Guidelines for Assuring Quality

of

Medical Microbiological Culture Media

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

2nd edition
July 2012

The Australian Society for Microbiology
bringing Microbiologists together

FOREWORD to the First Edition

The Media Quality Control Special Interest Group 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.

It fell upon members of the Australian Society for Microbiology, the only professional or learned society in Australia dealing specifically with issues in microbiology, to establish some guidelines. To that end, the Media Quality Control Special Interest Group established a working party to devise a set of guidelines and it was agreed that they should not be dissimilar in content to the standard, *Quality Assurance for Commercially Prepared MicrobiologicalCulture Media,* Document M22-A, published by the National Committee for Clinical Laboratory, Standards in the USA in 1990. Appropriate provision should be made for the forms of microbiological media used routinely in Australia and any other local idiosyncrasies and the guidelines should complement NATA Technical Note No. 4*, Guidelines for the Quality Management of Microbiological Media***,** in providing specific instruction as to how testing of media should be performed.

The working party has produced this document based on consensus. It is intended to offer guidance to medical microbiology laboratories of any size, whether they prepare media in-house, purchase it commercially, or obtain it from a central facility within their greater organization. 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.

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FOREWORD to the Second Edition

Much has happened in the sixteen years since the first edition of these Guidelines for medical microbiological culture media were released. A draft revision prepared (and in limited release) in 2001 was with the intent of incorporating medical mycological culture media. This amalgamated version of guidelines was abandoned, after further discussion and consultation concluded that a separate set of guidelines was more appropriate for the mycology media. Unfortunately, release of the separate mycology document was delayed longer than originally planned or intended; however, this has also allowed for further improvements to the document before its final release in 2012 (1).

In 2000 the Culture Media Special Interest Group (SIG) began collaboration with the Mycobacteria SIG to produce a separate document for assuring quality of solid media used in Australia for cultivation of medically important mycobacteria, and resulted in the release of guidelines in 2004. A second edition of the mycobacteria guidelines have now been released in 2012 (2).

Through 2002 to 2004, the Culture Media SIG also worked on producing a new set of guidelines for assuring quality of food and water microbiological culture media, and the guidelines were released in 2004. These filled a significant gap, and were also cited as the basis for converting the existing International Standards Organisation (ISO) technical specifications for quality control of culture media used in food microbiology (3), to a full ISO Standard that also incorporates media used in water microbiology (4). The Food and Water Guidelines have been revised and a second edition released in 2012 (5).

This new edition of the Guidelines for medical microbiological culture media aims to capture and reflect those changes that have occurred since the first edition, and to re-invigorate the document's relevance in quality control and quality assurance of medical microbiological culture media. This has resulted in an expanded, larger document than previously existed.

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

For matters pertaining to assuring quality of medical mycological culture media, and to solid mycobacteria media used in Australia, please refer to the guidelines produced for those topics (1, 2).

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

Please send to admin@theasm.com.au

Please include as the Subject Line: *Medical Microbiological Media - QC Guidelines 2012 – 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 Convenors will be carried forward to be included in the next edition.
Any suggested amendments that are not accepted, or questions arising, will be included as a supplementary Q&A in the next edition, including an explanatory response.

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1.0 Introduction

As emphasized by the National Association of Testing Authorities Australia (NATA), 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 this needs to be fully documented (6, 7).

1.1 Application

These guidelines are applicable to medical microbiology laboratories and commercial suppliers who 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 medical microbiological culture media and ultimately the quality of the microbiological services of the laboratory. They should be viewed in conjunction with other relevant documentation to implement a comprehensive QA program (6, 7, 8, 9).

1.2 Scope

These guidelines pertain primarily to medical microbiological culture media used for cultivation, isolation and identification of bacteria. They do not apply to media prepared specifically for:

a) testing the susceptibility of bacteria to antimicrobial agents
b) susceptibility testing of yeasts and fungi, and general mycological media.
c) isolation and susceptibility testing of mycobacteria.

For information relating to those areas, reference should be made to the appropriate ASM Guidelines and other relevant standards (1, 2, 10, 11, 12).

