38°C/ 18-24h	E.aerogenes WDCM00175			growth on filter	red colonies, possibly metallic sheen
			SP		P.aeruginosa WDCM00024			-	no red colonies
M-enterococcus agar - see Slanetz & Bartley medium	Enterococci - membrane filtration method	AS4276.9							
Milk agar with cetrimide	Pseudomonas aeruginosa - membrane filtration method	AS4276.13	SP	34-38°C/ 18-24h	P.aeruginosa WDCM00024			growth	colonies surrounded by zones of casein hydrolysis, pyocyanin (pigment) production
					P.fluorescens WDCM00115				no pigment, no clearing zones
m-PA-C agar	Pseudomonas aeruginosa - membrane filtration method	AS4276.13	P	41-42°C/ 40-48h	P.aeruginosa WDCM00024			P <sub>R</sub> ≥ 0.5	colonies typically 0.8 to 2.2mm diameter, flat, light outer rims and brownish to green-black centres.
			SP		E.coli WDCM00090			inhibited	-
Muller-Kauffmann tetrathionate novobiocin broth (MKTn)	salmonellae	AS4276.14 (ISO19250)	P	36-38°C/ 21-27h	Salmonella Hofit IMVS1799	ADD E.coli (WDCM00012 or WDCM00013) AND P.aeruginosa WDCM00025		Growth	recovery ≥10colonies on XLD / 2 <sup>nd</sup> medium of choice
			SE		E.coli WDCM00012 or WDCM00013			partial inhibition	≤ 100 colonies on TSA
					E.faecalis WDCM00009 or WDCM00087			inhibition	≤ 10 colonies on TSA
Nitrate motility medium	Clostridium perfringens - membrane filtration method	AS4276.17.1	SP	34-38°C/ 21-27h	C.perfringens WDCM00007			-	motility negative, nitrate positive
					C.sporogenes WDCM00008			-	motility positive, nitrate negative
Nutrient agar	Coliforms- membrane filtration method	AS4276.5	P	34-38°C/ 18-24h	E.aerogenes WDCM00175			P <sub>R</sub> ≥ 0.7	Characteristic colony according to each species
	coliforms, E.coli & thermotolerant coliforms MPN	AS4276.6			E.coli WDCM00090				
Oleandomycin polymyxin sulphadiazine perfringens (OPSP) agar	Clostridium perfringens	AS4276.17.1	P	34-38°C/ 44-52h AnO <sub>2</sub>	C.perfringens WDCM00007			P <sub>R</sub> ≥ 0.5	2-4mm black colonies
		AS4276.17.2	SE	34-38°C/ 44-52h AnO <sub>2</sub>	C.sporogenes WDCM00008			inhibition	-
R2A Agar	heterotrophic plate count	AS4276.3.2	P	34-38°C/ 40-48h		E.coli WDCM00012 or WDCM00013 B.subtilis WDCM00003		P <sub>R</sub> ≥ 0.7	
Rappaport-Vassiliadis soya (RVS) medium	salmonellae	AS4276.14 (ISO19250)	P	40.5-42.5°C/ 21-27h	Salmonella Hofit IMVS1799	ADD E.coli (WDCM00012 or WDCM00013) AND P.aeruginosa WDCM00025		Growth	recovery ≥10colonies on XLD / 2 <sup>nd</sup> medium of choice
			SE		E.coli WDCM00012 or WDCM00013			partial inhibition	≤ 100 colonies on TSA
					E.faecalis WDCM00009 or WDCM00087			inhibition	≤ 10 colonies on TSA

