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LABORATORY PROTOCOL

Diagnosis of human African trypanosomiasis by acridine orange fluorescence microscopy

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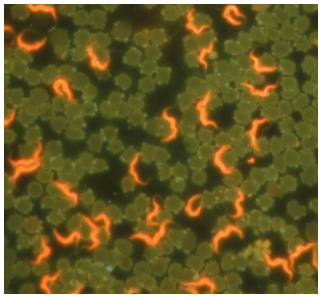
Scope

This laboratory protocol is to be used to diagnose human African trypanosomiasis (HAT) using blood smears stained with acridine orange viewed under an LED fluorescence microscope such as the Primo Star iLED microscope developed by Carl Zeiss and FIND.

Protocol

1. Prepare a stock solution of acridine orange (AO) at 1 mg/ml in distilled water.
2. Prepare a working solution of AO by diluting the stock solution 1:10 in phosphate buffered saline (PBS) pH 7.4. The final AO concentration in the working solution will be 0.1 mg/ml.
3. Prepare a thick blood smear on a microscopy slide. *Alternatively, a thin blood smear can also be used. Thin smears must be fixed with methanol.*
4. Allow the smears to dry for 20 minutes to ensure complete drying. If enough time is not allowed for proper drying, smears might be damaged during the staining and washing steps.
5. Flood the slides with freshly prepared AO working solution, and let them stain for 3 minutes.
6. Rinse the slides carefully with PBS and allow to air dry. Too much rinsing with PBS could reduce the intensity of staining on the parasites.
7. Do not use glycerol, cover slips or immersion oil.
8. Examine the slides under an LED fluorescence microscope using 400x magnification.
9. Slides should be examined for at least 10 minutes, or until parasites can be seen.



A thin blood smear showing trypanosomes stained with acridine orange (courtesy of Carl Zeiss).