

The Role of Colposcopy in Assessing Vaginal Irritation in Research

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PROCEEDINGS

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INTRODUCTION

Colposcopy of the vagina and cervix is receiving increasing application in the development of vaginal products. The goal of this type of colposcopy is the detection of epithelial changes that may increase the likelihood of HIV or other STD acquisition. In 1995, the World Health Organization published a manual entitled, "Manual for the Standardization of Colposcopy for the Evaluation of Vaginally Administered Products." In January 1999, the Contraceptive Research and Development Program (CONRAD) and the International Working Group on Microbicides (IWGM), in association with the Joint United Nations Program on HIV/AIDS (UNAIDS) convened a meeting in Washington, D.C., the goals of which were to: 1) review modifications of the WHO procedure that had been tried by various investigators; 2) review findings in women using and not using vaginal products; 3) consider alternatives to colposcopy; and 4) reach consensus on a revised procedure, terms, analysis, and future research. One result is the revised colposcopy procedure described in this updated manual. The procedure involves proper patient positioning, examination of the external genitalia under magnification, speculum examination of the cervix, fornices, and vaginal walls with the naked eye followed by lavage and speculum examination under magnification, and taking samples, as appropriate, for microscopic examination.¹

BACKGROUND

Colposcopy is used primarily to detect cancerous and pre-cancerous lesions of the cervix. However, the procedure provides a good light source and magnification, which can facilitate inspection of the vaginal surface as well. In the late 1980s and early 1990s, colposcopy began to be used to examine vaginal and cervical changes seen in studies of vaginal products, specifically spermicides and vaginal rings (Niruthisard 1991, Roddy 1993, Goeman 1995, Bounds 1993). The concern was raised that these changes could predispose a woman to infection, the most worrisome being, of course, HIV.

Before that concern could be addressed, it was necessary to devise a uniform procedure and terminology for carrying out colposcopy of the cervix and vagina in women not suspected of having cervical neoplasia so that findings from one study could be compared with those from another. The original WHO manual was designed to meet this need. The procedure called for examination of the external genitalia, cervix, vaginal fornices, and vaginal walls under low and high power magnification, with and without a green filter, and before and after application of acetic acid.

¹ A full report of the workshop proceedings, including a list of the participants, may be found on the CONRAD web site (www.conrad.org) and an abbreviated report in the following publication: Mauck CK, Baker JM, Birnkrant DB, Rowe PJ, Gabelnick HL. The use of colposcopy in assessing vaginal irritation in research: report from a conference. AIDS 2000; 14: (in press).

Findings were to be described using the following criteria: size; site; number; presence of edema, demarcation, peripheral reaction, and slough; and intactness of the epithelium and blood vessels. One of the following terms was to be used for each finding: ulcer, abrasion, ecchymosis, petechial hemorrhage, subepithelial hemorrhage and swelling, erythema, or edema.

Since this uniform procedure became available, a number of studies have been carried out in which colposcopy was used to evaluate the effect of using various products (see bibliography). It became evident from these trials that, because the significance of colposcopic findings is not known, subjecting volunteers to the discomfort and time involved in the original procedure may not be justifiable until the procedure, and the interpretation of its results, are further standardized.

Modifications of the original procedure have been tried by a number of investigators. In general, efforts have been made to simplify and shorten the length of the procedure from 30-40 minutes to 5-10 minutes. Most researchers have eliminated the use of acetic acid and the green filter as neither step is felt to make significantly more findings visible, and acetic acid interferes with the vascular evaluation (although certain protocols may retain acetic acid if, for example, changes associated with HPV are being evaluated). Pap smears and diagnostic tests for STD pathogens and other changes in vaginal flora have been added by some in an effort to correlate colposcopic findings with other cervical/vaginal conditions. Participants in the 1999 colposcopy workshop were able to achieve consensus on a revised version of the original procedure which is included in this manual.

THE ROLE OF COLPOSCOPY

Colposcopy in vaginal spermicide/microbicide research and in the development of mechanical barriers has, as its goal, the detection of epithelial changes in the vagina and cervix caused by the use of vaginal products. The clinical significance of such changes is not known, but it is theorized that they may increase the likelihood of STD/HIV acquisition either via the creation of portals of entry for microorganisms or via recruitment of target cells. Epithelial changes could also cause discomfort.

Factors unrelated to product use, such as age, time in cycle, frequency of intercourse, use of tampons, and the presence of vaginal infections may cause changes in the epithelium. Examples of factors that may affect colposcopic observations are shown in Table 1. In addition, the examiner's experience with pelvic examinations in general and colposcopy in particular will affect his or her ability to bring findings into view and describe them. Red-green color blindness will affect the ability to detect erythema.

TABLE 1

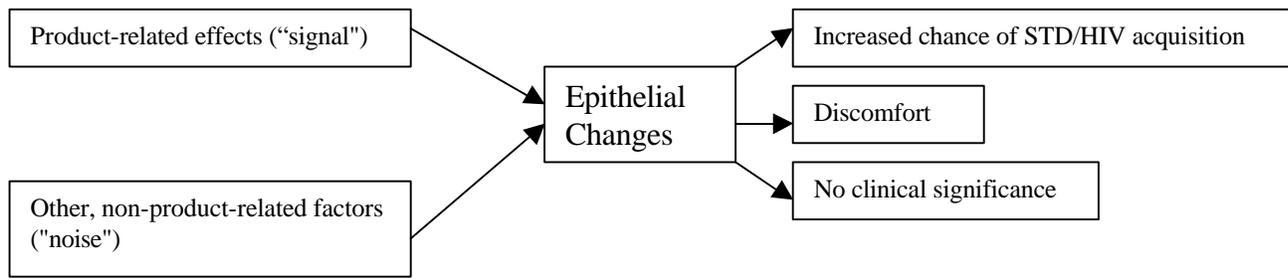
**FACTORS NOT ASSOCIATED WITH PRODUCT USE
THAT CAN AFFECT COLPOSCOPIC FINDINGS**

