How to use... eye swabs

Richard J Drew, Theresa S Cole, William Newman

ABSTRACT
Conjunctivitis is a very common presentation to general practitioners and general paediatricians. The investigation of conjunctivitis can be a significant cost to microbiology laboratories due to the high volume of samples that can be submitted, particularly from patients in the community. The key issue is to send eye swabs in clinical situations where it can make a difference to management, and limiting the use of eye swabs in routine cases of conjunctivitis which are likely to be due to viruses. For investigation of neonatal conjunctivitis we recommend sending a bacterial swab for routine culture, and also a swab for molecular detection of Chlamydia trachomatis and Neisseria gonorrhoeae. In older children with mild conjunctivitis no swab is necessary unless there is marked conjunctival injection. In this article we also highlight patient populations that require specialist tests to be sent as part of their assessment such as contact lens wearers and sexually active teenagers.

INTRODUCTION
Conjunctivitis is a very common presentation to general practitioners and general paediatricians. The estimated annual incidence in the USA for bacterial conjunctivitis is 12.5 per 1000, with direct and indirect costs totalling $589 million. The incidence in infants can be as high as 80/1000 person years as was shown in a study of Dutch general practitioners, which also determined that 80% of patients were being prescribed topical antimicrobials. The investigation of conjunctivitis and keratitis is a considerable cost for the National Health Service as a whole. Local outbreaks can have a considerable cost as was shown in an outbreak in a university campus which affected 67 patients and was estimated to cost between $66 000 and $120 000. The judicious use of investigations for both these disorders can result in reduced costs for laboratories, less work for clinicians in dealing with positive results and less anxiety for patients and their parents in waiting for laboratory results.

PHYSIOLOGICAL BACKGROUND
Bacterial conjunctivitis
Simple bacterial conjunctivitis is inflammation of the conjunctiva which is caused by bacterial infection. It presents with a mucopurulent discharge, in the absence of significant decreased visual acuity or eye pain. It is usually self-limiting, however in children there may be a need for topical ophthalmic antimicrobials, such as chloramphenicol, in persistent cases. If there is significant pain or loss of visual acuity then complications of the bacterial infection and other causes of ‘red eye’ need to be considered. In a 5-year audit of 138 paediatric ocular surface infections, the most common organisms recovered were Coagulase-negative Staphylococci (23.2%), Pseudomonas aeruginosa (9.4%) and Staphylococcus aureus (8%), which is similar to that found in a local audit in Alder Hey of eye swabs.
Gram stain images of *S. aureus* (Gram positive cocci) and *P. aeruginosa* are shown in figures 2A, B, respectively. The Alder Hey study shows that in the vast majority of eye swabs, skin commensals are recovered and thus the clinical utility of swabs in children with recent onset mild bacterial conjunctivitis is very limited. It also showed that only 1 of the 424 isolates that had chloramphenicol susceptibility testing performed was resistant to chloramphenicol. Thus if chloramphenicol treatment is failing in a child, then antimicrobial resistance is unlikely to be playing a role and other non-bacterial causes should be considered.

**Viral conjunctivitis**
Viral conjunctivitis presents with watery discharge in a red uncomfortable eye. There is typically less pus visible than with bacterial conjunctivitis. Often it presents when the patient has a concomitant upper respiratory tract infection, with the presence of preauricular lymphadenopathy. It can spread easily in hospital or childcare settings by contact spread. For this reason it is also important to remember good infection control practices when seeing these cases in the hospital environment to prevent nosocomial spread. The most common viruses implicated are adenovirus and herpes simplex virus (HSV). In most cases there is minimal limitation of visual acuity and there should only be minimal pain. Simple viral conjunctival infections are self-limiting and symptoms resolve in about 7 days. It is important to differentiate conjunctivitis from keratitis (box 2). Urgent processing of viral eye swabs is usually not needed; however in the setting of an institutional outbreak it would be important that eye swabs are processed quickly on the next working day to identify the causative organism.

**Neonatal conjunctivitis related to gonorrhoea and chlamydia**
Ophthalmia neonatorum is conjunctivitis that occurs within the first 4 weeks of life. It can be due to bacterial, viral or chlamydial causes. It is important to consider if the infection could be caused by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*, as it has implications for the treatment of the child and also for the parents, who would require a sexual health assessment. A study of 332 cases of neonatal conjunctivitis from Argentina showed that microbial growth was detected in about half of cases, with *C. trachomatis* detected in 7.8% of cases. Of cases in this study that had a bacterial growth, *H. influenzae* (16%) was the most common followed by *S. pneumoniae* (12.3%) and *S. aureus* (8.7%).

