Guidelines for Assuring Quality of Food and Water Microbiological Culture Media

Culture Media Special Interest Group
Australian Society for Microbiology

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FOREWORD

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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1.0 INTRODUCTION

1.1 Application

These Guidelines are applicable to food and water microbiology laboratories that manufacture or use microbiological culture media. They seek to offer direction to individuals who must implement procedures with the purpose of assuring the quality of food and water microbiological culture media, and ultimately the quality of the microbiological services of the laboratory. They should be used in conjunction with NATA Technical Note No. 4, *Guidelines for the Quality Management of Microbiological Media*, NATA Technical Note No. 5, Monitoring of Laboratory Steam Sterilizers, NATA's ISO/ IEC17025 Specific Requirements for Biological Testing and ISO/TS11133-2 *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* to implement a comprehensive quality assurance program for the manufacture and quality control of food and water microbiological culture media. These new guidelines will also be highly beneficial to pharmaceutical laboratories.

1.2 Scope

These guidelines pertain primarily to ready to use food and water microbiological culture media for isolation and identification of foodborne/waterborne microorganisms. Most of the media and microorganisms referred to in this document are those described by AS1766, AS4276 and AS3896 methods, which are the predominant methods used in food and water laboratories in Australia. Also included are the microorganisms recommended by ISO11133-2.

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 micro-organisms. (*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 prepared microbiological culture media are those facilities where ingredients are weighed, mixed, sterilized, dispensed and final products are labelled and packaged. This includes facilities that prepare media for sale outside their organization or for distribution within their organization, or for their own use.

User: Consumers of microbiological culture media.

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. For the purposes of this document, quality assurance encompasses those processes before, during and after the manufacture of microbiological culture media that verify the adequacy of the media for its intended purpose.

Quality Control: Operational techniques and activities that are used to fulfill the requirements for quality. For the purposes of this document, 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 of/Validated Methods: This is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. This means the collection of data that demonstrates the reproducibility of a specific property of a medium or process and must verify that, under usual testing 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 identical (to master) cultures obtained in the laboratory by a single sub-culture from the reference strain.

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

2.0 PROCESS QUALITY ASSURANCE

Process quality assurance is seen as an integral part of 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. These practices should include verification of raw material specifications, tests to verify a satisfactory level of freedom from contamination, demonstrate the correct performance of the medium when used in the usual or widely accepted manner, and ensure against significant physical imperfections that may compromise the utility of the media.

Performance of media listed in the accompanying tables should comply with expected results shown when tested according to methods suggested in these Guidelines, including microbial strains, specific incubation temperatures, times and atmospheres. The incubation conditions shown reflect those used for the enumeration, isolation and identification of specific indicator organisms or pathogens as described in AS1766, AS4276 and AS3896.

Some food and water laboratories have validated the use of media other than those listed in the accompanying tables. The lack of inclusion of these media is not meant to infer that they are unsuitable for use. However, the selection of microorganisms and incubation conditions should follow any technical recommendation provided by the manufacturer of the dehydrated culture media or papers published describing the development and applications of a particular medium.

2.1 Raw Materials

2.1.1 Storage

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

2.1.2 Management

Dehydrated media are usually purchased from commercial manufacturers in a powdered or granulated form in sealed containers. Supplements of different selective or diagnostic substances are supplied in either the lyophilized or liquid state. Purchases should be planned to encourage a regular turnover of stock (i.e. first in-first out). Further checks should include, rechecking of the seal, expiry date, date of first opening, visual assessment of contents of opened containers. Especially after opening a container, the quality of the dehydrated medium depends on the storage environment. (Preferably a cool, low light 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 or shows obvious changes in physical appearance should be discarded.

2.1.3 Lot Acceptance of Dehydrated Culture Media

(a) Enumeration Media: On receipt of a lot of dehydrated culture medium, the laboratory is expected to perform a quantitative recovery of the test micro-organisms and if appropriate, selective recovery of the negative control organisms.

(b) Selective Media (not used for enumeration): On receipt of a lot of dehydrated culture medium, the laboratory is expected to perform a semi-quantitative recovery of the test micro-organisms and (if appropriate) selective recovery of the negative control organisms.

(c) Non-Selective (not used for enumeration): On receipt of a lot of dehydrated culture medium, the laboratory is expected to perform semi-quantitative or qualitative recovery of the test micro-organisms.

This comprehensive testing regime for lot acceptance allows the laboratory to undertake a minimum approach to batch testing.

2.2 Finished Product

2.2.1 Physical Imperfections

Inspection for significant physical imperfections should include uneven distribution of media in Petri dishes (affecting colony size), variable amounts of medium in dishes/tubes/bottles (affecting haemolytic zone definition, shelf-life, consistency of colony size and recovery rate, etc.) colour, and gross deformation of the surface of media.

2.2.2 Chemical Imperfections

Final pH of the medium should be measured at ambient temperature using an appropriate pH meter, and should be in accordance with manufacturers' instructions. For solid media, appropriate gel strength needs to be checked and confirmed**.** NB: autoclaving may induce a pH shift, and needs to be taken into account when pH is adjusted before the sterilization process.

2.2.3 Sterility Testing and Sampling Plan

Testing for contamination shall include sampling, incubation and inspection of individual units from each batch produced. Incubation conditions should encompass storage (including transport) and in use testing parameters.

The sampling procedure applied conforms in part to ISO/TS11133-2 *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* (for batch sizes <100units) and also to Australian Standard 1199-1988, *Sampling Procedures and Tables for Inspection by Attributes* (for batch sizes > 100units). The sampling procedures recommended are summarised in Table 2 including notes on interpretation. Incubation of all media samples must be for a minimum period of 48-72 hours covering a range of temperatures (4 - 45ºC), summarized in Table 1 below. Use of inspected sterility samples to determine significant physical imperfections is acceptable.

Table 1: Suggested Conditions for Sterility Testing

Testing/Storage Incubation Temperatures	Minimum Sterility Incubation period
$4 – 8^0$C	10 –14 days
$20 - 25^0$C	2 – 5 days
$35 - 37^0$C	48 – 72 hours

There could be other conditions besides those stated above that may be appropriate.

TABLE 2: SAMPLING PLAN FOR MICROBIOLOGICAL CULTURE MEDIA

ACCORDING TO ISO/TS11133-2

Small Batches (<100 units): 1% or 1 unit from beginning and 1% or 1 unit from end of batch.

ACCORDING TO AS1199.1-2003

Double Sampling Plan (> 100 units): NORMAL SAMPLING PLAN, AQL=2.5, GENERAL INSPECTION LEVEL=1

Batch Size (units made)	Sample Number		1st Sample		2nd Sample	
	1st sample	2nd 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

Interpretation:

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 Ac and Re 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 (Ac), 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 (Re), the batch must be rejected.

Small Batches (<100 units): Based on ISO/TS11133-2 *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,* 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 the 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 must be rejected.

2.2.4 Volume Checks on Media

Some diluents or resuscitation broths are dispensed prior to sterilisation. To ensure that the final volumes are within specification it is necessary to dispense specific volumes that allow for volume loss during sterilisation. For example: 9.0ml 0.1% peptone diluents may have a specification of 9.0 ± 0.2 ml and from experience, volume losses of 0.1 – 0.3 ml are experienced with the autoclave. The laboratory sets the dispenser to discharge 9.2 ml so that if 0.3 ml is lost or 0.1 ml is lost the final volume should fall within the specification.

To verify that each batch of diluent prepared has the expected final volume, the sampling plan (Table 2) should be consulted to determine the number of units to be tested. Each unit should then be labelled and weighed empty, weighed again after the diluent has been dispensed, and weighed again after sterilisation and cooling down. With these weights it is possible to calculate the volume dispensed, the final volume and the volume lost in each unit. Then the sample plan needs to be re-consulted to determine whether the batch is acceptable or not.

In the case of 9ml diluents it is not possible to accurately verify the dispensed, sterile volumes by tipping the diluents out or by sucking up the volume in a pipette tip and discharging onto a tared balance. With larger volumes of 90ml, 100ml, 225ml etc better accuracy may be achieved with these procedures, but it is recommended that the weighing procedure offers the best accuracy and is not that difficult to perform.

3.0 CULTURES

3.1 Control Strains of Micro-organisms

The control strains listed in these Guidelines (see Appendix 1) are recommended. Alternative control strains should be equivalent in their performance to those recommended. In addition, it may be important to distinguish a control strain from a food/water isolate so that laboratory accidents (such as cross-contamination) can be readily recognised. *Salmonella Salford* is the preferred control strain for *Salmonella* testing. *S. Salford* is a rare strain in Australia and the detection of the *Salmonella* in a test sample will prompt laboratories to investigate their test procedures. Strains should be sourced and traceable to an internationally recognised culture collection, such as the American Type Culture Collection (Rockville, Maryland, USA), the National Collection of Type Cultures (London, UK), the Australian Collection of Microorganisms (Brisbane, Australia) or freeze dried cultures of organisms supplied by NATA approved or Australian Standards approved commercial suppliers. In some situations, freshly isolated “wild” cultures may be used, provided that these organisms have been fully identified and stocks of these cultures have been preserved for ongoing use. Cultures for which no subculture or handling history is available should not be used.

The cultures listed in the Appendix 1 reflect the minimal number of cultures that should be used to QC media. However 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 should be used. It is in such situations where the food and water testing laboratory may need to add wild cultures to its collection. In the case where the food and water testing laboratory purchases prepared media see the discussion in section 4.4.

