

Sexually Transmitted Diseases Practical

Microbiology 435's Teamwork
Reproductive Block



This document contains 3 cases two of them will show up in the exam

- Case 1: syphilis or herpes.
- Case 2: gonococcal urethritis or non-gonococcal urethritis.
- Case 3: bacterial vaginosis, candida vaginitis or chlamydia trachomatis.

Your answer for each case should be based on the organism characteristics that will be described in the exam so please make sure that you understand everything listed in this document. Remember: the cases' questions might change in the exam as always, but we tried to list all the possible questions.

كل الدعوات بالتوفيق والصلاح والبركات
لميس التميمي، فوزان العتيبي و زكي الوطنبان

Learning Objectives:

Name various **etiological agents** causing localized STD (sexually transmitted diseases)

Describe **the clinical presentations** of localized STD.

Discuss **the microbiological methods** used for Dx (diagnosis) of localized STD.

Outline **the management** of localized STD

-
- Important
 - Males notes
 - Females notes
 - Extra

Resources:

- 435 girls slides
- 435 boys slides
- 434 team work
- Dr.Alhazmi and Dr.Alsomali notes

We have systemic sexually transmitted diseases and localized STDs, localized STDs because signs and symptoms related to the site of inoculation (genital area) whereas the systemic STDs cause signs and symptoms not related to the genital area. Localized STDs are sub divided into two groups based on signs and symptoms. 1-localized STDs that are characterized by genital warts or ulcers, localized STDs that are characterized by discharge (urethral discharge or vaginal discharge), we have 3 cases to discuss these localized STDs.

Case 1

A 23-year-old alcoholic and drugs (cocaine) addict single male arrived from his trip to South East Asia four months ago. **He gave history of multiple sexual partners.** Two months ago, he developed ulcer (واحد من الطرق الممكنة للسؤال: يحددون خصائص هذه القرحة أو ممكن يجيبون) on his penis **which disappeared completely.** Full physical notes: rash on both his palms and his soles.



Note that: the presence of rash on palms and soles gives a clue of secondary syphilis; the ulcer was painless ulcer because it has not been noticed by the patient.

- What are the possible causes for his presentation (just ulcer) & How could you differentiate between them based on signs and symptoms of the patient?

Ulcer	Etiology	Ulcer characteristics	Lymphadenopathy (Bubo)	Systemic
Chancere maestro pizza 	Treponema Pallidum	 Dry, single painless and raised margin " deep indurated and clean base", it is the hard ulcer	Non tender, Inguinal	Depends on stage ulcer just in 1ry

Chancroid	Haemophilus Ducreyi	 Wet, painful multiple ulcers,	Inguinal tender	Present
Ulcerated Vesicles or (vesicles without ulcer)	Herpes Simplex Virus <u>2</u> Recurrent & more severe than HSV1	 Multiple shallow painful vesicles that may rupture to cause painful superficial ulcer	Occasionally present	In primary

- What are the investigations that you would like to order for him?
Explain how those investigations would help you?

In general the diagnosis of infectious diseases depends on 5 approaches: 1- microscopic 2- culture 3- detection of the organism antigen by immunofluorescence 4- detection of specific antibody against the organism antigen 5- molecular to detect the organism gene.

organism or causative agent	Microscopy	Culture	Direct Fluorescent Antibody (DFA)	Serology
Treponema Pallidum ¹	Dark Field microscopy; Motile Spirochetes ²	Not grown	 +	The test are divided into: 1- Screening: ³ RPR ⁴ & VDRL ⁵ 2- Confirmatory ⁶ : TPHA ⁷ MHA-TP ⁸ FTA.ABS ⁹
Haemophilu	Gram stain;	Selective	NA " not	NA "not applicable"

¹ We cannot stain it by gram stain or see it by light microscope. Why? Because it is too thin and below the resolution power of the light microscope. It can be seen by dark filed microscopy because it depends on the light reflection from the organism surface.

