Guidelines for Assuring Quality

of

Food and Water Microbiological Culture Media

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

3rd edition
2024

The Australian Society for Microbiology
bringing Microbiologists together

FOREWORD to the First Edition (2004)

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.

Major contributors to the First Edition:
Dr. Stuart Andrews
Mr. Peter Traynor
Mrs. Alida Scholtes
Mrs. Jill Anderson
Mr. Neil Shepherd
Mrs Agnes Tan
Members of the Victorian Branch of the Culture Media SIG

FOREWORD to the Second Edition (2014)

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, to a full ISO Standard that also incorporates media used in water microbiology.

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.

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.

Peter Traynor,
National Convenor,
Culture Media Special Interest Group,
Australian Society for Microbiology, Inc.

FOREWORD to the Third Edition (2024)

Despite the passing of a decade since the last edition of these Guidelines, the pleasing aspect is how well they have, during that time, maintained their overall robustness and 'fit-for-purpose' nature. The feedback from end users, as well as assessors involved in laboratory accreditation to the relevant Standards (notably ISO17025 (1,2)) has been positive throughout that time.

This new edition of the Guidelines for food and water microbiological culture media aims to capture and reflect relevant changes that have occurred since the release of the second edition. The third edition is a very modest update in the most part; the Appendices are where most changes have occurred.

ISO11133, first published as an International Standard in 2014, was complemented by amendments published in 2018 and 2020. AS5140, the Australian adoption of ISO11133, was published in 2019, reflecting performance requirements for not only ISO methods, but other Australian standards in food and water microbiology. In 2022, AS5140 was re-issued, to capture changes including the ISO11133 amendments, and updates to other Australian Standards. The ISO11133 parent document is, at time of this third edition, currently undergoing its periodic review. Due to ISO rules, and the devolution of media characteristics back to individual standards, the next release of ISO11133 will contain many fewer media described within. These ASM Guidelines will continue to complement all relevant Australian Standards, as well as AS5140.

As was already the case, 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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Peter Traynor, MASM MAIMS
National Convenor, Culture Media Special Interest Group,
Honorary Life Member, Australian Society for Microbiology, Inc.

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

Please include as the Subject Line:
Food and Water Microbiological Media - QC Guidelines 3rd 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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1.0 Introduction

ISO/IEC 17025 *General requirements for the competence of testing and calibration laboratories* (2) 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 (1,3).

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 (1,2,3,4). 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 AS5140 (ISO11133) 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, vials, 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 (QA): 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 (QC): The final inspection and testing of the finished product to ensure its compliance with predetermined performance criteria.

Quality Management System (QMS): a formalised system that documents processes, procedures, and responsibilities for achieving quality policies and objectives, to meet customer and regulatory requirements, and improve effectiveness and efficiency on a continuous basis.

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 regarding 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 AS5140 (ISO11133) (4).

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 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 references (4, 5) 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± 2°C is recommended) before inspection. Testing for contamination should always be undertaken when media is aseptically dispensed. However, where media is terminally sterilized a protocol may be established for release, based on 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 3 and 4) should be used. The cultures listed in the Appendices reflect the minimal cultures that should be used to QC media performance. 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 (7)). 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 -70ºC 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 -20ºC **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*

MASTER CULTURE
Freeze dried, -70ºC, -20ºC or less
Purity + Culture identity verified

(First Generation)**
Store for years

STOCK CULTURE (Multiple)
Frozen at -20ºC
Purity Check

(Second Generation)
Store 0.5 – 2.0 years

WORKING CULTURE
Non-Selective Media
at 4ºC or other storage temp.
Purity Check
Minimal confirmation (voluntary)

(Third Generation)
Store up to 4 weeks ***
Discard after this time

Standardised Cell Suspensions

Performance of Media Quality Control tests
or
Test Method Controls

* 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 Performance

The test procedure for culture media performance is recommended as follows:

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 performance 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:

$P_R = N_S / N_O$ 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 qualitative evaluations, visual checks are carried out and growth scores allocated (e.g., ‘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 as follows:

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

D_O is 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.
NOTE There are very few instances where a quantitative selectivity is actually required.

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 a current batch of a reference medium, with few exceptions. **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, but also with reference to Appendices 3 and 4 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 criterion 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 criterion 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.

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, 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. 10^1-10^2cfu ↓subculture Non-Inhibitory Medium (±Indicator)	-ve control org. 10^4-10^5 ↓subculture Non-Inhibitory Medium (±Indicator)	Mix(+ve &-ve) ↓subculture Non-Inhibitory Medium (±Indicator)	+ve control org. 10^1-10^2cfu ↓subculture Non-Inhibitory Medium (±Indicator)	-ve control org. 10^4-10^5 ↓subculture Non-Inhibitory Medium (±Indicator)	Mix(+ve &-ve) ↓subculture 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.

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

Fig.2

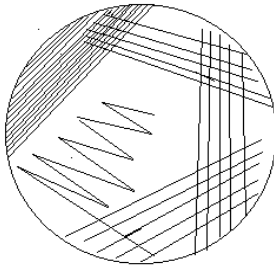
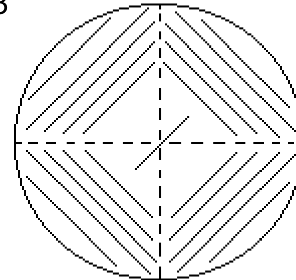


Fig.3



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:

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

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 criterion 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 criterion 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 are 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 (10µl) 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 (10µl) 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: ≥70% (wanted organism) nonselective medium
≥50% (wanted organism) selective medium
≤ 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 ≥70% (wanted organism) nonselective medium

≥50% (wanted organism) selective medium

≤ 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: (i) intended use; (ii) strains tested; (iii) testing method; (iv) the final pH of the medium; (v) the procedure used for testing for microbial contamination; (vi) expected performance characteristics; (vii) incubation temperature, period, and atmosphere (used to determine (vi)); (viii) storage conditions.

