



Guidelines for Assuring Quality of Medical Mycological Culture Media

*A jointly produced document of
Culture Media Special Interest Group
Mycology Special Interest Group
for the
Australian Society for Microbiology, Inc.*

2nd edition
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The Australian Society
for **Microbiology**
bringing Microbiologists together



FOREWORD to the First Edition

These Guidelines reflect the desire to promote a consistent, high-quality culture media product for the performance of medical mycology in Australia and recognizes the fact that quality assurance and quality control for this group of media is a complex issue.

The Culture Media Special Interest Group and the Mycology Special Interest Group of the Australian Society for Microbiology have collaborated to produce this document. Much of the work done within was produced by the Victorian branch of the Mycology SIG, and special acknowledgement must be given to the leadership of that group by Sue Coloe. The process began in recognition that the issue of quality assurance and quality control for mycology media had not been addressed within the 1996 *Guidelines for Assuring Quality of Medical Microbiological Culture Media* and left a gap that this document aims to fill. Initially, a 2001 draft revision of the 1996 Guidelines was prepared, with the intent of incorporating the mycology elements within. This was abandoned, after further discussion and consultation concluded that a separate set of guidelines was more appropriate for the mycology media.

Unfortunately, due to a range of factors, the release of this separate document was delayed longer than originally planned or intended; however, this has also allowed for further modifications of, and improvements to, the document so that it be further fine-tuned before release.

This document is particularly designed to aid end-users in defining their responsibilities for receiving and testing mycology media. The hope is that it will also assist producers and assessors in achieving a quality product that will be reflected in best-practice procedures for the mycology laboratory, delivery of the highest standards in microbiological laboratory practices, and delivery of optimal patient outcomes.

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FOREWORD to the Second Edition

Despite the passing of a decade since the last edition of these Guidelines, the pleasing aspect is how well they have, during that time, maintained their robustness and 'fit-for-purpose' nature. The feedback from end users, as well as assessors involved in laboratory accreditation to the relevant Standards (notably ISO17025 and ISO15189) has been positive throughout that time.

This new edition of the Guidelines aims to capture and reflect relevant changes that have occurred since the release of the first edition. Overall, the changes are modest. Again, this is reflective of the robust nature of how well the first edition has served its users. But changes do happen, and hence this new edition serves to reflect that. Special thanks to Dr Sarah Kidd for editorial input to this revision.

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

*Sincerely,
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Any suggestions for amendments or changes, questions arising, should be directed to the National Convenors of the two SIGs via email.

Please send to admin@theasm.com.au

Please include as the Subject Line:
Mycology Media QC Guidelines 2023 – Attention: Mycology, Culture Media SIG Convenors

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 (1, 2). In the case of mycology media, this issue can be more complicated than for general bacteriology media. A practical guideline on how this should be done is given in this document.

1.1 Application

These guidelines are applicable to medical mycology laboratories and commercial suppliers who manufacture or use mycological media. They seek to offer direction to individuals who must implement procedures with the purpose of assuring the quality of medical mycological culture media and ultimately the quality of the mycology services of the laboratory. They should be viewed in conjunction with other relevant documentation to help implement a comprehensive QA program (1, 2, 3, 4). They are designed to complement the overall strategy of media quality control.

1.2 Scope

These guidelines pertain primarily to medical mycological culture media used for the cultivation, isolation and identification of medically important yeasts and fungi.



1.3 Definitions

Manufacturer:

Manufacturers of medical mycological 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 mycological 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 mycological 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.



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.

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 AS1199.1-2003 (5) for sample size for sterility testing. The sampling procedures recommended are summarised in Appendix B including notes on interpretation.

Incubation of all samples must be for a minimum of 7 days at a suitable temperature before inspection.

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 for Yeasts and Fungi

It is suggested that the control strains specified in these guidelines (see Appendix A) should be used. Control strains should be cultures that exhibit typical microscopic, macroscopic, and biochemical characteristics of the species and verified by a reference laboratory, where possible (6). Use of cultures for which no subculture or handling history is available is discouraged.