FACTORS	EXAMPLES	MECHANISM
Endogenous hormonal factors	<ul style="list-style-type: none">▪ Age▪ Time in cycle	Hormonal changes may affect vaginal blood flow and epithelial thickness which in turn will affect the degree of erythema observed.
Exogenous hormonal factors	<ul style="list-style-type: none">▪ Use of hormonal contraception▪ Use of other steroid products	Hormonal changes may affect vaginal blood flow and epithelial thickness which in turn will affect the degree of erythema observed.
Anatomical factors	<ul style="list-style-type: none">▪ Vaginal and cervical dimensions▪ Uterine position▪ Parity	Anatomical factors and the location of a finding will affect the ease with which it is seen. Whether light falls on it directly or at an angle will affect its appearance.
Mechanical factors	<ul style="list-style-type: none">▪ Pattern of intercourse and/or masturbation▪ Use of intravaginal products such as tampons, barrier contraceptives, and condoms▪ Speculum injury▪ Stretching and drying of epithelium during examination	Mechanical stressors before or at the time of the examination may create findings that confound interpretation of those resulting from the new product.
Chemical factors	<ul style="list-style-type: none">▪ Use of intravaginal products such as spermicides, douches, and other chemical irritants▪ Cigarette smoking▪ Fluids introduced during the examination such as water, saline, or acetic acid	The use of chemical products other than the one under study may create findings that confound interpretation of those resulting from the new product.
Infections	<ul style="list-style-type: none">▪ Fungal, e.g. candida▪ Protozoal, e.g. trichomonas▪ Viral, e.g. herpes simplex, human papillomavirus▪ Bacterial, e.g. chlamydia, gonorrhea, syphilis, donovanosis	Infections may cause changes in blood flow and, therefore, erythema as well as fragility and disruption of the epithelium and blood vessels. Whether HIV has an effect on the epithelium is not known.

In studies in which the goal is to detect epithelial changes that are the result of product use, the

presence of epithelial changes that are not the result of product use can confound the results and lead to the unwarranted discontinued development of an otherwise promising product. Product-related changes may be likened to "signal" and non-product-related changes to "noise" (Figure1). A diagnostic procedure that best separates "signal" from "noise" is most desirable. Attaining this requires: 1) knowledge of factors which, when seen, may be attributed to product use ("signal"); 2) knowledge of all factors not associated with product use that can affect the cervical/vaginal epithelium ("noise") - this may include variability between observers; 3) knowledge of the epithelial changes which, regardless of their cause, either increase the likelihood of HIV acquisition or cause symptoms by themselves; and 4) knowledge of the best method to detect product-related, clinically significant epithelial changes. Attaining this knowledge requires a colposcopic procedure that is simple enough to be used in developing country settings, is objective enough to provide reproducible observations, and can produce data in a form suitable for appropriate statistical analysis.

FIGURE 1



SUMMARY OF PRESENTATION BY THE UNITED STATES FOOD AND DRUG ADMINISTRATION (FDA)

It is recognized that many readers of these proceedings work in countries outside the United States and that their development efforts are not subject to FDA review. Nevertheless, the perspective of the FDA may be of interest because regulatory agencies in other countries may look to the FDA for precedent and because U.S. funding agencies may require that funded studies be appropriate for FDA review.

There are no set FDA requirements for colposcopy. Both the Center for Devices and Radiological Health and the Center for Drug Evaluation and Research review studies involving colposcopy. The Obstetrics and Gynecology Devices Branch of the former reviews a variety of medical devices that are placed in the vagina or on the cervix, including barrier contraceptive devices (e.g., diaphragms, caps, etc.), vaginal pessaries and stents, menstrual tampons, and electro-optical sensors. Colposcopy studies in the past have revealed various problems with intravaginal device

use, such as abrasions & lacerations, fiber shedding, etc. Colposcopy studies may be linked with vaginal microbiological studies to see whether device use affects not just the epithelium but the vaginal microflora as well. Usually, colposcopy studies are done early in the product development, although depending on the device, its use characteristics, and study findings, additional colposcopy studies may be needed. Many of the methodologic recommendations given below for drug evaluation are equally valid for evaluation of intravaginal devices. Device developers are strongly encouraged to contact the Branch early in the product development to discuss appropriate study methodology.

Two groups within the Center for Drug Evaluation and Research at the FDA have considered colposcopy: the Division of Reproductive and Urological Drug Products (DRUDP), which regulates prescription drug contraceptives, and the Topical Microbicide Working Group (TMWG), an interdivisional group formed in 1998 to make recommendations about the development of topical microbicides. Although requirements will vary with the product, product use, and the indication(s) sought, both groups strongly recommend thorough colposcopy in Phase 1 and 2 trials. In addition, DRUDP recommends colposcopy in 10-15% of women in most Phase 3 topical contraceptive trials. The TMWG currently will evaluate the need for colposcopy on a case-by-case basis. The TMWG may consider a smaller subset (<10%) in large, adequately powered Phase 3 trials where earlier safety data are robust.

Phase 1 and 2 studies evaluate the product in a highly specified and controlled fashion. All studies should be blinded, if possible. Phase 1 and 2 studies should enroll healthy volunteers at low risk for STDs. Documentation of low risk should be confirmed by screening for common STDs, vaginitis and vaginosis. Vaginal products other than study products should not be used and volunteers should not have intercourse in Phase 1 trials. Control arms should include an untreated arm and a vehicle arm, if possible. Careful and accurate documentation of significant findings is critical for safety evaluations. Microbiologic diagnosis of all genital tract ulcerations should be sufficiently thorough to differentiate between infection and product effect. Epithelial disruption and inflammation are of interest to the TMWG because they may be associated with an increased transmission of HIV and STDs. Colposcopy should be done at baseline, at one or more interim time points, and at the end of study. Data from colposcopy should be as objective and reproducible as possible.

Different product volumes, concentrations, and dosing intervals should be studied. The goal of these early studies is to determine the toxicity of the product directly related to use. Dose effects provide compelling evidence. Therefore, dose-escalation (step-up) designs are also acceptable. Data should be collected on all women, whether symptomatic or not, on gross and colposcopic evidence of epithelial changes (disruption, edema, erythema, and vascular changes) and local infection. In women with symptoms that could be attributed to product use, colposcopy should be

done, even if not previously planned. Photographing findings is strongly encouraged to help ensure a permanent and reviewable record.

Phase 3 studies differ from Phase 1 and 2 studies in that Phase 3 studies are intended to evaluate the product in actual use with a sexually active population; colposcopy is recommended for a subset of the study population. These studies must, at a minimum, collect information on the amount of product used, the number of doses and the time intervals between doses, use of other vaginal products, and the effects of intercourse and the menstrual cycle. Use of other vaginal products is strongly discouraged because it could confound the interpretation of the data toward the goal of determining product efficacy and safety. Colposcopy findings and microbiological results should be correlated with product use, symptoms, subject age, hormone status, coitus, and biopsy results (if done).

Foreign data may be acceptable for FDA review provided they have relevance to a United States population and that the conduct of the clinical trials, as well as the handling of data and lab specimens, meets U.S. standards. Multicenter studies are, however, encouraged to incorporate some site(s) in the United States.

