Your stamp

The information in this booklet is given as a guideline only and is not intended to be exhaustive.
It in no way binds bioMérieux S.A. to the diagnosis established or the treatment prescribed by the physician.

bioMérieux sa
69280 Marcy l'Etoile
France
Tel.: (33) 04 78 87 20 00
Fax: (33) 04 78 87 20 90

www.biomerieux.com
www.biomerieux-diagnostics.com

BIOMÉRIEUX

EMPOWERING CLINICAL DECISIONS™

BACTERIOLOGY

CervicoVaginal

SPECIMENS

BIOMÉRIEUX

This booklet was compiled with the help of:
Doctor Bertille de Barbeyrac,
Doctor Sabine Pereyre
and Professor Cécile Bébéar
(Bacteriology Laboratory EA 3671,
University Victor Segalen, Bordeaux 2 and Bordeaux
University Hospital, France)

CervicoVaginal

SPECIMENS

Anatomophysiological summary

The genital tract is divided into two compartments:

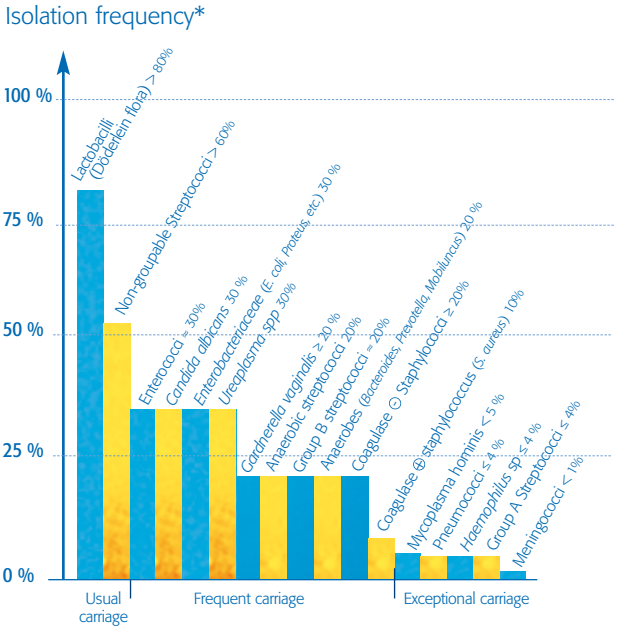
- the upper genital tract, which is bacteriologically sterile,
- the lower genital tract, which is continuously contaminated by flora from the skin and digestive tract.

During their genital activity period, the vaginal flora of women is composed of:

- Döderlein flora (Gram + lactobacilli),
- and many other bacterial species from vaginal colonization.

Vaginal commensal flora

In the absence of infection, for a woman during the genital activity period, the quantity of bacteria per gram of secretions is 10^6 to 10^9.
The isolation frequency of the various species is summarized below:



*According to bibliographic ref. 4 on page 13.