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1.3 Definitions

Manufacturer:
Manufacturers of medical 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 medical microbiological culture media who purchase or receive it from a physically separate location within or outside their organisation.

Quality Assurance:
Those processes before, during and after the manufacture of medical microbiological culture media that verify the adequacy of the media for its intended purpose.

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

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 must verify that, under usual conditions, the medium or process is reliable in producing the expected outcome.

Other definitions pertaining to preparation, quality control and quality assurance of microbiological media can be found in relevant standards (4).

2.0 Media Manufacturer Quality Assurance Practices

2.1 Requirements

Quality assurance practices should include tests to: verify freedom from contamination; demonstrate the correct performance of the medium when used in the usual or widely accepted manner; and ensure against significant physical or chemical imperfections (e.g. pH, gel strength) that may compromise the utility of the media.

Performance of media listed in Appendix A should comply with expected results shown when tested according to methods described in these Guidelines.

Media not listed in the Appendix should also be tested to demonstrate satisfactory performance and a low failure rate; minimum requirements would be the Quality Control guidelines provided by the manufacturers of dehydrated culture Media in their technical manuals, or other appropriate reference texts (8, 13).

2.2 Contamination and Significant Physical Imperfections

Testing for contamination shall include sampling, incubation and inspection of individual units from each batch produced.

The sampling procedures recommended are summarised in Appendix B including notes on interpretation.

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

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

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

2.3 Control Strains of Bacteria

The control strains specified in these guidelines (see Appendix A) should be used. The cultures listed in the Appendix reflect the minimal cultures that should be used to QC media. Control strains should be cultures that exhibit typical microscopic, macroscopic and biochemical characteristics of the species, and must be cultures that have been verified and validated and whose lineage is documented according to NATA requirements (7, 15). 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 microbiology laboratory may need to add wild cultures to its 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. 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 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.

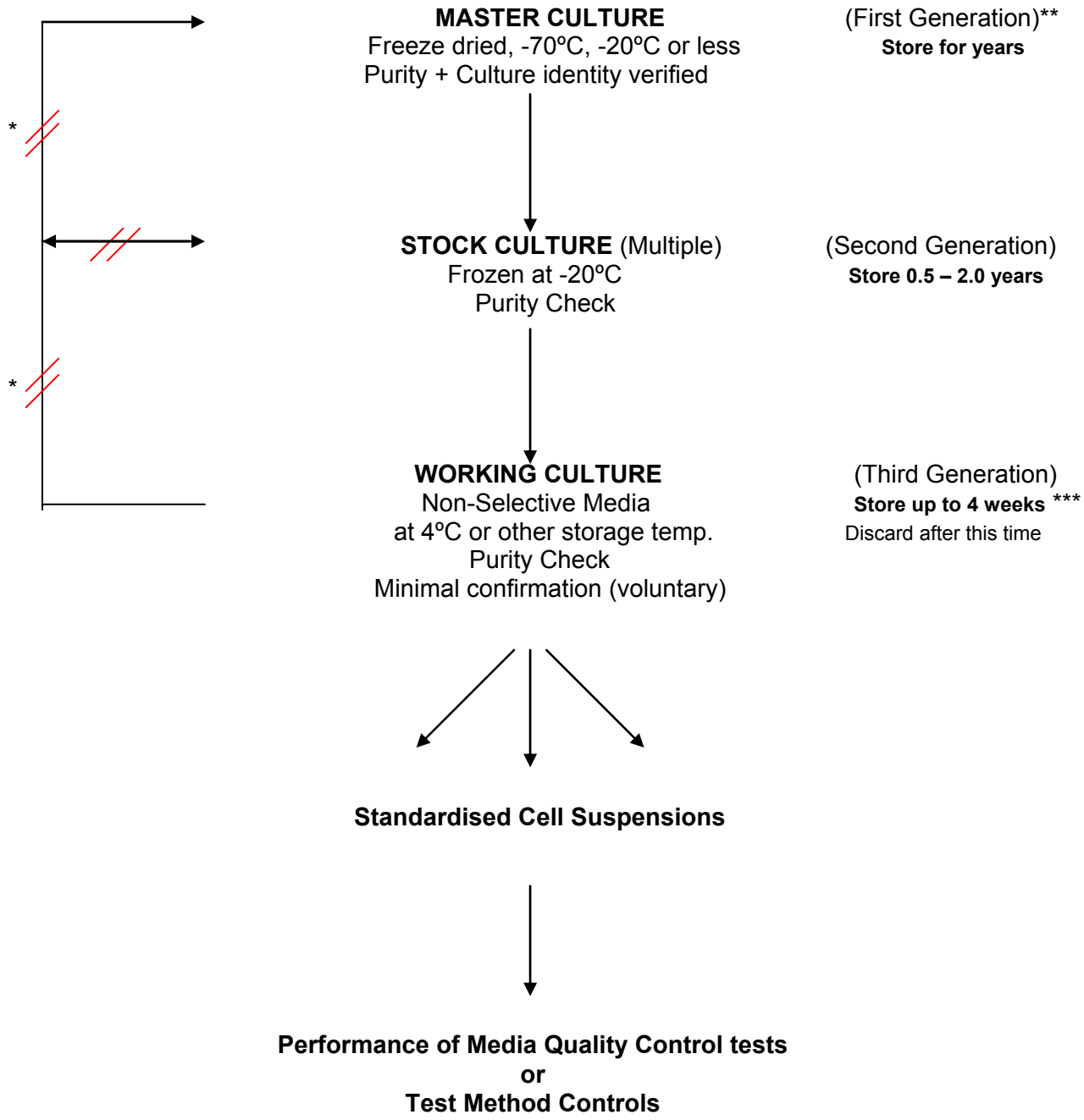
Concurrently with establishing the MASTER culture, the STOCK cultures should also be prepared. The STOCK cultures are usually glycerol broths or beads that are stored frozen. Sufficient vials should be prepared to last 3-12 months. The number of vials will be determined by the laboratory's usage rate. These "STOCK" cultures may be accessed 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 will be undermined if cultures are not appropriately managed to minimise 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 (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.