3.2 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. Cultures when received from a culture collection or other recognised (authorised) source should be preserved. 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 US Pharmacopoeia specifies a maximum of five passages from the culture received from a culture collection to the working culture. This limit may not always be possible for environmental or product isolates (wild strains). The most effective system for managing the culture collection is the hierarchical or tiered system that includes Master, Stock and Working cultures (NATA Technical Note No.14).

When a culture is first received by a laboratory it should be activated and tested for purity. 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 food and water laboratories, the diversity of bacteria, yeasts and moulds may necessitate the use of more than one system of preservation and the laboratory is advised to seek guidance from recognised publications or from culture collections.

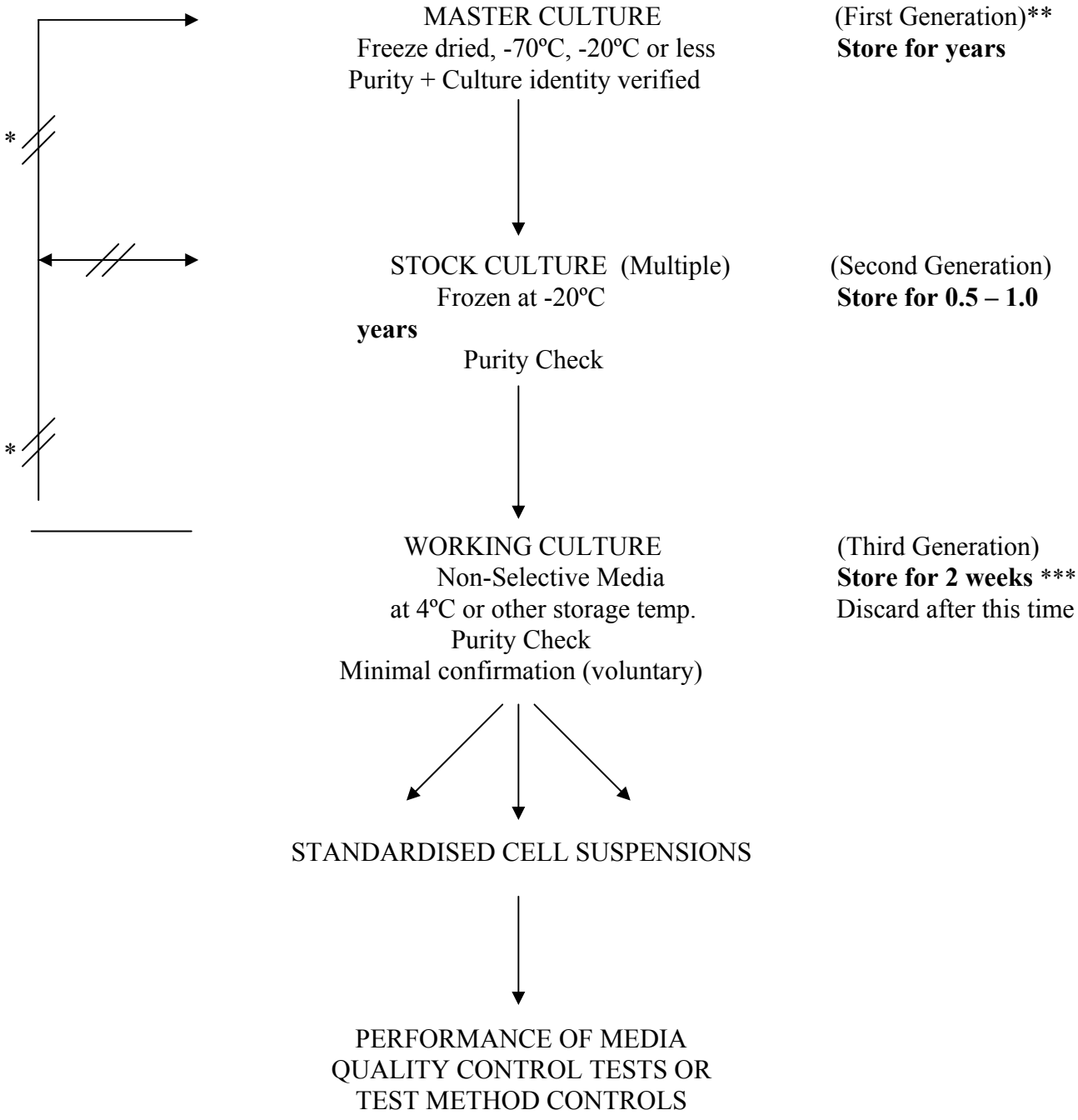
In addition to the purity check, and at the same time of preservation, the identity of the culture should be verified including the particular characteristics utilised for media growth performance checks. The preserved culture generated by this process is termed MASTER culture and should not be accessed 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 as often as once a week to prepare WORKING cultures which are used on a daily basis 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 generation removed from the received culture, the stock culture is therefore two generations removed and hence 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 however will be undermined if the original cultures are not acquired from a recognised culture collection where the authentic strains have been appropriately managed to minimise genetic change.

Figure 1: Maintaining a Culture Collection*



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* 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

Depending on the range of testing undertaken, the food/water laboratory will require a diverse range of microorganisms. The optimum storage conditions for each type of organism will differ and a variety of suspending media eg. Glycerol broth, skim milk will be required. It is essential to determine the optimum cell concentration in the suspension and the storage, temperature and gaseous conditions, to achieve maximum shelf life for each organism. This will obviously vary depending on the type of organism. It is also essential to establish the same parameters for the working cultures. Factors to be considered are the frequency of use and viability of the organism. The procedures for preservation, maintenance and subculture for all cultures in the collection must be well documented.

Ideally, MASTER cultures should be stored at -70ºC or freeze dried. However if these resources are not available, the MASTER should be stored in a dedicated freezer (operating at as close to -20ºC **or less** as possible) which is infrequently opened. 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.

Further details of what can be expected from a NATA accredited laboratory in terms of documentation of procedures and outcomes can be found in NATA's "*Supplementary Requirements for Accreditation in the Field of Biological Testing"*,(Section 5.6.3 'Reference standards and reference materials')

4.0 TEST PROCEDURE METHODOLOGY

The number of organisms used to assess the performance of a culture medium depends on whether the medium is selective or nutritive.

4.1 Preparation of Standardised Inocula

(i) Suspend 3-5 isolated colonies in a small volume of suitable culture medium and incubate to achieve exponential growth phase. Adjust the turbidity to give a basic suspension of approximately 10^7- 10^8 CFU/ml (equivalent to approximately a McFarland 0.5 turbidity standard). Alternatively with the more robust microorganisms prepare a $^1/_{10}$ dilution of an overnight broth culture which will give a similar viable cell number.

(ii) For testing the nutritive capacity of a medium, a dilute cell suspension should be prepared. The extent of dilution will be determined by whether a standard loop is used to deliver 10^3 - 10^4 CFU per streak plate, or an aliquot using a pipette is delivered to prepare a pour or spread plate to develop between 25-250 CFU/plate.

(iii) For testing the inhibitory capacity of a selective medium each plate should be inoculated with at least ten times more CFU than for a nutritive test (part ii). Hence this suspension needs to deliver 10^4 -10^5 CFU per streak plate, or 250 -2500 CFU per pour/ spread plate. For some selective media, the inhibitory capacity may be challenged with the equivalent aliquot from the original basic cell suspension of approximately 10^7 - 10^8 CFU/ml.

(iv) 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 10^2 CFU per unit of test medium. If the liquid medium is being challenged for its inhibitory capacity, heavier inocula of the order of $10^5 – 10^6$ CFU will normally be used. Broths should be subsequently subcultured to check correct inoculum.

4.2 Standard Method for Streak Plates

A standardised methodology must be used to distribute CFUs over the plate. Different streak plate techniques may be used, a 5 zone streak plate (Figure 2A) or a Mossel ecometric method (1980) (Figure 2B)

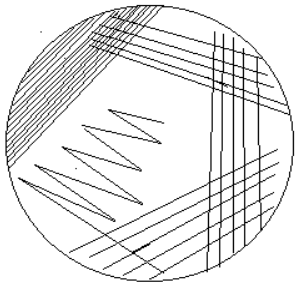

Fig.2A

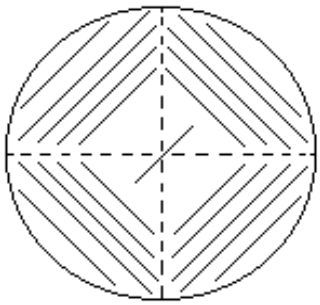

Fig.2B

Microbiologists all over the world have their "favourite" streak plate methods; however, each laboratory needs to standardise its method specifying the inoculum size, the number of zones/ lines and whether the loop is to be flamed between any of the sections. Then all of the operators must be trained to follow this procedure.

4.3 Incubation Conditions

Incubate the inoculated test media under conditions that are normally used for each of the food and waterborne pathogens or indicator microorganisms. This may include microaerobic, anaerobic or aerobic conditions at various temperatures such as 5ºC, 25ºC, 30ºC, 37ºC, 44ºC, 55ºC for the normal incubation period.

4.4 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. The use of a specific medium as reference medium is therefore mandatory for quantitative methods. For semi-quantitative or qualitative methods, the use of a specific reference medium helps to interpret results. The reference medium may be chosen from a previous lot/batch of the same media, a non-selective control medium, another suppliers' batch, etc.