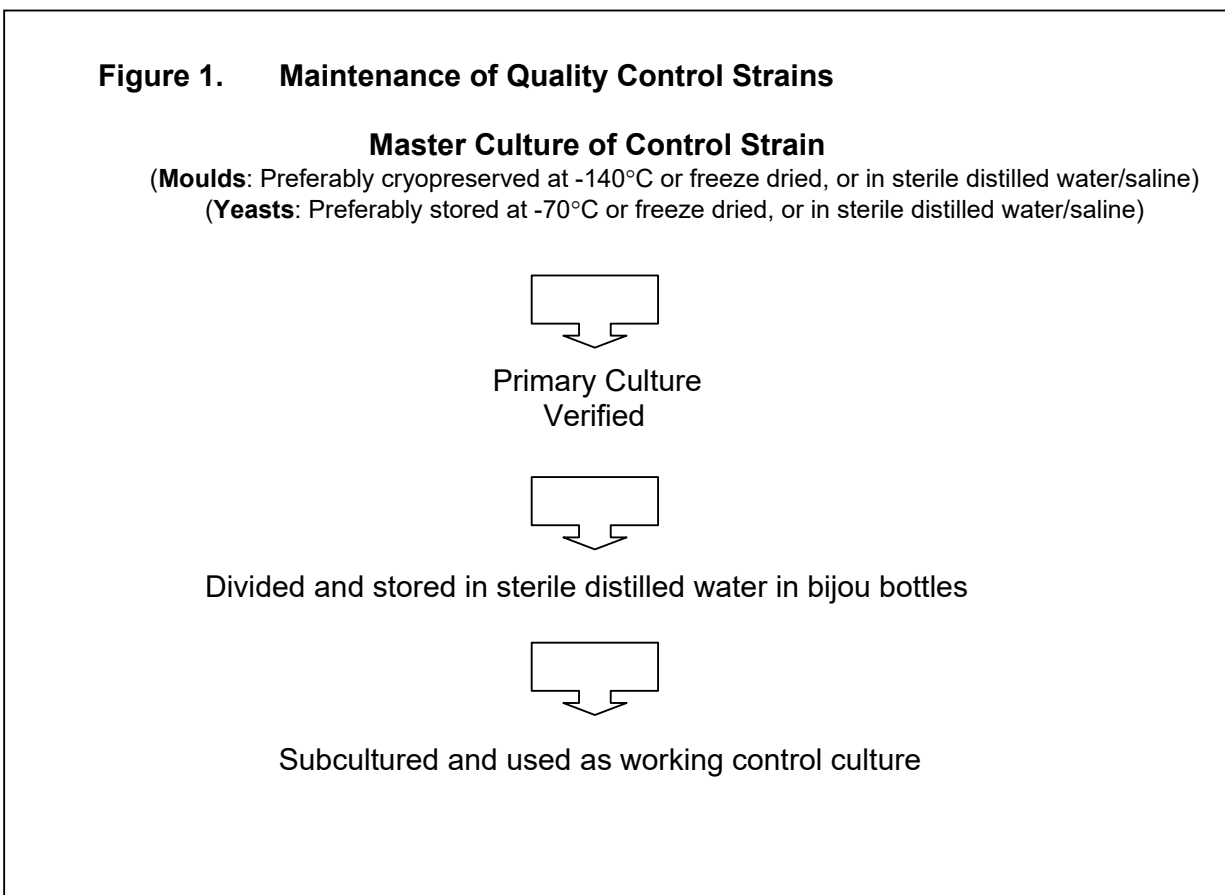


2.4 Working Control Cultures

When required, primary cultures should be generated from the master culture by inoculating a non-inhibitory medium capable of supporting the organism's growth and then incubating under appropriate conditions until adequate growth is observed. Once adequate growth has been obtained the primary culture should be:

- (i) Verified - confirming that the culture conforms to typical species identification; and
- (ii) Divided up and stored at room temperature in adequately labelled, glass bijou bottles containing distilled water or saline, capped, preferably with metal lids and sealed using parafilm.

Each culture can then be subcultured, to a maximum of 12 subcultures, as required, onto a medium capable of supporting the growth of the organism for quality control testing until it no longer exhibits characteristics typical of that fungus. (See Figure 1).





2.5 Test procedures for Mycological Culture Media

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

- Yeasts: A single colony streaked onto the agar using a bacteriological loop.
- Moulds: A relatively rigid wire, flamed to sterilise and cooled is used to remove a piece of thallus approximately 2-3 mm in size. Sterile 1µL disposable loops or sterile plastic needles may also be used. Care must be taken to remove as little of the underlying medium as possible. The mould is then inoculated onto the test agar by pressing it onto the agar firmly but not completely burying it.

The exception to this technique is when using profusely sporulating fungi (such as *Aspergillus fumigatus* etc.) which only require the sterile wire or needle to be touched onto the colony and the inoculum transferred to the medium being tested. The inoculating wire should be moistened by stabbing it into the medium prior to touching the colonies to be subcultured. For excessively sporulating moulds, a moistened sterile swab also works well.