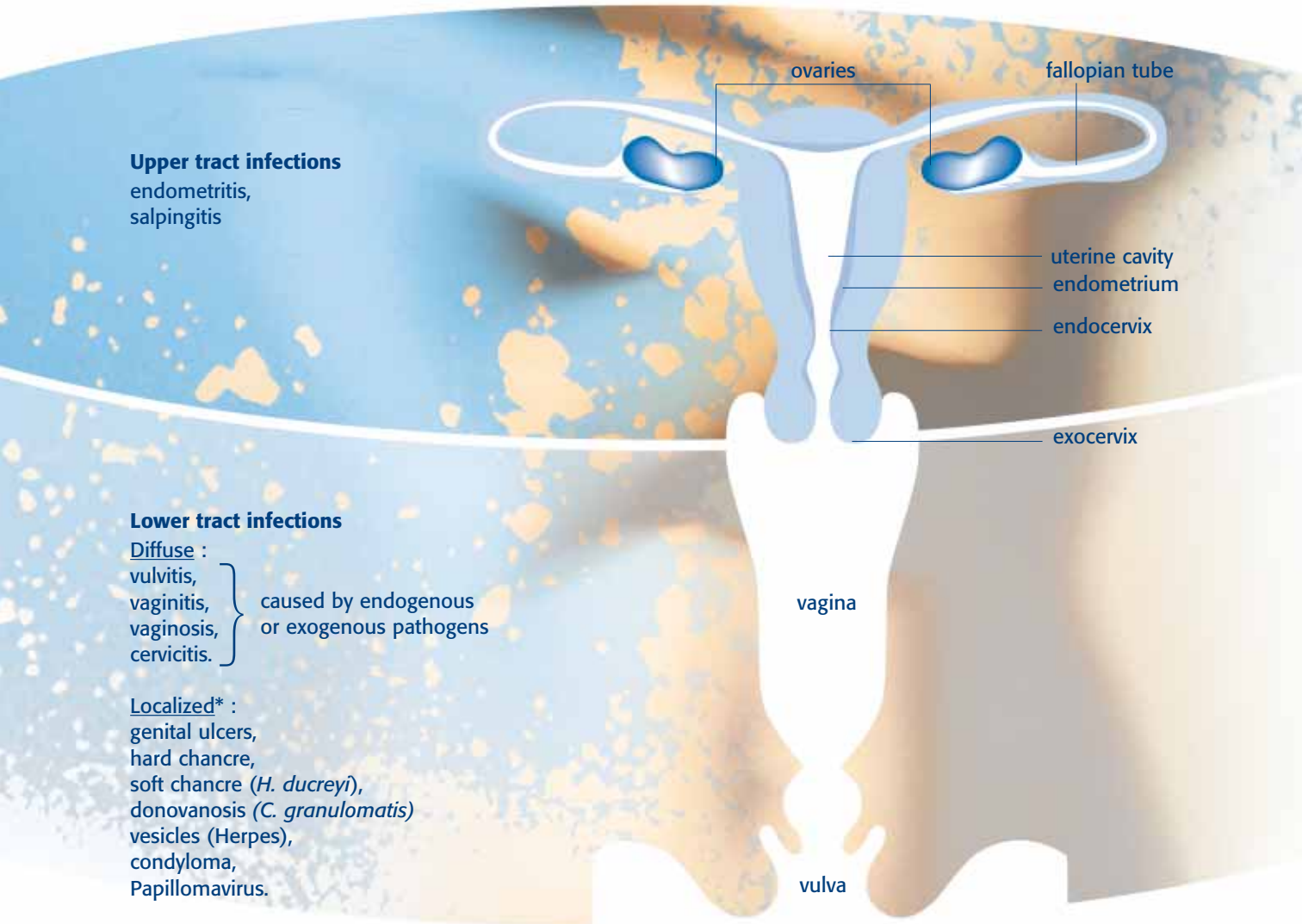
The vaginal ecosystem is influenced by **hormonal factors** which vary during the major stages of genital life (puberty, pregnancy, menopause, etc.) or are caused by an endocrine disease.

The vaginal commensal flora is abundant and varied. This makes specimen interpretation particularly difficult when commensal bacteria that have become pathogenic are concerned.

Female genital infections: pathogenic and clinical aspects

The analysis of a vaginal specimen consists in identifying, within the flora present:

- the organisms normally absent,
- the organisms normally present, but for which the relative quantity is abnormal. This is referred to as dysmicrobism.



Upper tract infections
endometritis,
salpingitis

Lower tract infections

Diffuse :
vulvitis,
vaginitis,
vaginosis,
cervicitis.
} caused by endogenous or exogenous pathogens

Localized* :
genital ulcers,
hard chancre,
soft chancre (*H. ducreyi*),
donovanosis *(C. granulomatis)*
vesicles (Herpes),
condyloma,
Papillomavirus.

* not discussed in this document.

Table of female genital infec tions

UPPER TRACT INFECTIONS

Pathologies	Infectious agent	Clinical signs		Context	Frequency
endometritis, salpingitis, etc.	*Chlamydia trachomatis* *Neisseria gonorrhoeae* *Bacteroides fragilis* and *Prevotella bivia* *Enterobacteriaceae* (*E. coli.*) Less frequent: streptococci B and other β-hemolytic and non β–hemolytic Streptococci, Enterococci, Staphylococci, *Ureaplasma spp, Mycoplasma hominis, Mycoplasma genitalium*	typical acute form with pelvic pain, fever, metrorrhagia, etc.	but also atypical forms and particularly relatively non-symptomatic, or asymptomatic forms	• <u>primary infections:</u> following an ascending infection (*Chlamydia*, gonococci, anaerobes, mycoplasmas) • <u>iatrogenic infections:</u> surgical context (endouterine examination, postpartum, curettage, IUD)	+

