

NATIONAL STANDARD METHOD

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

BSOP 28

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Specialist and Reference Microbiology Division

Association of Medical Microbiologists
Association of Medical Microbiologists



INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

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INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 2 of 33

Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

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INDEX

STATUS OF NATIONAL STANDARD METHODS	2
INDEX	3
AMENDMENT PROCEDURE	4
SCOPE OF DOCUMENT	5
INTRODUCTION	5
SEXUALLY TRANSMISSIBLE INFECTIONS	5
VAGINAL INFECTIONS (OTHER THAN STIs)	8
OTHER INFECTIONS OF THE FEMALE GENITAL TRACT	11
INFECTIONS (OTHER THAN STIs) OF THE MALE GENITAL TRACT	14
1.0 SAFETY CONSIDERATIONS	16
1.1 SPECIMEN COLLECTION	16
1.2 SPECIMEN TRANSPORT AND STORAGE	16
1.3 SPECIMEN PROCESSING.....	16
2.0 SPECIMEN COLLECTION	16
2.1 OPTIMAL TIME OF SPECIMEN COLLECTION	16
2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION	16
2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS	17
3.0 SPECIMEN TRANSPORT AND STORAGE	17
3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING.....	17
3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION	17
4.0 SPECIMEN PROCESSING	18
4.1 TEST SELECTION	18
4.2 APPEARANCE	18
4.3 MICROSCOPY	18
4.4 CULTURE AND INVESTIGATION	19
4.5 IDENTIFICATION	26
4.6 ANTIBIOTIC SUSCEPTIBILITY TESTING	26
5.0 REPORTING PROCEDURE	27
5.1 MICROSCOPY	27
5.2 CULTURE	28
5.3 ANTIBIOTIC SUSCEPTIBILITY TESTING	28
6.0 REPORTING TO THE HPA (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS)	28
REFERENCES	30

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 3 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

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AMENDMENT PROCEDURE

Controlled document reference	BSOP 28
Controlled document title	Standard Operating Procedure for the investigation of genital tract and associated specimens

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
5/ 03.05.05	4	4.1	1	Front page	Redesigned
			2	Status of document	Reworded
			4	Amendment page	Redesigned

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 4 of 33
Reference no: BSOP 28i4.1

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STANDARD OPERATING PROCEDURE FOR THE INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Types of specimens: High vaginal swab (HVS), vaginal discharge, vulval swab, labial swab, cervical swab, endocervical swab, penile swab, urethral swab and semen
Aspirates from: Bartholin's gland, fallopian tube, tubo-ovarian abscess, Pouch of Douglas fluid, genital ulcer swab, intra-uterine contraceptive device (IUCD), products of conception and screening swabs for *N. gonorrhoeae*

SCOPE OF DOCUMENT

This SOP describes the examination of genital specimens for the presence of *Neisseria gonorrhoeae*, yeasts, β -haemolytic streptococci and other specific target organisms (including *Trichomonas vaginalis*), and the microscopic diagnosis of bacterial vaginosis (BV).

INTRODUCTION

Appropriate specimens are often difficult to obtain, particularly from women, and incorrect or sub-optimal specimens are often received. It is important to avoid contamination with faecal flora during collection of specimens.

This SOP is laid out under the following headings:

Sexually transmitted infections (STIs)

Vaginal infections other than STIs

Other infections of the female genital tract

Infections (other than STIs) of the male genital tract

SEXUALLY TRANSMISSABLE INFECTIONS

A range of sexually transmissible organisms cause infections responsible for a large number of clinical syndromes. When a specific STI is diagnosed, it is recommended to screen for other infections. Screening has a role in helping to control gonorrhoea, syphilis, chlamydial infection, and human immunodeficiency virus (HIV) infection.

Gonorrhoea^{1,2}

N. gonorrhoeae causes a wide spectrum of clinical syndromes in men, women and neonates infants.

Local gonococcal infections in men most commonly present as symptomatic urethritis with a purulent urethral discharge and dysuria. The most common complication of this is acute epididymitis and, in rare cases, gonococcal urethritis can be complicated by gonococcal cellulitis, penile lymphangitis, or periurethral abscess.

Proper specimen collection is important to ensure optimal yield. The best specimen is expressed urethral exudate. In asymptomatic men a urethral swab is taken.

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 5 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

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Local gonococcal infections in women primarily affect the cervix. Gonococcal cervicitis is often asymptomatic, but it can cause an increased vaginal discharge, genital itching or dysuria. The urethra is frequently involved in women who have had a hysterectomy. The most important complication of gonococcal infection in women is pelvic inflammatory disease (PID), which may lead to infertility. Endocervical or urethral swabs are the preferred specimens.

Anorectal gonorrhoea may be an asymptomatic complication in women with cervical gonorrhoea. Women and homosexual men who participate in receptive anal intercourse with infected partners are at risk of developing anorectal gonorrhoea. Most men with anorectal gonorrhoea are asymptomatic though some develop symptomatic proctitis. Homosexually active men with symptomatic proctitis can be infected with a variety of other pathogens. Rectal specimens may be taken to seek *N. gonorrhoeae*, *Chlamydia trachomatis* and viruses³.

Asymptomatic mucosal infection can occur at any mucosal site such as the urethra, cervix, rectum and pharynx. Such infections are detected by screening patients presenting with appropriate histories, symptoms and signs suggesting exposure. Throat swabs are taken for screening for *N. gonorrhoeae* if there is a history of orogenital contact.

