

mini Anion Exchange Centrifugation Technique (mAECT)



The number of parasites in the blood of *Trypanosoma brucei rhodesiense* patients is usually high enough for them to be seen by direct examination of blood under a light microscope. However, *T. b. gambiense* and cerebrospinal fluid (CSF) parasites exist in such low numbers that they have to be concentrated before viewing.

The most sensitive method for detection of trypanosomes in blood that is commonly used is the mini Anion Exchange Centrifugation Technique (mAECT). The mAECT method is carried out in two stages, including chromatography then concentration and viewing. In the first step, the parasites are separated from venous blood in a gel column by anion exchange chromatography and collected in a sealed glass tube. The glass tube is sealed by passing the narrow end of a Pasteur pipette over a flame in a procedure that is potentially dangerous. After parasites are collected in the glass tube, it is centrifuged and the tip examined by microscopy. An alternative protocol involving centrifugation of 5 ml of venous blood and application of the resulting buffy coat into the mAECT column has been shown to result in higher sensitivity (Camara et al., 2010).

The mAECT has a number of limitations: it is expensive, time consuming, not easy to standardize, and cannot be used in most field situations where electricity is often lacking.

A partnership between FIND, the Institute of Tropical Medicine (ITM) in Antwerp and others improved the materials used in assembly of the mAECT kits, including the gel column, collector tube, holding racks and viewing chambers.

a) Gel column

In the standard column, the gel is retained in the injector by polyurethane filters that are 7 mm and 15 mm wide respectively (small lower and larger upper filter) as shown in Figure 1A below.

The polyurethane filters that were used in the old column were replaced with polyethylene ones of equal diameters as shown below in Figure 1B. Polyethylene is widely used for the fabrication of research and medical devices for filtration of gases and liquids, and is therefore easily available.



Figure 1A

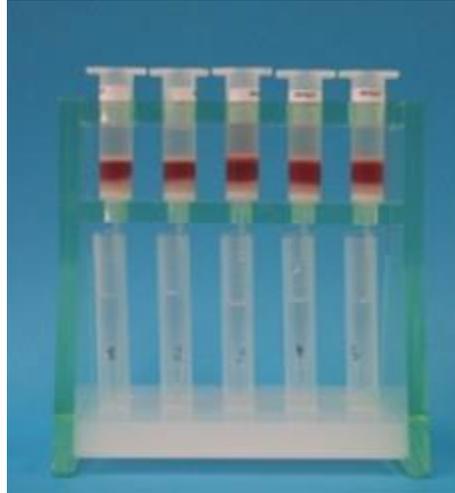


Figure 1B

Figure 1: (A) The old gel column used to filter trypanosomes from blood in the mAECT test. The gel is retained in the injector by two filters, a large upper one and a small lower one, seen here in yellow. (B) Filtration of blood using the new version of gel column. The gel is held in place by two white polyethylene filters of equal size (here shown just above and below the blood-stained gel), and mounted on a holding rack that allows easy collection of the filtrate in the collector tube below (Figure 2B).

b) Collector tube and viewing chamber

In a second step, the parasites are concentrated at the bottom of the sealed glass tube by low speed centrifugation. The tip of the glass tube is then examined for the presence of motile trypanosomes under low magnification microscopy (Figure 2A).

New, dual purpose models of collector tube (Figure 2B) and viewing chambers (Figure 2C) were developed, resulting in tremendous improvement in demonstration of parasites under a microscope. The collector tube can hold a large volume of liquid (up to 4 ml), has special features that enable it to fit into the new viewing chamber, and a specially designed tip that eliminates the need for a cover slip or water during viewing (Figure 2D).

This set-up is also used for viewing parasites in the modified single centrifugation (MSC) technique for examination of cerebrospinal fluid (CSF) during staging of HAT patients, and follow-up after treatment. Briefly, CSF is collected into the tube by gravity, directly from the lumbar puncture needle, then examined for the presence of trypanosomes under a microscope after low-speed centrifugation.