MODIFICATIONS OF AND ALTERNATIVES TO THE WHO COLPOSCOPY PROCEDURE

Modifications of the WHO procedure have been tried by a number of investigators. In general, efforts have been made to simplify and shorten the length of the procedure from 30-40 minutes to 5-10 minutes. The use of acetic acid and the green filter have been eliminated by most researchers as neither step is felt to make significantly more findings visible, and acetic acid interferes with the vascular evaluation. Pap smears and diagnostic tests for STD pathogens and other changes in vaginal flora have been added by some in an effort to correlate colposcopic findings with other cervical/vaginal conditions. A revised version of the WHO procedure as agreed upon by the participants at this meeting is included in these proceedings.

Colposcopy with digital video imaging has been proposed as a means of storing colposcopic images for later reference and analysis. It has not been suggested as a replacement for the WHO procedure but as an enhancement. It requires a camera, central processing unit with frame grabber, monitor, and software. It provides focused and standardized images which may be manipulated, archived, digitized, and annotated. Images may be read centrally, eliminating observer bias and improving quality control. Templates for data entry can be set up, reducing errors in reporting. Colposcopy with digital video imaging is being used in a number of NIH trials. New types of optical systems, such as fiberoptics, have also been proposed as an improvement on colposcopy.

Colposcopy, however, even at its best has a number of disadvantages. These include, among other things, special training for the investigator, a longer exam, equipment costs, and increased overall study costs. Some would argue that only findings of ulcers, sloughing, and generalized erythema are of generally recognized significance and that other findings detectable by colposcopy such as petechiae and microabrasions are not necessarily clinically significant (Elias 1997). An alternative to colposcopy is a careful clinical inspection without magnification using a good light source such as a head lamp or a traditional lamp with a flexible arm. Other alternatives include use of a "head loupe" with its 3X magnification or PATH's hand-held monocular Aviscope which is battery-operated and transportable but cannot record images. The Population Council has eliminated routine colposcopy from its Phase I microbicide trials. Gross visual assessment is used to detect edema, erythema, petechiae, ecchymoses, ulcers, and abrasions as in the WHO procedure, with the additional findings of epithelial bleeding, vesicles, bullae, friability, and vaginal or cervical epithelial disruption noted as well

Biopsies have been proposed in addition to colposcopy. However, studies carried out at St. Mary's Hospital in London on nonoxynol-9, dextrin sulfate, and docusate sodium have shown poor correlation between colposcopically observed erythema and inflammation on biopsy.

The process of using a less invasive technique such as the measurement of inflammatory markers secreted in vaginal fluid to evaluate epithelial inflammation is very attractive. White blood cells and proinflammatory cytokines can be measured in cervicovaginal lavages following inflammatory stimuli, and provide a quantitative endpoint of cervicovaginal inflammation as well as qualitative information concerning characteristics of the inflammatory process. Polymorphonuclear leukocytes as well as other white blood cell types involved in inflammatory processes (e.g. macrophages, T cells) can be enumerated in wet preps using enzyme substrates or in smears using immunohistochemical markers; concentrations of proinflammatory cytokines (IL-1, TNF- α , IL-6 and IL-8) and chemokines (MIP-1 α and β RANTES) can be determined by ELISA. All of these inflammatory markers are significantly elevated in cervicovaginal lavage specimens from women following multiple applications of N-9, and can provide sensitive and informative measures of genital tract inflammation for clinical trials of vaginal microbicides.

In vitro testing for inflammation using human vaginal and cervical epithelial cell lines also offers promise. Products can be cytotoxic resulting in the death of epithelial cells (as in ulcerations and erosions) with release of IL-1, or subtoxic, releasing IL-1, IL-6, IL-8, into cervicovaginal secretions. Release of IL-1 and IL-8 activates macrophages, PMN, dendritic cells, mast cells, and lymphocytes. Toxic or subtoxic effects may be accompanied by decreased production of mucosal anti-inflammatory mediators (PGE₂, SLPI). *In vitro* assessment of vaginal products could include a complex of endpoints including assays for cytotoxicity, irritation/inflammation, and epithelial

function.

Such a study has been done comparing nonoxynol-9 with other spermicides. The goals were to evaluate effects of these products on the release of proinflammatory markers by immortalized cervical and vaginal epithelial cells *in vitro*, to compare *in vitro* and *in vivo* data from human and animal models, and to identify soluble irritation markers for preclinical assessment of vaginal products. *In vitro* cytotoxicity and cytokine tests accurately discriminated between spermicides/vaginal microbicides that have a broad range of effects in the rabbit vaginal irritation test. Furthermore preliminary analysis shows that cytokine upregulation by N-9 *in vitro* mirrors elevated profiles detected in cervicovaginal lavages of N-9 treated women. Although the utility of using inflammatory markers to determine the safety or effectiveness of a microbicide is not proven, such an *in vitro* system may be a feasible means for obtaining preclinical information about the irritation potential of new compounds. It may be possible to have a subtoxic dose of a product that induces inflammation without disrupting the epithelium. It may also be possible to use measurement of other soluble factors to assess effects of vaginal products on other important cell functions such as antiviral immune defense.

AREAS REQUIRING RESEARCH

This topic was addressed by Working Group 2 in response to their assigned questions: "1)What research should be carried out on the colposcopy procedure itself? 2)What studies should be done to define expected colposcopic findings in women not using vaginal spermicides/microbicides and their relevance? 3)What modifications to the procedure are necessary because of anatomical variations in the woman as well as cyclic changes? 4)What studies should be done on the use of alternatives to colposcopy?"

Several areas have emerged as most urgently requiring more research. Broadly, they include: 1) The natural history of colposcopically observed cervical and vaginal findings; 2) Determination of which findings predispose to a more serious condition (i.e., which are "lesions" that will enhance transmission of pathogens); and 3) How these clinically significant findings can best be detected and assessed.

The "natural history" referred to in the first research area includes an assessment of which factors may precede the development of findings in women not using vaginal research products. If risk factors for the development of findings could be clearly identified, such as intercourse, type of contraception, time in cycle, menopausal status, tampon use, drying agent use, or cigarette smoking, these effects could be controlled for in future studies of vaginal products. In addition, differences between populations could be anticipated. A study in which women with and without proposed risk factors undergo colposcopy would be enlightening. Also needed is a study in which

