A New Partner

PATH 417
Case 2
The Microbiology Lab Questions
Pawan Dhaliwal
(Student #7)

The Case

21-year-old Naser G. recently hooked up with a new sexual partner. This morning he noticed a burning pain in his penis during urination followed by a greenish discharge. He immediately goes to the student health clinic. The clinic doctor asks Naser about his recent sexual history and he recounts how he had unprotected sexual intercourse with a new partner about one week ago. The new partner claimed that she did not have any sexually transmitted infections. The doctor asks Naser to provide a urine sample to send to the Microbiology Laboratory. The doctor prescribes antibiotics for him and counsels him on safe sex practices and on the importance of encouraging his new partner to come in for testing too.

Question 1: Narrowing in on the bacterial causes, what are the most common bacterial pathogens associated with this infectious scenario?

Based on information provided in the case, it is evident that Naser G. has a sexually transmitted infection (STI), causing urethritis (inflammation of the urethra). These are infections commonly spread by vaginal intercourse, anal and oral sex, and may be caused by bacteria, viruses and parasites.[1] Most STIs initially do not cause symptoms, which may be the reason why Naser's new partner claimed she did not have any STIs.[2] However, in this specific case, Naser is experiencing symptoms which have developed about a week after he had sex with a new partner. By doing a quick search of his symptoms, the following 3 most common bacterial pathogens associated with this infectious scenario are:

1. *Neisseria gonorrhoeae*
2. *Chlamydia trachomatis*
3. *Ureaplasma*

Neisseria gonorrhoeae (N. gonorrhoeae)[3]

- The causative agent of gonorrhoea
- Gram-negative, diplococcic bacteria
- Transmitted via sexual intercourse (oral, anal, vaginal)
- Can spread to eyes and throat
- Can be transferred to newborn infant from vaginal canal during birth
- Can lead to:
    - Uncomplicated gonococcal infection (UGI): bacteria colonize mucosa of lower anogential tract
    - Complicated gonococcal infection (CGI): bacteria ascend to normally sterile upper genital tract; uncommon but can result from untreated or poorly treated UGI
    - Disseminated gonococcal infection (DGI): bacteria enter blood system; rare
    - Blindness in neonates
- Treated with antibiotics
- Causes repeated infections due to:
    - Evasion of host immune response
    - Development of resistance to therapeutic agents
- Found in "core groups" of individuals:" female sex workers, ethnic minorities, and men who have sex with men (MSM)
- Symptoms appear 2 to 14 days after infection, and include:
    - For both men and women:
        - Pain or burning sensation during urination
        - Green, yellow or white discharge
    - For men:
        - Inflammation of foreskin
        - Pain or tenderness in testicles (rare)

Chlamydia trachomatis (C. trachomatis)[4]

- The causative agent of chlamydia
- Most common bacterial cause of STI's in the United States
- Transmitted via sexual intercourse (anal, oral, vaginal)
- Can spread to eyes and throat, and can be passed to infant during vaginal birth
- Obligate intracellular pathogen
- Gram-negative, ovoid, non-motile bacteria
- Has 18 serovars, which cause different complications:

    - A – C (including sub-serovars): cause trachoma which can lead to blindness
    - D – K:
        - In women: cervicitis, urethral infectious, pelvic inflammatory disease (PID), ectopic pregnancy, perihepatitis
        - In men: urethritis, epididymitis, proctitis, Reiter's syndrome, burning sensation when urinating, discharge which may be white, cloudy, or watery
        - Neonates: conjunctivitis, pneumonia
    - L1 – L3: lymphogranuloma venereum (LGV)
- Inhibits apoptosis of host cell during its replication cycle
- Treated with antibiotics
- Most clinical manifestations of *C. trachomatis* genital infections are "silent"
    - ~25 – 50% of all male chlamydia and ~70% of female chlamydia cases go completely unnoticed
    - If symptoms do occur, they appear within 1 to 3 weeks of initial exposure

Ureaplasma[5]

- Small, free-living bacteria found in normal flora of urogenital tract
- Typically sexually transmitted
- Unlike other bacteria, they lack a cell wall
- Round or coccobacillary in shape
- Symptoms include discharge, urinary frequency and urgency, and pain in men and women
- Fastidious organisms and thus difficult to culture
- Treated with tetracycline or erythromycins

In terms of prevalence, more infectious with *N. gonorrhoeae* and/or *C. trachomatis* are reported than infections due to *Ureaplasma*.[6] As such, I exclude *Ureaplasma* as the most likely pathogen causing Naser's infection.

N. gonorrhoeae and *C. trachomatis* cause infections which share many signs and symptoms with each other.[7] As such, both infections are indistinguishable from one another and laboratory testing is done to detect the presence of both organisms. However, for the sake of this question, I believe *N. gonorrhoeae* is the most likely pathogen causing Naser's infection. This is because chlamydial infections are known as "silent" infections as majority of patients do not present with symptoms. Furthermore, penile discharge due to chlamydia is white, cloudy or watery, whereas penile discharge in gonorrhoeal infection can be green. The latter is what Naser is experiencing, which further leads to me to believe *N. gonorrhoea* is the most likely pathogen in this infectious scenario.

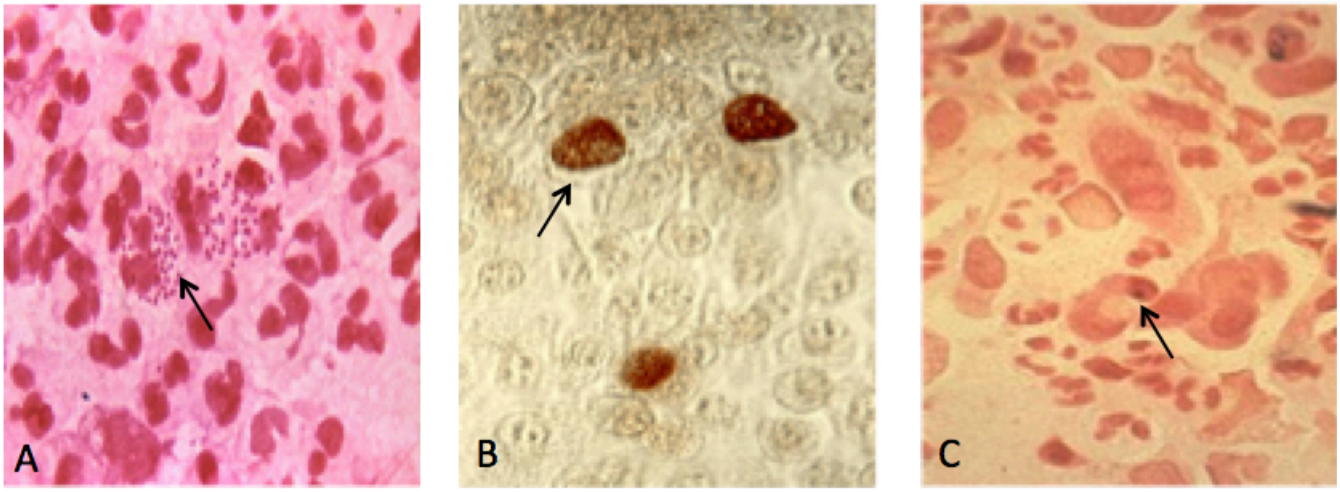

Figure 1: Microscope images of possible causative agents, indicated by arrows, of Naser's infection. (A) *N. gonorrhoeae* (B) *C. trachomatis* (C) *Ureaplasma*

Question 2: What samples are taken for lab testing? How important is the Microbiology Laboratory in the diagnosis of this disease?