Where compliance certificates are issued, such certifications should also include items (ii) to (vi).

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 several 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°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-week 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 (1,2).

Laboratories who obtain prepared culture media either from a commercial source or a central facility, that carries a compliance label should record:

- 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).
- Presence of bubbles.
- Presence of leakage.

4. 3 Remedial Action for Deficiencies Observed

Where significant defects are found, the users should notify the manufacturers providing all 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 (1,2).

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 microbial 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.

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

5.0 References

1. *General Accreditation Criteria. ISO/IEC17025 Standard Application Document.* Current edition. National Association of Testing Authorities (NATA), Sydney, Australia.
2. ISO17025:2017. *General requirements for the competence of testing and calibration laboratories*. 2017. International Standards Organisation, Geneva.
3. *General Accreditation Criteria. Media Preparation and Quality Control.* Current edition. National Association of Testing Authorities (NATA), Sydney, Australia.
4. AS5140 (ISO11133). *Microbiology of food, animal feed and water –Preparation, production, storage and performance testing of culture media.* Standards Australia, Sydney.
5. *Handbook of Culture Media for Food and Water Microbiology*. 3rd Edition. Edited by JEL Corry, Gordon DW Curtis and RM Baird 2011. Royal Society for Chemistry, https://doi.org/10.1039/9781847551450
6. 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.
7. *General Accreditation Criteria. Maintenance of Microbiological Reference Culture Collections (MRCCs).* Current edition. National Association of Testing Authorities, Sydney, Australia.

Appendix 1 Sampling Plan for Microbiological Culture Media

Small Batches (≤100 units): 1% or 1 unit from beginning and 1% or 1 unit from end of batch (4).

Double Sampling Plan (>100 units) NORMAL SAMPLING PLAN, AQL - 2.5, GENERAL INSPECTION LEVEL = 1 (6).

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
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

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* (5). 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.

APPENDIX 3
Batch Quality Control for Growth and Performance Testing of Media for Food Microbiology

Media	Microorganisms	Standard#	Function	Incubation	Control strains	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133		see Guidelines Section 2.7		
DILUENTS 0.1% peptone 0.1% peptone salt solution (PSS) PSS+BCP		AS5013.11.x (ISO6887-x)	D	20-25°C/ 45min-1hr	*Escherichia coli* WDCM00013 or 00012 *Staphyloccus aureus* WDCM00034	Quantitative	+/- 30% colonies vs time 0	n/a
Ringer's solution 1/4 strength		AS5013.20						
Agar Listeria according to Ottaviani and Agosti	*Listeria monocytogenes*	AS5013.24.1 (ISO11290-1 MOD) AS5013.24.2 (ISO11290-2 MOD)	P	36-38ºC/ 40-48h	*Listeria monocytogenes* WDCM00021 or 00109	Quantitative	$P_R \geq 0.5$	Blue-green colonies with opaque halo
			SE		*Escherichia coli* WDCM00013 or 00012 *Enterococcus faecalis* WDCM00087 or 00009	Qualitative	no growth	-
			SP		*Listeria innocua* WDCM00017		growth	Blue-green colonies withOUT opaque halo
Baird-Parker medium (B-P)	coagulase-positive staphylococci	AS5013.12.1 (ISO6888-1)	P	36-38ºC/22-50h	*Staphylococcus aureus* WDCM00034 or 00032	Quantitative	$P_R \geq 0.5$	Black/grey colonies, with clear halo
			SP		*Staphylococcus epidermidis* WDCM00036 *Staphylococcus saprophyticus* WDCM00159	Qualitative		Black/grey colonies, without clear halo
			SE	36-38ºC/46-50h	*Escherichia coli* WDCM00013 or 00012		no growth	-
Baird-Parker medium (B-P) containing rabbit plasma fibrinogen (RPF)	coagulase-positive staphylococci	AS5013.12.2 (ISO6888-2)	P	36-38ºC/22-50h	*Staphylococcus aureus* WDCM00034 or 00032	Quantitative	$P_R \geq 0.5$	Black/grey colonies, with opacity halo
			SP		*Staphylococcus epidermidis* WDCM00036 *Staphylococcus saprophyticus* WDCM00159	Qualitative		Black/grey colonies, without opacity halo
			SE	36-38ºC/46-50h	*Escherichia coli* WDCM00013 or 00012		no growth	
Brilliant Green Lactose Bile Broth	coliforms	AS5013.3 (ISO 4831) AS5013.4 (ISO4832)	P	29-31ºC/ 22-50h	*Escherichia 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
Buffered Peptone Water (BPW)	diluent	AS5013.11.1 (ISO6887-1)	D	20-25°C/ 45min-1hr	*Escherichia coli* WDCM00013 or 00012 *Staphyloccus aureus* WDCM00034	Quantitative	+/- 30% colonies vs time 0	na
	Listeria monocytogenes	AS5013.24.2 (ISO11290-2 MOD)		18-22ºC/ 55-65min	*Listeria monocytogenes* WDCM00021 or 00109			
	salmonellae	AS5013.10 (ISO6579)	NS	36-38ºC/ 16-20h	*Salmonella* Hofit IMVS 1799	Qualitative	Good growth	turbidity
	Cronobacter spp.	AS5013.13 (ISO22964 MOD)	NS	34-38ºC/ 16-20h	*Cronobacter sakazakii* WDCM00214 *Cronobacter muytjensii* WDCM00213			
CFC agar	*Pseudomonas* spp	AS5013.21 (ISO13720)	P	24-26ºC/40-48h	*Pseudomonas fluorescens* WDCM00115 *Pseudomonas fragi* WDCM00116	Quantitative	$P_R \geq 0.5$	-
			SE		*Escherichia coli* WDCM00013 or 00012	Qualitative	no growth	-

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

All incubation conditions are aerobic unless otherwise indicated.