Children with infection due to *C. trachomatis* present with a watery and on occasion mucopurulent discharge approximately 5–14 days after birth. It can persist, and is important because of the systemic effects of the infection and occasionally it can lead to corneal scarring and so it is important to detect cases. Treatment involves topical chloramphenicol and oral erythromycin for 2 weeks. Gonococcal conjunctivitis

![Figure 1](image-url)
usually presents earlier within the first 3 days of life. It is characterised by an extremely profuse purulent discharge, often with eyelid swelling such that the infant is unable to open the eyes and, which is not cleared by regular cleaning of the eye. This is a severe infection with the possibility of leading to corneal ulceration and requires prompt treatment. Thus it is important for the child and their parents that samples are taken as soon as the diagnosis is suspected and should be processed on the next working day.

The testing of children for gonorrhoea and chlamydia has significant implications for the parents should the test result be positive, and thus they should be informed that the tests are being sent. A recent study in Scotland showed that 4% of eye swabs tested by a multiplex PCR panel were positive for chlamydia. This study highlighted the importance of having a robust follow-up system in place for parents to ensure that treatment is not delayed. Treatment delays in the mother could lead to her developing pelvic inflammatory disease, while if the father is not treated he could reinfect the mother in the future.

**TECHNOLOGICAL BACKGROUND**

**Bacterial culture**

Swabs of the pus material should be taken before antimicrobial treatment is given. The sample should be sent to the laboratory in transport medium as soon as possible. The swab is then inoculated onto several agar plates for culture. A blood agar plate is used on which most bacteria will grow, however a chocolate (lysed blood) agar plate is also used for fastidious target organisms such as *N gonorrhoeae* and *H influenzae*. The agar plates are incubated for 40–48 h at 35–37°C in 5–10% CO₂. If growth is detected on an agar plate, then identification of the organisms is performed according to standard laboratory methods.

It is vital to ensure that clinical details are included with the sample request as in certain occasions additional agar plates will be inoculated. For neonates a gonorrhoea selective agar will also be used to enhance recovery of the organism. Samples from immunocompromised hosts and those with chronic blepharitis will have their swabs inoculated onto Sabouraud agar, which is a selective medium for yeasts. Finally if the clinical details mention surgery, trauma, or a more severe infection (keratitis, endophthalmitis, orbital cellulitis) an anaerobic plate will be inoculated.

If antibiotics have been started prior to the consideration of taking a swab and culture required one could consider stopping the antimicrobials for 48 h prior to taking the specimen, however specialist advice should be sought at an early stage. In complex cases appropriate investigation is assisted by the clinician discussing the case with the microbiology department especially in young children.

**Nucleic acid amplification tests**

Nucleic acid amplification tests are used to test for chlamydial, gonococcal and viral infections of the eye. These may not be performed in every microbiology laboratory as they are molecular tests and require specific laboratory facilities to minimise contamination of the samples. Specific swabs must be used which will be different from those used for regular bacterial culture. The advantage of NAAT tests are that they can detect organisms such as *C. trachomatis* and *N. gonorrhoeae* that are difficult to grow in the laboratory. For gonorrhea, the advantage is that viable organisms are not required and also that it is more sensitive than regular bacterial culture. These tests involve detecting a conserved region of DNA in the sample and then amplifying this DNA until it is detectable by an automated reader. A concern however is that occasionally an inhibitor may be present in the sample which prevents the amplification of the target DNA and thus no test result can be obtained.
**Box 2 Keratitis (bacterial, viral, protozoal)**

Keratitis is inflammation of the cornea with a breach in the epithelium and is a serious condition that warrants urgent investigation and treatment. The patient presents with a very red eye, and significantly there is pain and reduced vision. These children are very symptomatic; in bacterial keratitis there will likely be a mucopurulent discharge; however in viral and Acanthamoeba infection there is purfusse watering and little pus.

The main predisposing factors for keratitis are the presence of a herpes virus infection, eyelid disease (meibomian gland dysfunction) and trauma with a foreign body impacted upon the cornea. It is most often caused by bacteria (Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa) and viruses such as herpes simplex virus (HSV).

Three conditions deserve special mention

1. *P aeruginosa* because of the aggressive nature of the infections, this organism produces a protease which can cause a rapidly progressive keratitis. The following two conditions are disorders usually involving teenagers;

2. *Neisseria gonorrhoeae* is an aggressive organism which produces a protease, that can lead to a rapidly progressive keratitis.

3. *Acanthamoeba* which is a protozoal infection and is usually only found in contact lens wearers, which would be unusual before teenage years; however a significant number of very young children wear contact lenses due to other eye conditions such as treatment following congenital cataract removal.

**Box 3 Acanthamoeba**

Laboratory processing of samples for Acanthamoeba is difficult and time consuming. The specimen is added to the surface of the agar plate, which already has a lawn of *Escherichia coli* on it, and incubated at 30°C for up to 7 days and examined each day. Amoebae that are in the trophozoite stage can be seen to have made ‘train-tracks’ in the bacterial layer and this is suggestive of Acanthamoeba spp.

More recently PCR testing has been used for diagnosis of keratitis due to Acanthamoeba spp. This organism is often not initially considered and antibiotics have usually already been started. In this situation one should consider stopping the antimicrobials for 48 h prior to taking the specimen; provide the laboratory with the contact lenses which had been worn at the time the patient became symptomatic and the contact lens case to increase the likelihood of isolation of the organism.