Depending on the gender of the patient, the stage of the infection and site of infection, the samples taken for lab testing will differ. In general, samples taken for lab testing for STI's can be:

1. Swabs
2. Urine
3. Blood

Details important for collection, transport and/or storage:

Swabs

Swabs may be used to collect a sample of secretion or discharge from the infected site such as the urethra, penis, anus, throat, cervix or vagina. Care must be taken to avoid contamination with normal flora. Furthermore, excess mucus must be removed when swabbing urogenital areas.[8] Women should not douche or use vaginal creams prior to sample collection.[9] After collection, swabs are used to inoculate culture media or transport media suitable for testing.

N. gonorrhoeae[10]

Samples can be collected with either cotton-tipped swabs or with plastic disposable loops. To collect, wear suitable gloves and remove the swab from its tube. Swabs may either be wet or dry swabs, however for collection of wet surfaces, use dry swabs. After swabbing the test surface by rolling the swab lightly back and forth, insert the swab back in the tube and firmly close the cap and label the sample.

After collection, many clinics will inoculate the specimen directly onto gonococcal isolation medium (see question 3 below) and then incubate immediately until the sample can be transported to the lab.

In regards to transport, if the lab is far away from the clinic, the swab is instead place in transport medium such as Amie's or Stuart's and refrigerated prior to transfer. In this case, the swab should be used to inoculate gonococcal culture medium within 48 hours to achieve acceptable retrieval of organism.

C. trachomatis[11]

Care must be taken when collecting samples for chlamydial infections. As a general rule, swabs with wooden shafts should not be used because wood is toxic to *C. trachomatis*. For women, a cytobrush can be used to collect endocervical specimens, as they appear to collect more cells than swabs.

Once the sample is collected (using the same procedure described above), the sample is either: (1) cultured or (2) investigated using cytology. To culture, the specimen is forwarded to the lab in a special chlamydial transport medium such as 2SP (0.2 M sucrose-phosphate transport medium containing antibiotics). Broad-spectrum antibiotics must not be used in the transport media because they have activity against *C. trachomatis*.

For cytological investigation, smears are prepared (see question 3 below).

If storage is required, samples should be frozen at -70 °C.

Ureaplasma[12]

As with chlamydial infections, swabs with wooden shafts should not be used for collection because they inhibit growth of *Ureaplasma* organisms. After collection using the procedure detailed above, specimens should be

transported to the lab in a specialized liquid transport system, such as 10-B broth, or using the method described for *C. trachomatis* above. The specimens should be frozen until laboratory testing is available.

Urine[13]

For all 3 candidate bacterial pathogens mentioned above, first-void urine (FVU) specimens from men and women can be used for lab testing. FVU is the first 10 ml to 30 ml of urine, and specimens should be obtained after the patient has waited 1 to 2 hours from last urinating. Women must take care not to douche or use vaginal creams. Only the first 10 ml to 30 ml of urine is required as larger volumes of urine may result in specimen dilution and consequently a reduction in test sensitivity. Once 10 ml to 30 ml of urine have been collected in a sterile container, 2 ml are transferred into urine specimen transport tube using disposable pipette. The specimen should then be sealed in a plastic bag, with patient information, and maintained at 2- 8 °C during transport. If lab testing is not immediately available, the specimen must be refrigerated immediately. Testing should be done within 24 hours of refrigeration for accurate results.

It is important to note that urine is not an optimal specimen for detection of gonorrhoeal or chlamydial infections in women because urine does not travel through the infection site, which is usually the cervix in women.

In this case, however, the patient is a male and therefore urine is an appropriate specimen as it travels through the infection site, the urethra.

Blood/Serum[14]

Blood or serum specimens are not recommended samples for diagnosing infections by the candidate pathogens. However, for gonorrhoeal infections, blood specimens may be used in cases of DGI. For chlamydial infections, serum specimens are not recommended because the host immune responses against the pathogen are often short lived or are due to past infections. For *Ureaplasma*, serum samples may be used as they contain IgG antibodies specific for these microbes.

Importance of Microbiology Laboratory

The Microbiology Laboratory is paramount in a number of matters. Firstly, it is important in the diagnosis because lab tests will help to conclusively identify the causative agent(s) for the bacterial infection. Without knowing the causative agents, it is impossible to know their antibiotic susceptibilities and therefore it is difficult to prescribe appropriate medication. Incorrect prescription of antibiotics may not effectively eliminate the infection, and may contribute to the emergence of antibiotic resistance strains.

Secondly, the Microbiology Laboratory is important to prevent further transmission of the infection and to provide effective therapy to the patient. If the patient is unaware of lab test results, or is not screened in the lab, they may continue to engage in unsafe sex practices and transmit the infection onto their partners. More so, it is important that the patient know the pathogenic cause of their infection to prevent future complications. For example, chlamydial infectious may result in pelvic inflammatory disease (PID) if left untreated in women.[15] In men, untreated gonorrhoeal infections may result in epididymitis.[16]

Question 3: Explain the tests that will be performed on the sample in order to detect all of the bacterial pathogens that may be causing this disease.

The following tests can be performed on collected samples to detect all of the bacterial pathogens that may be causing this disease, except where noted. As mentioned before, the symptoms of gonorrhoeal and chlamydial infections are indistinguishable. As such, dual laboratory testing is done to detect the presence of both pathogens.

Laboratory Tests

1. Culture tests
    a. Gram stain
    b. Biochemical Tests
    c. Antibiotic Susceptibility Tests
2. Non-culture tests
    a. Enzyme Immunoassays (EIAs)
    b. Direct Fluorescent Antibody (DFA) and Staining
    c. Molecular Tests
        - Nucleic Acid Hybridization (NAH) Tests
        - Nucleic Acid Genetic Transformation Tests
        - Nucleic Acid Amplification Tests (NAAT)
    d. Serology Tests

Point-of-care Tests

1. Leukocyte Esterase Test (LET)

Laboratory-Based Tests

1. Culture Tests

For culture tests, sample transport must ensure organisms remain viable.[17]

N. gonorrhoeae[18]

Samples are streaked on a selective medium if specimens are from non-sterile sites, or on non-selective (e.g. chocolate agar) medium if specimens are from sterile sites. Selective gonococcal isolation medium consists of Thayer-Martin or Martin-Lewis plates, which have a GC agar base enriched with: peptones; starch; iron; growth factors; antimicrobial and antifungal agents to prevent overgrowth of normal flora for rectal and endocervical smears; and antibiotics to inhibit Gram-positive and other Gram-negative organisms. Conditions required for growth of *N. gonorrhoeae* include warm, moist conditions: high humidity (>90%) with 5 – 7% carbon dioxide at 36 °C for a minimum of 48 hours.

C. trachomatis[19]

As mentioned previously, *C. trachomatis* is an obligate intracellular pathogen. As such, cell culture for this pathogen involves inoculating a confluent monolayer of susceptible cells. Once retrieved at the laboratory, specimens should be refrigerated or frozen at -70 °C if they cannot be processed within 24 hours after collected. To culture, clinical specimens should be inoculated onto cycloheximide-treated monolayer cultures of McCoy cells or other appropriate cells. Inoculation involves staining the glycogen of infected cells with iodine, followed by centrifugation of cell homogenates onto the cell monolayer followed by incubation for 48 to 72 hours.

