# Aim

To describe the processing of fluids from eye specimens (swabs, corneal scrapings, intra-ocular fluid/pus).

# Principle

Common mild eye infections include conjunctivitis (conjunctiva) and blepharitis (eyelid). Less common and more severe infections include keratitis (cornea) and endophthalmitis (infection inside the eye ball). Infection may also occur around the eye: dacryoadenitis (lacrimal gland), dacrocystitis (lacrimal duct), canaliculitis (lacrimal puncta and canaliculi), and orbital/periorbital cellulitis.

The range of organisms causing eye infections is wide. This SOP focuses on common infections (blepharitis, conjunctivitis, keratitis, endophthalmitis, and orbital/periorbital cellulitis).

* Blepharitis: *Staphylococcus aureus,* skin organisms (*S. epidermidis*, diphtheroids, *Propionibacterium acnes*).
* Conjunctivitis: *S. aureus, Streptococcus pneumoniae, Haemophilus influenzae,* Group A, C, and G beta-haemolytic streptococci, *Neisseria* spp. (*N. meningitidis, N. cinerea*), *Moraxella* spp., anaerobes, *Chlamydia trachomatis*, viruses.
* Neonatal conjunctivitis may also be caused by: *N. gonorrhoeae, H. parainfluenzae,* Group B beta-haemolytic streptococcus, *Enterococcus* spp., coliforms, *Pseudomonas aeruginosa*.
* Orbital/periorbital cellulitis: *S. aureus, H. influenzae,* streptococci, anaerobes, *P. aeruginosa.*
* Keratitis: staphylococci, streptococci, pseudomonads (contact lens-associated), coliforms, acanthamoebae (contact lens-associated / trauma), microsporidia (HIV positive patients), fungi, viruses (adenovirus, HSV, VZV).
* Endophthalmitis:
	+ Surgery-related: staphylococci, streptococci, *P. acnes,* diphtheroids, *P. aeruginosa,* coliforms, fungi, mycobacteria.
	+ Trauma-related: *Bacillus cereus*, streptococci, *Clostridium* spp., fungi.
	+ Endogenous: *S. aureus,* streptococci, coliforms, *Bacillus* spp., yeasts, fungi.

# Method

## Specimen collection

Specimens should be collected using sterile swabs and placed into Amies transport medium (+/-charcoal). Ideally corneal scrapings and intra-ocular fluid should be collected by the ophthalmologist and processed at the patient’s side. The inoculated agar plates should be transferred to the lab without delay.

## Specimen transport and storage

Swab specimens should ideally be stored and transported in sealed plastic bags. Laboratory processing should occur as soon as possible after specimen collection. Specimens should be refrigerated if delays in processing over two hours are unavoidable.

## Specimen processing

### Reception

Log the specimen in the appropriate specimen book and assign a specimen number.

### Microscopic examination

After inoculating the appropriate agar plates, prepare a smear of the specimen and Gram stain.

For corneal scrapes, and other specimens if requested, perform a KOH preparation to identify fungal filaments (SOP MID-001).

If requested, or TB suspected, also prepare a smear for ZN stain.

### Culture

Inoculate and incubate culture media as indicated in Table 1.

**Table 1.** Culture media, conditions, and target organisms

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| **Swabs** |
| **Clinical / Gram strain** | **Standard media** | **Incubation** | **Cultures read** | **Target organism(s)** |
| **Temp (°C)** | **Atmosphere** | **Time** |
| All | Blood agar | 35 – 37 | 5 – 10% CO2 | 40 - 48h | Daily | β-haemolytic streptococci*S. aureus**Haemophilus* spp.*Neisseria* spp.*S. pneumoniae*Yeasts |
| Chocolate agar | 35 – 37 | 5 – 10% CO2 | 40 - 48h | Daily |
| MacConkey agar | 35 – 37 | Air | 40 - 48h | Daily | EnterobacteriaceaePseudomonads |
| Neonate | GC agar | 35 – 37 | 5 – 10% CO2 | 40 - 48h | Daily | *N. gonorrhoeae* |

