Production of HES : adult *H. polygyrus* excretory-secretory product ES

1. Parasite Recovery

The life cycle of *H. polygyrus bakeri* is maintained in male CBA x C57BL/6 F1 mice orally gavaged with 600 L3 larvae. At day 14 the small intestines are placed, in Petri dishes of warm Hanks' BSS medium, two per dish. The intestines are then opened longitudinally using scissors and the luminal surface gently scraped using the edge of a microscope slide to dislodge worms bound to the gut wall.

The loose material from 4-8 Petri dishes, without the gut walls, is then placed in a Baermann apparatus, consisting of a muslin layer fixed near the top of a glass funnel with paper clips, immersed in HBSS (Sigma(. The base of the funnel is connected via plastic tubing to a removable glass tube.

The Baermann apparatus is incubated at 37°C for ~2 hrs, agitating the contents above the muslin layer after 1 hour.

Typically, from 12 mice, 3 ml of packed adult worms (~3000 parasites) collect in the glass tube at the base of the apparatus. The glass tube is disconnected (over a sink!) and the contents transferred to a 50 ml plastic conical tube, in which 6 washes of worms sedimented under gravity, using 50 ml HBSS, are performed.

The parasites are then transferred, in a sterile flow cabinet, to a fresh sterile 50 ml tube, and washed 6 times under unit gravity in 50 ml of RPMI1640 supplemented with 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma). During this time the worms are counted (removing 20 μl volumes with a sterile yellow tip that has been cut with a sterile scalpel to enlarge the aperture).

A final step involves standing the worms at RT° in 10 ml RPMI1640-Pen-Strep to which 10% Gentamicin (Gibco 15710) has been added for 20 mins.. Parasites are then washed a further 12 times under unit gravity in 50 ml of RPMI1640 and placed in culture as described below to generate *H. polygyrus* excretory-secretory product (HES).

2. Parasite Culture

Adult *H. polygyrus* worms are incubated at approximately 75-100 worms/ml for 21 days in serum-free parasite culture medium:

500 ml RPMI1640 (Gibco 42401)
20 ml 25% sterile glucose (Sigma G5400, to 1%)
5 ml 200 mM L-glutamine (Gibco 25030, to 2 mM)
5 ml 100x penicillin (to 100 U/ml) streptomycin (to 100 μg/ml) (Gibco15140)
5 ml 100x gentamycin.

Cultures are set up in 15 ml medium in T25 (25cm2) flasks (eg Corning 430639).
After 24 hrs, and subsequently every 3-4 days the supernatants are removed from the parasites and replaced with fresh media. If cultures are set up on a Thursday, remove the 24-hour supernatant on Friday and change the medium on subsequent Tuesdays and Fridays.

3. Recovered Medium

At each point of medium replacement, collect the spent supernatant under sterile conditions. Centrifuge culture medium to spin down any released eggs (400 g for 5 mins). Keeping in the sterile hood, pass supernatant through 0.22 μm filter (Millipore Miilex-GP SLGP033RS), label with date and store short-term at –20°C.

4. Preparation of HES

To prepare one batch of HES, pool 1000-1600 ml of culture supernatants, excluding any collected in the first 24 hours. Concentrate by diafiltration into PBS over a 3,000 MW filter (Millipore Cat No PLBC04310) in an Amicon stirred ultrafiltration device (Millipore). Reduce original volume to ~5 ml, fill chamber with sterile PBS (not home-made - Sigma endotoxin-tested D8537) and re-concentrate twice. Take 2 μl samples to measure by Nanodrop, using extinction co-efficient OD280 1.0 = 1 mg/ml. Aim to concentrate close to 1 mg/ml. Do not return sample to main batch!
The final concentrated batch is then also measured by Bradford assay for final protein concentration.

Adhesion of proteins to the membrane represent a significant source of reduced yield. Losses can be reduced by re-use of the membrane, if the membrane is kept sterile in 20% ethanol at 4°C, but processing times become slower with re-use.

Routinely, 1.5 L of supernatant is diafiltrated to 4 ml of final product of 1 mg/ml. Assign a batch number, label with date and concentration, and store at –80°C.

Statistics

- Each mouse yields around 300 adult worms
- Routine cycle infects 20 mice, generating 6000 worms cultured in 5 x 15 ml flasks
- Each cycle needs 12,000 larvae for infection
- Each set of cultures of 5 flasks produces 450 ml of raw supernatant collected on days 4, 8, 11, 15, 18 and 21 (15mlx5x6)
- Pooling 3 batches x 450 ml (1350 ml) produces approx 3 mg of HES, representing the product of 18000 worms (~167 ng/worm) over 20 days (~8 ng/worm/day)

Written by:
Kara Filbey
Yvonne Harcus
Rick Maizels
Elaine Robertson