² the samples are taken either from the ulcer of from the lymph nodes

³ Screening tests may show us a false positive that means that some of the positive results are not syphilis but other diseases. These test are not specific because they use no treponomal antigen to detect the antibodies so positive by non- specific should be confirmed by the specific.

⁴ "Rapid Plasma Regain" test

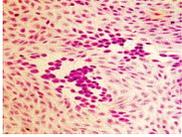
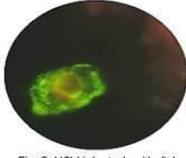
⁵ " venereal disease research laboratory" test

⁶ The confirmatory test are specific for the treponemal antigen, it is not important to perform all of them, depends on what do you have in the lab.

⁷ " Treponema pallidum hemagglutination assay" test

⁸ " microhemagglutination assay for Treponema pallidum antibodies" test

⁹ "fluorescent treponemal antibody absorption" test.

s Ducreyi	gm-ve small bacilli & pus cell	media and Needs x factor	applicable"	
Herpes Simplex Virus 2	Electron microscope -NA ¹⁰	Produce cytopathic effect ¹¹ in cell culture 	+  Fig. 3. HSV-infected epithelial cell from skin lesion (DFA)	IgM IgG

- **What is the most common method used to diagnose herpes?**

Direct immunofluorescence by detection of hsv 2 antigens "the presence of apple green fluorescence indicates positive results

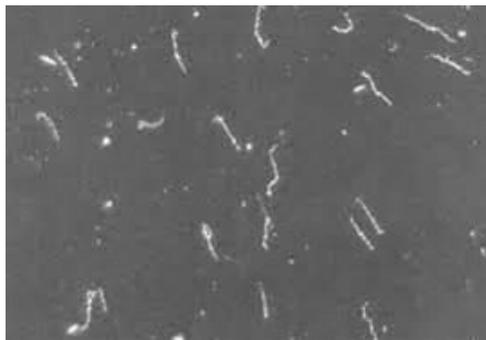
- **other method used to diagnose herpes?**

culture take 1 to 2 days

PCR can used most rapid sensitive method usually used in congenital herpes but can used in any herpes type ether congenital or not

- **Describe the organism under the microscope "darkfield**

microscopy"? in syphilis sample is taken from ulcer in 1ry or condyloma lata in 2ry



- Very thin and coiled spirochetes.

- **What is the likely diagnosis and the stage of the disease in this case?**

Secondary syphilis

- **What is the most common method used to diagnose syphilis?**

- **Serology**¹² " الإجابة يجب أن تكون مفصلة كما سبق طرحه في الجدول أعلاه "

¹⁰ Not applicable anymore because it is time consuming

¹¹ Cytopathic effect: change of the morphology of the infected cell culture line and the cell will be full of the inclusion bodies 1 to 2 day culture we use soap of vesicle we open head of it then take soap from inside then should put it in transport media

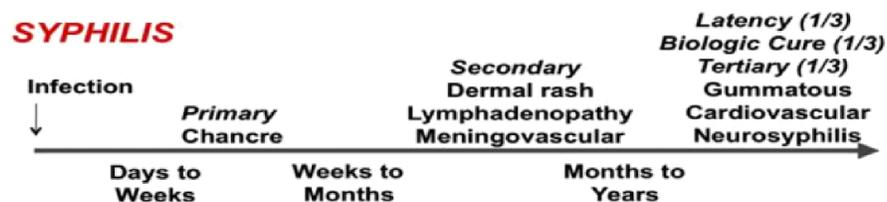
¹² مهم جداً جداً

- **What are the benefits of the non-specific serology tests RPR?**

- Screening and monitoring the patient's response of the treatment (they will be negative after effective treatment) in contrast of the confirmatory tests which will remain positive for life even after effective treatment.
- Example 1: negative for RPR, Positive for TPHA → the patient has acquired the syphilis infections and has been treated.
- Example 2: positive for RPR and positive for TPHA → he still has the syphilis infection.

