Public Health England

NHS

UK Standards for Microbiology Investigations

Investigation of Genital Tract and Associated Specimens

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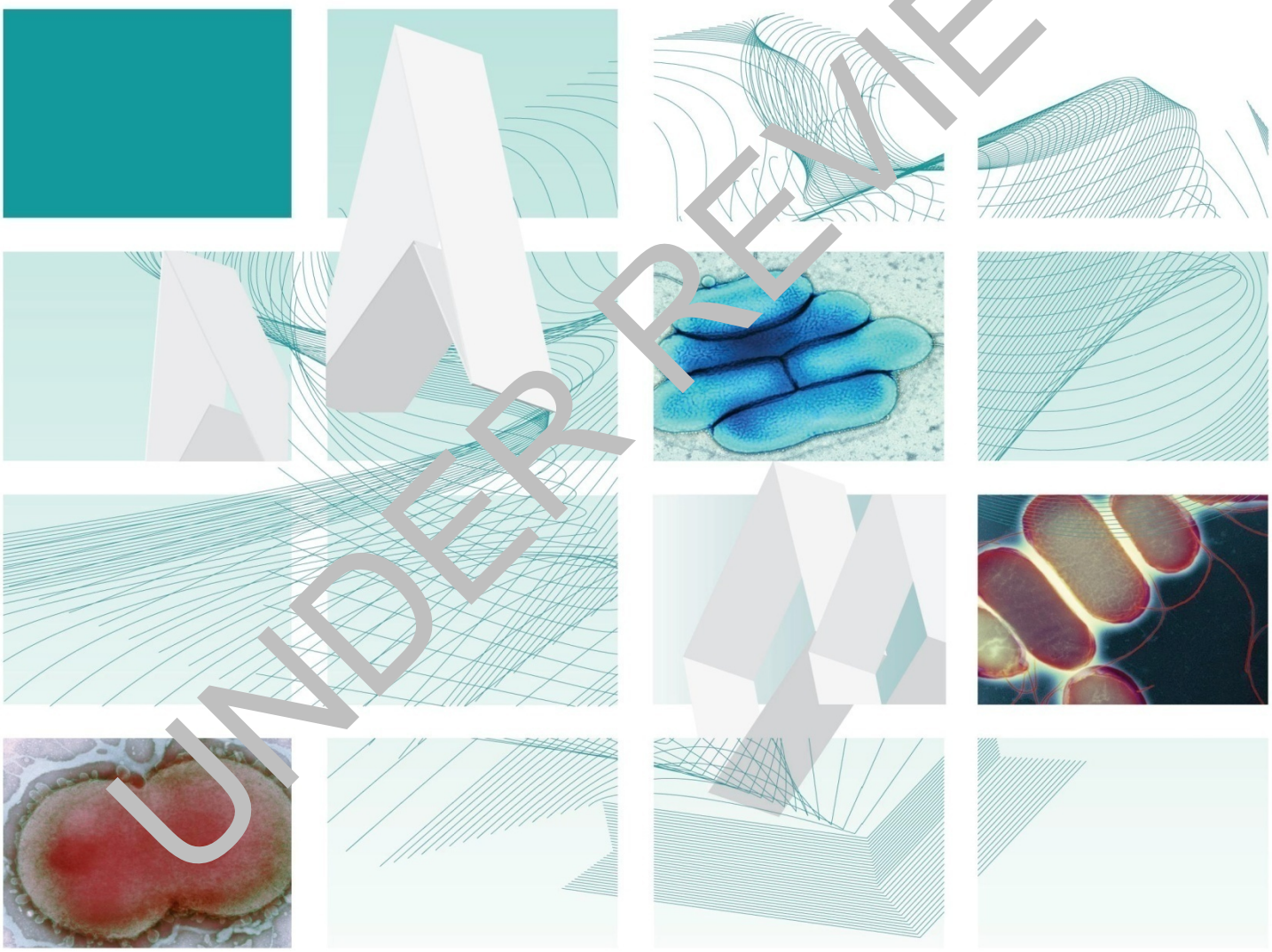

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Acknowledgments

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Logos correct at time of publishing.