Disseminated gonococcal infection (DGI) is a rare complication of gonorrhoea. This presents with one or more of the following⁴:

- arthralgia (joint pain)
- asymmetric polyarthritis
- rash (often pustular)
- myalgia (muscle pain)
- septic arthritis
- tenosynovitis (inflammation of a tendon sheath)

In most cases of DGI, mucosal infection is present but can be asymptomatic. All potential mucosal sites should be screened for *N. gonorrhoeae*. Blood cultures and examination of fluids such as joint fluids may be useful in diagnosis. Dermatitis as a result of DGI is characterised by a small number of skin lesions that are located mainly on the extremities. DGI may be complicated when seeding of the heart valves or meninges results in gonococcal endocarditis or meningitis. The most common manifestation of DGI is the arthritis-dermatitis syndrome⁵. DGI resulting in meningitis is very rare⁶.

Sexual transmission of *Neisseria meningitidis* may also cause lower genital infections in both men and women but asymptomatic colonisation usually results.

Media for the isolation of *N. gonorrhoeae* may become overgrown with yeasts. In addition, it has been demonstrated that *C. albicans* may produce a soluble factor which may inhibit the growth of *N. gonorrhoeae*⁷. Therefore, this SOP recommends the use of selective media containing antifungal agents.

Trichomoniasis⁸, caused by the flagellate protozoan, *T. vaginalis*, is almost always acquired through sexual contact. Presenting symptoms include an increased vaginal discharge, pruritus and dysuria. An erythematous, friable cervix with punctate areas of exudate (strawberry cervix) is pathognomonic of *T. vaginalis*. Long-term carriage may occur, with symptoms not appearing for years after the initial sexual contact. TV infection in pregnancy has been associated with low birth weight and preterm delivery⁹. The prevalence of *T. vaginalis* remains at a constant low level in cases seen in GUM clinics¹⁰.

Various techniques^{11,12} including culture followed by microscopy^{13,14}, direct microscopy and immunodiagnostic methods for the detection of *T. vaginalis* have been compared^{13,15}. Microscopy has been the most practicable means of diagnosis for routine screening^{16,17}. Microscopy of a wet

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 6 of 33

Reference no: BSOP 28i4.1

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preparation is highly specific and easily performed, but it fails to detect 30-50% of TV infections (even when undertaken close to the patient^{12,14}) compared to culture which is regarded as the "gold standard"¹⁴. Detection using films stained with acridine orange has been found to be only slightly more sensitive than unstained wet preparations¹³.

Conventional culture methods are slow and labour-intensive, but a method utilising microtitre trays read with an inverted microscope has been described which is cost effective without any loss in sensitivity¹⁴.

This SOP recommends selective culture from patients with clinically suspected *T. vaginalis* infection, with other diagnosed or suspected STI, in pregnancy, when requested and in other groups according to local protocols. Local protocols may vary depending on local prevalence.

Genital ulcers are most commonly caused by¹⁸:

- herpes simplex virus (HSV)
- *C. trachomatis* (lymphogranuloma venereum)
- *Treponema pallidum* (syphilis)
- *Calymmbacterium granulomatis* (granuloma inguinale)
- *Haemophilus ducreyi* (chancroid)

Investigations for HSV, *T. pallidum* and *C. trachomatis* are not covered by this SOP.

Staphylococcus aureus and Lancefield group A streptococci may also cause tender pustules resembling ulcers on genitalia, as well as inguinal lymphadenopathy and soreness¹⁸.

Chancroid is an important cause of genital ulceration in the tropics, and its incidence increased dramatically in North America during the late 1980's. It is caused by *H. ducreyi* which enters via a break in the epithelium¹⁹. Chancroid ulcers are vascular, painful and the granulomatous base bleeds easily.

Lesions occur on and around the genitalia^{18,20}. As well as genital ulcers, painful inguinal lymphadenopathy (buboes) can develop in about 50% of cases¹⁹. Asymptomatic carriage appears to be rare. Infection rarely presents as urethritis alone without any genital ulcers²¹.

The incidence of chancroid is reportedly increasing in many areas, although diagnosis is often made on clinical grounds alone and may thus be inaccurate¹⁹. Chancroid, in common with other sexually transmitted diseases, is thought to be an important co-factor in the transmission of HIV in the tropics²².

Examination of Gram stained material from genital ulcers has poor sensitivity and specificity^{19,20}. Results from immunofluorescence and molecular techniques are encouraging but need further evaluation²⁴.

Isolation of *H. ducreyi* is comparatively difficult²² and requires selective agar media^{19,23,24}, although isolation rates of up to 80% have been reported.

C. granulomatis²⁵ infection is a rare condition found only in certain parts of the tropics. This organism has rarely been grown in vitro and culture is not routinely practicable. It is demonstrated by performing Giemsa or Wright stains on scrapings from the edge of the ulcer. It is an encapsulated Gram-negative bacterium. The organisms or "Donovan bodies" appear as a cluster of blue or black bodies with a "safety pin" morphology found within PMNs. The primary lesion begins as an indurated nodule that erodes to form a granulomatous, heaped ulcer. Lesions occur on the folds of the scrotum, thighs, labia and vagina.

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 7 of 33

Reference no: BSOP 28i4.1

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Genital warts is a venereal infection caused by human papillomavirus (HPV)²⁶. Sub-clinical carriage of HPV is common and greatly exceeds the prevalence of visible warts²⁷. They may occur as flat warts which may progress to carcinoma in situ, or may occur as a papillary projection above the skin with a rich capillary bed. In women, warts are located most frequently in the posterior introitus and labia and less commonly in the perianal area. In uncircumcised men the prepuce is the most common site of infection²⁷.

Children are also at risk of acquiring sexually transmissible infections^{28,29-34}. Although the presence of a sexually transmitted organism beyond the neonatal period is highly suggestive of sexual abuse, and this possibility should always be investigated, exceptions do exist. Rectal or genital infection with *C. trachomatis* among young children may be the result of perinatally acquired infection and may persist for as long as 3 years. Similarly, anogenital warts may be present in prepubertal children as a consequence of perinatal transmission, or of autoinoculation from common hand warts. Bacterial vaginosis has been identified among both abused and non-abused children.