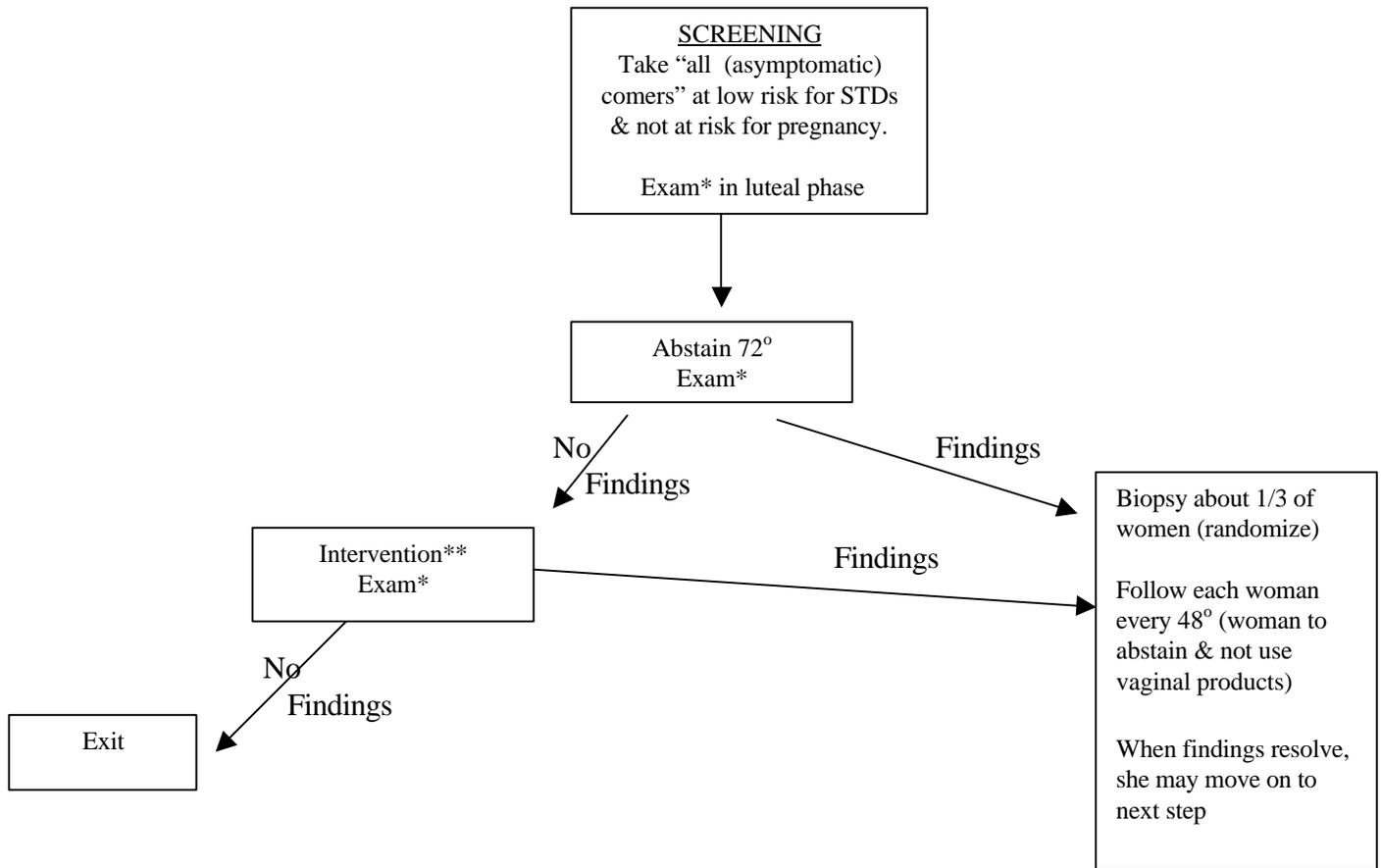
women with well-characterized cervical and vaginal findings are followed up to determine whether findings disappear and, if so, at what rate, or whether they evolve into other types of findings. The effects on the epithelium of repeated colposcopic examinations would have to be determined.

The second research area may be most expeditiously addressed prospectively in animal studies or in a high risk population in which women are examined and the subsequent rate of HIV seroconversion or other STD transmission among women with findings is compared with the rate among those without findings.

The third research area could be addressed by comparing the revised WHO modification as outlined in these proceedings with some of the suggested alternatives to colposcopy, such as visualization with the naked eye or hand-held scope or assessment of inflammatory markers. Such a trial could be done at several centers to allow evaluation of intra- and interobserver variability among research colposcopists. Colposcopy with digital video imaging could be assessed as a means of creating a permanent record of the image for later reference and as a training device. An analysis of the costs and benefits of various assessment techniques would be useful.

PROPOSED RESEARCH DESIGN

The study design below is suggested as one that begins to address the natural history of findings and to compare different means of detecting findings.



Objectives:

- 1) Determine incidence of findings in asymptomatic population at screening and retrospectively correlate them with recent history of intercourse, tampon use, intravaginal product use, smoking, contraceptive use, age, parity, etc.
- 2) Determine sensitivity and specificity of inflammatory markers, naked eye exam and other alternatives as compared with colposcopy
- 3) Determine natural history of findings by observing them until resolved.
- 4) Determine whether various interventions (intercourse, tampon use, spermicide use) are associated with the development of findings.
- 5) Evaluate the correlation between colposcopic findings and biopsies taken from the area with findings, and between colposcopic findings and biopsies from uninvolved areas in women with findings. Consider comparing biopsies from these uninvolved areas with biopsies from women without findings.

In order to evaluate the interaction of interventions in #4 above and various assessment techniques (lavage, naked eye exam, etc.), each assessment technique must be used at each time point.

* Exam to consist of 1) cervicovaginal lavage for inflammatory markers followed by 2) naked eye exam w/ good light source followed by 3) exam with head loupe and or hand held scope followed by 4) colposcopy (digital if possible to allow central reading).

** Intercourse, tampon use, or spermicide use as directed.

COLPOSCOPY TO BE USED IN VAGINAL PRODUCT DEVELOPMENT

This topic was addressed by Working Group 3 in response to their assigned question, "Do different stages of drug product development and different product types warrant differences in colposcopic procedures? If so, make appropriate recommendations." It was also addressed in a later meeting of key members of that group, held on June 2, 1999.

Although much is not known about the causes and natural history of findings seen on colposcopy and their implications, colposcopy as adapted for use in the vagina as well as the cervix is currently the most widely used method for the assessment of epithelial effects of new product use. An objective means of detecting epithelial effects is necessary since patient symptoms have not been shown to correlate with visible epithelial changes. Future research will determine whether it truly is necessary to look for findings not visible to the naked eye, whether digital data recording offers advantages over standard colposcopy with paper and/or photographic records, and whether visualization of any kind is superior to other techniques such as measurement of cytokine levels. It is important to remain flexible concerning colposcopy recommendations before it is known whether colposcopic findings are meaningful. On the other hand, it is important not to omit requirements for safety testing if such testing could provide useful data and potentially protect users from harmful products.

The earliest studies in product development should be done in such a way as to minimize the effects of confounding variables so that the effects of the product being tested are most discernable. Such studies should enroll women who are abstinent during the study, who use no other vaginal products, and who have no colposcopic findings at baseline. It is recommended that a dose-escalation study be carried out over at least a two week period of time. Colposcopy, ideally with photodocumentation, should be carried out on all subjects within 12-24 hours of product administration at three time points: baseline, interim, and end of study. Women with findings at the interim visit should not be discontinued from the study. Incorporation of an N-9 control arm should be considered. The women in the earliest studies should be at low risk for STDs, and verification should be done by appropriate diagnostic testing.

