



The laboratory investigation of vaginal discharge

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The female genital tract has a complex microbial flora which varies with site, age of the patient, and hormonal background. Likewise, the quality and quantity of cervical and vaginal secretions vary with many factors including age, phase of the menstrual cycle, use of oral contraceptive, pregnancy, and presence of an intrauterine contraceptive device. Vaginal discharge may arise from the vagina, cervix, or upper genital tract. There are many non-infective causes of vaginal discharge; however, it is the role of the diagnostic microbiology laboratory to determine the presence of recognised microbial pathogens and disturbances of the normal flora.

The normal flora of the vagina changes according to circulating oestrogen levels. When oestrogen is present, the vaginal epithelium contains glycogen which favours colonisation by lactobacilli and other acid tolerant bacteria, which metabolise glycogen to lactic acid. This results in a pH of 4.5 or less. Other bacteria commonly present in large numbers include anaerobic streptococci, diphtheroids, coagulase negative staphylococci, and α haemolytic streptococci. Other common commensals which can act as pathogens include *Candida* spp, *Staphylococcus aureus*, β haemolytic streptococci including *Str agalactiae*, and *Actinomyces* spp. In bacterial vaginosis the concentration of *Gardnerella vaginalis*, *Mobiluncus* spp, anaerobic gram negative rods (for example, *Bacteroides* spp, *Prevotella* spp, and *Porphyromonas* spp) and *Mycoplasma hominis* increases, compared with the lactobacilli.

The vaginal secretions of prepubertal children and postmenopausal women are more alkaline and contain a varied bacterial flora dominated by coagulase negative staphylococci, diphtheroids, α haemolytic streptococci, lactobacilli, non-haemolytic streptococci, anaerobic Gram negative rods, and *Escherichia coli*.

Collection of specimens

Investigation of vaginal discharge in sexually active adult women should involve the collection of both endocervical and high vaginal swabs. Low vaginal swabs are to be avoided as they are likely to be contaminated with perineal/faecal flora. High vaginal swabs (HVS) should be placed in transport medium to prevent drying

(important for *M hominis*) and to allow the survival of anaerobes. For the identification of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* endocervical swabs are required. Failure to isolate *N gonorrhoeae* from an HVS does not exclude gonorrhoea. There is evidence that vaginal swabs may be suitable for the detection of *C trachomatis* using molecular techniques (for example, PCR or LCR), but further work is needed before this can be recommended.

To collect an endocervical swab the cervix should be gently wiped with a sterile swab before a further cotton swab is inserted into the cervix and rotated 360°. The addition of charcoal to the transport medium (Bibby Sterilin, Stone, Staffordshire, UK) improves the survival of the gonococcus but, ideally, cervical smears should be prepared and warmed media inoculated at the bedside. Rectal, urethral, and pharyngeal swabs may also be taken for culture of *N gonorrhoeae* if indicated.

Harvesting of endocervical cells from the squamocolumnar junction is required for chlamydial culture, detection of chlamydial antigen, or chlamydial DNA by PCR or LCR. After wiping the cervix with sterile gauze or a swab, a sterile swab (not charcoal impregnated) should be inserted into the cervical canal, rotated, and then wiped across the surface of the cervix if ectropion is present.

Swabs for chlamydial cell culture which cannot be processed within 24 hours must be stored at -70°C , and not at -20°C . Endocervical swabs for specific immunofluorescence are spread directly onto a glass slide and air dried.

Intrauterine devices for culture should be submitted dry, in a sterile container.

Assessment of specimens

Processing of the specimens in the laboratory depends on the specimen type, referral source, age of the patient, and the clinical details given. All too often the latter are illegible, inadequate, or abbreviated. Specimens from cases of assault or suspected sexual abuse must have a laboratory chain of evidence form completed, documenting the passage of the specimen from the clinic to the laboratory.

