

Guidelines for Assuring Quality of Medical Microbiological Culture Media

*Media Quality Control Special Interest Group
Australian Society for Microbiology*

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FOREWORD

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 Microbiological Culture 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.

Media Quality Control Special Interest Group Committee

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1.0 INTRODUCTION

1.1 Application

These Guidelines are applicable to medical microbiology laboratories that manufacture or use microbiological culture media. They seek to offer direction to individuals who must implement procedures with the purpose of assuring the quality of medical microbiological culture media, and ultimately the quality of the microbiological services of the laboratory. They should be used in conjunction with NATA Technical Note No. 4, *Guidelines for the Quality Management of Microbiological Media*, to implement a comprehensive quality assurance program for the manufacture and quality control of medical microbiological culture media.

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 (even though many of the principles incorporated here may be applied)
- c) isolation and susceptibility testing of mycobacteria.

For information relating to these areas reference should be made to the appropriate Australian Society for Microbiology Special Interest Groups (SIGs) and relevant standards.

1.3 Definitions

Manufacturer: Manufacturers of medical microbiological culture media are those facilities where ingredients are weighed, mixed, sterilized, dispensed and final products are labelled and packaged. This includes facilities who prepare media for sale outside their organization or for distribution within their organization, 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 organization.

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 testing conditions, the medium or process is reliable in producing the expected outcome.

2.0 MANUFACTURER QUALITY ASSURANCE PRACTICES

2.1 Requirements

Quality assurance practices are seen as an integral part of the manufacture of medical microbiological culture media. They should include tests to verify a satisfactory level of freedom from contamination, demonstrate the correct performance of the medium when used in the usual or widely accepted manner, and ensure against significant physical imperfections that may compromise the utility of the media.

Performance of media listed in Table 1 should comply with expected results shown when tested according to methods suggested in these Guidelines, including microbial strains, incubation temperature, time and atmosphere. An incubation temperature of $35 \pm 2^{\circ}\text{C}$ should be used in all cases unless otherwise specified. Incubation conditions shown in Table 1 should not be inferred to be optimal for the isolation of the respective strains from clinical material.

Each batch of media not listed in Table 1 should also be tested to demonstrate satisfactory performance and a low failure rate. A suggested minimum requirement would be the Quality Control guidelines provided by the manufacturers of Dehydrated Culture Media in their technical manuals or appropriate reference texts (e.g. Manual Clinical Microbiology).

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. Refer NATA Technical Note 5, *Monitoring of Laboratory Steam Sterilisers*.

TABLE 1

**Control Microorganisms for Growth Performance Testing
of General Purpose Media for Medical Microbiology**

MEDIUM	INCUBATION CONDITIONS ¹	ORGANISMS (ATCC) ²	ACCEPTABLE RESULTS
SOLID MEDIA			
Anaerobic Blood Agars (non-selective)	Anaerobic, 24-48 h	<i>B. fragilis</i> (25285) <i>C. perfringens</i> (13124) <i>P. anaerobius</i> (27337)* <i>B. levii</i> (29147)* <i>F. nucleatum</i> (25586)*	Growth Growth, haemolysis Growth Growth Growth
Anaerobic Blood Agars (selective: neomycin, nalidixic acid, phenyl ethyl alcohol)	Anaerobic, 24-48 h	<i>B. fragilis</i> (25285) <i>P. anaerobius</i> (27337)* <i>P. mirabilis</i> (12453) <i>B. levii</i> (29147)*	Growth Growth Inhibition (partial to complete) Growth
Anaerobic Blood Agars (selective: neomycin + vancomycin, nalidixic acid + vancomycin)	Anaerobic, 24-48 h	<i>B. fragilis</i> (25285) <i>P. mirabilis</i> (12453) <i>B. levii</i> (29147)* <i>E. faecalis</i> (29212)	Growth Inhibition (partial to complete) Growth Inhibition (partial to complete)
Blood Agars (non-selective)	Aerobic or CO ₂ , 24 h	<i>S. pyogenes</i> (19615) <i>S. pneumoniae</i> (6305) <i>E. coli</i> (25922)	Growth, β-haemolysis Growth, α-haemolysis Growth
Blood Agars (selective: colistin + nalidixic acid)	Aerobic or CO ₂ , 24 h	<i>S. aureus</i> (25923) <i>P. mirabilis</i> (12453) <i>P. aeruginosa</i> (27853)	Growth Inhibition (partial or complete) Inhibition (partial or complete)
Blood Agar (selective: gentamicin)		<i>S. pyogenes</i> (19615) <i>P. aeruginosa</i> (27853) <i>S. aureus</i> (25923)	Growth, β-haemolysis Inhibition (partial or complete) No growth
Blood Agar (selective: neomycin, nalidixic acid, phenyl ethyl alcohol)		<i>S. pyogenes</i> (19615) <i>S. aureus</i> (25923) <i>P. mirabilis</i> (12453)	Growth, β-haemolysis Growth Inhibition (partial or complete)

