Protocols for Requesting and Interpreting

Molecular Tests in Infectious Diseases of the Lower Genital Tract

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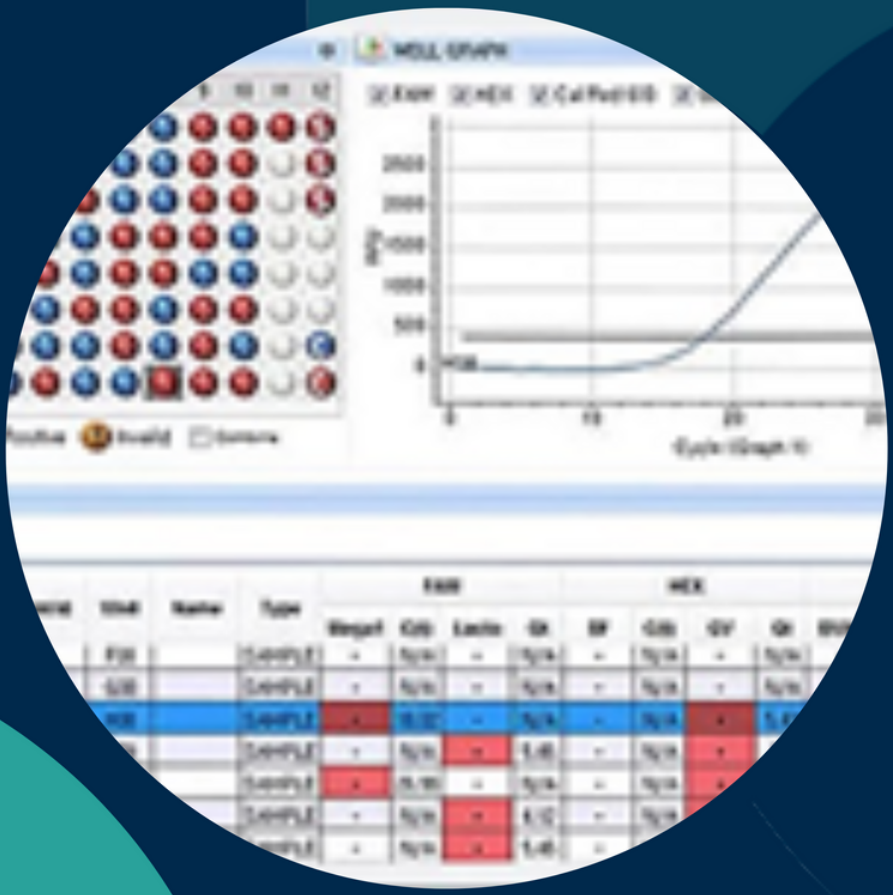

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This material was developed by the clinical staff of Prof. Eleutério Laboratory to contribute to the knowledge of physicians currently in the research area of infectious agents of anogenital diseases.

Protocols for Requesting and Interpreting Molecular Tests in Infectious Diseases of the Lower Genital Tract.

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Summary

Introduction.

The diseases that affect the lower genital tract of women and men are diverse and with variable frequency. Often the diagnosis cannot be given based only on the clinic and requires complementary tests.

Until the beginning of the 21st century, many of the complementary tests that helped clinicians in the etiological diagnosis of diseases, especially infectious ones, were performed using morphological laboratory methods and culture.

Despite the adequate provider's training in university and in medical residences, the breadth of knowledge, and the speed that has been experienced in recent times, the evolution of this knowledge sometimes can lead to particular difficulty in understanding each technology of complementary diagnostic method, its indications, and limitations.

The purpose of this protocol is, through the knowledge and experience of the authors who have been working in the area for a long time, to collaborate with those who work in the lower genital tract to understand the indications and interpretations of the methods available.

Most Common Diseases of the Lower Genital Tract

We can divide the lower genital tract diseases in a compartmentalized one. The diseases affect the vulva, vagina, and/or cervix. We will focus on infectious conditions since biomolecular methods are formatted especially for identifying bacteria, fungi, and viruses.

