

MANUAL FOR THE STANDARDIZATION
OF COLPOSCOPY FOR THE EVALUATION
OF VAGINAL PRODUCTS

UPDATE 2004

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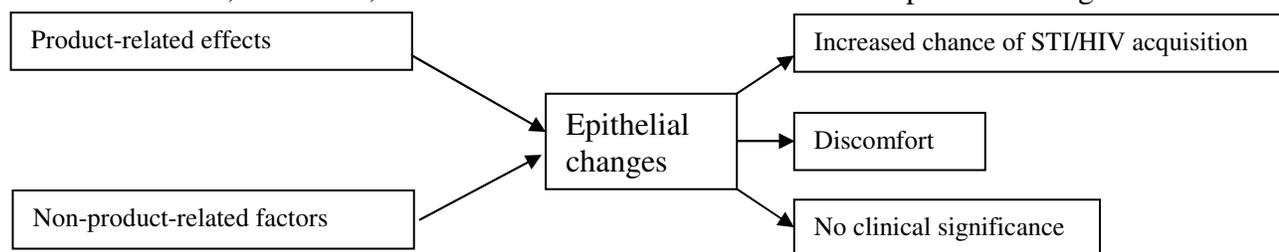
INTRODUCTION

Colposcopy of the vagina and cervix has become a standard technique for the safety assessment of vaginal products. The goal of this type of colposcopy is the detection of epithelial changes that may increase the likelihood of acquisition of HIV or other sexually transmitted infections (STI). In 1995, the World Health Organization convened a meeting to address the technique and nomenclature for this type of colposcopy and published a manual entitled "Manual for the Standardization of Colposcopy for the Evaluation of Vaginally Administered Products."¹ This was revised in conjunction with an expert review convened by CONRAD and WHO in 1999. The revised manual, referred to as "Update 2000," shortened the procedure by eliminating the use of a green filter and acetic acid, and simplified recording of findings by limiting what was recorded to just the intactness of the epithelium and blood vessels, eliminating the use of descriptors such as "erythema," "abrasion," etc.²

A third conference of experts was recently convened by CONRAD and WHO. The meeting was entitled "Assessing Inflammation and Epithelial Integrity in Vaginal Product Research" and took place on November 19-21, 2003, in Punta Cana, Dominican Republic. It was attended by 68 participants from 13 countries, and produced this second updated manual ("Update 2004") for the colposcopy procedure, as well as separately published proceedings. The conference addressed the colposcopy procedure as well as other visual and non-visual means of assessment. This Update 2004 manual addresses only the colposcopy procedure. Other assessment procedures are addressed in the conference proceedings. The major differences between Updates 2000 and 2004 are the reintroduction of descriptors (Table 1), clarification of recording non-colposcopic findings, and redesigning of the Data Collection Form.

THE ROLE OF COLPOSCOPY IN ASSESSING NEW VAGINAL PRODUCTS

In general, the sequence of events by which a vaginal product could affect STI/HIV acquisition is shown in the figure below. The central feature is "epithelial changes," which could include breaks in the epithelium, inflammation, or other not well-characterized changes. These epithelial changes may be product-related, but may also result from non-product-related factors, such as mechanical trauma from intercourse, exposure to semen, use of other vaginal products, endogenous or exogenous hormonal factors, infections, or the examination carried out to detect epithelial changes.



¹ *Manual for the Standardization of Colposcopy for the Evaluation of Vaginally Administered Products*. Geneva: WHO, 1995

² *WHO/CONRAD Manual for the Standardization of Colposcopy for the Evaluation of Vaginal Products, Update 2000*. Geneva: CONRAD/WHO, 2000

Epithelial changes could lead to an increased risk of STI, including HIV, cause discomfort without increasing the risk of infection, or be of no clinical significance. It is hypothesized that epithelial changes could increase the risk of infection by two mechanisms. First, mechanical disruption of the epithelium and/or underlying blood vessels could create portals of entry for pathogens and/or an increase in availability of target cells. In a study of nonoxynol-9, it was found that the hazard ratio for HIV infection among women with a grossly visible epithelial breach was twice that of women without such a break.³ Second, inflammation could loosen tight junctions between cells, release enzymes that destroy tissue, attract target cells, and increase the viral load in genital secretions by activation of latent virus in mucosal immune cells. The purpose of colposcopy is to identify signs of mechanical disruption.

Since the second conference, the benefits but also the limitations of colposcopy have become clearer. Standard colposcopy requires reliable electricity, expensive equipment, and specific training, all of which increase the cost and complexity of vaginal product studies. A study which compared different means of assessment shed light on the value of colposcopy over naked eye examination, the amount of variation between observers, and two different methods of magnified observation.⁴ The development of a photo atlas for colposcopy has helped to standardize documentation of findings,⁵ but the decision of what to record is still very often subjective. Colposcopic assessment yields a large number of observations but it is not well established which are the most important when evaluating product safety. Colposcopy should be primarily used as a screening tool for products in the early phases of development. More research is needed to better understand which colposcopic findings indicate risk.

