GUIDELINES FOR THE DIAGNOSIS OF VAGINOSIS-VAGINITIS IN PRIMARY CARE OF WOMEN IN FERTILE AGE OR MENOPAUSE

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2.1.- Concept of Vaginal Dysfunction (VD): Vaginosis, Vaginitis, and vaginosis/vaginitis simultaneous condition .

The perception of signs and symptoms at the level of the lower genital tract is the most frequent cause of medical visits of pregnant and non-pregnant women in fertile age, and with minor degree, but significant importance in menopausal women.

In addition a considerable number of women make non-medical consultations in pharmacies and another even higher number of women bear their condition either without any treatment or using "home" treatments.

The morphological study of the Balance of Vaginal Content (Balance del Contenido Vaginal:BACOVA in Spanish) shows that up to 50% of pregnant and non-pregnant asymptomatic women present significant alterations in at least one of two basic vaginal functions. A significant percentage (15 to 30%) of women who consult for symptoms (in the absence of obvious signs) of vaginal itching, burning sensation, or dyspareunia, show no morphological alterations in the vaginal content (1).

The great magnitude of the problem of VD, which includes symptomatic and asymptomatic patients, but all exposed to an increase in sexual and reproductive health risks, requires a joint effort of the biomedical staff, health-care providers, organized social groups and the National Health Service, to optimize the primary attention of vaginosis/vaginitis in women in fertile age and menopause.

The first stage is to improve and coordinate the **diagnosis of VD.** The signs and symptoms compatible with VD are presented individually or associated in an arbitrary and disjointed manner. The most frequent are: itching, burning sensation, irritation, odor, abnormal vaginal discharge, edema in the vulvovaginal area, vulvodynia, dysuria, dyspareunia and/or pain in the pelvic region.

The signs and symptoms mentioned above, either individually or together, are associated with a significant number of pathologies of the female genital tract. They are not pathognomonic for diagnosis of vaginosis, vaginitis or any specific etiology. They only allow establishing, in a presumptive way, the state of possible VD (1, 2). Multiple studies conclude that a reliable diagnosis cannot be made on the basis of history or physical examination along (1, 11)

A very efficient laboratory study has been developed. (1 to 7). The morphological study of the BACOVA (Annex I), which integrates two basic vaginal functions evaluation. The systemic regulation of the vaginal microbiota using Nugent score (NS) (5) and the simultaneous determination of the Vaginal Inflammatory Response (VIR) (8), allow the precise diagnosis of the two most

highly frequent vaginal pathologies: Vaginosis and Vaginitis and with a high predictive value, cases in which both alterations are present (1).

RIV is established with a high efficiency counting procedure for leucocytes in de vaginal content.

Vaginosis is defined, based on the alteration of the healthy vaginal ecosystem and demonstrated absence of VIR (3, 5, 9).

Vaginitis shows presence of significant VIR in the vaginal content, **with or without alterations of the** healthy vaginal ecosystem (1, 6, 9).**Vaginitis, with or without simultaneous vaginosis.**

The etiology of vaginosis is not definitely clear (10). However, there is agreement that the metabolic factors include a systemic imbalance of the "estrogen factor" and/or an alteration in the innate proinflammatory response as a stage previous to the alterations in the complex function (sexual/reproductive) of the vagina (1). Till now the most conspicuous detection of these alterations is the change in the balance of the healthy vaginal ecosystem. A decrease in the relative amount of Lactobacilli and a simultaneous increase in the endogenous anaerobic microbiota (1, 5, 9,10). Internationally accepted bacterial morphotypes count proposal by Nugent (5) is the gold standard (7) . Nugent Score have been incorporated in BACOVA,, but there are others variations of the same basic methodology with valid results (16).

In addition to induce the reduction o lactobacilli and promoting the relative growth of the native anaerobic microbiota of the vagina, the state of vaginosis or **primary vaginal dysfunction** significantly increases the colonization of opportunistic bacteria in the vaginal content of all women in fertile age/menopausal, independently of whether they are sexually active or not. At the same time, it increases the risk of acquiring and transmits sexually transmitted infections (STIs) in those who are sexually active (1).

So far, no specific infectious etiology of vaginosis has been found (10). Common bacteria of the vaginal content, such as *Gardnerella vaginalis, Atopobium vaginae, Mycoplama* spp.*, Ureaplasma* spp., *Prevotella* spp., *Clostridium* spp., *Leptotrichia* spp., *Megasphaera* spp.*,* and many others (1), can become aggressive depending on the degree of the systemic alterations manifested in the function of the vaginal epithelium. The increase in the relative amount of these species and their eventual aggressiveness depends on the insufficiency of the epithelium previously damaged by the systemic primary vaginal dysfunction. The ability of some of these bacteria to develop biofilms has added important material of controversy in the discussion of the problem of relapses in patients with Bacterial Vaginosis (1,16). In fact, until the present, no etiologic factor of Bacterial Vaginosis can be assigned to a specific microorganism.

This have been confirmed with the recent research of the microbiome of vaginal content, in which 280 Operational Taxonomic Units (OTUS), including a significant number of non cultivated species, have been identified (1).

The controversial results of antibiotic treatment could be explained accepting that the real etiology of BV is not infective.

Vaginitis as a basic diagnostic evidence, requires the presence of VIR in the vaginal content, and the etiology could or could not be infectious.

As exceptions, with very low frequency in fertile age, VIR could be associated with atrophic non-infectious vaginitis.

Its significant increase of leucocytes in the vaginal content is a strong sign of the vaginal, cervical and/or upper urogenital infection. There are few specific infectious agents that produce real vaginitis, but two of them, Yeast and Trichomonas, have a universal and high prevalence.

The morphological study of the BACOVA relating the Nugent score (NS) and VIR allows identifying five **Basic Vaginal States (BVS).** These states are diagnosed by a methodology accessible to low-complexity laboratories and the results provide the highest current predictive value (1, 3-6). BACOVA provides the most efficient laboratory report that ensures rational therapeutic clinical follow-up of DV, in the primary health care of both women in fertile age and menopausal women.

The predictive value (positive and negative) of the syndromic diagnosis to distinguish the state of vaginosis or vaginitis does not exceed 50% (1,2) and is even less efficient to define cases in which both functions are altered.

pH and amine odor also do not contribute efficiently to improve syndromic diagnosis of vaginosis, vaginitis and vaginosis/vaginitis simultaneous cases.

We developed BACOVA supporting the previous results of Donders (6) about the definition of a new syndrome: Aerobic Vaginitis (1). Most of the typical cases of Aerobic Vaginitis are included in BVS V (Microbial Non- Specific Vaginitis).

As a regular practice BV is diagnosed only by the 7 to 10 NS, but 5 to 10% of all this cases adds evidence of RIV, what require a clinical and therapeutic different follow up.

2.2.- Basic Vaginal States (BVS)

The NS is expressed from 0 to 10, where a value of 0-3 indicates normal microbiota (most Lactobacilli), a value of 4-6 indicates intermediate microbiota (relative decrease in Lactobacilli and rest of residents bacteria overgrowth) and a value of 7-10 indicates a significant alteration in the vaginal microbiota (Lactobacilli practically disappear and practically absolute presence of the rest of native mainly anaerobic microbiota.).