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

Media	Microorganisms	Standard#	Function	Incubation	Control strains	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133		see Guidelines Section 2.7		
Chromogenic Cronobacter agar (CCI)	Cronobacter spp.	AS5013.13 (ISO22964 MOD)	P	40.5-42.5ºC/ 22-26h	*Cronobacter sakazakii* WDCM00214 Cronobacter muytjensii WDCM00213	Qualitative	Good growth	small to medium blue green colonies on CCI
			SP		*Enterobacter cloacae* WDCM00083		growth	colonies do not have green or blue-green colour
			SE		*Staphylococcus aureus* WDCM00032 or 00034		no growth	-
Cronobacter selective broth (CSB)	Cronobacter spp.	AS5013.13 (ISO22964 MOD)	P	40.5-42.5ºC/ 22-26h	*Cronobacter sakazakii* WDCM00214 AND *Staphylococcus aureus* WDCM00032 or 00034	Qualitative	Growth	CSB yellow; small to medium blue green colonies on CCI
					Cronobacter muytjensii WDCM00213 AND *Staphylococcus aureus* WDCM00032 or 00034			
			SE		*Staphylococcus aureus* WDCM00032 or 00034		inhibition or no growth	CSB remains purple
CT-SMAC agar	*Escherichia coli* O157	AS5013.26 (ISO16654, MOD)	P	36-38ºC/18-24h	*Escherichia coli* WDCM00014	Qualitative	growth	translucent colonies, pale yellow-brown ~1mm
			SE		*Staphylococcus aureus* WDCM00032 or 00034		no growth	
			SP		*Escherichia coli* WDCM00013 or 00012		inhibition or no growth	pink colonies (where growth occurs)
Dichloran 18% Glycerol agar (DG18)	yeasts & moulds	AS5013.29	P	24-26ºC/ 5d	*Saccharomyces cerevisiae* WDCM00058 *Wallemia species* *see also AS5140*	Quantitative	$P_R \geq 0.5$	Characteristic colonies according to each species
			SE		*Escherichia coli* WDCM00013 or 00012 *Bacillus subtilis subsp. spizizenii* WDCM00003	Qualitative	no growth	-
Dichloran Rose Bengal Chloramphenicol agar (DRBC)			P		*Saccharomyces cerevisiae* WDCM00058 *Candida albicans* WDCM00054 *Aspergillus brasiliensis* WDCM00053	Quantitative	$P_R \geq 0.5$	Characteristic colonies according to each species
			SE		*Escherichia coli* WDCM00013 or 00012 *Bacillus subtilis subsp. spizizenii* WDCM00003	Qualitative	no growth	-
EC broth	*E.coli*	AS5013.15 (ISO7251)	P	43-45ºC/22-50h	*Escherichia coli* WDCM00013 or 00012	Qualitative	growth	Gas production and turbidity
			SE		*Pseudomonas aeruginosa* WDCM00025		no growth	-
EE Broth (Enterobacteriaceae Enrichment broth)	Enterobacteriaceae	AS5013.8.1 (ISO21528-1)	P	36-38ºC/22-26h	*Escherichia coli* WDCM00013 or 00012 AND *Enterococcus faecalis* WDCM00087 or 00009	Qualitative	*E. coli* growth on VRBGA	Pink to red colonies, ± precipitation halo
			SE		*Enterococcus faecalis* WDCM00087 or 00009		no growth on TSA	na

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

All incubation conditions are aerobic unless otherwise indicated.

APPENDIX 3
Batch Quality Control for Growth and Performance Testing of Media for Food Microbiology

Media	Microorganisms	Standard#	Function	Incubation	Control strains	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133		see Guidelines Section 2.7		
Fraser Broth	*Listeria monocytogenes*	AS5013.24.1 (ISO11290-1 MOD)	P	36-38ºC/ 46-50h	*Listeria monocytogenes* WDCM00021 or 00109 AND *Escherichia coli* WDCM00013 or 00012 AND *Enterococcus faecalis* WDCM00087 or 00009	Qualitative	> 10 colonies on Agar Listeria according to Ottaviani and Agosti	Blue green colonies with opaque halo
			SE		*Escherichia coli* WDCM00013 or 00012		no growth	-
					Enterococcus faecalis WDCM00087 or 00009		<100 colonies on TSA	-
Giolitti-Cantoni Broth	coagulase-positive staphylococci	AS5013.12.3 (ISO6888-3)	P	36-38ºC/ 22-50h (tubes sealed with agar plug)	*Staphylococcus aureus* WDCM00034 or 00032 AND *Escherichia coli* WDCM00013 or 00012	Qualitative	>10 staph colonies on Baird-Parker or RPF	Characteristic colonies according to each medium
			SE	36-38ºC/ 46-50h (tubes sealed with agar plug)	*Escherichia coli* WDCM00013 or 00012		no growth	-
Half Fraser Broth	*Listeria monocytogenes*	AS5013.24.1 (ISO11290-1 MOD)	P	29-31ºC/ 22-26h	*Listeria monocytogenes* WDCM00021 or 00109 AND *Escherichia coli* WDCM00013 or 00012 AND *Enterococcus faecalis* WDCM00087 or 00009	Qualitative	> 10 colonies on Agar Listeria according to Ottaviani and Agosti	Blue green colonies with opaque halo
			SE		*Escherichia coli* WDCM00013 or 00012		no growth	-
					Enterococcus faecalis WDCM00087 or 00009		<100 colonies on TSA	-
Heart Infusion Broth (BHIB)	coagulase-positive staphylococci	AS5013.12.1 (ISO6888-1)	NS	36-38ºC/ 22-26h	*Staphylococcus aureus* WDCM00034	Qualitative	turbidity	-
Lactose gelatin medium	*Clostridium perfringens*	AS5013.16 (ISO7937)	SP	36-38ºC/ 22-26h, anaerobic	*Clostridium perfringens* WDCM00007	Qualitative	growth	gas, yellow colour, gelatin liquefaction
					Hafnia alvei WDCM00095			no colour change or red, no gelatin liquefaction
Lactose-sulfite medium	*Clostridium perfringens*	AS5013.16 (ISO7937)	SP	46ºC/ 18-24h, waterbath	*Clostridium perfringens* WDCM00007	Qualitative	growth	Durham tube gas; black precipitate
					Clostridium sporogenes WDCM00008			Durham tube gas; NO black precipitate
Lauryl Tryptose Broth (LTB) (Lauryl Sulphate Broth LSB)	coliforms	AS5013.3 (ISO 4831)	P	29-31ºC/ 22-50h	*Escherichia coli* WDCM00012 *Klebsiella aerogenes* WDCM00175	Qualitative	turbidity & gas in Durham tube	Gas production and turbidity
			SE		*Enterococcus faecalis* WDCM00087 or 00009		no growth	n/a
	Escherichia coli	AS5013.15 (ISO7251)	P	36-38ºC/22-50h	*Escherichia coli* WDCM00013 or 00012	Qualitative	turbidity & gas in Durham tube	Gas production and turbidity
			SE		*Enterococcus faecalis* WDCM00087 or 00009		no growth	n/a
Lysine Decarboxylase Broth (LDC Broth)	salmonellae	AS5013.10 (ISO6579)	SP	36-38ºC/ 21-27h oil overlay in tube	*Salmonella* Hofit IMVS 1799	Qualitative	Good Growth	turbidity and purple/ pale purple
Minerals Modified Glutamate agar (MMGA)	β- D-glucuronidase positive *Escherichia coli*	AS5013-19.1 (ISO16649-1)	P	36-38ºC/ 22-26h	*Escherichia coli* WDCM00013 or 00012	Qualitative	acid production	colour change to yellow
			SP		*Enterococcus faecalis* WDCM00087 or 00009		no growth	-