4.4.1. Productivity

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

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

$$P_R = N_S / N_O$$

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 ≥ 100cfu.

For semi-quantitative methods, the scores of different sectors of a plate streaked with either method described in 4.2 are summed to obtain the Growth Index G_I. G_I vary by culture medium. It is therefore important to compare them with previous indices and/or G_I of a reference medium and to ensure that variations are not excessive. The range of culture medium-specific variations can also be determined once sufficient experience of the method has been gained.

For qualitative evaluations, visual checks are carried out and growth scores allocated.

4.4.2. Selectivity

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

The Selectivity Factor S_F is calculated quantitatively as follows:

$$S_F = D_O - D_S \qquad (S_F \text{ is expressed in log 10 units})$$

where

D_O is the difference between the highest dilution showing growth of at least 10 colonies of the reference medium.

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

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

4.4.3. Specificity (Physiological Characteristics)

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

4.5 Growth Recovery of Control Microorganisms

For lot/batch control of culture medium and nutritive ingredients for culture media, as appropriate, growth should be assessed:

- quantitatively
- semi-quantitatively
- qualitatively

4.5.1 Quantitative Recovery (Typically used for Raw Materials Testing)

(i) Non-Selective Solid Medium

Verification of each new lot/batch of non-selective medium (e.g. Plate Count Agar) used to enumerate microorganisms is made by comparison to previous batches of the same media or a current batch of a non-selective medium (reference media such as TSA). Perform viable counts on both the test and reference medium and compare the results as described in 4.5.1. The counts for both media should be compared and the P_R calculated.(Counts should be similar). Each laboratory needs to set its own acceptance/rejection criteria (which should be at least 70% recovery) and with reference to Appendix 1. Furthermore, the medium also needs to be assessed for typical morphology and colony size to complete the performance evaluation on the medium.

(ii) Selective Solid Medium

Verification of each new lot/batch of selective medium (e.g. Violet Red Agar, Bacillus Cereus Selective Agar, Baird Parker etc) used to enumerate specific indicator organisms or pathogens is made by comparison to a current batch of a non-selective medium (reference media such as TSA) Perform viable counts using both positive and negative control microorganisms on both the test and reference medium and compare results as described in 4.5.1. The counts on both media should be compared and the P_R calculated. It is expected that at least 70% of the positive test organisms will be recovered on the selective medium compared to the non-selective medium.
It is also relevant to demonstrate the capacity of the test medium to suppress the negative control organism. The dilutions which support the growth of a least 10 colonies on both the selective test and non-selective reference medium is recorded. It is expected that there will be a difference of greater than two ten fold dilutions between the dilution showing growth on the non-selective medium compared to the selective.

For example when *E. coli* is being tested as a negative control organism on Baird Parker medium

Medium	**-1**	**-2**	**-3**	**-4**	**-5**	**-6**	**-7**	**-8**
TSA						TNTC	55	5
BP	120	13	1	-	-	-	-	-

The key dilutions are 10^{-7} on the non-selective and 10^{-2} on the selective test medium and there is a difference of five ten fold dilutions.

Each laboratory needs to establish its own acceptance/rejection criteria with reference to Appendix 1. Furthermore the medium also needs to be assessed for typical colony morphology, colony size and biochemical responses to complete the performance evaluation on the medium.

Note: Comparison with a previous lot/batch of the same 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.

(iii) Non-Selective Liquid Medium

Between 10-100 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. Acceptance/rejection criteria need to be developed by the laboratory when the method has been standardised.

(iv) Selective Liquid Medium

This procedure will apply to broths used for Most Probable Numbers determinations, selective enrichment broths etc. Inoculation of the positive control at 10-100cfu and is required into both the test medium and a non-selective reference broth (TSB or BHI). At the same time the negative control at >1000cfu needs to be inoculated into second set of the same two media. Finally a third set of media needs to be inoculated with a mixture of both positive and negative controls in exactly the same ratio as the individual controls. All broths are incubated for the times and temperatures used in the method. Then, remove an aliquot from each broth and spread/streak or enumerate on a non-inhibitory medium (this may need to contain an indicator to differentiate the positive and negative organisms when present in the mixture). After incubation, count the positive and negative organisms from both selective and non-selective broths and determine the percentage recovery for the positive control and the degree of inhibition for the negative control organism. For the mixed culture, the percentage recovery of the positive organism must not be compromised. The laboratory needs to develop its own acceptance/rejection criteria based on the exact methodology followed.

Example: Selective Liquid Medium Testing

Test Medium			Non Selective Reference Medium		
+ve control org. 10-100CFU	-ve control org. 1000CFU	Mix(+ve &-ve)	+ve control org. 10-100CFU	-ve control org. 1000CFU	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; Determine % Inhibition of –ve organism; % Recovery from mix must equal from +ve alone.					

4.5.2. Semi-Quantitative Recovery (Typically used for batch testing)

(i) Non-Selective Solid Medium

Verification of each new lot/batch of non-selective medium is made by comparison to previous batches of the same media or a current batch of a non-selective medium (reference media such as TSA). Prepare standard inocula and use either of the streak plate methods described previously on both the test and reference medium and compare the results as described in 4.5.1. The growth (e.g. number of streak lines or quadrants grown) for both media should be compared and the growth index G_I calculated or determined.(Growth should be similar). Each laboratory needs to set its own acceptance/rejection criteria and with reference to Appendix 1. Furthermore, the medium also needs to be assessed for typical morphology and colony size to complete the performance evaluation on the medium.

(ii) Selective Solid Medium

Verification of each new lot/batch of selective medium is made by comparison to a current batch of a non-selective media (reference media such as TSA). Prepare standard inocula and use either of the streak plate methods described previously on both the test and reference medium and compare the results as described in 4.5.1. The growth of the positive control (e.g. number of streak lines or quadrants grown) for both 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 non-selective medium is recorded as the Absolute Growth Index (*AGI*), whilst the number of streak lines on the selective medium is recorded as the Relative Growth Index (*RGI*). The % Relative Growth Index is calculated as follows:

$$\%RGI = {}^{RGI}/_{AGI} \times 100$$

Typically for the positive control, greater than 70% should be achieved for the %RGI.
This process should also be repeated for the negative control microorganism to demonstrate the selectivity of the medium. In most cases you would expect the %RGI to be less than 25%.
Each laboratory needs to set its own acceptance/rejection criteria and with reference to Appendix 1. Furthermore, the medium also needs to be assessed for typical morphology, colony size and biochemical responses to complete the performance evaluation on the medium.

Note: Comparison with a previous lot/batch of the same medium is discouraged because of the possibility of insidious decline or performance standards. Example given previously.

(iii) Non-Selective Liquid Medium

Between 10-100 cfu of the test organism is inoculated into the test broth, incubated and then a standard aliquot is removed to enumerate by semi-quantitative methods to demonstrate the recovery of an adequate number of test organisms. Acceptance/rejection criteria need to be developed by the laboratory when the method has been standardised.

(v) Selective Liquid Medium

Inoculate a test broth with a mixture of positive and negative control microorganisms and another test broth with just the negative control. After incubation, a standard loop (10µl) of the mixture is plated out onto a selective medium for the positive organism and a standard loop (10µl) of the negative control 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.

4.5.3. Qualitative Recovery

(i) Solid Media

Standardised streaking techniques and inocula are used. Test microorganisms are streaked on to both test and reference media. The growth on the plates after incubation is assessed and recorded as follows: zero 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 displays typical appearance, size, morphology and if appropriate biochemical response of colonies. The growth of the unwanted microorganisms should be partly (wek or 1), or completely inhibited (zero or 0).

(ii) Liquid Media

Standard inocula of working cultures are directly inoculated into the medium being tested and reference broth using a 1µl loop. 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.

The score of the wanted microorganisms should be good turbidity or 2. (Note 1: Liquid media may need to be carefully shaken before interpreting turbidity). Other characteristics such as gas formation, colour change etc can be assessed by this method. (Note 2: Media with turbid ingredients cannot be tested by this method).

4.6 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.

4.6.1 Interpretation of Quantitative Recovery Results

Productivity: >70% (wanted organism) or < 25% (unwanted organism)
Selectivity: >2 (Log)
Specificity: Reject if fails to produce typical colonial morphology, size or biochemical response or Reject if fails to suppress background flora.

4.6.2 Interpretation of Semi-Quantitative Recovery Results

%RGI: >70% (wanted organism) or 25% (unwanted organism)
Specificity: Reject if fails to produce typical colonial morphology, size or biochemical response or Reject if fails to suppress background flora or reject if PR/%RGI unsatisfactory.

4.6.3 Interpretation of Qualitative Recovery Results

Type of growth: good growth (wanted organism) or zero/weak growth (unwanted organism)
Type of turbidity: good turbidity (wanted organism) or zero/very light turbidity(unwanted organism)
Specificity: Reject if fails to produce typical colonial morphology, size or biochemical response or Reject if fails to suppress background flora or reject if PR/%RGI unsatisfactory.

5.0 PACKAGING, TRANSPORT, STORAGE AND SHELF LIFE OF PREPARED MEDIA

5.1 Packaging, Transport and Storage

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

Prepared media should be stored in unopened or resealable packages at 2-8°C unless documented validation experiments have 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.

Where transportation of media occurs, appropriate packaging and modes of transportation should be used to ensure against exposure to potentially detrimental conditions. (see Section 5.2).

