Sputum

PRINCIPLE

The organisms that may be found in a sputum include the migrating larvae of Ascaris, Strongyloides, hookworm, the eggs of *Paragonimus*, *Echinococcus* hooklets, *Pneumocystis**, *E. histolytica*, *E. gingivalus* and *Cryptosporidium*. In a *Paragonimus* infection the sputum may be viscous and tinged with brownish flecks, which are clusters of eggs ("iron filings") and may be streaked with blood.

SPECIMEN

Two types of sputum may be submitted: 1) expectorated sputum; and 2) induced "deep" sputum. Deep sputum samples should not contain saliva or other products of the mouth. Induced sputa are collected by pulmonary or respiration therapy staff collecting samples. The induction protocol is critical for success of the procedure, and well trained individuals are mandatory for the recovery of organisms. If possible, sputum samples should be submitted immediately after collection. KOH is added if the sputum is thick however it should be avoided if you are looking for *Entamoeba spp*. or *T. tenax*. If examination has to be delayed for any reason, the sputum should be fixed in 10% formalin to preserve helminth eggs or larva or SAF fixative to be stained later for protozoa.

REAGENTS

3% KOH,
phosphate buffer, pH 7.2,
Giemsa and Haematoxylin stains (commercial products, VWR)

PROCEDURE

1. Examine a direct wet preparation (saline or iodine) using low and high dry power.
2. Remove a 1ml portion of the sample and place it in a 15ml tube.
3. Add 1ml of 3% KOH.
4. Incubate at room temperature for 15 minutes
5. Add 2mls of phosphate buffer.

6. Centrifuge the material for 5 minutes at 500g.
7. Use the sediment to prepare wet mounts and smears for Giemsa staining.

QUALITY CONTROL

- Make sure that the saline, phosphate buffer, and 3% KOH are free from contamination.
- Include a control slide if Giemsa Stain is used. Red cells should stain grayish, white cell nuclei stain red-purple and cytoplasm stains bluish.
- Ensure that the microscope has been calibrated in the last year or when ever the optics have been changed and that the results of the calibration are displayed on the microscope base.
- Care should be taken not to confuse *E. gingivalus* which can be found in the mouth/siliva, with *E. histolytica* which could result in an incorrect suspicion of pulmanary abcess. *E. gingivalus* will usually contain ingested polymorphonuclear leukocytes (PMNs), while *E. histolytica* may contain ingested red cells.
- see QC comments associated with hematoxylin staining.
- If *Cryptosporidium* is suspected then acid fast staining or monoclonal antibody techniques normally used for detection in stools can be used.

REPORT

Report the species and developmental stage of any parasites seen.

LIMITATIONS OF PROCEDURE

- Care must be taken to ensure that the sputum sample is not contaminated from environmental sources containing organisms.

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REFERENCES

Garcia LS, Bruckner DA. Diagnostic Medical Parasitology. 3rd Edition.ASM Press, Washington DC. Pp676-684 1997.