EduPath

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UPDATE ON LABORATORY TESTING OF OPHTHALMOLOGY SAMPLES

Key considerations

- A key issue is the small volume of sample that is available. It is not feasible to perform all tests on all samples whilst maintaining optimum sensitivity.
- Therefore, it is the clinician's responsibility to prioritise testing for each individual patient. If in any doubt, it is best to speak to a microbiologist at the outset, otherwise there may not be a sufficient sample for the last tests on the list, and "after requesting" of additional tests may not be possible.
- Samples should be optimally collected and transported to the laboratory.

Eye packs consisting of agar plates, microscopy slide and slide holder as well as a blood culture bottle for bedside inoculation of corneal scrapings, are available.

Choice of possible diagnostic tests

1. Microscopy, culture and sensitivity

For conjunctival swabs, corneal scrapings, corneal biopsies, vitreous and aqueous fluids and particularly for vitreous and aqueous fluids, microscopy and culture have limited sensitivity and specificity and are slow, taking up to 2-3 weeks. One option to increase sensitivity and limit contamination is to inoculate such fluids directly into paediatric blood culture bottles.

2. Molecular tests

For conjunctival swabs, corneal scrapings, corneal biopsies, vitreous and aqueous fluids.

These are tests with high sensitivity and specificity and a quick time-to-result, but the risk of contamination during collection or processing means that false positive results are possible.

Vitreous or aqueous fluids can each be collected in one tube. Please note, that if multiple PCRs are requested, the sensitivity of these tests may be compromised as 200µl of vitreous or aqueous fluid is required per test.

A disadvantage is that no organism is available for drug susceptibility testing.

Options for molecular testing:

- Broad range PCR: 16S rRNA (pan-bacterial) PCR for bacteria (including mycobacteria) and ITS (pan-fungal) PCR for fungi.
  - Amplification of relatively conserved sequences present in all bacteria/fungi, followed by sequencing to distinguish the small variations present in different bacteria/fungi. This should detect any bacteria/fungi present.
  - Turnaround time is 5 working days after receipt of specimen in molecular laboratory.
  - Limited experience with eye fluids.
  - Negative result may be due to the effect of inhibitors in eye fluid.

- Targeted PCR: detects presence or absence of specified organisms.
  - CMV, Enterovirus, HSV 1+2, VZV, *Streptococcus pneumoniae* PCR panel (all these organisms are detected simultaneously in a combination panel)
  - EBV PCR
  - Adenovirus PCR
  - Rubella PCR
  - *Toxoplasma gondii* PCR
  - *Acanthamoeba PCR*
  - TB PCR (GeneXpert MTB/RIF)

3. Serology

Conditions such as syphilis, toxoplasmosis and sarcoidosis are best diagnosed by serology, done on serum. Testing of intraocular fluids for antibodies is not recommended.

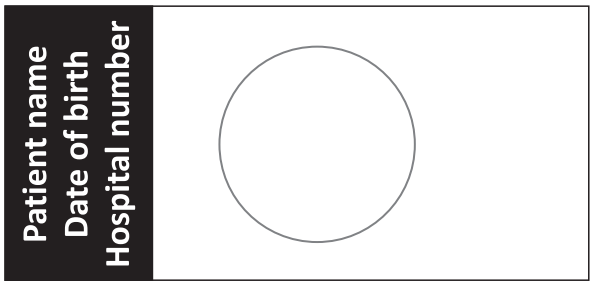
Specimen collection

i. Collection of corneal scrapings for bacterial and fungal culture

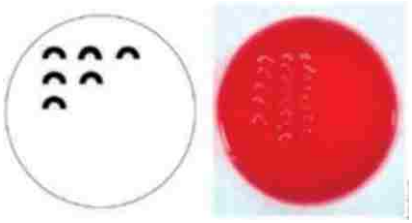
- Collect specimens before antimicrobial therapy, where possible, using aseptic technique.
- Corneal scrapings should be of sufficient quantity to make a visible deposit on a microscope slide and to inoculate culture plates. If insufficient specimen to make an impression smear and to inoculate plates, cultures should be prioritised.
- Collect corneal scrapings from the advancing edge of the ulcer by scraping multiple areas of ulceration and suppuration with a sterile spatula or sterile needle, using short firm strokes in one direction.
- Obtain at least three to five scrapings per cornea.