It is convenient to divide specimens into those requiring a full culture and those requiring a screening culture. A screening culture will

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Table 1 Indications for full culture

Postoperative Pregnancy	Following termination of pregnancy, hysterectomy Premature labour, prolonged rupture of membranes, spontaneous rupture of membranes, antepartum haemorrhage, threatened abortion. Shirodkar suture <i>in situ</i>
Postnatal discharge or fever Pelvic inflammatory disease	Salpingitis, endometritis, endometriosis, intrauterine device <i>in situ</i>
Children	Full culture should always be undertaken

include selective plates for *N gonorrhoeae*, *Candida* spp, and β haemolytic streptococci; and microscopy for *Trichomonas vaginalis* and bacterial vaginosis. Culture of vaginal swabs in cases of trichomonal infection and bacterial vaginosis does not yield significantly more information than microscopy alone. Full culture is indicated in the clinical situations shown in table 1.

Microscopy

In those specimens designated to receive screening culture only, a wet preparation is undertaken, unless the clinical details state "vaginosis" or "Gardnerella," when a Gram stain and wet preparation are made. Specimens receiving "full culture" are Gram stained routinely. When cervical and high vaginal

swabs are received, the HVS is used for wet preparation.

WET PREPARATIONS

Vaginal fluid is added to a drop of saline on a slide, and covered with a coverslip. Wet preparations are checked for the presence of *T vaginalis*, yeast cells, pus cells, epithelial cells, and clue cells. Clue cells are vaginal epithelial cells covered with numerous, short coccobacilli. In addition lactobacilli will be absent or reduced in number when clue cells are present. If clue cells are seen on the wet preparation a confirmatory Gram stain is made. The relative numbers of epithelial and pus cells can be helpful. Culture for *T vaginalis* will yield few additional positives.

Bacterial vaginosis

Bacterial vaginosis is a common cause of vaginal discharge in which there is disturbance of the normal vaginal flora without inflammation of the vagina. Figure 1A shows the normal lactobacilli predominant flora, and fig 1B the typical appearance of Gram variable coccobacilli and clue cells in bacterial vaginosis. There is overgrowth of organisms normally present in small numbers, and a reduction or absence of lactobacilli. Bacterial vaginosis is diagnosed when three out of four of the following are present:

- abnormal, thin, homogeneous vaginal discharge;
- vaginal pH > 4.5 (NB, pH of cervical mucus is 7). The presence of blood, serum, or vaginal preparations may affect the pH;
- positive amine test;
- presence of clue cells.

Vaginal pH should be measured in the clinic by applying narrow range pH papers to a drop of discharge on a glass slide. The amine test is then performed by adding a drop of 10% potassium hydroxide to the vaginal discharge. A positive test is signified by a transient fishy odour, caused by the liberation of aromatic amines. The presence of clue cells is the most sensitive and specific marker of bacterial vaginosis. Culture does not form the basis of a diagnosis of bacterial vaginosis. The routine culture of specimens for *G vaginalis* should be discouraged. If pus cells are seen on microscopy, an additional diagnosis should be sought. The full significance of bacterial vaginosis is gradually unfolding and currently it is recognised as a risk factor in:

- preterm labour/late miscarriage;
- ascending infection of the female genital tract;
- endometritis following caesarean section;
- vaginal cuff cellulitis following abdominal hysterectomy;
- neonatal sepsis.