REVISED PROCEDURE FOR COLPOSCOPY IN THE DEVELOPMENT OF NEW VAGINAL PRODUCTS

The steps to be followed in carrying out the revised colposcopy procedure appear on page 6. The terminology to be used for recording colposcopic findings appears in Table 1. Photographs of colposcopic findings follow Table 1, and a sample Data Collection Form follows the photographs. The entire manual is available on the CONRAD and WHO websites, on CD-ROM, and in print (see inside front cover). The Data Collection Form may be edited as needed for individual study protocols.

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

³ Van Damme L, Ramjee G, Alary M, Vuylsteke B, Chandeying V, Rees H, Sirivongrangson P, Mukenge-Tshibaka L, Ettiegne-Traore V, Uaheowitchai C, Karim SS, Masse B, Perriens J, Laga M; COL-1492 Study Group. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. *Lancet*. 2002;360:971-7. Erratum in: *Lancet* 2002;360:1892.

⁴ Ballagh SA, Mauck CK, Henry D, Archer DF, Abercrombie T, Callahan M, Gabelnick H. A Comparison of Techniques to Assess Cervicovaginal Irritation and Evaluation of the Variability between Two Observers. Submitted.

⁵ Bollen LJM, Kilmarx PH, Wiwatwongwana P. *Photo Atlas for Microbicide Evaluation*. Bangkok: Thailand MOPH – U.S. CDC Collaboration, 2002

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. Since the colposcopic observations are subjective and difficult to interpret in isolation, it is highly desirable that the same colposcopist make the observations on all women in a center, in particular the observations before and after product use for an individual woman. In addition, a similar placebo or control vaginal product can be used by certain women so that the colposcopist is not aware of the exact product to which the woman has been exposed. Such masking will add objectivity to the study and improve the value of the research.

Either a plastic or metal speculum can be used during the colposcopic examination, as long as it is clean (sterilized, if reusable) and has been inspected to ensure that it is free of rough edges that could cause epithelial injury. The type of speculum used will be determined by cost and patient acceptability, with the final choice either specified in the protocol or made by the site. The protocol should specify the lubricant that may be applied to the speculum, if any. As stated in Step #6 of the colposcopy procedure, a dry swab may be used to remove excess fluid from the posterior blade of the speculum. At no time during the colposcopic examination should a dry swab be applied directly to the epithelium, as this may cause injury. Only large swabs moistened with saline may be applied to the epithelium.

Data should be collected on the type of findings, their location, size, and number, and whether they persist or progress. Findings should be summarized by participant and should include categorization by location and type of finding. Photography is desirable for documentation, quality assurance, and/or independent blinded review of findings.

The use of terms (Table 1) to describe findings makes communication between investigators easier, and terms can be used to double-check what is recorded regarding intactness of the epithelium and blood vessels. Disruption of the epithelium should be recorded as superficial or deep. Superficial epithelial disruption does not penetrate into the subepithelial tissue. Deep epithelial disruption penetrates into and exposes the subepithelial tissue and possibly blood vessels. If bleeding from the finding is present, the disruption should be recorded as deep even if it appears that only a minimal amount of tissue has been lost. The assessment of the depth of disruption is subjective, even at the level of magnification provided by colposcopy. This is one of the recognized limitations of the procedure.

When a finding involves more than one anatomical area, the percent of each area occupied by the finding should be recorded. Five categories should be used: none, 1-25%, 26-50%, 51-75%, and 76-100%. This is preferred over recording the percent of a finding present in each anatomical area. For example, it is 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 on the anterior fornix. Findings involving more than one anatomical area should be included in the tallies of each area, recognizing that the sum of the findings in each anatomical area may be greater than the total number of individual findings. Very

small findings that appear to result from a single insult (e.g. a group of petechiae or tiny abrasions) can be grouped and recorded as the area covered by the group.

The definition of severity of colposcopic findings has not been standardized, but the following scale is used by CONRAD and WHO. Colposcopic findings are considered mild in severity if both the epithelium and the blood vessels are intact. Colposcopic findings are considered severe if they involve deep disruption of the epithelium. All other findings are considered moderate. The sample Data Collection Form in this manual does not have a place to record severity, since it can be derived from other recorded variables. It should be noted that grading of the severity of colposcopic findings does not take into account the presence or absence of symptoms.