Subsequent early studies should include a vehicle or placebo arm as well as a no-treatment arm. If an active control is used, N-9 is recommended since it is the most well-studied vaginal compound and permits clinical correlation of findings, given previous and current microbicide trials. Administration to women having intercourse who can act as their own controls in a cross-over design should be considered. Different product volumes, concentrations and dosing intervals should be considered and exposure to the product for several months should eventually be studied. Assessing the effect of the applicator should be considered as well as inclusion of women with baseline colposcopic findings not involving complete disruption of the epithelium.

When later, larger studies are done to demonstrate efficacy, colposcopy may or may not be required depending on the demonstration of acceptable safety results of earlier studies. If colposcopy is required, it can be performed either in a subset of the larger study or as a smaller parallel study. An interim analysis can be conducted after a pre-determined number of subjects complete the study, with elimination of further colposcopy if results are reassuring. Consideration should be given to the possible biasing effects of treating a subset differently from the rest of the study population. Information on the amount of product used, the number of doses, the time intervals between doses, use of other vaginal products, intercourse, and menses should be recorded. Use of other vaginal products is discouraged because of its confounding effect. The study design of efficacy trials in general must support the eventual labeling of a product: when the product should be applied, the number of applications relative to the frequency of acts of intercourse, etc.

PROCEDURAL DETAILS

The term "colposcopy" should be understood to mean magnified visualization of the vagina and cervix. Use of a colposcope, per se, is not necessary as long as the following criteria are met by whatever technique is used:

- a) Ability to magnify at 4 to 10X;
- b) Self-contained (integrated) light source;
- c) Binocular or monocular optics; and
- d) Ability to photodocument findings, if possible. (Photographs or other pictorial records should be calibrated to permit comparison of records obtained at different centers and using different magnification factors.)

The research colposcopy procedure to be used should be standardized at an investigators' meeting that precedes the start of the study. The people carrying out the colposcopy examinations in

preliminary research studies must have experience in colposcopy in general and must demonstrate, prior to the start of the study, that they have competence in the research colposcopy procedure specified in the protocol. In later research studies, in addition to having investigators with the required experience in colposcopy at the centers taking part in the research, someone should also be appointed to take responsibility for the overview of the colposcopy being carried out during the study.

In all studies, data should be collected on the number of findings, their location, size, and type, whether they persist or progress, and, if applicable, the effect of dose. Findings should be tallied by

subject and should include categorization by location and type of finding.

When a finding involves more than one anatomical area, the percent of each anatomical area that is occupied by the finding should be recorded. This is preferred over recording the percent of a finding that may be found in each of the anatomical areas. For example, it is felt to be more informative to record that a finding involved half of the anterior cervical trunk and one third of the anterior fornix than to record that two thirds of the finding was located on the anterior cervical trunk and one third of it on the anterior fornix. Findings involving more than one anatomical area should be included in the tallies of each area, recognizing that the resulting tallies of anatomical areas, when taken together, will add up to more than the tally of individual findings made regardless of location. Very small findings (e.g. petechiae) should be grouped and recorded as the area covered by the group.

REVISED WHO PROCEDURE FOR COLPOSCOPY IN THE DEVELOPMENT OF NEW VAGINAL PRODUCTS

This topic was addressed by Working Group 1 in response to their assigned question, "Choose one of the presented procedures and modify it to represent the optimal procedure to be tried in future research. Include recommendations on procedure, findings to be documented, and nomenclature to use for findings." A comparison of the original and the revised WHO procedures may be found in Appendix A.

1. **PATIENT POSITIONING:**
The subject should lie on a soft examination table in the lithotomy position with leg supports so as to enable the perineum and vulva to be inspected. At all times, the comfort and privacy of the woman should be ensured.
2. **COLPOSCOPIC EXAMINATION OF EXTERNAL GENITALIA:**
Using appropriate magnification (usually 4-10X), examine the external genitalia. Record findings. (See Note A.)
3. **INSERTION OF SPECULUM:**
Use a speculum with sufficiently long blades to permit adequate visualization of the vagina and cervix. If necessary, apply a small amount of the lubricant specified in the study protocol to the external blades. Gently insert and open the speculum so as to prevent trauma and enable the cervix and upper vagina to be seen clearly. (See Note B.)
4. **NAKED EYE EXAMINATION OF VISIBLE EPITHELIUM:**
Naked eye inspection of visible epithelial surfaces should be performed without manipulation. Record findings. (See Note A.)

5. **WET PREPARATION:**

If a wet preparation, pH, or microbiological tests are performed, the sample should be obtained after the speculum is placed and initial visual examination is made, but prior to lavage. The sample should be taken from the vaginal pool or lateral vaginal wall (or as directed by the protocol) away from any apparent abnormal areas. The area from which the wet preparation is taken should be excluded from the subsequent examination, or findings should be noted as "probably iatrogenic - wet preparation site."

6. **LAVAGE:**

Using a small (3-4 cc) bulb syringe, lavage the cervix and vaginal walls with normal saline to remove mucus and cellular debris. Avoid contact between the tip of the syringe and the epithelium. The lateral fornices may be lavaged without manipulation by directing the stream into them. Aspirate the fluid with the tip of the syringe against the inner surface of the posterior blade of the speculum. Use dry swabs only to remove obscuring fluid from the posterior blade that cannot be removed by aspiration. Do not use dry swabs in any other manner and do not permit contact between the syringe or the dry swabs and the epithelium. Record any observations not noted on previous naked eye examination. (See Note C.)

7. **COLPOSCOPIC EXAMINATION OF CERVIX:**

Inspect the cervix under appropriate magnification (usually 4-10X) and record findings. (See Note A).

8. **COLPOSCOPIC EXAMINATION OF FORNICES:**

Under appropriate magnification (usually 4-10X), examine the anterior, right lateral, left lateral, and posterior fornices and adjacent cervical trunk and record findings. If necessary, slightly manipulate speculum so that fornices may be adequately visualized. The lateral fornices are best exposed by placing a saline-moistened swab into the contralateral fornix and pressing toward the head and laterally. For example, to view the right lateral fornix, place the moistened swab into the left lateral fornix and press gently toward the woman's head and left side. A dry swab should never be used. (See Note D.)

9. **COLPOSCOPIC EXAMINATION OF VAGINA:**

To examine the rest of the vagina, slowly withdraw the speculum with the blades moderately open, refocusing as needed. Record findings. (See Note A.)

Inspect the cervix under appropriate magnification (usually 4-10X) and record findings. (See Note A).

