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Guidelines for laboratory diagnosis of *Neisseria gonorrhoeae* in East-European countries

Part 1. Gonorrhoea, sampling and microscopy for diagnosis

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INTRODUCTION

The present guidelines aim to provide comprehensive and precise information regarding the sexually transmitted infection (STI) gonorrhoea and laboratory diagnosis of the aetiological agent, i. e. *Neisseria gonorrhoeae* (the gonococcus), in East-European countries. The recommendations contain important and crucial information for both physicians and laboratory staff working with STIs and/or STI-related issues. For the different East-European countries, minor national adjustments of the present guidelines may be needed to oblige the requirements, lack of accessibility of, for example, some chemical reagents and equipment, and laws in each specific country.

European Union's case definition of gonorrhoea:
Clinical description: Clinical picture compatible with gonorrhoea, e.g., urethritis, cervicitis, or salpingitis.
Laboratory criteria for diagnosis:
- isolation of *Neisseria gonorrhoeae* from a clinical specimen
- detection of *N. gonorrhoeae* antigen or nucleic acid
- demonstration of Gram-negative intracellular diplococci in an urethral smear from a male.

Gonorrhoea is a mandatorily notified STI. The causative bacteria *N. gonorrhoeae* are Gram-negative coffee bean-shaped diplococci, approximately 1.25–1.60 μm long and 0.7–0.8 μm wide, which mainly are arranged in pairs with the concave sides towards each other. The obligate pathogen *N. gonorrhoeae* is mainly transmitted from infected individuals by direct human-to-human contact between the mucosal membranes of the urogenital tract,

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anal canal, or the oropharynx during sexual activities. However, neonates can be infected during passage through the birth canal if the mother has urogenital gonorrhoea.

In men, the infection is most frequently manifested as acute urethritis, while in women it most often causes endocervicitis. Though, anorectal and/or oropharyngeal gonorrhoea, often asymptomatic, are common among men who have sex with men (MSM, i. e. homosexual and bisexual men) and can also be identified in heterosexual individuals. Overall, asymptomatic infection is common, i. e. up to 10–20% of infected men and approximately 50% of infected women. The main symptoms, clinical manifestations of uncomplicated gonorrhoea, and complications associated with infection are shown in Tables 1–3.

In Table 4, clinical and other anamnestic indications for testing of gonococcal infection are described.

Table 1. **Main symptoms associated with gonococcal infection**

Patients	Symptoms
Women	– vaginal and/or cervical mucopurulent discharge – dysuria – lower abdominal pain – dyspareunia – pain and discharge from the rectum
Men	– purulent urethral discharge – dysuria – pain in the urethra – pain in perineum radiating into the rectum – pain and discharge from the rectum
Neonates	– eye lesions – rhinitis – vulvovaginitis

Table 2. **Clinical manifestations of uncomplicated gonococcal infection**

Patients	Clinical manifestations
Women	– cervicitis – urethritis – bartholinitis
Men	– urethritis – balanoposthitis
Men and women	– pharyngitis – proctitis – conjunctivitis
Children	– urethritis – vaginitis – conjunctivitis – pharyngitis – proctitis
Neonates and babies	– ophthalmia neonatorum – vulvovaginitis

Furthermore, risk groups that would benefit from routine screening, e. g., using the DNA/RNA-based method, of gonococcal infection include: persons engaged in commercial sex, MSM, pregnant women, and sexually active young people (less than 25 years of age) with multiple sexual partners.

For each patient, the following should be stated in an appointment card for testing:

- patient's identification (country-specific)
- patient's age
- patient's gender
- anatomical sampling site
- time and date of sampling
- anamnestic data, clinical presentation, and eventual suggestive diagnosis
- requested laboratory diagnostic method
- if culture, state also if antibiotic susceptibility testing should be performed.

Methods for diagnosis of gonococcal infection include:

- microscopy: detection of Gram-negative intracellular diplococci in stained preparations from mainly male urethra or conjunctiva
- culture: isolation of *N. gonorrhoeae* from infected sites
- nucleic acid (DNA/RNA) detection and immunological/antigenical methods: detection of *N. gonorrhoeae* specific nucleic acid sequences or gonococcal antigens.

