TB-Beads
TBRP Instructions for Use
(Issue 22.2)

A. Intended use. For the concentration of mycobacteria including *Mycobacterium tuberculosis* from at least 1 ml of thinned sputum prior to diagnosis by microscopy, culture, PCR or immuno analysis. For *in vitro* use only.

B. Warning and precautions. To be used by trained personnel only. Use within an appropriate bio-safety cabinet. Wear appropriate protective clothing including gloves and laboratory coats. Dispose of all potentially infectious waste in a safe and responsible manner.

C. Kit contents:

TBRPB	1 * 500 ml	TB-Bead Solution.
TBEB9	1 * 10 ml	TB Elution Buffer.
TBSR	1 * 10 ml	TB Slide Reagent.

Do not use beyond stated expiration date. Store all reagents at room temperature.

D. Accessory reagents
Magnet to capture TB-Beads.

E. Preparation before kit use.

Solutions required

Thinning Solution
This is the Decontamination/Thinning Solution used by most laboratories. Normally this is 2-4% (w/v) sodium hydroxide (2-4 gm dissolved in 100 ml of mycobacteria-free water). This solution is stable for many months at room temperature. Many laboratories add 1.45% (w/v) sodium citrate and 0.5% (w/v) NALC for more effective thinning. Once NALC is added, this solution can be used for up to 24 hours which allows two consecutive days of work.

Wash Solution – 10 mM NaOH
This is 0.04% (10mM) sodium hydroxide. This can be made by dissolving 0.4 g sodium hydroxide in 1 litre of sterile mycobacteria-free distilled water. Alternatively, stock sodium hydroxide can be diluted appropriately.

Preparation of slides for microscopy
Slides should be labelled well in order to avoid mix ups. If a comparison is to be made between the direct smear and the TB-Bead concentrated sample, a further direct smear should be performed from the sputum, dried and stained.

Equipment required but not supplied
Magnet to capture TB-Beads. This can be supplied; please enquire.

F. Protocol
Before proceeding, see Preparation before Kit use above.
Note: The TB-Beads concentration procedure concentrates the sample into approximately 250-350µl of eluate (this can vary, depending on the strength of the magnet being used). Therefore, for microscopy analysis, little or no benefit will be derived from sputum samples of less than ~500µl. i.e. in this case the sample ends up in about the same elution volume as the original sputum volume. Ideally, 1ml to 5ml of sputum should be used which will give a concentration factor of 4-10 fold.

1. Thin the sputum sample with sodium hydroxide by adding an equal volume of Thinning Solution (see above) to the sample. Mix and incubate for 15-20 min. Use a container appropriate for the volume of sputum to be tested. For example, for larger volumes, use sputum pots and for smaller volumes, use microfuge tubes.
2. Make a note of the approximate volume of thinned sputum to be used in the capture. **Note:** variable volumes of thinned sputum can be used (between 1-10 ml). The assay will be more sensitive if more sputum is used but we recommend a maximum volume of 10 ml of thinned sputum should be used.
3. To the thinned sputum, add an equal volume of TB-Bead Solution. **Note:** make sure that the beads are resuspended before use (shake the stock bottle). It is not important if you do not add exactly an equal volume of TB-Beads. The capture of the mycobacteria **will not** be affected if the volume of the TB-Bead Solution slightly exceeds or is slightly less than the volume of thinned sputum **but if the volume of the TB-Bead Solution is considerably less than that of the thinned sputum, the capture may be inefficient**.
4. Leave the tubes for 2 minutes at room temperature to enable the mycobacteria to be captured to the beads. **Note:** a longer capture time will not affect the capture.
5. Place the containers onto magnets and leave to allow the beads to be captured to the magnet which usually takes less than 1 min. Thicker sputum samples may take longer.

6. Leaving the container on the magnet, pour the liquid to waste. Remove the container from the magnet immediately after pouring off the supernatant.

7. Add a volume of Wash Solution and re-suspend the beads. **Note:** the exact volume of the Wash Solution is not essential but the volume used should be **at least** the original volume of thinned sputum. **It is essential** that the beads do not remain in the Wash Solution for longer than 5 minutes as the bacilli will begin to elute. Therefore, re-suspend the beads in the Wash Solution and proceed to step 8 as quickly as possible. At this step we **do not** recommend batch processing i.e. adding Wash Solution to all samples being processed before proceeding to step 8. Instead, we recommend that samples should be washed and carried through to step 8 individually.

8. Capture the beads again on the magnet and pour off as much liquid as will easily drain away. **Note:** Some liquid will remain. Remove the container from the magnet immediately after pouring off supernatant.

9. Finally, add 100µl Elution Buffer to the tubes in order to elute the bound mycobacteria

   Note: some liquid will carry over with the beads so the final volume may be 200-350µl which means that the Elution Buffer is diluted 3-5 fold. If performing analysis on small volumes of sputum, with processing in microfuges, for example, dilute the Elution Buffer 4-fold before use and use 50 µl for elution).

   Re-suspend the beads well in this Elution Buffer and leave for about 5 minutes. In practice a pipette or pastette can be used to add a drop(s) of Elution Buffer.

Analysis can now be performed on the eluted solution which contains the eluted mycobacteria. **Note:** it is important that the eluate is not diluted prior to microscopy or the microscopy will be less sensitive.

For microscopy
Capture the Beads in the container using the magnet. To each slide to be used, add 10 µl of TB Slide Reagent to the area where the smear is to be made. Add 50 µl of the bead-free eluate from step 9 above to the slide and mix well with the TB Slide Reagent; a faint white precipitate will form. Allow the liquid to dry (slides can be warmed to aid drying). Heat fix and stain with ZN or auramine as normal.

For culture and/or nucleic acid analysis (including PCR)
First use the eluate to prepare the slide for microscopy (see above). If more eluate is needed for multiple analysis, 250-500µl sterile mycobacteria-free water can now be added to the eluate and mixed. Capture the beads using the magnet and use the bead-free supernatant for the culture and/or nucleic acid analysis.
If using the eluate for molecular analysis, take 250µl of the eluate and centrifuge in a microfuge (small centrifuge) for 5 min at 12,000 rpm. After centrifugation, remove the liquid being careful not to disturb the pellet. Re-suspend the pellet in 50µl of liquid appropriate for the DNA release method being used - please see the guidance provided on our website www.microsens.co.uk on our Product Resources page.

G. Controls

It is recommended to keep stocks of sputum known to be smear positive to act as a control for the TB-beads kit.

H. Sensitivity and Specificity

The TB-Bead kit for the concentration of mycobacteria from thinned sputum has been demonstrated to have similar sensitivity and specificity as mycobacteria concentrated from thinned sputum by centrifugation as tested by auramine O acid-fast microscopy and culture[1-3].

1. Liu J, Sun Z-Q,Peia H et al. Increased case finding of tuberculosis from sputum and sputum deposits after magnetic bead concentration of mycobacteria. J Microbiol Methods 2013; 93(2):144-7. DOI: http://dx.doi.org/10.1016/j.mimet.2013.02.010.
2. Ghodbane R, Drancourt M. Magnetic bead protocol for culturing Mycobacterium tuberculosis from Sputum samples. J Clin Microbiol 2013; 51(5): 1578. 2013. DOI: http://dx.doi.org/10.1128/JCM.03428-12.
3. Ohkuma M, Ikeda K, Obayashi K, et al. Evaluation of TB-beads assay utilizing the technique of magnetic beads--an innovative assay method for detection of acid fast bacilli. Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi. 2012;23(1):1-9.