BIOMEDICAL RESEARCH INSTITUTE
NIH NIAID Schistosomiasis Resource Center

9410 Key West Avenue
Rockville, MD 20850
Ph (301) 329-0412
www.afbr-bri.org

Preparation of *in vitro* schistosomules

Authors: Fred Lewis, PhD and André Miller

Introduction
A number of methods are used to transform cercariae into the next developmental stage, the schistosomule. Two of the more commonly used laboratory methods are presented here. Currently the Schistosomiasis Resource Center prefers transformation of cercariae by vortexing.

A. Transformation of cercariae by vortexing
Equipment
Refrigerated centrifuge

Materials and reagents
S. mansoni cercariae
RPMI cell culture medium
Percoll gradient suspension*
50 mL plastic, conical centrifuge tubes
250 mL tissue culture flasks

Procedure
- Place cercariae in a 50mL centrifuge tube and place on ice for 30min.
- Centrifuge the tube for 2 minutes at 100 x g and 4°C.
- With a pipette, withdraw and discard all but the bottom 3 mL from the top of the pellet.
- Add 3mL RPMI
- Cap the tube and vortex 45 seconds at high speed.
- Place tube on ice for 3min, then vortex as before.
- Vortex a total of 3 times, resting cercaria for 3 minutes after each vortex.
- Gently pipette the cercarial suspension onto 40 mL of the pre-made Percoll suspension.
- Centrifuge for 15 minutes at 500 x g and 4°C.
- Withdraw and discard the top 40 mL of the suspension.
- Wash with RPMI twice. Resuspend the pellet and add 5-10 ml RPMI to 50 mL final volume.
- Centrifuge for 5 minutes at 100 x g and 4°C.
- Wash the final pellet twice, using the above procedures, with RPMI.
- Place the resulting organisms into 250 mL tissue culture flasks in 100 mL RPMI at a density of approximately 500 organisms/mL and incubate at 37°C in a 5% CO_2 incubator.

*Formula for the Percoll medium
24 mL Percoll
4 mL RPMI
1.5 mL penicillin-streptomycin (100X penicillin 100X streptomycin)

1 mL of 1 M HEPES in 0.85% NaCl
9.5 mL distilled water

B. Transformation of cercariae by needle and syringe

Equipment

Refrigerated centrifuge

Materials and reagents

S. mansoni cercariae
RPMI
50 mL plastic, conical centrifuge tubes
10 mL plastic syringes with 22 gauge disposable hypodermic needles

Procedure

- Place 10 mL of the cercarial suspension into a 50 mL plastic centrifuge tube
- Using a 10 mL syringe with a 22 gauge needle, fill the syringe and repeatedly pass it through the needle (10-15 times)
- Allow the cercariae to settle for 3 minutes, then withdraw and discard all but the lowest 3 mL of the suspension
- Add RPMI to the resulting pellet and purify by Percoll gradient separation, as described above.

Comments

Cercariae will begin the transformation into schistosomules following different stimuli, the most important of which is their placement into culture medium. The two mechanical procedures, described above, combined with Percoll separation, will yield a clean preparation by separating the cercarial tails from the bodies. Schistosomules prepared by the above procedures and incubated at 37ºC will gradually undergo morphological and physiological changes. By 24 hours in culture, the organisms will resemble (in most respects) cercariae that have penetrated and resided in the skin for about 1 hour.

References

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For further technical information, contact André Miller in the Schistosomiasis Resource Center at ***amiller@afbr-bri.org***