Infectious Diseases of the Vulva

1. Vulvovestibulitis

Clinical condition	Possibility of infection	Biomolecular method for diagnosis
Itching, irritation, cracking	Candidiasis Tínea	Multiplex panel for Candida

2. Genital ulcer

Clinical condition	Possibility of infection	Biomolecular method for diagnosis
Painless single ulcer	Sífilis (*T. pallidum*)	multiplex panel for genital ulcer
Multiple, small, coalescing ulcers	Herpex simples	multiplex panel for genital ulcer
Small ulcers with inguinal adenopathy	Lymphogranulma venereum (*Chlamydia trachomatis*)	multiplex panel for genital ulcer
Friable ulcer with serpiginous edges	Chancroid (*Haemophilus ducreyi*)	multiplex panel for genital ulcer

As you can see in the table above, the clinical diagnosis is more challenging for most diseases. Of course, a typical case of herpes can make the clinician feel at ease about its diagnosis. However, there are situations where the diagnosis deserves a complementary test. The search for an etiological diagnosis without using multiplex platforms can be much more complex and frustrating due to low sensitivity of traditional tools, such as examining Tzanck cells in smears of ulcers to diagnose genital herpes.

When to indicate a genital ulcer panel?	Nonspecific ulceration.
How to collect the sample to be analyzed?	Material from the lesion (bottom and edge) must be collected with a delicate brush (provided by the laboratory), and the tip of this brush must be detached and fixed in a fixative medium provided by the laboratory (the same as cytology in liquid medium [SurepathTM or Thinprep®]).
How to request the exam?	The request must be forwarded with the patient's data and clinical findings. The order must be for a multiplex panel for genital ulcers. It is not necessary to specify the pathogen, but it is essential to know which ones are included in the probe. They are usually: *T. pallidum*, HSV 1, HSV2, *Haemophilus ducreyi, Chlamydia trachomatis*, *Varicella-zoster virus*, and *Cytomegalovirus*.
How to interpret the exam?	The identified pathogen(s) will likely be associated with the ulcer. However, clinical correlation is essential.

3. Genital wart

Indeed, the most frequent infectious genital wart is condyloma acuminatum, caused by human papillomavirus (HPV). Most are benign and easily diagnosed. However, it may eventually be necessary, due to some characteristic of the lesion, to research the infectious agent, that is, the HPV, and its genotype.

When to indicate HPV panel?	We are faced with a nonspecific or exuberant picture.
How to collect the sample to be analyzed?	The material should be collected from the lesion with a delicate brush (supplied by the laboratory), and the tip of this brush should be detached and fixed in a fixative medium provided by the laboratory (the same as liquid cytology [SurepathTM or Thinprep®])
How to request the exam?	The proposal must be forwarded with the patient's data and clinical findings. The order must be PCR for HPV in a multiplex panel with genotyping.
How to interpret the exam?	The first thing is to see if the test was positive for HPV. The identification of the specific genotype is carried out by the platform, which allows ruling out or confirming the participation of high-risk HPV.

We reiterate that the HPV test in cases of genital warts is an exception and should only be done in cases of a difficult diagnosis.

Infectious Diseases of the Vagina

The vaginal compartment is extraordinarily complex and may have clinical manifestations from a specific infection, but mainly due to an imbalance in its microbiome, called dysbiosis.

The complaint is predominantly vaginal discharge, which soils underwear of variable colors and associated symptoms such as genital itching and odor. Considering only clinical findings for diagnosing infections (vaginitis) and dysbiosis (vaginosis and candidiasis) is at least frivolous and dangerous. The risk of error is very high. If we get the diagnosis wrong, we will undoubtedly have a fruitless and frustrating treatment.

Usually, these conditions can be diagnosed by established morphological methods, such as direct examination, Gram-stained bacterioscopy, and even the Papanicolaou test. However, there are situations where it is necessary to know better which agents are associated with the conditions, especially when there is persistence.