- **What are the investigations that you would like to order for him? Explain how those investigations would help you? (Syphilis case)**

- 1- Microscopy: dark field microscopy. How? It will show motile spirochetes.
- 2- DFA how? Will show positive results.
- 3- **Serology how? We will perform screening methods first (RPR) and (VDRL) if positive results → do the confirmatory tests : TPHA ,MHA-TP and FTA.ABS also the screening tests (RPR) will help us to monitor the patient's response to the therapy.**



- **If the secondary syphilis not treated what are the possible consequences?**

- 1/3 latency¹³
- 1/3 biologic cure¹⁴
- 1/3 tertiary¹⁵ (gummatous –skin and bones, cardiovascular and neurosyphilis)
- neurosyphilis appear in tertiary except in HIV patient appear in 2ry syphilis

- **Briefly outline the management of this patient.**

- **Patient:**
 - **Benzathine penicillin IM---allergy Doxycycline**
 - Counseling and Education about the route of transmission and complications.
 - tested for other STD especially **HIV**
- **Look for the partner.**

¹³ Positive for screening and confirmatory tests.

¹⁴ No signs and symptoms negative for screening tests and positive only for confirmatory tests either because of treatment or spontaneous cure.

¹⁵ Noninfectious stage.

2ry Syphilis	
presentation	rash on palms and soles, history of ulcer and sexual relationship
diagnosis	Dark Field microscopy serology Screening by RPR & Confirmation by FTA.ABs or MHA-TP if sample was biopsy silver stain
treatment	Benzathine penicillin IM---allergy Doxycycline

Case 2

A 35-year-old married male presented to the emergency room complaining of dysuria for the last 24-hours and noted some "pus-like" drainage in his underwear and the tip of his penis.

- What is the most likely diagnosis?

Urethritis¹⁶

	Organisms	Urethritis
Gonococcal Urethritis 	Neisseria gonorrhoeae	Purulent discharge
Non-gonococcal urethritis 	Chlamydia trachomatis	Mucopurulent
	Others <ul style="list-style-type: none"> • Trichomonas vaginitis • Mycoplasma 	

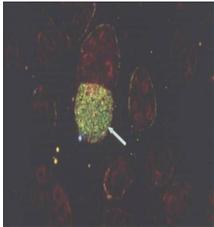
¹⁶if they do not add a picture or clues write just urethritis but more likely **in the exam they will add a picture or description then you should answer the questions based on your understanding for the schedule.**

- **What investigations do you like to order for him? Explain how those investigations would help you?**

if the gram stain shows pus cells with organisms → gonococcal urethritis.

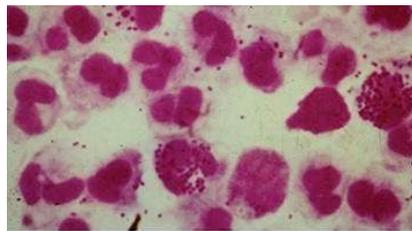
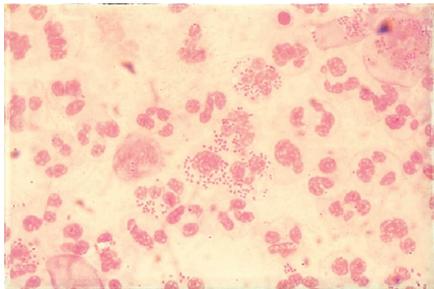
if the gram stain shows pus cells without organisms → non- gonococcal urethritis.