Figure 1: Maintaining a Culture Collection*



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

2.5 Test Procedures for Culture Media

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

a. Suspend three to five isolated colonies in a small volume of suitable medium and incubate 4-5 hours to achieve an exponential growth phase, or use growth from an 18-24 hour culture of the quality control organism (8). 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 must be supported by validated data, generated by the laboratory using the method.

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 10^2 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 subcultured to check correct inoculum.

e. Incubate the inoculated test media under conditions that are normally used for clinical specimens. This may include a humidified atmosphere with elevated carbon dioxide ($C0_2$) levels, or atmospheres suitable for anaerobic or microaerophilic microorganisms. Normally the incubation period will be 18-24 hours or 40-48 hours at 35 $\pm$ 2°C, depending on the medium being tested.

2.6 Parameters to be Measured in Test Procedures

For the interpretation of the results of the tested media, it is necessary to have tools which enable the comparison of the amount of growth. 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 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 $\geq$ 100cfu.

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

2.6.2 Selectivity

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

For quantitative methods, the Selectivity Factor S_F is calculated quantitatively 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 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.

NOTE e.g: if D_0 10^{-4} = $\log_{10}$ 4.0 and D_S 10^{-3} = $\log_{10}$ 3.0 then the selectivity factor S_F = 1.0

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

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

2.6.3 Specificity

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

2.7 Growth Recovery of Control Microorganisms

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

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

2.7.1.1 Non-selective solid media

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 reference medium. Perform viable counts on both the test and reference medium and compare the results as described in 2.6.1. The counts for both media should be compared and the P_R calculated. Each laboratory needs to set its own acceptance/rejection criteria, (but with an acceptance criteria of at least 70% recovery) and with reference to Appendix A. Furthermore, 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

Verification of each new lot/batch of selective medium used to enumerate specific pathogens is made by comparison to a previously passed 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 2.6.1. The counts on both media should be compared and the P_R calculated. It is expected that at least 50% 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 S_F of non-target microorganisms on most selective media should be at least 2.