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Media	Microorganisms	Standard[#]	Function	Incubation	Control strains	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133		see Guidelines Section 2.7		
Modified semi-solid Rappaport Vassiliadis agar (MSRV)	salmonellae	AS5013.10 (ISO6579)	P	40.5-42.5ºC/ 21-27h	*Salmonella* Hofit IMVS 1799	Qualitative	Grey-white turbid zone extending from point of inoculation	Optional: characteristic salmonellae colonies on subculture to XLD
			SE		*Escherichia coli* WDCM00013 or 00012		possible growth at point of inoculation, no turbid zone	-
					Enterococcus faecalis WDCM00087 or 00009		no growth	-
Mueller-Kauffmann Tetrathionate Broth with novobiocin (MKTTn)	salmonellae	AS5013.10 (ISO6579)	P	36-38ºC/ 21-27h	*Salmonella* Hofit IMVS 1799 AND *Escherichia 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		*Escherichia coli* WDCM00013 or 00012		Partial inhibition ≤ 100 colonies on TSA	-
					Enterococcus faecalis WDCM00087 or 00009		< 10 colonies on TSA	-
MYP agar (Mannitol egg-Yolk Polymyxin)	*Bacillus cereus*	AS5013.2 (ISO7932, MOD)	P	29-31ºC/ 21-48h	*Bacillus cereus* WDCM00001	Quantitative	$P_R \geq 0.5$	pink colonies with precipitation halo
			SE	29-31ºC/ 40-48h	*Escherichia coli* WDCM00013 or 00012	Qualitative	no growth	-
			SP		*Bacillus subtilis subsp. spizizenii* WDCM00003		-	yellow colonies without precipitation halo
Nitrate motility medium	*Clostridium perfringens*	AS5013.16 (ISO7937)	SP	36-38ºC/ 22-26h, anaerobic	*Escherichia coli* WDCM00013 or 00012	Qualitative	growth	motility positive: diffuse growth from stab point
					Clostridium perfringens WDCM00007			motility negative: growth at stab point, no diffusion nitrate positive: red colour after reagents added
					Clostridium sporogenes WDCM00008			nitrate negative: no red colour after reagents added; red colour after addition of zinc dust
Nutrient agar	salmonellae	AS5013.10 (ISO6579)	P	36-38ºC/ 22-26h	*Salmonella* Hofit IMVS 1799	Qualitative	good growth	n/a
	Enterobacteriaceae	AS5013.8.1 (ISO21528-1) AS5013.8.2 (ISO21528-2)			*Escherichia coli* WDCM00013 or 00012			
Plate Count Agar (PCA)	total aerobic count	AS5013.14.3 AS5013.5 (ISO4833) ^	P	29-31ºC/ 69-75h	*Escherichia coli* WDCM00013 or 00012 *Staphylococcus aureus* WDCM00034 ^ add *Bacillus subtilis subsp. spizizenii* WDCM00003	Quantitative	$P_R \geq 0.7$	-
Preston agar	Campylobacter	AS5013.6	P	41-43ºC/ 40-48h, microaerobic	*Campylobacter jejuni* WDCM00005 *Campylobacter coli* WDCM00072	Qualitative	growth	smooth, flat, translucent, colourless to grey colonies spreading along the streak line
			SE		*Escherichia coli* WDCM00013 or 00012 *Staphylococcus aureus* WDCM00034		inhibition	-
Preston Broth with antibiotic supplement	Campylobacter	AS5013.6	P	41-43ºC/ 40-48h, microaerobic	*Campylobacter jejuni* WDCM00005 *Campylobacter coli* WDCM00072	Qualitative	growth	growth on subculture on selective medium
			SE		*Escherichia coli* WDCM00013 or 00012 *Proteus mirabilis* WDCM00034		inhibition	inhibited or no growth on subculture on selective medium