Culture

The procedure for handling specimens to receive a screening culture is shown in table 2. For specimens receiving full culture, a Gram

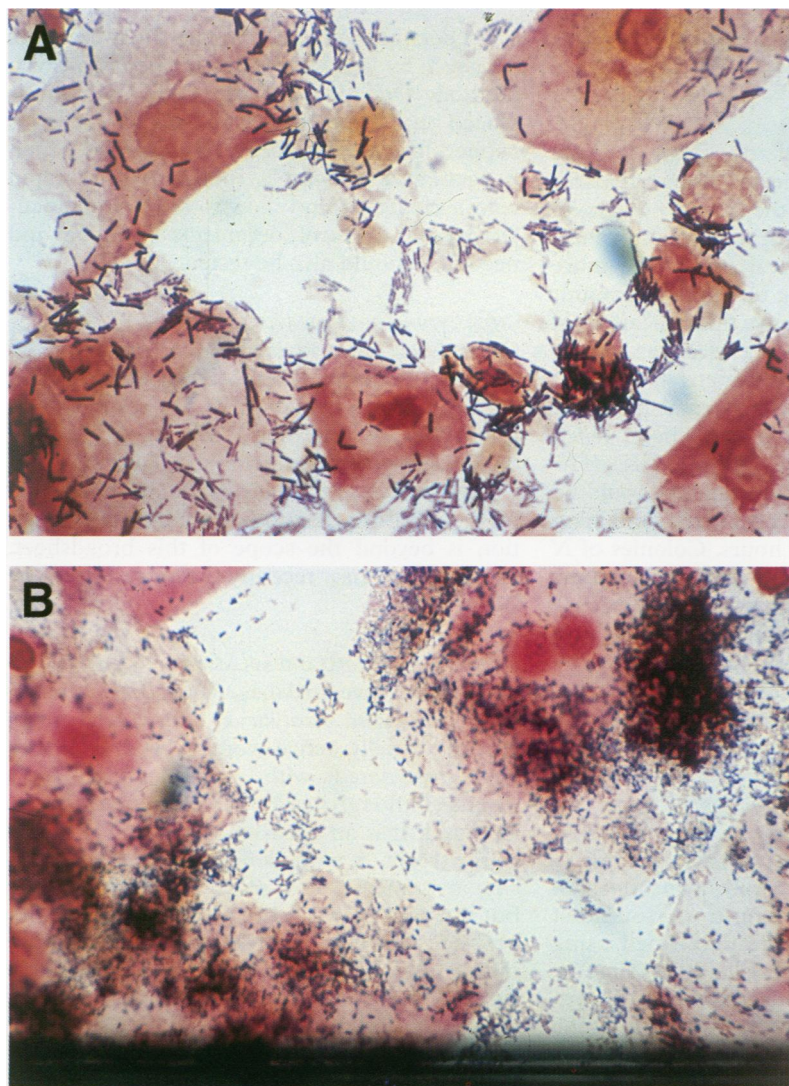


Figure 1 Normal lactobacillus predominant vaginal flora (A) and typical Gram variable coccobacilli and clue cell of bacterial vaginosis (B). (Original magnification $\times 1000$.)

Table 2 Investigation of vaginal discharge: screening culture

Diagnosis	Perform	Special requirements	Observe for
Routine screening culture	Wet preparation ¼ sabouraud plate ¼ selective gonococcal medium	5% CO ₂ , humidity	<i>T vaginalis</i> , yeasts, clue cells, pus cells <i>Candida</i> spp. <i>N gonorrhoeae</i>
Preoperative swabs Vaginosis "Gardnerella"	As above plus nalidixic acid blood agar Wet preparation Gram stain ¼ sabouraud plate ¼ selective gonococcal medium	Anaerobic incubation	As above and β haemolytic streptococci As above Clue cells <i>Candida</i> spp. <i>N gonorrhoeae</i>
Vaginal prolapse	Wet preparation Sabouraud plate	5% CO ₂ , humidity	As above <i>Candida</i> spp.