1 The recommended duration of incubation for all media listed is not intended as a recommendation of the maximum incubation time for clinical specimens.

2 ATCC is a registered trademark of the American Type Culture Collection, Rockville, Md, USA.

* Larger laboratories and commercial manufacturers may include organisms marked with asterisks.

TABLE 1 (continued)

Control Microorganisms for Growth Performance Testing
of General Purpose Media for Medical Microbiology

MEDIUM	INCUBATION CONDITIONS ¹	ORGANISMS (ATCC) ²	ACCEPTABLE RESULTS
Campylobacter Agar	Microaerophilic, 48 h	<i>C. jejuni</i> (33291) <i>E. coli</i> (25922)	Growth Inhibition (partial or complete)
Chocolate Agar	CO ₂ , 24-48 h	<i>N. gonorrhoeae</i> (43069) <i>H. influenzae</i> (10211)	Growth Growth
CIN (Yersinia) Agar	Aerobic, 24-48 h	<i>Y. enterocolitica</i> (9610) <i>E. coli</i> (25922) <i>E. faecalis</i> (29212) <i>Ps. aeruginosa</i> (27853)	Growth, bull's eye colony, red centre, clear periphery Inhibition (partial or complete) Inhibition (partial or complete) Inhibition (partial or complete)
CLED Agar	Aerobic, 24-48 h	<i>E. coli</i> (25922) <i>P. mirabilis</i> (12453) <i>S. aureus</i> (25923) <i>S. pyogenes</i> (19615)	Growth, yellow colonies Growth, swarming inhibited (partially or completely) at 24h Growth Growth
<i>Clostridium difficile</i> Agars (selective)	Anaerobic, 24-48 h	<i>C. difficile</i> (43593) <i>E. faecalis</i> (29212) <i>P. mirabilis</i> (12453)	Growth Inhibition (partial or complete) Inhibition (partial or complete)
CYE/BCYE Agars (non-selective)	Aerobic, 24-48 h	<i>L. pneumophila</i> (33152) <i>L. longbeachae</i>	Growth Growth
CYE/BCYE Agars (selective)	Aerobic, 24-48 h	<i>L. pneumophila</i> (33152) <i>L. longbeachae</i> <i>S. aureus</i> (25923) <i>E. coli</i> (25922)	Growth Growth Inhibition (partial or complete) Inhibition (partial or complete)
MacConkey Agar (without crystal violet)	Aerobic, 24 h	<i>E. coli</i> (25922) <i>P. mirabilis</i> (12453) <i>S. typhimurium</i> (10428) <i>E. faecalis</i> (29212) <i>S. aureus</i> (25923)	Growth, pink colonies Growth, colourless, swarming inhibited Growth, colourless colonies Growth Growth, pink colonies

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TABLE 1 (continued)

Control Microorganisms for Growth Performance Testing
of General Purpose Media for Medical Microbiology