Ureaplasma[20]

These bacteria may be plated on A7 agar plates, which allow for the culture, semi-quantitative enumeration and morphological identification of *Ureaplasma*.

One advantage of culture tests is that they allow for further specimen analysis by means of Gram stain, biochemical tests and antibiotic susceptibility testing. Isolates are chosen from culture plates for these additional tests.

A) Gram stain

N. gonorrhoeae

A presumptive identification of *N. gonorrhoeae* isolates can be made with a Gram stain, which is done by creating smears on glass slides. The Gram stain separates bacteria into 2 groups on the basis of cell wall composition: 1) Gram-positive bacteria and 2) Gram-negative bacteria.[21] Figure 2 shows the process of the Gram stain.

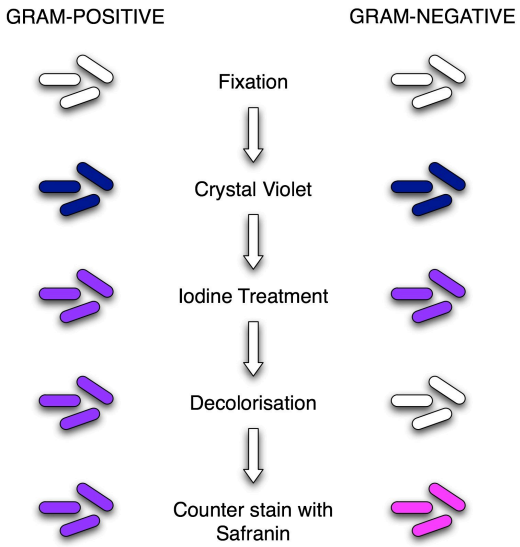

Figure 2: Gram stain process

Gram staining differences can be attributed to the differences in cell wall composition of Gram-positive and Gram-negative bacteria. Gram-positive bacteria contain a thick layer of peptidoglycan (PG) in their cell wall with numerous teichoic acid cross-linking. This thick cell wall retains the crystal violet stain even after the decolourization stage. Gram-negative bacteria, however, have a thin layer of PG in their cell wall covered by an outer membrane. In the decolourization step, alcohol degrades the outer membrane and the thin layers of PG in the cell wall are unable to retain the crystal violet stain. Gram-negative bacteria appear red/pink due to retention of a counterstain.

Once stained, the slides can be viewed under a microscope and the lab technician can note morphological characteristics.

C. trachomatis

Gram staining is typically not done because *C. trachomatis* is an intracellular pathogen and therefore difficult to stain. However, *C. trachomatis* is classified as a Gram-negative organism as it most closely resembles other Gram-negative organisms when stained with the Gram stain.[22]

Ureaplasma

Gram staining cannot be used to visualize these organisms since they lack a cell wall.[23]

B) Biochemical Tests

Only for *N. gonorrhoeae*

An oxidase test detects the presence of cytochrome c oxidase, and can also be used alongside a Gram stain to make a presumptive diagnosis for gonorrhoea.[24] Cytochromes are iron - containing hemoproteins and in aerobic respiration, they transfer electrons to oxygen to form water.

If cytochrome oxidase is present in the sample, it will oxidize the reagent tetramethylphenylendiamine, which is used as an artificial electron acceptor, to a purple or dark blue end product known as indophenol. If the enzyme is not present, the reagent remains colorless.

Other biochemical tests detect the presence of preformed enzymes which utilize carbohydrates. One such test for *N. gonorrhoeae* is the QuadFERM+ Test.[25] This test is comprised of a plastic strip composed of a cupule containing carbohydrate-free medium control and cupules containing carbohydrate substrates to detect acid production from glucose, maltose, lactose and sucrose. QuadFERM+ also includes DNase and beta-lactamase tests. Organisms which produce acid from carbohydrates will produce enough acid to exceed the buffering capacity of phenol red indicator, which will change color from red (alkaline) to yellow (acid).

C) Antibiotic Susceptibility Testing

N. gonorrhoeae, Ureaplasma

In this test, bacteria are inoculated in rich media broth and incubated overnight (Step 1 in Figure 3).[26] After overnight incubation, the rich media broth is added to broth solutions containing differing concentrations of antibiotics and incubated overnight (step 2). After incubation, cultures are checked for growth and the minimum inhibitory concentration (MIC) is noted (step 3). The MIC is the concentration of antibiotic that prevents visible growth of bacteria.[27]

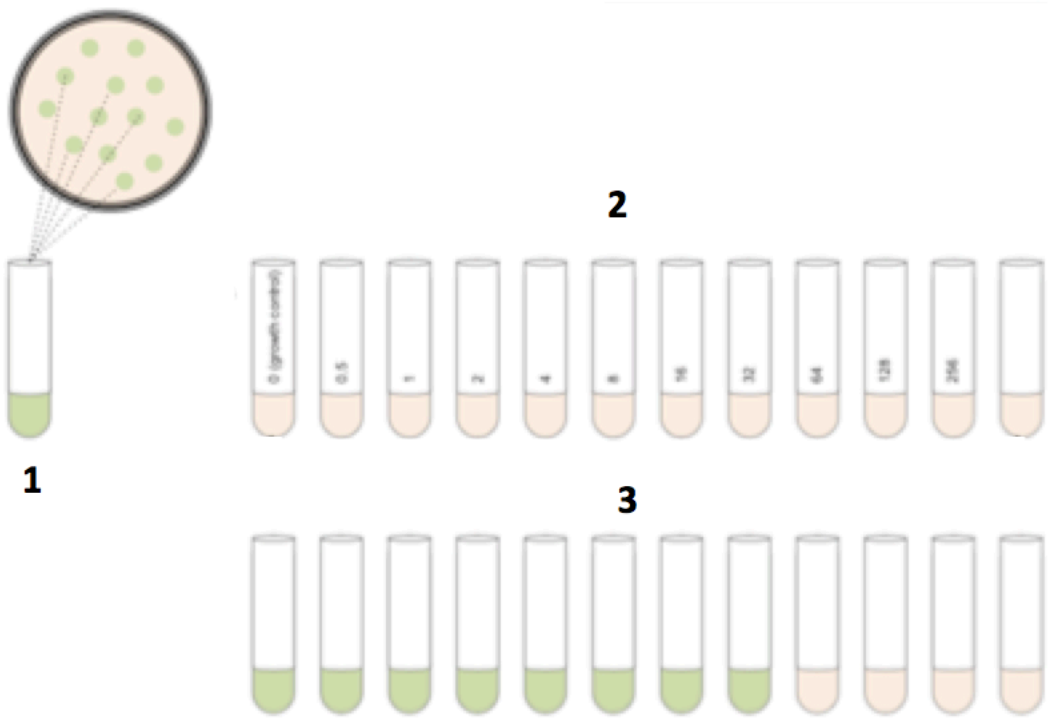

Figure 3: Antibiotic susceptibility testing using broth dilution method

C. trachomatis

Antimicrobial susceptibility testing is typically performed in cell culture with increasing concentrations of antibiotics.[28] Drug efficacy is then determined by staining cells with fluorescently labeled anti-chlamydial antibodies and microscopically enumerating the intracellular chlamydial inclusions.

2. Non-culture Tests

A) Enzyme Immnoassays (EIA)[29]

N. gonorrhoeae

There are no EIA tests available for *N. gonorrhoeae* due to high performance costs.

