Methods of specimen collection for the diagnosis of STIs

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Many of the landmark discoveries with regard to the etiology, pathogenesis and epidemiology of sexually transmitted infections (STIs) occurred with the use of various diagnostic techniques many years ago.[1] However, the concept of providing comprehensive laboratory services for the diagnosis of STIs has surfaced relatively recently. The correct method of specimen collection helps in achieving desirable goals in the laboratory diagnosis of STIs. If simple precautions are taken, it will avoid spurious results. The collection of specimens and use of the appropriate swab and transport media are vital in the success of tissue culture.

Principles to be followed while collecting specimens:[1]

1. Communication with laboratory staff to discuss collection, transport and testing of specimens.
2. All procedures should be performed while wearing appropriate protective gear.
3. Avoid contamination by indigenous commensal flora.
4. Adequate volumes of each specimen should be collected.
5. All specimens should be labeled correctly with the patient's name, hospital number and source, date and time of collection.
6. Leakproof containers should be used.
7. Optimal transport conditions should be followed as many of the organisms are fastidious.

The common laboratory diagnostic procedures that can be done in the outpatient department are:[2]

1. Dark-field microscopy-Syphilis
2. Gram staining for gonorrhea, non-gonococcal urethritis, chancroid, bacterial vaginosis
3. Tzanck smear for herpes genitalis, donovanosis, molluscum contagiosum
4. Wet mount for trichomoniasis
5. KOH wet mount for candidiasis
6. Bubo aspiration and smear for LGV and chancroid

DARK-FIELD MICROSCOPY

Dark-field examination is most productive in primary, secondary and early congenital syphilis, when moist lesions containing large numbers of treponemes (e.g., chancres, condylomata lata, umbilical cord or mucous patches) are present.[2-4] Aspirate from enlarged regional lymph nodes and cervical and vaginal specimens can also be used, but oral lesions are avoided as even an experienced observer may find it difficult to differentiate *T. pallidum* from saprophytic spirochetes. The specimen for dark-field microscopy consists of serous fluid that contains *T. pallidum*, but should be free of erythrocytes, other organisms and tissue debris.

Method of specimen collection for dark-field microscopy[2-4]

- Observe universal safety precautions.
- To clean the lesion only if it is encrusted or obviously contaminated.
- Use only tap water or physiological saline (without antibacterial additives). Antiseptics or soaps should not be used as they may kill the treponemes. Use minimal amounts of liquid for cleaning as large amounts may dilute and reduce the yield of the organisms.

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- Gently abrade the lesion with dry gauze, wipe away any blood-stained exudate and apply gentle pressure until only clear serum exudes.
- Collect the specimen directly on a cover slip or a clean glass slide by pressing directly onto the lesion.
- For cervical and vaginal lesions, identify the lesion by speculum examination, clean with saline, abrade with a gauze pad held in suitable forceps. Collect the serous exudates using a bacteriological loop or Pasteur pipette and transfer to a slide.
- If material is not sufficient, mix with a drop of saline.
- Seal the edges of the cover slip with petroleum jelly.
- Examine immediately.
- If negative at first, dark-field examination should be repeated daily for at least three consecutive days.

GRAM STAINING

Sterile cotton, calcium alginate, dacron rayon or polyethylene terephthalate (PET) swabs with plastic or aluminium shaft or bacteriological loop can be used for collecting the specimen.[2,3] Gram staining is useful for the diagnosis of gonococcal and non-gonococcal urethritis, mucopurulent cervicitis, chancroid, bacterial vaginosis and candidal infections.

(A) Gonorrhea

The choice of the specimen depends on the age, sex, sexual habits and clinical presentation of the patient.

(i) Heterosexual men --urethra, first void urine (FVU)
(ii) Homosexual men--urethra, rectum, oropharynx
(iii) Women
- Primary site-endocervical canal
- Secondary sites-urethra, vagina, rectum, oropharynx

Two swabs should always be collected--one for direct microscopy and one for culture. Transport media should be used if the laboratory is not in the vicinity of the clinic.