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Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	10/26.04.17
Issue no. discarded.	4.5
Insert Issue no.	4.6
Section(s) involved	**Amendment**
Page 32.	Change to the scoring for *Mobiluncus* morphotypes to match ref. 72 (>30/hpf =**2** not 4; 5-30/hpf =**2** not 3; 2-4/hpf = 1 not 2).
Page 33.	Change to the interpretation of the scoring to match ref. 72 (Abnormal: indicative of BV when Total score ≥**7** not 6).

Amendment No/Date.	9/08.12.14
Issue no. discarded.	4.4
Insert Issue no.	4.5
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Reporting Procedure.	Due to a transcription error during transfer to the PHE template, some text regarding Nugent's criteria score was removed. This information has been re-instated in the document.

Amendment No/Date.	8/24.04.14
Issue no. discarded.	4.3
Insert Issue no.	4.4
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template

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	to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	7/18.12.12
Issue no. discarded.	4.2
Insert Issue no.	4.3
Section(s) involved	**Amendment**
Whole document.	Minor formatting amendments.
2.5.3.	Section number amended in table.

Amendment No/Date.	6/11.07.12
Issue no. discarded.	4.1
Insert Issue no.	4.2
Section(s) involved	**Amendment**
Whole document.	Document presented in a new format. The term "CE marked leak proof container" replaces "sterile leak proof container" (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC1,2. Edited for clarity. Reorganisation of [some] text. Minor textual changes.
Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.

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References.	Some references updated.

Amendment No/Date.	5/03.05.05
Issue no. discarded.	4
Insert Issue no.	4.1
Section(s) involved	**Amendment**
Front page.	Redesigned.
Status of document.	Reworded.
Amendment page.	Redesigned.

Amendment No/Date.	4/15.12.03
Issue no. discarded.	3.1
Insert Issue no.	4
Section(s) involved	**Amendment**
Whole document.	Text revision.

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UK SMI[#]: Scope and Purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post-analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

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laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of Document

Type of Specimen

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

Scope

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

This SMI should be used in conjunction with other SMIs.

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 SMI 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 Transmissible 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

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.

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Local gonococcal infections in women

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

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 viruses[3].

Asymptomatic mucosal infection

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 history, 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)

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

- 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 syndrome[5]. DGI resulting in meningitis is very rare[6].

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 SMI recommends the use of selective media containing antifungal agents.

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Trichomoniasis[8]

Trichomoniasis is caused by the flagellate protozoan, *T. vaginalis*; it 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. *T. vaginalis* infection in pregnancy has been associated with low birth weight and preterm delivery[9]. The prevalence of *T. vaginalis* remains at a constant low level in cases seen in GUM clinics[10].

Various techniques including culture followed by microscopy, direct microscopy and immunodiagnostic methods for the detection of *T. vaginalis* have been compared[11-15]. Microscopy has been the most practicable means of diagnosis for routine screening[16,17]. Microscopy of a wet preparation is highly specific and easily performed, but it fails to detect 30-50% of *T. vaginalis* infections (even when undertaken close to the patient) compared to culture which is regarded as the 'gold standard'[12,13]. Detection using films stained with acridine orange has been found to be only slightly more sensitive than unstained wet preparations[14].

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 sensitivity[13].

This SMI 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

Genital ulcers are most commonly caused by[18]:

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

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

Staphylococcus aureus and Lancefield group A streptococci may also cause tender pustules resembling ulcers on genitalia, as well as inguinal lymphadenopathy and soreness[18].

Chancroid

Chancroid is an important cause of genital ulceration in the tropics, and its incidence increased dramatically in North America during the late 1980s. It is caused by *H. ducreyi* which enters via a break in the epithelium[19]. 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 cases[19].

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Asymptomatic carriage appears to be rare. Infection rarely presents as urethritis alone without any genital ulcers[21].

The incidence of chancroid is reportedly increasing in many areas, although diagnosis is often made on clinical grounds alone and may thus be inaccurate[19]. Chancroid, in common with other sexually transmitted diseases, is thought to be an important co-factor in the transmission of HIV in the tropics[22].

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 evaluation[23].

Isolation of *H. ducreyi* is comparatively difficult and requires selective agar media, although isolation rates of up to 80% have been reported[19,22-24].