The number of leukocytes present in the vaginal content is simultaneously determined at the same sample. BACOVA give priority to RIV evaluation and leucocytes number is counted in wet mount, Gram and Giemsa smears. The cutoff value that indicates significant or non-significant VIR depends on the microscopy magnification used. (Annex BACOVA report).

Based on the relationship of these two criteria, five BVS can be distinguished (Table I). This constitutes fundamental information for clinical/ therapeutic decision-making in the management of VD (vaginosis/vaginitis) of women in fertile age and menopause.

The BACOVA high predictive value, enrich at the same report, valuable information about morphological detection of yeast, trichomonas, uncommon vaginal bacterial morphotypes and clue and other epithelial cells that have to be absent in a normal vaginal content.

Table I

BASIC VAGINAL STATES (BVS) OF WOMEN IN FERTILE AGE

Basic Vaginal State (BVS)	Acronym	NUGENT SCORE (NS) OF THE TYPICAL MICROBIOTA	VAGINAL INFLAMMATORY RESPONSE (VIR)
I.- NORMAL MICROBIOTA	NM	0 - 3	NO
II.- NORMAL MICROBIOTA + VIR	NM +VIR	0 - 3	YES
III.- INTERMEDIATE MICROBIOTA	IM	4 - 6	NO
IV.-BACTERIAL VAGINOSIS	BV	7 - 10	NO
V.- MICROBIAL NON-SPECIFIC VAGINITIS	MNV	4 - 10	YES

These five states of vaginal function, one of which fits every woman in fertile age, are diagnosed with practically 100% of predictive value (positive and negative) and allow a safe follow-up of the patient. The syndromic diagnosis alone does not reach acceptable predictive values to distinguish between these basic vaginal states and their additional variables (1, 2).

Menopausal women

The value of normal microbiota in a woman in fertile age is 0-3. In normal menopausal women, this value is 0-5 because there is a **physiological decrease in Lactobacilli with no increase in anaerobic microbiota.**

Thus, we have for menopausal women the same five BVS, which differ only in the cutoff values of the NS established in each case, defined (Table II).

Table II

BASIC VAGINAL STATES (BVS) OF MENOPAUSAL WOMEN

Basic Vaginal State (BVS)	Acronym	NUGENT SCORE (NS) OF THE TYPICAL MICROBIOTA	VAGINAL INFLAMMATORY RESPONSE (VIR)
I.- NORMAL MICROBIOTA	NM	0 - 5	NO
II.- NORMAL MICROBIOTA + VIR	NM +VIR	0 -5	YES
III.- INTERMEDIATE MICROBIOTA	IM	6	NO
IV.- BACTERIAL VAGINOSIS	BV	7 - 10	NO
V.- MICROBIAL NON-SPECIFIC VAGINITIS	MNV	6 - 10	YES

2.3.-BACOVA adds predictive Variables to the BVS detected.

The BVS establish the vaginal functional profile, normal, vaginosis or vaginitis with practically 100% of positive and negative predictive value. A unique, morphological study is able to help in differentiated an important percentage of cases that do not need vaginal treatment and define the follow up of vaginosis (Two levels of intensity), vaginitis (in normal strogenic women) and with special importance the cases in witch an immune hormonal alteration (vaginosis) and vaginitis simultaneously occurs

BACOVA allows simultaneously, morphological detection of yeasts (1, 3, 12), trichomonas (1,3,13), atypical bacterial morphotypes (1,3), and/or unusual epithelial cells (1,3). It is of significant positive predictive value in the case of Yeast and Trichomonas, that also distribute with different relative frequencies for each BVS (1).

During the fertile age, cases of atrophic vaginitis are much less frequent (11). In these cases, BACOVA provides **predictive values of alert**. Because there is a deep study of presence or no of VIR, and a priority in detection of the presence of “round” cells, normally absent in vaginal content.

3.- Basic Vaginal States (BVS), some practical data.

The BVS are defined on the basis of the results derived from the morphological analysis of the vaginal content, based on a sample with minimal operative problems and not invasive. (Annex 4).

The basic information report is expressed with **two numbers**, which makes it **predictive**, avoiding all unnecessary and inaccurate description.

The first number is the NS, which expresses the **quantitative relationship** between Lactobacilli and the rest of the natural microbiota of the vaginal content. In fact, it indirectly evidences the normal, intermediate, or altered vaginal epitelial function derived of hormone/cytokine balance of the woman. The state is independent of any specific infectious factor. Complete absence of VIR have to be demonstrated at the same time.

The relative proportion Lactobacilii/anaerobic species move drastically after first menstruation, with minimal variations during normal menstrual cycle (NS 0 at ovulatory time and 3 near m menstruation) and finally a drastic decrease of lactobacilli (wiyjout grow of anaerobic bacteria) at menopausal time, following hormonal variations.

As mentioned above, the NS is expressed from 0 to 10.

The scale of interpretation of the NS is a reliable indicator of the hormone state (estrogen factor) **only applicable in the study of women in fertile age.** As discussed before, to define the state of normal microbiota in menopausal women, the cutoff values of the NS are different. (Table 1 and 2)

The increase in the NS in intermediate microbiota (BVS III), bacterial vaginosis (BVS IV) and microbial non-specific vaginitis, (BVS V) is proportional to the intensity and duration of the own systemic problems of each woman, strongly associated with factors of psychological stress (15).

The second number, which is essential to build the BVS diagnosis, is the expression of the number of leukocytes in the vaginal content, which is interpreted as an indicator of the VIR degree. Traditionally, the two following

cutoff values are used (1,8): a value of 10 leukocytes per field, when the reading is performed with 400 X magnification (usually with wet mount and Giemsa stain readings), and a value of 5 leukocytes per field, when the reading is performed with 1000 X magnification (reading in Gram stain). Both have a similar predictive value. The most accurate report is: **number of leukocytes per epithelial cell per microscopic field, in which more than one means significant inflammatory state in the vaginal content** (8).

BACOVA report the three results when necessary.

If the results are in the cutoff limits, obtained in direct readings of leukocytes per field in wet mount, Gram and Giemsa, the definitive value should be confirmed and reported as leukocytes per epithelial cell per field in Giemsa. We established that, independently of the values obtained in the wet mount examination, Gram, Giemsa stain, the leukocyte/epithelial cell ratio should be **mandatory in the study of pregnant women** (1, 8).

In the vast majority of cases, but no always, the presence of VIR implies a reaction to aggressive states induced by virulence factors of the microorganisms.

It should be taken into consideration that VIR can occur by direct action on the vaginal epithelium and/or come from a state of infectious aggression located in the endocervix or other regions of the genital or urinary tract.

It has been recently shown that the use of condoms increases the relative frequency of BVS II, with VIR, which may be attributed to certain types of spermicides (1).

3.1.- Basic Vaginal States (BVS) basic application.

Consolidation of the of the initial syndromic evaluation.