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		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133		see Guidelines Section 2.7		
Rappaport-Vassiliadis soya peptone (RVS) broth	salmonellae	AS5013.10 (ISO6579)	P	40.5-42.5ºC/ 21-27h	*Salmonella* Hofit IMVS 1799 AND *Escherichia 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		*Escherichia coli* WDCM00013 or 00012		Partial inhibition ≤ 100 colonies on TSA	-
					Enterococcus faecalis WDCM00087 or 00009		< 10 colonies on TSA	-
Salt tolerance medium	enteropathogenic *Vibrio* species	AS5013.18.1 (ISO21872-1)	SP	34-38ºC/46-50h	*Vibrio parahaemolyticus* WDCM00185	Qualitative		
0% NaCl							NO growth	-
6% NaCl							growth	-
10% NaCl							NO growth	-
Skirrow agar	Campylobacter	AS5013.6	P	41-43ºC/ 40-48h, microaerobic	*Campylobacter jejuni* WDCM00005 *Campylobacter coli* WDCM00072	Qualitative	good growth	smooth, flat, translucent, colourless to grey colonies spreading along the streak line
			SE		*Escherichia coli* WDCM00013 or 00012 *Staphylococcus aureus* WDCM00034		inhibition	-
STAA agar	*Brocothrix thermosphacta*	AS5013.22 (ISO13722)	P	22-25ºC/ 44-52h	*Brochothrix thermosphacta* WDCM00071	Qualitative	growth	-
Sulphite Cycloserine (SC) agar	*Clostridium perfringens*	AS5013.16 (ISO7937)	P	36-38ºC/ 18-22h, anaerobic	*Clostridium perfringens* WDCM00007 or 00080	Quantiitative	$P_R \geq 0.5$	black colonies
			SE		*Escherichia coli* WDCM00013 or 00012	Qualitative	no growth	-
Thioglycollate medium	*Clostridium perfringens*	AS5013.16 (ISO7937)	P	36-38ºC/ 18-24h	*Clostridium perfringens* WDCM00007	Qualitative	good growth (turbidity)	-
Thiosulphate citrate bile salts sucrose (TCBS) agar	*enteropathogenic Vibrio species*	AS5013.18.1 (ISO21872-1)	P	36-38ºC/ 21-27h	*Vibrio parahaemolyticus* WDCM00185	Qualitative	good growth	blue-green colonies
					Vibrio furnissii WDCM00186			yellow colonies
			SE		*Escherichia coli* WDCM00012 or 00013		inhibition	-
Tryptone Bile X (TBX) agar	β- D-glucuronidase positive *Escherichia coli*	AS5013-19.1 (ISO16649-1)	P	43-45ºC/18-24h	*Escherichia coli* WDCM00013 or 00012	Qualitative	growth	blue colonies
			SE		*Enterococcus faecalis* WDCM00087 or 00009		no growth	-
			SP		*Citrobacter freundii* WDCM00006 *Pseudomonas aeruginosa* WDCM00025		-	white to green-beige colonies
modified Tryptone soya broth (mTSB)	*Escherichia coli* O157	AS5013.26 (ISO16654, MOD)	P	36-38ºC/ 18-24h	*Escherichia coli* WDCM00014	Qualitative	good growth	-

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

All incubation conditions are aerobic unless otherwise indicated.

APPENDIX 3

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

Media	Microorganisms	Standard#	Function	Incubation	Control strains	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in ISO11133		see Guidelines Section 2.7		
Tryptone soya yeast extract agar (TSYEA)	*Listeria monocytogenes*	AS5013.24.1 (ISO11290-1 MOD) AS5013.24.2 (ISO11290-2 MOD)	P	36-38ºC/ 18-24h	*Listeria monocytogenes* WDCM00021 or 00109	Qualitative	good growth	-
Tryptone soya yeast extract broth (TSYEB)				24-26ºC/ 18-24h			turbidity	
Tryptone water	*Escherichia coli*	AS5013.15 (ISO7251)	SP	44ºC/ 46-50h	*Escherichia coli* WDCM00012 or 00013	Qualitative	Growth	Indole +ve after addition of indole reagent
					Klebsiella aerogenes WDCM00175		Growth	Indole -ve after addition of indole reagent
Violet Red Bile agar (VRBA)	coliforms	AS5013.4 (ISO4832)	P	29-31ºC/22-26h	*Escherichia coli* WDCM00013 or 00012	Quantitative	$P_R \geq 0.5$	purple 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	AS5013.8.1 (ISO21528-1) AS5013.8.2 (ISO21528-2)	P	36-38ºC/22-26h	*Escherichia coli* WDCM00013 or 00012	Quantitative	$P_R \geq 0.5$	pink to red colonies, with or without precipitation halo
			SE		*Enterococcus faecalis* WDCM00087 or 00009	Qualitative	inhibition	n/a
XLD agar	salmonellae	AS5013.10 (ISO6579)	P	36-38ºC/ 21-27h	*Salmonella* Hofit IMVS 1799	Qualitative	good growth	red colonies black centres
			SE		*Escherichia coli* WDCM00013 or 00012		limited or poor growth	yellow colonies
					Enterococcus faecalis WDCM00087 or 00009		no growth	-

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

All incubation conditions are aerobic unless otherwise indicated.