5.2 Shelf Life of Prepared Media

All prepared media should be marked with an expiry date. This should be validated under the conditions of packaging, storage and transportation that will prevail under normal circumstances. In addition, the date of manufacture should be indicated (i.e. on product, packaging or Product Specification)

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 transportation 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.

5.2 Shelf Life of Prepared Media (cont'd)

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 = 110plates / 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^{o}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 the operator and the standard techniques may have week to week variation.

Using quantitative or semi-quantitative 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 the reference medium remains fresh (but not constant). 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 may need to 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 needs to be included in the testing protocol. This could be done either during the x number of weeks testing period or after determining the shelf life under ideal conditions.

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 is not constant, but the operator, inoculation techniques, incubation conditions, control/reference batch and recording of results are constant.

Using quantitative or semi-quantitative 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 may need to 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.

6.0 USER QUALITY ASSURANCE PRACTICES

6.1 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 3 years.

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 (see 6.2) 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.

6.2 Physical Inspection of Plates/Tubes

Users of commercially prepared media, or media supplied to satellite laboratories on a non-commercial basis (i.e. within one organization), should undertake a brief inspection of the media on receipt in their laboratory. Examination should include each of the following:

- integrity of packaging
- broken or cracked Petri dishes
- quality and accuracy of labelling
- expiry date
- condensation in Petri dishes
- dehydration (split or retracted medium, dry surface)
- discolouration or haemolysis
- sloped or uneven filling of Petri dishes
- contamination
- gel strength
- crystalline pattern on surface of medium (indicative of freezing)
- pitted surface or large bubbles
- presence of leakage

6.3 Reviewing of the Media Quality Control Control Certificates

If performance testing is not going to be done, it is essential to review the Q.C certificate checking that the correct microorganisms have been tested, by the correct protocol, the extent of growth recorded, the biochemical responses, the colour intensity of colonies and other relevant parameters recorded and are within the specifications for the product. If the certificate is incomplete ring the supplier before filing the QC certificate, so that you can be assured that all of the criteria parameters have been tested for.

Nonetheless, it is recommended that users of commercially prepared media continue to monitor each batch of media on a random basis. Upon any failure of a medium either on quality control performance tests or in-use monitoring, a return to the monitoring of each batch must be undertaken until reliability is re-established.

6.4 Remedial Action for Deficiencies Observed

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

a. product affected (catalogue number or plate identification code)
b. batch number and expiry date
c. date received by user
d. detailed description of problem or deficiency

Whenever possible, samples of the defective medium 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 records. (Refer Section 6.1)

6.5 Discussions with Supplier over Choices of Test Microorganisms

Many of the larger media suppliers are providing quality media to medical, food, water, pharmaceutical and environmental laboratories. In most cases the Q.C control organisms used are those most appropriate to the predominant users. If the test organisms used are not appropriate for your end use, then you need to discuss with the supplier your preferred choice of microorganisms and determine if they are able to include them in their QC procedures. If this is not possible, then on receipt of the medium, a monitoring program will need to be implemented to include the additional test microorganisms.

Example:XLD
XLD may be tested with *Salmonella, Shigella, E .coli and Enterococcus faecalis* and meet all the QC specifications. However if during the analysis of some meat products for *Salmonella* on XLD, *Proteus* is frequently present you should be assured that there is a reasonable discrimination between *Proteus* and *Salmonella* on XLD after 18-24 hours incubation at 37°C. If the supplier is unable to add *Proteus* to the test organisms, then on receipt of the medium, the laboratory should do the additional testing

Example: *Listeria* Selective Media
When using *Listeria* selective medium for examination of minimally processed vegetables and salads, the inclusion of *Bacillus pumilis* or a similar species may be included to help discriminate *Listeria* from some of the soil microorganisms that may grow through the enrichments.

It is recommended that when a user is establishing a relationship with a media supplier that the applications of the media are discussed so both parties are clear of the expectations being placed on each medium.

7.0 CONCLUDING COMMENTS

These guidelines have been prepared by a team of interested individuals, and that team is aware of the diversity of sizes and facilities that exist in food and water microbiology laboratories in Australia. It also recognizes the need for establishment of a minimum set of procedures to be followed by all manufacturers and users of food and water microbiological culture media so that good microbiological practices will flow on to an improved quality of food safety in Australia.

This document is a consensus document and as such must make some compromises. It should be read and applied as a guide to minimum requirements and procedures. Certainly, laboratories that wish to seek superior standards of microbiological practice are encouraged to do so within the context of good science. That is, all practices should be tested through a properly designed and documented validation process in order to verify that they do what they are purported to do in a reliable and reproducible manner.

As this is a consensus document, comments are welcomed and will be considered in the future reviews of this document. Future committees will endeavour to issue updates to this document on a needs basis.

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APPENDIX 1
Batch Quality Control for Growth and Performance Testing of Media for Food and Water Microbiology

Media	Microorganisms	Standard#	Function	Incubation	Aust.Std QC strains*	ISO11133-2 QC strains^	Method of Control	Criteria	Characteristic reactions
		(# = where media first cited)	see footnotes		(* 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 4.5		
FOOD MICROBIOLOGY									
0.1% peptone	total aerobic count	AS5013.x-2004	D	20-25°C/ 45 min		E.coli ATCC25922 or 8739 S.aureus ATCC25923	Quantitative	+/- 50% colonies at time 0	n/a
0.1% peptone salt solution	total aerobic count	AS5013.x-2004	D	20-25°C/ 45 min		E.coli ATCC25922 or 8739 S.aureus ATCC25923	Quantitative	+/- 50% colonies at time 0	n/a
0.1% peptone water + 3% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	D	20-25°C/ 45 min	V.parahaemolyticus NCTC10884 or UQM2200		Quantitative	+/- 50% colonies at time 0	-
Alkaline peptone water	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SE	37°C/ 6h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth on subculture to TCBS	-
Baird-Parker medium (B-P) containing egg yolk	coagulase-positive staphylococci	AS5013.12.1-2004	RGI	37°C/ 24-48h		S.aureus ATCC 25923 or 6538	Quantitative	see main text section 4.5.1	Black shiny colonies, opaque zones surrounded by clear zones
			SP	37°C/ 24-48h		S.epidermidis ATCC 12228	Qualitative		Black colonies, not shiny, no clearing
			SE	37°C/48h		E.coli ATCC 25922 or 8739	semi-quantitative	Inhibition	Large brown-black colonies if growh occurs
Baird-Parker medium (B-P) containing rabbit plasma fibrinogen (RPF)	coagulase-positive staphylococci	AS5013.12.2-2004	RGI	37°C/ 24-48h		S.aureus ATCC 25923 or 6538	Quantitative	see main text section 4.5.1	Black/grey colonies, with opacity halo
			SP	37°C/ 24-48h		S.epidermidis ATCC 12228	Qualitative		
			SE	37°C/48h		E.coli ATCC 25922 or 8739	semi-quantitative	Inhibition	Black/grey colonies, without opacity halo
Brain Heart Infusion Broth (BHIB)	coagulase-positive staphylococci	AS5013.12.x-2004	NS	37°C/ 24h		S.aureus ATCC 25923	Qualitative	Growth	tube coagulase positive
Brilliant Green Lactose Bile Broth	coliforms	AS5013.3-2004	SE	30°C/ 48h OR 37°C/ 48h		E.coli ATCC 25922 or 8739 Citrobacter freundii ATCC43864	semi-quantitative	recovery as per main text section 4.5.2	Gas production and turbidity
			SE	30°C/ 48h OR 37°C/ 48h		Enterococcus faecalis ATCC 29212 or 19433	Qualitative	no growth	n/a
Bromocresol purple cellobiose broth +3% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SP	37°C/ 96h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	no fermentation
			SP	37°C/ 96h	*E. aerogenes* ATCC13048 (not listed in this specific Std but suitable for intended task described here)		Qualitative	growth	Fermentation of cellobiose
Buffered glucose broth +3% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SP	37°C/ 18-24h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	Voges-Proskauer -ve
			SP	37°C/ 18-24h	*S.aureus* ATCC25923*(not listed in this specific Std but suitable for intended task described here)*		Qualitative	growth	Voges-Proskauer +ve

Function
D= dilution
P=productivity
SE=selectivity
SP=specificity
NS=nonselective
RGI=Relative Growth Index