NOTES:

A) Photography is not required but may be desirable for documentation, quality assurance, and/or independent blinded review of findings. Some means of standardizing assessment, such as by placement of a plastic disk of known diameter and color near the finding, should be used if possible. Baseline photography is especially helpful if subsequent examinations are separated by time or by multiple other examinations.

B) The length and axis of the vagina, position of the uterus, and least traumatizing type/size of speculum should be recorded on the source document during the first examination for reference at later examinations. This information should be reviewed prior to subsequent examinations to reduce the chance of causing iatrogenic injury.

C) Some protocols may require collection of lavage fluid for measurement of inflammatory markers. Note also that if the product obscures findings, it should be lavaged away as completely as possible using a medium specified in the protocol, and as much of the epithelial surface examined as possible.

D) At no time should a dry swab be used during examination of either the cervix or the vagina, as this may traumatize the epithelium of either surface. Large swabs moistened with non-bacteriostatic saline should be used.

DESCRIPTION OF FINDINGS

The nomenclature to use for findings has been simplified. The results of the colposcopic examination should be documented by recording the following for each numbered finding:

1) Epithelium:

- Integrity:
 - Intact
 - Disrupted
 - Superficial
 - Deep: Complete disruption is now called “deep” and exposes stroma and possibly blood vessels. A bleeding area should be considered “deep”.

Color: normal, slightly red, red, white, other (including pale)

2) Blood vessels:

- Integrity:
 - Intact

- Disrupted

The Case Record Forms should include specific mention of the cervix, its trunk, the four fornices, and each of the two halves of the vagina. (See "Recording of Findings".)

The terms in italics in Table 2 are from the original WHO procedure. They have been replaced with the descriptors in bold.

TABLE 2
DOCUMENTATION OF FINDINGS*

	Blood vessels intact	Blood vessels disrupted
Epithelium intact	<i>Erythema (color red or slightly red)</i> <i>Edema (color pale)</i>	<i>Ecchymosis</i> <i>Petechiae</i> <i>Petechial hemorrhage</i>
Epithelium disrupted – superficial	<i>Abrasion</i> <i>Ulcer</i>	<i>Abrasion</i> <i>Ulcer</i>
Epithelium disrupted – deep	<i>Abrasion</i> <i>Ulcer</i>	<i>Abrasion</i> <i>Ulcer</i>

- C Findings previously called "erythema" or "edema" should be described as an area with intact blood vessels and epithelium. Erythema should be distinguished from edema by the former having a red or slightly red color and the latter being pale.
- C Findings previously called "ecchymosis," "petechiae," and "petechial hemorrhage" should be described as an area with disrupted blood vessels and intact epithelium. (The size of these and all findings should continue to be recorded.)
- C Findings previously called "abrasion" or "ulcer" should be described as an area with either intact or disrupted blood vessels and superficially or deeply disrupted epithelium, as appropriate.

* Findings referred to as "peeling" or "deepithelialization" by some researchers should be recorded as an area with superficially disrupted epithelium and intact blood vessels.

RECORD OF COLPOSCOPIC FINDINGS

1. Finding number
(if previously reported, use same finding number)

2. Size of entire finding
 1: <5 mm 2: 5-10 mm
 3: >10 mm

3. Epithelium/surface
 0=Intact (**go to Q.5**) 1=Not intact

4. If epithelium not intact, indicate depth.....
 1=Superficial 2=Deep

5. Color of epithelium.....
 1=Normal 2=Slightly red
 3=Red 4=White
 5=Other --> specify: _____

6. Blood vessels
 0=Intact 1=Not intact

7. Is any part of the finding located on the external genitalia
 0=No (**go to Q.9**) 1=Yes

8. For each of the following parts of the external genitalia, indicate if it is involved in the finding.
 0=No, and 1=Yes.
 If YES, indicate percent of area involved in the finding*.

INVOLVED % OF AREA

a) Right Labia Majora	<input type="checkbox"/>	<input type="checkbox"/>
b) Left Labia Majora	<input type="checkbox"/>	<input type="checkbox"/>
c) Right Labia Minora	<input type="checkbox"/>	<input type="checkbox"/>
d) Left Labia Minora	<input type="checkbox"/>	<input type="checkbox"/>
e) Clitoris/prepuce	<input type="checkbox"/>	<input type="checkbox"/>
f) Vestibule	<input type="checkbox"/>	<input type="checkbox"/>
g) Perineum	<input type="checkbox"/>	<input type="checkbox"/>
h) Other -->	<input type="checkbox"/>	<input type="checkbox"/>

specify: _____

9. Is any part of the finding located on the cervix.....
 0=No (**go to Q.11**) 1=Yes

10. For each of the following parts of the cervix, indicate if it is involved in the finding.
 0=No, and 1=Yes.
 If YES, indicate percent of area involved in the finding*.

INVOLVED % OF AREA

a) Anterior cervical trunk	<input type="checkbox"/>	<input type="checkbox"/>
b) Posterior cervical trunk	<input type="checkbox"/>	<input type="checkbox"/>
c) Right lateral cervical trunk..	<input type="checkbox"/>	<input type="checkbox"/>
d) Left lateral cervical trunk ...	<input type="checkbox"/>	<input type="checkbox"/>
e) Cervical face	<input type="checkbox"/>	<input type="checkbox"/>

11. Is any part of the finding located in the vaginal fornix
 0=No (**go to Q.13**) 1=Yes

12. For each of the following parts of the vaginal fornix, indicate if it is involved in the finding.
 0=No, and 1=Yes.
 If YES, indicate percent of area involved in the finding*.

INVOLVED % OF AREA

a) Anterior	<input type="checkbox"/>	<input type="checkbox"/>
b) Posterior	<input type="checkbox"/>	<input type="checkbox"/>
c) Right lateral	<input type="checkbox"/>	<input type="checkbox"/>
d) Left lateral	<input type="checkbox"/>	<input type="checkbox"/>

13. Is any part of the finding located on the vaginal wall
 0=No (**end here**) 1=Yes

14. For each of the following parts of the vaginal wall, indicate if it is involved in the finding.
 0=No, and 1=Yes.
 If YES, indicate percent of area involved in the finding*.

INVOLVED % OF AREA

a) Anterior, proximal half.....	<input type="checkbox"/>	<input type="checkbox"/>
b) Anterior, distal half	<input type="checkbox"/>	<input type="checkbox"/>
c) Posterior, proximal half.....	<input type="checkbox"/>	<input type="checkbox"/>
d) Posterior, distal half	<input type="checkbox"/>	<input type="checkbox"/>
e) RT lateral, proximal half	<input type="checkbox"/>	<input type="checkbox"/>
f) RT lateral, distal half	<input type="checkbox"/>	<input type="checkbox"/>
g) LFT lateral, proximal half....	<input type="checkbox"/>	<input type="checkbox"/>
h) LFT lateral, distal half	<input type="checkbox"/>	<input type="checkbox"/>

***PERCENT CODES:**

1= 1% to 25%	2= 26% to 50%
3= 51% to 75%	4= greater than 75%

MEETING AGENDA

JANUARY 21, 1999 - SCIENTIFIC SESSIONS

8:00 - 8:30 Continental breakfast

8:30 - 8:40 Welcome, introductions - Henry Gabelnick, Ph.D.