Each laboratory needs to establish its own acceptance/rejection criteria with reference to Appendix A. 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 (see 2.8.1.2).

2.7.1.3 Non-selective liquid media

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.

2.7.1.4 Selective liquid media

Between 10-100 cfu of the test organism is inoculated into both the test medium and a non-selective reference broth (TSB or BHI). At the same time the negative control organism at ≥1000cfu needs to be inoculated into a 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.

Figure 2: Selective Liquid Medium Testing

Test Medium			Non Selective Reference Medium		
+ve control org. 10-100cfu ↓subculture	-ve control org. ≥1000cfu ↓subculture	Mix(+ve &-ve) ↓subculture	+ve control org. 10-100cfu ↓subculture	-ve control org. ≥1000cfu ↓subculture	Mix(+ve &-ve) ↓subculture
Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)	Non-Inhibitory Medium (±Indicator)
Count	Count	Count Both Org.	Count	Count	Count Both Org.
Determine: % Recovery of the +ve organism % Inhibition of –ve organism % Recovery of the +ve organism from mix must 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 (4).

2.7.2.1 Non-selective solid media

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 a standardised streak plate method on both the test and reference medium and compare the results as described in 2.6.1. 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. Each laboratory needs to set its own acceptance/rejection criteria (but with an acceptance criteria of at least 70% recovery) and with reference to Appendix A. Furthermore, the medium also needs to be assessed for typical morphology and colony size to complete the performance evaluation on the medium.

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.

2.7.2.2 Selective solid media

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 a standardised streak plate method on both the test and reference medium and compare the results as described in 2.6.1. 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 50% 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 A. 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 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.

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. The growth of unwanted microorganisms should be reduced (weak growth or 1), or completely inhibited (no growth or 0).

2.7.2.3 Non-selective liquid media

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 qualitative 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.

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 may need to 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 Interpretation of Quantitative Recovery Results

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 Interpretation of Qualitative Recovery Results

%RGI: >70% (wanted organism) nonselective medium
>50% (wanted organism) selective medium
< 25% (unwanted organism)

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
Reject if fails to suppress background flora.

2.9 Reporting Quality Assurance Data to Users

Manufacturers testing medical microbiological culture media according to these Guidelines may affix 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 must detail intended use and storage conditions, strains tested, testing method, incubation temperature, period and atmosphere, the final pH of the medium and the procedure used for testing for contamination.
If compliance certificates are issued, such certifications must also include the strains tested and their performance, incubation temperature, period and atmosphere, the final pH of the medium and the procedure used for testing for contamination.

3.0 Packaging, Transport and Storage

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

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

Prepared media should be stored in such a way as to minimise moisture loss and provide protection against physical and microbial contamination, as well as against light-induced damage and thermal damage. Prepared media should be stored in unopened or resealed packages at 2-8^{o}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 indicated (this may be on the product, or on the packaging, or on the conformity certificate).
Validations of expiry dates should be based on evaluations of the performance of samples of each type of medium according to these guidelines. Where media is prepared commercially or for distribution outside the manufacturing laboratory, such validations should include simulated transportation phase(s) in the storage/testing protocol. Such simulated phases should reflect the least favourable conditions likely to be encountered during transportation. Conditions to which the media are exposed during transport should be evaluated using suitable measuring devices i.e. temperature indicator or electronic monitor.

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

Validation of Shelf Life Example: Method 1

Prepare a batch of the medium to be shelf life validated. This should be of a size that will allow testing with a number of different microorganisms per x number of weeks. (10 organisms for 10 weeks = 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 med*ium *is constant but the operator and the standard techniques may have week to week variation.*

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 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 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.

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 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 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.

4.0 User Quality Assurance Practices

4.1 General Requirements

Laboratories who receive prepared media accompanied by a media quality control certificate should retain these certificates in an appropriate file for a minimum of 3 years (7).
Laboratories who obtain prepared culture media either from a commercial source or a central facility, that carries a compliance label should record the following data in a log book or similar.