DIFFUSE LOWER TRACT INFECTIONS

Pathologies	Infectious agent	Clinical signs Leucorrhea	 Associated signs	Context	Frequency
vulvovaginitis	*Candida albicans*	whitish, granular	pruritus, vaginal burning, dyspareunia, vulvovaginal edema	oral contraception, antibiotic therapy, pregnancy, diabetes, local washing with antiseptic (acidic)	+++
	Trichomonas vaginalis	greenish, malodorous	burning, dyspareunia, dysuria	sexual transmission, low estrogen (alkalinization of vaginal environment)	+
vaginosis	*Gardnerella vaginalis* alone or associated with anaerobes (*Mobiluncus, Bacteroides* and *Prevotella* in 80% of cases) and *Mycoplasma hominis*	grayish, foamy, malodorous (characteristic fish-like odor) vaginal pH > 4.5	few inflammatory signs		++
cervicitis, cervicovaginitis	*Chlamydia trachomatis*	blood-tinged if present	often asymptomatic, hemorrhagic cervicitis	• STI (sexually transmitted infection) • systematic examination • detected due to complication (salpingitis, sterility evaluation, etc.) • infected partner • risk factor (age, change of partner, multiple partners)	++
	Neisseria gonorrhoeae	yellowish if present	often asymptomatic	STI detected on examination	rare
	Mycoplasma genitalium		often asymptomatic	detected on examination	

When to take a cervicovaginal specimen

In the presence of clinical signs of infection

- signs of vulvovaginitis: leucorrhea, pruritus, dyspareunia, dysuria, etc.
- suspected upper tract infection: pelvic pain, with or without fever, metrorrhagia.
- systematic gynecological examination showing signs of infection: cervicitis, etc.

In pregnant women to detect bacteria causing materno-fetal and neonatal infections

- systematic screening for carriage of Group B Streptococci (GBS) is recommended in the late stages of pregnancy, between 34 and 38 weeks of amenorrhea.[7]

For systematic screening

in the case of an STI-carrying partner or at-risk behaviour.

Important recommendations

- **In the event of antibiotic therapy, observe an interval of:**
  - 4 weeks for *C. trachomatis*,
  - 1 week for other micro-organisms,
  before sampling. If this is not possible, inform the laboratory.
- **Refrain from washing in the 24 hours before sampling.**
- **Avoid menstrual periods.**
- **Sample with a speculum, without lubricant.**
- **Note: the appearance of the leucorrhea, cervix and vaginal mucosa.**
- **Use 2 swabs (alginate or Dacron) per site**
  - one for the direct examination,
  - the other for the cultures (transport medium).
- **Send to laboratory without delay.**
- **Specify the sampling site on the prescription and any specific medical context.**

How to take a cervicovaginal specimen

As a general rule, it is important to note that the reliability of a cervicovaginal specimen result depends on compliance with the sampling procedures, the shipping conditions of the specimen to the laboratory and the information provided by the prescribing physician.
(see diagram on pages 8 and 9)

Sampling sites:

These are determined by the clinical signs and must include at least the vagina and endocervix.
Depending on the context, the specimen may also be taken from the vulva, urethra, anus, Skene's and Bartholin's glands, etc.

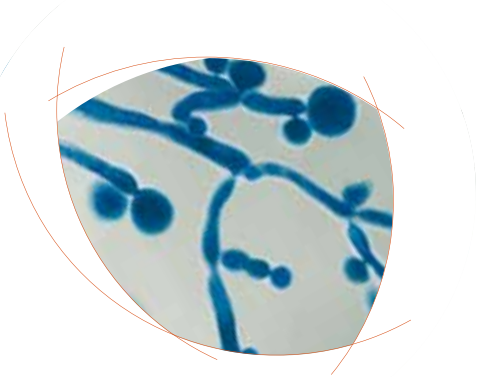
Vaginal specimen:

- on vaginal wall and in posterior cul-de-sac,
- sample as much of the secretions as possible.

Endocervical specimen:

- after thorough cleansing of the exocervix with a sterile compress soaked in saline solution.
- insert the swab into the endocervical cavity.

Note: do not take endocervical specimens from pregnant women.



C. albicans

Cervicovaginal sampling

All cervicovaginal specimens not collected in a laboratory must be shipped in a transport medium. Accompanied by 2 smears on non-fixed slides, they must be shipped without delay.