C. trachomatis

In EIA tests, antigens, such as chlamydial lipopolysaccharide (LPS), present in chlamydial elementary bodies, are detected with a monoclonal or polyclonal antibody that has been labelled with an enzyme.

This principle behind this test is detailed in Figure 4.

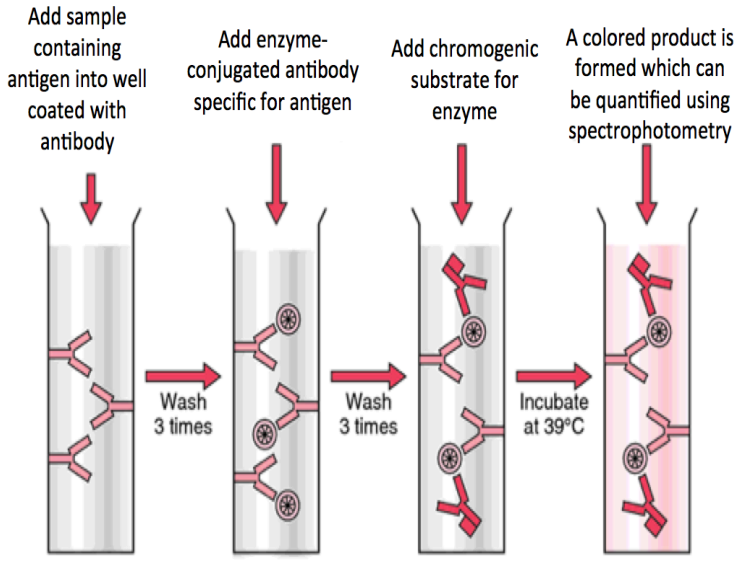

Figure 4: Principle of Enzyme Immunoassays

B) Direct Fluorescent Antibody (DFA) and Staining[30]

Neisseria gonorrhoeae, C. trachomatis

Antibodies tagged with fluorescent molecules may bind to L-antigen of *N. gonorrhoeae*, or to chlamydial antigens such as lipopolysaccharide (LPS) or the major outer membrane protein (MOMP) molecule. Identification is done via fluorescence microscopy. This procedure is detailed in Figure 5.

Instead of using fluorescein-labeled antibodies for visualization, stains may also be used visualize *C. trachomatis*. These stains will bind to chlamydial inclusions and therefore impart color to them. Such stains include Giemsa, Haematoxylin & Eosin (H&E), and Papanicolaou. For staining, a smear is prepared and then is air-dried, fixed with absolute methanol for at least 5 minutes and then dried again. It is then covered the desired stain for at least 1 hour. The slide is rapidly rinsed in 95% ethanol to remove excess dye and then dried and examined microscopically.

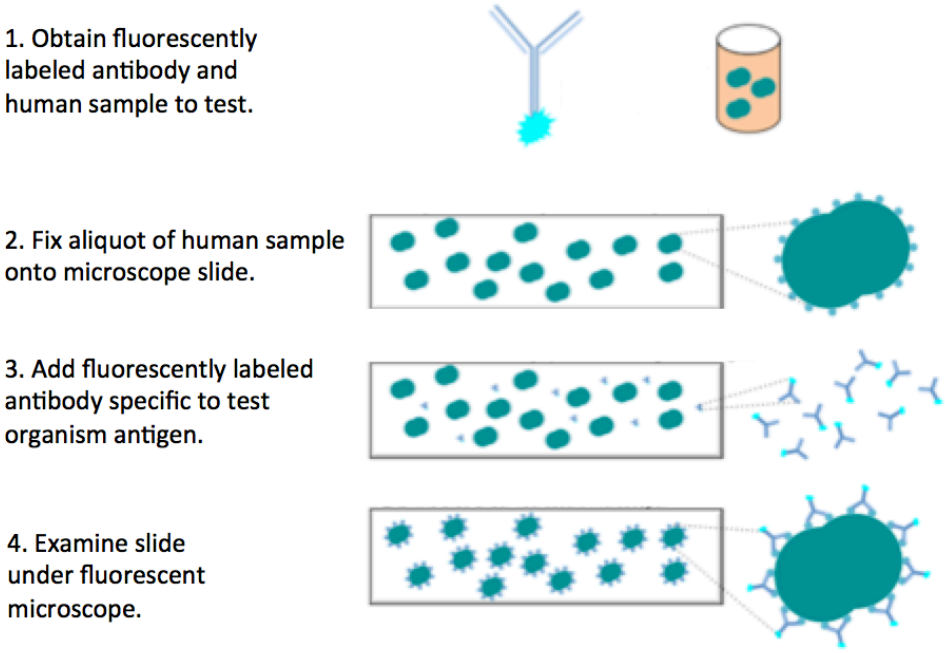

Figure 5: Direct Fluoresent Antibody Principle

C) Molecular Tests[31]

These tests require extraction and purification of genetic material. Extraction involves: cell lysis or cell disruption via chemical and physical methods to expose the genetic material within; removal of membrane lipids via detergents or surfactants; removal of proteins by proteases; removal of RNA with RNases or of DNA with DNases. Purification usually involves ethanol precipitation to remove detergents, proteins, salts and reagents used during cell lysis step.

- Nucleic Acid Hybridization (Nucleic Acid Probe) Tests

  In these tests, a labeled DNA or RNA probe (Figure 6) that is complementary to a specific sequence of *C. trachomatis*, *N. gonorrhoeae*, or *Ureplasma* genetic material hybridizes with any complementary genetic material (DNA or RNA) that is present in the specimen, resulting in fluorescence detection. Examples of such tests include the Gen-Probe hybridization assays for chlamydial and gonorrhoeal infections (Figure 7).

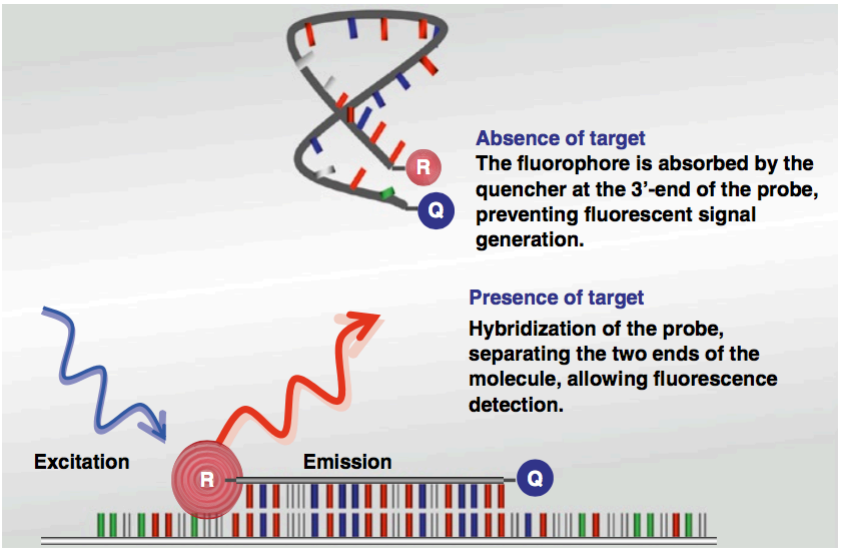

Figure 6: Probe Design in Nucleic Acid Hybridization and Nucleic Acid Amplification Tests

