Acanthamoeba on agar

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.

On receipt of culture: These amoebae are cystformers and may have encysted whilst in transit. To excyst the culture, subculture onto fresh medium as detailed below.

ACDP Hazard Gp: 2 – biological agent that may cause human disease and which might be a hazard to laboratory workers but is unlikely to spread to the community. Laboratory exposure rarely produces infection and effective prophylactics or effective treatment is available. Please see attached Safety Data Sheet.

Culture Medium: The amoebae are grown on Non-Nutrient agar plates (NN) with a food source, at CCAP we are using a non-pathogenic strain of *Escherichia coli*.
Media recipes can be found on our website: www.ccap.ac.uk/index.php/media-recipes/

Lighting: not required

Light Cycle: -

Temperature: 8 degrees C

Sub Interval: 6 months (at CCAP, may vary depending on environment)

Culture Vessel: Universal bottles.

Culture Method:

Preparation of *E. coli* plates

Using a sterile swab, the bacteria from an established culture are spread over the entire surfaces of nutrient agar plates. The plates are incubated at 20 degrees C for a week, at this temperature *E. coli* will have grown up to an adequate lawn of bacteria (if the *E. coli* is needed in a shorter time-frame it can be grown at 37 degrees C).

Subculturing *Acanthamoeba*

Acanthamoeba is grown on agar slopes in Universal bottles. An older culture is observed using a stereoscopic microscope, and high density areas are marked. These marked areas will be used as a guide to cut blocks for transfer to the new media. New agar slopes are spread with *E. coli* using a microbiology disposable loop, and the cut agar blocks placed, amoeba-side down, on top of the bacteria so the amoebae can feed on it. To minimise drying out of the agar and reduce the possibility of contaminants entering the bottle, the lid is sealed with a narrow strip of parafilm.

Watch the video: Subculturing cyst-forming amoebae on Vimeo

All procedures involving transfer of *Acanthamoeba* are performed within a microbiological Class II Biosafety cabinet. The scalpel used for cutting the agar blocks is immersed in 70% ethanol and heated inside the cabinet using a Bunsen burner. Use strict aseptic techniques throughout.

Hazard Group 2 Safety data sheet

To accompany cultures assigned to hazard group 2
(The CCAP does not supply organisms of hazard groups 3 or 4)

Information on Supplied Cultures as required under COSHH regulations and HSW Act s.6 (4)(c)

The strain(s) supplied (as listed on the enclosed Delivery Note) are Risk Group 2 organisms under EU Directive 90/679/EEC Classification of Biological Agents to be adopted by the Advisory Committee on Dangerous Pathogens (ACDP) Categorisation of biological agents, 4 edition. These are biological agents that may cause human disease and which might be a hazard to laboratory workers but are unlikely to spread to the community. Laboratory exposure rarely produces infection and effective prophylactics or effective treatment is available.

Avoid all contact with the organism, growth media or materials on which they have grown. To avoid these possible hazards, and reduce the risk in handling, normal aseptic microbiological techniques should be employed. All parcels containing microorganisms should be opened in a laboratory with containment level 2 as described by The Advisory Committee on Dangerous Pathogens 1995 (Categorisation of pathogens according to hazard and categories of containment. London: HMSO). Any work that may result in the creation of an aerosol containing the organism must be carried out in an appropriate microbiological safety cabinet.

CONTAINMENT LEVEL 2

Containment level 2 is suitable for use with agents of in Hazard Group 2. Laboratory personnel must receive instruction and training in handling pathogens and an appropriate standard of supervision of the work must be maintained.

1. The laboratory should be easy to clean. Bench surfaces should be impervious to water and resistant to acids,alkalis, solvents and disinfectants.
2. Access to the laboratory should be limited to laboratory personnel and other specified persons.
3. There should be adequate space (24m3) in the laboratory for each worker.
4. If the laboratory is mechanically ventilated, an inward airflow into the laboratory must be maintained by extracting room air to atmosphere.
5. The laboratory must contain a wash hand basin which should be located near the laboratory exit. Taps should be of a type which can be operated without being touched by hand.
6. An autoclave for the sterilisation of waste materials must be readily accessible, normally in the same building as the laboratory.
7. The laboratory door should be closed when work is in progress.
8. Laboratory coats or gowns, which should be side or back fastening, must be worn in the laboratory and removed when leaving the laboratory suite. Separate storage (pegs) must be provided in the laboratory suite for this clothing.
9. Eating, drinking, smoking, storing of food and applying cosmetics must not take place in the laboratory.
10. Mouth pipetting must not take place.
11. Hands must be disinfected or washed immediately when contamination is suspected, after handling infective materials, and also before leaving the laboratory.

12. In general, work may be conducted on the open bench, but care must be taken to minimise the production of aerosols. For manipulations such as vigorous shaking or mixing and ultrasonic disruption etc., a microbiological safety cabinet (Class 1 or Class II) or equipment which is designed to contain the aerosol must be used. The cabinet must exhaust to the outside air or to the laboratory air extract system (see para 34, 14(b) of ACDP Categorisation of pathogens and their containment levels).
13. Effective disinfectants must be available for routine disinfection and immediate use in the event of spillage.
14. Bench tops should be disinfected after use.
15. Used laboratory glassware and other materials awaiting sterilisation must be stored in a safe manner. Pipettes if placed in disinfectant must be totally immersed.
16. Material for autoclaving must be transported to the autoclave in robust containers, without spillage.
17. All waste materials must be made safe before disposal or removal to the incinerator.
18. All accidents and incidents must be immediately reported to, and recorded by, the person responsible for the work.

Opening cultures: all parcels containing microorganisms must be opened in a laboratory by trained personnel and, ideally, in a cabinet that will prevent inhalation of aerosols.

Details of suitable media, incubation temperatures for the growth of the strains, and any known special hazards, are supplied with the strains.

Transport: if the materials are to be transported to another laboratory they should be packaged with enough absorptive material to absorb all contents of the containers in case of breakage. They should be placed in containers that will prevent breakage. If they are to be sent abroad they must be placed in packaging conforming to current international regulations and all regulations of the recipient country must be followed. Cultures should not be forwarded to third parties outside your company.

Disposal: all cultures, media and containers should be sterilised by autoclaving at 121°C for 15 min before disposal by suitable means such as incineration.

Procedures in case of spillage: if the culture is spilt or its container broken, thoroughly wet with a disinfectant, such as 4% sodium hypochlorite, and allow 30 min before swabbing up and transferring into a container for autoclaving.