This transport medium must be able to keep the organisms alive for the time required for their culture, e.g.:

PORTAGERM AMIES AGAR + SWAB

- 24 hours to 48 hours for a large number of micro-organisms,
- 24 hours for fastidious bacteria such as *Neisseria gonorrhoeae*, *Streptococcus pneumoniae* and anaerobes.

UROGENITAL MYCOPLASMAS
(R1 reagent: resuspension fluid):

- 5 hours at 18-25°C • 48 hours at 2-8°C • > 48 hours at -70°C

Specimen collection 1

In vagina (posterior cul-de-sac)

Mucosal secretions or swabbing

- Aerobic and anaerobic commensal bacteria
- Vaginosis-related bacteria (*G. vaginalis, M. hominis*…)
- Pyogenic bacteria in abnormal quantities
- Yeasts
- *Trichomonas*
- *C. trachomatis*
- *N. gonorrhoeae*

after cleansing of exocervix

Specimen collection 2

In endocervix

Endocervical secretions and cells after elimination of endocervical secretions and scraping of mucosa

Specific detection of *C. trachomatis, N. gonorrhoeae* and *M. genitalium* and according to context (upper tract infection, pathological pregnancy) : *Ureaplasma* spp. and *M. hominis*

How to interpret a cervicovaginal specimen

Reading a cervicovaginal specimen result should make it possible to:
- confirm infection,
- identify the causative infectious agent(s),
- determine, if required, the antibiotic susceptibility of the organism involved.

1 Cytology

Direct examination is an essential stage.
Fresh mounting and May-Grünwald-Giemsa (MGG) staining are used to observe *Trichomonas*.
The nature and quantity of the cells present must be taken into consideration.

- Polynuclear cells indicate infection if present in an abundant quantity (vaginitis or vulvovaginitis).
- Epithelial cells show the presence of desquamation, frequently detected in vaginosis in the form of "clue cells" (bacteria-coated epithelial cells).
- *Trichomonas vaginalis.*

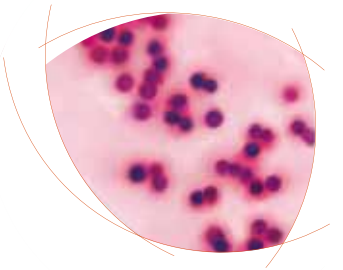
2 Identification of infectious agent

- Gram staining is used to :
  - evaluate the Döderlein flora, its abundance or replacement by a more or less polymorphous flora,
  - observe any dysmicrobism,
  - assess the presence of specific species, such as *Mobiluncus*,
  - observe the presence or absence of yeasts (and assess their abundance), and filaments,
  - observe intraleukocytic Gram-negative cocci (*N. gonorrhoeae*).

- Cultures, or specific detection by gene amplification, are used to isolate or identify the infectious agents: bacteria and yeasts.

If an infectious agent normally absent from the vaginal flora is detected, it must always be taken into consideration, irrespective of the quantity: *Neisseria gonorrhoeae, Chlamydia trachomatis* and *Mycoplasma genitalium*.

For other organisms, identification is only significant when associated with their quantification and predominant presence.
- pneumococci, group A and B streptococci, *S. aureus, N. meningitidis, M. hominis* > 10^4 CCU (color-changing unit)/ml.



N. gonorrhoeae

Antibiotic susceptibility testing

- **Essential** for some pathogenic species such as *Neisseria gonorrhoeae* due to the frequency of antibiotic resistance. β-lactamase testing must be performed systematically for this species.

- **May also be determined by the clinical context:**
  - Urogenital mycoplasmas (susceptible to tetracyclines, macrolides and related antibiotics as well as fluoroquinolones).
  - Group B streptococci (80% cycline-resistant strains, 5% macrolide-resistant strains).

  * *M. hominis* : naturally resistant to 14- and 15-member ring macrolides.
  Ureaplasma spp. : naturally resistant to lincosamides

- **Not performed for:**
  - *C. trachomatis* (susceptible to tetracyclines, macrolides and fluoroquinolones),
  - *M. genitalium,*
  - *Gardnerella vaginalis* (susceptible to imidazoles), *Mobiluncus*.

As a general rule, any strain considered to be pathogenic must undergo antibiotic susceptibility testing in order to guide or validate the treatment or for epidemiological purposes.

