CULTIVATION OF INTESTINAL AND UROGENITAL PROTOZOA

PRINCIPLE

Culturing intestinal protozoa confirms their presence and provides material for our research activities. Culturing in the presence of an antibiotic helps to control the inevitable bacteria present in the stool sample.

SPECIMEN

Fresh stool sample.

Robinson's Culture Technique for *E. histolytica*

REAGENTS

BR (see appendix)
0.5% erythromycin,
BRH (see appendix)

PROCEDURE

This is considered to be a non-routine procedure therefore it should only be performed by experienced personnel.

To each bottle of agar slope, add 1.5 ml "BR", 4 drops (0.12 ml) of 0.5% erythromycin and 10 mg of starch

1. Add about 50 mg of fresh stool to the culture bottle, mix with the overlay and incubate 24 hours at 37^{o}C.

2. On the second day and again on the fourth day examine the culture for *E. histolytica*.

3. If required, further subcultures are made in 3 ml volumes of the culture medium.

QUALITY CONTROL

- Make sure that the agar slope is not outdated or contaminated and that the correct temperature is maintained.
- Ensure that the microscope has been calibrated in the last year and that the results of the calibration are displayed on the microscope base.

REPORT

The presence of growing entamoeba.

AUTHOR

Ian Crandall

REFERENCES

Gillespie, S.H. and Hawkey, P.M. Medical Parasitiology: A Practical Approach. IRL Press New York 1994 pp119-136

Robinson, G.L. *Trans. R. Soc. Trop. Med. Hyg*. **62:** 285 1968

Free-Living Amoebae

PRINCIPLE

Free living amoebae can be opportunists and cause infections in humans. *Naegleria fowleri* is rare, but can cause fatal primary amoebic meningo-encephalitis when it is present in the CSF. Acanthamoeba feed on bacteria, however they can also thrive in some body sites, such as the eye. Diagnosis is made by finding the trophozoite or cyst forms of these organisms.

SPECIMEN

CSF sample
contact lens
corneal scrapings.

PROCEDURE

This is considered to be a non-routine procedure therefore it should only be performed by experienced personnel.

1. Place the specimen in the center of two agar plates pre-coated with *E. coli* (ATTC). Incubate one plate at 37^{o}C and the other plate at room temperature.
2. Observe the plates daily for 7 days, using a low power objective of an inverted microscope. Examine the agar surface for bacterial trials or tracks which indicate the presence of migrating amoebae.
3. Amoebae can be stained with Giemsa stain to aid in identification.

QUALITY CONTROL

- Make sure that the agar plates are not outdated or contaminated and that the correct temperature is maintained.
- Ensure that the microscope has been calibrated in the last year and that the results of the calibration are displayed on the microscope base.

REPORT

The species of any amoebae observed.

AUTHOR

Ian Crandall

REFERENCES

Gillespie, S.H. and Hawkey, P.M. Medical Parasitology: A Practical Approach. IRL Press New York 1994 pp131-136

Visvesvara, G.S. Laboratory Diagnosis of Infectious Disease. Springer New York 1988

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