All incubation conditions are aerobic unless otherwise indicated

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		(# = where media first cited)	see footnotes		(* 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 4.5		
Buffered Listeria Enrichment Broth (BLEB)	*Listeria monocytogenes*	AS/NZS 1766.2.15-1998	SE	37ºC/ 48h		#*Listeria monocytogenes* ATCC 13932 and ATCC 19111 #(no orgs specified in either standards for this media)	semi-quantitative	recovery as per main text section 4.5.2	Grey-black colonies with sunken centre, surrounded by black halo on Oxford
		AS/NZS 1766.2.15-1998	SE	37ºC/ 48h		#E.coli ATCC25922 or 8739 E.faecalis ATCC29212 or 19433 #as above	semi-quantitative	recovery as per main text section 4.5.2	inhibited/ no growth on subculture on selective medium
Buffered Peptone Water (BPW)	salmonellae	AS5013.10-2004	NS	37ºC/ 16-20h		Salmonella Typhimurium ATCC14028 S.Enteritidis ATCC13076	Qualitative	Good growth	turbidity
			NS	37ºC/ 16-20h			Qualitative	Good growth	turbidity
Decarboxylase Broth base	salmonellae	AS5013.10-2004	NS	37ºC/ 24-48h oil overlay in tube		Salmonella Typhimurium ATCC14028 S.Enteritidis ATCC13076	Qualitative	growth	turbidity and yellow
Dichloran 18% Glycerol agar (DG18)	yeasts & moulds	AS1766.2.2-1997	RGI	25ºC/ 7days		*fungal species to be determined *S.cerevisiae ATCC 9763	semi-quantitative	recovery as per main text section 4.5.2	Characteristic colonies according to each species
			SE	25ºC/ 7days		*E.coli ATCC 25922 *B.subtilis ATCC6633	Qualitative	inhibition	n/a
Dichloran Rose Bengal Chloramphenicol agar (DRBC)	yeasts & moulds	AS1766.2.2-1997	RGI	25ºC/ 5days in dark		Candida albicans ATCC10231 Aspergillus niger ATCC16404 Penicillium spp to be determined S.cerevisiae ATCC 9763	semi-quantitative	recovery as per main text section 4.5.2	Characteristic colonies according to each species
			SE	25ºC/ 5days in dark		E.coli ATCC 25922 B.subtilis ATCC6633	Qualitative	inhibition	n/a
EC broth	coliforms/ E.coli	AS5013.15-2004	SE	37ºC/ 48h		E.coli ATCC 25922 or 8739	Semi-quantitative	Gas $\geq$ 1/3 fill of tube recovery as per main text section 4.5.2	Gas production and turbidity
			SE	44-44.5ºC/48h		E.coli ATCC 25922 or 8739	Semi-quantitative	Gas $\geq$ 1/3 fill of tube recovery as per main text section 4.5.2	Gas production and turbidity
			SE	44-44.5ºC/48h		Pseudomonas aeruginosa ATCC 27853	Qualitative	no gas production	turbidity, no gas production
Fraser Broth	*Listeria monocytogenes*	AS/NZS 1766.2.16.1-1998	SE	30ºC/ 24h		*L.mono* ATCC19111 OR L.mono ATCC 13932 AND E.coli ATCC25922 or 8739 AND E.faecalis ATCC29212 or 19433	semi-quantitative	recovery as per main text section 4.5.2	Grey-black colonies with sunken centre, surrounded by black halo on Oxford
			SE	30ºC/ 24h		E.coli ATCC25922 or 8739 E.faecalis ATCC29212 or 19433	semi-quantitative	recovery as per main text section 4.5.2	-
Giolitti-Cantoni Broth	coagulase-positive staphylococci	AS5013.12.3-2004	SP	37ºC/48h tubes sealed with agar plug 2-3cm thick		S.aureus ATCC 25923 or 6538	Qualitative	Growth	Black discolouration and/or black precipitate

Function
D= dilution
P=productivity
SE=selectivity
SP=specificity
NS=nonselective
RGI=Relative Growth Index

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		(# = where media first cited)	see footnotes		(* 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 4.5		
			SP	37ºC/48h tubes sealed with agar plug 2-3cm thick		S.epidermidis ATCC 12228	Qualitative	poor growth	n/a
			SE	37ºC/48h tubes sealed with agar plug 2-3cm thick		S.aureus ATCC 25923 or 6538 S.epidermidis ATCC 12228	semi-quantitative	recovery as per main text section 4.5.2	Black shiny colonies, opaque zones surrounded by clear zones
Half Fraser Broth	*Listeria monocytogenes*	AS/NZS 1766.2.16.1-1998	SE	30ºC/ 24h		*L.mono* ATCC19111 OR L.mono ATCC 13932 AND E.coli ATCC25922 or 8739 AND E.faecalis ATCC29212 or 19433	semi-quantitative	recovery as per main text section 4.5.2	Grey-black colonies with sunken centre, surrounded by black halo on Oxford
			SE	30ºC/ 24h		E.coli ATCC25922 or 8739 E.faecalis ATCC29212 or 19433	semi-quantitative	recovery as per main text section 4.5.2	-
Lactose-sulfite medium	*Clostridium perfringens*	AS5013.16-2004	SP	37ºC/ 24h, anaerobic		C.perfringens ATCC 13124 or 12916	Qualitative	-	growth, gas production, presence of blackening (iron sulphide precipitate)
Lauryl Tryptose Broth (LTB)	coliforms	AS5013.3-2004 AS5013.15-2004	SE	30ºC/ 48h OR 37ºC/ 48h		E.coli ATCC 25922 or 8739 Citrobacter freundii ATCC43864	semi-quantitative	recovery as per main text section 4.5.2	Gas production and turbidity
			SE	30ºC/ 48h OR 37ºC/ 48h		Enterococcus faecalis ATCC 29212 or 19433	Qualitative	no growth	n/a
Lysine Decarboxylase Broth (LDC Broth)	salmonellae	AS5013.10-2004	SP	37ºC/ 24-48h oil overlay in tube		Salmonella Typhimurium ATCC14028 S.Enteritidis ATCC13076	Qualitative	Good Growth	turbidity and purple/ pale purple
Minerals Modified Glutamate agar (MMGA)	*Escherichia coli* in bivalve molluscs	AS/NZS 1766.2.12-2002	RGI	37ºC/ 18-24h	E.coli NCTC9001or ATCC11775 or NZRM3309 or ACM1803		semi-quantitative	recovery as per main text section 4.5.2	yellow colonies
			RGI	37ºC/ 18-24h	Enterobacter aerogenes NCTC10006 or ATCC13048 or NZRM798 or ACM4982		semi-quantitative	recovery as per main text section 4.5.2	colourless transparent colonies
			SP	37ºC/ 18-24h	Pseudomonas aeruginosa ATCC 27853		Qualitative		
Mollers decarboxylase broth base with 1% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	NS	37ºC/ 96h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	turbidity and yellow
Mollers decarboxylase broth base with 1% NaCl and arginine	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SP	37ºC/ 96h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	turbidity and yellow
			SP	37ºC/ 96h	V.fluvialis		Qualitative	growth	turbidity and purple
Mollers decarboxylase broth base with 1% NaCl and lysine	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SP	37ºC/ 96h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	turbidity and purple
			SP	37ºC/ 96h	V.fluvialis		Qualitative	growth	turbidity and yellow
Mollers decarboxylase broth base with 1% NaCl and ornithine	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SP	37ºC/ 96h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	turbidity and purple

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Media	Microorganisms	Standard#	Function	Incubation	Aust.Std QC strains*	ISO11133-2 QC strains^	Method of Control	Criteria	Characteristic reactions
		(# = where media first cited)	see footnotes		(* 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 4.5		
			SP	37ºC/ 96h	V.fluvialis		Qualitative	growth	turbidity and yellow
Mueller-Kauffmann Tetrathionate Broth with novobiocin (MKTTn)	salmonellae	AS5013.10-2004	SE	37ºC/ 24h		Salmonella Typhimurium ATCC14028 or S.Enteritidis ATCC13076 AND E.coli ATCC25922 or 8739 AND P.aeruginosa ATCC27853	semi-quantitative	recovery as per main text section 4.5.2	recovery on XLD and other medium of choice
			SE	37ºC/ 24h		E.coli ATCC25922 or 8739 E.faecalis ATCC29212 or 19433	semi-quantitative	recovery as per main text section 4.5.2	minimal or no growth on XLD and other medium of choice
Nutrient agar	salmonellae	AS5013.10-2004	NS	37ºC/ 18-24h		Salmonella Typhimurium ATCC14028 S.Enteritidis ATCC13076	Qualitative	growth	n/a
Nutrient agar +3% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	NS	37ºC/ 18-24h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	n/a
ONPG Broth	salmonellae	AS5013.10-2004	SP	37ºC/ 18-24h		Salmonella Typhimurium ATCC14028 S.Enteritidis ATCC13076	Qualitative	growth	colourless and turbidity
Oxford agar	*Listeria monocytogenes*	AS/NZS 1766.2.15-1998	RGI	37ºC/ 48h		*Listeria monocytogenes* ATCC 13932 and ATCC 19111	semi-quantitative	recovery as per main text section 4.5.2	Grey-black colonies with sunken centre, surrounded by black halo
			SE	37ºC/ 48h		E.coli ATCC25922 or 8739 E.faecalis ATCC29212 or 19433 C.albicans ATCC10231	Qualitative	inhibition	-
PALCAM agar	*Listeria monocytogenes*	AS/NZS 1766.2.16.1-1998	RGI	37ºC/ 48h		*Listeria monocytogenes* ATCC 13932 and ATCC 19111	semi-quantitative	recovery as per main text section 4.5.2	Grey-green to balck colonies with sunken centre, surrounded by black halo
			SE	37ºC/ 48h		E.coli ATCC25922 or 8739 E.faecalis ATCC29212 or 19433	Qualitative	inhibition	-
Plate Count Agar (PCA)	total aerobic count	AS5013.1-2004 AS5013.5-2004	RGI	30ºC/ 72h		E.coli ATCC25922 /8739 S.aureus ATCC6538 /6538P B.subtilis ATCC6633	semi-quantitative	recovery as per main text section 4.5.2	
Polymyxin pyruvate egg-yolk mannitol bromothymol blue agar (PEMBA)	*Bacillus cereus*	AS1766.2.6-1991	RGI	37ºC/ 24h	Bacillus cereus UQM 1978	*Bacillus cereus ATCC11778 *listed for QC of MYP agar	semi-quantitative	recovery as per main text section 4.5.2	distinct blue colonies surrounded by zone of precipitin, approx. 5mm diameter, crenated, irregular
			SE	37ºC/ 48h		*E.coli ATCC 25922 or 8739	Qualitative	total inhibition	-
			SP	37ºC/ 48h		*Bacillus subtilis ATCC 6633	Qualitative	-	3mm yellow-green colonies without precipitin zone, sometimes clearing
Preston agar	Campylobacter	AS5013.6-2004	RGI	42ºC/ 48h, microaerobic	C.jejuni NCTC11351 C.coli NCTC 11366	C.coli ATCC43478 C.jejuni ATCC33291 or 29428	semi-quantitative	recovery as per main text section 4.5.2	smooth, flat, translucent, colourless to grey colonies spreading along the streak line
			SE	42ºC/ 48h, microaerobic		E.coli ATCC25922 or 8739 S.aureus ATCC25923	Qualitative	inhibition	-