1. Bacterial vaginosis (BV)

It is an anaerobic dysbiosis with variable participation of bacteria. The clinic and morphology give its laboratory diagnosis. Nevertheless, morphology has its limitations when it is necessary to identify which species are associated with bacterial vaginosis. Today, specific research on bacteria may allow the elaboration of adequate treatment with less recurrence.

When to indicate bacterial vaginosis panel?	In the face of a persistent or recurrent condition, trace the bacterial "signature".
How to collect the sample to be analyzed?	Medial, lateral vaginal wall material should be collected with a delicate brush (supplied by the laboratory), and the tip of this brush should be detached and fixed in a fixative medium provided by the laboratory (the same as liquid cytology [SurepathTM or Thinprep®])
How to request the exam?	The request must be forwarded with the patient's data and clinical findings. The order must be a multiplex panel for bacterial vaginosis.
How to interpret the exam?	The result of the exam brings the individual identification of several anaerobic bacteria (*Atopobium vaginae,* Bacteria associated with bacterial vaginosis 2 (BVAB2), *Bacteroides fragilis, Gardnerella vaginalis, Megasphaera* Type 1 and *Mobiluncus* spp.). *Megasphaera* and *Mobiluncus* may suggest a greater risk of persistence when treated traditionally.

2. Vaginal candidiasis

This is the second most frequent diagnosis in cases of vaginal discharge. It is essential to understand that the presence of fungus is not necessarily linked to vaginitis. Fungi can be commensal. For this reason, it is crucial to request research on these agents in cases of vaginitis, especially those associated with pruritus. In most cases, the condition is considered simple and easy to treat. However, some conditions are complicated, and more than identifying the yeast, it is imperative to identify the species for proper treatment. Complicated candidiasis is associated with immunosuppression, pregnancy, and recurrent conditions. Under these conditions, requesting culture or research by PCR on a multiplex platform is indicated. The culture is cheaper; however, the patient usually must go to the laboratory to collect the sample. On the other hand, PCR allows using the cytology material in a liquid medium, which has already been collected, so there is no need to go to the laboratory.

When to indicate Candida panel?	Cases of complicated candidiasis, such as recurrent cases.
How to collect the sample to be analyzed?	Medial, lateral vaginal wall material should be collected with a delicate brush (supplied by the laboratory), and the tip of this brush should be detached and fixed in a fixative medium provided by the laboratory (the same as liquid cytology [SurepathTM or Thinprep®])
How to request the exam?	The request must be forwarded with the patient's data and clinical findings. The order must be a multiplex panel for Candida.
How to interpret the exam?	The test result brings the individual identification of Candida species (*Candida albicans, Candida dubliniensis, Candida glabrata, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis*).

3. Mixed vaginitis

Mixed vaginitis is a condition in which two or more pathogens are identified. One of the most frequent associations are bacterial vaginosis and candidiasis. Although it may seem paradoxical, sometimes mutual encounters can occur (in about 15 to 20% of vaginosis), and treatment must be simultaneous. It is vital to ensure the fungus has a pathogenic action, not just an incidental finding of a commensal yeast. Simultaneous research with two panels can be requested. However, only the vaginosis panel can help to identify the bacterial signature since the panel cannot give the impression of Candida of pathogenic action.

4. Trichomoniasis

It is a sexually transmitted parasite, primarily asymptomatic. As it is a sexually transmitted, the certainty of diagnosis can help avoid diagnostic errors that can have high psychological and legal costs.