Specimens for forensic or medico-legal investigations are outside the remit of this SOP and should be processed according to local protocols. It is advisable to use a "chain of evidence" procedure when processing specimens from possible cases of sexual assault or abuse. In such cases, appropriate specimens may be taken for investigation for *C. trachomatis*, *N. gonorrhoeae*, and the presence of *T. vaginalis* and clue cells³⁵.

VAGINAL INFECTIONS (OTHER THAN STIS)

Normal vaginal flora consists of a wide range of organisms³⁶⁻³⁹ including *Lactobacillus* species, streptococci, enterococci and coagulase-negative staphylococci. Anaerobes, such as *Bacteroides* species and anaerobic cocci, *Gardnerella vaginalis*, yeasts, coliforms, *Ureaplasma urealyticum* and *Mycoplasma* species may also be present as part of the normal flora, but they have also been incriminated in vaginal infections.

Vaginal candidosis occurs when alterations in the vaginal environment allow yeasts (which are often present as commensal organisms in the vagina), to proliferate. Increased levels of oestrogens promote their growth. Yeast overgrowth is often seen in the following conditions:

- after antimicrobial therapy
- diabetes mellitus
- immunosuppression
- obesity
- pregnancy
- use of oral contraceptives

Although *Candida albicans* is isolated in 80-90% of cases of vaginal candidosis, other yeasts account for

10-15% of cases and include⁴⁰:

- *Candida krusei*
- *Candida kefyr*
- *Candida tropicalis*
- *Candida glabrata*

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 8 of 33
Reference no: BSOP 28i4.1

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Cases commonly present with pruritus, dysuria and a whitish discharge, although sometimes there is just mucosal erythema and soreness. Infections with species other than *albicans* may result in treatment failure and subsequent persistent infections.

This SOP recommends routine culture of all vaginal, endocervical and urethral swabs for yeasts.

Vaginitis⁴¹ can be caused by *Candida* species and *T. vaginalis*. In children, infections caused by β -haemolytic streptococci⁴² and *S. aureus* are common. Lancefield group A streptococci also cause vaginitis and purulent vaginal discharge in adults⁴³.

Atrophic vaginitis⁴⁴ is a rare condition usually associated with the elderly. The majority of women with mild to moderate atrophy are asymptomatic. Reduced endogenous oestrogen causes the epithelium to thin, contributing to a reduction in lactic acid production and an increase in vaginal pH. This change causes overgrowth with mixed flora and the disappearance of lactobacilli. The vaginal discharge contains polymorphonuclear leucocytes and small round basal epithelial cells.

Vulvovaginitis is mainly seen in prepubertal females, but may affect women of any age. It may be associated with poor hygiene, skin irritation due to soaps, or with streptococcal throat carriage. Symptoms include irritation, soreness and discharge. Causative organisms include^{18,45-47}:

- Lancefield group A streptococcus
- *Staphylococcus aureus*
- *C. albicans*
- *Haemophilus influenzae*
- *N. gonorrhoeae*

Other unusual organisms may cause vulvovaginitis⁴⁸ including *Salmonella* and *Shigella* species. Threadworm infestation may predispose to vulvovaginitis (see BSOP 31).

Bacterial vaginosis (BV)^{49,50} is characterised by an increase in anaerobes and a decrease in *Lactobacillus* species.

BV has been regarded in the past as a harmless abnormality. However it is now considered to be associated with a variety of genital tract infections and complications including^{49,51}:

- amnionitis
- postpartum endometritis and fever
- preterm labour and low birth weight
- premature rupture of membranes (PROM)
- post vaginal hysterectomy sepsis
- pelvic inflammatory disease (PID)
- urinary tract infections

BV may be diagnosed clinically if three of the following four criteria are fulfilled⁵²:

- grey-white, thin homogenous discharge

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 9 of 33

Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

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- vaginal secretions pH > 4.5
- positive amine odour test (release of fishy amine odour when vaginal secretion is mixed with 5-10% potassium hydroxide)
- presence of clue cells on microscopic examination

A normal vaginal flora is associated with the presence of *Lactobacillus* species alone or in the presence of small numbers of *G. vaginalis* morphotypes. The shift in vaginal flora associated with BV is characterised by a decrease in numbers of lactobacilli which are replaced by a mixed flora of aerobic, anaerobic and microaerophilic species⁵³. A diverse group of organisms is involved, many of which are difficult to grow. Organisms associated with BV include^{49-50,51,53}:

- *Prevotella* species
- *G. vaginalis*
- *Mobiluncus* species
- *Peptostreptococcus* species
- *Mycoplasma hominis*

Although *G. vaginalis* is encountered consistently and in large numbers in women with BV, the organism can also be isolated from as many as 60% of asymptomatic women. Direct examination of vaginal secretions is more relevant for the diagnosis of BV than is the isolation of *G. vaginalis* from these specimens⁵⁴.

Examination of Gram-stained films is reported to be useful in the diagnosis of BV⁵⁰ and standard criteria for morphotypes have been described^{54,55}. In typical smears from patients with BV, clue cells are accompanied by a mixed flora consisting of very large numbers of small Gram-negative rods (predominantly *Prevotella* species) and Gram-variable rods and coccobacilli (predominantly *G. vaginalis*) in the absence of larger Gram-positive rods (*Lactobacillus* species). Curved Gram-variable rods (*Mobiluncus* species) may be also be present.

Clue cells are epithelial cells to which Gram-variable rods are attached in large numbers, obscuring the cell border. They are reported as being highly specific (almost 100%) but not as sensitive as using other aspects of a Gram stain to detect BV⁵⁶. Using Amsel's original criteria, clue cells are only significant if two of the other three criteria (grey-white discharge, pH >4.5 and a positive amine test) are fulfilled.