8:40 - 8:50 Overview of meeting - Marianne Callahan

8:50 - 9:05 History of colposcopy in research - Christine Mauck, M.D.

9:05 - 9:10 Discussion

9:10 - 9:40 FDA perspective - Daniel Davis, M.D.

9:50 - 10:00 Discussion

10:00 - 10:15 Difficulties in colposcopy data analysis - Christine Mauck, M.D.

10:15 - 10:20 Discussion

10:20 - 10:40 Break

10:40 - 12:00 Clinical trials in which colposcopy has been used - comments on colposcopic technique:

- **Baseline colposcopic findings in women enrolling in clinical trials - Jay Baker, M.D.**
- C **Colposcopy observations in sexually active women -Harold Nash, Ph.D.**
- C **Phase I and II trials in Antwerp, Phase II trial in developing countries - Lut Van Damme, M.D.**
- C **Phase I and II trials of microbicides in the U.K. - Val Kitchen, M.D.**

12:00 - 12:20 Discussion

12:20 - 1:30 Lunch

1:30 - 1:55 Clinical trials in which colposcopy has been used - comments on colposcopic technique (continued):

- C **Buffergel trial - Ken Mayer, M.D./Jeff Peipert, M.D.**

1:55 - 2:00 Discussion

2:00 - 2:20 Colposcopy for safety/toxicity assessment: feasibility in developing country settings - Jean Anderson, M.D.

2:20 - 2:25 Discussion

2:25 - 2:45 The research colposcopy exam: confounding variables - Jay Baker, M.D.

2:45 - 2:50 Discussion

2:50 - 3:10 - Break

3:10 - 4:40 - Alternatives to colposcopy in assessing irritation from new products

a) **Gross visual assessment - Christa Coggins, M.P.H., Lut Van Damme, M.D.**

b) **Evidence of inflammation**

- C **Cytokines as markers of vaginal irritation: *in vitro* models - Raina Fichorova, M.D., Ph.D.**

c) Cytokines as markers of vaginal irritation: *in vivo* assessment - Deborah Anderson, Ph.D.

c) Digital colposcopy - Daron Ferris, M.D.

d) Correlation of colposcopy and biopsy - Val Kitchen, M.D.

4:40 - 5:00 Discussion

5:00 - 5:05 Introduction to working group questions - Marianne Callahan

5:05 Adjourn

JANUARY 22 - WORKING GROUP SESSIONS

8:00 - 8:30 Continental breakfast

8:30 - 9:00 Orientation

(To include brief going over of all groups' questions, describing and answering questions about how day will progress)

9:00 - 11:45 Working group sessions

(Groups to take a break when it's convenient. Groups to come up with first draft of answers to their questions)

12:00 - 1:00 Lunch

1:00 - 1:45 Working group sessions

(Groups will finalize first draft of answers to questions)

2:00 - 3:45 Presentations of working groups to plenary session

(Designated speakers from each group will present conclusions of group using overheads. Audience will give feedback.)

3:45 - 4:00 Break

4:00 - 4:45 Groups will revise drafts using audience feedback and information from other groups

4:45 - 5:00 Summary and plans for follow-up

(The main conclusions of the meeting will be summarized and plans for follow-up will be outlined. Follow-up may include distribution of final drafts from groups including a new modification of the WHO procedure for research colposcopy, designation of a central group for keeping interested parties abreast of other groups' research, and planning of follow-up meeting)

LIST OF WORKING GROUPS AND THEIR QUESTIONS

1. Guidelines for colposcopy procedure, terminology, equipment and training:

- C Choose one of the presented procedures and modify it to represent the optimal procedure to be tried in future research. Include recommendations on procedure, findings to be documented, and nomenclature to use for findings.
- C Make recommendations as to the equipment that should be used for colposcopy - minimum requirements and useful "bells and whistles".
- C What sort of training should practitioners undergo who wish to perform colposcopy in research and have findings that may be compared with those others might get? Include such things as screening for red-green color blindness.

Participants:

- C Blumenthal - Chair
- C Mauck - Rapporteur
- C Baker
- C Lu
- C Drucker
- C Ferris
- C Rowe
- C Davis
- C Thau
- C Alvarez
- C Brache
- C Anderson, Jean
- C MacKay
- C Van Damme
- C Peipert
- C Thomas

2. Research needs and alternatives to colposcopy:

- C What research should be carried out on the colposcopy procedure itself?
- C What studies should be done to define expected colposcopic findings in women *not* using vaginal spermicides/microbicides and their relevance?
- C What modifications to the procedure are necessary because of anatomical variations in the woman as well as cyclic changes?
- C What studies should be done on the use of alternatives to colposcopy?

Participants:

C Mayer - Chair
C Coggins - Rapporteur
C Callahan
C Moench
C Barnhart
C Harrison
C Fichorova
C Blanchard
C Anderson, Deborah
C Rosenberg
C Linton
C Berlin
C Welch
C Apter
C Ballagh
C Wright
C Maslankowski

3. Effect of different stages of drug product development and different product types:

- C Do different stages of drug product development and different product types warrant differences in colposcopic procedures? If so, make appropriate recommendations.
- C What FDA guidelines would be helpful for different stages of product development?

Participants:

C Upmalis - Chair
C Vogelsong - Rapporteur
C Hitchcock
C Weiner
C Kilmarx
C Hamilton
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ATTACHMENT A:
COMPARISON OF ORIGINAL AND MODIFIED WHO COLPOSCOPY PROCEDURES

Original WHO procedure

Modified WHO procedure

1. The subject should lie on a gynecological couch either in the lithotomy position or with leg stirrups so as to enable the perineum and vulva to be inspected.
2. Using low power (X4-10) magnification and no filter, examine the external genitalia. Note and photograph any positive findings on the external genitalia including the perineum, peri-anal area, and the mucosal lining of the introitus.
3. Using higher power (X16-25) magnification and green filter, re-examine the external genitalia and note and photograph any positive vascular findings.
4. Gently insert a speculum of appropriate size moistened with warm saline into the vagina so as to enable the cervix and upper vagina to be seen clearly.
5. Open the speculum blades carefully to prevent trauma and

1. **PATIENT POSITIONING:**
The subject should lie on a soft examination table in the lithotomy position with leg supports so as to enable the perineum and vulva to be inspected. At all times, the comfort and privacy of the woman should be ensured.
2. **EXAMINATION OF EXTERNAL GENITALIA:**
Using appropriate magnification (usually 4-10X), examine the external genitalia. Record findings. (See Note A.)
3. **INSERTION OF SPECULUM:**
Use a speculum with sufficiently long blades to permit adequate visualization of the vagina and cervix. If necessary, apply a small amount of the lubricant specified in the study protocol to the external blades. Gently insert and open the speculum so as to prevent trauma and enable the cervix and upper vagina to be seen clearly. (See Note B.)