The diagnostic value of the information attained during the first visit (presumptive syndromic diagnosis) increases significantly with the diagnosis of the BVS generated on solid evidence of the true state of the vaginal content. This increase in the diagnostic value is important for clinical/ therapeutic decision-making.

The primary accurate identification of normal, typical vaginosis, vaginitis and mixed condition allows, according with sings/symtoms, deciding whether or not initiate treatment or indicate a precise microbiological and/or cytological complementary studies if necessary. (1).

Associated prevalent etiologies

With a positive predictive value of 65-75%, BACOVA reports the morphological detection of yeast and/or trichomonas (conventional vaginitis), which are additional supportive criteria that orient the follow-up.

Yeast vulvovanitis accumulates in BVS II and symptomatic trychomoniasis in BVS V.

An important number of cases of BVS I (Normal Microbiota) shows presence of yeast, besides exceptions this cases do not need treatment.

BVS are Independent of pH and Fishy odor study.

BACOVA does not incorporate the determination of pH and/or of amine odor. If these criteria are available from the first visit (and/or at the time of sample collection), they will efficiently contribute to the follow-up decision, especially in the case of BVS II (NM + VIR), in which confirming the pH is

essential. Normal pH is compatible with yeast infection and increased pH with possible cervicitis.

The points below describe concepts that optimize the clinical interpretation once the BVS has been established.

BVS I.- NORMAL MICROBIOTA (NM)

The term normal microbiota refers to a number of Lactobacilli higher than the rest of common bacterial morphotypes, which generates a NS of 0-3, and absence of VIR. These two criteria define morphologically the state of normal microbiota. Normal vaginal function is accompanied with normal pH and absence of amine odor. However, there are cases in which an increase in pH is detected without any morphological abnormal criteria (4).

Exceptionally, trichomonas, atypical bacterial morphotypes and/or unusual epithelial cells may be detected in BVS I. Although yeast, is detected in at least 10% of the BVS I cases, their presence does not determine yeast vulvovaginitis. In this case, the morphological intrinsic state of yeasts should be verified and the determination of the VIR should be adjusted, especially in the cutoff limits and leukocytes/epjthelial cells/per field have to be done..

BVS II.-NORMAL MICROBIOTA WITH SIGNIFICANT VAGINAL INFLAMMATORY RESPONSE (NM + VIR)

This state refers to a number of Lactobacilli higher than the rest of common bacterial morphotypes, which generates a NS of 0-3, but presence of VIR, indicated by the significant increase in the number of leukocytes in the vaginal content. This functional state requires immediate consideration because of its high risk, especially in pregnancy.

It is mandatory to confirm vaginal pH (Annex I). If vaginal pH is normal and the BACOVA report detects yeasts (independently of the morphological evidences) yeast vaginitis should be considered. In the case that BACOVA does not detect yeasts, with normal pH, among other possible decisions derived from the clinical study, culture should be attempted before discarding yeast vaginitis.

BACOVA detect up to 50% of Yeast vulvovaginitis in the total cases accumulated in BVS II.

If the pH is high, the possibility of yeast vaginitis decreases significantly, and cervicitis and/or infection of the upper genital tract or eventual urinary infection should be investigated. If the pH is high, the presence of vaginal trichomoniasis is also possible.

This BVS II with elevated pH is associated with infection by Chlamydia trachomatys, mostly in women under 25 years of age (1).

Recently a significant relative increase in BVS II was detected in a sub group of condom users women (1).

BVS III.-INTERMEDIATE MICROBIOTA (IM)

In this state, the vaginal content shows a decrease in Lactobacilli and a relative increase in bacterial morphotypes mostly corresponding to anaerobic species, which is expressed in the report with a NS of 4-6. This state is defined by this intermediate NS and the absence of VIR. It actually indicates an

unpredictable evolutionary state, in which the woman is recovering the normal state, moving towards a BVS IV (bacterial vaginosis), or maintaining an intermediate state of chronic systemic imbalance.

The presence of clue cells (see item 7.3.1.1 b) **generally suggests a progressive state towards** bacterial vaginosis**. In fact, their presence, as proposed by Lanzafame (1) determines an increase of two points in the real NS obtained.**

The pH (Annex II) and the amine odor (Annex III) may or may not be altered.

BVS IV.-BACTERIAL VAGINOSIS (BV)

The state of the vaginal content that defines the typical Bacterial Vaginosis is the minimal presence or total absence of Lactobacilli and near-absolute dominance of bacterial morphotypes corresponding to the vagina native species (mainly anaerobic). The report is expressed with a NS of 7-10. The definition of BV **requires the demonstrated absence of VIR**.

Studies have shown that the bacterial species present in the normal vaginal content are more than 250, including 18 species of Lactobacilli. Quantitative abnormal relationships between them are conditioned, *prima facie*, by the metabolic changes occurring in the vaginal epithelium primarily altered by hormonal and metabolic deficiencies. To date, no infectious etiology has been assigned to BV, so requesting additional studies of specific microbial agents for diagnosis and/or follow-up of BV is unnecessary (1,2).

A normal pH virtually discards only typical cases of BV (14). In most cases of microbial non-specific vaginitis, but also in some cases of normal microbiota, pH is also high (4). Amine odor provides a positive and negative predictive value of 80%. Remember that in a significant number of cases, amine odor is one of the main reasons for consultation.

BACOVA does not require the determination of pH and/or amine odor to define BV. About 15% of samples from oligo- or asymptomatic women studied have shown a state of BV (morphologically detected), without alteration of either criterion (1).

BVS V.- MICROBIAL NON-SPECIFIC VAGINITIS (MNV)

This BVS V includes those cases that show an altered ratio between Lactobacilli and the rest of the typical vaginal microbiota, This BVS V includes a NS of 4-10, accompanied by significant VIR. This state includes the cases defined as Aerobic Vaginitis by Donders (6), but because of the heterogeneity of oxygen requirement by the involved vaginal and exogenous microbiota, the term aerobic must be discussed.

Typical microbial agents of other niches such as the intestine, mouth, skin, or environment, can be found colonizing the vaginal environment. It is very difficult to attribute an specific etiology in these cases.

It is essential to consider that the VIR detected can be originated in the vaginal epithelium (conventional vaginitis) or respond to infections in other levels of the genital or urinary tract. It is thus necessary to discriminate between the cases with a NS of 4-6 from those with a NS of 7-10 within this BVS V (1). In the group with a NS of 7-10, colonization and/or aggression by yeasts is virtually absent. The permissiveness induced by the primary vaginal dysfunction

(imbalance of the normal microbiota) generates a high risk to all STIs, including a significant increase frequency of association of BVS V with vaginal trichomoniasis (1, 13).

The pH is high in most cases. Amine odor is not always present.

In all cases, the rational use of additional studies from a specialized laboratory (level D) must be forced.

As it was mentioned before most cases of atrophic vaginitis coul be present en BVS V.

3.2.-Conventional vaginitis

Based on their frequency, yeast vulvovaginitis and trichomoniasis need to be prioritized. These are morphologically detectable and their frequencies distribute with clear differences in two BVS.

Yeast in BVS II and trichomones in BVS V.