Table 3. **Complications associated with gonococcal infection**

Patients	Complications
Women	- Bartholin apostasis/abscess *Pelvic inflammatory disease (PID):* – endometritis – salpingitis – tubo-ovarian abscesses – pelvic peritonitis – perihepatitis – ectopic pregnancy – infertility
Men	– epididymo-orchitis – prostatitis – phimosis – paraphimosis – urethral stricture – lymphadenitis – infertility
All patients (systemic complications)	*Disseminated gonococcal infection (DGI):* – arthritis – dermatitis – tenosynovitis – endocarditis – myocarditis – pericarditis – meningitis

Table 4. **Clinical and anamnestic indications for testing of gonococcal infection**

Patients	Indications
Men	– complaints of purulent or mucopurulent discharge from the urethra, symptoms of dysuria – epididymal and/or testal pain – pain and discharge from the rectum, signs of proctitis – inflammatory changes in the urethral opening, paraurethral ducts – symptoms consistent with acute prostatitis
Women	– inflammatory diseases of the urogenital system, purulent or mucopurulent vaginal discharge, symptoms from the endocervical canal, adnexitis, proctitis, vulvovaginitis, cervicitis, inflammation of pelvic organs – genital irritation, dysuria, lower abdominal pain – infertility, habitual abortions, premature births – pregnant women: • 1st examination should be carried out at the first antenatal visit • 2nd examination – at 27–30 weeks • 3rd examination – at 36–40 weeks – women not examined prior to hospitalisation at gynaecologic in-patient facilities, before prescription of antimicrobial therapy – complications during the puerperal period (5–6 days after childbirth)
Neonates	– purulent conjunctivitis and/or vulvovaginitis (should gonococcal aetiology of conjunctivitis and/or vulvovaginitis be confirmed, the parents should be examined)
Children (girls)	– symptoms of vulvovaginitis, vaginitis
Other indications	– sexual contact with a person with diagnosed gonorrhoea – examination for other STIs – detection of trichomoniasis (prior to and after its treatment) – examination of persons of decreed professions during mandatory pre-employment examination and periodical medical examinations in compliance with approved regulatory documents – examination of patients in cases of sexual abuse

Table 5. **Samples and methods recommended for gonorrhoea diagnostics**

Sampling site	Method of diagnostics	Comments
Urethra (in men)	Microscopy (methylene blue stain and/or Gram's stain)	– useful diagnostic method for symptomatic men. Sensitivity and specificity in symptomatic men is >90% and >95%, respectively*.
	Culture	– is applied for definitive confirmation of diagnosis (isolation and identification of *N. gonorrhoeae*) and for asymptomatic men – if optimised conditions, sensitivity and specificity up to 100%* – is necessary for determination of sensitivity to antibiotics.
	Nucleic acid (DNA/RNA) and immunologic analyses (detection of specific nucleic acid or antigen)	– applied to first-catch urine specimens or urethral swabs mainly as a screening method with further confirmation using culture.
Endocervix/urethra (in women)	Culture	– preferred diagnostic method – if optimised conditions, sensitivity and specificity up to 100%* – is necessary for determination of sensitivity to antibiotics – is mandatory for examination of children and women in menopause.

Table 5 (continued)

Sampling site	Method of diagnostics	Comments
Endocervix/urethra (in women)	Microscopy (methylene blue stain and/or Gram's stain)	– not recommended because of the low sensitivity and insufficient specificity*.
	Nucleic acid and immunologic analyses (detection of nucleic acid or antigen)	– should be applied mainly as a screening method with further confirmation using culture.
Pharynx/tonsils/ conjunctiva/rectum	Culture	– preferred diagnostic method – if optimised conditions, sensitivity and specificity up to 100%* – is necessary for determination of sensitivity to antibiotics.
	Nucleic acid and immunologic analyses (detection of nucleic acid or antigen)	– should be applied only as a screening method with further confirmation by culture. No DNA/RNA-based method has yet proven to comprise sufficient specificity for extragenital specimens.
Conjunctiva (neonates)	Microscopy (methylene blue stain and/or Gram's stain)	– the method has high sensitivity and specificity for neonates but is not applied in adults*.
Urine (men and women)	Nucleic acid and immunologic analyses (detection of nucleic acid or antigen)	– should be applied mainly as a screening method with further confirmation using culture.
Vulva/vagina (girls)	Culture of vaginal swab	– preferred diagnostic method – is necessary for determination of sensitivity to antibiotics.

