Myco DDR™ Trident™ 3.0%

Mycobacterium Digestion and Decontamination Reagent Set

CE IVD 15C 30C

INTENDED USE

The IMMY MycoDDR reagent set contains all the required reagents for the Digestion and Decontamination of clinical specimens for Mycobacterium spp. diagnosis.

SUMMARY and EXPLANATION

Specimens that are suspected to contain normal, transient, or contaminating bacterial flora should be subjected to a chemical decontamination process that allows for effective recovery of the mycobacteria.(1) The digestion and decontamination procedure and reagents utilized in this set are based on those described by Kubica et.al. (2) The mucolytic compound N-acetyl-L-cysteine (NALC) is combined with a sodium hydroxide:sodium citrate solution to digest the mucus while the high pH of the sodium hydroxide kills any contaminating bacteria. The high pH of this solution can also kill mycobacterium after 15-20 minutes making the timing of the digestion-decontamination process critical.

After the digestion/decontamination process, it is equally critical that the solution be brought back to a neutral pH as quickly as possible. The 3.0% NaOH Reagent A includes a pH indicating reagent that changes from blue at basic pH to colorless at near neutral pH. This allows the laboratory technologist to visually titrate the solution using the included Neutralization Buffer B. The resulting solution is subjected to centrifugation and decanted. The resulting specimen sedimentation pellet is re-suspended in Resuspension Buffer C.

WARNINGS and PRECAUTIONS

- For In Vitro Diagnostic Use Only
- Precautions should be taken to avoid cross-contamination between specimens. In a retrospective study, 16% of 140 cases of multi-drug resistant tuberculosis were the result of laboratory cross-contamination (3)

REAGENT PRECAUTIONS

- The 3.0% NaOH Reagent A contains sodium hydroxide, a caustic chemical. Please take care when working with this solution.
- Standard biohazard precautions should be employed when working with clinical specimens that have the potential to contain viable tuberculosis cells to prevent contamination or infection of other samples or laboratory personnel.

REAGENTS PROVIDED

Quantities According to REF number				
REF	NALC (# 300 mg vials)	3.0% NaOH Reagent A (# 60mL bottles)	Neutral. Buffer B (# 30mL Bottles)	Resuspens. Buffer C (# 3mL bottles)
DDR012-3.0	1	1	12	12
TBNN1010-3.0	10	10	None	None
TBP300-1	1	None	None	None
TBP300-5	5	None	None	None
TBNN11-3.0	1	1	None	None
TBPN67-60	None	None	60	None
TBPN67-500	None	None	1 x 500mL bottle	None
TBRB30-60	None	None	None	60

MATERIALS NOT PROVIDED

- **Centrifuge**
- **Vortex Mixer**
- **Sterile Pipettes**
- **Microscope Slides**
- **Centrifuge Tubes**
- **TB Media**

REAGENT PREPARATION

Note: The 3.0% NaOH Reagent A will remain active for 72 hours after the addition of the NALC reagent. For best results, please discard any remaining reagent after this time period.

1. Loosen the cap on the 3.0% NaOH Reagent A vial.
2. With the plastic safety sleeve still attached, carefully break off the top of the glass ampoule containing the NALC Powder.
3. Add the NALC Powder to the 3.0% NaOH Reagent A vial. It is not necessary to rehydrate any residual NALC powder that may remain in the ampoule at this time.

REAGENT STABILITY AND STORAGE

The MycoDDR reagents contained in this package are stable until the labeled expiration date when stored at 15-30 C.

After mixing the 3.0% NaOH Reagent A and the NALC Powder, store any unused portion at 2-8 C for up to 72 hours. Do not freeze or heat above 30 C. Allow the product to come to ambient temperature prior to use.

SPECIMEN COLLECTION AND PROCESSING

Clinical specimens should be collected and transported to the laboratory according to established protocols and standards. Please refer to your local institutional guidelines for the required collection and transport procedures.

All specimens should be handled according to CDC/NIH guidelines or local institution guidelines for any potentially infectious human serum, blood or other body fluids. Prior to discarding, sterilize specimen containers and other contaminated materials by autoclaving.