- Order of specimen preparation: microscopy slide followed by culture plates.
  Use a different needle to take each specimen.

- Specimen collection for microscopy:



  - º Draw a circle on the slide and place the specimen within the circle as shown in the figure above.
  - º Prepare smears by applying scrapings in a gentle circular motion over a clean microscope slide.
  - º Label the slide with the patient's name, date of birth, and hospital number.
  - º Air-dry the slide and place it in the slide transport holder.
- Inoculating culture media:



  - º Inoculate each set of scrapings, in the following order, onto a chocolate agar, blood agar and sabouraud dextrose agar with chloramphenicol using a C-formation for each scraping as shown in the figure above.
  - º Sellotape the lid of the plate to the base around the perimeter.
  - º Specimens should be transported and processed as soon as possible. If processing is delayed, it is preferable to keep samples at room temperature.

ii. Corneal scrapings for molecular testing can be treated as fluids as described previously

iii. Acanthamoeba testing

- Corneal biopsies and scrapings are the best specimen types for the detection of Acanthamoeba species.

Contact lenses, cases and solutions can be cultured for Acanthamoeba species, but these are not optimal samples and therefore have limited relevance. Please note that contact lenses, cases and solutions will not be returned to the patient. The blade used for corneal scrapings can also be submitted in a sterile screw cap container (without saline), but again, these are not optimal samples.

- All specimens submitted for testing will receive both culture and PCR.
- Turnaround time is up to 14 days after receipt of specimen in molecular laboratory.

Procedure for Ophthalmology Specimen Collection in Theatre

1 Procedure for the Ophthalmologist:

1.1 Please inform the laboratory preferably with at least 24 hours' notice of the corneal scraping to be done in theatre.

1.2 Case to be discussed with one of the Microbiologists to enable the correct specimens to be obtained and also the correct tests to be ordered.

1.3 Contact should be made with the Microbiologist on call for full discussion. Telephone number 012 6440891.

1.4 Microbiologist to liaise with laboratory to dispatch an ophthalmology box to the rooms or theatre where the procedure is to take place.

1.5 An information leaflet is to be included into the box to explain the procedure to the doctor.

1.6 Eye fluid (humor) to be injected directly into the blood culture bottle.

Instruction leaflet for the obtaining of corneal scrapings in cases of keratitis.

1. The tip of the needle used to scrape the cornea is to be inoculated into the yellow blood culture bottle. Withdraw a few millilitres of the culture media into the syringe and re- introduce into the blood culture bottle without withdrawing the needle. This will ensure that any material on the tip of the needle will be transferred into the media.
2. Also roll the tip of the needle across the glass slides provided to deposit material on the slides. Transfer the glass slides to the plastic container provided and close.
   - Use a second needle tip to inoculate the various plates of agar.
   - Request fungal culture should the clinical presentation necessitate this.

Please contact one of the Microbiologists at Vermaak / Pathcare Laboratories for any further discussion or questions.

Compiled by Dr Shareef Abrahams, Dr Colleen Bamford (Pathcare) and adapted by Dr Louis Marcus Vermaak / Pathcare.

Further Reading:

1. Garcia, L.S. ed., 2010. Clinical microbiology procedures handbook (Vol. 1). American Society for Microbiology Press.
2. Leck, A., 2009. Taking a corneal scrape and making a diagnosis. Community eye health/International Centre for Eye Health, 22(71), pp.42-3.
3. Public Health England. (2017). Investigation of Bacterial Eye Infections. UK Standards for Microbiology Investigations. B 2 Issue 6.1. https://www.gov.uk/uk- standards-formicrobiology-investigations-smi-qualityand-consistency-in-clinical- laboratories.