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

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

4.2 Physical Inspection of Plates/Tubes, Bottles

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

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

4. 3 Remedial Action for Deficiencies Observed

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

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

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

4. 4 Performance Monitoring

It is recommended that users of commercially prepared media monitor performance of the following types of media (8):
Blood-containing media selective for *Campylobacter spp.*
Media selective for pathogenic *Neisseria spp.*
Media for isolation of *Haemophilus spp*
Bordetella pertussis media
Testing should include nutrient and inhibitory performance, but not contamination.

Laboratories are also strongly encouraged to confirm the ability to support growth for *all* media used for recovery of *fastidious* organisms or those organisms with unique growth requirements (8).
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 must be undertaken until reliability is re-established.
Other commercially prepared media (from suppliers that meets all of the relevant conditions of technical accreditation (7)) need not be retested.

5.0 References

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15. NATA policy Circular 34. *Maintenance of Microbiological Reference Culture Collections (MRCC).* 2011. National Association of Testing Authorities, Sydney, Australia.

Appendix A: Recommended control strains & acceptance criteria for growth performance testing of medical microbiological culture media

Medium	Incubation	Organisms[1]	Acceptance criteria
▪ Anaerobic blood agar (nonselective)	35-37°C AnO_2 24-48hrs	*Bacteroides fragilis* NCTC 9343; ATCC® 25925™ *Peptostreptococcus anaerobius* NCTC 11460; ATCC® 27337™	Growth Growth
▪ Anaerobic blood agar (*selective agars including neomycin, naladixic acid, phenyl ethyl alcohol*)	35-37°C AnO_2 24-48hrs	*Bacteroides fragilis* NCTC 9343; ATCC® 25925™ *Peptostreptococcus anaerobius* NCTC 11460; ATCC® 27337™ *Proteus mirabilis* ATCC® 12453™	Growth Growth Inhibition (partial to complete)
▪ Anaerobic blood agar (*selective agars including neomycin+vancomycin, naladixic acid +vancomycin*)	35-37°C AnO_2 24-48hrs	*Bacteroides fragilis* NCTC 9343; ATCC® 25925™ *Proteus mirabilis* ATCC® 12453™ *Enterococcus faecalis* WDCM 00087	Growth Inhibition (partial to complete) Inhibition (partial to complete)
▪ Blood agar (nonselective)	35-37°C O_2 or CO_2 24hrs	*Streptococcus pyogenes* NCTC 12696; ATCC® 19615™ *Streptococcus pneumoniae* ATCC® 6305™ *Escherichia coli* WDCM 00013; NCTC 12241	Growth, β-haemolysis Growth, α-haemolysis Growth
▪ Blood agar (*selective agar including colistin & naladixic acid*)	35-37°C O_2 or CO_2 24hrs	*Staphylococcus aureus* WDCM 00034; NCTC 12981 *Proteus mirabilis* ATCC® 12453™ *Pseudomonas aeruginosa* WDCM 00025; NCTC 12903	Growth Inhibition (partial to complete) Inhibition (partial to complete)
▪ Blood agar (*selective agar including gentamicin*)	35-37°C O_2 or CO_2 24hrs	*Streptococcus pyogenes* NCTC 12696; ATCC® 19615™ *Pseudomonas aeruginosa* WDCM 00025; NCTC 12903 *Staphylococcus aureus* WDCM 00034; NCTC 12981	Growth, β-haemolysis Inhibition (partial to complete) No growth

1. Organisms listed were chosen based on demonstration of quality control parameters (growth, selectivity, colonial morphology). Additional microorganisms may be selected to challenge specific media types
WDCM is the World Data Centre for Microorganisms. See www.wdcm.org
ATCC is the American Type Culture Collection. See www.atcc.org

NCTC is the National Collection of Type Cultures of the UK Health Protection Agency www.hpacultures.org.uk

Appendix A: Recommended control strains & acceptance criteria for growth performance testing of medical microbiological culture media