APPENDIX 4
Batch Quality Control for Growth and Performance Testing of Media for Water Microbiology

Media	Microorganisms	Standard#	Function	Incubation	QC strains	Method of Control	Criteria	Characteristic reactions
		(# = current issue)	see footnotes	as recommended/listed in Std or in AS5140		see Guidelines Section 2.7		
DILUENTS saline solution, 0.1% peptone, 0.1% peptone salt solution(PSS), phosphate buffer solution, Ringer's solution 1/4 strength		AS4276.1 (ISO8199 MOD)	D	20-25°C/ 45min-1hr	*Escherichia coli* WDCM00013 or 00012 *Staphylococcus aureus* WDCM00034 or 00035	Quantitative	+/- 30% colonies vs time 0	n/a
Alkaline peptone water	*Vibrio cholerae*	AS4276.15	NS	34-38ºC/ 18-24h	*Vibrio cholerae* WDCM00203	Qualitative	>10 colonies recovery on TCBS	yellow colonies
Baird-Parker medium (B-P) containing egg yolk	coagulase-positive staphylococci including *Staphylococcus aureus* - membrane filtration method	AS4276.20	P	35-37ºC/ 24-48h	*Staphylococcus aureus* WDCM00035	Quantitative	$P_R \geq 0.5$	Black shiny colonies, opaque zones surrounded by clear zones
			SP		*Staphylococcus epidermidis* NCTC6513	Qualitative	growth	Black colonies, not shiny, no clearing
			SE	35-37ºC/46-50h	*Escherichia coli* WDCM00012 or 00013	Qualitative	Inhibition	-
BCYE	Legionella	AS3896 AS5132	P	34-38ºC/ 2-5d	*Legionella pneumophila* WDCM00107 AND *Tatlockia micdadei* ATCC®33218™/NCTC11371 OR *Fluoribacter bozemanae* NCTC11368/ATCC®33217™	Quantitative	$P_R \geq 0.7$	colonies 1-2mm diameter, grey-white, circular, smooth, raised with entire edge, ground-glass appearance
BCYE +BMPA BCYE + MWY BCYE + antibiotics	Legionella	AS3896 AS5132	P	34-38ºC/ 2-5d	*Legionella pneumophila* WDCM00107 AND *Tatlockia micdadei* ATCC®33218™/NCTC11371 OR *Fluoribacter bozemanae* NCTC11368/ATCC®33217™	Quantitative	$P_R \geq 0.5$	colonies 1-2mm diameter, grey-white, circular, smooth, raised with entire edge, ground-glass appearance
			SE	34-38ºC/ 72h	*Pseudomonas aeruginosa* WDCM00024	Qualitative	total or partial inhibition	-
BCYE-GVPC	Legionella	AS3896 AS5132	P	34-38ºC/ 2-5d	*Legionella pneumophila* WDCM00107 AND *Tatlockia micdadei* ATCC®33218™/NCTC11371 OR *Fluoribacter bozemanae* NCTC11368/ATCC®33217™	Quantitative	$P_R \geq 0.5$	colonies 1-2mm diameter, grey-white, circular, smooth, raised with entire edge, ground-glass appearance
			SE	34-38ºC/ 72h	*Pseudomonas aeruginosa* WDCM00024	Qualitative	total or partial inhibition	-
					Enterococcus faecalis WDCM00009 or 00087		total inhibition	-
					Escherichia coli WDCM00012 or 00013		total or partial inhibition	-
Blood agar + neomycin	*Clostridium perfringens*	AS4276.17.1 AS4276.17.2	P	34-38ºC/ 21-27h	*Clostridium perfringens* WDCM00007	Qualitative	Growth	large spreading colonies
			SE		*Escherichia coli* WDCM00090		inhibition	-

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

All incubations are aerobic unless otherwise indicated

APPENDIX 4
Batch Quality Control for Growth and Performance Testing of Media for Water Microbiology

Media	Microorganisms	Standard# (# = current issue)	Function (see footnotes)	Incubation (as recommended/listed in Std or in AS5140)	QC strains	Method of Control (see Guidelines Section 2.7)	Criteria	Characteristic reactions
Bolton Broth	thermophilic campylobacters	AS 4276.19	P	41-42ºC/ 40-48h, microaerobic (5-6%O_2, 10% CO_2)	*Campylobacter jejuni* WDCM00005 or 00156	Qualitative	growth	>10 colonies growth on subculture on mCCDA
					Campylobacter coli WDCM00004 or 00072			
			SE		*Escherichia coli* WDCM00090		inhibition	inhibited/ no growth on subculture on mCCDA
Buffered peptone water	salmonellae	AS4276.14 (ISO19250)	NS	34-38ºC/ 16-20h	*Salmonella* Hofit IMVS1799	Qualitative	Good growth	turbidity
Campylobacter agar mCCDA	thermophilic campylobacters	AS 4276.19	P	41-42ºC/ 40-48h, microaerobic (5-6%O_2, 10% CO_2)	*Campylobacter jejuni* WDCM00005 or 00156	Qualitative	growth	flat, greyish colonies spreading along the streak line
					Campylobacter coli WDCM00004 or 00072			
			SE		*Escherichia coli* WDCM00090		total or partial inhibition	-
Chromogenic coliform agar (CCA)	*Escherichia coli* and coliform bacteria - membrane filtration method for waters with low bacterial background flora	AS4276.22	P	43.5-44.5ºC/ 46-50h	*Escherichia coli* WDCM00012 or 00013 or 00090	Quanitative	$P_R \geq 0.5$	Dark blue to violet colonies
					Klebsiella aerogenes WDCM00175			Pink colonies
			SP		*Pseudomonas aeruginosa* WDCM00024	Qualitative	growth	colourless colonies
Chromogenic *E.coli* /coliform selective agar	Coliforms, *Escherichia coli* and thermotolerant coliforms—Membrane filtration method	AS4276.5	P	34-38ºC/ 21-24h	*Escherichia coli* WDCM00012 or 00013 or 00090	Quanitative	$P_R \geq 0.5$	Blue colonies
					Klebsiella pneumoniae			Pink colonies
			SP		*Pseudomonas aeruginosa* WDCM00024	Qualitative	-	no blue or pink colonies
			P	43.5-44.5ºC/ 20-22h	*Escherichia coli* WDCM00012 or 00013 or 00090	Quanitative	$P_R \geq 0.5$	Blue colonies
					Klebsiella pneumoniae			Pink colonies
			SP		*Pseudomonas aeruginosa* WDCM00024	Qualitative	-	colourless colonies
Differential reinforced clostridial medium (DRCM)	*Clostridium perfringens* MPN	AS4276.17.2	P	34-38ºC/ 44-52h AnO_2	*Clostridium perfringens* WDCM00007	Qualitative	growth	blackening of the medium
			SP		*Clostridium sporogenes* WDCM00008			no blackening
EC Broth + MUG	Coliforms, *Escherichia coli* and thermotolerant coliforms - Membrane filtration method, MPN	AS4276.5 AS4276.6	P	43.5-44.5ºC/ 46-50h	*Escherichia coli* WDCM00090 or 00179	Qualitative	growth	turbidity, gas production and fluorescence
			SP		*Klebsiella aerogenes* WDCM00175			turbidity, no gas production or fluorescence
			SE		*Pseudomonas aeruginosa* WDCM00024		no growth	-
EHS medium with ONPG and MUG	Examination of coliforms and *Escherichia coli*—MPN using enzyme hydrolysable substrates	AS4276.21 (ISO9308-2 MOD)	P	34-38ºC/ 18-22h	*Escherichia coli* WDCM00013 or 00090	Quantitative	$P_R \geq 0.7$	ONPG +ve, MUG +ve
					Klebsiella pneumoniae WDCM00206			ONPG +ve, MUG -ve
			SE		*Pseudomonas aeruginosa* WDCM00024 or 00025	Qualitative	inhibition	ONPG -ve, MUG -ve