Attention!

** The sensitivity and specificity of each method described above are greatly dependent on the quality of sampling, correct type of specimen, transportation of samples, performance of the utilized method, and interpretation of results.*

– In suspected cases of disseminated gonococcal infection (DGI), blood cultures in addition to urogenital and extragenital specimens should be obtained!

SAMPLING

The physician or laboratory technician who samples the clinical material should assure:

- sampling of the most suitable specimens based on the clinical manifestations and diagnostic method to use
- high-quality sample collection
- ideally, sterile cotton swabs are used for sampling for microscopic examination and dacron swabs for culture of the bacteria
- special marking on the specimen container/slide indicating, like in the patient's appointment and medical cards, the patient's identification, gender, age, time and date of sampling, and anatomical site sampled
- for testing by culture and DNA/RNA-based methods, i. e. nucleic acid amplification tests (NAATs; e. g., polymerase chain reaction (PCR) and hybridisation-based assays), sampled material is placed in tubes with an appropriate transportation medium, sterile container/large tube (for urine) or, for culture, if possible, directly inoculated on a plate (Petri dish) containing selective culture medium
- avoiding a delay of sample transportation
- transportation is carried out at a definite temperature for each method.

The quality of laboratory testing results depends on the clinical presentation of the patient at the time for sampling. Samples are most informative if they are collected under the following conditions:

- the patient has not received antimicrobial treatment during the latest 7–8 days (ten days for DNA/RNA-based methods) prior to examination and sampling
- local treatment has not been provided during the previous 48–72 hours
- in women, samples from the genital tract should preferably not be taken during active menstruation
- the patient has not urinated or had sexual intercourse at least 1–2 hours before sampling
- samples from the most suitable anatomical sites based on the clinical manifestations and chosen diagnostic method are essential (Table 5).

It should be greatly emphasized that systemic treatment with antimicrobial agents can have a significant effect on the results of diagnostic testing and highly reduce the diagnostic value.

Regarding sampling and preparation of slides for microscopic examination, the following is important to keep in mind:

- in men, a specimen from the urethral meatus should be taken prior to all other urethral samples
- in women, a specimen from the endocervical canal should be taken prior to other cervical specimens, after taking precautions to reduce contamination with vaginal flora
- in pre-pubertal girls, material from the vagina has to be sampled prior to all other vaginal samples
- microscope glass slides should be dry, clean and not scratched
- slides should either be marked by pencil (on frosted slides) or by a long-lasting marker not obliterated by the subsequent staining process
- swabs should always be rolled with a light pressing across the surface of the slide in one direction only and a sample deposited as a thin homogeneous layer of clinical material solely on one side of the slide
- if the quantity of the material is small or it is necessary to place more than one sample that have been taken from various sites from the same patient on one slide, the sample should be placed closer to the centre of the slide at pre-marked sites
- after sample application on the slide, the applied clinical material should be dried at ambient temperature, in avoidance of contact with other slides.

Attention!

In some countries, material for microscopic examination must be taken on two glass slides (one for Gram stain and the second for methylene blue stain).

Tools utilized for sampling of different specimens:

- plastic bacteriological loop (1 μl)
- sterile cotton or dacron swabs on wooden or plastic shafts (throat swabs)
- sterile cotton or dacron swabs on fine aluminium shafts (nasopharyngeal swabs)
- special brushes (for some DNA/RNA-based methods)
- sterile cotton wool
- vaginal speculum
- rectal speculum/proctoscope.

Sampling of clinical material in men

Sampling from urethra

Preparation prior to sampling:

- In presence of discharge:
  - clean the head of the penis and the area of the external urethral meatus using a sterile swab moistened with physiological saline; retract the prepuce of the penis to prevent contamination when taking the specimen.
- In absence of free discharge:
  - the patient should massage the urethra with light sliding movements from the base of the penis towards the tip prior to taking a meatal or urethral specimen.

Sampling procedure:

- Collect exudates directly on a sterile swab. If no exudates are obtained, gently introduce a sterile cotton or dacron swab on a fine aluminium shaft into the urethral meatus (0.5-2 cm) and leave in place for 5–10 seconds.

Attention!