Medium	Incubation	Organisms[1]	Acceptance criteria[2]
▪ Blood agar (*selective agars including neomycin, naladixic acid, phenyl ethyl alcohol*)	35-37°C O_2 or CO_2 24hrs	*Streptococcus pyogenes* NCTC 12696; ATCC® 19615™ *Staphylococcus aureus* WDCM 00034; NCTC 12981 *Proteus mirabilis* ATCC® 12453™	Growth, β-haemolysis Growth Inhibition (partial to complete)
▪ Burkholderia cepacia agar	35-37°C O_2 24-48hrs	*Burkholderia cepacia* NCTC 10661; ATCC® 17759™ *Pseudomonas aeruginosa* WDCM 00025; NCTC 12903 *Staphylococcus aureus* WDCM 00034; NCTC 12981 *Proteus mirabilis* ATCC® 12453™	Growth, colour, morphology Inhibition (partial to complete) Inhibition (partial to complete) Inhibition (partial to complete)
▪ Campylobacter agar	42°C microaerophilic 48hrs	*Campylobacter jejuni* WDCM 00005; NCTC 13367 *Escherichia coli* WDCM 00013; NCTC 12241	Growth Inhibition (partial to complete)
▪ Chocolate agar	35-37°C CO_2 24-48hrs	*Neisseria gonorrhoeae* ATCC® 43069™ *Haemophilus influenzae* NCTC 13377; ATCC® 10211™	Growth Growth
▪ Chromogenic agar - MRSA	35-37°C O_2 24hrs	*Staphylococcus aureus* MRSA ATCC® 33591™ *Staphylococcus aureus* WDCM 00034; NCTC 12981 *Staphylococcus aureus* WDCM 00035 *Escherichia coli* WDCM 00013; NCTC 12241 *Pseudomonas aeruginosa* WDCM 00025; NCTC 12903	Growth, colour, morphology No growth No growth No growth No growth

1. Organisms listed were chosen based on demonstration of quality control parameters (growth, selectivity, colonial morphology). Additional microorganisms may be selected to challenge specific media types
   WDCM is the World Data Centre for Microorganisms. See www.wdcm.org
   ATCC is the American Type Culture Collection. See www.atcc.org
   NCTC is the National Collection of Type Cultures of the UK Health Protection Agency www.hpacultures.org.uk
2. Chromogenic colony colour may vary between suppliers – check manufacturers' specifications

Appendix A: Recommended control strains & acceptance criteria for growth performance testing of medical microbiological culture media

Medium	Incubation	Organisms[1]	Acceptance criteria[2]
▪ Chromogenic agar – UTI	35-37°C O_2 24hrs	*Escherichia coli* WDCM 00013; NCTC 12241 *Enterococcus faecalis* WDCM 00087 *Staphylococcus aureus* WDCM 00034; NCTC 12981 *Klebsiella pneumoniae* WDCM 00097; NCTC 9633 *Proteus mirabilis* ATCC® 12453™	Growth, colour, morphology Growth, colour, morphology Growth, colour, morphology Growth, colour, morphology Growth, colour, morphology
▪ Chromogenic agar - VRE	35-37°C O_2 24-48hrs	*Enterococcus faecalis* WDCM 00085; NCTC 13379 *Enterococcus faecalis* WDCM 00087 *Enterococcus faecium* NCTC 12202 *Staphylococcus aureus* WDCM 00034; NCTC 12981 *Escherichia coli* WDCM 00013; NCTC 12241	Growth, colour, morphology No growth Growth, colour, morphology No growth No growth
▪ CIN (Yersinia) agar	35-37°C O_2 24-48hrs	*Yersinia enterocolitica* ATCC® 9610™ *Escherichia coli* WDCM 00013; NCTC 12241 *Enterococcus faecalis* WDCM 00087 *Pseudomonas aeruginosa* WDCM 00025; NCTC 12903	Growth, bull's eye colony Inhibition (partial to complete) Inhibition (partial to complete) Inhibition (partial to complete)
▪ CLED agar	35-37°C O_2 24-48hrs	*Escherichia coli* WDCM 00013; NCTC 12241 *Staphylococcus aureus* WDCM 00034; NCTC 12981 *Proteus mirabilis* ATCC® 12453™	Growth, yellow colonies Growth Growth, swarming inhibited at 24h
▪ *Clostridium difficile* agars (selective)	35-37°C AnO_2 48hrs	*Clostridium difficile* ATCC® 43593™ *Enterococcus faecalis* WDCM 00087 *Proteus mirabilis* ATCC® 12453™	Growth Inhibition (partial to complete) Inhibition (partial to complete)