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APPENDIX 4
Batch Quality Control for Growth and Performance Testing of Media for Water Microbiology

Media	Microorganisms	Standard# (# = current issue)	Function (see footnotes)	Incubation (as recommended/listed in Std or in AS5140)	QC strains	Method of Control (see Guidelines Section 2.7)	Criteria	Characteristic reactions
Exeter Broth (Modified)	thermophilic campylobacters	AS 4276.19	P	41-42ºC/ 40-48h, microaerobic (5-6%O_2, 10% CO_2)	*Campylobacter jejuni* WDCM00005 or 00156	Qualitative	growth	>10 colonies growth on subculture on mCCDA
					Campylobacter coli WDCM00004 or 00072			
			SE		*Escherichia coli* WDCM00090		inhibition	inhibited/ no growth on subculture on mCCDA
Heart Infusion Broth (BHIB)	coagulase-positive staphylococci including *Staphylococcus aureus* - membrane filtration method	AS4276.20	NS	35-37ºC/ 18-24h	*Staphylococcus aureus* WDCM00035	Qualitative	Growth	-
Improved Formate Lactose Glutamate medium (IFLG) (also known as Minerals Modified Glutamate Medium)	Coliforms, *Escherichia coli* and thermotolerant coliforms - MPN	AS4276.6	P	34-38ºC/ 18-24h	*Escherichia coli* WDCM00090	Qualitative	growth	acid production
					Klebsiella aerogenes WDCM00175			
			SP		*Pseudomonas aeruginosa* WDCM00024			no acid production
Lysine decarboxylase broth	salmonellae	AS4276.14 (ISO19250)	SP	34-38ºC/ 16-20h	*Salmonella* Hofit IMVS1799	Qualitative	Growth	turbidity, purple to pale purple
					Citrobacter freundii WDCM00006			turbidity, yellow
MacConkey agar	Coliforms, *Escherichia coli* and thermotolerant coliforms - MPN	AS4276.6	P	34-38ºC/ 18-24h	*Escherichia coli* WDCM00090	Qualitative	growth	red/pink colonies
					Klebsiella aerogenes WDCM00175			red/pink colonies
			SP		*Pseudomonas aeruginosa* WDCM00024			pale/colourless colonies
mEI agar	Enterococci - membrane filtration method	AS4276.9	P	40.5-41.5ºC/ 22-26h	*Enterococcus faecalis* WDCM00009 or 00087 or 00176	Quanitative	$P_R \geq 0.5$	colonies >0.5mm with blue halo
			SE		*Staphylococcus aureus* WDCM00032 or 00034	Qualitative	inhibition	-
					Escherichia coli WDCM00012 or 00013 or 00090			
MI agar	Coliforms, *Escherichia coli* and thermotolerant coliforms - Membrane filtration method	AS4276.5	P	34-38ºC/ 21-24h	*Escherichia coli* WDCM00012 or 00013 or 00090	Quanitative	$P_R \geq 0.5$	Blue colonies with or without fluorescence
					Klebsiella aerogenes WDCM00175			colourless fluorescent colonies
			SP		*Pseudomonas aeruginosa* WDCM00024	Qualitative	-	no blue colonies, no fluorescence
Milk agar with cetrimide	*Pseudomonas aeruginosa* - membrane filtration method	AS4276.13	SP	34-38ºC/ 18-24h	*Pseudomonas aeruginosa* WDCM00024	Qualitative	growth	clear zones around colonies, pigmented colonies
					Pseudomonas fluorescens WDCM00115			no clearing zones, no pigment
m- heterotrophic plate count agar (m-HPC)	heterotrophs	AS4276.3	P	34-38ºC/ 40-48h	*Escherichia coli* WDCM00012 or 00013 or 00090 *Bacillus subtilis subsp spizizenii* WDCM00003	Quanitative	$P_R \geq 0.7$	

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

All incubations are aerobic unless otherwise indicated

APPENDIX 4
Batch Quality Control for Growth and Performance Testing of Media for Water Microbiology