Method of collection of the specimen

In men

a) Urethral swab
- Collect specimen at least 2 hours after urination as voiding decreases the amount of exudates.
- Retract the prepuce, clean the tip of the meatus with normal saline and collect the pus directly onto a glass slide or sterile swab in case of frank urethral discharge.
- If no urethral discharge is seen, milk / strip the urethra from the root of the penis to the glans and collect the discharge as above.
- If no discharge is obtained, insert a sterile cotton tipped or thin calcium alginate swab with a flexible wire shaft or a bacteriological loop 2-3 cm into the urethra and rotate for 5-10 seconds
- If there is no evidence of urethritis on examination, but there is a history of contact, ask the patient to hold the urine overnight and then milk / strip the urethra and collect the discharge if any. If no discharge is obtained, insert a swab and collect the specimen as above.

b) Urine
- The first 10-15 ml of the early morning first void urine is collected in a sterile plastic container with a wide mouth and processed immediately.

In women

a) Endocervical swab
- Cervical specimens are not collected in prepubertal girls since gonococci in this age group involve the vagina and not the cervix.
- No antiseptics, analgesics or lubricants should be applied.
- A sterile vaginal speculum moistened with warm water is inserted in the vagina and the ectocervix is visualized.
- After cleaning the ectocervix using forceps with a sterile cotton swab, insert a sterile swab 2-3 cm into the endocervical canal, rotate and move from side to side for 5-10 seconds and withdraw.

b) Urethral swab
- Same method as for men, except that the urethra is massaged against the pubic symphysis from its proximal end towards the meatus if no pus is visible.

c) Vaginal swab
- Prepubertal / unmarried girls and women who have undergone hysterectomy.
- Vaginal swab or vaginal tampon may be used to obtain the specimen.
- Using a speculum, swab the posterior fornix with a sterile swab in women.
- Collect the specimen without a speculum in prepubertal girls.

Both sexes

a) Rectal swab
- If recent anal intercourse is admitted, a proctoscope is inserted, followed by a swab stick inserted 3 cm into the anal canal, rotating it for 10 seconds to collect the exudates / mucus / muco-pus from the crypts just inside

the anal ring. If fecal contamination occurs, discard and collect a fresh specimen.

b) Pharyngeal swab
- If orogenital contact with an infected person is suspected, a specimen is collected from the tonsillar crypts and the bed of the pharynx in both sexes.

(B) Non-gonococcal urethritis / cervicitis

Specimen is collected in the same manner as for gonorrhea, but as discharge may be scanty, samples are collected after holding the urine for 3-4 hours.

(C) Chancroid
- Specimens are collected from the undermind edge or the base of the ulcer. Organisms are usually demonstrable in the aspirate from an intact bubo.
- Wipe the lesion with saline gauze followed by dry gauze (thorough cleaning not essential) to remove the superficial debris and crusts. Roll a sterile swab in one direction beneath the undermined edge of the ulcer. Re-roll the swab in the reverse direction at 180° on a clean glass slide to maintain the arrangement of the bacteria.
- Use appropriate transport media for cultures

(D) Bacterial vaginosis

Specimen is collected from the posterior or lateral wall of the vagina with a sterile swab soaked in saline.

TZANCK SMEAR/ GIEMSA STAIN

Giemsa stain can be used in the diagnosis of genital herpes, molluscum contagiosum, donovanosis and chancroid.[2,3]

(A) Genital herpes[2,3]

(i) Scrapings from blister / vesicle / ulcer base for Tzanck smear
- vesicle (< 72 h old) - open with an 18G hypodermic needle on one side, drain the fluid, fold the roof back and scrape the undersurface of the roof and floor with a curette or scalpel. The vesicle fluid may be sent for culture, where facilities are available.
- ulcer – cotton-tipped swab on a wire shaft is used.

(ii) Women with genital harpes, swab ectocervix and junction of ecto and endo cervix for Tzanck smear and culture.

(iii) Asymptomatic women-use a single swab premoistened with saline to rub the clitoral hood, labia minora and majora, perineum and perianal region for culture.

(iv) Men without vesicles-swab urethra and meatus for Tzanck smear and culture.

(v) Asymptomatic neonates-use swabs premoistened with saline; one each from the conjunctiva, mouth, around the lips, external auditory canal, umbilicus, axillae, and groins for culture.

(B) Donovanosis[5]
- Wipe the lesion with saline gauze, followed by dry gauze. Remove a small piece of tissue from the border of a well-defined ulcer using a curette / forceps / edge of a safety razor blade. Place this specimen on a clean grease-free microscopic glass slide and crush the specimen between two clean slides (Rajam and Rangiah method).
- Alternatively, a crush biopsy specimen may be used (Greenblatt and Barfield method).
- Impression smears from the lower surface of the biopsy specimen may also be used.
- The specimen is air-dried and stained with Giemsa or Leishman stain.