2.6 Interpretation of Results

A medium's performance is regarded as satisfactory if all test strains grow or are inhibited as is appropriate for the medium being tested, and colonial morphology and reactions produced in the medium are typical for the organism on that particular type of medium.

2.7 Reporting Quality Results to Users

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

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



3.0 Packaging, Transport and Storage

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

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

Verification of this should be undertaken (2, 10).

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

3.1 Shelf Life of Prepared Media

All prepared media should be marked with an expiry date. This should be validated under the conditions of packaging, transportation and storage that will prevail under normal circumstances. Examples for determination of shelflife are provided in the ASM Guidelines (3). 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.



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 (2, 10).

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

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

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

4.2 Physical Inspection of Plates/Tubes, Bottles

Users of commercially prepared media, or media supplied 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 or excessive moisture
- Discolouration
- Sloped or uneven filling of Petri dishes
- Contamination
- Crystalline pattern on surface of medium (indicative of freezing)
- Presence of bubbles
- Presence of leakage



4.3 Remedial Action for Deficiencies Observed

Where significant defects are found the users should notify the manufacturers providing all 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 (2, 10).



5.0 References

1. *General Accreditation Criteria. ISO/IEC17025 Standard Application Document.* Current edition. National Association of Testing Authorities (NATA), Sydney, Australia.
2. *General Accreditation Criteria. ISO15189 Standard Application Document.* Current edition. National Association of Testing Authorities (NATA), Sydney, Australia.
3. *Guidelines for Assuring Quality of Medical Microbiological Culture Media.* Current edition. Culture Media Special Interest Group, Australian Society for Microbiology, Inc.
4. M22-A3. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard –* Third edition. 2004. Clinical and Laboratory Standards Institute (CLSI), Pennsylvania.
5. AS1199.1-2003 (ISO2859-1:1999). *Sampling Procedures for Inspection by Attributes. Part 1: sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection.* 2003. Standards Australia, Sydney.
6. *General Accreditation Criteria. Maintenance of Microbiological Reference Culture Collections (MRCCs).* Current edition. National Association of Testing Authorities, Sydney, Australia.
7. AS5140:2022 (ISO11133:2014, MOD). *Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media.* Standards Australia, Sydney.
8. Kidd, S., C. Halliday, D. Ellis. *Descriptions of Medical Fungi.* 4th edition. 2023. CAB International., Wallingford, Oxfordshire, United Kingdom. doi:10.1079/9781800622340.0000.
9. Snyder, J.W., R.M. Atlas, & M.T. LaRocco. Ch.113. Reagents, Stains and Media: Medical Mycology. 2011. In: *Manual of Medical Microbiology*, 10th edition. ASM Press, Washington.
10. ISO15189:2022. *Medical laboratories – Requirements for quality and competence.* 2022. International Standards Organisation, Geneva.

**Appendix A: Recommended control strains and acceptance criteria for growth performance testing of mycology culture media**

Medium (8, 9)	Incubation ¹	Organisms ²	Acceptance criteria
• Birdseed agar (Caffeic acid agar)	26-30°C	<i>Candida albicans</i> <i>Cryptococcus neoformans</i> or <i>Cryptococcus gattii</i>	Growth, white colonies Growth, brown colonies
• Heart Infusion agar	30°C	<i>Nocardia asteroides</i>	Growth
• Heart Infusion agar with blood	30°C	<i>Candida albicans</i> <i>Aspergillus fumigatus</i> or <i>Aspergillus brasiliensis</i>	Growth, white yeast Growth, Blue/Green colony Growth, white colony black conidia
• Heart Infusion agar with antibiotics	30°C	<i>Candida albicans</i> <i>Aspergillus fumigatus</i> or <i>Aspergillus brasiliensis</i> <i>Escherichia coli</i> WDCM 00013 ³	Growth, white yeast Growth, Blue/Green colony Growth, white colony black conidia Inhibited
• CGB (Canavanine, glycine, Bromothymol blue) agar	26-37°C	<i>Cryptococcus neoformans</i> <i>Cryptococcus gattii</i>	No growth; or minimal growth (yellow/light green) Agar turns blue
• Chromogenic Candida agar	30°C * or 35-37°C* <small>(*check manufacturers' specifications)</small>	<i>Candida albicans</i> <i>Pichia kudriavzevii</i> (formerly <i>Candida krusei</i>) <i>Candida parapsilosis</i> * <i>Nakaseomyces glabratus</i> (formerly <i>Candida glabrata</i>)* <i>Candida tropicalis</i> <i>Candida auris</i> <small>(*where colonial morphology is the same, either or both may be used)</small>	Growth, colour* and morphology Growth, colour* and morphology Growth, colour* and morphology Growth, colour* and morphology Growth, colour* and morphology Growth, colour* and morphology <small>(*colour may vary between suppliers – check manufacturers' specifications)</small>
• Cornmeal Tween 80 agar	26-28°C	<i>Candida albicans</i>	Growth: pseudohyphae, blastoconidia, terminal thick-walled vesicles (chlamydoconidia)

1. All media are incubated aerobically

2. 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.

