SALUBRIS, Inc.
GATEWAY TO HEALTH, WORLDWIDE

DECOCENT®

DECONTAMINATION AND CONCENTRATION KIT FOR MYCOBACTERIAL CULTURE, MICROSCOPY AND MOLECULAR METHODS

Catalogue #: DCC010, DCC050 **Instructions for Use** **For In Vitro Diagnostic Use**

Product's name:
DECOCENT®

Product's intended use:
DECOCENT® is a sample decontamination and concentration kit. It permits the processing of samples for microscopy, culture and molecular methods, for identification and isolation of mycobacteria. It is intended for in vitro diagnostic use.

General information:
DECOCENT® is a kit that enables to apply an improved version of Kubica ("sodium hydroxide - N-acetyl-L-cysteine decontamination and concentration) method".[1,2,3] It is designed to eliminate the disadvantages of the Kubica method. Thanks to its pH indicator and its original neutralization buffer, it allows better and easy adjustment of the final pH of the processed sample. ***DECOCENT®*** provides all the materials needed for processing the samples. It eliminates the problem of cross-contamination while saving time and effort.

Principles of the procedure:
Clinical samples like sputum contain many microorganisms other than mycobacteria. Processing with a solution containing sodium hydroxide decontaminates the samples by killing many microorganisms susceptible to sodium hydroxide, while mycobacteria, which are resistant to alkaline pH, survive. Sodium hydroxide also decreases viscosity of the sputum and thus liquefies the samples. This facilitates the sedimentation of bacilli during centrifugation.

Ingredients:
- Specially formulated decontamination solution containing NaOH and a pH indicator.
- Neutralization solution containing a specially formulated buffer solution.

Cautions and warnings:
FOR IN VITRO DIAGNOSTIC USE.
Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards. Specimen preparation must be done in a biological safety cabinet.
DECOCENT® has been designed to minimize risks associated with mycobacterial testing. However, to further reduce the risks of accidental exposure to infectious agents, additional precautions should be taken. At a minimum, specimen manipulation should be done in a contained environment with controlled access, which has a tuberculosis exposure control plan. The locations should have surfaces that can be easily decontaminated using an appropriate topical disinfectant. Pathogenic microorganisms including Hepatitis B Virus and Human Immunodeficiency Virus (HIV) may be present in specimens. Universal precautions and local laboratory guidelines should be followed in handling all items contaminated with blood or body fluids. If a tube is found to be leaking or is accidentally broken during collection or transport, use the established procedures in your facility for dealing with mycobacterial spills. At a minimum, universal precautions should be employed.

General safety precautions:
- Always wear masks and gloves when working with potential biohazard material.
- Work in a laminary flow cabin, biosafety level II, when pipetting the samples.
- Never use mouth pipetting.
- A refrigerated centrifuge with airtight swinging buckets is recommended for sedimenting bacteria to minimize aerosols.
- Use only conical centrifuge holders adapted to the shape of the sampling tubes.
- If spills of the contaminated material occur, disinfect with 2.5% hypo-chloride solution.
- If decontamination or neutralization solution contacts skin, eyes or mucosal surfaces, wash immediately and thoroughly with water and seek immediate medical help.
- At a minimum, universal precautions should be employed.
- Tubes should be discarded in an appropriate manner.

Storage instructions:
Store at room temperature, in a dry place, in its original box.

List of materials provided:
List of materials for processing one sample:
- 10 mL of decontamination solution in polypropylene tube.
- 10 mL of neutralization solution in polypropylene tube.

In ***DECOCENT®*** DCC010 each cardboard box contains 75 sets of the materials listed above.
In ***DECOCENT®*** DCC050 each cardboard box contains 52 sets of the materials listed above as well as 52 plastic centrifuge tubes.

List of materials that are not provided:
- Centrifuge tubes (DCC010)
- (Refrigerated) centrifuge
- Vortex
- Automatic pipettors
- Sterile pipette tips
- Disinfectant solution

Indications of instability or deterioration: ***DECOCENT®*** kits should not be used if above indicated volumes are not present in each tube or if there is turbidity or sediments in the solutions.