1. Organisms listed were chosen based on demonstration of quality control parameters (growth, selectivity, colonial morphology). Additional microorganisms may be selected to challenge specific media types
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   NCTC is the National Collection of Type Cultures of the UK Health Protection Agency www.hpacultures.org.uk
2. Chromogenic colony colour may vary between suppliers – check manufacturers' specifications

Appendix A: Recommended control strains & acceptance criteria for growth performance testing of medical microbiological culture media

Medium	Incubation	Organisms[1]	Acceptance criteria
▪ *Haemophilus* agar (selective) (chocolate + bacitracin)	35-37°C CO_2 48hrs	*Haemophilus influenzae* NCTC 13377; ATCC® 10211™ *Streptococcus pyogenes* NCTC 12696; ATCC® 19615™	Growth Inhibition (partial to complete)
▪ Legionella CYE agar (nonselective)	35-37°C O_2 24-48hrs	*Legionella pneumophila* NCTC 11192; ATCC® 33152™ *Legionella longbeachae*	Growth Growth
▪ Legionella CYE agar (selective)	35-37°C O_2 24-48hrs	*Legionella pneumophila* NCTC 11192; ATCC® 33152™ *Legionella longbeachae* *Escherichia coli* WDCM 00013; NCTC 12241 *Staphylococcus aureus* WDCM 00034; NCTC 12981	Growth Growth Inhibition (partial to complete) Inhibition (partial to complete)
▪ MacConkey agar (without crystal violet)	35-37°C O_2 24hrs	*Escherichia coli* WDCM 00013; NCTC 12241 *Enterococcus faecalis* WDCM 00087 *Staphylococcus aureus* WDCM 00034; NCTC 12981 *Salmonella* Typhimurium WDCM 00031; NCTC 12023 *Proteus mirabilis* ATCC® 12453™	Growth, pink colonies Growth Growth, pink colonies Growth, colourless colonies Growth, colourless colonies, swarming inhibited

1. Organisms listed were chosen based on demonstration of quality control parameters (growth, selectivity, colonial morphology). Additional microorganisms may be selected to challenge specific media types
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Appendix A: Recommended control strains & acceptance criteria for growth performance testing of medical microbiological culture media

Medium	Incubation	Organisms[1]	Acceptance criteria
▪ MacConkey agar (with crystal violet)	35-37°C O_2 24hrs	*Escherichia coli* WDCM 00013; NCTC 12241 *Enterococcus faecalis* WDCM 00087 *Staphylococcus aureus* WDCM 00034; NCTC 12981 *Salmonella* Typhimurium WDCM 00031; NCTC 12023 *Proteus mirabilis* ATCC® 12453™	Growth, pink colonies Inhibition (partial to complete) Inhibition (partial to complete) Growth, colourless colonies Growth, colourless colonies, swarming inhibited
▪ Sorbitol MacConkey agar	35-37°C O_2 24-48hrs	*Escherichia coli* WDCM 00014 or ATCC® 35150™ *Escherichia coli* WDCM 00013; NCTC 12241 *Staphylococcus aureus* WDCM 00034; NCTC 12981	Growth, colourless colonies Growth, pink colonies Inhibition (partial to complete)
▪ Sorbitol MacConkey agar (with antibiotics)	35-37°C O_2 24-48hrs	*Escherichia coli* WDCM 00014 or ATCC® 35150™ *Escherichia coli* WDCM 00013; NCTC 12241 *Proteus mirabilis* ATCC® 12453™	Growth, colourless colonies Growth, pink colonies Inhibition (partial to complete)
▪ Mannitol Salt agar	35-37°C O_2 24-48hrs	*Staphylococcus aureus* WDCM 00034; NCTC 12981 *Staphylococcus epidermidis* WDCM 00036; NCTC 13360 *Escherichia coli* WDCM 00013; NCTC 12241	Growth, yellow colonies Growth, pink colonies Inhibition (partial to complete)
▪ Media for pathogenic *Neisseria* spp (selective)	35-37°C O_2 24-48hrs	*Neisseria gonorrhoeae* ATCC® 43069™ *Proteus mirabilis* ATCC® 12453™ *Enterococcus faecalis* WDCM 00087 *Candida albicans* ATCC® 10231™	Growth Inhibition (partial to complete) Inhibition (partial to complete) Inhibition (partial to complete)

