Instructions for Use

REF - BM1314 **TB Buffer (pH 4.5)**

Bottled Media

1. Intended Use

For *in vitro* diagnostic use. BM1314 TB Buffer (pH 4.5) is primarily used for neutralising sputum specimens during processing.

2. Composition*

Ingredient	g/L
Potassium dihydrogen orthophosphate	140.0
Phenol red solution (0.2%)	3.8ml

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Most clinical specimens submitted for acid-fast bacilli isolation are contaminated with more quickly developing commensal microbial flora [(1)]. Additionally, acid-fast bacilli may be retained in respiratory secretions and not released for culture until the material is liquefied [(2)]. Respiratory specimens, such as sputum, contain mucin which may trap microorganisms. Decontamination and digestion of the mucous components kills contaminating normal flora and allows slower growing mycobacteria to grow. Timely neutralisation with sodium hydroxide prevents the potential loss of mycobacteria caused by high pH levels of decontaminants, resulting in the preservation of more viable organisms.

BM1314 is suitable for use in conjunction with Sodium Hydroxide (4%) (e.g., E&O BM1322).

4. Principle

Potassium dihydrogen orthophosphate acts as a buffer and neutralises any alkaline reagents used in the treatment of the specimen. Phenol red is used as a pH indicator.

5. Physical Characteristics

	Appearance and Colour	pH
Medium	Yellow liquid	4.5 ± 0.2

6. Materials Provided

BM1314 TB Buffer (pH 4.5) can be provided in the formats detailed below. Each bottle is ink-jet printed or labelled with (abbreviated) product name, product code, lot number and expiry date.

Product Code	Product Format
BM1314-P030-10	50 x 10ml in Polycarbonate Universals
BM1314-P030-14	50 x 14ml 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

BM1314 TB Buffer (pH 4.5) is suitable for the testing of the following specimens:

- Clinical Specimens: Specimens could 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 accepted 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

Clinical Specimens [(1)]

- Add an equal volume of Sodium Hydroxide (4%) (E&O BM1322) to 3-5ml of specimen in a sterile centrifuge tube.
- Mix the contents thoroughly to digest.
- Allow the sodium hydroxide to act for 15 minutes with periodic vortexing of the specimen during this time. Do not
- expose specimen for longer than 15 minutes as this may result in reduced recovery of mycobacteria.
- Neutralise the specimen with BM1314 TB Buffer (pH 4.5). The yellow colour of the buffer will disappear when neutralisation is complete.
- Centrifuge at 3000rpm for 15 minutes.

- Discard the supernatant leaving 1ml to re-suspend the pellet. Alternatively, discard all the supernatant and re-suspend the pellet in 1ml of 0.067M phosphate buffer (pH 6.8).

Inoculate 0.2ml to required culture media (e.g. E&O BM8300 Non-acidified Lowenstein Jensen Pyruvate Medium).

10. Quality Control

Tested for sterility only. Incubate two samples at 37 ± 1°C for 72 hours and 20 ± 5°C for 72 hours. After 48 hours, subculture 10µl of one sterility broth (one from the 20 ± 5°C set and one from the 37 ± 1°C set) onto E&O PP0120 Columbia Blood Agar and incubate at the respective temperatures for 18-24 hours.

To test for neutralisation, BM1314 TB Buffer (pH 4.5) is used in conjunction with E&O BM1322 Sodium Hydroxide (4%). Add BM1322 to BM1314 and mix well. The yellow colour should disappear as the BM1314 is neutralized. Check the pH of the mixture: pH spec of BM1322/BM1314 mixture = 7.0 ± 0.2

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 fully verify sterility of BM1314 TB Buffer (pH 4.5), samples were incubated at 37 ± 1°C for 72 hours and 20 ± 5°C for 72 hours and subcultured onto Columbia blood agar before being incubated for a further 37 ± 1°C for 18-24 hours and 20 ± 5°C for 18-24 hours. No growth was observed, and samples were concluded to be sterile. Additionally, pH neutralisation testing resulted in final mixture pH values ranging from 6.8-7.2. Therefore, it can be concluded that BM1314 TB Buffer (pH 4.5), meets criteria to be used in microbial testing. Trend analysis data available upon request.

12. Limitations

- Additional biochemical, serological, or molecular tests should be performed on sub-culture isolates to confirm identity.
- Large volumes of sodium hydroxide solution may overcome the neutralisation capacity of BM1314.
- Insufficient BM1314 may fail to neutralise sodium hydroxide.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory 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.

Refer to E&O BM1314 Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM1314 in the original bottle with the lid tightly closed at between 2 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. London: Standards Unit

2. Kubica, G., Dye, W, Cohn, M. and Middlebrook, G. (1963) Sputum digestion and decontamination with N-acetyl-L-cysteinesodium hydroxide for culture of mycobacteria. Am Rev Respir Dis., 87, pp.775–779.

Version History*

001 18/04/2024 - New document created

002 10/03/2026 - Added Limitations in line with product device file. Formatting changes.

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

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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