MEDIUM	INCUBATION CONDITIONS ¹	ORGANISMS (ATCC) ²	ACCEPTABLE RESULTS
MacConkey Agar (with crystal violet)	Aerobic, 24 h	<i>E. coli</i> (25922) <i>P. mirabilis</i> (12453) <i>S. typhimurium</i> (14028) <i>E. faecalis</i> (29212) <i>S. aureus</i> (25923)	Growth, pink colonies Growth, colourless, swarming inhibited Growth, colourless colonies Inhibition (partial or complete) Inhibition (partial or complete)
Mannitol Salt Agar	Aerobic, 24-48 h	<i>S. aureus</i> (25923) <i>S. epidermidis</i> (12228) <i>P. mirabilis</i> (12453)	Growth, colonies with yellow zones at 48 h Growth, colonies have red zones at 48 h Inhibition (partial or complete)
Mannitol Salt Agar + oxacillin	Aerobic, 24-48 h	<i>S. aureus</i> (25923) MRSA strain <i>P. mirabilis</i> (12453)	Inhibition Growth, colonies have yellow zones at 48 h Inhibition (partial or complete)
Media for pathogenic <i>Neisseria</i> spp. (selective)	CO ₂ , 24-48 h	<i>N. gonorrhoeae</i> (43069) <i>P. mirabilis</i> (43071) <i>E. faecalis</i> (29212) <i>C. albicans</i> (60193)	Growth Inhibition (partial or complete) Inhibition (partial or complete) Inhibition (partial or complete)
Media for salmonella /shigellae (selective)	Aerobic, 24 h	<i>S. typhimurium</i> (14028) <i>S. flexneri</i> (12022) <i>E. faecalis</i> (29212) <i>E. coli</i> (25922)	Growth Growth Inhibition (partial or complete) Inhibition (partial or complete)
LIQUID MEDIA			
Anaerobic broth media (thioglycollate medium with or without indicator)	Aerobic, 24-48 h	<i>P. anaerobius</i> (27337) <i>S. pyogenes</i> (19615) <i>B. fragilis</i> (25285)	Growth Growth Growth

1 The recommended duration of incubation for all media listed is not intended as a recommendation of the maximum incubation time for clinical specimens.

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* Larger laboratories and commercial manufacturers may include organisms marked with asterisks.

TABLE 1 (continued)

Control Microorganisms for Growth Performance Testing
of General Purpose Media for Medical Microbiology

MEDIUM	INCUBATION CONDITIONS ¹	ORGANISMS (ATCC) ²	ACCEPTABLE RESULTS
Cooked Meat Medium	Aerobic, 24-48 h	<i>P. anaerobius</i> (27337)	Growth
		<i>S. pyogenes</i> (19615)	Growth
		<i>B. fragilis</i> (25285)	Growth
Enrichment Broths for enteric organisms (Selenite F Broth, RV Broth)	Aerobic, 18-24 h	<i>S. typhimurium</i> (14028)	Growth on subculture to selective medium
		<i>S. sonnei</i> (9290)	Growth on subculture to selective medium (may be slightly inhibited)
		<i>E. coli</i> (25922)	Inhibition (partial or complete)
Nutrient broths (non-selective: BHI Broth, Soybean Casein Digest Broth, etc.)	Aerobic, 24-48 h	<i>E. coli</i> (25922)	Growth
		<i>S. aureus</i> (25923)	Growth

1 The recommended duration of incubation for all media listed is not intended as a recommendation of the maximum incubation time for clinical specimens.

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* Larger laboratories and commercial manufacturers may include organisms marked with asterisks.

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 procedure applied should conform to Australian Standard 1199-1988, *Sampling Procedures and Tables for Inspection by Attributes*. The sampling procedures recommended are summarised in Table 2 (single plan for smaller manufacturers and a double plan for larger manufacturers) including notes on interpretation. Incubation of all samples must be for a minimum period of 48 hours at a suitable temperature ($30 \pm 2^\circ\text{C}$ is recommended) before inspection. Use of inspected sterility samples to determine significant physical imperfections is acceptable.