Bibliography

1. Baker C.J. Groupe B Streptococcal infections. Infections in perinatology. 1997, 24, 59-70.
2. Bébéar C., Bébéar C.M. Infections humaines à mycoplasmes. 2007. Revue Française des Laboratoires. N°391;63-69
3. Bébéar C.M., De Barbeyrac B., Pereyre S., Bébéar C. Mycoplasmes et chlamydiae : sensibilité et résistance aux antibiotiques. Revue Française des Laboratoires. 2007. 391; 77-85
4. Blond M.H., Poulain P., Gold F., Bingen E., Watier H., Quentin R. Infection bactérienne maternofoetale. Gynécologie Obstétrique. 2005. 28-90.
5. De Barbeyrac B., Clerc M., Raherison S., Bébéar C.M., Bébéar C. Infections humaines à Chlamydiae : diagnostic biologique et épidémiologique. Revue Française des Laboratoires. 2007. 391;71-76.
6. Gorbach S.L., Bartlett J.G., Blacklow N. R. Infectious Diseases. W.B. Saunders Company. 2004 .
7. CDC - Morbidity and Mortality Weekly Report, Recommendations and Reports, August 16, 2002 / Vol. 51 / No. RR-11, Prevention of Perinatal Group B Stretococcal Disease, Revised Guidelines from CDC.

bioMérieux's Solutions

Culture-based Tests

Routine testing

Culture media

N. gonorrhoeae	*Streptococcus / Staphylococcus*	*Gardnerella vaginalis*	Enterobacteriaceae & Non-Enterobact.	Yeasts	*Mycoplasma*
				C albicans	
Chocolate +PolyViteX VCAT3	Columbia CNA + 5% sheep blood detection of GB Streptococcus, *S. aureus* and *Listeria monocytogenes*	Gardnerella agar	Mac Conkey	chromID™ Candida	Mycoplasma IST 2
Chocolate + PolyViteX	Columbia or Trypcase-Soy + blood	Columbia or	BCP	Sabouraud chloram. genta 2	
	chromID™ S. aureus	Trypcase-Soy + blood			
Gonoline DUO 2					

Identification and Resistance testing — API/ATB

N. gonorrhoeae	*Streptococcus / Staphylococcus*	*Gardnerella vaginalis*	Enterobacteriaceae & Non-Enterobact.	Yeasts	*Mycoplasma*
				C albicans	
API NH	API® 20 STREP	API® 20 STREP	API 10 S	API CANDIDA	Mycoplasma IST 2
	RAPID ID 32 STREP	API CORYNE	API 20 E	API 20 C AUX	
	API STAPH	RAPID ID 32 STREP	RAPID 20 E	ID 32 C	
	ID 32 STAPH		ID 32 E		
			RAPID ID 32 E		
			API 20 NE		
			ID 32 GN		
	ATB™ STREP 5		ATB G-5	ATB FUNGUS 3	
	ATB ENTEROC 5		ATB UR 5		
	ATB STAPH 5		ATB PSE 5		
	NCCLS Standard		RAPID ATB E 4		
			NCCLS Standard		

VITEK® 2

NH Identification card	GP Identification card	NH Identification card	GN Identification card	YST Identification card

Specific testing

Culture media

Anaerobes	*Haemophilus*	*Listeria*	*Group B Streptococcus*
Schaedler + blood	Haemophilus Chocolate 2	Columbia CNA + blood	Todd Hewitt broth + antibiotics
			chromID™ Strepto B, Granada agar and tubes
	Chocolate PolyViteX agar	Columbia	Columbia or Trypcase-Soy + 5% sheep or horse blood
		or Trypcase-Soy+ blood	

Identification and Resistance testing — API/ATB

Anaerobes	*Haemophilus*	*Listeria*	*Group B Streptococcus*
API 20 A	API NH	API LISTERIA	API 20 STREP
RAPID ID 32 A			RAPID ID 32 STREP
			API STAPH
			ID 32 STAPH
ATB ANA	ATB HAEMO		ATB STREP 5
NCCLS Standard	*NCCLS Standard*		ATB ENTEROC 5
			ATB STAPH 5
			NCCLS Standard

VITEK 2

	NH Identification card	GP Identification card

Other Identification Tests

Chlamydiae
Chlamydia direct IF

Molecular Offer

- NucliSENS® tests use real-time NASBA® amplification technology and nucleic acid BOOM® extraction technology
- NucliSENS Easy Q® HSV
- NucliSENS Basic Kit for home-brew real-time NASBA applications (www.basickit-support.com)

NOTES