(C) Molluscum contagiosum[2,3]
- Compress the lesion to extrude the cheesy material or use a small curette to remove the top of a papule.
- Crush the specimen between two clean grease-free microscopic slides and stain.

KOH MOUNT

This test may be used for the diagnosis of genital candidiasis and bacterial vaginosis.[2,3]
- Under speculum examination, the specimen is collected with a cotton or polyester swab from the wall of the posterior fornix. The skin surrounding the genitals is also scrapped.
- In men, the swab is moistened with saline and the glans surface is scrapped.
- The specimen is mixed with a drop of 10-20% KOH on a glass slide, covered with a cover slip and examined under high power (40x lens).

WET MOUNT

This is a simple diagnostic procedure commonly used to visualize trichomonads, but can also demonstrate candida and organisms responsible for bacterial vaginosis.[1-3]
- Under speculum examination, the vaginal swab is collected from the posterior fornix using a sterile swab or bacteriological loop. In men, the urethra is sampled with a cotton wool or polyester swab.

- The specimen is mixed with 1 ml of body-temperature saline in a test tube or directly mixed with a drop of normal saline on a slide. Using warmed saline or warming the slide enhances the motility of the trichomonads.

BUBO ASPIRATION

Non-fluctuant bubo (for syphilis):[6]

In cases in which an antibiotic or other antiseptic lotion or cream has been applied on the primary sore or in which the sore is healing or is hidden in the terminal portion of the urethra or under a phimotic prepuce, diagnostic puncture of lymph nodes is particularly useful. By this technique, 0.1 ml of sterile normal saline is injected into an enlarged regional lymph node before aspiration. The enlarged node is first steadied between finger and thumb with the skin stretched over it. A hypodermic needle attached to a small syringe containing 0.1 ml saline is introduced through the skin along the long axis of the node and plunged well into its body. Movement of the syringe and needle in various directions will confirm that the needle is in the correct position. The saline is then injected into the node and after further movement of the needle to encourage flow of lymph, aspiration is performed. The fluid material so obtained is used for dark-field microscopy.[6]

Fluctuant bubo (for LGV, chancroid):[2]

- The patient is made to lie in the supine position and the area is painted with (povidone iodine)
- The bubo is aspirated from the non-dependent fluctuant part with a 16-18G needle with a 10 or 20 ml syringe till most of the fluid is aspirated.

METHOD OF SPECIMEN COLLECTION FOR IMMUNOFLUORESCENCE

Immunofluorescence techniques are used primarily in the diagnosis of syphilis and herpes genitalis.[3] For the detection of *Treponema pallidum* (Direct fluorescent antibody test-Treponema pallidum "DFA-Tp"), lesion exudates, tissues, body fluids or secretions are taken onto slides, dried and fixed with acetone and then stained. For herpes genitalis, the specimen is directly taken from the lesions or centrifuged deposits from transport media containing the specimen. For chancroid, gonorrhea, chlamydia and trichomoniasis, immunofluorescence has been described but is not routinely used.

METHODS FOR POLYMERASE CHAIN REACTION (PCR)

The type of specimen taken is less critical for PCR and neither refrigeration nor rapid transport is required. PCR and ligase chain reaction (LCR) give good results even in first-catch urine samples or self-administered vaginal swabs for *Chlamydia trachomatis*. The combined detection of gonorrhea and *Chlamydia trachomatis* has been achieved on urethral discharge. Multiplex genital ulcer PCR can detect *Treponema pallidum, Haemophilus ducreyi* and *Herpes simplex virus* types 1 and 2 from genital sore exudate or tissue.[1]

TRANSPORT MEDIA FOR CULTURE

In general, transport must be as rapid as possible, avoiding excesses of temperature.[1-3] The ideal transportation temperature for Chlamydia (sucrose phosphate transport media in cryo vials) is 4°C, whereas ambient room temperature is recommended for *N. gonorrhoeae* (nutritive media containing carbon dioxide-Transgrow/Jembec or nonnutritive media-Stuarts/Amies). Plastic or metal shafts are better than cotton-tipped swabs on wooden sticks for obtaining the specimen for chlamydia and mycoplasma. For herpes, the specimen is to be placed in 1 ml of viral transport medium and stored at 4°C till inoculation into tissue culture media. For storage more than 48 hours, the sample may be frozen at -70°C. Whittington / Kupferberg medium is used as transport medium for *Trichomonas vaginalis*.

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