Gram staining (using the criteria of Nugent⁵⁴ or Hay⁵⁵) of vaginal smears is the most sensitive method for the laboratory diagnosis of BV^{50,52} as it detects both clue cells and the disturbance in bacterial morphotypes associated with BV. It is not necessary to see clue cells to make a diagnosis of BV. One of the key features is the absence of typical lactobacilli and their replacement with Gram-variable or Gram-negative rods³⁹. However, one study⁵⁷ found that acridine orange or wet preparations are more sensitive methods for detecting clue cells than the Gram stain. Detection of clue cells alone, whilst highly specific for BV, is not as sensitive as detection of different morphotypes by the Gram stain⁵⁶.

This SOP recommends the examination of all vaginal swabs from women of child bearing age for the presence of BV by Gram film.

Toxic shock syndrome (TSS)⁵⁸ is an acute multi-system illness characterised by fever, hypotension, erythematous rash, diarrhoea and desquamation of the skin upon recovery. TSS is caused by a toxin produced by *S. aureus*. Isolation of a toxin-producing *S. aureus* from a mucous membrane is strong support for a positive diagnosis. There is a TSS-like illness caused by Lancefield group A streptococci.

TSS can be associated with:

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 10 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

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- tampon use
- childbirth or other surgical wound infection
- contraceptive devices
- cervico-vaginal colonisation with *S. aureus*

Lancefield group B streptococcus normally colonises the vagina in many women. In pregnancy this organism can infect the amniotic fluid (see BSOP 26) which can lead to neonatal sepsis, pneumonia and meningitis (see BSOP 23). According to local protocol, patients judged at high risk for the development of group B streptococcal infection may be screened for carriage. Optimum yield will be achieved by selective/enrichment procedures applied to swabs obtained from the vagina and the anorectum⁵⁹⁻⁶¹.

Conditions considered to confer a high risk of infection include:

- fever in labour
- premature labour
- PROM
- previously infected baby

Listeria monocytogenes may cause serious infection in pregnant women, neonatal infants and patients who are immunocompromised^{62,63}. In pregnant women septicaemia caused by *L. monocytogenes* presents as an acute febrile illness that may affect the fetus⁶². This may lead to systemic infection (granulomatosis infantisepticum), stillbirth and neonatal meningitis. Products of conception, placenta and neonatal screening swabs should be examined for this organism (processing neonatal screening swabs is described in BSOP 23). Routine culture of vaginal swabs for *L. monocytogenes* is not usually performed although it may be useful in suspected cases⁶⁴. Blood cultures are indicated. Serological investigations have no place in the diagnosis of listeriosis⁶³.

Septic abortion may result in serious maternal morbidity and may be fatal⁶². Uterine perforation, presence of necrotic debris, and retained placental products can lead to infection. Most infections are polymicrobial and involve anaerobes.

Clostridial sepsis complicating abortion is potentially lethal. *Clostridium* species are part of the normal vaginal flora in some women.

OTHER INFECTIONS OF THE FEMALE GENITAL TRACT

Bartholinitis⁶² is inflammation of the Bartholin glands, the small mucus-producing glands on each side of the vaginal orifice of adult women. Two stages of infection occur. The first stage is acute infection of the duct and lining of the gland. The second stage is abscess formation in which the gland is obstructed.

Causative organisms of Bartholin's gland infections include^{62,65}:

- anaerobes
- *N. gonorrhoeae*
- streptococci
- Enterobacteriaceae

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 11 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

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- *C. trachomatis*
- *H. influenzae*
- *S. aureus*
- other *Neisseria* species
- *M. hominis*

Mucopurulent cervicitis is inflammation of cervical columnar epithelium. Causative organisms include⁶⁶:

- *C. trachomatis*
- HSV
- *N. gonorrhoeae*

Other organisms such as *U. urealyticum*, *M. hominis* and those linked with BV have not been consistently associated with mucopurulent cervicitis, suggesting only a weak association or their dependence on the presence of other organisms⁶⁶.

Cervicitis is important as it provides a source of pathogenic organisms which may infect the endometrium and endosalpinx. Ascent during pregnancy can cause chorioamnionitis, premature rupture of membranes, puerperal and neonatal infections.

Gram stained smears are used to evaluate the presence of polymorphonuclear leucocytes⁶⁷. Mucopurulent cervicitis is characterised by the presence of an endocervical exudate containing PMNs. A visible yellow discharge is produced.

Endometritis is inflammation of the endometrium, the inner lining of the uterus. Organisms that may cause this infection include:

- *C. trachomatis*
- *N. gonorrhoeae*
- *Mycobacterium tuberculosis*
- HSV

Postpartum endometritis - most infections are caused by vulvovaginal flora that ascend into the uterus. Infections are often polymicrobial and caused by⁶⁸.

- β -haemolytic streptococci
- *S. aureus*
- enterococci
- anaerobes
- *C. trachomatis*
- Enterobacteriaceae
- *G. vaginalis*

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 12 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

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- *M. hominis*

Risk factors include:

- amniotic fluid infection
- caesarean delivery
- invasive fetal monitoring
- prolonged rupture of membranes
- vaginal examinations

Appropriate specimens include a swab of the lower uterine segment or the cervix.

Salpingitis is inflammation of the uterine (fallopian) tube. Infection is sometimes polymicrobial involving⁶⁹:

- *C. trachomatis*
- *N. gonorrhoeae*
- mixed anaerobic, facultative anaerobic and aerobic bacteria
- *M. hominis*

Specimens from the fallopian tubes are superior to endocervical swabs. Endocervical swabs may be useful but require more careful interpretation. Acute salpingitis can result in sequelae such as chronic abdominal pain and an increased risk of ectopic pregnancy.

