

***Blepharisma* spp.**

Note: The culturing conditions below are not necessarily the optimal growth conditions for each strain, as much variation is found between strains, and cultures are not always kept in optimal growth conditions at CCAP for practical reasons. There may be more info in the individual strain data on the website.

Storing the cultures in natural daylight at room temperature should also be fine, providing they are kept out of direct sunlight.

On receipt of culture: cultures should be subcultured into fresh sterile medium as described below, ideally within a few days of receipt. If the culture vessel is very full on receipt and subculturing cannot be done immediately, we advise transferring half of the culture to a sterile container to provide air space.

ACDP Hazard Gp: 1 - Non pathogenic / non hazardous. Unlikely to cause human disease.

Culture Medium: SES (Soil extract with added salts) with 1 or 2 boiled wheat grains.

Media recipes can be found on our website: www.ccap.ac.uk/index.php/media-recipes/

Lighting: Low light

Light Cycle: -

Temperature: 20 degrees C

Sub Interval: 5 weeks (at CCAP, may vary depending on environment)

Culture Vessel: Tissue culture flask (30ml medium with 2 wheat grains added) or small petri dishes (1 wheat grain added).

Culture Method:

Blepharisma americanum CCAP 1607/1 – cells are pink in colour and quite large, this ciliate usually eats bacteria, but can be cannibalistic, this makes the food vacuole red.

Blepharisma hyalinum CCAP 1607/4 and *Blepharisma* sp. CCAP 1607/6 – cells are smaller than and not as pink as *B. americanum*.

Subculturing:

Choose one dense culture from existing stocks, the state of the culture is ascertained by microscopic examination. Aseptically prepare two new dishes/tissue culture flasks for each strain – fill the petri dishes approx. half full, add 30ml to each 50ml tissue culture flask.

Gently swirl old culture until the grain is in the middle, take up approx. 1ml with a sterile pipette from around the grain and put into the new dish/flask. Add fresh boiled wheat/barley grains to encourage bacterial growth. Seal with parafilm and incubate at 20 degrees.

Use strict aseptic techniques throughout and if possible carry out all subculturing within a laminar flow cabinet (particularly important for axenic strains).