1. Organisms listed were chosen based on demonstration of quality control parameters (growth, selectivity, colonial morphology). Additional microorganisms may be selected to challenge specific media types
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Appendix A: Recommended control strains & acceptance criteria for growth performance testing of medical microbiological culture media

Medium	Incubation	Organisms[1]	Acceptance criteria
▪ Media for salmonellae and shigellae (selective)	35-37°C O_2 24hrs	*Salmonella* Typhimurium WDCM 00031; NCTC 12023 *Shigella flexneri* WDCM 00126; NCTC 12698 *Escherichia coli* WDCM 00013; NCTC 12241 *Enterococcus faecalis* WDCM 00087	Growth Growth Inhibition (partial to complete) Inhibition (partial to complete)
▪ TCBS agar	35-37°C O_2 24-48hrs	*Vibrio parahaemolyticus* WDCM 00037 *Vibrio furnisii* WDCM 00186 *Proteus mirabilis* ATCC® 12453™ *Escherichia coli* WDCM 00013; NCTC 12241	Growth, blue/green colonies Growth, yellow colonies Growth, yellow/green, black centres Inhibition (partial to complete)

1. Organisms listed were chosen based on demonstration of quality control parameters (growth, selectivity, colonial morphology). Additional microorganisms may be selected to challenge specific media types
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Appendix A: Recommended control strains & acceptance criteria for growth performance testing of medical microbiological culture media

Medium	Incubation	Organisms[1]	Acceptance criteria
▪ Anaerobic broth media including cooked meat medium	35-37°C O_2 48-96hrs	*Bacteroides fragilis* NCTC 9343; ATCC® 25925™ *Peptostreptococcus anaerobius* NCTC 11460; ATCC® 27337™ *Streptococcus pyogenes* NCTC 12696; ATCC® 19615™	Growth Growth Growth
▪ Enrichment broths for enteric organisms (selective)	35-37°C O_2 18-24hrs	*Salmonella* Typhimurium WDCM 00031; NCTC 12023 *Shigella sonnei* ATCC® 9290™ *Escherichia coli* WDCM 00013; NCTC 12241	Growth on subculture Growth on subculture Inhibition (partial to complete)
▪ Group B streptococci broths (selective)	35-37°C O_2 18-24hrs	*Streptococcus agalactiae* NCTC 8181; ATCC® 13813™ *Escherichia coli* WDCM 00013; NCTC 12241 *Proteus mirabilis* ATCC® 12453™	Growth on subculture Inhibition (partial to complete) Inhibition (partial to complete)
▪ Nutrient broths (nonselective)	35-37°C O_2 24-48hrs	*Escherichia coli* WDCM 00013; NCTC 12241 *Staphylococcus aureus* WDCM 00034; NCTC 12981	Growth Growth

1. Organisms listed were chosen based on demonstration of quality control parameters (growth, selectivity, colonial morphology). Additional microorganisms may be selected to challenge specific media types
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Appendix B Sampling Plan for Medical Microbiological Culture Media

Small Batches (≤100 units): 1% or 1 unit from beginning and 1% or 1 unit from end of batch (3, 4).

Double Sampling Plan (>100 units) NORMAL SAMPLING PLAN, AQL - 2.5, GENERAL INSPECTION LEVEL = 1 (14)

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): Based on ISO/TS11133-2 (3), 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 must 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 must be rejected.

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