Guidelines for Assuring Quality of Solid Media used in Australia for the Cultivation of Medically Important Mycobacteria

A jointly produced document of
Culture Media Special Interest Group
Mycobacteria 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 solid media product for the performance of medical mycobacteriology in Australia and recognizes the fact that quality assurance and quality control for this group of media is a highly complex issue. Overseas-published experiences document the importance and the specialized nature of quality assurance and quality control of mycobacteria media (1,2).

The Culture Media Special Interest Group (CMSIG) and the Mycobacteria Special Interest Group (MSIG) of the Australian Society for Microbiology have collaborated to produce this working document. William Chew (on behalf of MSIG) and Tom Olma (on behalf of the CMSIG) produced the initial draft of these guidelines in July 2000. The process began in recognition that the issue of quality for mycobacteria media had not been addressed, due in part to the time taken to grow mycobacteria and controversial issues such as parallel versus concurrent testing. Mycobacterium testing was, for this very reason, left out of the 1996 *Guidelines for Assuring Quality of Medical Microbiological Culture Media* (3) and left a gap that this document will now fill.

This document follows wide consultation within the field. It is particularly designed to aid end-users in defining their responsibilities for receiving and testing mycobacteria media, *and the text has been italicized to highlight key areas*. The hope is that it will also assist producers and assessors in achieving a quality product that will be reflected in quality outcomes for the mycobacteriology laboratory.

The issue of quality control of liquid mycobacteria media has been deliberately omitted as most liquid media used in Australia to culture mycobacteria today is commercially prepared overseas, with manufacturers making their own recommendations for end-user testing.

On this occasion, Frank Haverkort (MSIG) and Peter Traynor (CMSIG) were the co-ordinators. Contributions have been gratefully received from:

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FOREWORD to the Second Edition

With some minor amendments, updates and changes, the Second Edition of this document incorporates changes that have occurred in processes, documentation, and references since the First Edition, as well as dealing with minor editorial matters.

These Guidelines were well received at the time of their launch and the reviews received from end-users were most favorable. They have been referenced in the *Guidelines for Australian Mycobacteriology Laboratories* (4). Nonetheless, we recognize that there will always be further room for improvement, and that the likelihood of other changes occurring over time will lead to a need for further revisions and new editions in the future. To this end we welcome all feedback and suggestions, for change and improvement, from all end-users of the Guidelines both within and outside the Society.

We trust that this document will further enable your delivery of the highest standards in microbiological laboratory practices, and best-practice delivery of optimal patient outcomes.

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National Convenor,
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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:
Mycobacteria Media QC Guidelines 2012 – Attention: Mycobacteria, 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 (5, 13). In the case of (solid) mycobacteria media this issue is more complicated than for general bacteriology media. A practical guideline on how this should be done is given in this document.

2.0 Application

These guidelines are applicable to laboratories that use mycobacterial culture media in Australia. They should be viewed in conjunction with other relevant documentation to help implement a comprehensive QA program (5, 6, 7, 13). They are designed to complement the overall strategy of media quality control.

If a medium is used on-site from the point of manufacture no additional testing is required. *If, however, it is used off-site then end-users need to assure themselves that the above elements have been met and they should, periodically, review the reliability (performance) of such media as outlined here*.

Those accredited by NATA as manufacturers of prepared media are already responsible for ensuring that their accreditation extends to the media in question: for the initial assessment of the media's suitability to a particular requirement and for full quality control assessment according to regulated requirements.

If laboratories receive their mycobacteria media from a manufacturer not accredited by NATA as a media manufacturer (and this includes ISO 9000 certified manufacturers) they are required to carry out a quality control evaluation as outlined below.

3.0 Scope

These guidelines pertain primarily to medical mycobacteria culture media produced for the cultivation, isolation and identification of medically important mycobacteria within Australia.

4.0 Media Quality Assurance Requirements

4.1 Media received from NATA-accredited manufacturer

Manufacturers will provide users with a quality control report or a compliance label on each batch of media. The products should be marked with the product name, batch number, date manufactured and expiry date & storage conditions.

A logbook detailing the type of media, batch number and date received must be maintained.

On an ongoing basis, most media from these manufacturers will require only visual examination by user laboratories. Some types however, e.g. selective media, require the monitoring of *all* batches - until sufficient data have been generated to assure the end-user of the reliability of the product . At such time, the frequency of testing may be reviewed and reduced. All records relating to media quality control need to be retained by the laboratories for three years (13).