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Preston Broth with antibiotic supplement	Campylobacter	AS5013.6-2004	SE	42ºC/ 48h, microaerobic	C.jejuni NCTC11351 C.coli NCTC 11366	C.coli ATCC43478 OR C.jejuni ATCC33291 or 29428 AND E.coli ATCC25922 or 8739 AND P.mirabilis ATCC29906	semi-quantitative	recovery as per main text section 4.5.2	growth on subculture on selective medium
			SE	42ºC/ 48h, microaerobic		E.coli ATCC25922 or 8739 P.mirabilis ATCC29906	Qualitative	inhibition	inhibited/ no growth on subculture on selective medium
Rappaport-Vassiliadis soya peptone (RVS) broth	salmonellae	AS5013.10-2004	SE	42ºC/ 18-24h		Salmonella Typhimurium ATCC14028 or S.Enteritidis ATCC13076 AND E.coli ATCC25922 or 8739 AND P.aeruginosa ATCC27853	semi-quantitative	recovery as per main text section 4.5.2	recovery on XLD and other medium of choice
			SE	42ºC/ 18-24h		E.coli ATCC25922 or 8739 E.faecalis ATCC29212 or 19433	semi-quantitative	recovery as per main text section 4.5.2	minimal or no growth on XLD and other medium of choice
Salt tolerance medium	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997							
0% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SP	37ºC/ 48h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	NO growth	-
			SP	37ºC/ 48h	V.cholerae		Qualitative	growth	-
8% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SP	37ºC/ 48h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	-
			SP	37ºC/ 48h	V.cholerae		Qualitative	NO growth	-
11% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SP	37ºC/ 48h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	NO growth	-
			SP	37ºC/ 48h	V.cholerae		Qualitative	NO growth	-
Sheep blood agar	*Listeria monocytogenes*	AS/NZS 1766.2.15-1998	SP	37ºC/ 48h	*L.mono* NCTC11994 or ACM4986		Qualitative	growth & B-haemolysis	narrow clear light zone around colonies
			SP	37ºC/ 48h	*L.innocua* NCTC11288 or ATCC33090 or ACM4984		Qualitative	growth & no haemolysis	
			SP	37ºC/ 48h	*L.ivanovii* NCTC11846 or ATCC19119 or ACM4985		Qualitative	growth & B-haemolysis	wide zone around colonies
Skirrow agar	Campylobacter	AS5013.6-2004	RGI	42ºC/ 48h, microaerobic	C.jejuni NCTC11351 C.coli NCTC 11366	C.coli ATCC43478 C.jejuni ATCC33291 or 29428	semi-quantitative	recovery as per main text section 4.5.2	smooth, flat, translucent, colourless to grey colonies spreading along the streak line
			SE	42ºC/ 48h, microaerobic		E.coli ATCC25922 or 8739 S.aureus ATCC25923	Qualitative	inhibition	-
Thioglycollate medium	*Clostridium perfringens*	AS5013.16-2004	NS	37ºC/ 24h		C.perfringens ATCC 13124	Qualitative		good growth (turbidity)
Thiosulphate citrate bile salts sucrose (TCBS) agar	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	RGI	37ºC/ 18h	V.parahaemolyticus NCTC10884 or UQM2200		semi-quantitative	recovery as per main text section 4.5.2	blue-green colonies 3-5mm
			RGI	37ºC/ 18h	V.cholerae non-O1 NCTC4711		semi-quantitative	recovery as per main text section 4.5.2	yellow flat colonies 2-3mm
Triple Sugar Iron agar +3% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	SP	37ºC/ 18-24h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	lac/suc -ve, H2S -ve, no gas

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			SP	37ºC/ 18-24h	V.cholerae non-O1 NCTC4711		Qualitative	growth	suc +ve, H2S -ve, no gas
Tryptone Bile agar	*Escherichia coli* in bivalve molluscs	AS/NZS 1766.2.12-2002	RGI	44.0-44.5ºC/18h	E.coli NCTC9001or ATCC11775 or NZRM3309 or ACM1803		semi-quantitative	recovery as per main text section 4.5.2	growth & indole positive
			SE	44.0-44.5ºC/18h	Enterobacter aerogenes NCTC10006 or ATCC13048 or NZRM798 or ACM4982		Qualitative	-	inhibition, indole negative
Tryptone Soy Polymyxin Broth	*Bacillus cereus*	AS1766.2.6-1991	SE	30ºC/ 48h	Bacillus cereus UQM 1978		semi-quantitative	recovery as per main text section 4.5.2	turbidity
			SE	30ºC/ 48h		*E.coli ATCC 25922 or 8739	Qualitative	no growth on subculture to PEMBA	no turbidity
Tryptone soya yeast extract agar (TSYEA)	*Listeria monocytogenes*	AS/NZS 1766.2.15-1998	NS	37ºC/ 24h		*Listeria monocytogenes* ATCC 13932 or ATCC 19111	Qualitative	growth	Blue or blue-grey colonies when viewed with oblique (45º) lighting
Tryptone soya yeast extract broth (TSYEB)	*Listeria monocytogenes*	AS/NZS 1766.2.15-1998	NS	20-25ºC/ 8-24h		*Listeria monocytogenes* ATCC 13932 and ATCC 19111	Qualitative	growth	turbidity
Tryptone water	E.coli	AS1766.2.3-1992	SP	44-44.5ºC/24h	E.coli ATCC11775 or NCTC9001 or UQM1803		Qualitative	Growth	Indole +ve after addition of indole reagent
			SP	44-44.5ºC/24h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or UQM1976		Qualitative	Growth	Indole -ve after addition of indole reagent
Tryptone water + 3% NaCl	*Vibrio parahaemolyticus*	AS/NZS 1766.2.9-1997	NS	42ºC/ 18-24h	V.parahaemolyticus NCTC10884 or UQM2200		Qualitative	growth	-
Tryptose Sulphite Cycloserine (TSC) agar without egg yolk	*Clostridium perfringens*	AS5013.16-2004	RGI	37ºC/ 24h, anaerobic		C.perfringens ATCC 13124 or 12916	semi-quantitative	recovery as per main text section 4.5.2	2-4mm black colonies
			SE	37ºC/ 24h, anaerobic		E.coli ATCC 25922 or 8739	Qualitative	inhibition	no growth
Violet Red Bile agar (VRBA)	coliforms	AS5013.4-2004	RGI	30ºC/ 24h		E.coli ATCC 25922 or 8739	semi-quantitative	recovery as per main text section 4.5.2	0.5-2mm colonies, dark purple-red, surrounded by purple halo
			SE	30ºC/ 24h		Enterococcus faecalis ATCC 29212 or 19433	Qualitative	inhibition	n/a
			SP	30ºC/ 24h		Ps.aeruginosa ATCC 27853	Qualitative	n/a	colourless to beige colonies
XLD agar	salmonellae	AS5013.10-2004	SP	37ºC/ 18-24h		Salmonella Typhimurium ATCC14028 S.Enteritidis ATCC13076	Qualitative	Good Growth	red colonies black centres
			SE	37ºC/ 18-24h		E.coli ATCC25922 or 8739 E.faecalis ATCC29212 or 19433	Qualitative	limited or poor Growth	yellow colonies

