Instructions for Use

REF - BM1301 **Oxalic Acid (3%)**

Bottled Media

1. Intended Use

For *in vitro* diagnostic use only. BM1301 Oxalic Acid (3%) is a reagent used in the digestion and decontamination of sputum samples [1] for subsequent cultivation of *Mycobacteria* spp. It is particularly useful for specimens contaminated with *Pseudomonas* spp.

2. Composition*

Ingredient	g/L
Oxalic Acid	30

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Most clinical specimens submitted for *Mycobacterium* spp. isolation are contaminated with more quickly growing commensal microbial flora [2]. Additionally, *Mycobacterium* spp. may be retained in respiratory secretions and not released for culture until the sample is liquefied [3]. In 1930, Corper and Uyei reported successful use of an oxalic acid technique for digestion and decontamination of clinical materials prior to medium inoculation [4].

4. Principle

Oxalic acid inactivates or kills contaminating microorganisms in clinical samples such as sputum. It also liquifies such samples releasing *Mycobacterium* spp. for culture. Mycobacteria are more resistant to the effects of oxalic acid and are culturable after the acid/neutralisation process. BM1301 Oxalic Acid (3%) is particularly useful for samples that may be contaminated with pseudomonads [3].

5. Physical Characteristics

	Appearance and Colour	pH
Medium	Colourless liquid	N/A

6. Materials Provided

BM1301 Oxalic Acid (3%) is available in the formats detailed below. Each bottle is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

Product Code	Product Format
BM1301-P030-2	50 x 2ml in Polycarbonate Universals
BM1301-P030-3	50 x 3ml in Polycarbonate Universals
BM1301-P030-10	50 x 10ml in Polycarbonate Universals
BM1301-P030-20	50 x 20ml in Polycarbonate Universals

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms.

8. Specimens

BM1301 Oxalic Acid (3%) is suitable for use with the following specimens:

- Clinical Specimens: Specimens may include sputum, endotracheal or tracheal aspirates, and nasopharyngeal aspirates/secretions. A sputum sample should appear thick and mucoid or clear but with purulent grains. Clear saliva or nasal discharge is not acceptable as a specimen. Ideally, a sputum specimen should have a volume of approximately 5 - 10ml.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing

9. Test Procedures and Interpretation of results

The choice of the most suitable method and length of decontamination will vary with specimen type and the expected level of contaminants [1]. Laboratories should treat samples according to the level of contamination expected in the sample.

Example:

Add an equal volume of BM1301 Oxalic Acid (3%) to specimen in a sterile centrifuge tube. Mix the contents thoroughly and let stand for 30 minutes with further mixing to ensure digestion of the sample.

Centrifuge at 3000g for 15 to 20 minutes. Pour off supernatant into a splash-proof discard container filled with a suitable disinfectant.

Neutralise the specimen with 2% sodium hydroxide solution (E&O BM1318) or resuspend in 1-2ml of 0.067M phosphate buffer (E&O BM1359).

Culture treated samples according to local, national, or international standards or guidelines being followed.

10. Quality Control

Tested for sterility only. Incubate a sample of BM1301 at 37 ± 1°C for 48 hours and a second sample at 20 ± 5°C for 48 hours then subculture 10µl of each sample to separate Columbia Agar with 5% Horse Blood plates (E&O PP0120). Incubate the subculture plates at the respective temperature for 18-24 hours.

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To verify sterility of BM1301 Oxalic Acid (3%), sterility testing as described in Section 10 was carried out. No growth was observed so it was concluded that samples were sterile.

12. Limitations

- Over exposure to BM1301 Oxalic Acid (3%) may damage target organisms and reduce numbers recovered or prevent growth

13. Precautions and Warnings

This product is considered hazardous.

Refer to BM1301 Material Safety Data Sheet. **Conduct full COSHH assessment for the local laboratory.**

Causes serious eye damage, harmful if swallowed, and harmful in contact with skin. Wear protective gloves/protective clothing/eye protection/face protection as determined by the local COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

14. Storage conditions and Shelf life

Store BM1301 upright in the original bottle with the lid tightly closed at between 15 and 30°C. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use media if it is contaminated, discoloured or out of date. Additionally, do not use medium if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Public Health England (2020) UK Standards for Microbiology Investigations: Investigation of specimens for *Mycobacterium* species. SMI B40; Issue 7.3. London: Standards Unit.
2. Kubica, G., Dye, W, Cohn, M. and Middlebrook, G. (1963) Sputum digestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria. Am Rev Respir Dis., 87, pp.775–779.
3. Kent, P. and Kubica, G. (1985) Public Health Mycobacteriology: A Guide for the Level III Laboratory. Minnesota: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control.
4. Corper, H., and Uyei, N. (1930) Oxalic acid as a reagent for isolating tubercle bacilli and a study of the growth of acid-fast nonpathogens on different mediums with their reaction to chemical reagents. J Lab Clin Med., 15(4), pp.348-369.
5. Owusu, E., Newman, M., Akumwena, A., Bannerman, E., and Pluschke, G. (2017) Evaluating decontamination protocols for the isolation of Mycobacterium ulcerans from swabs. BMC Microbiol., 17(1), pp.2

Version History*

001 16/08/23 - New Document Created

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

EC REP

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place