Issues may arise when a NATA-accredited manufacturer (Biological Testing) cannot perform medical performance testing on-site and requests a Mycobacterium Reference Laboratory (MRL) to perform these specialist tests. Although the MRL may be accredited for Medical Testing it may not be so for performing media QA. Clearly, NATA endorsements need to be carefully applied.

4.2 Media received from non-NATA accredited suppliers

All laboratories receiving media from these facilities will be required to:

a) *confirm that a full quality control evaluation has been carried out, and*
b) *conduct performance tests against acceptance/ rejection criteria regards physical appearance, sterility and performance as outlined in these Guidelines.*

4.3 Revalidation of expiry dates

All prepared media will be marked with an expiry date. *This should be validated under the conditions of packaging, storage and transportation that will prevail under normal circumstances*. Revalidation should be done whenever significant changes to the usual conditions of packaging, storage and transportation or to the formulation of the medium occur.

5.0 Parallel versus Concurrent QA Testing

Due to the slow-growing nature of mycobacteria it is not always practical to test each batch of manufactured media prior to release; concurrent testing (using the media while QA is underway) has become an accepted practice. Before this practice can be adopted however, the manufacturer must have shown well-documented ability to produce reliable media that achieves the required outcomes.

Media that is released prior to full QA having been performed needs to be clearly identified and identifiable and documentation of batch numbers against sample usage is essential. In the event of media failure the manufacturer may not be able to supply mycobacterial media until the validation and verification procedures have been satisfied and re-verified. This may take some time. End-users need to have contingencies in place to deal with such situations, especially when that media has been used on clinical samples. If no parallel liquid culture medium is present for instance, this may include notifying the requesting doctor of the time delay and repeating the culture using alternative or replacement media.

Because concurrent testing can lead to potentially damaging situations, it is appropriate to at least consider parallel testing. Having good housekeeping practices in place, which allow an adequate lead-time to QA quarantined media (say one month from the placement of the order) could still make this a viable option if the manufacturer has proven reliability.

6.0 Control Strains for Mycobacteria Media

Appendix A lists suggested control strains and acceptance/ rejection criteria. The organisms have been selected to challenge the media in question. Of primary concern is the ability to grow *Mycobacterium tuberculosis*; there are, however, other significant non-tuberculous mycobacteria that have special growth requirements. *Mycobacterium malmoense* has a narrow pH tolerance and is commonly recovered in Europe but less so in Australia (9, 10); *M. haemophilum* requires haemin or ferric ammonium citrate; *M. bovis* has a preference for pyruvate.

Laboratories that culture for mycobacteria should have media capable of growing all of these organisms (1, 10). Good quality control of primary isolation media is essential and experience has highlighted the folly of performing this process poorly (11). Control strains must be cultures that have been verified and validated and whose lineage is documented according to NATA requirements (4, 12, 13). A number of MRLs in Australia have culture collections that can comply with these NATA requirements. Use of cultures for which no lineage history is available is unacceptable.

6.1 Master, Stock and Working Control Cultures

The concept of culture hierarchy ("generations") needs to be clearly understood when dealing with control strains. Also, time-lines require consideration when dealing with slowly growing mycobacteria (see Appendix B).

In this scenario the culture received from the authorised supplier will be considered to be "generation zero". The first generation (G1, master culture) is that derived from this G-zero material and is the source from which all subsequent stock and working cultures are made. *Upon receipt from the culture collection, and each subsequent occasion this first generation master stock culture is accessed, the strain needs to be verified for purity and expected characteristics. All accessioning and validation details must be documented and retained.*

Growth from this verified G1 culture is (concurrently) used to prepare back-up stock using a storage system that minimizes change and allows long term viability of the organism. This may be by freeze-drying or super-cold freezing (preferably at -70^0C or colder) of glycerol broths or beads. This supply should not be accessed frequently.

6.1 Master, Stock and Working Control Cultures (cont'd)

Subcultures from this first generation culture become the second generation (G2) *stock* and *working* storage cultures for use in the laboratory. *No working culture must ever be used to create new stock, nor be further sub-cultured; they are used once for their intended purpose and then discarded*. Sufficient *stock vials* should be prepared to last 12 months and are usually glycerol broths or beads that are stored frozen (-20^{0}C or colder). The number prepared will be determined by the laboratory's usage rate. The colder the storage temperature the longer the storage time and some stock at least should be held for long-term storage. *Working cultures* are typically beads stored frozen (-20^{0}C or colder) and the purity and identity of the organism (verified using simplified confirmatory tests, for example, typical colonial morphology) should be checked as they are used. Once sub-cultured and grown, the solid media vials may be stored on the bench.