It is not recommended to take urethral samples in children of prepubertal age. Exudates are collected solely from the urethral meatus with a small swab or urine is obtained for testing by DNA/RNA-based methods. If sampling from the preputial bag is required, a sterile cotton/dacron swab is used.

Sampling of clinical material in women

Sampling from endocervix

Preparation prior to sampling:

- insert a vaginal speculum, which may be prelubricated with warm water, and thoroughly clean the external cervical os to remove contaminating vaginal discharge by using sterile cotton wool or a large swab.

Sampling procedure:

- gently introduce a sterile cotton or dacron swab on a fine aluminium shaft into the endocervical canal (2 cm) and rotate it for 5–10 seconds.

Attention!

Endocervical specimens are not taken in girls of prepubertal age. Should gonorrhoea be suspected in this age, specimens should be sampled from the vestibule of the vagina!

Sampling from urethra

Preparation prior to sampling:

- *In case of abundant discharge:*
  - clean the external opening with a sterile swab.
- *In absence of free discharge:*
  - lightly massage the urethra.

Sampling procedure:

- gently introduce a sterile cotton or dacron swab on a fine aluminium shaft into the urethral opening (0.5–2 cm) and leave in place for 5–10 seconds.

Sampling from vagina (mainly in girls of prepubertal age)

Sampling procedure:

- a sterile cotton or dacron swab on a fine aluminium shaft is carefully introduced through the hymeneal opening (no speculum should be used) into the vagina and material is taken from the posterior wall of the vagina, avoiding cervical discharge.

Sampling from vestibular and paraurethral glands

Preparation prior to sampling:

- thoroughly clean the vaginal vestibule or the area of the external urethral opening with a sterile swab
- massage the Bartholin's glands with a gloved forefinger introduced into the vagina, and at the same time place the index finger of the other hand over the excretory duct of the gland
- material from paraurethral ducts (if they are affected) is received during careful massage of the front part of the urethra.

Sampling procedure:

- the discharged effluent is collected on a sterile cotton or dacron swab.

Sampling of clinical material in both men and women

Collection of urine

Sampling procedure:

- the patient should collect the first 10–20 ml (first catch) of the freely voided urine.

Attention!

A patient should not have urinated or had sexual intercourse during the latest 1–2 hours prior to sampling.

Sampling from conjunctiva

Preparation prior to sampling:

- if purulent discharge is observed, it should be removed with a sterile swab.

Sampling procedure:

- the inferior eyelid is retracted and a sterile cotton or dacron swab (for neonates, thin swabs on a fine aluminium shaft are used) is moved across the surface of the inferior palpebral conjunctiva towards the median corner of the eye.

Sampling from rectum

Clinical material from the rectum ought to be sampled:

- in patients who have had anal sexual intercourse or if this is suspected
- in case of manifestations of proctitis (subjective and objective), including mucopurulent discharge from the anus
- in pregnant women and children undergoing examination for gonorrhoea
- in cases of skin inflammation around the anus
- in cases of peri-anal condylomata
- in cases of thickened anal folds.

Sampling procedure:

Clinical material is obtained from the rectum either under visual control using a proctoscope or a rectal speculum or by the 'blind' method – from the anal canal, the former being more effective.

- A sterile cotton or dacron swab on a wooden or plastic shaft is introduced 3 cm into the anal canal, and material is obtained from all walls of the rectum with circular movements during 10 seconds.

Attention!

If faecal contamination is observed on the swab, this should be discarded and another swab used to obtain the specimen.

Sampling from oropharynx

Oropharyngeal specimens ought to be sampled in patients who have had oral sexual intercourse or if this is suspected.

Sampling procedure:

- a sterile cotton or dacron swab on a fine aluminium shaft is moved across the posterior pharyngeal wall above the inferior edge of the soft palate and across the tonsillar surface.

Attention!

In suspected cases of disseminated gonococcal infection (DGI), blood cultures in addition to urogenital and extragenital specimens should be obtained!

TRANSPORTATION OF SAMPLES

Transportation of glass slides for microscopy

- Accurately marked glass slides with applied specimens are placed in an airtight transportation container and are transported to a laboratory accompanied by the filled in appointment card for testing of each patient (see above).
- If the slides cannot be transported within 24 hours, the slides should, after application of specimen and drying, be individually fixed with 96% ethyl alcohol for 3 minutes. Flame fixation is not to recommend as this may affect the result of microscopy. The appointment card of each patient should indicate also the method of fixation used.