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WATER MICROBIOLOGY									
Alkaline peptone water	*Vibrio cholerae*	AS4276.15 - 1999	NS	36 ± 2ºC/ 18-24h	V.cholerae non-O1 NCTC4711 or ATCC14730 or ACM2934		Qualitative	Growth	turbidity
Asparagine broth	Pseudomonads - estimation of most probable number (MPN)	AS4276.10 - 1995	SP	21ºC/ 48h	P.fluorescens NCTC10038 or ATCC13525 or ACM441 P.aeruginosa NCTC10332 or ATCC10145 or ACM495		Qualitative	Growth	turbidity with or without pigment production
			SP	21ºC/ 48h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or ACM1976		Qualitative	inhibited	
BCYE	Legionella	AS3896 - 1998	SE	36ºC/ 72h	*L.pneumophila* sgp1 ATCC43111 or NCTC11404		semi-quantitative	recovery as per main text section 4.5.2	colonies 1-2mm diameter, white, glistening, circular, smooth, raised with entire edge
BCYE-BMPA	Legionella	AS3896 - 1998	SE	36ºC/ 72h	*L.pneumophila* sgp1 ATCC43111 or NCTC11404		semi-quantitative	recovery as per main text section 4.5.2	colonies 1-2mm diameter, white, glistening, circular, smooth, raised with entire edge
			SP	36ºC/ 72h	*P.aeruginosa* ATCC10145 or NCTC10332		Qualitative	inhibition	-
BCYE-GVPC	Legionella	AS3896 - 1998	SE	36ºC/ 72h	*L.pneumophila* sgp1 ATCC43111 or NCTC11404		semi-quantitative	recovery as per main text section 4.5.2	colonies 1-2mm diameter, white-grey-blue, ground-glass appearance, circular, smooth, raised with entire edge
			SP	36ºC/ 72h	*P.aeruginosa* ATCC10145 or NCTC10332		Qualitative	inhibition	-
BCYE-MWY	Legionella	AS3896 - 1998	P	36ºC/ 72h	*L.pneumophila* sgp1 ATCC43111 or NCTC11404		semi-quantitative	recovery as per main text section 4.5.2	colonies 1-2mm diameter, white, with slightly discernible green colouration, glistening, circular, smooth, raised with entire edge
			SP	36ºC/ 72h	*P.aeruginosa* ATCC10145 or NCTC10332		Qualitative	inhibition	-
Bile aesculin agar	Faecal streptococci - estimation of most probable number (MPN)	AS4276.8 - 1995	SE	37ºC/ 24h	E.faecalis NCTC775 or ATCC19433 or ACM2517		semi-quantitative	recovery as per main text section 4.5.2	small colonies producing blackening of the medium
			SP	37ºC/ 24h	S.aureus ATCC25923		qualitative		small colonies without blackening of the medium
bismuth sulphite agar	salmonellae	AS4276.14 - 1995	SP	35 or 37ºC/ 48h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		Qualitative	Good Growth	Black colonies metallic sheen, sometimes staining of surrounding medium
			SP	35 or 37ºC/ 48h	Citrobacter freundii NCTC9750 or ATCC8090		Qualitative	limited or poor Growth	inhibited or dull green or brown colonies with no metallic sheen
Brain heart infusion agar (BHIA)	Faecal streptococci - estimation of most probable number (MPN)	AS4276.8 - 1995	NS	37ºC/ 48h, 44ºC/ 48h	E.faecalis NCTC775 or ATCC19433 or ACM2517		Qualitative	Growth	catalase negative colonies recovered from BHIB

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Brain heart infusion broth (BHIB)	Faecal streptococci - estimation of most probable number (MPN)	AS4276.8 - 1995	NS	37ºC/ 24h	E.faecalis NCTC775 or ATCC19433 or ACM2517		Qualitative	Growth	turbidity, growth on subculture on BHIA
Buffered peptone water	salmonellae	AS4276.14 - 1995	NS	35 or 37ºC/ 16-20h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		Qualitative	Good growth	turbidity
Cetrimide B agar	Pseudomonads - estimation of most probable number (MPN)	AS4276.10 - 1995	SE	27ºC/ 48h	P.fluorescens NCTC10038 or ATCC13525 or ACM441 P.aeruginosa NCTC10332 or ATCC10145 or ACM495		semi-quantitative	recovery as per main text section 4.5.2	raised, convex, bright green, translucent colonies, may fluoresce under UV light, may be pigmented
Decarboxylase broth base	salmonellae	AS4276.14 - 1995	NS	35 or 37ºC/ 18-24h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		Qualitative	Growth	turbidity and yellow
Differential reinforced clostridial medium (DRCM)	*Clostridium perfringens*	AS4276.17.2 - 2000	SE	36 ± 2ºC/ 48h	C.perfringens NCTC 8237 or ACM2516 or NZRM20		semi-quantitative	recovery as per main text section 4.5.2	blackening of medium
			SP	36 ± 2ºC/ 48h	B.cereus NCTC9945 or ACM1978 or NZRM773		Qualitative	-	no blackening of medium
EC Broth	Thermotolerant coliforms and *Escherichia coli* - estimation of most probable number (MPN)	AS4276.6 - 1995	SE	44ºC/ 48h	E.coli ATCC11775 or NCTC9001 or ACM1803		semi-quantitative	recovery as per main text section 4.5.2	turbidity and gas production
			SP	44ºC/ 48h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or ACM1976		Qualitative	-	turbidity, no gas production
				37ºC/ 48h	E.coli ATCC11775 or NCTC9001 or ACM1803		semi-quantitative	recovery as per main text section 4.5.2	turbidity and gas production
Eosin Methylene Blue agar (EMB)	Coliforms-estimation of most probable number (MPN)	AS 4276.4-1995	SP	37ºC/ 24h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or ACM1976		Qualitative	Good growth	4-6mm, raised, often mucoid,pink/lavender possibly with grey-brown centres, metallic shhen usually absent
			SP	37ºC/ 24h	Pseudomonas aeruginosa NCTC10332 or ATCC10145 or ACM495		Qualitative	Growth	translucent, colourless pinpoint colonies
			SP	37ºC/ 24h	E.coli ATCC11775 or NCTC9001 or ACM1803		Qualitative	Good growth	2-3mm dark purple centres, green metallic sheen in reflected light
Hugh & Leifson's oxidation /fermentation (O/F) medium	Pseudomonads - estimation of most probable number (MPN)	AS4276.10 - 1995	SP	21ºC/ 96h	P.fluorescens NCTC10038 or ATCC13525 or ACM441 P.aeruginosa NCTC10332 or ATCC10145 or ACM495		Qualitative	-	growth (with colour change) in open tube only
			SP	21ºC/ 96h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or ACM1976		Qualitative	-	growth in open AND sealed tubes

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Improved Formate Lactose Glutamate medium (IFLG) (also known as Minerals Modified Glutamate Medium)	Coliforms-estimation of most probable number (MPN)	AS 4276.4-1995	RGI	37ºC/ 48h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or ACM1976		semi-quantitative	recovery as per main text section 4.5.2	acid production
			SP	37ºC/ 48h	Pseudomonas aeruginosa NCTC10332 or ATCC10145 or ACM495		Qualitative	growth	no acid production
			RGI	37ºC/ 48h	E.coli ATCC11775 or NCTC9001 or ACM1803		semi-quantitative	recovery as per main text section 4.5.2	acid and gas production
Lactose peptone water	Coliforms-estimation of most probable number (MPN)	AS 4276.4-1995	SP	37ºC/ 48h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or ACM1976		Qualitative	growth	acid production
			SP	37ºC/ 48h	Pseudomonas aeruginosa NCTC10332 or ATCC10145 or ACM495		Qualitative	-	no acid production
Lactose-gelatin medium	*Clostridium perfringens*	AS4276.17.1 - 2000	SP	36 ± 2ºC/ 24h	C.perfringens NCTC 8237 or ACM2516 or NZRM20		Qualitative	Growth	gelatin liquefaction, acid & gas production
			SP	36 ± 2ºC/ 24h	B.cereus NCTC9945 or ACM1978 or NZRM773		Qualitative	Growth	gelatin liquefaction, NO acid production
Lysine decarboxylase broth	salmonellae	AS4276.14 - 1995	SP	35 or 37ºC/ 18-24h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		Qualitative	Growth	turbidity, purple to pale purple
			SP	35 or 37ºC/ 18-24h	Citrobacter freundii NCTC9750 or ATCC8090		Qualitative	Growth	turbidity, yellow
Mannitol selenite cystine broth	salmonellae	AS4276.14 - 1995	SE	35 or 37ºC/ 48h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		semi-quantitative	recovery as per main text section 4.5.2	recovery on BSA/XLD
			SE	35 or 37ºC/ 48h	Citrobacter freundii NCTC9750 or ATCC8090		semi-quantitative	recovery as per main text section 4.5.2	minimal or no growth on BSA/XLD
Membrane lauryl sulfate agar	Coliforms- membrane filtration method	AS4276.5-1995	RGI	37ºC/ 24h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or ACM1976		semi-quantitative	recovery as per main text section 4.5.2	yellow colonies
			SP	37ºC/ 24h	Pseudomonas aeruginosa NCTC10332 or ATCC10145 or ACM495		Qualitative	-	no yellow colonies
			RGI	37ºC/ 24h	E.coli ATCC11775 or NCTC9001 or ACM1803		semi-quantitative	recovery as per main text section 4.5.2	yellow colonies
Membrane lauryl sulfate broth	Coliforms- membrane filtration method	AS4276.5-1995	SE	37ºC/ 24h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or ACM1976		semi-quantitative	recovery as per main text section 4.5.2	turbidity and acid production
			SP	37ºC/ 24h	Pseudomonas aeruginosa NCTC10332 or ATCC10145 or ACM495		Qualitative	-	no acid production
			SE	37ºC/ 24h	E.coli ATCC11775 or NCTC9001 or ACM1803		semi-quantitative	recovery as per main text section 4.5.2	turbidity and acid production