C. granulomatis[25]

C. granulomatis 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.

Genital warts

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 warts[27]. 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 infection[27].

Children are also at risk of acquiring sexually transmissible infections[28-34]. 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 prenatally acquired infection and may persist for as long as three years. Similarly, anogenital warts may be present in pre-pubertal children as a consequence of prenatal 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 SMI, 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 cells[35].

Vaginal Infections (other than STIs)

Normal vaginal flora

Normal vaginal flora consists of a wide range of organisms including *Lactobacillus*

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species, streptococci, enterococci and coagulase negative staphylococci[36-39]. 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

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 include[40]:

- *C. krusei*
- *C. kefyr*
- *C. tropicalis*
- *C. glabrata*

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

Vaginitis[41]

Vaginitis can be caused by *Candida* species and *T. vaginalis*. In children, infections caused by β-haemolytic streptococci and *S. aureus* are common[42]. Lancefield group A streptococci also cause vaginitis and purulent vaginal discharge in adults[43].

Atrophic vaginitis is a rare condition usually associated with the elderly[44]. 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

Vulvovaginitis is mainly seen in pre-pubertal females, but may affect women of any age. It may be associated with poor hygiene, skin irritation due to soaps, or with

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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 vulvovaginitis, including *Salmonella* and *Shigella* species[48]. Threadworm infestation may predispose to vulvovaginitis (see [B 31 – Investigation of Specimens other than Blood for Parasites]).

Bacterial vaginosis (BV)

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

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 fulfilled[52]:
    - grey-white, thin homogenous discharge
    - 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 species[53]. A diverse group of organisms is involved, many of which are difficult to grow. Organisms associated with BV include[49-51,53]:

- *Prevotella* species
- *G. vaginalis*
- *Mobiluncus* species

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- *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 specimens[54].

Examination of Gram stained films is reported to be useful in the diagnosis of BV and standard criteria for morphotypes have been described[50,54,55]. 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 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 BV[56]. 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 Nugent or Hay) of vaginal smears is the most sensitive method for the laboratory diagnosis of BV as it detects both clue cells and the disturbance in bacterial morphotypes associated with BV[50,52,54,55]. 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 rods[39]. However, one study found that acridine orange or wet preparations are more sensitive methods for detecting clue cells than the Gram stain[57]. Detection of clue cells alone, whilst highly specific for BV, is not as sensitive as detection of different morphotypes by the Gram stain[56].

This SMI 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)

Toxic shock syndrome (TSS) is an acute multi-system illness characterised by fever, hypotension, erythematous rash, diarrhoea and desquamation of the skin upon recovery[58]. 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:

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

Lancefield group B streptococcus

Lancefield group B streptococcus normally colonises the vagina in many women. In pregnancy this organism can infect the amniotic fluid (see B 26- Investigation of Fluids

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from Normally Sterile Sites) which can lead to neonatal sepsis, pneumonia and meningitis. 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 anorectum[59-61].

Conditions considered to confer a high risk of infection include:

- fever in labour
- premature labour
- premature rupture of membranes (PROM)
- previously infected baby

Listeria monocytogenes

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 fetus[62]. 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. Routine culture of vaginal swabs for *L. monocytogenes* is not usually performed although it may be useful in suspected cases[64]. Blood cultures are indicated. Serological investigations have no place in the diagnosis of listeriosis[63].

Septic abortion

Septic abortion may result in serious maternal morbidity and may be fatal[62]. 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

Bartholinitis

Bartholinitis 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*
- *C. trachomatis*
- *H. influenzae*
- *S. aureus*

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- *other Neisseria* species
- *M. hominis*

Mucopurulent cervicitis

Mucopurulent cervicitis is inflammation of cervical columnar epithelium. Causative organisms include[41]:

- *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 organisms[41].

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 leucocytes[66]. Mucopurulent cervicitis is characterised by the presence of an endocervical exudate containing PMNs. A visible yellow discharge is produced.