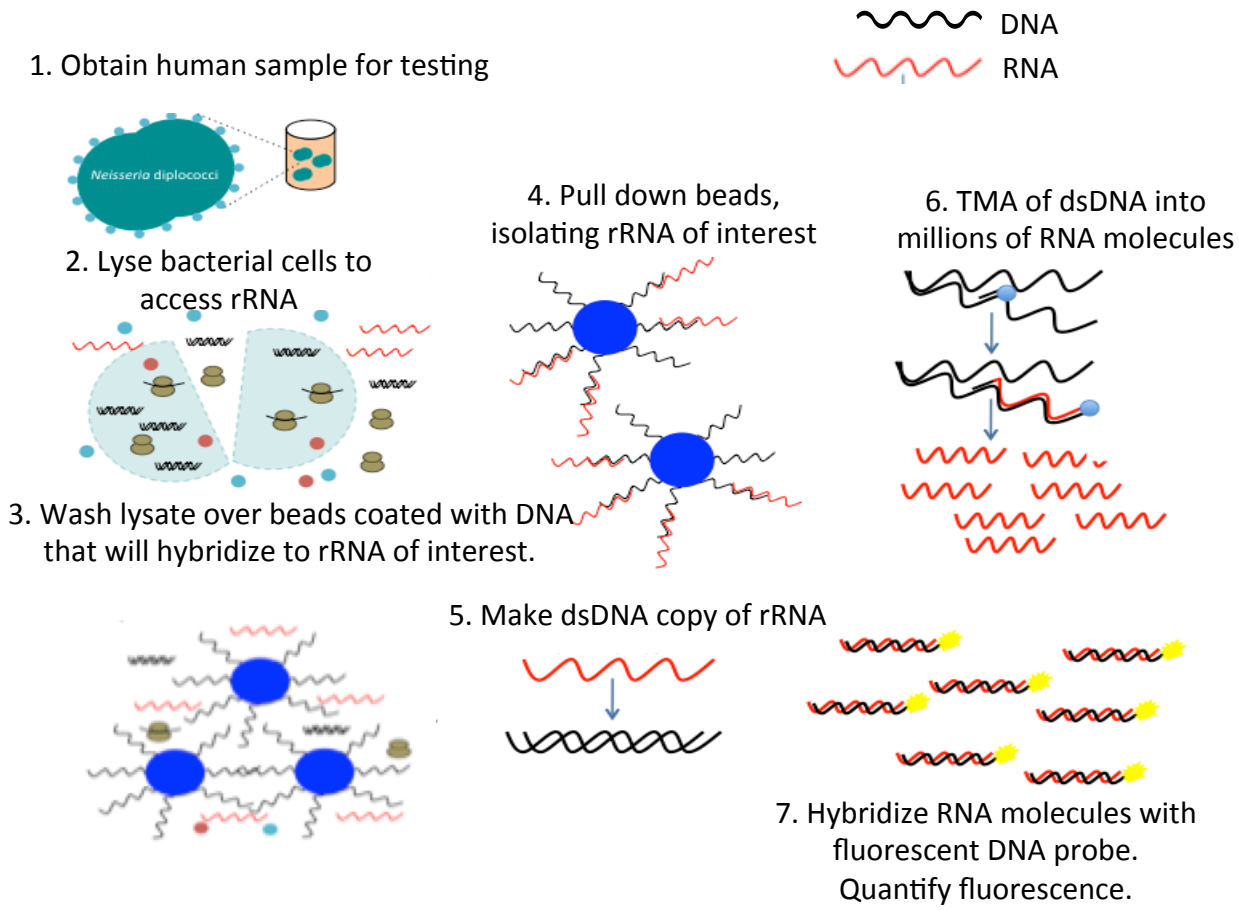

Figure 7: In the Gen-Probe assay, a labelled DNA probe that is complementary to a specific sequence of *C. trachomatis* or *N. gonorrhoeae* rRNA hybridizes with any complementary rRNA that is present in the specimen.

- Nucleic Acid Genetic Transformation Tests

  For *N. gonorrhoeae*

  A gonococcal mutant available from a commercial test kit acquires the ability to grow when transformed by DNA extracted from patient specimen containing *N. gonorrhoeae*.

- Nucleic Acid Amplification Tests (NAATs)

  The NAAT method detects specific target sequences of genetic material (DNA or RNA) of the infection-causing bacteria by amplifying the genetic material so a detection system can identify the presence of the bacteria. Amplification may be achieved by PCR, strand displacement amplification (SDA), transcription-media amplification (TMA), ligase chain reaction (LCR) or real-time PCR depending on the commercial test (Table 1).

Table 1: Amplification strategies used by commercial tests and the microbial target sequences they amplify.

Commercial Test	Amplification Strategy	Target Sequences	
		N. gonorrhoeae	***C. trachomatis***
Roche Amplicor®	PCR	201 base pair sequence within cytosine methyltransferase gene	DNA sequences in the cryptic plasmid that is found in >99% of strains of *C. trachomatis*
Abbot LCx®	LCR	48 base-pair sequence in Opa genes	
Becton Dickinson BDPProbeTec™ ET	SDA	DNA sequence within multicopy pilin gene-inverting protein homologue	
Gen-Probe APTIMA™	TMA	16S rRNA	23S rRNA

D) Serology Tests[32]

C. trachomatis

Serology tests are not useful for screening *C. trachomatis* infections. This is in part due to the long-lasting antibodies produced during previous chlamydial infections which cannot be distinguished from antibodies produced in current infections.

Ureplasma

Blood samples can be subjected to agglutination tests, in which antibodies and their specific antigens are mixed.[33]

Point-of-Care Tests

These are single tests performed while a patient awaits results, and can be performed within 30 minutes. These rapid, or stat tests, are useful in making decisions regarding additional testing or treatment when the patient first presents to the clinic.

1. Leukocyte Esterase Test (LET)[34]

This test can only be used for male patients, as urine does not pass through the infection site in women.

Although many point-of-care tests are available, one common test is the LET which is a dipstick test that is applied to urine specimens to check for urinary tract inflammation and the presence of white blood cells in urine. The LET detects esterase, an enzyme released by white blood cells. If esterase is present, a color change will occur on the dipstick that is then compared against a chromatic scale provided by the manufacturer.

Question 4: For each potential pathogen, what are the expected results from these tests?

Laboratory Based Tests

1. Culture Test Results

N. gonorrhoeae. *C. trachomatis* and *Ureasplasma*

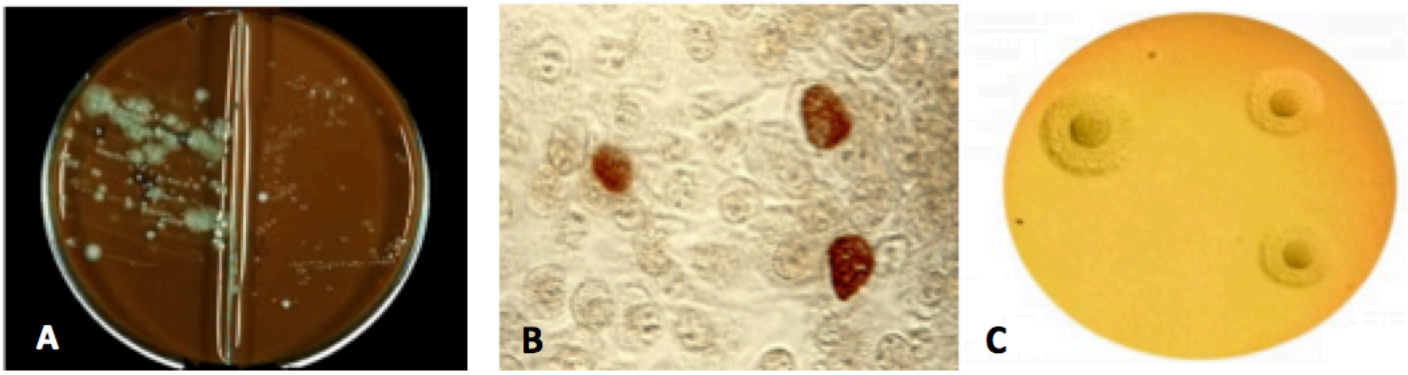

Figure 8: Culture test results. (A) *Neisseria gonorrhoeae* (B) *Chlamydia trachomatis* (C) *Ureaplasma*