When all the G2 *working* ampoules are used go back to the G2 *stock* and sub-culture to create third generation (G3) stock and working cultures, and so on. The use of colour co-ordinated caps and work sheets may be useful for tracking within the system. No more than 5 generations (passages) *at any level* is recommended as genetic changes may begin to appear (4, 12, 13). This means that if the supplier provided you with a G1-culture (instead of G zero) you must *still* limit total generations to a maximum of 5.

Laboratory practice must provide sufficient time for the slow division rate of mycobacteria. Cultures grown and maintained on solid media will of necessity be kept longer than other microorganisms. Shelf life should however be set to an arbitrary limit of around 3 months. This will allow a serviceable life of 2 months after allowing one month for growth. Anticipating demand and ensuring adequate supply needs must become part of good laboratory housekeeping.

7.0 Testing of Sterility and Physical Properties

The manufacturer needs to conform to AS1199.1-2003 (8) for sample size for sterility testing. The sampling procedures recommended are summarised in Appendix D including notes on interpretation. Incubation at 36^{o}C must be for a minimum of 7 days and at 30-32^{o}C for 3 weeks to exclude fungal contamination.

Inspection for significant physical imperfections should include:

- uneven distribution of media;

- variable amounts of medium in petri dishes/tubes/bottles;

- color;

- consistency;

- gross deformation of the surface of the media.

(Simple tests such as: touching the media surface with an inoculating loop; gently hitting a slope into the palm of your hand, may be useful to gauge consistency.)

8.0 Test Procedures for Determining Performance

The following procedures are recommended to determine the ability of mycobacteria to grow on manufactured media:

Using at least a Class l Biological Safety Cabinet and observing all due safety precautions for dealing with Class ll organisms, make a suspension equivalent to 0.5 MacFarland in distilled water. Use approximately 0.2ml to inoculate the test media. Sloped media should be spread over an area of the media approximately 2cm x 2cm and incubated under appropriate test conditions. Plate media may be ecometrically plated.

Maximum incubation period is normally limited to 3 weeks and any media that cannot support the growth of mycobacteria within this time is deemed unsatisfactory.

For performance testing, random samples of media from each batch are chosen. *The sample size need only be that number required to actually perform the test* .

9.0 Interpretation and Reporting of Results

Testing outcomes should be reported in a format that also details:

1 the physical properties of the media
(i.e. final pH (*but only if you have measured this)*, color, consistency).
Refer to Appendix C. and

2 the Reference Strains tested (including their identifying reference number) measure of the observed result should be given.

Each feature is then rated as either 'accepted' or 'rejected' against expected growth outcomes (see Appendix A). An overall 'acceptance' or 'rejection' of the batch of media is then made against the all observed results, interpreted in terms of the purpose for which it was designed.

The form should carry a statement that the testing of the media complies with the requirements of this document. The form should ideally comply with other NATA requirements (document control, Logo, testing and authorizing signatures, etc), however unless the testing laboratory is NATA certified to perform QA on mycobacteria media, the NATA endorsement cannot be used.

Absolute quantification of growth outcomes (i.e., 'measured' growth on the new media over 'measured' growth on a previously QA'd batch, expressed as a percentage) has not been formally established for mycobacteria media. Qualitative measures provide satisfactory information and, for purposes of standardization, the following guide should be used to assess growth:

0	No Growth
1+	Scanty, barely discernable growth; colonies are countable
2+	Dense but discrete growth; colonies not countable
3+	Confluent, abundant growth

10.0 References

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5. ISO/IEC17025. Field Application Document. Biological testing. *Supplementary requirements for accreditation.* 2009. National Association of Testing Authorities (NATA), Sydney, Australia.
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7. M22-A3*. Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard* – Third edition.2004. Clinical and Laboratory Standards Institute (CLSI), Pennsylvania.
8. 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.
9. Katila, M.L., J. Matilla, and E. Brander . 1989. Eur. J. Clin. Infect. Dis. **8** : 998-1000.
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11. Haverkort, F. 2004. Microbiology Australia **25** (4): 23-25.
12. NATA policy Circular 34. *Maintenance of Microbiological Reference Culture Collections (MRCC).* 2011. National Association of Testing Authorities, Sydney, Australia.
13. AS4633 (ISO15189). Field Application Document. Medical testing. *Supplementary requirements for accreditation.* 2009. National Association of Testing Authorities (NATA), Sydney, Australia.
14. ISO/TS11133-2:2003. *Microbiology of food and animal feeding stuffs –Guidelines on preparation and production of culture media –Part 2: Practical guidelines on performance testing of culture media.* International Standards Organisation, Geneva.