ممکن أجبیب لك التشیخیص فوق وأقول what do you expect in the gram stain

	Organisms	Smear/Culture	Immunological tests	molecular testing
Gonococcal Urethritis ¹⁷	Neisseria gonorrhoeae	Gram-ve diplococci & pus cell /Selective media		+ve (Gold Standard)
Non-gonococcal urethritis	Chlamydia trachomatis obligatorily intracellular organism can not be grown on cell culture so we use McCoy cell culture (not MacConkey)	Pus cell without organisms/McCoy Cell culture	 <ul style="list-style-type: none"> - DFA(positive for chlamydia) ,apple green fluorescence - ELISA - Rapid test 	+ve(Gold Standard) for screening

¹⁷ it is important to understand that the green apple fluorescence indicates ALWAYS positive result but the diagnosis depends on what antibody we have added. in this case we added chlamydia antibody.

	Others	Wet mount;	EIA	+ve
	- Trichomonas vaginalis	pus &TV/ Culture		
	- Mycoplasma	Pus cell / Special media culture	EIA	+ve



Source: Faud AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: <http://www.accessmedicine.com> Copyright © The McGraw-Hill Companies, Inc. All rights reserved.

- provided the gram stain for our patient's sample, can you describe it?
- gram negative diplococci with pus cells
- Base on the finding, what is the most likely diagnosis?
- gonococcal urethritis

طبيب إذا ما كان فيه الاورقنزم --- chlamydia trachomatis urethritis¹⁸

what is the selective agar for Neisseria Gonorrhoea ? thayer Martin “ a chocolate agar”



- **Neisseria gonorrhoeae will grow as non hemolytic colonies on chocolate agar**
- **Briefly outline the management of this patient?**

-Ceftriaxone¹⁹

if the infections is combination (neisseria gonorrhoea and chlamydia trachomatis)--->

tetracycline , erythromycin or azithromycin

-Screen for other STDs.

-Partner should be treated as well.

	Gonococcal Urethritis	Non-gonococcal urethritis
causative agent	Neisseria gonorrhoeae	Chlamydia trachomatis
presentation is discharge (Urethritis, cervicitis)	Purulent whitish yellowish some time with granules	Mucopurulent
sample	squeeze penis take discharge if no come take from inside women use speculum cervical sample	
first we do Gram stain	kidney shape intracellular gram - diplococci within pus cell	pus cell without any organism
other test to diagnose	culture on thayer-martin agar or chocolate agar oxidase + Urine suap PCR glucose fermentation	McCoy cell culture (rarely done) oxidase + Urine suap PCR
treatment	ceftriaxone cefixime also treat partner	doxycycline or clarithromycin
discharge come again after treatment	may co-infection with C.T OR resistance	

¹⁹ why chlamydia is resistant to ceftriaxone? because it does not have cell wall nor peptidoglycan ;)

Case 3

A 24-year-old female noted vaginal itching and irritation with a discharge. Previously, she developed a yeast infection that was treated with over-the-counter medications and resolved. Thinking that this was recurrence, she again self-treated. This time, however, the symptoms did not resolve. She presented to her family physician for management. On examination there is a bad odor along with a frothy discharge and strawberry cervix. Swab of the secretions was taken in order to perform tests.



"strawberry cervix" is associated with trichomonas.