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| **Corneal scraping or pus / intra-ocular fluid** |
| **Clinical / Gram strain** | **Standard media** | **Incubation** | **Cultures read** | **Target organism(s)** |
| **Temp (°C)** | **Atmosphere** | **Time** |
| All | Blood agar | 35 – 37 | 5 – 10% CO2 | 40 - 48h | Daily | Any organism |
| Chocolate agar | 35 – 37 | 5 – 10% CO2 | 40 - 48h | Daily | Any organism |
| MacConkey agar | 35 – 37 | Air | 40 - 48h | Daily | EnterobacteriaceaePseudomonads |
| Sabouraud agar | 35 – 37 | Air | 40 - 48h | Daily | Fungi |
| Blood agar+ MTZ disc | 35 – 37 | Anaerobic | 40 - 48h | ≥40h | Anaerobes |

# Interpretation

Record the semi-quantitative growth of each colony type (i.e. +/- to ++++).

## Minimum level of identification in the laboratory

In general significant isolates should be identified as fully as possible (i.e. to species level): potentially significant organisms are summarised in SOP MID-004.

Intra-ocular fluid and corneal scrapings:

* All organisms should be followed up and fully identified as per and sterile fluid culture.

For eye swabs:

* Yeasts should be reported to the “yeasts” level in swabs.
* Coliforms should be to the “coliforms” level: identification and antimicrobial susceptibility testing is not normally required unless heavy and pure.
* Non-*P. aeruginosa* pseudomonads should be reported to the “pseudomonads” level: antimicrobial susceptibility testing is not normally required unless heavy and pure.
* In the absence of foreign material (e.g. suture), coagulase negative staphylococci, diphtheroids, and non-pneumococcal alpha-haemolytic streptococci should be considered as contaminating skin flora.

## Antimicrobial susceptibility testing

All significant isolates should have antimicrobial susceptibilities determined according to SOP MIC-001.

## Reporting

Gram stain results: WBC and organisms detected.

Culture:

* Intra-ocular fluid / corneal scrapings: Presence of significant isolates or absence of growth.
* Swabs: Presence of significant isolates (e.g. *S. aureus*); no significant growth / mixed growth of doubtful significance may be used; absence of growth.

# Quality assurance

Media and identification tests should be quality controlled according to the relevant SOP.

# Limitations

Prior antimicrobial use may result in negative cultures.

# References

1. Health Protection Agency, UK SOP B2: Investigation of eye swabs and canalicular pus (Issue 5.2; March 2012).
2. Health Protection Agency, UK SOP B52: Investigation of intraocular fluids and corneal scapings (Issue 5.1; August 2012).
3. Hawkey, P and Lewis, D. Medical Bacteriology. 2nd Edition (2004). Oxford University Press.

# Synopsis / Bench aid



# Risk assessment

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| **COSHH risk assessment - University of Oxford COSHH Assessment Form** |
| **Description of procedure**Culture of eye specimens | **Substances used**Variable, depending on organism cultured (may include Gram stain reagents; 3% hydrogen peroxide (catalase test); N,N,N',N'-tetramethyl-1,4-phenylenediamine (oxidase test); sodium deoxycholate (bile solubility test); bioMerieux API reagents) |
| **Quantities of chemicals used**Small | **Frequency of SOP use**Daily |
| **Hazards identified**1. Autoclaved liquid2. Potentially infectious material in sample 3. Potentially pathogenic bacteria4. Chemical exposure form bacterial identification tests | **Could a less hazardous substance be used instead?** No |
| **What measures have you taken to control risk?** 1. Training in good laboratory practices (GLP)2. Appropriate PPE (lab coat, gloves, eye protection)3. Use of biosafety cabinet for reading of plates / follow-up of BSL-3 organisms (e.g. *B. pseudomallei*) |
| **Checks on control measures**Observation and supervision by senior staff |
| **Is health surveillance required?**No | **Training requirements:**GLP |
| **Emergency procedures**:1. Report all incidents to Safety Adviser2. Use eyewash for splashes3. Clean up spills using 1% Virkon or chemical spill kit | **Waste disposal procedures**:1. Sharps discarded into appropriate rigid containers for incineration2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration3. Chemical waste disposed of according to manufacturer’s instructions |