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M-enterococcus agar	Faecal streptococci - membrane filtration method	AS4276.9 - 1995	SE	37ºC/ 48h	E.faecalis NCTC775 or ATCC19433 or ACM2517		semi-quantitative	recovery as per main text section 4.5.2	pink to maroon colonies
			SP	37ºC/ 48h	S.aureus ATCC 25923		Qualitative	-	pale or colourless colonies if growth
Milk agar	*Pseudomonas aeruginosa* - estimation of most probable number (MPN)	AS4276.12 - 1995	RGI	37ºC/ 24h	P.aeruginosa NCTC10332 or ATCC10145 or ACM495		semi-quantitative	recovery as per main text section 4.5.2	colonies surrounded by zones of hydrolysis
			SP	37ºC/ 24h	E.coli ATCC11775 or NCTC9001 or ACM1803		Qualitative	-	no hydrolysis
MIX agar	Aeromonas	AS4276.18 - 2001	SE	30ºC/ 18h anaerobically then 36ºC/ 18h aerobically	A.hydrophila ATCC7966 or NCTC8049 or NZRM804		semi-quantitative	recovery as per main text section 4.5.2	circular, convex, blue-green to green-brown colonies 1-3mm diameter
			SP	30ºC/ 18h anaerobically	P.aeruginosa NCTC10332 or ATCC10145 or ACM495		Qualitative	-	no growth
m-PA-C agar	*Pseudomonas aeruginosa* - membrane filtration method	AS4276.13 - 1995	SE	41.5ºC/ 24h	P.aeruginosa NCTC10332 or ATCC10145 or ACM495		semi-quantitative	recovery as per main text section 4.5.2	colonies typically 0.8 to 2.2mm diameter, flat, light outer rims and brownish to green-black centres.
			SP	41.5ºC/ 24h	E.coli ATCC11775 or NCTC9001 or ACM1803		Qualitative	inhibited	
Mueller-Hinton agar	Aeromonas	AS4276.18 - 2001	NS	30ºC/ 48h	A.hydrophila ATCC7966 or NCTC8049 or NZRM804		Qualitative	-	resistance to 30ug cephalothin disc
Nitrate motility medium	*Clostridium perfringens*	AS4276.17.1 - 2000	SP	36 ± 2ºC/ 24h	C.perfringens NCTC 8237 or ACM2516 or NZRM20		Qualitative	-	growth only in and along the stab line and development of red colour after adding solutions A&B (indicating NO_2 formed)
			SP	36 ± 2ºC/ 24h	B.pumilis		Qualitative	-	growth beyond the stab line (motility +ve) and nitrite negative
Nutrient agar	Coliforms-estimation of most probable number (MPN)	AS 4276.4-1995	NS	37ºC/ 18-24h	Enterobacter aerogenes ATCC 13048 or NCTC10006 or ACM1976		Qualitative	growth	n/a
ONPG broth	salmonellae	AS4276.14 - 1995	SP	35 or 37ºC/ 18-24h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		Qualitative	Growth	S.salford ONPG -ve Salmonella IIIb ONPG +ve
			SP	35 or 37ºC/ 18-24h	Citrobacter freundii NCTC9750 or ATCC8090		Qualitative	Growth	ONPG +ve
Plate Count Agar (PCA)	total aerobic count	AS4276.3.1-1995	RGI	21ºC/ 72h, 37ºC/ 48h		E.coli ATCC25922 /8739 S.aureus ATCC6538 /6538P B.subtilis ATCC6633	semi-quantitative	recovery as per main text section 4.5.2	
Preston agar	Campylobacter	AS 4276.2.19 -2001	SE	42ºC/ 24-72h, microaerobic (5-6%O_2, 10% CO_2)	C.jejuni NCTC11351 or ACM3393 or NZRM2397		semi-quantitative	recovery as per main text section 4.5.2	smooth, flat, translucent, colourless to grey colonies spreading along the streak line
			SP	42ºC/ 24-72h, microaerobic (5-6%O_2, 10% CO_2)	E.coli ATCC11775 or NCTC9001 or ACM1803 or NZRM3309		Qualitative	inhibition	-

Function
D= dilution
P=productivity
SE=selectivity
SP=specificity
NS=nonselective
RGI=Relative Growth Index

All incubation conditions are aerobic unless otherwise indicated

APPENDIX 1
Batch Quality Control for Growth and Performance Testing of Media for Food and Water Microbiology

Media	Microorganisms	Standard#	Function	Incubation	Aust.Std QC strains*	ISO11133-2 QC strains^	Method of Control	Criteria	Characteristic reactions
		(# = where media first cited)	see footnotes		(* 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 4.5		
Preston Broth with antibiotic supplement	Campylobacter	AS 4276.2.19 -2001	SE	42ºC/ 24-72h, microaerobic (5-6%O_2, 10% CO_2)	C.jejuni NCTC11351 or ACM3393 or NZRM2397		semi-quantitative	recovery as per main text section 4.5.2	growth on subculture on selective medium
			SP	42ºC/ 24-72h, microaerobic (5-6%O_2, 10% CO_2)	E.coli ATCC11775 or NCTC9001 or ACM1803 or NZRM3309		Qualitative	inhibition	inhibited/ no growth on subculture on selective medium
Rappaport-Vassiliadis (RV) medium	salmonellae	AS4276.14 - 1995	SE	42ºC/ 48h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		semi-quantitative	recovery as per main text section 4.5.2	recovery on BSA/XLD
			SE	42ºC/ 48h	Citrobacter freundii NCTC9750 or ATCC8090		semi-quantitative	recovery as per main text section 4.5.2	minimal or no recovery on BSA/XLD
Salt tolerance medium	*Vibrio cholerae*	AS4276.15 - 1999							
0% NaCl tryptone water	*Vibrio cholerae*	AS4276.15 - 1999	SP	36 ± 2ºC/ 16-24h	Vibrio fluvialis NCTC11218 or ACM2944 or NZRM2605		Qualitative	no growth	-
			SP	36 ± 2ºC/ 16-24h	V.cholerae non-O1 NCTC4711 or ATCC14730 or ACM2934		Qualitative	Growth	-
3% NaCl tryptone water	*Vibrio cholerae*	AS4276.15 - 1999	SP	36 ± 2ºC/ 16-24h	V.cholerae non-O1 NCTC4711 or ATCC14730 or ACM2934		Qualitative	Growth	
			SP	36 ± 2ºC/ 16-24h	Vibrio fluvialis NCTC11218 or ACM2944 or NZRM2605		Qualitative	Growth	
8% NaCl tryptone water	*Vibrio cholerae*	AS4276.15 - 1999	SP	36 ± 2ºC/ 16-24h	V.cholerae non-O1 NCTC4711 or ATCC14730 or ACM2934		Qualitative	no growth	
			SP	36 ± 2ºC/ 16-24h	Vibrio fluvialis NCTC11218 or ACM2944 or NZRM2605		Qualitative	Growth	
Sodium azide medium	Faecal streptococci - estimation of most probable number (MPN)	AS4276.8 - 1995	SE	37ºC/ 48h	E.faecalis NCTC775 or ATCC19433 or ACM2517		semi-quantitative	recovery as per main text section 4.5.2	turbidity and acid production
			SP	37ºC/ 48h	S.aureus ATCC 25923		Qualitative	-	no acid production
sucrose peptone water	salmonellae	AS4276.14 - 1995	SP	35 or 37ºC/ 18-24h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		Qualitative	Growth	no production of acid
			SP	35 or 37ºC/ 18-24h	Citrobacter freundii NCTC9750 or ATCC8090		Qualitative	Growth	production of acid
Thiosulphate citrate bile salts sucrose (TCBS) agar	*Vibrio cholerae*	AS4276.15 - 1999	SE	36 ± 2ºC/ 16-24h	V.cholerae non-O1 NCTC4711 or ATCC14730 or ACM2934		semi-quantitative	recovery as per main text section 4.5.2	Smooth flat yellowish-brown colonies, surrounded by yellow zones in medium
			SP	36 ± 2ºC/ 16-24h	E.coli ATCC11775 or NCTC9001 or ACM1803		Qualitative	inhibited	
Tryptone water	Thermotolerant coliforms and *Escherichia coli* - estimation of most probable number (MPN)	AS4276.6 - 1995	NS	44-44.5ºC/24h	E.coli ATCC11775 or NCTC9001 or ACM1803		Qualitative	Growth	Indole +ve after addition of indole reagent

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Media	Microorganisms	Standard#	Function	Incubation	Aust.Std QC strains*	ISO11133-2 QC strains^	Method of Control	Criteria	Characteristic reactions
		(# = where media first cited)	see footnotes		(* 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 4.5		
Tryptose Sulphite Cycloserine (TSC) agar without egg yolk	*Clostridium perfringens*	AS4276.17.1 - 2000	RGI	36 ± 2ºC/ 48h anaerobic	C.perfringens NCTC 8237 or ACM2516 or NZRM20		semi-quantitative	recovery as per main text section 4.5.2	2-4mm black colonies
			SE	36 ± 2ºC/ 48h anaerobic	B.cereus NCTC9945 or ACM1978 or NZRM773		Qualitative	inhibition	no growth
urea agar	salmonellae	AS4276.14 - 1995	SP	35 or 37ºC/ 18-24h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		Qualitative	Growth	no production of urease
			SP	35 or 37ºC/ 18-24h	Citrobacter freundii NCTC9750 or ATCC8090		Qualitative	Growth	production of urease
XLD agar	salmonellae	AS4276.14 - 1995	SE	35 or 37ºC/ 24h	Salmonella salford IMVS1710 Salmonella IIIb IMVS1679		semi-quantitative	recovery as per main text section 4.5.2	S.salford red colonies black centres Salmonella IIIb yellow colonies possibly with black centres
			SE	35 or 37ºC/ 24h	Citrobacter freundii NCTC9750 or ATCC8090		semi-quantitative	recovery as per main text section 4.5.2	yellow colonies possibly with black centres

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