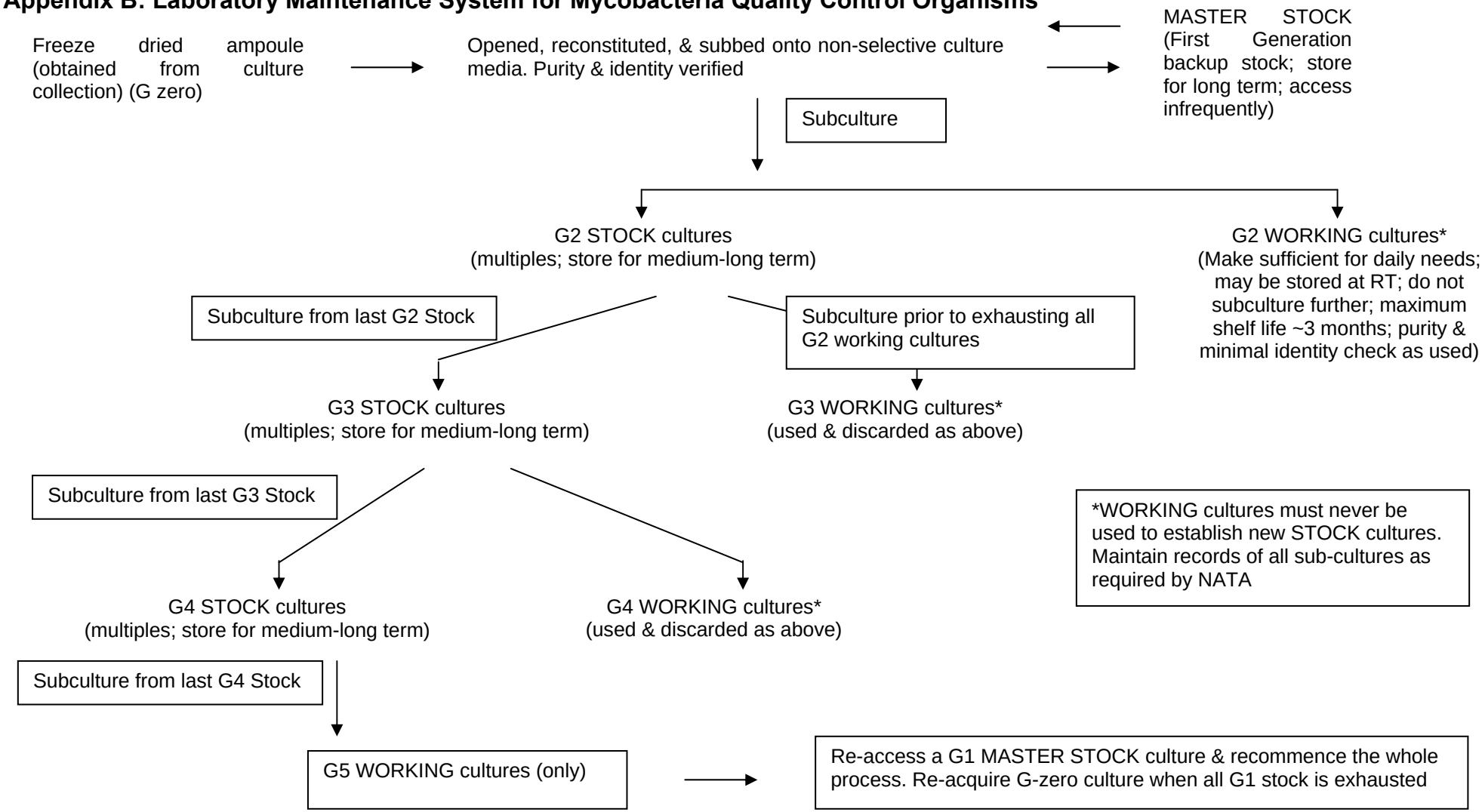
Appendix A: Recommended control strains and acceptance criteria for growth performance testing of solid mycobacteria media