Attention!

If the recommendations regarding sampling and transportation are not complied with (e. g., broken or not marked slides, slides glued together, or absence of material on a slide), the specimens will not be subjected to microscopic examination.

Transportation of inoculated agar plates or samples for culture

Gonococci are very fastidious and fragile bacteria, due to this fact non-compliance with the recommendations concerning transportation can significantly affect the success of culture, i. e. the probability of identification of viable gonococci decreases considerably.

The following transportation conditions should be strictly followed:

- after sampling, specimens should be placed in an appropriate non-nutritive transportation medium containing charcoal (if charcoal-coated swabs are not used) such as Stuart, Modified-Stuart or Amies buffered semisolid medium (Copan) or inoculated on a nutritive transportation culture

medium, e. g., Jembec, the Gono-Pak, the InTray GC system, or Transgrow (or transportation medium that is comparable, validated and certified for this purpose in the specific country) or, ideally, immediately inoculated on selective culture medium (see Guidelines for Laboratory Diagnosis of *Neisseria gonorrhoeae* in East-European Countries – Part 2), optimally also on non-selective medium

- an accurately labelled transportation tube or a plate with an inoculated specimen is placed in a container containing a humid atmosphere of 5–10% CO_2 and transported to the laboratory accompanied by the filled in appointment card for testing of each patient (see above)
- specimens for culture should be delivered to the laboratory as soon as possible following inoculation (optimally within 2 hours)
- the specimens can be kept in the non-nutritive transportation medium in refrigerator for 24 (absolute maximum 48) hours
- the material should not be transported to the laboratory at temperatures below 18°C.

Sampling and transportation of samples for diagnosis using DNA/RNA-based methods

Recommended clinical material should be sampled with appropriate tools (e. g. special brushes or swabs) and transported in correct transportation tubes according to the instructions of the manufacturer of each specific assay. The manufacturer may supply sampling tools and an appropriate transportation medium. If the sampling and transportation procedure are not described in the instructions or if in-house tests are used, sampling is performed as described above and transportation is executed as described below.

Transportation:

- swab samples, in e. g. 2-SP transportation medium, and urine specimens should mainly be transported under refrigeration
- swab samples in e. g. 2-SP transportation medium may be stored at 5 ± 3°C for up to seven days post collection or at –70°C for up to two months
- urine samples are stable for 24 hours at ambient temperature. Urine samples that will not be processed within 24 hours of collection may be stored at 5 ± 3°C, but must be processed within 7 days. Otherwise, the urine samples may be stored at –20°C or lower for up to two months
- all specimens should be placed in appropriate transportation tubes/containers and transported to the laboratory accompanied by the filled in appointment card for testing of each patient (see above).

Attention!

If frozen at –70°C, the samples may be stored up to two months (at least). However, this has to be confirmed in the instructions of the manufacturer of each specific DNA/RNA-based test.

MICROSCOPY FOR DIAGNOSIS OF *N. GONORRHOEAE*

Advantages:

- simple transportation conditions
- ease of performance
- quickness of obtaining results
- low cost
- high sensitivity and specificity for urethral samples in symptomatic men (Table 5).

Disadvantages:

- low sensitivity for cervical samples, and pharyngeal as well as rectal specimens are not recommended to use (Table 5)
- low sensitivity for early diagnosis, asymptomatic infection, and test of cure
- subjectivity of the test, i. e. the results greatly depend upon the experience of the microscopist, type of specimen, quality of specimen, quality of staining, the microscope used, etc.
- microscopic diagnosis is usually not sufficient for medico-legal purposes
- mainly used to obtain a presumptive diagnosis.

Preparation of slides prior to staining

Fixation at the laboratory

- *For staining with aniline dyes:*
- If a non-fixed preparation is delivered to the laboratory:
  - the preparation is fixed by passing the slide through a burner flame three times.
- *Storage of slides:*
- Slides with a fixed preparation may be stored at ambient temperature for several days.

Attention!

During fixation in a burner flame, it is extremely important not to overheat the slide, as cells in the preparation may be destroyed and the preparation will become suboptimal or even unsuitable for testing.