Media	Microorganisms	Standard# (# = current issue)	Function see footnotes	Incubation as recommended/listed in Std or in AS5140	QC strains	Method of Control see Guidelines Section 2.7	Criteria	Characteristic reactions
m-PA-C agar	*Pseudomonas aeruginosa* - membrane filtration method	AS4276.13	P	41-42ºC/ 40-48h	*Pseudomonas aeruginosa* WDCM00024	Quanitative	$P_R \geq 0.5$	colonies typically 0.8 to 2.2mm diameter, flat, light outer rims and brownish to green-black centres.
			SE		*Escherichia coli* WDCM00090	Qualitative	inhibition	-
Muller-Kaufmann tetrathionate novobiocin broth (MKTTn)	salmonellae	AS4276.14 (ISO19250)	P	36-38ºC/ 21-27h	*Salmonella* Hofit IMVS1799 AND *Escherichia coli* WDCM00012 or 00013 AND *Pseudomonas aeruginosa* WDCM00025	Qualitative	Growth	recovery ≥ 10colonies on XLD / 2nd medium of choice
			SE		*Escherichia coli* WDCM00012 or 00013		partial inhibition	≤ 100 colonies on TSA
					Enterococcus faecalis WDCM00009 or 00087		inhibition	≤ 10 colonies on TSA
Nutrient agar	heterotrophs	AS4276.3	P	34-38ºC/ 40-48h	*Escherichia coli* WDCM00012 or 00013 or 00090 *Bacillus subtilis subsp spizizenii* WDCM00003	Quanitative	$P_R \geq 0.7$	
Oleandomycin polymyxin sulphadiazine perfringens (OPSP) agar	*Clostridium perfringens*	AS4276.17.1 AS4276.17.2	P	34-38ºC/ 18-24h AnO_2	*Clostridium perfringens* WDCM00007	Quanitative	$P_R \geq 0.5$	2-4mm black colonies
			SE		*Escherichia coli* WDCM00012 or 00013	Qualitative	inhibition	-
Plate Count Agar (PCA)	heterotrophs	AS4276.3	P	34-38ºC/ 40-48h	*Escherichia coli* WDCM00012 or 00013 or 00090 *Bacillus subtilis subsp spizizenii* WDCM00003	Quanitative	$P_R \geq 0.7$	
R2A Agar	heterotrophs	AS4276.3	P	34-38ºC/ 40-48h	*Escherichia coli* WDCM00012 or 00013 or 00090 *Bacillus subtilis subsp spizizenii* WDCM00003	Quanitative	$P_R \geq 0.7$	
Rappaport-Vassiliadis soya (RVS) medium	salmonellae	AS4276.14 (ISO19250)	P	40.5-42.5ºC/ 21-27h	*Salmonella* Hofit IMVS1799 AND *Escherichia coli* WDCM00012 or 00013 AND *Pseudomonas aeruginosa* WDCM00025	Qualitative	Growth	recovery ≥ 10colonies on XLD / 2nd medium of choice
			SE		*Escherichia coli* WDCM00012 or 00013		partial inhibition	≤ 100 colonies on TSA
					Enterococcus faecalis WDCM00009 or 00087		inhibition	≤ 10 colonies on TSA

Function D= dilution
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SP=specificity NS=nonselective

All incubations are aerobic unless otherwise indicated

APPENDIX 4
Batch Quality Control for Growth and Performance Testing of Media for Water Microbiology

Media	Microorganisms	Standard# (# = current issue)	Function (see footnotes)	Incubation (as recommended/listed in Std or in AS5140)	QC strains	Method of Control (see Guidelines Section 2.7)	Criteria	Characteristic reactions
salt tolerance media	*Vibrio cholerae*	AS4276.15	SP	34-38ºC/ 18-24h	*Vibrio cholerae* WDCM00203	Qualitative		-
Tryptone water + 0% NaCl							growth	
Tryptone water + 3% NaCl							growth	
Tryptone water + 8% NaCl							no growth	
Selenite cystine broth	salmonellae	AS4276.14 (ISO19250)	P	34-38ºC/ 16-20h	*Salmonella* Hofit IMVS1799	Qualitative	Growth	recovery on XLD / 2nd medium of choice
			SE		*Citrobacter freundii* WDCM00006		partial inhibition	≤ 100 colonies on TSA
					Enterococcus faecalis WDCM00009		inhibition	≤ 10 colonies on TSA
Thiosulphate citrate bile salts sucrose (TCBS) agar	*Vibrio cholerae*	AS4276.15	SE	34-38ºC/ 18-24h	*Vibrio cholerae* WDCM00203	Qualitative	growth	Smooth flat yellowish-brown colonies, surrounded by yellow zones in medium
			SP		*Escherichia coli* WDCM00012 or 00013 or 00090		inhibition	-
Tryptone soya agar (TSA)	reference	various	P	34-38ºC/ 18-24h	*Escherichia coli* WDCM00090 *Enterococcus faecalis* WDCM00087 *Pseudomonas aeruginosa* WDCM00024	Quanitative	$P_R \geq 0.7$	-
Tryptose Sulphite Cycloserine (TSC) agar without egg yolk	*Clostridium perfringens*	AS4276.17.1 AS4276.17.2	P	34-38ºC/ 44-52h AnO_2	*Clostridium perfringens* WDCM00007	Quanitative	$P_R \geq 0.5$	2-4mm black colonies
			SE		*Escherichia coli* WDCM00012 or 00013	Qualitative	inhibition	-
Urea agar	salmonellae	AS4276.14 (ISO19250)	SP	34-38ºC/ 16-20h	*Salmonella* Hofit IMVS1799	Qualitative	Growth	urease negative
					Proteus mirabilis WDCM00023			urease positive
XLD agar	salmonellae	AS4276.14 (ISO19250)	P	34-38ºC/ 21-27h	*Salmonella* Hofit IMVS1799	Qualitative	growth	reddish transparent colonies black centres
			SE		*Escherichia coli* WDCM00012 or 00013		growth or partial inhibition	yellow colonies
					Enterococcus faecalis WDCM00009 or 00087		inhibition	-
Yeast Extract agar (YEA)	heterotrophs	AS4276.3	P	34-38ºC/ 40-48h	*Escherichia coli* WDCM00012 or 00013 or 00090 *Bacillus subtilis subsp spizizenii* WDCM00003	Quanitative	$P_R \geq 0.7$	

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All incubations are aerobic unless otherwise indicated

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