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

TABLE 2: SAMPLING PLAN FOR MICROBIOLOGICAL CULTURE MEDIA**ACCORDING TO AS1199-1988**

NORMAL SAMPLING PLAN, AQL=2.5, GENERAL INSPECTION LEVEL=1

Batch Size (units made)	Sample Number		1st Sample		2nd Sample	
	1st	2nd	Ac	Re	Ac	Re
Single Sampling Plan (< 150 units)						
5 - 15	2		0	1		
16 - 25	3		0	1		
26 - 90	5		0	1		
91 -150	5		0	1		
Double Sampling Plan (> 150 units)						
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	4	4	5
3201 - 10000	50	50	2	5	6	7

Interpretation:

Small Batches (<150 units): Based on AS1199-1988 a single sample plan is recommended as being the most cost effective option for sampling small batches of media. When sterility testing small batches it is more economical to reject the batch and prepare a new one than devote time and resources to repeat testing. If the number of contaminated/defective items in the sample is zero, the batch may be accepted. If the number of contaminated/defective items in the sample is equal to or greater than one, the batch must be rejected.

Large Batches (>150 units): A double normal sampling plan provides for a second set of samples to be taken where larger lots are prepared and fail to be accepted after the first sample is examined. If, after inspection of the initial sample, the number of contaminated items lies between the Ac and Re levels, a second sample may be taken and tested. If the cumulative total of contaminated items, i.e. first sample plus second sample, is equal to or less than the second sample level of acceptance (Ac), the batch may be accepted. If however, the cumulative total of contaminated items, i.e. first sample plus second sample, is equal to or greater than the second sample level of rejection (Re), the batch must be rejected.

2.3 Control Strains of Bacteria

The control strains specified in these Guidelines (see Table 1) should be used. Strains should be sourced directly from an internationally recognised culture collection, such as the American Type Culture Collection (Rockville, Maryland, USA), the National Collection of Type Cultures (London, UK) or the Australian Collection of Microorganisms (Brisbane, Australia). Use of cultures for which no subculture or handling history is available is strongly discouraged.

2.4 Maintenance of Master Cultures of Control Strains

Maintenance of master cultures to be used for Quality Control purposes must be standardised in a manner that will minimize the opportunity to contaminate or alter the growth characteristics. It is recommended that NATA Technical Note No. 14, *Maintenance and Preservation of Microbial Cultures in a Laboratory Culture Collection*, be referred to for acceptable methods of maintaining stocks of control strains. The method selected should be compatible with the laboratory's resources. Phenotypic and genotypic changes in cultures may occur through repeated subculture and exposure to physical and/or chemical conditions that induce alterations. Management of the laboratory culture collection shall follow guidelines provided by the technical note and should include regular verification of the identity, viability and purity of each strain. This especially should be performed each time a strain is introduced into the collection.