There is also no place to record whether a finding has worsened from one examination to the next, as this determination can be made retrospectively, but before unblinding, by evaluation of the following parameters. One suggestion for doing this is to base “worsening” on any of the following: a) an increase in size of one or more levels (i.e., <5 mm to 5-10 mm; 5-10 mm to >10 mm; <5 mm to >10 mm); b) a change in color of normal to red or white to red; c) an increase in epithelial disruption by one or more levels (i.e. intact to not intact/superficial; not intact/superficial to not intact/deep; intact to not intact/deep); d) the new occurrence of blood vessel disruption; or e) an increase in the percentage of an anatomical location that is involved (e.g. from code 1 to 3 or from code 2 to 4 or from not involved to any level of involvement). If a finding has worsened in some ways and improved in others, those parameters most likely to represent risk of HIV/STI infection, such as epithelial disruption, should be given the most weight.

Normal findings such as gland openings, Nabothian cysts, mucus retention cysts, Gartner’s duct cysts, skin tags, atrophic changes, blood vessel changes other than disruption, and anatomic variants may be recorded on a source document but not on a document that is entered into the database.

Cervical ectopy should be recorded and entered into the database, on a form that is separate from the colposcopy Data Collection Form, recognizing that its significance for disease transmission has not been conclusively demonstrated.

Findings that are not normal but are not considered colposcopic findings (e.g. bleeding from the cervical os, condylomata, copious cervical discharge, cervical polyps, foreign bodies) should be recorded on the source document. Such findings that are present before product use do not need to be entered into the database. Those that change or are first seen after product use begins should be recorded as an adverse event on a form that is separate from the colposcopy data collection form, entered into the database, and followed according to standard clinical procedures.

The presence and source of blood seen on the naked eye examination should be recorded on a form that is separate from the colposcopy Data Collection Form and entered into the database. Such bleeding could be: a) from the cervical os (and recorded retrospectively as intermenstrual or

depending on cycle day and the investigator's judgment);⁶ b) from an abnormality that is not considered a colposcopic finding, such as a cervical polyp; or c) from no obvious source: for example, blood that is easily removed with no further evidence of bleeding from any source. Intermenstrual bleeding, bleeding associated with new or changed findings described in the preceding paragraph, and bleeding from no obvious source should be considered adverse events.

The presence of blood seen in association with a colposcopic finding should be recorded as a colposcopic finding involving deep disruption of the epithelium (even if the apparent degree of epithelial disruption is minimal) and disrupted blood vessels.

Certain aspects of studies are not part of the colposcopy procedure itself, but may affect it or be affected by it. For example, Pap smears should be done at screening to rule out neoplastic changes of the cervix requiring further investigation. Sufficient time should be allowed to elapse between a Pap smear and the subsequent colposcopy to allow healing of the epithelium. Although there are no definitive data on how long this takes, it is generally agreed that 3 days should be sufficient. Most study protocols for safety evaluation of vaginal products require that women with abnormal Pap smears be excluded and that sufficient time be allowed for the microinjury caused by the procedure to have resolved before any exposure to test product. Diagnosing and treating reproductive tract infections, e.g. yeast vaginitis, genital herpes, and trichomoniasis, should be done at screening since these infections can cause epithelial damage. If pustules, ulcerations, vesicles, or bullae are found during a study, they should be sampled and examined in potassium hydroxide for fungal elements. In addition, Polymerase Chain Reaction for the *Herpes simplex* virus, and testing for syphilis via fluorescent treponemal antibody and/or darkfield microscopy should be performed and the results recorded. Bimanual exams, if performed, should be done after colposcopy is completed.

⁶ Colposcopy should be avoided during menstruation, in order to avoid attributing bleeding to use of the product. For this reason, protocols for expanded safety studies usually include a follow-up period of no more than 14 days and schedule the interval of product use to fall between menses.

STEPS TO BE CARRIED OUT WHEN PERFORMING COLPOSCOPY IN THE DEVELOPMENT OF NEW VAGINAL PRODUCTS

Prior to the examination: Everything needed for the procedure should be in place before the study participant is brought into the room. This includes working equipment, spare bulbs, and adequate specula that have been inspected to make sure there are no rough edges that could induce epithelial injury. A Standard Operating Procedure written by each site helps ensure that these steps are taken.

1. PARTICIPANT POSITIONING:

The participant should lie on a soft examination table in the lithotomy position with leg supports so that the perineum and vulva can be inspected. At all times, the physical and emotional comfort and privacy of the woman should be ensured.

2. NAKED EYE AND COLPOSCOPIC EXAMINATIONS OF EXTERNAL GENITALIA:

Examine the external genitalia with the naked eye and record findings. Then, using appropriate magnification (usually 4-10X), examine the external genitalia again and record findings.

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 protocol to the external surfaces of the blades. Gently insert and open the speculum so as to prevent trauma and position it so that the cervix and upper vagina can be seen clearly.

The position of the cervix relative to the vagina and the 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.