3.2.1 Yeast vulvovaginitis

Yeast vulvovaginitis refers to the vaginal and/or vulvovaginal inflammatory state induced by yeast. In Argentina, yeast vulvovaginitis is caused mainly by various species of the genus Candida, with prevalence of *Candida albicans*. In the case of typical yeast vulvovaginitis, the pH of the vaginal content is normal and the balance of Lactobacilli and anaerobic microbiota (NS) also remains normal.

Yeast vulvovaginitis is significantly associated with BVS II (NS of 0-3 and significant VIR). Yeasts are regular members of the microbiota of the mouth, intestine, skin and vagina. The presence of yeasts in the vaginal content, determined by morphology, culture and/or gene amplification, is necessary but not sufficient for the diagnosis of yeast vulvovaginitis. Independently of the context of signs and symptoms, it is necessary to prioritize the presence of VIR (in this case, real vaginitis) and as an important additional criterion, detection of morphological changes in yeasts (formation of pseudohyphae and blastospores (18)

BACOVA considers that the presence of yeasts in the vaginal content is significant when it is accompanied by VIR. If there are morphological changes, the diagnosis is reinforced.

There is evidence that the adherence and aggression of yeasts to the epithelium and skin of the vulvovaginal area is favored by a systemic (immune-hormonal) disorder of the patient and not necessarily by a specific yeast virulence factor. Recent studies indicate that there are changes in the receptors of epithelial cells that allow the adherence of yeasts (19). This new environment induces the morphological changes and the inflammatory state response (1).

These immune-hormonal alterations that condition vulvovaginitis are different from those that generate bacterial vaginosis. The association of yeasts in the BVS IV of bacterial vaginosis is minimal or null. There is evidence of the participation of factors of psychological stress in the sensitivity of women to recurrent yeast vulvovaginitis (1,12).

3.2.2 Vaginal trichomoniasis (***Trichomonas vaginalis)***.

The presence of Trichomonas determined by morphology, culture and/or gene amplification is generally distributed with mayor prevalence in BVS V, being the latter usually accompanied with significant VIR and high pH.

VIR is not detected in a small number of cases associated with a reduced number of oligo or asymptomatic women in which, trichomonas are found in BVS I and BVS III and BVS IV (1).

Vaginal discharge is present up to 70% of confirmed cases. Although varying in consistence, the classically described frothy yellow discharge occurs only in 10 to 25 % of women (1).

When the infection becomes chronic, symptoms decrease and secretions lose their purulent appearance due to the decrease in the number of parasites and leukocytes, the increase in epithelial cells, and the establishment of a mixed bacterial microbiota. A large number of parasites are necessary for signs and symptoms to appear. In up to 2% women, a small number of parasites may be present in patients without symptoms, with a normal vaginal pH and a normal microbiota (e.g. BVS I), which can be interpreted as a carrier status.

This case and control post treatment need culture or PCR study in order to detect trichomonas.

Unlike considerations referred for yeasts, if trichomonas are detected, immediate treatment should be indicated in all cases, regardless of the VIR and/or symptoms, because trichomoniasis is a STI. The intermediate microbiota, bacterial vaginosis and microbial non-specific vaginitis states favor the colonization by aggressive trichomonas.

In women urethral infection is present in 90% episodes although the urinary trac is the sole site of infection in less than 5% of cases (17). Tis accord with frequent detection of trichomones in urine sediment study.

3.3. Other pathologies associated with vaginal dysfunction

A number of other diseases of the genital tract generate alterations in the vaginal function or develop signs and symptoms compatible with Vaginal Dysfunction, but without alterations of basic vaginal functions.

Because of their low frequency and other diagnostic algorithms, they are not analyzed in this Guideline for Primary Health Care. With much lower prevalence in the fertile age, these pathologies are associated with atrophic problems (11). Contact vaginitis, allergic vaginitis, Desquamative Inflammatory vaginitis, erosive liquen planus and others (19,20), in general, these cases fall mainly under BVS V and require immediate specialized study.

Patients with atrophic vaginitis may have an abnormal vaginal discharge, dryness, itching, or dyspareunia. Even with a very low frequency in childbearing women it is necessary to be alert and more in menopausal women. A basic diagnostic help is the morphological detection of RIV and parabasal or intermediate cells in vaginal content. BACOVA incorporate Giemsa study in order to increase the RIV diagnostic accuracy and detection the “round” cells it is a very important alert. The pH is high and amine odor negative.(11).

Corollary

The five BVS described are detected by the morphological study of the balance of vaginal content (BACOVA) in all women in fertile age and menopause women, with practically 100% of positive and negative predictive value. They

are actually a postulate of morphological bases, independent of anamnesis or any defined clinical signs and symptoms.

The syndromic diagnosis, even accompanied by the determination of pH and amine odor, does not distinguish all the BVS with acceptable predictive values. These five BVS are extremely useful for the follow-up and immediate therapeutic behaviors. Adding morphological criteria to the BVS detected, by means of BACOVA and/or by data arising from the clinical study (mainly vaginal speculoscopy), will indicate whether or not specific treatment or rational selected specialized studies are necessary.

The most common variables that BACOVA adds to the BVS definition are the detection of yeast and/or trichomonas, which contributes with 65 to 75% of positive predictive value. The negative predictive value is very low.
The report of atypical bacterial morphotypes and unusual epithelial cells has only predictive values of alert. In the case of positive detection, the presence of these types of cells is of great importance to orient the need of specialized studies. The negative predictive value is null.

4. Dimension, social impact and vulnerability of the problem of vaginal dysfunction and associated risks in Argentina.

4.1. Dimension of the problem

In virtually all sanitary health regions of Argentina (1), vaginal dysfunction affects between 20 and 50% of all women in fertile age, thus constituting one of the diseases of greatest dimension (no less than two million women daily), of which 50% are asymptomatic (1).

The results achieved so far still do not allow establishing reliable figures for the prevalence of vaginal dysfunction in menopausal women (1).

In their overall concept (vaginosis/vaginitis), these pathologies generate direct damage that ranges from being asymptomatic to leading to a noticeable loss of the quality of life of the woman affected.

4.2 Social impact

Although these pathologies generate risk of damage in all women with special prevalence in sexual and reproductive health, the social impact of the problem is practically null.

They are associated with increased risks for STIs, infertility, severe gestational problems and puerperal and neonatal infections.

In central countries, vaginal dysfunction is a priority, as revealed by Guidelines of specific procedures (1, 11,17).

4.3. Vulnerability of the problem of VD

Regarding the vulnerability of the problem of VD, this Guideline describes methodologies of high predictive value for its diagnosis in primary health care.

The treatment of Bacterial Vaginosis is a universal problem without definitive answers.

In Argentina, the control of vaginosis is offered in prenatal follow up, but no diagnosis methodology have been indicated yet. The possibility to include BACOVA at the time of prenatal screening for Group B streptococci (35-37 weeks) to obtain complete information of the vaginal function is being currently discussed.

Prevention, except that related to infection by *Trichomonas vaginalis*, which fits into that applied to STIs, is currently null for the rest..