Pelvic inflammatory disease (PID) is the term used to refer to endometritis, salpingitis, pelvic peritonitis or a combination of these. Symptoms include dyspareunia, intermenstrual bleeding and lower abdominal cramps.

Many women who develop PID suffer long term sequelae such as:

- chronic pelvic pain
- ectopic pregnancy
- infertility
- pyosalpinx (collection of pus in a fallopian tube)
- tubo-ovarian abscess (TOA)

PID is often a polymicrobial illness^{70,71}. Women with gonococcal PID may also be infected with *C. trachomatis*.

The preferred specimens for diagnosis of PID are aspirates collected from a fallopian tube or a TOA, or peritoneal fluid (processing peritoneal fluid is described in BSOP 26). Swabs of pus or fluid are acceptable but where possible pus or fluid samples should be sent. These are processed in the same manner as pus/fluid.

Organisms that cause PID include:

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 13 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

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- *C. trachomatis*
- *N. gonorrhoeae*
- anaerobes
- Lancefield group B streptococcus
- other streptococci
- *Escherichia coli*
- *G. vaginalis*
- *Actinomyces israelii*
- *M. hominis*
- *H. influenzae*

Intrauterine contraceptive devices (IUCDs) - the presence of an IUCD may be associated with PID⁷². Infections may be polymicrobial with the isolation of both Gram-positive and Gram-negative aerobic and anaerobic organisms. *Actinomyces* species, particularly *A. israelii*, may be significant isolates. This SOP recommends that IUCDs are only cultured where there are clinical indications of PID or other inflammatory conditions.

INFECTIONS (OTHER THAN STIS) OF THE MALE GENITAL TRACT

Prostatitis is inflammation of the prostate. Acute or chronic infection may be caused by Enterobacteriaceae, *C. trachomatis*, *N. gonorrhoeae* and streptococci. *Cryptococcus neoformans* may be isolated from the prostate in patients who are HIV positive and this is an important site for persistence and a potential origin for relapse. Diagnosis is made by examining voided and midstream urine specimens as well as expressed prostatic secretions (see BSOP 41).

Epididymitis is inflammation of the epididymis. It may occur as a result of trauma or chemical irritation associated with urine reflux, or more usually as a complication of urethral or urinary infections. Diagnosis is usually made by examining urine or urethral swabs. Organisms that cause infection include⁷³.

- *C. trachomatis*
- Enterobacteriaceae
- *N. gonorrhoeae*
- pseudomonads
- *M. tuberculosis*

Orchitis is inflammation of the testis. It is usually as a result of a blood-borne viral infection, the most common being mumps. Bacterial infection usually occurs as a result of contiguous spread. Diagnosis is made by examining urine. Causative organisms include⁷³:

- Enterobacteriaceae
- pseudomonads
- staphylococci
- streptococci
- *M. tuberculosis*

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 14 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

Balanitis is inflammation of the glans penis⁷⁴.

Balanoposthitis is inflammation of the prepuce and the glans penis.

Irritation due to smegma, urethral discharge or other agents can play a role in the aetiology of these two conditions.

Organisms that cause balanitis and balanoposthitis include⁷⁴⁻⁷⁷:

- yeasts
- HSV
- Lancefield group A streptococcus
- *S. aureus*
- Lancefield group B streptococcus
- anaerobes

Candida species may be isolated in cases of penile thrush.

Urethritis in men is mainly caused by⁷³:

- *N. gonorrhoeae*
- *C. trachomatis*
- *U. urealyticum*

Haemophilus species such as *H. influenzae* and *H. parainfluenzae* have been isolated from urethral discharge⁷⁸.

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 15 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

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1.0 SAFETY CONSIDERATIONS⁷⁹⁻⁸⁹

1.1 SPECIMEN COLLECTION

N/A

1.2 SPECIMEN TRANSPORT AND STORAGE

Pus, fluids and IUCDs

Sterile leakproof container in a sealed plastic bag

Swabs

Sealed plastic bag

1.3 SPECIMEN PROCESSING

Containment Level 2

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet

Refer to current guidance on the safe handling of all organisms documented in this SOP

The above guidance should be supplemented with local COSHH and risk assessments

Compliance with postal and transport regulations is essential

2.0 SPECIMEN COLLECTION

2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

Before antimicrobial therapy where possible

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION⁹⁰

Genital tract swabs

Cervical and high vaginal swabs should be taken with the aid of a speculum. It is important to avoid vulval contamination of the swab. For *Trichomonas*, the posterior fornix, including any obvious candidal plaques should be swabbed. If pelvic infection, including gonorrhoea, is suspected, the cervical os should be swabbed

For the specific diagnosis of BV, it is recommended that an air-dried smear of vaginal discharge is sent in addition to the swab

Separate samples should be collected into appropriate transport media for detection of viruses or *C. trachomatis*

High vaginal swabs

After the introduction of the speculum, the swab should be rolled firmly over the surface of the vaginal vault. The swab should then be placed in Amies transport medium with charcoal

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 16 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

Cervical swabs

After introduction of the speculum to the vagina, the swab should be rotated inside the endocervix. The swab should then be placed in Amies transport medium with charcoal

Urethral swabs

Contamination with micro-organisms from the vulva or the foreskin should be avoided. Thin swabs are available for collection of specimens

The patient should not have passed urine for at least 1 hour. For males, if a discharge is not apparent, attempts should be made to "milk" exudate from the penis. The swab is gently passed through the urethral meatus and rotated. Place the swab in Amies transport medium with charcoal

Intrauterine contraceptive devices (IUCDs)

The entire device should be sent

Rectal swabs

Rectal swabs are taken via a proctoscope

Throat swabs

Throat swabs should be taken from the tonsillar area and/or posterior pharynx avoiding the tongue and uvula