Media	Incubation	Organisms	Acceptance criteria
General purpose, non-antibiotic containing media *e.g.:*			
▪ Lowenstein-Jensen (glycerol)	35-37°C	Standard set: *M.tuberculosis* ATCC® 25177™ (H_{37}Ra) or ATCC® 27294™ (H_{37}Rv) (NCTC 13144) *M.intracellulare* ATCC® 13950™ (NCTC 13025) *M.fortuitum* ATCC® 6841™ (NCTC 10394) *M.malmoense* ATCC® 29571™ (NCTC 11298) (optional)	2+ to 3+ growth within 21 days
▪ Gerloff's Egg Media ▪ Egg Yolk Agar ▪ Lowenstein-Jensen plus pyruvate		Standard set plus *M.bovis* ATCC® 19210™ (NCTC 10772)	
General purpose media containing antibiotics e.g.:			
▪ Mitchison's LJ plus Mitchison's antibiotic mixture ▪ Gerloff with NVAP ▪ Other variations	35-37°C	Standard set (± *M.bovis* ATCC®19210™ (NCTC 10772))	2+ to 3+ growth within 21 days
		Plus: *Ps.aeruginosa* WDCM 0025 *K.pneumoniae* WDCM 0097 *E.faecalis* WDCM 0087 *C.albicans* ATCC® 90028™	0 to 1+ growth after 48 hours
Media designed for the isolation of *M.haemophilum* e.*g.:*			
▪ Lowenstein-Jensen plus Pyruvate & FAC ▪ Kovac's B83 agar	35-37°C	Standard set	2+ to 3+ Growth within 21 days
	30-32°C	Plus: *M.haemophilum* ATCC® 29548™ (NCTC 11185)	
Plate media e.*g.:*			
▪ Middlebrook 7H9, 10 or 11 agar	35-37°C	Standard set	2+ to 3+ Growth within 21 days

Underlined number denotes the Type Strain
ATCC is a registered trademark of the American Type Culture Collection, Rockville, Md, USA www.atcc.org
NCTC is the National Culture Type Collection of the UK Health Protection Agency www.hpacultures.org.uk
WDCM is the World Data Centre for Microorganisms. See www.wdcm.org
NVAP: Naladixic acid, vancomycin, amphotericin, polymyxin
FAC: ferric ammonium citrate

These are recommended requirements. Additional microorganisms may be selected to challenge specific media types

Appendix B: Laboratory Maintenance System for Mycobacteria Quality Control Organisms



Appendix C: Example - Mycobacteria Media Quality Control report

HEADER: *TESTING LABORATORY LOGO*

MYCOBACTERIA MEDIA QUALITY ASSURANCE REPORT

MEDIA MANUFACTURER:

MEDIA NAME: Lowenstein-Jensen with pyruvate

Batch Number:……..Date Prepared…….. Expiry Date:…… Date Tested:……..…..

	Acceptance criteria	**Actual Result**	**Accept/ Reject**
pH	7.6 – 8.0		Accept☐ Reject☐
Colour	Light green (+/- slightly yellow)		Accept☐ Reject☐
Consistency	Firm		Accept☐ Reject☐
Growth characteristics after 3 weeks incubation at 36°C: (grade growth –,1+ to 3+)			
M.tuberculosis NCTC 13144 #1	2+/3+		Accept☐ Reject☐
M.tuberculosis NCTC 13144 #2	2+/3+		Accept☐ Reject☐
M.intracellulare NCTC 13025 #1	2+/3+		Accept☐ Reject☐
M.intracellulare NCTC 13025 #2	2+/3+		Accept☐ Reject☐
M.bovis NCTC 10772 #1	2+/3+		Accept☐ Reject☐
M.bovis NCTC 10772 #2	2+/3+		Accept☐ Reject☐
M.malmoense NCTC 11298 #1	2+/3+		Accept☐ Reject☐
M.malmoense NCTC 11298 #2	2+/3+		Accept☐ Reject☐
Uninoculated	No growth		Accept☐ Reject☐
Uninoculated	No growth		Accept☐ Reject☐

Comments:

The testing of this media complies with the requirements of the document, "Guidelines for Assuring Quality of Solid Media used in Australia for the Cultivation of Medically Important Mycobacteria, July 2012".

The media has been tested and found to be ACCEPTABLE / NOT ACCEPTABLE for its intended purpose.

Tested by:……Date:…/…/… Authorized by:……Date:…/…/…

Footer: *© Microbiology* *Form: MTP\xxxx_QA* *Issue #: 1* *Effective date: 11/12/13* *Authorised by: ……..*

Appendix D Sampling Plan for Mycobacteria Culture Media

Small Batches (≤100 units): 1% or 1 unit from beginning and 1% or 1 unit from end of batch (14).

Double Sampling Plan (>100 units) NORMAL SAMPLING PLAN, AQL - 2.5, GENERAL INSPECTION LEVEL = 1 (8)

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 (14), 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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