4.4. Associated Risks of vaginal dysfunction

Gynecological risk

- Pelvic inflammatory disease
- Sterility/ infertility
- Postoperative gynecological infections
- Co-factor for lower genital tract carcinogenesis
- Transmission/acquisition of HIV and other STIs

Obstetric and perinatal complications

- Fertility
- Abortions (1st and 2nd trimester)
- Premature births
- Premature rupture of membranes
- Chorioamnionitis
- Puerperal infections
- Post-cesarean infections
- Neonatal infections
- Late sequelae in the newborn

  The analysis of the relationship between vaginal dysfunction (VD) and gynecological-obstetrical risk is discussed in the Support Module

4.5.- Levels of primary care.

This Guideline aims, primarily, to contribute to organize the primary attention of vaginal dysfunction starting with the uniform application of diagnosis management.

It is imperative that complementary laboratory studies (pH and amine odor) and the microscopic study of the vaginal content (BACOVA) be available for the primary health care of all women in fertile age, especially of pregnant women and menopause.

The fact that some sectors of primary health care do not have yet the rational support of a laboratory, we include an elemental level of attention that in a near future must be completed at least with pH and amine odor detection.

The actual situation needs to consider three levels of primary attention:

- **Level A**.- Syndromic diagnosis: when there is no possibility to apply any complementary support.
- **Level B**.- Syndromic diagnosis together with the relative support provided by the determination of pH and/or amine odor, which is possible and necessary to be carried out during medical consultation,

- **Level C**.- Syndromic diagnosis, eventual determination of the pH and/or amine odor and application of the BACOVA, which allow the highest diagnostic predictive value in the management of VD, in primary health care of women in fertile age and menopause.

In places with no conventional laboratories available and/or by decision of the physician, the microscopic study can be carried out within the medical visit. Observing at least the wet mount preparation of the vaginal content, the predictive value of the diagnosis of the BVS is incomplete but significantly positive.

Anyone interested in analyzing this possibility should consult the Medical Area at www.fba.org.ar PROSAR.
Free inscription workshops for training are offered in all Sanitary Regions of the country.

Level D, which is not developed in this Guideline, refers to the availability of benefits that require specialists in the microbiological and/or cytological field.

Proposals or consults in the area of level D can also be made at prosar@fba.org.ar (mentioning medical area).

5. Objectives of the Guideline.

5.1.-To update and propose joint coordinated actions (clinical/laboratory), including scientifically proved positive cost/benefit service. Optimize the primary health care quality and ensure equity in the coverage in all sanitary regions of the country.

5.2.-To use a unified system of request of laboratory studies and standardized reports that guarantee the real predictive value of the studies and and generate information of epidemiological value.

5.3.-To optimize the early detection of the most prevalent pathologies, to offer immediate safe primary health care and/or rationally orient towards the requirement of specialized level of care.

5.4.-To provide a solid added value in the early suspicion of sexually transmitted infections and other pathologies of the lower genital tract**.**

5.5.-To provide, in a parallel and continuous way, a site for consultation and participatory opinion, open to anyone who may be interested, to support the effective use of the Guideline and ensure its annual updates, by rational assessment of its application, scientific advances and the opinion of active users (Medical area. Sexual and Reproductive Health Program. www.fba.org.ar PROSAR).

6.-CONTEXT

6.1. Target readers

The Guideline is directed to all biomedical professionals, especially those involved in primary health care. Open free at the www.fba.org.ar (PROSAR/CONSENSOS)

6.2. Target population and health care levels considered in this Guideline.

The target population includes all pregnant and non-pregnant symptomatic women between post-menarche and pre-post-menopause.

Asymptomatic women in fertile age or menopause that have access to regular controls or perform risk assessment prior to interventions in the Genital Tract.

6.3 Nomenclature

The proposals in this Guideline require an adaptation of the nomenclature of basic benefits at the clinical consultation and laboratory, which must be recognized for the purpose of their incorporation to the nomenclature system and regulation of a rational fee.

6.3.1. In the framework of medical care

A single fee must be established for the determination of vaginal pH and amines test. Similarly, a fee must also be established for the morphological study of vaginal content (wet mount) carried out by the physician.

6.3.2. In the framework of the laboratory.

The study of the BACOVA covers the clinical requirement for primary detection of vaginal dysfunction, validated by all the studies carried out at the national and international levels (3, 7).

BACOVA has been incorporated into the single biochemical nomenclature of the Unified Confederation of Biochemistry of Argentina (CUBRA).

7. Real levels to diagnose vaginal dysfunction that have to be considered in the actual situation.

The experience of the past four years reflected in various reports arising from the application of the Guideline, scientific presentations and national and international publications (1) confirms the need to incorporate the morphological study of the BACOVA in the primary care of symptomatic or asymptomatic women in fertile age, especially pregnant ones (1, 8).and menopause women.

Following the structure of the previous edition and being the lack of access to complementary studies still a problem in a great part of the current fragmented system of health, three levels of primary care (A, B and C) are proposed.

Level D, which involves the availability of specialized services, is mentioned but not discussed.

AT ALL LEVELS
(A, B, C and D)
Clinical study, Anamnesis,
Speculoscopy,
Basic recommendation

The risk of sexually transmitted infections (STIs) should be evaluated without exceptions; in case of high cervical secretion/inflammation, specialized microbiological and/or cytological laboratory tests (level D) are necessary and the exploration of chlamydia and/or gonococci IS ESSENTIAL.

Contact with more than one partner in the last six months should be considered a high risk factor for STIs.

7.1.-Level A

LEVEL A
Clinical study, Anamnesis,
Speculoscopy,
STI risk assessment

This level does not reach acceptable predictive values to differentiate the more frequent variables of vaginal dysfunction.
At least determination of pH and amines test, must be incorporated.

How should patients be evaluated in the absence of microscope?
Level A shows a basic strategic that obviously have to be only accepted as an emergency situation.
Unfortunately there is still a regular practice in many places.
In places in which the possible application of microscopy morphology is still remote, at least pH and amines tests (Level B) have to be done for the medical professional staff. .
Numerous point of care determinations for diagnosis of Bacterial Vaginosis and Trichomonas are offered. Besides the elevated cost, the exact role in

current diagnostic algorithms is unclear, their use should be considered only when a microscope is unavailable.(11)
Among several proposals pH and amines detection have demonstrated positive cost benefit.

7.2.-Level B

LEVEL B
Clinical study, Anamnesis, Speculoscopy, STI risk assessment, determination of pH, and amines test

When unable to access the microscopic study, these two tools improve the predictive value for some specific syndromes (Annex 2 and Annex 3).