Endometritis

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

Postpartum endometritis most infections are caused by vulvovaginal flora that ascends into the uterus. Infections are often polymicrobial and caused by[67]:

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

Risk factors include:

- amniotic fluid infection

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- caesarean delivery
- invasive foetal monitoring
- prolonged rupture of membranes
- vaginal examinations

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

Salpingitis

Salpingitis is inflammation of the uterine (fallopian) tube. Infection is sometimes polymicrobial involving[68]:

- *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)

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[69,70]. 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 B 26 - Investigation of Fluids from Normally Sterile Sites). 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:

- *C. trachomatis*
- *N. gonorrhoeae*
- anaerobes
- Lancefield group B streptococcus

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- other streptococci
- *Escherichia coli*
- *G. vaginalis*
- *Actinomyces israelii*
- *M. hominis*
- *H. influenzae*

Intrauterine contraceptive devices (IUCDs)

Intrauterine contraceptive devices (IUCDs) - the presence of an IUCD may be associated with PID[71]. 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

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 B 41 - Investigation of Urine).

Epididymitis

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 include[72]:

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

Orchitis

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 include[72]:

- Enterobacteriaceae
- pseudomonads
- staphylococci

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- streptococci
- *M. tuberculosis*

Balanitis

Balanitis is inflammation of the glans penis[73].

Balanoposthitis

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 include[73-76]:

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

Candida

Candida species may be isolated in cases of genile thrush.

Urethritis in men is mainly caused by[72]:

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

Haemophilus species such as *H. influenzae* and *H. parainfluenzae* have been isolated from urethral discharge[77].

Technical information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity, where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

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Specimen Containers[78,79]

SMIs use the term, “CE marked leak proof container,” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes.”

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1 Safety Considerations[78-94]

1.1 Specimen Collection, Transport and Storage[78-83]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Collect swabs into appropriate transport medium and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[78-94]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[86].

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

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

2 Specimen Collection

2.1 Type of Specimens

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

2.2 Optimal Time and Method of Collection[95]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[95].

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.

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

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium[96-100].

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

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

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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[97].

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[97].

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 one 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[97].

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[95]

Fluids and pus – preferably a minimum volume of 1mL.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

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3 Specimen Transport and Storage[78,79]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[95].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[95].

4 Specimen Processing/Procedure[78,79]

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 and are not recommended for optimal results[50,52]. 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 or Hay criteria (see section 5.1).

Culture for TV

Perform on specimens with clinically suspected *T. vaginalis* or other sexually transmitted disease (see 4.3.1), 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 and are not recommended for optimal results[14,15].

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

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4.3.1 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 10min.

Decant the supernatant leaving approximately 0.5mL.

Resuspend the deposit in the remaining fluid.

4.3.2 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 bijou 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 40-48hr.

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 2[13]

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 40-48hr.

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

Note: 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.

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4.4 Microscopy

4.4.1 Standard

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

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

Microscopy for BV

Vaginal swabs.

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

Either

Perform Gram stain and apply Nugent or Hay criteria (see section 5.1)[101].

Or

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

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 one drop of the centrifuged deposit (see section 4.5), 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.

4.4.2 Supplementary

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.

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.

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Note: Methods for staining procedures are contained in separate SMIs.

4.5 Culture and Investigation

Swabs

Inoculate each agar plate with swab (see Q 5 - Inoculation of Culture Media for Bacteriology).

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 4.3.1 or neat specimen see Q 5 - Inoculation of Culture Media for Bacteriology).

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 Q 5 - Inoculation of Culture Media for Bacteriology).

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

Products of conception

Using a sterile pipette inoculate each agar plate with homogenised specimen (see Q 5 - Inoculation of Culture Media for Bacteriology).

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

4.5.1 Culture media, conditions and organisms

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	16-24hr	16-24hr	*S. aureus* Lancefield Groups A, C and G streptococci Other organisms may be significant (see 4.6.1) eg Lancefield group B streptococci in pregnancy
	Sabouraud agar	35-37	air	40-48hr†	≥40hr	Yeasts
Urethral swabs Cervical swabs	Blood agar*	35-37	5-10% CO_2	16-24hr	16-24hr	*S. aureus* Lancefield Groups A, C and G streptococci Other organisms may be significant (see 4.6.1)
	Sabouraud agar	35-37	air	40-48hr	≥40hr	Yeasts

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	GC selective agar with antifungal agent	35-37	5-10% CO_2	40-48hr	≥40hr	*N. gonorrhoeae*

Note: 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.