Table 3 Investigation of vaginal discharge: full culture

Diagnostic group	Possible pathogens	Suggested media and special requirements
Postoperative	<i>Staph aureus</i> <i>Listeria monocytogenes</i> <i>Str pyogenes</i>	Blood agar, 5% CO ₂
Postpartum Pregnancy Pelvic inflammatory disease	<i>Str agalactiae</i> <i>Cl perfringens</i> <i>Bacteroides</i> spp coliforms <i>N gonorrhoeae</i> <i>Mycoplasma hominis</i>	Neomycin blood agar, metronidazole disc Anaerobic incubation MacConkey ¼ selective gonococcal medium in 5% CO ₂ Mycoplasma (not required for elderly and post-hysterectomy patients), incubate anaerobically 72 hours
IUCD	As above plus <i>Actinomyces</i> spp	<i>Actinomyces</i> selective medium incubated anaerobically up to 12 days
Children	<i>Str pyogenes</i> , <i>H influenzae</i> , <i>Str pneumoniae</i> <i>Candida</i> spp <i>N gonorrhoeae</i>	Chocolate agar Sabouraud medium Selective gonococcal medium

stain is prepared and plates are inoculated as shown in table 3.

NEISSERIA GONORRHOEAE

A purulent endocervical discharge is only apparent in 10–20% of infected women. Microscopy and culture remain the basis for diagnosis of gonorrhoea. In women with gonorrhoea, Gram stains of endocervical smears are more difficult to interpret owing to a greater amount of debris, and are less sensitive than smears from the male urethra. Culture on a selective medium is essential for cervical specimens to prevent overgrowth of the plate with normal flora (for example, VCNT blood agar, GC base; Difco Laboratories, West Molesey, Surrey, UK). The agar should be fresh, and specimens should be incubated in a moist atmosphere containing 5–10% CO₂ for 24 to 48 hours. Colonies of *N gonorrhoeae* are 0.5 to 2 mm in diameter, shiny and grey. Suspect colonies which are oxidase positive should be Gram stained. Gram negative diplococci should be confirmed as gonococci by sugar utilisation tests or by a coagglutination test, for example Phadebact® (Launch Diagnostics, Longfield, Kent, UK).

Sensitivity testing of *N gonorrhoeae*

Resistance to penicillin is common. Tetracycline resistance is increasing, with establishment of the TETm plasmid. Ciprofloxacin resistant strains are emerging from Southeast Asia. Disc testing should be performed using penicillin 0.25 U, penicillin 1 U, cefuroxime 30 µg, tetracycline 10 µg, spectinomycin 10 µg, and nalidixic acid 0.03 µg. If there is no zone against the nalidixic acid disc, the test is repeated, including a ciprofloxacin 1 µg disc in addition (low volume (0.1 µg) ciprofloxacin discs are no longer readily available).

β-Lactamase production can most easily be assessed by the chromogenic cephalosporin method. One drop of nitrocefin solution is placed on a piece of filter paper. One colony is rubbed into the nitrocefin impregnated paper. β-Lactamase activity is indicated by a rapid colour change from yellow to red in the inoculated area. Control organisms (positive and negative) should also be tested.

CHLAMYDIA TRACHOMATIS

Chlamydiae are incapable of growth in a cell-free medium. Diagnosis depends on cell culture, antigen detection, or detection of chlamydial DNA. Serology is not helpful in diagnosing genital infection in adults.

Description of the various methods available for non-culture diagnosis of chlamydial infection is beyond the scope of this broadsheet. The subject has recently been reviewed by Black.¹

MYCOPLASMAS AND UREAPLASMAS

The genital mycoplasmas, *Mycoplasma hominis* and *Ureaplasma urealyticum*, are widely prevalent in sexually active women. *M hominis* has been associated with pelvic inflammatory disease, although its role as a prime pathogen is disputed. It is an occasional cause of postpartum pyrexia. A pathogenic role for *U urealyticum* in women is even more difficult to define, but the organism is associated with respiratory problems in low birthweight or premature infants. *M genitalium* may be involved in some cases of pelvic inflammatory disease but it is extremely difficult to isolate. Routine culture for these organisms probably cannot be justified by most diagnostic laboratories, but a brief description is given here to aid those who attempt to do so in the situations described

above. *M hominis* and *U urealyticum* may be isolated using a selective medium (for example, Oxoid GMP mycoplasma medium) which is incubated in a moist atmosphere for 72 hours. This can be incubated microaerobically or anaerobically, but in practice a moist environment is easier to maintain anaerobically. Growth is detected by examining the agar plate directly under a plate microscope. *M hominis* colonies appear as "fried eggs" 50 to 500 µm in size, while *U urealyticum* colonies appear smaller and denser and are 50 to 100 µm in size. Culture of *U urealyticum* yields brown colonies in the presence of manganous salts when viewed by transmitted light.