In Figure 8A growth of gonococci is visible. The left side of the plate contains chocolate agar (non-selective medium) whereas the right side of the plate contains chocolate agar and antibiotics to inhibit growth of normal flora.[35]

Growth of characteristic intracytoplasmic inclusion bodies is seen in Figure 8B. The inclusion bodies will contain reticulate bodies unique to chlamydial infections.[36]

Cellular morphology of *Ureaplasma* (Figure 8C) will be noted as round or coccobacillary and colonies are 330 nm in diameter. Cells are bounded by a plasma membrane only. Colonial morphology will present with small colonies, 15 to 60 μm in diameter.[37]

A) Gram stain

N. gonorrhoea and *C. trachomatis* are classified as Gram-negative organisms since they appear red/pink after staining.[38]

Lack of Gram stain results for *Ureaplasma* can be attributed to the lack of a cell wall in these organisms.[39]

B) Biochemical Tests

Table 2 shows the expected results of various tests for *N. gonorrhoeae*.

Table 2: Biochemical test results for *Neisseria gonorrhoeae*. (G: glucose; M: mannose; F: fructose; L: lactose; S: sucrose)

Test	**Carbohydrate Utilization**					**DNase**	**Oxidase**	**Nitrate Reduction**
	G	**M**	**F**	**L**	**S**			
Result	+	-	-	-	-	-	+	-

In terms of carbohydrate utilization, *N. gonorrhoeae* only uses glucose (G). All other carbohydrate tests, including mannose (M), fructose (F), lactose (L) and sucrose (S) are negative (Table 2 and Figure 9).[40] Other biochemical tests, including presence of DNase or beta-lactamase, are negative (Figure 9). The oxidase test (Figure 10) is

positive (indicated by colorless reagent turning dark blue/purple) and is used in the presumptive diagnosis of *N. gonorrhoeae*.[41]

Figure 9: Biochemical test results for *Neisseria gonorrhoeae*. (G: glucose; M: mannose; F: fructose; L: lactose; S: sucrose

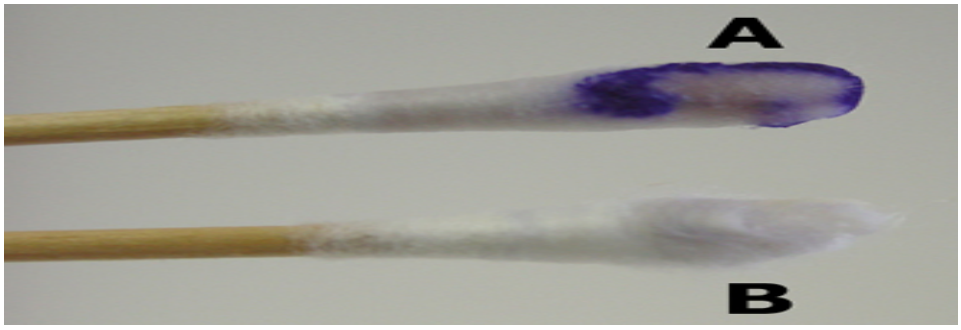

Figure 10: A positive oxidase test result is expected for *Neisseria gonorrhoeae (*A*)*. A negative oxidase test result is shown in (B).

C) Antibiotic Susceptibility Testing Results

As resistance to antibiotics is a dynamic phenomenon, the results in Table 3 provide only a partial list of antibiotics to which the candidate pathogens are susceptible or resistant to. [42, 43, 44]

Table 3: Antibiotic Susceptibilities of *N. gonorrhoeae, C. trachomatis* and *Ureaplasma*

Pathogen	**Resistant to:**	**Susceptible to:**
N. gonorrhoeae	Penicillins Tetracyclines Macrolides Spectinomycin Quinolones	Third-generation cephalosporins
C. trachomatis	Drug resistance is rare in these organisms	Azithromycin Doxycycline Erythromycin Ofloxacin
Ureaplasma	Beta-lactams (these organisms lack a cell wall, which is the target of these antibiotics)	Azithromycin Doxycycline

2. Non-culture Test Results

A) Enzyme Immunoassays (EIA)

A positive result (i.e. bacteria are present in sample) will be indicated by a color change in the wells of the microtiter plate and quantification by spectrophotometry. A negative result will be indicated by a lack of color change.[45]

B) Direct Fluorescent Antibody and Staining

A positive result using DFA for *N. gonorrhoeae* is shown in Figure 11.

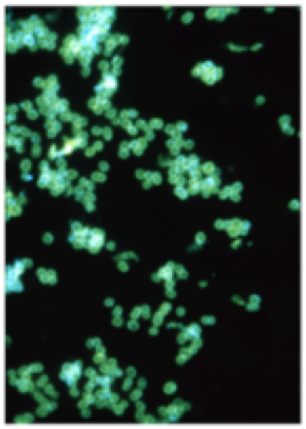

Figure 11: Positive identification of *N. gonorrhoeae* using DFA procedure.

If *C. trachomatis* is present in sample, then fluorescence will be observed when using a fluorescent microscope.[46] Figure 12A shows the fluorescent staining of an inclusion body using DFA.

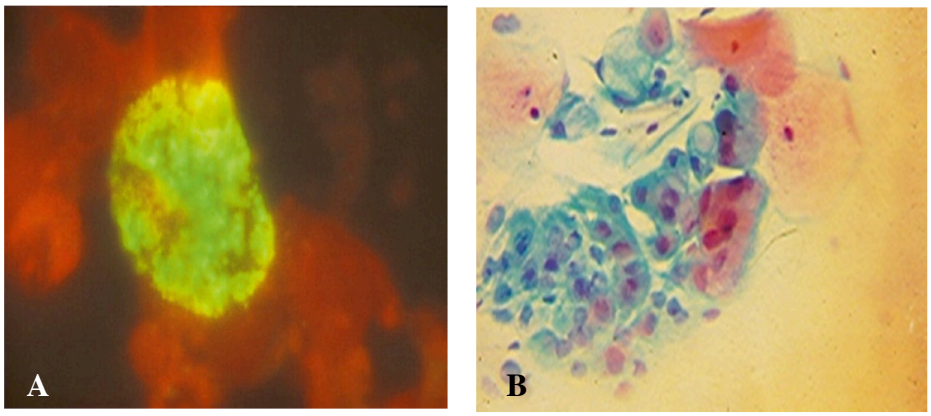

Figure 12: Direct fluorescent antibody staining (A) and Papanicolaou staining of *C. trachomatis* (B).

Figure 12B shows the staining of chlamydial elementary bodies with Papanicolaou stain.[47] Also present in the smear are white blood cells, such as neutrophils, which function to fight the infection. Dead neutrophils form pus, leading to the presence of discharge at infection sites.

