THE PATHCARE NEWS

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 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.
    - Eppendorf tubes containing sterile phosphate-buffered saline (PBS) are available for transport of small volume samples for PCR (vitreous fluids, aqueous fluids or corneal scrapings).

      These samples should be placed in special orange "CSF for Molecular Testing Only" bags that are designed to prevent inadvertent opening of samples in unsterile areas of the laboratory.
    - Eye packs consisting of agar plates, microscopy slide and slide holder for bedside inoculation of corneal scrapings are available.

      Please contact the local Pathcare depot or the Pathcare Park (PCP) media laboratory (021 596 3510) 24 to 48 hours prior the procedure if possible, to arrange for the availability of eye packs.
    - A special ophthalmology request form is also available.

Choice of possible diagnostic tests

1. Microscopy, culture and sensitivity

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

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 Eppendorf tube. Please note, that if multiple PCRs are requested, the sensitivity of these tests may be compromised as 200 ul 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)

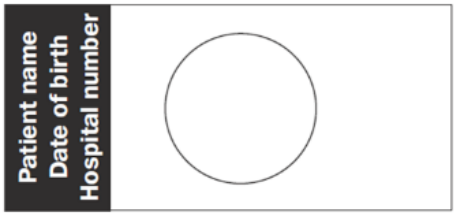
3. Serology

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

Appendix: 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 Kimura 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 or, if using a Kimura scalpel, flame the scalpel between samples.
- Specimen collection for microscopy:



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

  - 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.
  - Sellotape the lid of the plate to the base around the perimeter.
  - 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. Corneal or conjunctival swabs for molecular testing: use orange Sigma swab in liquid Amies transport media.

iv. *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.

References

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-quality-and-consistency-in-clinical-laboratories.