SPECIMEN PROCESSING PROCEDURE

1. Place clinical specimens (in 50 mL centrifuge tubes) in an appropriate biosafety hood prior to processing.
2. Loosen, but do not remove, the caps on each of the individual specimen tubes. It is important to only have one specimen open at a time and to prevent any interchanging of caps
3. Working with one specimen at a time, aseptically pipet a volume of prepared 3.0% NaOH Reagent A/NALC reagent **(See Reagent Preparation Section)** as outlined in the table below:

SAMPLE VOL	*3.0% NaOH Volume to Add*
1-9 mL	Equal to Sample Volume
9-10 mL	9 mL
>10 mL	Split specimen in half and process separately. Combine pellets

4. Tighten the caps on the centrifuge tube and vortex each for approximately 30 seconds.
5. Allow each specimen to incubate at room temperature for 15-20 minutes, vortexing briefly every 5 minutes.
6. After the incubation step, remove a cap from a single specimen tube and slowly begin to pour the contents of a Neutralization Buffer B vial into the tube. Observe the color of the liquid in the tube and stop pouring once the color of the solution has changed to clear or colorless.
7. Discard any remaining Neutralization Buffer B after use.
8. Repeat steps 6 & 7 for each individual specimen, using a separate vial of Neutralization Buffer B on each. Do not reuse the vial on multiple specimens as this can lead to cross contamination and erroneous results.
9. Tighten the cap on each tube.
10. Centrifuge each tube for 15 minutes at 3000 x g.
11. Return the specimen tubes to the biosafety hood.
12. Slowly pour off all of the supernatant into a splash-proof container partially filled with an appropriate disinfectant or into a separate disposable discard tube to prevent cross-contamination.
13. Using a sterile transfer pipette, add approximately 0.5 - 1.0 mL of an individual vial of Resuspension Buffer C to the pellet and mix to resuspend.
14. Prepare the appropriate smears for Acid Fast Staining and/or inoculate culture media according to laboratory protocols.
15. Add an additional 1-2 mL of Resuspension Buffer C (According to laboratory volume requirements) to the pellet using a sterile transfer pipette.
16. Follow the manufacturer's recommendations for any additional diagnostic procedures on the resuspended pellets

QUALITY CONTROL

Visually inspect buffers to ensure that they are clear and colorless, with the exception of the 3.0% NaOH Reagent A solution, which should have a blue color. Discard any reagents that show precipitation, turbidity or cloudiness.

PROCEDURE NOTES

Specimens that are consistently contaminated with Pseudomonas species may require an additional oxalic acid treatment as outlined in Kent & Kubica (2).

LIMITATIONS

It is necessary to accurately follow the procedure as outlined above. Inaccurate timing, buffering, decanting, etc. can lead to loss of viable Mycobacterium spp. and flawed culture results.

EXPECTED RESULTS

The recovery of viably Mycobacterium spp. organisms can be expected if present in the clinical sample and processed according to this package insert.

PERFORMANCE CHARACTERISTICS

Specimens submitted for routine mycobacterium testing were simultaneously processed using the IMMY Myco-DDR system and Company A's system. To date, 99 total specimens have been tested, 38 pulmonary specimens (sputum, BAL, pleural fluid), and 61 miscellaneous specimens (tissue and wounds). Of the 99 specimens, 10 tested positive for mycobacterium. Overall agreement between IMMY and Company A is excellent (kappa=0.878, 95% CI: 0.712-1.00).

IMMY / Co. A	Mycobacterium Positive	Mycobacterium Negative
Mycobacterium Positive	8	0
Mycobacterium Negative	2	89

REFERENCES

1. **Cernoch, P.L., R.K. Enns, M.A. Saubolle, and R.J. Wallace, Jr.** 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coord. ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
2. **Kent, P., Kubica, G.P.**, Public Health Mycobacteriology: A Guide for the Level III Laboratory. Centers for Disease Control and Prevention (CDC). 1985.
3. **Small, P.M., N.B. McClenny et.al.,** Molecular Strain Typing of Mycobacterium tuberculosis to confirm cross-contamination in the mycobacteriology laboratory and modification of procedures to minimize occurrence of false-positive cultures. J. Clin. Microbiol. 31(7):1677-1682.

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INTERNATIONAL SYMBOL USAGE

15C 30C	Storage 15-30° C	LOT	Lot Number
	Manufactured by	REF	Reference Number
	Expiration Date	IVD	In Vitro Diagnostics
CE	Conforms to European Union Requirements	Σ	Sufficient for "#" Tests

3.0% NaOH

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