When to indicate PCR for Trichomonas?	Cases of suspected infection or screening for the population at risk.
How to collect the sample to be analyzed?	Material from the mid-lateral vaginal wall should be collected with a delicate brush (supplied by the laboratory), and the tip of this brush should be detached and fixed in a fixative medium provided by the laboratory (the same as liquid cytology [SurepathTM or Thinprep®]).
How to request the exam?	The request must be forwarded with the patient's data and clinical findings. The order must be PCR for *Trichomonas vaginalis**. * *Trichomonas vaginalis* is included in the STI panel. Thus, collecting the cervical canal and requesting the STI panel may be more cost-effective.
How to interpret the exam?	The result will be positive or negative for *Trichomonas vaginalis.*

Infectious Diseases of the Uterine Cervix

1. HPV

The greatest importance of HPV is its association with squamous intraepithelial lesions, especially high-grade lesions (HSIL), which are the true precursors of cervical cancer. Today we know that types of HPV have different risks for HSIL and cancer. Hence the importance of identifying the genotype and its persistence since these are important risk factors.

When to indicate PCR for HPV with genotyping?	The most important world associations have established the following indications for the investigation of HPV genotypes in the uterine cervix: 1. Screening for precursor lesions of cervical cancer (secondary prevention of cervical cancer) 2. Post-treatment of intraepithelial lesions of the uterine cervix (cure criterion) 3. Cases with atypical squamous cells of undetermined significance (ASC-US). 4. Cases with cytology of atypical glandular cells*. 5. Cases of abnormal cytology and normal colposcopy*. * Non-consensual indication. The studies established the following indications for investigating HPV genotypes in the anus: 1. Screening in men who have sex with men. 2. Screening in women with high-grade squamous intraepithelial lesions. 3. Screening in women with associated HPV lesions in at least three sites and 4. Women with immunosuppression.
How to collect the sample to be analyzed?	The material should be collected from the uterine cervix or anus (according to indication) with a delicate brush (supplied by the laboratory), and the tip of this brush must be detached and fixed in a fixative medium provided by the laboratory (the same as for cytology in liquid medium [SurepathTM or Thinprep®]).
How to request the exam?	The request must be forwarded with the patient's data and clinical findings. The request must be for HPV research with genotyping. There is an option for only high-risk (14 types) or high and low-risk (28 types).
How to interpret the exam?	The result will be positive or negative for HPV by identifying the genotypes:For high risk only: HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 66, HPV 68.For low-risk and high-risk HPV:High risk: HPV 16, HPV 18, HPV 26, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 53, HPV 56, HPV 58, HPV 59, HPV 66, HPV 68 , HPV 69, HPV 73, HPV 82.Low risk: HPV 11, HPV 40, HPV 42, HPV 43, HPV 44, HPV 54, HPV 6, HPV 61, HPV 70.

Infectious Diseases of the Endocervix (cervicitis)

Most cases of cervicitis are asymptomatic. However, the consequences are serious, such as infertility, chronic pelvic pain, repeated pregnancy loss, premature rupture of membranes, premature delivery, recurrent vaginitis, and vaginosis, and increased risk for cervical cancer.

Among the most frequent pathogens are *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis*. The need for screening, especially for the first two, is well-established in young and pregnant women.

When to indicate STI panel?(STI: sexually transmitted diseases)	Annual screening in women under 25 and women with risk behavior. Pregnancy screening (first and third trimesters).
How to collect the sample to be analyzed?	Material from the cervix must be collected (but it can even be from the vaginal introitus due to the high sensitivity of the method) with a delicate brush (supplied by the laboratory), and the tip of this brush must be detached and fixed in a fixative medium provided by the laboratory (same as liquid cytology [SurepathTM or Thinprep®]).
How to request the exam?	The request must be forwarded with the patient's data and clinical findings. The order must be a multiplex panel for STI.
How to interpret the exam?	The test result identifies the following microorganisms: *Chlamydia trachomatis, Mycoplasma genitalium,Mycoplasma hominis*, Neisseria gonorrhoeae, Trichomonas vaginalis, Ureaplasma parvum*, Ureaplasma urealyticum**. * These micro agents do not need treatment as they can be commensal. Only for *U. parvum*, studies suggest treatment during pregnancy due to the risk of poor prognosis. For others (*C. trachomatis, M. genitalium, N. gonorrhoeae and T. vaginalis)* the treatment and approach of sexual partnership is imperative.

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