Equipment and materials required for microscopic examination:

- quality controlled, binocular microscope with the ×1000 possibility of magnification
- immersion oil
- medical gloves
- 1% methylene blue solution
- set of chemical reagents to perform Gram staining
- 96% ethyl alcohol
- alcohol lamp or gas burner
- timer
- special glass slides
- device for staining prepared slides
- pipette
- a glass, 200 ml

- blotting paper
- vessels for collection and treatment of slides
- disinfecting solutions.

Methylene blue stain

Methylene blue stain allows detection of inflammation and evaluation of the morphology and location of cellular material and microorganisms on a slide. Both aqueous and alcohol methylene blue solutions may be used. Utilization of alcohol methylene blue solution, e. g., according to Loeffler, allows reducing the fixation time without decreasing the staining quality.

Procedure for staining with aqueous methylene blue solution:

- the slide with the specimen is air-dried and fixed by immersing for 1-2 minutes into 96% ethyl alcohol
- the fixed slide is dried and 1% aqueous methylene blue solution is applied for 1–2 minutes (depending on the thickness of preparation)
- the slide is rinsed thoroughly with running cold water and finally air-dried.

Procedure of Loeffler's methylene blue staining:

- the slide with the specimen is air-dried
- the slide is placed in a glass with 1% methylene blue dye according to Loeffler for 10–15 seconds
- the slide is rinsed by immersing into tap water
- finally, the slide is air-dried or carefully dried using blotting paper.

Gram staining

Using Gram staining, the chemical structure of the bacterial cell wall, i. e. presence or absence of teichoic acids and the amount of murein, may be indicated. Bacteria can retain the complex of crystal violet and iodine and, consequently, are resistant to alcohol decolouration, i. e. Gram-positive, or they are decolourised, i. e. Gram-negative. Consequently, Gram staining allows also identification of, e. g., Gram-negative intracellular diplococci such as suspected gonococci.

Gram staining procedure:

- the slide is air-dried and fixed over a burner flame for some seconds
- the fixed slide is overlaid with a 1% aqueous solution of crystal violet for 1 minute
- the slide is rinsed with tap water
- lugol solution is applied for approximately 1 minute
- the slide is rinsed with tap water
- the slide is discoloured with 96% ethyl alcohol until the thinnest sections of the smear become pale-grey or until the fluid flowing over the smear becomes colourless. This usually takes approximately 10–30 seconds, depending on the thickness of the smear. Excessive discolouration should be avoided as this can change the apparent outcome of the staining procedure
- the slide is immediately rinsed with tap water
- the slide is overlaid with an aqueous solution of 1% neutral red (or safranin) for 1 minute
- the dye is removed, the slide rinsed with running water and, finally, air-dried or carefully dried using blotting paper.

Quality control

When performing the staining procedures, every lot of slides should contain control slides with reference strains of known Gram-positive and Gram-negative bacteria.

Sources of errors in microscopic examination:

- improper types of specimens (Table 5)
- incorrect sampling of the clinical material (see Sampling) may result in a too limited or low-quality specimen that is impossible to interpret
- use of a pencil not intended for marking slides may contaminate the preparation during the process of fixation and staining
- improper application of the material to the slide may result in damage of cells and, consequently, their morphology may be destroyed
- insufficient fixation may result in removal of clinical material from a slide during the process of staining
- overheating during fixation may result in appearance of coloured artefacts and damage the cells
- incorrect, too old or not quality-assured chemical reagents are used for staining
- use of lugol solution after expiry date (usually the solution can be kept for 90 days if stored in the dark at ambient temperature) may distort the results
- in the case of overdecolouration, Gram-positive microorganisms may appear to be Gram-negative
- in the case of underdecolouration, Gram-negative microorganisms may appear to be Gram-positive
- staining reagents contaminated with other microorganisms may give erroneous results
- failure in set up, alignment or quality control of the microscope
- microscopist with insufficient experience.

Microscopic examination of stained preparations

In the process of microscopic examination, preparations stained with methylene blue and Gram stain are evaluated in succession. Consequently, conclusions are not entirely based on the results of only one specimen examination.