C) Molecular Test Results

- Nucleic Acid Hybridization (NAH) Test

  If bacterial genetic material is present, the labeled probe will produce a quantifiable signal. For example, the probe may be radioactively labelled and thus autoradiography can be used to quantify the signal of the probe if bacterial genetic material is present.

- Nucleic Acid Amplification Test (NAAT)

  Amplification of specific bacterial genes will be detected.

- Nucleic Acid Genetic Transformation Test

  Will see growth of mutant gonococci if successfully transformed with gonorrhoeal DNA from specimen.

D) Serology Tests

If IgG antibodies present in blood sample, they will clump, or agglutinate, when subject to agglutination tests.

Point-of-Care Test Results

1. Leukocyte Esterase Test

Will see violet color on urine dipstick if white blood cells are present in urine of male patient.[48] White blood cells are present to fight off infection caused by potential pathogens.

Summary of Questions 3 and 4

Test	Principle of Test	Result
Culture	Specimens are streaked on media capable of supporting bacterial growth (e.g. Thayer-Martin for *N. gonorrhoeae* or A7 agar for *Ureaplasma)* For *C. trachomatis,* cell culture is required.	Positive: • growth of bacterial colonies on media • for *C. trachomatis,* characteristic intracytoplasmic inclusions will form in infected cells which can be stained with labelled antibodies or with Giemsa, H&E, Papanicolaou Negative: • no bacterial growth present • susceptible cells not infected in cell culture
Gram strain	Bacterial smear is prepared on glass slide to which Gram stain is added	Gram-negative: • *N. gonorrhoeae* and *C. trachomatis* appear red/pink No reaction for *Ureplasma* since they lack a cell wall
Biochemical tests	Bacterial cells are subjected to a variety of reagents to check for presence of preformed enzymes	*N. gonorrhoeae* is oxidase+ as can be seen with color change of reagent from clear to purple/dark blue *N. gonorrhoeae* utilizes glucose
Antibiotic susceptibility	Bacterial cells are subjected to broth cultures containing differing concentrations of antibiotics	See Table 3
Enzyme Immunoassays	Human test sample is subjected to antibodies immobilized to solid phase to detect bacterial antigens	Positive: • Tagged antibodies will bind to antigen and upon addition of chromogenic substrate will lead to production of color which can be quantified by spectrophotometry Negative: • No development of color after chromogenic substrate added
Direct Fluorescent Antibody	Use of fluorescently labeled antibodies that are specific for bacterial antigens	Positive: • Fluorescence detected using fluorescence microscopy Negative: • No fluorescence seen under fluorescent microscope

Molecular Tests: • Nucleic Acid Hybridization (NAH) and Nucleic Acid Amplification Test (NAAT) • Nucleic Acid Genetic Transformation (NAGT)	NAH • Use of specific labeled probes complementary to bacterial genetic material NAAT: • Use of various amplification techniques to increase copy numbers of bacterial genetic material NAGT: • Use of gonococcal mutant that grows when transformed with DNA of *N. gonorrhoea* from patient sample	Positive: • NAH: hybridization occurs. Fluorescence detected. • NAAT: amplification occurs and is picked up by detection system. • NAGT: growth of mutant bacteria Negative: • NAH and NAAT: no detection of signal • NAGT: no growth of mutant gonococcal bacteria
Serology Tests	Detection of specific antibodies in blood samples using agglutination	Positive: • Agglutination occurs Negative: • Absence of agglutination
Leukocyte Esterase Test	One of the many tests available on urine dipstick to detect the presence of the enzyme esterase, which is released by white blood cells.	Positive: • Reagent on dipstick will turn violet (indicates presence of esterase and therefore white blood cells in urine) Negative: • No color change on urine dipstick

REFERENCES

[1] Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Retrieved

[2] Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Retrieved February 6, 2016.

[3] Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Page 77. Retrieved February 6, 2016.

[4] Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Page 91. Retrieved February 6, 2016.

[5] Horner, P., Thomas, B., Gilroy, C. B., Egger, M., & Taylor-Robinson, D. (2001). Role of Mycoplasma genitalium and Ureaplasma urealyticum in Acute and Chronic Nongonococcal Urethritis. *Clinical Infectious Diseases, 32*(7), 995-1003. Retrieved February 6, 2016.

[6] Chlamydia Statistics. (2015). Retrieved February 06, 2016, from http://www.cdc.gov/std/chlamydia/stats.htm

[7] Gonorrhea - CDC Fact Sheet. (2016). Retrieved February 06, 2016, from http://www.cdc.gov/std/gonorrhea/stdfact-gonorrhea.htm

[8] How To Collect Swab Samples For Microbiological Testing. (n.d.). Retrieved February 06, 2016, from http://www.moldbacteriaconsulting.com/indoor-air-quality/microbial-sampling/how-to-collect-swab-samples-for.html

[9] How To Collect Swab Samples For Microbiological Testing. (n.d.). Retrieved February 06, 2016, from http://www.moldbacteriaconsulting.com/indoor-air-quality/microbial-sampling/how-to-collect-swab-samples-for.html

[10] Gonorrhea Testing. (n.d.). Retrieved February 06, 2016, from https://labtestsonline.org/understanding/analytes/gonorrhea/tab/sample/

[11] Chernesky, M. A. (2002). Chlamydia trachomatis diagnostics. *Sexually Transmitted Infections, 78*(4), 232-234.

[12] Ureaplasma Infection Workup. (n.d.). Retrieved February 06, 2016, from http://emedicine.medscape.com/article/231470-workup

[13] Preanalytical Guidelines and Resources. (n.d.). Retrieved February 06, 2016, from http://www.specimencare.com/main.aspx?cat=711

[14] Gonorrhea - CDC Fact Sheet. (2016). Retrieved February 06, 2016, from http://www.cdc.gov/std/gonorrhea/stdfact-gonorrhea.htm

[15] Chlamydia - CDC Fact Sheet. (2014). Retrieved February 06, 2016, from http://www.cdc.gov/std/chlamydia/stdfact-chlamydia.htm

[16] Gonorrhea - CDC Fact Sheet. (2016). Retrieved February 06, 2016, from http://www.cdc.gov/std/gonorrhea/stdfact-gonorrhea.htm

[17] Laboratory Tests for Venereal Diseases. (n.d.). Retrieved February 06, 2016, from http://www.labome.com/method/Laboratory-Tests-for-Venereal-Diseases.html

[18] Thayer, J. D., & Martin, J. J. (1964). A Selective Medium for the Cultivation of N. gonorrhoeae and N. meningitidis. *Public Health Reports (1896-1970), 79*(1), 49.

[19] Chernesky, M. A. (n.d.). The laboratory diagnosis of *Chlamydia trachomatis* infections. Retrieved February 06, 2016, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2095010/

[20] Shepard, M. C., & Lunceford, C. D. (n.d.). Differential agar medium (A7) for identification of Ureaplasma urealyticum (human T mycoplasmas) in primary cultures of clinical material. Retrieved February 06, 2016, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC274365/

[21] Gram Staining. (n.d.). Retrieved February 06, 2016, from http://serc.carleton.edu/microbelife/research_methods/microscopy/gramstain.html

[22] Johnson, R. E. (2002). *Screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections --- 2002*. Atlanta, GA: Centers for Disease Control and Prevention.