DETERMINATION OF pH AND AMINES TEST
SUMMARY TABLE

Criteria / Presumptive vaginal functional state	pH*	Amines test	Abnormal vaginal secretion**
Compatible with normal state* **	**= 0 < 4.5 normal pH reduces but does not exclude the possibility of IM or BV**	**Negative**	**Absent**
Profile associated with IM, BV and MNV, without being able to discriminate among them because of the lack of evidence of VIR	**> 4.5.**	**Generally Positive**	**Homogeneously grayish**
Vaginal trichomoniasis	**> 4.5**	**Often positive**	**Foamy yellowish**
Yeast Vulvovaginitis	**= 0 < 4.5**	**Negative**	**Whitish with lumps**

*There are cases with high pH in which no altered morphological criteria of the vaginal content are detected (4).
** In 90% of cases with evident vaginal dysfunction there is not evidence of vaginal discharge. The frequent solicitude "culture of the flow" should be replaced by “microscopy study of the vaginal content”.
***This profile “hides” cases which present NM (NS = 0-3), normal pH and a significant VIR, compatible with yeast vulvovaginitis.
This confirms the fundamental importance of establishing the presence of VIR (leukocytes quantitative morphology).

Limitations:
With regard to the aspect of the vaginal discharge, the table refers to typical cases, but the predictive value (positive and negative) of the secretion is very low. In fact, in BV, 50% of the cases are asymptomatic and the increase in the vaginal discharge is evident in not more than 10% of the symptomatic cases detected by microscopic morphology.

The determination of pH and amine odor does not confirm the diagnosis, but when negative, it helps to exclude typical cases of BV, MNV and IM (14).
The limitations generated by the measurement of pH and amines odor in the overall framework of the diagnosis of vaginal dysfunction are discussed in detail in the Support Module (1).

IMPORTANT COMENT
A mentioned procedure in all international Guidelines for BV diagnosis is the "clinical" AMSEL integration.
Ph and amines odor had been evaluated before and without microscopy helps partially in Bacterial Vaginosis diagnosis orientation.
Vaginal discharge have heavy limitations. The specificity of homogeneous discharge, in pregnancy has been questioned because many pregnant women experience increase in vaginal discharge.
Changes in vaginal discharge in Trichomones, generates big interference in AMSEL interpretation. .
Finally, detection and quantitative evaluation of clue cells, even only in wet mount, needs a microscope and in using only wet mounth recognition of clue cells a solid experienced operator is needed..

If the clinical group is able to complete de clue cells study as AMSEL requires, during the women visit, there is mandatory that complete the wet mount counting leucocytes at least.

AMSEL along do not distinguish several different vaginal states, including cases of Aerobic Vaginitis.

7.3.-Level C.

LEVEL C
Clinical study, Anamnesis, Speculoscopy,
STI risk assessment, determination of pH, and amines test

Balance of Vaginal content (BACOVA**)**

7.3.1.-Additional aspects in the application of level C

WARNING

When anamnesis and the inspection of the cervix (suspicion of cervicitis) show no indication for the need to request a diagnostic study of specific pathogens, the cost/benefit relationship of any study of isolation and/or detection of any kind of microbial agent is negative until the real picture of the basic vaginal state of the woman is known. In cases of significant VIR, the need or not of specialized microbiological and/or cytological studies should be considered immediately, supported by the report of other morphological criteria and mainly by the update of the syndromic profile.

8.- Valuable data added to the five BVS defined.

Balance del Contenido Vaginal (BACOVA)

The 2012 Manual of Procedures is available free at the web page of the Argentine Biochemistry Foundation (Fundación Bioquímica Argentina): www.fba.org.ar / PROSAR/Consensos.

Given the positive importance provided by the experience in the use of BACOVA, everything related to the collection of samples is detailed in in this Guideline. Annex IV.

Within each of the BVS here described, there are some additional factors that help to define more accurately the most common syndromes and orient therapeutic behaviors and follow-up.

8,1.- Presence of clue cells

The detection of clue cells is of great importance. Up to a NS of 6, the presence of clue cells adds two points to the real NS obtained (3). In the case of an IM state of vaginal dysfunction, the clue cells presence shows a tendency to evolve to BV.

8.2.- Atypical bacterial morphotypes

With some exceptions, atypical bacterial morphotypes have a very low predictive value and do not constitute a criterion defining follow-up decisions with certainty.

Vaginal contents with significant expression of morphotypes compatible with Gram-positive cocci, corynebacteria, enterobacteria, and Actinomyces, which reflect in general, vaginal colonization after alteration of the primary vaginal function, are found with relative frequency.

The BVS should be considered. The presence of a**typical bacterial morphotypes** In the cases of NM, IM and even BV (absence of VIR), is not indication of vaginitis. In the presence of VIR, regardless of the NS, complementary studies have to be done according to level D. Obviously those belomging to the BVS V, are compatible with Aerobic Vaginitis cases (17).

8.3.- Yeast

BACOVA generates a predictive, not descriptive, report. When the the morphological study is compatible with yeast vulvovaginitis, BACOVA reports: **significant presence of yeasts**.

When the presence of yeasts is accompanied by a clear negative VIR status, independent of NS and shows the typical morphology of yeasts without

vegetative morphological alterations, BACOVA reports: **non-significant presence of yeasts.**

8.4. –Trichomonas

The single detection of Trichomonas in the current state of interpretation means “infection”. Independently of other factors such as VIR or pH, the general rule indicates treatment.

8.5- Atypical epithelial cells detected in vaginal content.

The detection of clue cells is of main importance, because they are indicators of great predictive value of the state of vaginal dysfunction that causes BV.

BACOVA is a study required to define the BVS. Also, the detection of yeast and/or Trichomonas, with positive predictive values of 65 to 75%, adds a high cost/benefit value.

Cells that should not be present in the normal vaginal content may be detected in a regular sample from the vaginal posterior formix. BACOVA incorporate an integral study. Added to conventional wet mount and Gram study we incorporate Giemsa staining of vaginal content.

All professionals authorized to carry out clinical analyses have the ability to recognize the morphological differences between typical exfoliated epithelial cells from atypical cells, which are primary identified as "round" cells. The report acts as alert and only specialized professionals can inform about diagnostics details (1)

The report of the presence of **atypical epithelial cells in the vaginal content** by BACOVA only has the **value of "alert**" and never refers to cytological diagnosis of any kind (unless the professional is a specialist in cytology).

The experience analyzed in several training workshops has shown that the addition of the reading of the vaginal contents using Giemsa staining considerably increases the possibility of detecting indicator cells of advanced inflammatory alterations and/or compatible with viral infection (Koiocytes/Virocytes) or atrophic or proliferative states. The alert report allows the clinician to take early decisions to request confirmatory studies at level D.

Comparatively, the case is similar to that raised by the PAP. The PAP is not requested upon the suspicion of an infection by Trichomonas or yeast or to assess the balance of the vaginal content. However, the PAP report frequently confirms the presence of vaginal trichomoniasis or "Gardnerella", indicating major alert in the therapeutic clinical management of the patient.

As a result of preliminary results, the problem of the complementary study of the Giemsa, open new aggregated value to vaginal content study, had deepened. The possibility of detecting atypical cells in the vaginal content increases significantly when taking an additional sample with a wooden spatula and fixing the preparation with alcohol (1). The recently developed methodology of the study of the Vaginal Inflammatory Response (VIR) should be applied. The group led by Dr. Luis Palaoro continuously develops workshops in this regard. The possibility to participate and the working material are available at (www.fba.org.ar PROSAR / ERIGE and/or erige@fba. org.ar)

The Giemsa incorporation is very important, because permit de detection of cells alteration, but also confirm the the results obtained in wet mount/Gram, and several times permit the detection of trichomonas/yeast. Giemsa reading define the diagnosis of presence or not of RIV.