Slides with methylene blue stain or Gram stain are evaluated under two magnifications – i. e. ×100 and ×1000. Microscopic examination should commence with screening of the entire slide at low magnification (×100) which allows detection of clinical material on the slide, evaluation of the quality of the sample, and selection of areas on the slide for a more detailed examination at a higher magnification (×1000). Areas

on the slide where the specimen is applied in a thin layer and optimally leucocytes are present in monolayers should be identified. In almost every specimen there are regions that are unsuitable for diagnosis owing to overlaying of cells. Subsequent microscopic examination at a higher magnification (×1000) enables detection and evaluation of the inflammatory reaction as well as presence and morphotypes of different micro-organisms.

At a magnification of ×1000 (under oil immersion), leucocytes should be counted in at least five fields. Special attention should be paid to the search of intracellular diplococci in polymorphonuclear (segmented nucleus) leucocytes.

Microscopic evaluation of stained smears

The microscopic diagnosis of gonococci is based on three main characteristics:

- cell morphology and arrangement
- location
- colour.

Only the identification of **all three characteristics** makes it possible to provide a presumptive diagnosis of gonorrhoea. If any of these characteristics is absent, culture of the bacteria is required!

The gonococci are mainly identified in pairs, i. e. as **diplococci**, with the **coffee-bean-shaped** bacterial cells arranged with the concave sides opposing each other.

Location of gonococci is of crucial significance in the microscopic diagnosis of gonorrhoea. Most often gonococci are located **inside mainly polymorphonuclear leucocytes but also apparently inside/on epithelial cells.** If only extracellular diplococci are identified, culture should be performed to obtain a definitive diagnosis.

The colour of Gram-stained gonococci is **pink-red**. The **nuclei of leucocytes and epithelial cells are coloured violet.**

The diagnosis of urethritis in men is based on detection of four or more polymorphonuclear leucocytes per field at a magnification of ×1000.

In case of cervicitis, the quantity of polymorphonuclear leucocytes is increased (more than 10 per field at a magnification of ×1000).

When testing vaginal specimens, the number of leucocytes varies depending on the organism present, on the day of the menstrual cycle, the presence of an intrauterine contraceptive device, etc. Therefore, it is recommended to use both the criteria for diagnosing mucopurulent cervicitis, i. e. presence of clinical manifestations and inflammatory nature of a cervical sample.

The diagnosis of urethritis in women is confirmed by detection of more than 10 polymorphonuclear leucocytes per field at a magnification of ×1000. If there is a significant inflammation of the vagina and/or cervix, the urethral specimen may be 'contaminated' with discharge (squamous epithelium cells, leucocytes, vaginal microflora).

Evaluation of methylene blue stained smears

The following is visible in the course of microscopic examination:

- cell nuclei, coloured blue
- cytoplasms, coloured blue of various intensity
- bacterial microflora, coloured blue of various intensity.

Methylene blue stain is indicative and enables to estimate the morphology and location of cells and micro-organisms, including extra- and intracellular diplococci, in a smear.

Microscopy of a sample from urethra in men

The following morphological objects can be detected:

- mucous (blue-violet amorphous mass/beams, which form the image background)
- squamous and in some cases columnar (cuboidal) epithelium of the urethra
- polymorphonuclear leucocytes
- blue microorganisms
- erythrocytes (rarely)
- spermatozoa (rarely).

Microscopy of a sample from endocervical canal in women

The following morphological objects can be detected:

- cervical mucous (blue-violet amorphous mass/beams)
- columnar epithelial cells from the cervical canal (in most cases, only nuclei are visible, which are oval, monomorphously coloured, and as large as leucocytes)
- polymorphonuclear leucocytes
- blue microorganisms
- erythrocytes (rarely)
- spermatozoa (rarely).

Attention!

If the endocervical canal has been incorrectly sampled, vaginal epithelial cells, microorganisms and leucocytes flowing from the vagina are often detected, indicating that the sample has a limited value for the evaluation of the endocervical component.

Microscopy of a sample from vagina in children

The following morphological objects may be detected:

- mucous (blue-violet amorphous mass/beams)
- normal squamous epithelial cells (dark-blue nucleus; the edges of the cells are clear)
- polymorphonuclear leucocytes
- blue microorganisms
- dense blue yeast-like fungi (pseudomycelium, yeast cells)
- trichomonads (cells with foamy cytoplasm and a dark-blue nucleus located in peripheral parts)
- erythrocytes (rarely)
- spermatozoa (rarely).