[23] Ureaplasma Infection. (n.d.). Retrieved February 06, 2016, from http://emedicine.medscape.com/article/231470-overview

[24] Acid Detection Test. (2008). Retrieved February 06, 2016, from http://www.cdc.gov/std/gonorrhea/lab/tests/acid.htm

[25] Acid Detection Test. (2008). Retrieved February 06, 2016, from http://www.cdc.gov/std/gonorrhea/lab/tests/acid.htm

[26] Laboratory Tests for Venereal Diseases. (n.d.). Retrieved February 06, 2016, from http://www.labome.com/method/Laboratory-Tests-for-Venereal-Diseases.html

[27] Laboratory Tests for Venereal Diseases. (n.d.). Retrieved February 06, 2016, from http://www.labome.com/method/Laboratory-Tests-for-Venereal-Diseases.html

[28] Mourad, A., Sweet, R. L., Sugg, N., & Schachter, J. (1980). Relative resistance to erythromycin in Chlamydia trachomatis. *Antimicrobial Agents and Chemotherapy, 18*(5), 696-698.

[29] Laboratory Tests for Venereal Diseases. (n.d.). Retrieved February 06, 2016, from http://www.labome.com/method/Laboratory-Tests-for-Venereal-Diseases.html

[30] Laboratory Tests for Venereal Diseases. (n.d.). Retrieved February 06, 2016, from http://www.labome.com/method/Laboratory-Tests-for-Venereal-Diseases.html

[31] Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Pages 111 - 117. Retrieved February 6, 2016.

[32] Chernesky, M. A. (n.d.). The laboratory diagnosis of *Chlamydia trachomatis* infections. Retrieved February 06, 2016, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2095010/

[33] Latex agglutination test: MedlinePlus Medical Encyclopedia. (n.d.). Retrieved February 07, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003334.htm

[34] Leukocyte esterase urine test: MedlinePlus Medical Encyclopedia. (n.d.). Retrieved February 06, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003584.htm

[35] Neisseria gonorrhoeae bacteria. (n.d.). Retrieved February 06, 2016, from http://www.microbiologyinpictures.com/neisseria gonorrhoeae.html

[36] Chernesky, M. A. (n.d.). The laboratory diagnosis of *Chlamydia trachomatis* infections. Retrieved February 06, 2016, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2095010/

[37] Shepard, M. C., & Lunceford, C. D. (n.d.). Differential agar medium (A7) for identification of Ureaplasma urealyticum (human T mycoplasmas) in primary cultures of clinical material. Retrieved February 06, 2016, from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC274365/

[38] Johnson, R. E. (2002). *Screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections --- 2002*. Atlanta, GA: Centers for Disease Control and Prevention.

[39] Ureaplasma Infection. (n.d.). Retrieved February 06, 2016, from http://emedicine.medscape.com/article/231470-overview

[40] Acid Detection Test. (2008). Retrieved February 06, 2016, from http://www.cdc.gov/std/gonorrhea/lab/tests/acid.htm

[41] Acid Detection Test. (2008). Retrieved February 06, 2016, from http://www.cdc.gov/std/gonorrhea/lab/tests/acid.htm

[42] Jaffe, H. W., Biddle, J. W., Thornsberry, C., Johnson, R. E., Kaufman, R. E., Reynolds, G. H., & Wiesner, P. J. (1976). National Gonorrhea Therapy Monitoring Study. *New England Journal of Medicine N Engl J Med, 294*(1), 5-9.

[43] Mourad, A., Sweet, R. L., Sugg, N., & Schachter, J. (1980). Relative resistance to erythromycin in Chlamydia trachomatis. *Antimicrobial Agents and Chemotherapy, 18*(5), 696-698.

[44] Kenny, G. E., & Cartwright, F. D. (1994). Susceptibilities of Mycoplasma hominis, Mycoplasma pneumoniae, and Ureaplasma urealyticum to new glycylcyclines in comparison with those to older tetracyclines. *Antimicrobial Agents and Chemotherapy, 38*(11), 2628-2632.

[45] Laboratory Tests for Venereal Diseases. (n.d.). Retrieved February 06, 2016, from http://www.labome.com/method/Laboratory-Tests-for-Venereal-Diseases.html

[46] Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Page 112. Retrieved February 6, 2016.

[47] Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Page 124. Retrieved February 6, 2016.

[48] Leukocyte esterase urine test: MedlinePlus Medical Encyclopedia. (n.d.). Retrieved February 06, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003584.htm

FIGURES

Figure 1A:

Campbell, S. (n.d.). SEX ALERT: Dozens infected with UNTREATABLE gonorrhoea amid fears disease could sweep UK. Retrieved February 06, 2016, from http://www.express.co.uk/life-style/health/606078/untreatable-gonorrhoea-antibiotic-resistant-Leeds-sexually-transmitted-disease-STD-alert

Figure 1B:

Chlamydia trachomatis. (n.d.). Retrieved February 06, 2016, from https://en.wikipedia.org/wiki/Chlamydia_trachomatis

Figure 1C:

Ureaplasma (T-Mycoplasma; Ureaplasma parvum). (2014, September). Retrieved February 6, 2016, from https://lookfordiagnosis.com/mesh_info.php?term=ureaplasma&lang=1

Figure 2:

Gram Staining: Principle, Procedure and Results - microbeonline. (2015). Retrieved February 06, 2016, from http://microbeonline.com/gram-staining-principle-procedure-results/

Figure 3:

Laboratory Tests for Venereal Diseases. (n.d.). Retrieved February 06, 2016, from http://www.labome.com/method/Laboratory-Tests-for-Venereal-Diseases.html

Figure 4:

Enzyme-linked immunosorbent assay. (n.d.). Retrieved February 6, 2016, from http://medical-dictionary.thefreedictionary.com/enzyme-linked immunosorbent assay

Figure 5:

Laboratory Tests for Venereal Diseases. (n.d.). Retrieved February 06, 2016, from http://www.labome.com/method/Laboratory-Tests-for-Venereal-Diseases.html

Figure 6:

Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Page 115. Retrieved February 6, 2016.

Figure 7:

PaceHybridization. (n.d.). Retrieved February 06, 2016, from http://chlamydiae.com/twiki/bin/view/Diagnostics/PaceHybridization

Figure 9A:

http://www.pharmacology2000.com/Antibacterial/gon3.htm

Figure 9B:

Chlamydia trachomatis. (n.d.). Retrieved February 06, 2016, from https://en.wikipedia.org/wiki/Chlamydia_trachomatis

Figure 9C:

ElitechGroup : Overview. (n.d.). Retrieved February 06, 2016, from http://www.elitechgroup.com/corporate/products/market-segment/microbiology/bacteriology/mycoplasma/current-range/a7-agar-ref66/overview

Figure 10:

Acid Detection Test. (2008). Retrieved February 06, 2016, from http://www.cdc.gov/std/gonorrhea/lab/tests/acid.htm

Figure 11:

MicrobLog: Microbiology Training Log. (n.d.). Retrieved February 06, 2016, from http://microblog.me.uk/135

Figure 12:

Laboratory Tests for Venereal Diseases. (n.d.). Retrieved February 06, 2016, from http://www.labome.com/method/Laboratory-Tests-for-Venereal-Diseases.html

Figure 13A:

Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Page 112. Retrieved February 6, 2016.

Figure 13B:

Gross, G., & Tyring, S. K. (2011). *Sexually transmitted infections and sexually transmitted diseases*. Page 124. Retrieved February 6, 2016.