8.-REFERENCES

1.- Módulo de Apoyo a la GUIA PRÁCTICA INTEGRAL (Clínica-Laboratorio) DE DIAGNÓSTICO DE VAGINOSIS-VAGINITIS en la ATENCIÓN PRIMARIA de la MUJER EN EDAD FÉRTIL" Actualización 2011. www.fba.org.ar PROSAR/ CONSENSOS

2.- Landers DV,Wisenfeld HC, Heine RP,Krohn MA, Hillier SL. Predictive value of the clinical diagnosis of lower genital tract infection in women. Am J Obstet Gynecol 2004, 190(4)1004-10.

3.- Di Bartolomeo S., Offner G., Ojeda M., Valle S., Leonino A., de Torres RA. Balance del Contenido Vaginal (BACOVA) valor de la expresión numérica en el diagnóstico de Vaginosis Bacteriana (VB) Obstetricia y Ginecología Latinoamericanas. 2002. 60:175-183

4.- Di Bartolomeo S, Leonino A P, Rodríguez Fermepin M, de Torres RA. Balance del Contenido Vaginal (BACOVA) en el Diagnóstico Diferencial De Vaginosis-Vaginitis. Reacción Inflamatoria Vaginal (Riv) En Embarazadas Sintomáticas. Acta Bioquímica Clínica Latinoamericana.2007

5.- Nugent IR, Krohn Ma, Hillier M. Reliability of diagnosing bacterial vaginosis is improved by a standarized method of Gram stain interpretation .J.Clin.Microbiol. 1991;29:297-301.

6.- Donders GG, Vereeken A, Bosman E, Dekeersmaecker A, Salembier G, Spitz B. Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis:aerobic vaginitis. BJOG, 2002, 109(1):34-43.

7.- Forsum U,Jacobson T,Larsson PG,Smith H,Beverly A,Bjorneren A, Carlsson B,Csango P, Donders G, Hay P,Ion C,Kene F,McDonald H, Moi H, Platz-Chrisdtensen JJ, Schwbke J.
An international study of the interobserver variation between interpretations of vaginal smears criteria of bacterial vaginosis.APMIS 2002, 110: 811-818

8.- Geisler WM, Yu S, Vanglarik M, Schwebke JR. Vaginal leucocyte counts in women with bacterial vaginosis:relation to vaginal and cervical infections. Sex Transm Infect 2004; 80;401-5

9.- Spiegel CA. Bacterial Vaginosis. Clinical Microbiology Reviews . 4(4):485-502. 1991

10.- Marazzo JM. A (persistent/tly) enigmatic ecological mystery:bacterial vaginosis. J Infect Dis 2006, 193(11):1475-7.

11.- American College of Obstetricians and Gynecologists (ACOG)Practice Bulletin Clinical Management Guidelines. Vaginitis . n172 May 2006 Obstet Gyneco ,2006, 107: 1195-1206.

12.- Fidel JR, Sobel JD. Immunopathogenesis of recurrent vulvovaginal candidiasis. Clinical Microbiol Rev, 1996,9:335-48

13.-Perazzi B,Menghi C, Copolillo E, Gatta C, Eliseth Co, Vay C, Mendez O, Malamud H, De Torres RA, Famiglietti A. Investigación de Trichomonas vaginalis durante el embarazo mediante diferentes metodologías. Rev Argent Microbiol, 2007, 39:99-104

14.-Tilli M, Orsini A, Alvarez MM, Almuzara M, Gallardo E y Mormandi JO. ¿La presencia del pH normal descarta el diagnóstico de Vaginosis Bacteriana? DST J Bras Doenças Sex Transm, 2005, 17(2) 117-120

15.-Nansell TR,Riggs MA, Yu KF, Andrews WV,Schwebke JR, Klebanoff MA. The association of psychological stress and bacterial vaginosis in a longitudinal cohort. 2006. Am J Obstet Gynecol , 194(2):381-6

16.-Hay P. Recurrent bacterial vaginosis. 2009; Curr Opn Infect Dis 22(1): 82-8

17- Sherrand J,Donderrs G, White D, Skov JennsenJ.European (IUSTI/WHO) Guideline on the management of vaginal discharge, 2011. International Journal of STD & AIDS 2011:: 22; 421-429

18.- Yang, Y. L. (2003). Virulence factors of Candida species. Journal of Microbiology Immunology and Infection, 36(4), 223-228.

19.- Sobel, Jack D. Vulvovaginal Candidiasis, Desquamative Inflammatory Vaginitis, and Atrophic Vaginitis. Sexually Transmitted Diseases, 2012, vol. 16, p. 72-84

20.-Nyirjesy P, Leigh RD, Mathew L, Lev-Sagie A, Culhane JF. Chronic vulvovaginitis in women older than 50 years: analysis of a prospective database. J Low Genit Tract Dis. 2012 ;16(1):24-9.

ANNEXES

The Manual of Procedures for Balance del Contenido Vaginal BACOVA is available at www.fba.org.ar, under PROSAR / CONSENSOS. Details of the procedures and expanded discussion of the problems of interpretation for clinical decision-making are described in the Support Module (1).

The methodological advance added to improve VIR diagnosis can be consulted at erige@fba.org.ar.
This general material can be requested from prosar@fba.org.ar (subject: medical area).

ANNEX 1

BACOVA

Details related to sample collection for BACOVA are described in Annex IV.

REPORT MODEL

STUDY OF THE BALANCE OF VAGINAL CONTENT AND OF THE VAGINAL INFLAMMATORY RESPONSE:

• Preanalytical data:

Last name:		First name	Date
Age:	DLMP	Pregnant: YES ○ NO ○	Symptomatic: YES ○ NO ○
Time of sample collection: Time of sample processing: Validation of the sample:			

- **Study of the balance of the vaginal microbiota (NS):**

Examination by Gram staining

Nugent score		
clue cells	YES □	NO □
Final Nugent score (correction of Lanzafame)		

- **Study of the vaginal inflammatory response (VIR)**:

Method used	Number of leukocytes	Cutoff point. Leukocytes
Leukocytes / field (1000X) reading in Gram		5
Leukocytes / field (400 X) reading in Fresh/Giemsa		10
Leukocytes /epithelial cell/field (400 X) reading in Giemsa		1

DIAGNOSIS OF THE BASIC VAGINAL STATE (BVS)

- **Added criteria**

Presence of atypical cells	YES	NO
Red blood cells		
Other atypical cells:		

- **Study of conventional Vaginitis**

Criteria analyzed	YES	NO
Presence of Trichomonas		

Yeasts
Presence YES ○ NO ○
Inflammatory response YES ○ NO ○
Altered morphology YES○ NO ○

- **Report of atypical bacterial morphotypes:**

- **Observations:**

CONCLUSION:

ANNEX 2

Basic details for the determination of the vaginal pH

The use of paper strips ensures an excellent result, **but it is essential to standardize the procedure**. The strips must be of excellent quality, with an appropriate margin (pH 3.6 to pH 6.0) and stored in a dry place at normal temperatures, avoiding sunlight. To obtain the vaginal content sample, it is convenient to use a "trimmed toothpick" or small plastic coffee stirrer. The strip of paper must always be of the same type and of course disposable.