Microscopy of a sample from urethra in women

The following morphological objects may be detected:

- mucous (blue-violet amorphous mass/beams)
- squamous and in some cases columnar epithelium of the urethra
- polymorphonuclear leucocytes
- blue microorganisms
- erythrocytes (rarely).

Attention!

The urethral preparation can be highly contaminated with discharge content (squamous epitelium cells, leucocytes, and vaginal microflora) from the vagina and/or endocervical canal and may, consequently, not be suitable for evaluation.

Evaluation of Gram-stained smears

There are no principal differences in the evaluation of preparations stained with Gram stain and methylene blue stain (see above). However, Gram staining in addition enables to distinguish Gram-negative (pink-red) from Gram-positive (blue-violet) bacteria. Consequently, intracellular Gram-negative diplococci such as gonococci may be identified.

In case of correct staining, a smear should be red-violet (the thinnest areas are red and the thickest are violet). The nuclei of eukaryotic cells (leucocytes, epithelial cells) should partially keep the basic violet colour (i. e. they should be coloured violet in the centre and orange-red in the peripheral parts), whereas gonococci located in leucocytes and in/on epithelial cells should stain pink-red.

In case of incorrect staining, it is necessary to repeat either sampling or staining. If the decolouration step of the staining procedure is not sufficient, leucocytes and epithelial cell nuclei are coloured dark violet and Gram-negative bacteria may retain the violet stain, i. e. all bacteria will be interpreted as Gram-positive. In this case, it is necessary to repeat the decolouration step with alcohol, to dry and re-stain with neutral red. In contrast, if the decolouration step is too long, Gram-positive microorganisms can be stained orange-red and incorrectly interpreted as Gram-negative.

Overall, in a correctly Gram-stained smear the following morphological objects may be detected:

- mucous (reddish amorphous mass/beams, which form the background of the preparation)
- epithelial cells (in most cases, only nuclei are visible that are oval/round and monomorphously brilliant violet coloured)
- polymorphonuclear leucocytes (nuclei are coloured violet and the cytoplasm is pale-pink)
- gonococci (pink-red; located extracellularly and intracellularly in polymorphonuclear leucocytes and in/on epithelial cells)
- other bacteria (Gram-negative and Gram-positive)
- yeast-like fungi (pseudomycelium, yeast cells of brilliant violet colour)
- erythrocytes (rarely)
- spermatozoa (rarely).

Laboratory conclusions based on results using microscopy

- Gram-negative intracellular diplococci within polymorphonuclear leucocytes, morphologically resembling *N. gonorrhoeae*, were not detected.
- Gram-negative intracellular diplococci within polymorphonuclear leucocytes, morphologically resembling *N. gonorrhoeae*, were detected.

Attention!

In all cases when a positive result is obtained, the stained smears (methylene blue and Gram-stained preparations) should be stored in the laboratory for at least 3 months.

The clinical presentation and results of microscopic examination of stained urethral smears solely are sufficient for a definitive diagnosis of gonorrhoea only in symptomatic men.

When testing women, children, in cases of sexual abuse, oropharyngeal, conjunctival or rectal specimens, culture of N. gonorrhoeae, with subsequent species confirmatory assays, is required in order to establish a definitive diagnosis of gonorrhoea.

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NEISSERIA GONORRHOEAE LABORATORINĖS DIAGNOSTIKOS PROTOKOLAS RYTŲ EUROPAI. 1 dalis. Gonorėjos apibrėžimas, mėginių paėmimas bei mikroskopijos panaudojimas diagnostikoje

Pirmoje straipsnio dalyje pateikiama *N. gonorrhoeae* laboratorinės diagnostikos metodika, aprašomi optimalūs mėginių paėmimo būdai bei instrumentai, taip pat mėginių transportavimo sąlygos tolesniam mikroskopijos, kultūros bei nukleino rūgščių tyrimui. Aptariami lyties takų mėginių mikroskopijos metodai bei veikimas. Protokolas parengtas tarptautinių lyties bei reprodukcinės sveikatos ir teisių bei lyties takų infekcijų diagnostikos grupių iniciatyva. Projektą remia Švedijos tarptautinio vystymosi kooperacijos agentūra SIDA, projekto vadovas Marius Domeika.