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Media	Microorganisms	Standard <sup>#</sup>	Function	Incubation	Aust.Std QC strains*	ISO11133 QC strains <sup>A</sup>	Method of Control	Criteria	Characteristic reactions	
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133	(*as listed in the Australian Standard; OR, suggested strains where no equivalents listed in Australian Standards)	<sup>A</sup> where listed for these media; not all strains required as a minimum	see Guidelines Section 2.7			
Salt tolerance medium										
0% NaCl tryptone water	Vibrio cholerae	AS4276.15	SP	34-38°C/ 18-24h	V.cholerae WDCM00203		Qualitative	Growth	-	
3% NaCl tryptone water				34-38°C/ 18-24h	V.cholerae WDCM00203		Qualitative	Growth	-	
8% NaCl tryptone water				34-38°C/ 18-24h	V.cholerae WDCM00203		Qualitative	no growth	-	
Selenite cystine broth	salmonellae	AS4276.14 (ISO19250)	SE	34-38°C/ 16-20h	Salmonella Hofit IMVS1799		Qualitative	Growth	recovery on XLD / 2 <sup>nd</sup> medium of choice	
					Citrobacter freundii WDCM00006		Qualitative		minimal or no growth on XLD/ 2 <sup>nd</sup> medium of choice	
Slanetz & Bartley medium (m-enterococcus agar)	Enterococci - membrane filtration method	AS4276.9 (ISO7899-2)	P	34-38°C/ 40-48h	E.faecalis WDCM00009	E.faecalis WDCM00009 , (WDCM00087, WDCM00176)	Quantitative	P <sub>R</sub> ≥ 0.5	pink to maroon colonies	
						E.faecium WDCM00177 or WDCM00178	Quantitative	P <sub>R</sub> ≥ 0.5	pink to maroon colonies	
					SE	34-38°C/ 40-48h	S.aureus WDCM00032 or WDCM00034	Qualitative	inhibition	-
					E.coli WDCM00012 or WDCM00013					
Thiosulphate citrate bile salts sucrose (TCBS) agar	Vibrio cholerae	AS4276.15	SE	34-38°C/ 18-24h	V.cholerae WDCM00203		Qualitative	growth	Smooth flat yellowish-brown colonies, surrounded by yellow zones in medium	
			SP			E.coli WDCM00012 or WDCM00013 or WDCM00090		inhibited	-	
Tryptone bile agar (TBA)	coliforms, E.coli & packaged water - membrane filtration method	AS4276.22	P	34-38°C/ 18-24h	E.coli WDCM00090		Qualitative	growth	(indole +ve)	
					E.aerogenes WDCM00175				(indole -ve)	
			SP		P.aeruginosa WDCM00024				(indole -ve)	
Tryptone soya agar (TSA)		various	P	34-38°C/ 18-24h	E.aerogenes WDCM00175	E.faecalis WDCM00087	Quantitative	P <sub>R</sub> ≥ 0.7	Characteristic colony according to each species	
						P.aeruginosa WDCM00024				P.aeruginosa WDCM00024
						E.coli WDCM00090				E.coli WDCM00090
Tryptose Sulphite Cycloserine (TSC) agar without egg yolk	Clostridium perfringens	AS4276.17.1 AS4276.17.2	P	34-38°C/ 44-52h AnO <sub>2</sub>	C.perfringens WDCM00007	C.perfringens WDCM00007 (WDCM00080, WDCM00174)	Quantitative	P <sub>R</sub> ≥ 0.5	2-4mm black colonies	
			SE	34-38°C/ 44-52h AnO <sub>2</sub>	C.sporogenes WDCM00008	B.subtilis WDCM00003	Qualitative	inhibition	-	
urea agar	salmonellae	AS4276.14 (ISO19250)	SP	34-38°C/ 16-20h	Salmonella Hofit IMVS1799		Qualitative	growth	no production of urease	
						Citrobacter freundii WDCM00006				production of urease
XLD agar	salmonellae	AS4276.14 (ISO19250)	P	34-38°C/ 21-27h	Salmonella Hofit IMVS1799		Qualitative	growth	reddish transparent colonies black centres	
			SE			E.coli WDCM00012 or WDCM00013			growth or partial inhibition	yellow colonies
						E.faecalis WDCM00009 or WDCM00087			inhibition	-
Yeast Extract agar (YEA)	heterotrophic plate count	AS4276.3.1 (ISO6222)	P	34-38°C/ 40-48h		E.coli WDCM00012 or WDCM00013 B.subtilis WDCM00003	Quantitative	P <sub>R</sub> ≥ 0.7		

All incubations are aerobic unless otherwise indicated

Italics not used for ease of reading

Function  
P=productivity  
SP=specificity

D=dilution  
SE=selectivity  
NS=nonselective



## Appendix 4

### Recommended control strains & numbering: World Data Centre for Microorganisms (WDCM).

The World Data Centre for Microorganisms was produced to enable broader and easier access to the reference strains listed by the ISO TC 34 SC 9 Joint Working Group 5 and by the Working Party on Culture Media of the International Committee on Food Microbiology and Hygiene (ICFMH-WPCM) in their publication *Handbook of Culture Media for Food and Water Microbiology*. It fulfils a need expressed by these bodies for a unique system of identifiers for strains recommended for use in quality assurance.

The World Federation of Culture Collections (WFCC) and the WDCM have initiated a system that will help users find local sources of the reference strains by citing all collections and providing contact details and the collection's unique reference. Future publications of ISO and ICFMH-WPCM will cite the WDCM reference number for each strain and the WDCM catalogue provides the collection acronyms and strain numbers of the relevant strains so that they may be found.

Important links:

WDCM website <http://refs.wdcm.org/home.htm>

WDCM updates <http://refs.wdcm.org/history.htm>

WDCM pdf latest release see <http://refs.wdcm.org/home.htm> and latest version.



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