Measurement of vaginal pH varies by whether the sample is taken from the vaginal formix (recommended), vaginal wall or near cervix os, where pH is higher than real vaginal pH. Also elevated pH is associated to recent presence of semen.

The market also offers products that can be more comfortable for the determination of pH, but their efficiency is not higher than that of paper strips (1).

ANNEX 3

Determination of amine odor

One 'drop' of the vaginal content is placed on a slide or similar flat surface, covered with a drop of KOH 10%, homogenized slightly with a small plastic or glass disposable coffee stirrer (stable in alkaline medium) and sniffed through the nose, at a distance no greater than 10 to 15 cm, so as to be able to recognize the "fishy" odor. The starting limit detection of amine odor is purely subjective.

ANNEX 4

Sample collection

The problem of collecting and transporting the sample is one of the most critical points in an attempt to standardize the study of vaginal dysfunction through BACOVA.

Here we explicit fundamental aspects of the ideal requirements and suggest viable options. In all cases in which the use of variables, should be expressed clearly in the sample collection sheet, because of its eventual level of impact on the interpretation.

Annex 4.1.-Condition of absolute exclusion

The sample should not be taken during the three days before, during or within five days after menstruation.

Annex 4.2.-Transport in Stuart medium

Although it limits the possibility of wet mount reading, the validity of the BACOVA study has been determined in a sample transported in Stuart medium (1) with the aim to determine the NS and the VIR. The NS is obtained in the same way as in conventional sampling, but the VIR should be evaluated

exclusively by the number of leukocytes per epithelial cell per microscopic field in Giemsa staining.

Annex 4.3.-Information to the patient.

The information provided to the patient, which should be preferably printed, or in lack of that, verbally explained, must assure that the patient has understood the type of study to which she will be subjected and the importance of complying with the instructions.

Informed consent

The patient should know and accept that BACOVA requires taking a sample from the vaginal posterior formix, with the use of a speculum, but without any kind of puncture. The patient must request all the clarification concerning her obligations and give her consent and commitment to comply with the instructions.

Basic standards for sample collection

a. Sexual abstinence for 48 hours prior to sample collection.
b. The patient should not use any local vaginal treatment for five days prior to sample collection, except for medical indication, which will be reported in the sample collection sheet. All medication that the patient is taking must be recorded in the sample collection sheet and be explicit in the report.
c. The patient should not use vaginal douches, tampons, or any intimate cosmetic for five days prior to sample collection.
d. On the day of sample collection, the previous hygiene must be only of the external genital region without using any additive other than common soap.
e. In case of abundant vaginal discharge ("flow"), the patient can use a feminine sanitary napkin until the time of sample collection.
f. There are no restrictions on the emission of urine, but it is preferable to urinate an hour before sample collection.
g- Whenever possible, samples should be collected in the ovulatory menstrual cycle stage and at the same time of the day, in case of follow-up of a patient.

If the sample is collected in the clinical area, this information should be agreed upon with the patient and decide the right time for sample collection. This should be informed along with the sample collection sheet.

In the case of referring the patient for sample collection to the laboratory area, when issuing the order for the study, the conditions required should be indicated. In this case, the actual conditions of the patient must be verified at the time of sample collection and documented in the sample collection sheet.

Although in cases the ideal conditions are not met in full, it has been shown that they do not annul the validity of the correct interpretation of the criteria that define the BVS. It is essential to clarify which variable has not been fulfilled both in the report and in the sample collection sheet.

Sample collection includes a large number of variables, especially those related to the further transport of samples. A permanent effort should thus be made by all the actors in the primary care of women to improve the ethical and technical conditions of sample collection from all patients, independently of the social care system to which they belong.

Annex 4.4.-Minimum data of the patient and validation of the sample

The minimum data of the patient that must inevitably accompany the sample and for which we recommend using a pre-printed form are indicated in the report model described above (Annex I). and repeated below. We insist on the need to indicate any alternative that modifies the ideal sampling instructions for report validation.

Sample collection sheet

<table>
<tr><td colspan="2">Last name:</td><td colspan="2">First name</td><td>Date</td></tr>
<tr><td>Age:</td><td>DLMP</td><td colspan="2">Pregnant: YES○
NO ○</td><td>Symptomatic: YES○
NO ○</td></tr>
<tr><td colspan="5">Time of sample collection:
Time of sample processing:
Validation of the sample:
Information on any variable that do not agree with Basic rules.</td></tr>
</table>

Annex 4.5.-Operative Sample collection

Annex 4.5.1-Biosafety

Make sure that all biosafety requirements are controlled during sample collection, transport and processing.

Annex 4.5.2.-Basic procedure for sample collection

The patient will be placed in gynecological position and once the speculum is correctly inserted, with sufficient lighting, the position of the cervix will be located and the possibility of direct access to the virtual space below it, (vaginal posterior formix) will be verified.

In the absence of any kind of obvious anatomical anomaly, a swab will be introduced and material of secretion accumulated on that site will be taken with rotating movements (without pressing on the vaginal mucous membranes). Avoid touching the cervix, walls of the speculum or the outer vaginal region when withdrawing the swab. Even in conditions of a small volume of accumulated fluid in the vaginal posterior formix, "scraping" of the vaginal mucosa should be avoided.

Annex 4.5.3-Swabs

The swab to be used requires no special features but must be sterile to ensure biosafety. The standardization of the type of swab must be guaranteed, so as to ensure uniformity of all studies and especially the same quality, quantity and molding of the cotton wool used. The grip may be made of wood, plastic or metal.

Two swabs, kept in scrupulously clean, sterile, two test tubes will be used for each sampling. After taking the first sample, the swab should be placed in the tube. The other swab will be used to collect another sample of the vaginal

posterior formix and then placed in another tube containing 0.5 ml of sterile saline solution.
Both tubes must be perfectly identified and sent together to the processing area, together with the elementary data of the woman in the sample collection sheet.

Annex 4.6.-Sample transport

Verify that the outside label of the tubes coincides with reliable identification data of the patient under study and sent to the laboratory processing area. Ideally, the sample should be transported immediately. If this is not possible, the sample should be stored at room temperature (20 +/- 5 °C) avoiding cooling or overheating.

Ideally, the sample should be processed in fresh (swab in the tube with 0.5 ml of saline solution), within no more than one hour from sample collection.

Corollary on sample collection

As mentioned above, sample collection is the most difficult problem to standardize. Several works have shown that sample collection involves several important variables that may generate variables of high predictive value for the purpose of establishing the morphological diagnosis of the state of vaginal function. Among the most important because of its practical application is collecting the sample without a speculum. This and other variables, are being subjected to comparative studies (1).

Any criticism, suggestion and/or consultation are of great importance to optimize the objectives of this Guideline.

The Guideline of procedures is updated every December, based on the open participation of all who may be interested and the evaluation of scientific advances.