4.5.1 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	*Trichomonas* medium	35-37	air	40-48hr	≥40hr	*T. vaginalis*
Intra-uterine death Septic abortion Miscarriage Balanitis Balanoposthitis Epididymitis† Orchitis	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35-37	anaerobic	48hr	≥40hr	Anaerobes
	CLED agar	35-37	air	≥16hr	≥16hr	Enterobacteriaceae Pseudomonads
?Listeriosis Intra-uterine death Septic abortion Miscarriage	*Listeria* selective agar	35-37	air	40-48hr	daily	*Listeria*
<10 years old	Chocolate agar	35-37	5-10% CO_2	40-48hr	daily	*H. influenzae*
?Actinomyces (clinically indicated or suggested by microscopy)	Blood agar supplemented with metronidazole and nalidixic acid	35-37	anaerobic	10d	≥40hr, at 7d and 10d	*Actinomyces*
?chancroid‡	*H. ducreyi* selective agar	33-34	5-10% CO_2	5d	5d	*H. ducreyi*

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

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

†urine specimens may be investigated for these conditions (see B 41 - Investigation of Urine).

‡often a clinical diagnosis - refer to local protocols.

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4.5.1 Culture media, conditions and organisms (continued)

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	40-48hr	≥40hr	*N. gonorrhoeae*
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	40-48hr	≥40hr	Yeasts

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

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4.5.1 Culture media, conditions and organisms (continued)

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	Chocolate agar	35-37	5-10% CO_2	40-48hr	daily	*H. influenzae*
Bartholin's abscess Pyosalpinx Products of conception	Blood agar	35-37	5-10% CO_2	40-48hr	daily	*S. aureus* Streptococci Enterobacteriaceae
Infected IUCD Other inflammatory conditions	Fastidious anaerobe agar	35-37	anaerobic	5d	≥40hr and at 5	Anaerobes
	GC selective agar with antifungal agent	35-37	5-10% CO_2	40-48hr	daily	*N. gonorrhoeae*
For these situations add the following:						
Clinical details/ conditions	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
		Temp °C	**Atmos**	**Time**		
? Actinomyces (clinically or suggested by microscopy)	Blood agar supplemented with metronidazole and nalidixic acid	35-37	anaerobic	10d	≥40hr, at 7d and 10d	*Actinomyces*
If microscopy suggestive of mixed infection	Neomycin fastidious anaerobe agar with metronidazole 5mg disc	35-37	anaerobic	5d	≥40hr and at 5d	Anaerobes
	CLED agar	35-37	air	16-24hr	≥16hr	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		35-37	air	continuous monitoring (minimum 40-48hr)	N/A	Any organism

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broth Subcultured as appropriate at ≥40hr on to the standard media					
	35-37	air	40-48hr	daily	
	35-37	as above	as above	as above	

Note: a growth of any organism may be significant.

*follow manufacturer's recommendations.

Other organisms for consideration: *C. trachomatis*, *Mycoplasma* and *Ureaplasma* species, *Mycobacterium* species (B 40 - Investigation of Specimens for *Mycobacterium* species) and viruses.

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

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

Organisms may be further identified if this is clinically or epidemiologically indicated.

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

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Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should, be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting Procedure

5.1 Microscopy

Gram films

Report on yeasts, 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 magnification) or of Hay[54]:

Nugent's criteria[54]

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

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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 ≥7	abnormal: indicative of BV	

Hay's criteria[72]

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

Wet preparations or acridine orange films.

Report on WBCs, yeasts and trichomonads seen.

Note: 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: 16-72hr.

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: 16-72hr stating, if appropriate, that a further report will be issued.

Supplementary investigations: see appropriate SMIs.

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5.3 Antimicrobial Susceptibility Testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

6 Notification to PHE[102,103] or Equivalent in the Devolved Administrations[104-107]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified verbally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[104,105], Wales[106] and Northern Ireland[107].

It may be appropriate for laboratories to report isolates of Group B streptococci to the Ante-natal Clinics according to local protocols for the reporting of the carriage of these isolates.

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