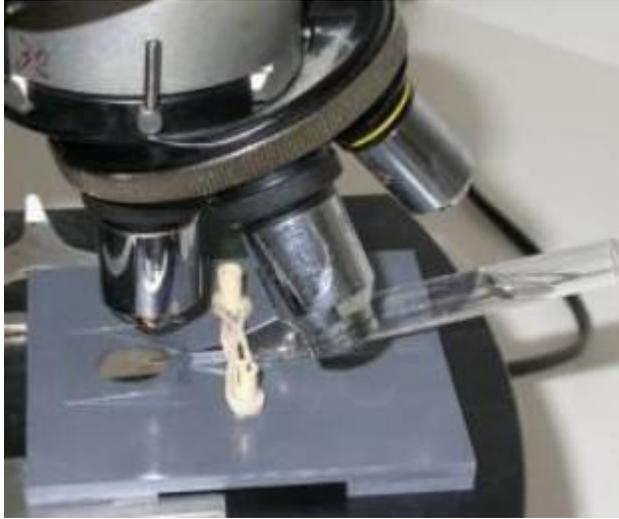


Figure 2A



Figure 2B

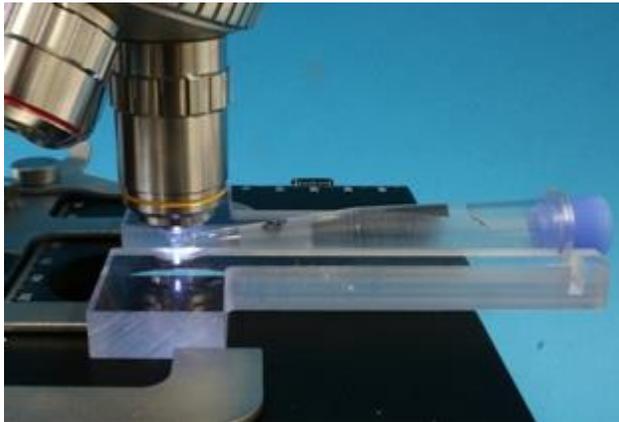


Figure 2C

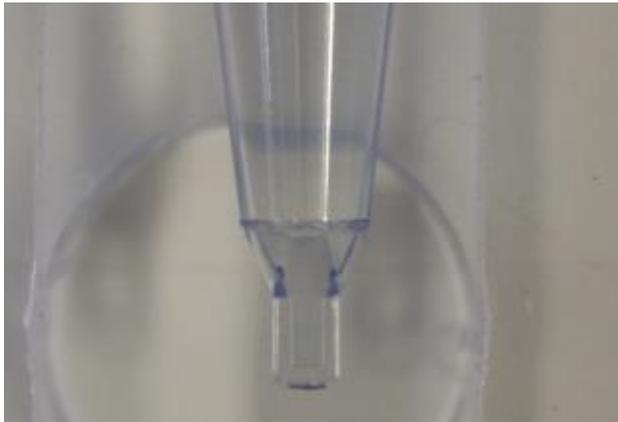


Figure 2D

Figure 2: (A) Old model of glass tube and viewing chamber that was used when examining samples for the presence of trypanosomes. The tube is held onto the chamber with a rubber band and the tip examined by low magnification microscopy. (B) The new model of collector tube (size: 10 cm) used for examining blood and CSF for trypanosomes in the mAECT and MSC techniques. The tube can hold a large volume of fluid, increasing the chances of seeing parasites when they exist at a low concentration. (C) The new set-up for viewing trypanosomes under a microscope in the mAECT and MSC techniques. The collector tube rests inside a groove in the viewing chamber, such that its narrow end is directly below the objective lens of a microscope. (D) Enlarged view of the tip of the collector tube as seen under the microscope. It comprises of a chamber with two flat surfaces between which the trypanosomes are seen.