3. WDCM is the World Data Centre for Microorganisms. See www.wdcm.org

**Appendix A: Recommended control strains and acceptance criteria for growth performance testing of mycology culture media**

Medium (8, 9)	Incubation ¹	Organisms ²	Acceptance criteria
• Czapek Dox agar	26-30°C	<i>Aspergillus fumigatus</i> or <i>Aspergillus brasiliensis</i>	Growth, blue-green colonies Growth, white colony black conidia
• Dermatophyte Test agar <i>(incorporating antibacterials & cycloheximide)</i>	26-28°C	<i>Candida albicans</i> <i>Trichophyton interdigitale</i> or <i>Trichophyton mentagrophytes</i> <i>Escherichia coli</i> WDCM 00013 ³ <i>Saccharomyces cerevisiae</i>	Growth, yellow colonies Growth, medium turns red No growth No growth
• Dermasel agar <i>(incorporating antibacterials & cycloheximide)</i>	26-28°C	<i>Trichophyton interdigitale</i> or <i>Trichophyton mentagrophytes</i> <i>Escherichia coli</i> WDCM 00013 ³ <i>Saccharomyces cerevisiae</i>	Growth, white colony No growth No growth
• Lactrimel agar	26-28°C	<i>Trichophyton rubrum</i>	White colonies with wine red reverse pigment
• Lactrimel agar with chloramphenicol & gentamicin	26-28°C	<i>Trichophyton rubrum</i> <i>Escherichia coli</i> WDCM 00013 ³	White colonies with wine red reverse pigment No growth
• Littman Oxgall agar	26-28°C	<i>Trichophyton interdigitale</i> <i>Trichophyton mentagrophytes</i> <i>Trichophyton rubrum</i>	Growth, raised white colony Growth, grey-white colony, yellow-brown reverse Growth, raised grey to whitish colony
• Malt Extract agar	26-28°C	<i>Trichophyton mentagrophytes</i> <i>Aspergillus brasiliensis</i> or <i>Aspergillus fumigatus</i> or <i>Aspergillus flavus</i>	Growth, white colony Growth, white mycelia, black spores Growth, white mycelium, blue-green conidia Growth, white mycelium, yellow-green conidia

1. All media are incubated aerobically

2. 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.

3. WDCM is the World Data Centre for Microorganisms. See www.wdcm.org

**Appendix A: Recommended control strains and acceptance criteria for growth performance testing of mycology culture media**

Medium (8, 9)	Incubation ¹	Organisms ²	Acceptance criteria
<ul style="list-style-type: none"> Mycobiotic agar (Mycosel agar) 	26-28°C	<i>Trichophyton interdigitale</i> or <i>Trichophyton mentagrophytes</i> <i>Saccharomyces cerevisiae</i> <i>Escherichia coli</i> WDCM 00013 ³	Good growth No growth No growth
<ul style="list-style-type: none"> 1% Peptone agar 	26-28°C	<i>Trichophyton mentagrophytes</i> <i>Trichophyton interdigitale</i> <i>Trichophyton rubrum</i>	Growth, granular colony Growth, downy colony Growth, downy colony
<ul style="list-style-type: none"> Potato Dextrose agar 	26-28°C	<i>Trichophyton interdigitale</i> or <i>Trichophyton mentagrophytes</i> <i>Trichophyton rubrum</i> <i>Aspergillus brasiliensis</i> or <i>Aspergillus fumigatus</i> or <i>Aspergillus flavus</i>	Growth, cream-white colony, no reverse pigment Growth, white colony with wine red reverse pigment Growth, white mycelia, black spores Growth, white mycelium, blue-green conidia Growth, white mycelium, yellow-green conidia
<ul style="list-style-type: none"> Rice agar with Tween 80 	26-28°C	<i>Candida albicans</i>	Growth with thick-walled vesicles (chlamydoconidia)
<ul style="list-style-type: none"> Rice grain medium 	26-28°C	<i>Microsporum canis</i>	Growth, white aerial mycelium
<ul style="list-style-type: none"> Sabouraud Dextrose agar 	26-28°C	<i>Saccharomyces cerevisiae</i> <i>Aspergillus brasiliensis</i>	Growth, white yeast-like colony Growth, blackened colony