**Box 4 What are the clinical presentations with suspected infection that warrant urgent investigation?**

Prompt testing and investigation should be performed in the following groups of patients:

- Neonates presenting with profuse discharge from the eye
- Neonates whose mothers have been diagnosed with chlamydia or gonorrhoea, which was not treated at the time of delivery
- Persistent discharge and conjunctivitis beyond 3 days, particularly if associated with reduced visual acuity
- Contact lens wearers who have persistent discharge and conjunctival infection
- Any child with a persistent purulent discharge from the eye, with sexually transmitted diseases considered if relevant.

Most virology laboratories now offer a multiplex PCR test for the detection of viruses that can cause conjunctivitis, such as adenovirus and HSV. These are cheaper as two or more viruses can be sought in a single PCR run from one sample. A limitation of performing multiplex PCR is that some sensitivity can be lost when two or more targets are sought at the same time. This should not however have clinical implications in practice as usually the viral load is high in samples taken from children with conjunctivitis.

PCR technology can also be used for the detection of *P aeruginosa* from corneal samples from patients with keratitis. A single-centre study showed that an inhouse assay had a specificity of 95% when targeting the ecfX PA gene of *P aeruginosa*, and 100% when targeting the 16 s rRNA gene of the organism. Acanthamoeba should also be considered as a potential cause (box 3).
regular blood agar plate. These guidelines state that *C. trachomatis* should also be considered in children with conjunctival exudates. Many laboratories provide combined molecular tests for *C. trachomatis* and *N. gonorrhoeae*, which are advantageous as *C. trachomatis* cannot be grown in the routine laboratory and also *N. gonorrhoeae* can be fastidious, meaning that the organism may die during transport to the laboratory if there is a delay of several hours (box 4).

In children presenting with some pus discharge and a white eye (no conjunctival injection), is it necessary to send swabs to identify the causative organism?

For children over a month of age with watery discharge or even a sticky discharge and no conjunctival injection there is no need to send any swabs. If swabs are sent they are likely to grow commensal skin flora and thus do not impact on patient management. Some clinicians may feel they have to treat the child if there is a positive bacterial growth, and so it is more prudent not to test in this situation. Most commonly this is due to congenital nasolacrimal duct obstruction or simple viral conjunctivitis. Reassurance should be given to parents and there is no need to treat with antibiotics, either topically or orally.

If the child attends again with persistent discharge and injection beyond 2–3 days, then swabs can be sent for viral PCR (for adenovirus and HSV) and bacterial culture. If the child wears contact lenses then swabs, the contact lenses and samples of the used contact lens fluid should be sent for investigation for Acanthamoeba, either by direct plating or if available by PCR testing.

It is also important to consider that gonorrhoea or chlamydia could be causing the infection in sexually active teenagers that may present with profuse discharge, or children of any age in whom sexual abuse is suspected. Although these cases will be rare presenting to paediatricians, it is important that sexually transmitted diseases are considered. The child should be investigated as per the previous guide for children, but samples should also be sent for PCR for *N. gonorrhoeae* and *C. trachomatis*. If positive the child should be referred for a full sexual health screen and social services should be informed if required.

### Clinical bottom line

- A swab for bacterial culture and a swab for Nucleic Acid Amplification Testing (NAAT) testing for *C. trachomatis* and *N. gonorrhoeae* should always be sent in neonates with a red eye and persistent or purulent conjunctivitis.
- It is not necessary to send a swab for bacterial culture in children presenting with minimal discharge and no conjunctival injection.
- Viral conjunctivitis can be persistent and swabs should be sent for PCR detection of adenovirus and herpes simplex virus.
- In children with persistent conjunctivitis and red eye, a swab for bacterial culture and viral PCR should be sent.
- In contact lens wearers, Acanthamoeba should be considered as a possible pathogen, and the laboratory should be contacted in advance so that the required agar plates can be prepared in advance.

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**Table 1** Summary of investigations that should be sent in each category of patient that presents with conjunctivitis

<table>
<thead>
<tr>
<th></th>
<th>Bacterial swab</th>
<th>Viral swab</th>
<th>Specimen for Acanthamoeba</th>
<th>Swab for Chlamydia trachomatis and Neisseria gonorrhoeae PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal conjunctivitis</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Child with conjunctivitis and white eye</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Child with conjunctivitis and red eye (1st presentation)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Child with conjunctivitis and red eye (2nd presentation)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Contact lens wearer</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Any child with severe profuse discharge</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

TATE more rapid treatment of children who have conjunctivitis secondary to *N. gonorrhoeae* and *C. trachomatis*. A recent trial of a point-of-care molecular test for use in low-resource countries has had quite disappointing results, which showed that the rate of false positives greatly increased when the atmospheric temperature increased over 31°C and humidity increased over 11%.13

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**REFERENCES**


Answers to the questions on page 160

1. b
2. a
3. c
4. a
5. d
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