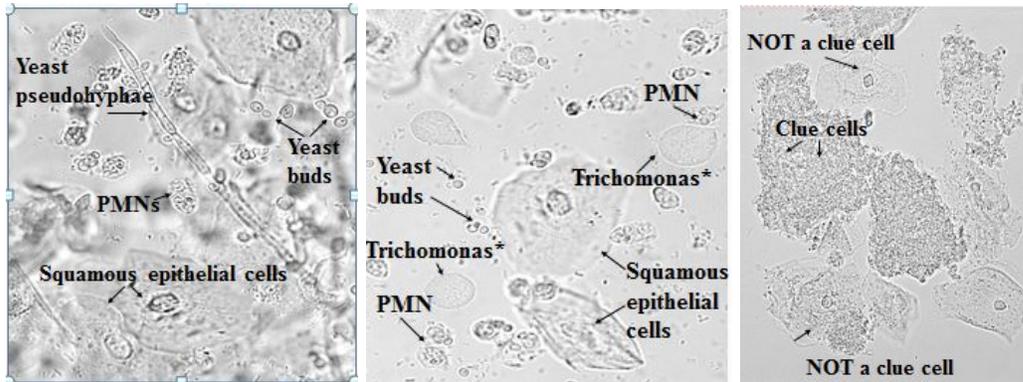
- **What are the possible causes for her presentation?**
 - **Bacterial vaginosis**²⁰
 - **Candida vaginitis**
 - **Trichomoniasis**²¹
 - Allergic vaginitis
 - Chlamydia trachomatis *mostly vaginal discharge due to cervicitis*
 - Neisseria gonorrhoeae *mostly vaginal discharge due to cervicitis*
- **What investigations would you like to order for her? Explain how those investigations would help you?**
 - **Ph how? if it is more than 4.5 indicates bacterial vaginosis and trichomonas vaginalis. but if below 4.5 indicates candida vaginalis**
 - **Whiff test how? if it produces bad smell → indicates positive results (from anaerobic organisms *KOH will alkalize the amines produced from anaerobic organisms*) positive with bacterial vaginosis and trichomonas**
 - **microscopic examination either by gram stain or Wet prep**²² **how?** clue cells → bacterial vaginosis, pseudohyphae → candida vaginalis. moving vagella →

²⁰ mainly by gardnerella vaginitis due to alteration of Ph and the lactobacilli may present in a low number of absent

²¹ caused by trichomonis vaginalis. it is a protozoal parasite.

²² it is the normal saline with the vaginal discharge

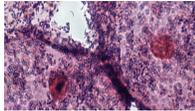
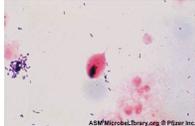
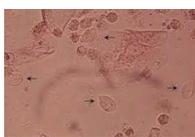
trichomonis vaginalis.



“candidia vaginlais” “trichomonis vaginilis” “bacterial vaginosis”

(the picture and you describe what you see) note that the squamous epithelial cells are from the normal vaginal wall. hint: the trachelomonas vallagella are pear- like shape

- What investigations would you like to order for her? Explain how those investigations would help you?

	PH	Whiff test	Gram stain / Wet prep	Culture	Immunologic/ molecular test
Bacterial vaginosis	>4.5	+++	Clue cells 	Not helpful	DNA Probe (gardnerella vaginalis)
Candida vaginitis	<4.5	-	Yeast and pseudohyphae 	Candida	DNA Probe
Trichomonas vaginitis	>4.5	+/-	 	Motile Trophozoites	EIA DNA Probe

²³ note that there is gram negative bacilli at the background these are NOT the pseudo hypha يا جماعة these are the normal flora

			 <p>Trichomonas flagella moving</p> 		
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- **Base on the finding, what is the most likely diagnosis?**
trichomonas vaginitis

- **Briefly outline the management this case?**

Management:

-Metronidazole

-Husband should be treated

- screen the partner and screening for other sexually transmitted diseases.

-Patient Counselling and Education

	causative agent	presentation	diagnosis	treatment
vaginosis (Bacterial)	Gardnerella vaginalis or Mobiluncus	discharge greyish whitesh fishy odor (usually after inter course)	1-gram stain clue cells, gram -, gram variable bacilli & ↓ gram + 2-3out 4 features	Metronidazole
vginanitis (fungal or parasite)	fungal (Candida albicans)	discharge white thich small amount itching & satellite lesions	1- wet prep (budding yeast + pseudohyphae if no pseudohyphae may Glabrata) 2-culture	fluconazole
	parasite (trachomonis vaginalis)	discharge frothy greenish	1-wet prep organism has flagella 2-culture	Metronidazole

- **What are the organisms that you would like to screen for in any patient presented with any STD?**

- **HIV**
- Herpes
- syphilis
- the twins: neisseria gonorrhoeae and chlamydia trachomatis. TB and brucella
- hepatitis B & hepatitis C²⁴.

²⁴ if a drug abuser but usually we check it bc it is tansmitted sexally