Fluids and pus

These are taken from the fallopian tubes, tubo-ovarian and Bartholin's abscesses etc during surgery

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

Fluids and pus – preferably a minimum volume of 1ml

3.0 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible⁹¹

Ideally, inoculation of specimens for *N. gonorrhoeae* is made directly to culture media at the bedside and incubated without delay. Transport time should be as short as possible^{92,93}

For *H. ducreyi* direct inoculation of media ensures optimal recovery⁹⁴

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Swabs should be transported in Amies transport medium with charcoal⁹⁵

If processing is delayed, refrigeration is preferable to storage at ambient temperature⁹⁶. Delays of over 48h are undesirable

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 17 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

4.0 SPECIMEN PROCESSING

4.1 TEST SELECTION

Investigation for *Chlamydia* and viruses may also be performed on genital tract specimens with the appropriate swabs and transport media for the organism under investigation

Microscopy for BV

Either

A Gram stained film of the vaginal discharge is the recommended method of detecting BV

Or

Acridine orange films or wet preparations may be used for the detection of clue cells, but are not as sensitive as the Gram film^{50,52} and are not recommended for optimal results. If clue cells or other abnormalities (such as lack of lactobacilli and/or the presence of numerous small rods) are present, then a new smear should be made (preferably from the vaginal discharge), Gram stained, and examined applying Nugent's or Hay's criteria (see section 5)

Culture for TV

Perform on specimens with clinically suspected *T. vaginalis* or other sexually transmitted disease (see 4.4.2), and all pregnant women. Routine screening may be justified in areas of higher prevalence

Microscopy for TV

Acridine orange films or wet preparations may be used for the detection of TV when routine screening is required. However, these methods are less sensitive than culture^{13,15} and are not recommended for optimal results

4.2 APPEARANCE

N/A

4.3 MICROSCOPY

4.3.1. Standard

Note 1: A direct, thin smear from the patient's exudate/discharge is the preferred specimen

Note 2: Smears made from swabs in charcoal transport medium are not ideal for examination of specimens where gonorrhoea is suspected

(a) Microscopy for BV

Vaginal swabs from females of childbearing age with a diagnosis of vaginal discharge

Either

Perform Gram stain⁹⁷ and apply Nugent's or Hay's criteria (see section 5)

Or

Acridine orange stained smear or wet preparation for clue cells with a Gram stained smear to confirm the presence of clue cells

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 18 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

(b) Microscopy for gonorrhoea

Cervical, endocervical, and female urethral smears and all male urethral specimens from suspected *N. gonorrhoeae* or known *N. gonorrhoeae* contact (unless previously performed in GUM clinic)

Prepare a thin smear on a clean microscope slide for Gram staining

Aspirates, fluids and pus (or swabs of these)

Using a sterile pipette place 1 drop of the centrifuged deposit (see section 4.4.1), or neat specimen if there is insufficient to centrifuge, on a clean microscope slide

Spread this with a sterile loop to make a thin smear for Gram staining

Screening swabs for *N. gonorrhoeae*

Microscopy may be performed in the GUM clinic

IUCDs

Rub the surface of the IUCD thoroughly with a sterile swab, previously moistened with sterile water or saline. After inoculation of all agar plates, prepare a thin smear on a clean microscope slide for Gram staining. If any pus or exudate is present prepare the smear from this

4.3.2. Supplementary

(a) Wet preparation for the detection of TV

After inoculation of all agar plates, prepare a wet prep by rotating the swab (or placing a drop of vaginal discharge) on a clean microscope slide

Place a coverslip over the wet inoculum and examine with a low power objective

(b) Acridine orange film for the detection of TV

After inoculation of all agar plates, prepare a thin smear on a clean microscope slide for acridine orange staining

Note 3: Methods for staining procedures are contained in separate SOPs

4.4 CULTURE AND INVESTIGATION

4.4.1. Pre-treatment

Products of conception

Grind or homogenise specimen with a sterile tissue grinder (Griffiths tube or unbreakable alternative), sterile scissors and petri dish, or a pestle and mortar. The addition of a small amount of sterile, filtered water, saline, peptone or broth will aid the homogenisation process.

All grinding or homogenisation must be performed in a microbiological safety cabinet

Aspirates, fluids

If sufficient sample is received, centrifuge at 1500 x g for 10mins

Decant the supernatant leaving approximately 0.5ml

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 19 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

Resuspend the deposit in the remaining fluid

4.4.2. Specimen processing

Culture for TV

Perform on specimens with clinically suspected *T. vaginalis* or other sexually transmitted infection

Method 1

Place the swab into a bijoux bottle containing *Trichomonas* culture medium. This should be performed after inoculation of culture plates unless a separate swab is sent, because the medium contains antimicrobial agents

Incubate in air at 35-37°C for 40-48h

Do not mix the culture after incubation. Withdraw some of the deposit from the bottom of the bottle with a pipette

Place a drop on a clean microscope slide and over-lay with a coverslip

Examine for the presence of motile trichomonads with a low power objective

Method 2¹⁴

Pipette 100µl of *Trichomonas* culture medium to each well of a 96-well flat-bottomed microtitre tray

Carefully swirl the genital swab in the appropriately labelled well

Add a further 200µl culture medium to each well, cover with clear microplate sealer and incubate in air at 35-37°C for 40-48h

Examine at 16h - 48h, without removing the seal, for the presence of motile trichomonads with an inverted microscope under the low power objective

Note 4: Do not remove microplate seal after application as cross contamination of wells may occur. Because of this, it is advisable to perform culture by this method as a batch towards the end of the working day. Microscopy should be performed through the seal

Swabs

Inoculate each agar plate with swab (see BSOP 54)