ACTINOMYCOSIS

Actinomyces israelii may cause infection in association with an intrauterine contraceptive device (IUCD). The IUCD should be inspected for sulphur granules using a plate microscope. If present a granule should be crushed between two microscope slides, heat fixed, and Gram stained to look for branching Gram positive filaments. In the absence of diagnostic sulphur granules the IUCD is used to inoculate selective agar (brain-heart infusion agar with defibrinated horse blood, nalidixic acid, and metronidazole) and is then itself incubated in fastidious anaerobic broth (FAB; Bioconnections, Bardsey, Yorkshire, UK) for five days. The broth is then subcultured onto another of the selective plates. Both sets of plates should be incubated anaerobically and examined at 5, 7, 9, and 12 days. Thick plates (> 4 mm) should be prepared to minimise drying during the prolonged incubation.

Special situations

CHILDREN

Vulvovaginitis is a common condition of prepubertal girls and presents with dysuria, soreness, or itching. The most common bacterial causes are *Str pyogenes* and non-capsulate *Haemophilus influenzae*.² Other causes include candida, *Str pneumoniae*, *Staph aureus*, other β haemolytic streptococci, *N gonorrhoeae*, and *C trachomatis*. Non-bacterial causes to consider are retained foreign body and threadworm infection. In children vaginal swabs only will be taken and these should always be submitted for full culture. The prepubertal vaginal epithelium can be infected with *N gonorrhoeae* and *C trachomatis* and this specimen is adequate for diagnosis. If a chlamydial infection is a possibility in a child, cell culture for *C trachomatis* should be undertaken at a centre experienced in this technique. The report of Royal College of Physicians on child sexual

abuse confirms the legal requirement for cell culture, pointing out that immunofluorescence and immunoassays have not been validated in prepubertal children.³ This report further states that, if a non-culture method is used, treatment should be delayed until the result is available, as any positive result should be confirmed by taking a further sample for cell culture. It is therefore highly desirable that the preferred samples are taken at initial consultation.

POSSIBLE LEGAL CASES

If specimens are taken from patients of any age where sexual assault or abuse is considered, or if the diagnosis of a sexually transmitted disease is considered in a child, a chain of evidence form should be initiated by the clinician who took the specimens. All persons thereafter who handle the specimen (including transport to the laboratory, subsequent cultures, and results) should document their involvement. Specimens for *C trachomatis* from these cases require culture.

Reporting of significant isolates

Heavy growths of *Staph aureus*, β haemolytic streptococci, and mixed anaerobes should be reported. Heavy growths of coliforms may be significant in certain circumstances, for example retained products of conception. Scanty or mixed growths of *Staph aureus* and non-group-A β haemolytic streptococci are not usually reported. An exception to this is *Str agalactiae*, which should be reported when isolated from an HVS taken around the time of childbirth because of its importance as a cause of neonatal sepsis.

- 1 Black CM. Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. *Clin Microbiol Rev* 1997;10:160-84.
- 2 Cox RA. *Haemophilus influenzae* an underrated cause of vulvovaginitis in young girls. *J Clin Pathol* 1997;50:765-8.
- 3 Royal College of Physicians. *Physical signs of sexual abuse in children*, 2nd ed. London: Royal College of Physicians, 1997. (Appendix 4b gives advice on the collection of microbiological specimens.)

Further reading

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