1. All media are incubated aerobically

2. 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 and acceptance criteria for growth performance testing of mycology culture media

Medium (8, 9)	Incubation ¹	Organisms ²	Acceptance criteria
<ul style="list-style-type: none"> Sabouraud Dextrose agar with chloramphenicol, gentamicin and cycloheximide (actidione) 	26-28°C	<i>Saccharomyces cerevisiae</i> <i>Trichophyton interdigitale</i> or <i>Trichophyton mentagrophytes</i>	No growth Growth, white colony
		<i>Escherichia coli</i> WDCM 00013 ³	No growth
<ul style="list-style-type: none"> Sabouraud + 5% Salt agar 	26-28°C	<i>Trichophyton interdigitale</i> or <i>Trichophyton mentagrophytes</i>	Growth, buff colony
		<i>Trichophyton rubrum</i>	Minimal growth, white colony
<ul style="list-style-type: none"> Trichophyton agars #1 #2 #3 #4 	26-28°C	<i>Trichophyton tonsurans</i>	Minimal growth Minimal growth Good growth Good growth
<ul style="list-style-type: none"> Trichophyton agars #1 #2 #3 #4 	26-28°C	<i>Trichophyton interdigitale</i> or <i>Trichophyton mentagrophytes</i>	Good growth Good growth Good growth Good growth
<ul style="list-style-type: none"> Urea glucose agar 	26-28°C	<i>Trichophyton interdigitale</i> or <i>Trichophyton mentagrophytes</i> or <i>Cryptococcus neoformans</i>	Positive hydrolysis
		<i>Trichophyton rubrum</i> or <i>Escherichia coli</i> WDCM 00013 ³	Negative hydrolysis* <small>(*if incubated >7days, <i>T. rubrum</i> will go positive)</small>

1. All media are incubated aerobically

2. 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.

3. WDCM is the World Data Centre for Microorganisms. See www.wdcm.org



Appendix B Sampling Plan for Mycological Culture Media

Small Batches (≤ 100 units): 1% or 1 unit from beginning and 1% or 1 unit from end of batch (7).

Double Sampling Plan (>100 units) NORMAL SAMPLING PLAN, AQL - 2.5, GENERAL INSPECTION LEVEL = 1 (5)

Batch Size (units made)	Sample Number		1 st Sample		2 nd Sample	
	1 st sample	2 nd 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 AS5140 (ISO11133) (7), 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.



Appendix C

Points arising from the first draft of the mycology media guidelines, and responses from the working party.

Why not list ATCC strain numbers of fungi in these guidelines?

ATCC strains were deemed expensive (by the original working party); (in their opinion) the filamentous fungi may be unstable and atypical.

(Editorial note: end-users may source ATCC cultures, or from another reference collection, such as NCPF, or NCYC. Refer to (6) for more information).

Why not take QC inoculum from water culture to minimise carry over of nutrients?

- (i) The QC inoculum is taken from a plate so that a visual check for contamination may be made.
- (ii) Water culture may not be viable.

Why not use quantitative methods, similar to that used for bacteria, for yeasts?

Working party felt that this was not necessary.

Yeasts store well at -70°C and not many laboratories have access to freeze drying equipment.

This temperature has been added to guidelines.

Freeze drying is not suitable for all dermatophytes. Liquid nitrogen is used at ATCC and IMI, where the curators consider it to be the only method which ensure long term viability of stored fungi.

Unfortunately, few laboratories have access to liquid nitrogen facilities.

Does the time of storage of the working control need to be stated?

Time of storage will vary depending on the organisms. Once typical features of the organisms on the medium being tested are no longer present, the working culture should be discarded.

No recommendations as to the number of subcultures which can be made from the control strains. The number of subcultures which can be made from the control strains will depend on the species of fungus.

Can the working culture control be subcultured?

No

What records are required for subculturing? Suggestion - use for approximately 3 months then discard and start afresh.

This would be acceptable but would depend on the fungal species.

What would invalidate a Master culture?

- (i) Contamination
- (ii) Non-viability
- (iii) Loss of typical features

Will the inoculation procedures described give too heavy an inocula for primary isolation media?

No

Technical and editorial changes to the second edition

Throughout: grammar amended and improved where needed and appropriate.

Section 5. references: updates to references and citations.

Appendix A: updates to nomenclature of yeasts and fungi. Additional organisms added to some media for quality performance testing.