For the isolation of individual colonies, spread inoculum with a sterile loop

Aspirates, fluids

With a sterile pipette, inoculate each agar plate with centrifuged deposit (see section 4.4.1) or neat specimen (see BSOP 54)

For the isolation of individual colonies, spread inoculum with a sterile loop

IUCDs

Rub the surface of the IUCD thoroughly with a sterile swab. Inoculate each agar plate with the swab (see BSOP 54)

For the isolation of individual colonies, spread inoculum with a sterile loop

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Products of conception

Using a sterile pipette inoculate each agar plate with homogenised specimen (see BSOP 54)

For the isolation of individual colonies, spread inoculum with a sterile loop

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 21 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

4.4.3 Culture media, conditions and organisms
All genital swabs except STI screen, IUCDs
For all specimens:

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
All HVS	Blood agar*	35-37	5-10% CO ₂	16-24h	16-24h	<i>S. aureus</i> Lancefield Groups A, C and G streptococci Other organisms may be significant (see 4.5.1) eg Lancefield group B streptococci in pregnancy
	Sabouraud agar	35-37	air	40-48h†	≥40h	Yeasts
Urethral swabs Cervical swabs	Blood agar*	35-37	5-10% CO ₂	16-24h	16-24h	<i>S. aureus</i> Lancefield Groups A, C and G streptococci Other organisms may be significant (see 4.5.1)
	Sabouraud agar	35-37	air	40-48h	≥40h	Yeasts
	GC selective agar with antifungal agent	35-37	5-10% CO ₂	40-48h	≥40h	<i>N. gonorrhoeae</i>

Other organisms for consideration - *T. vaginalis*, *C. trachomatis*, *Mycoplasma* species and viruses

*may be replaced by Staph/strep selective agar ONLY in patients with vaginal discharge

†incubation may be extended to 5 days; in such cases plates should be read at ≥40h and left in the incubator/cabinet until day 5

Note 5: If a vaginal swab is received in combination with a cervical and urethral swab, include standard media only with the vaginal and urethral swabs and add supplementary media as appropriate for the cervical swab

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 22 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

4.4.3 Culture media, conditions and organisms (continued)

For these situations add the following:

Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Clinically suspected TV STD Pregnancy	<i>Trichomonas</i> medium	35-37	air	40-48h	≥40h	<i>T. vaginalis</i>
Intra-uterine death Septic abortion Miscarriage Balanitis Balanoposthitis Epididymitis† Orchitis	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35-37	anaerobic	40-48h*	≥40h	Anaerobes
	CLED agar	35-37	air	≥16h	≥16h	Enterobacteriaceae Pseudomonads
?Listeriosis Intra-uterine death Septic abortion Miscarriage	<i>Listeria</i> selective agar	35-37	air	40-48h	daily	<i>Listeria</i>
<10 years old	Chocolate agar	35-37	5-10% CO ₂	40-48h	daily	<i>H. influenzae</i>
? <i>Actinomyces</i> (clinically indicated or suggested by microscopy)	Blood agar supplemented with metronidazole and nalidixic acid	35-37	anaerobic	10d	≥40h, at 7d and 10d	<i>Actinomyces</i>
?chancroid‡	<i>H. ducreyi</i> selective agar	33-34	5-10% CO ₂	5d	5d	<i>H. ducreyi</i>

Other organisms for consideration - *T. vaginalis*, *C. trachomatis* (BSOP 56), *Mycoplasma* species and viruses

*incubation may be extended to 5 days; in such cases plates should be read at ≥40h and left in the incubator/cabinet until day 5

†urine specimens may be investigated for these conditions (see BSOP 41)

‡often a clinical diagnosis - refer to local protocols

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 23 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

4.4.3 Culture media, conditions and organisms

All STI screening swabs

For all specimens:

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
?STI	GC selective agar with antifungal agent	35-37	5-10% CO ₂	40-48h	≥40h	<i>N. gonorrhoeae</i>

For these situations add the following:

Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
?STI (if required by local protocol)	Sabouraud agar	35-37	air	40-48h	≥40h	Yeasts

Other organisms for consideration: *T. vaginalis*, *C. trachomatis*, *Mycoplasma* species, *T. pallidum* and viruses

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 24 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

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4.4.3 Culture media, conditions and organisms

Aspirates/pus and swabs from tubo-ovarian abscess (TOA), fallopian tube, Pouch of Douglas (PoD), Bartholin's gland, IUCD and surgical specimens. For all specimens:

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
PID Salpingitis TOA Bartholin's abscess Pyosalpinx Products of conception Infected IUCD Other inflammatory conditions	Chocolate agar	35-37	5-10% CO ₂	40-48h	daily	<i>H. influenzae</i>
	Blood agar	35-37	5-10% CO ₂	40-48h	daily	<i>S. aureus</i> Streptococci Enterobacteriaceae
	Fastidious anaerobe agar	35-37	anaerobic	5d	≥40h and at 5d	Anaerobes
	GC selective agar with antifungal agent	35-37	5-10% CO ₂	40-48h	daily	<i>N. gonorrhoeae</i>

For these situations add the following:

Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
? <i>Actinomyces</i> (clinically or suggested by microscopy)	Blood agar supplemented with metronidazole and nalidixic acid	35-37	anaerobic	10d	≥40h, at 7d and 10d	<i>Actinomyces</i>
If microscopy suggestive of mixed infection	Neomycin fastidious anaerobe agar with metronidazole 5mg disc	35-37	anaerobic	5d	≥40h and at 5d	Anaerobes
	CLED agar	35-37	air	16-24h	≥16h	Enterobacteriaceae

Optional media	Incubation			Cultures read	Target organism(s)
	Temp °C	Atmos	Time		
Either: Non-supplemented or supplemented blood culture bottles* or Supplemented brain heart infusion broth Subcultured as appropriate at ≥40h on to the standard media	35-37	air	continuous monitoring (minimum 40-48h)	N/A	Any organism
	35-37	air	40-48h	daily	
	35-37	as above	as above	as above	