2.5 Working Control Cultures

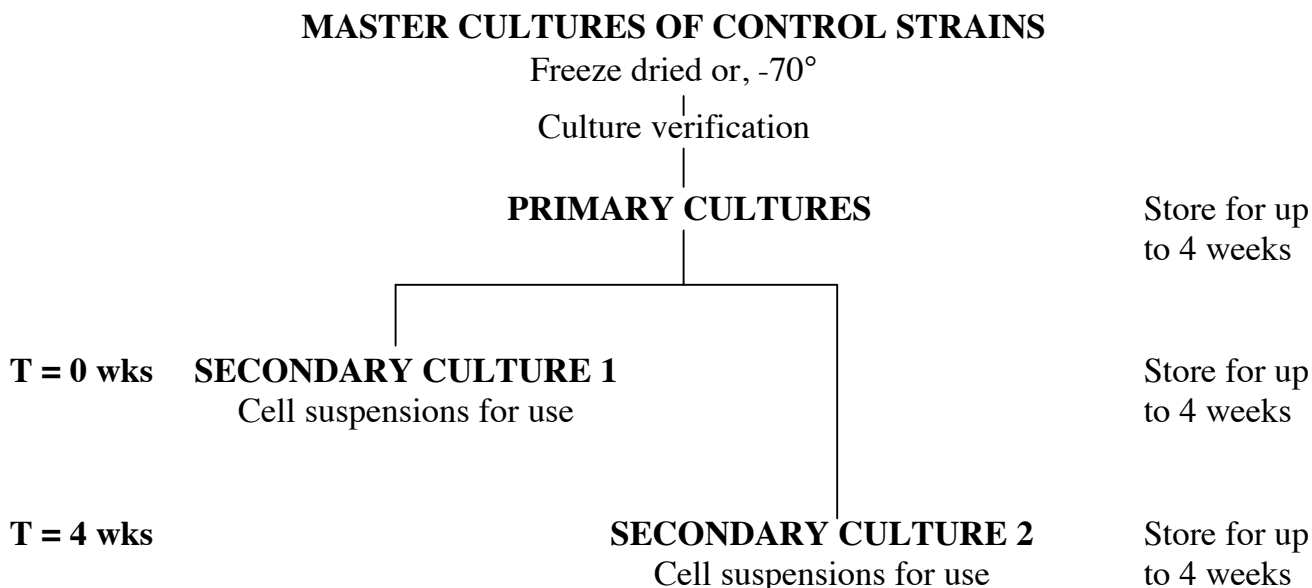
When required, primary cultures should be generated from master cultures of control strains (see Figure 1) by thawing or reconstituting, depending on how the laboratory has implemented culture maintenance (see Section 2.4). Thawed or reconstituted master cultures of control strains are streaked on to a non-inhibitory nutrient medium capable of supporting the organism's growth, and incubated under appropriate conditions until adequate growth is observed.

No more than two serial subcultures (secondary cultures) of a primary culture should be prepared as further subcultures increase the likelihood of phenotypic or genotypic change. After a maximum of two subcultures, the primary culture must be replaced from the master cultures of control strains (see Section 2.4).

Secondary cultures should be used to prepare cell suspensions for dilution and inoculation of test media (see Section 2.6). Primary cultures and secondary cultures may be stored at 2-8°C for up to four weeks.

While no more than two serial subcultures of a primary culture should be made, storage times for secondary cultures and suspensions (not dilutions) at 2-8°C is flexible to the extent that individual laboratories must validate their storage routine if they do not wish to faithfully follow the protocol described above.

Figure 1 “Maintenance of Quality Control Strains”



After 8 weeks Secondary culture 2 is discarded and a new Primary culture is prepared from the Master cultures.

2.6 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 sterile soybean casein digest medium or equivalent and incubate four to five hours to achieve an exponential growth phase. Adjust the turbidity to approximate a McFarland 0.5 turbidity standard (refer to NCCLS Standard M2-A4 for preparation and use of turbidity standard). This basic suspension should contain approximately 1×10^7 to 1×10^8 CFU/ml. Alternatively, use a thawed frozen culture suspension initially adjusted to give this count or other internally validated methodology.
- b. For testing of the nutritive capacity of a medium, dilute the cell suspension 1:10 in sterile normal saline, and inoculate each test plate with a $1 \mu\text{L}$ calibrated or disposable loop loaded with the diluted suspension to provide 1×10^3 to 1×10^4 CFU/plate. A standardised methodology should be used to distribute CFUs over the plate to generate isolated colonies, e.g. the Clayton (1977) dipstrip method or the Mossell (1980, 1983) ecometric method. However if isolated colonies are not achieved, use a ten-fold lighter inoculum. Use of modifications to published methods or unpublished 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 1µL calibrated or disposable loop loaded with the undiluted cell suspension to provide 1×10^4 to 1×10^5 CFU/plate.
- d. For testing the performance of liquid media, dilute the cell suspension 1:100 and make a further 1:100 dilution of this. Inoculate each tube with 10µL of the final dilution to provide an inoculum of approximately 100 CFU. Adjustment of this inoculation procedure may be required if a heavier or lighter inoculum is normally required for the medium being tested.
- 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 (CO₂) levels, or atmospheres suitable for anaerobic or microaerophilic microorganisms. Normally the incubation period will be 18-24 hours or 40-48 hours at $35 \pm 2^\circ\text{C}$, depending on the medium being tested.