(Wet mount done in Step #5 below)

- bring the cervix into view.
6. If any abnormal vaginal or cervical discharge is seen, perform a wet mount, obtain vaginal pH, and obtain a sample for microbiology.
 7. Initial naked eye observation should be performed noting the general state of the cervix and upper vagina.
 - a) Hyperemia which might indicate vaginitis
 - b) Congenital abnormalities
 - c) Purulent exudate which might indicate endocervical infection
 - d) Bleeding which might make further examination impossible
 - e) Macroscopic condylomata
 - f) Cervical ulceration which might suggest HSV infection
 - g) Mucus retention cysts
 - h) Signs of chronic or acute trauma
 - I) Foreign bodies
 - j) Atrophic changes
 - k) Hyperkeratosis
 4. **EXAMINATION OF VISIBLE EPITHELIUM:**
Naked eye inspection of visible epithelial surfaces should be performed without manipulation. Record findings. (See Note A.)
 5. **WET PREPARATION:**
If a wet preparation, pH, or microbiological tests are performed, the sample should be obtained after the speculum is placed and initial visual examination is made, but prior to lavage. The sample should be taken from the vaginal pool or lateral vaginal wall (or as directed by the protocol) away from any apparent abnormal areas. The area from which the wet preparation is taken should be excluded from the subsequent examination or findings should be noted as "probably iatrogenic - wet prep site."
 6. **LAVAGE AND RECORDING OF ANY NEW OBSERVATIONS:**
Using a small (3-4 cc) bulb syringe, lavage the cervix and vaginal walls with normal saline to remove mucus and cellular debris. Avoid contact between the tip of the syringe and the epithelium. The lateral fornices may be lavaged without manipulation by directing the stream into them. Aspirate the fluid with the tip of the syringe against the inner surface of the posterior blade of the speculum. Use dry

8. Using low power (X4-10) magnification and no filter, examine the cervix with regard to the position of the squamocolumnar junction and any surface irregularity in the transformation zone. Locate and describe the transformation zone (completely or incompletely visualized, degree of ectopy, presence or absence of edema, areas of thickened epithelium, Nabothian cysts, endocervical gland openings, etc.). Photograph any significant findings.
9. In a dabbing fashion, gently use a saline-moistened swab to remove any mucus from the cervix. Avoid twisting the swab or rolling it over the surface of the cervix. Still using low power and no filter, describe the cervix as in 8.
10. Using higher-power magnification and green filter, examine the vasculature of the cervix and note any positive findings (dilation, branching patterns, hairpin patterns, etc.)
11. Slowly and gently pull back on the speculum (to approximately 3 to 4 cm) to allow visualization of the vaginal fornices.

swabs only to remove obscuring fluid from the posterior blade that cannot be removed by aspiration. Do not use dry swabs in any other manner and do not permit contact between the syringe or the dry swabs and the epithelium. Record any observations not noted on previous naked eye examination. (See Note C.)

7. **EXAMINATION OF CERVIX:** Inspect the cervix under appropriate magnification (usually 4-10X) and record findings. (See Note A.)

11. Using a saline-moistened swab, apply pressure to the same side of the cervix as the fornix to be viewed. This will facilitate inspection of the fornix. Perform a systematic naked eye examination of the anterior, right lateral, left lateral, and posterior fornices, noting any positive findings.
12. Using low-power magnification, examine the vaginal fornices and photograph any significant findings.
13. Gently reposition the speculum so that the cervix is again in full view.
14. Apply 3-5% acetic acid by gentle irrigation of the surface of the cervix. Wait 30 seconds and reexamine the cervix as in 8 above. Note and photograph any changes in the findings (either positive or negative) from the previous examination.
15. Using higher magnification and green filter, re-examine the cervical vasculature as in 10.
16. Gently pull back on the speculum just enough to visualize all the vaginal fornices and repeat the examination as in 13 and 14, above.

8. **EXAMINATION OF FORNICES:**
Under appropriate magnification (usually 4-10X), examine the anterior, right lateral, left lateral, and posterior fornices and adjacent cervical trunk and record findings. If necessary, slightly manipulate speculum so that fornices may be adequately visualized. The lateral fornices are best exposed by placing a saline-moistened swab into the contralateral fornix and pressing toward the head and laterally. For example, to view the right lateral fornix, place the moistened swab into the left lateral fornix and press gently toward the woman's head and left side. A dry swab should never be used. (See Note D.)

17. To examine the middle and lower thirds of the vagina, slowly withdraw the speculum with the blades slightly open so as to separate the vaginal walls, refocusing the colposcope continuously. Note and photograph any relevant finding.
18. Repeat 18 after application of acetic acid.
19. Re-examine the perineal area, the introitus and urethra using the colposcope at X10 magnification, after application of acetic acid. Note and photograph any relevant finding.

NOTE:

- a) At no time should a dry swab be used during examination of either the cervix or the vagina, as this may traumatize the epithelium of either surface. Use large swabs preferably.
- b) Record size and site of condylomata
- c) If an endocervical purulent discharge is present, take the appropriate sample for microbiology before application of acetic acid.

9. **EXAMINATION OF VAGINA:**
To examine the rest of the vagina, slowly withdraw the speculum with the blades moderately open, refocusing as needed. Record findings. (See Note A.)

NOTES:

- A) Photography is not required but may be desirable for documentation, quality assurance, and/or independent blinded review of findings. Some means of standardizing assessment, such as by placement of a plastic disk of known diameter and color near the finding, should be used if possible. Baseline photography is especially helpful if subsequent exams are separated by time or by multiple other exams.
- B) The length and axis of the vagina, position of the uterus, and least traumatizing type/size of speculum should be recorded on the source document during the first examination for reference at later examinations. This information should be reviewed prior to subsequent exams to reduce the chance of causing iatrogenic injury.
- C) Some protocols may require collection

of lavage fluid for measurement of inflammatory markers. Note also that if the product obscures findings, it should be lavaged away as completely as possible using a medium specified in the protocol, and as much of the epithelial surface examined as possible.

D) At no time should a dry swab be used during examination of either the cervix or the vagina, as this may traumatize the epithelium of either surface. Use large swabs moistened with non-bacteriostatic saline.