Note 6: a growth of any organism may be significant

*follow manufacturers recommendations

Other organisms for consideration: *C. trachomatis*, *Mycoplasma* and *Ureaplasma* species, *Mycobacterium* species (BSOP 40) and viruses

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 25 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

4.5 IDENTIFICATION

4.5.1. Minimum level

<i>Actinomyces</i>	"actinomycetes" level
Anaerobes	"anaerobes" level
β -haemolytic streptococci	Lancefield group level
Other streptococci and enterococci	genus level
Enterobacteriaceae	"coliforms" level
<i>Haemophilus</i>	species level
<i>Listeria</i>	species level
<i>Neisseria</i>	species level
Pseudomonads	"pseudomonads" level
<i>S. aureus</i>	species level
Yeasts	"yeasts" level

Organisms may be further identified if clinically or epidemiologically indicated

4.5.2. Referral to Reference Laboratories

Listeria species for confirmation of identity

Actinomyces species for identification

N. gonorrhoeae for confirmation of identity, typing (medico-legal cases) and susceptibility testing

N. gonorrhoeae for all antibiotic resistant isolates (and strains with a reduced susceptibility to penicillin)

Isolates associated with outbreaks and where epidemiologically indicated

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem or anomaly that requires elucidation

4.6 ANTIBIOTIC SUSCEPTIBILITY TESTING

Refer to SOP on Susceptibility Testing (BSOP 45)

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 26 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

5.0 REPORTING PROCEDURE

5.1 MICROSCOPY

(a) Gram films

Report on yeasts and WBCs if present and on the presence or absence of intracellular Gram-negative diplococci

Report on organisms seen in films from aspirates/pus (local reporting procedures should be followed on reporting of organisms seen in other specimens)

Report on clue cells if present and whether microscopy is suggestive of BV according to the criteria of Nugent (number of organisms per high power, oil immersion field (hpf) at approximately x1000 magnification⁵⁴) or of Hay:

Nugent's criteria⁵⁴

Numbers of <i>Lactobacillus</i> morphotypes seen	Score	Numbers of <i>Gardnerella</i> and <i>Prevotella</i> morphotypes seen	Score	Numbers of <i>Mobiluncus</i> morphotypes seen	Score
>30/hpf	0	>30/hpf	4	>30/hpf	4
5-30/hpf	1	5-30/hpf	3	5-30/hpf	3
2-4/hpf	2	2-4/hpf	2	2-4/hpf	2
1/hpf	3	1/hpf	1	1/hpf	1
none	4	none	0	none	0

Code each morphotype separately according to numbers of organisms seen as indicated in the table above and add individual scores together. Interpret scores as follows:

Total score 0-3 normal

Total score 4-6 intermediate: suggestive of BV Assess with clinical criteria and send repeat to confirm

Total score ≥ 6 abnormal: indicative of BV

Hay's criteria⁷³

Grade I normal predominantly *Lactobacillus* morphotypes

Grade II intermediate mixed *Lactobacillus* and other morphotypes. Assess with clinical criteria and send repeat to confirm if necessary

Grade III abnormal few or absent *Lactobacillus* morphotypes, but greatly increased number of *G. vaginalis* and other bacterial morphotypes. Suggestive of BV

Note 7: A vaginal smear should be requested on any swab that is suggestive of BV or if examination for BV is specifically requested

(b) Wet preparations or acridine orange films

Report on WBCs, yeasts and trichomonads seen

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 27 of 33
Reference no: BSOP 28i4.1

This SOP should be used in conjunction with the series of other SOPs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

Note 8: A negative microscopy result does not exclude the possibility of TV infection

5.1.1. Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically

Written report: 16 - 72h

5.2 CULTURE

Report clinically significant organisms isolated **or**

Report other growth (eg normal flora isolated) **or**

Report absence of specific pathogens **or**

Report absence of growth

The absence of *N. gonorrhoeae* in vaginal swabs should not be reported as these are not the specimen of choice for the isolation of *N. gonorrhoeae*. Recommendations on the appropriate specimen type should be included in the report

Also, report results of supplementary investigations

According to local protocols for reporting the carriage of Group B Streptococci, it may be appropriate for laboratories to report isolates of Group B streptococci to the Ante-natal Clinic

5.2.1. Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically

Written report: 16 - 72h stating, if appropriate, that a further report will be issued

Supplementary investigations: see appropriate SOPs

5.3 ANTIBIOTIC SUSCEPTIBILITY TESTING

Refer to SOP on Susceptibility Testing (BSOP 45)

6.0 REPORTING TO THE HPA⁹⁸ (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS)

Refer to the following:

Individual SOPs on organism identification

Health Protection Agency publications:

"Reporting to the CDR: A guide for laboratories"

"Hospital infection control: Guidance on the control of infection in hospitals"

Refer to current guidelines on CDSC and COSURV reporting

Local guidelines

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 28 of 33
Reference no: BSOP 28i4.1

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Report all isolates of *N. gonorrhoeae* and *L. monocytogenes*

It may be appropriate for laboratories to report isolates of Group B streptococci to the Antenatal Clinic as according to local protocols for the reporting of the carriage of these isolates

INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 29 of 33
Reference no: BSOP 28i4.1

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INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 30 of 33

Reference no: BSOP 28i4.1

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Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 31 of 33
Reference no: BSOP 28i4.1

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INVESTIGATION OF GENITAL TRACT AND ASSOCIATED SPECIMENS

Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 32 of 33
Reference no: BSOP 28i4.1

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Issue no: 4.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory. Page 33 of 33
Reference no: BSOP 28i4.1

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