Instructions for use:
Sputum and body fluids other than urine:
1- Samples like sputum, broncho alveolar fluid, gastric lavage fluid, pleural, pericardial, or peritoneal fluids may be processed. **For microscopy:** before starting the decontamination and concentration process, make a direct smear of the sample on a slide and let it dry. This dry smear improves adhesion of the concentrated specimen to the slide and increases the sensitivity of microscopic examination.
2- Transfer a maximum volume of 10 mL of the sample from the collection cup into a centrifuge tube (included in DCC050).
3- **Empty all** of the red decontamination solution into the centrifuge tube containing the sample and close the cap securely.
4- Homogenize the sample by vortexing.
5- Leave at room temperature for 15 minutes. In laboratories with high contamination rates, this decontamination time may

DECOCENT®

be extended up to 20 minutes.

6- **Empty all** of the transparent neutralization solution into the centrifuge tube in one movement. Close the cap securely.
7- Mix the sample by vortexing. The formation of an orange to pinkish color will indicate that the pH is adjusted properly.
8- Spin the tube for 15 minutes at 2000 x g in a centrifuge. Using a refrigerated centrifuge with airtight buckets will minimize killing mycobacteria due to heat formation.
9- Carefully discard the supernatant into a container with disinfectant solution. Leave approximately 2 mL of fluid over the sediment.
10- Resuspend the sediment by vortexing. This suspension can now be used for microscopy, culture, and molecular diagnostic methods.

Urine samples:

It is recommended to obtain early morning urine to increase the chance of recovery of mycobacteria.

1- Transfer the urine sample from the collection cup into the centrifuge tube. The tube can be filled up to the 50 mL line.
2- Spin the tube in a centrifuge for 15 minutes at 2000 x g. Using a refrigerated centrifuge with airtight buckets will minimize killing mycobacteria due to heat formation.
3- Discard the supernatant according to the safety rules of your laboratory, leaving approximately 2 mL of concentrated sample.
4- Continue processing the urine sample, according to the instructions for *Sputum and body fluids other than urine*, starting from step 3.

Quality control:

Positive control: Respiratory secretions spiked with mycobacteria.
Negative control: Respiratory secretions spiked with *Escherichia coli* and *Staphylcoccus aureus*.

Description of the amounts of reagents necessary, and the parameters of time and temperature:

The only reagents required are those included in the kit. The whole procedure takes between 30 to 45 minutes. The procedure is performed at room temperature. It is recommended to spin in a centrifuge at 4°C to minimize killing of mycobacteria and minimize aerosol formation.

Time restrictions:

Decontamination time must be from 15 to 20 minutes. Shorter decontamination times may increase the contamination rate. Extension of decontamination time may decrease the number of living mycobacteria.

Limitations of the procedure:

Some organisms other than mycobacteria may survive the decontamination process. If recurrent contamination occurs from a certain specimen, increase decontamination time appropriately.

Bibliography:

1- Kubica GP, Dye WE, Cohn ML, Middlebrook G. Sputumdigestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria. 1963. Am.Rev. Respir. Dis. 87:775-779.
2- N-acetyl-L-cysteine-sodium hydroxide method for liquefaction and decontamination of specimens. Bailey &Scott's Diagnostic Microbiology, Ninth Edition. Mosby-Year Book Inc. St. Louis, MO. USA. 1994, p:600.
3- Heifets LB, Good RC. Current laboratory methods for the diagnosis of tuberculosis. In "Tuberculosis" Ed. BloomBR.ASM Press, Washington D.C. 1994. 85-110.

Manufacturer:
SALUBRIS, Inc.
One Boston Place, Suite 2600
Boston, MA 02108, U.S.A.
Phone: (617) 960-9698
Fax: (617) 249-0803

Authorised representative:
SAS DIAGNOTIS
2, Clos de Toussue, 27300
Menneval – FRANCE
Phone: +33 232 44 28 12

Catalogue number:
DCC010, DCC050

CE