2.7 Interpretation of Results

Parallel growth performance testing of batches of media against "control" batches of the same sort of medium is not necessary, if a semi-quantitative means of recording results of growth performance testing is employed. Continuous monitoring with adequate documentation of results for all batches made should provide a growth performance history for each formulation. This then allows an assessment to be made of the performance of the test batch.

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.

2.8 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, or products are not covered by these guidelines, customers should be supplied with a Product Specification. The specification must detail intended use and storage condition, 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 certificates 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, STORAGE AND SHELF LIFE OF PREPARED MEDIA

3.1 Packaging, Transport and Storage

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

Prepared media should be stored in unopened or resealable packages at 2-8°C unless documented validation experiments have been conducted on samples of each medium type to demonstrate that storage under alternative conditions is not detrimental to its performance when tested according to these guidelines.

Where transportation of media occurs, appropriate packaging and modes of transportation should be used to ensure against exposure to potentially detrimental conditions. (see Section 3.2).

3.2 Shelf Life of Prepared Media

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

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

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.

Laboratories who obtain prepared culture media either from a commercial source or a central facility, that carries a compliance label should record the following data (see 4.2) in a log book or similar:

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

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

4.2 Physical Inspection of Plates/Tubes

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

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

4.3 Performance Monitoring

It is recommended that users of commercially prepared media continue to monitor each batch of the following types of media (Wilkinson,1992), according to Section 2 of these Guidelines or by another validated method.

- Media selective for *Campylobacter spp.*
- Media selective for pathogenic *Neisseria spp.*
- Blood agar containing colistin and nalidixic acid
- Selective agar for anaerobic isolations
- Media for isolation of *Clostridium difficile*
- Media for isolation of *Haemophilus spp*
- Selective media for isolation of enteric pathogens

However, if the laboratory is 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. An example of a revalidation procedure can be found in NCCLS Approved Standard M2-A5, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 5th Edition.

Commercially prepared media (including media obtained from a central source that distributes to satellite locations on a non-commercial basis), other than the media listed above, need not be retested provided that the media is obtained from sources that employ the quality control criteria recommended in Section 2 of this document and assure the purchaser that the criteria have been met (see Section 2.8). Testing should include nutrient and inhibitory performance, but not contamination.

4.4 Remedial Action for Deficiencies Observed

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

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

Whenever possible, samples of the defective 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 records. (Refer Section 4.1)

5.0 CONCLUDING COMMENTS

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

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

As this is a consensus document, comments are welcomed and will be considered in the process of moving from this First standard to future revisions. Future committees will endeavour to issue updates to this document every two years.

Your comments should be sent to:

**Convenor/Secretary
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DRAFT COMMENTS AND REVIEW PANEL RESPONSE

This section includes those comments arising from the September 1994 draft document. Respondents comments are shown, with the review panels responses shown in italics.

Respondents	Review Panel
Media QC - SIG, Melbourne	P. Mugg
NATA	I. Wilkinson
Mr J Glasson, IMVS	H. Papazaharoudakis
Mr D Robb, South West Pathology Service	
Mr D Winwood, Micro Diagnostics	
Mr K Sherlock, Sullivan and Nicolaides	

Q: Section 2.1 Should some guidance be given for the quality control of media not included in Table 1?

A: *Yes, as this document is intended to provide a minimum standard, it is suggested that the quality control guidelines provided in the technical manual published by the suppliers of dehydrated culture media be consulted.*

Q: Table 1 *S. pyogenes* should also be used to quality control Blood Agar Selective (Nalidixic Acid + Colymicin)

A: **S. pyogenes* is significantly more resistant to nalidixic acid than *S. aureus*. Therefore, if the incorrect (excess) quantity of nalidixic acid is added to the medium, *S. aureus* will be inhibited whilst *S. pyogenes* may not be affected.*

Q: Table 1 Should *S. aureus* be included in the quality control of Blood Agar Selective (Gentamicin)?

A: *As this media is primarily employed in the isolation of *S. pyogenes* from samples contaminated with gram negative bacilli, the inclusion of *S. aureus* which generally will not grow is not considered to be of any benefit.*

Q: Table 1 Why include *S. typhimurium* as a control organism in the quality control of MacConkey Agar without crystal violet?

A: *Some laboratories employ these media for the isolation of *Salmonella sp* particularly from enrichment broth subcultures.*

Q: Table 1 GC Media. Is it necessary to include *S. epidermidis* as a quality control organism?

A: *The panel felt that the inclusion of *E. faecalis* was sufficient to cover inhibition of gram positive organisms and therefore, *S. epidermidis* has been dropped.*

Q: Table 2 The sampling plan is felt to be excessive for small batches of media.

A: *The NATA Technical Note No.4 provided an alternative sampling plan also based on AS1199. The interpretation of this standard is not an easy task, therefore we employed the services of a statistician to clarify which interpretation was correct. Based on their advice, it appears that both plans are equally valid depending upon the criteria used to establish the plan. The panel has therefore decided that in order to ensure both consistency between the two documents and to provide a more acceptable sampling plan for small batches, the original sampling plan presented in the NATA Technical Note should be adopted. This plan provides for a single sampling plan for small batches (< 150 units) and a double sampling plan for larger batches (>150 units). This decision was partially based on the fact that for smaller batches it would be more cost effective to discard a suspect batch than to resample.*

Q: Section 2.4 Do control strains need to be validated every 12 months?

A: *It was felt that if strains are maintained in a lyophilised form, it was excessive to expect them to be validated every 12 months.*

Q: Section 2.6 (b) Could alternative plating techniques be employed?

A: *Yes. The key issue is that a procedure be established which is :*

- a. Reproducible*
- b. Produces isolated colonies*

Any referenced or validated method may be employed.

Q: Section 2.6 Why complicate the inoculation methods by employing 2 different size loops?

A: *As an alternative, a 1µl loop could be used to subculture from the undiluted broth rather than a 10µl sample from the 1:10 dilution. This will conform to NATA requirements.*

Q: Section 2.8 Is it necessary that we receive a quality control certificate with each batch of media?

A: *It is recognised that the issuing of certificates with every batch of every product creates a considerable volume of paperwork which does not appear to serve any useful purpose. This issue has been discussed with NATA who have indicated that they will accept compliance labels attached to products as an alternative to written quality control reports provided that the supplier provide each customer with a once only product specification.*

This specification should include the following information:

- *Formulation*
- *Intended use*
- *Storage conditions/ shelf life*
- *QC procedure and expected results.*

Q: Section 3.2 Should all prepared media be labelled with both a date of preparation and an expiry date?

A: *The panel agrees that both are important pieces of information. However, to supply both pieces of information on the product was felt to be excessive and would be difficult to achieve when the labelling must also include the product name and lot number. It was felt that both dates should be provided by a supplier but may be provided on external packaging or quality control report.*

Q: Section 4.2 How can a small one or two person laboratory, often multi disciplinary be expected to perform the level of quality control suggested for those items highlighted?

A: *While it is recognised that this places an additional burden on smaller laboratories, it is believed that the survey data presented by Ms I Wilkinson, ASM 1992 and 1994 supports the need for the monitoring of these selected products. After discussions with a number of groups including NATA it is felt that 2 alternatives should be provided:*

- *A monitoring system be developed based on abbreviating the testing methods described in Table 1. This basic quality control should include a minimum of one positive and one negative control strain and should be applied to each new batch of product delivered OR,*
- *Laboratories monitor the performance of the selected media either by performing quality control on each batch received or by in use performance monitoring until they can demonstrate reliable performance. Once this is achieved the level of monitoring may be reduced. If however, failure to perform or variability in performance is noted, a revalidation program must be implemented. Once a return to reliable performance is demonstrated, the level of monitoring may be reduced again.*

This philosophy of validation followed by periodic sampling and revalidation if problems arise is the approach employed in industries such as the pharmaceutical industry. With regards to what is sufficient testing to validate or re validate, discussions with our statistician are continuing.