4. NAKED EYE EXAMINATION OF VISIBLE EPITHELIUM:

Naked eye inspection of visible epithelial surfaces should be performed without manipulation. Record findings.

5. AUXILIARY VAGINAL TESTS:

If a vaginal specimen, such as a wet preparation, pH test, or vaginal microbiological test is collected, 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 syringe, gently 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. Do not permit contact between the syringe and the epithelium. Dry swabs may be used to remove obscuring fluid from the posterior blade that cannot be removed by aspiration. (Do not use dry swabs in any other manner during the colposcopic exam.)

If the product obscures findings, it should be lavaged away as gently and completely as possible using a medium specified in the protocol. All unobscured epithelial surfaces should be examined. If lavage alone does not adequately remove the study product, a saline-soaked swab may be used. Record any observations not noted on previous naked eye examination.

Some protocols may require collection of lavage fluid for measurement of inflammatory markers. If this is felt to be of higher priority than collection of vaginal specimens, it may be collected first; this should be specified in the protocol.

7. COLPOSCOPIC EXAMINATION OF CERVIX:

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

8. AUXILIARY CERVICAL TESTS:

Cervical specimens are generally collected after colposcopic examination of the cervix since their collection is likely to induce minor trauma that may be erroneously attributed to product use.

9. 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. Additional irrigation and/or slight manipulation of the speculum may be necessary to clearly visualize the fornices. The lateral fornices are best exposed by placing a saline-moistened swab in the contralateral fornix and pressing toward the head and laterally. For example, to view the right lateral fornix, place a saline-soaked swab in the left lateral fornix and press gently toward the woman's head and left side. Record findings.

10. COLPOSCOPIC EXAMINATION OF VAGINA:

To examine the rest of the vagina, move the colposcope to bring the lateral vaginal walls into focus. Slowly withdraw the speculum, relaxing the blades as necessary for the comfort of the woman and refocusing as needed, to view the anterior and posterior vaginal walls. Record findings.

The results of the colposcopic examination should be documented using the terms in Table 1 and by recording the status of the epithelium and blood vessels for each numbered finding.

TABLE 1. TERMINOLOGY FOR COLPOSCOPIC FINDINGS

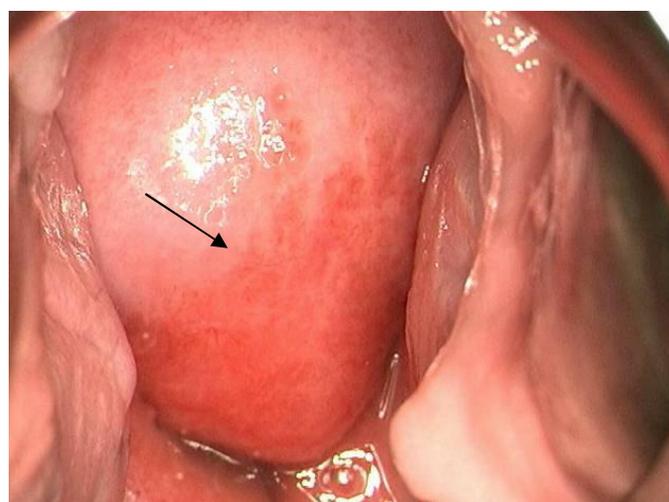
Term	Status of epithelium*	Status of blood vessels	Comments	
Erythema	Intact	Intact	Distinguished by color (erythema being redder than normal, edema either normal or paler than normal, and grossly white findings being white). Grossly white findings are sharply demarcated whereas edema and erythema may be sharp or diffuse.	
Edema	Intact	Intact		
Grossly white finding	Intact	Intact		
Petechiae	Intact	Disrupted	≤ 3mm	Color of finding is red or purple.
Ecchymosis	Intact	Disrupted	> 3mm	
Peeling	Disrupted, superficial	Intact	Fragment of disrupted epithelium may remain attached to the area from which it has peeled off. Generally has well demarcated outline. Underlying epithelium looks normal.	
Ulcer	Disrupted, superficial or deep	Intact or disrupted	May include sloughing at base. Generally round or oval with sharply demarcated outline. Superficial ulcers are more accurately called erosions.	
Abrasion	Disrupted, superficial or deep	Intact or disrupted	Distinguished from other findings in this class by diffuse or poorly demarcated outline	
Laceration	Disrupted, superficial or deep	Intact or disrupted	Sharply demarcated linear finding. Includes fissures. Lacerations appear to be the result of trauma. Fissures appear to be linear “pulling apart” or wearing away of tissue.	

*Superficial epithelial disruption does not penetrate into the subepithelial tissue. Deep epithelial disruption penetrates into and exposes the subepithelial tissue and possibly blood vessels. If bleeding from the finding is present, the disruption should be recorded as deep.

PHOTOGRAPHS - 1



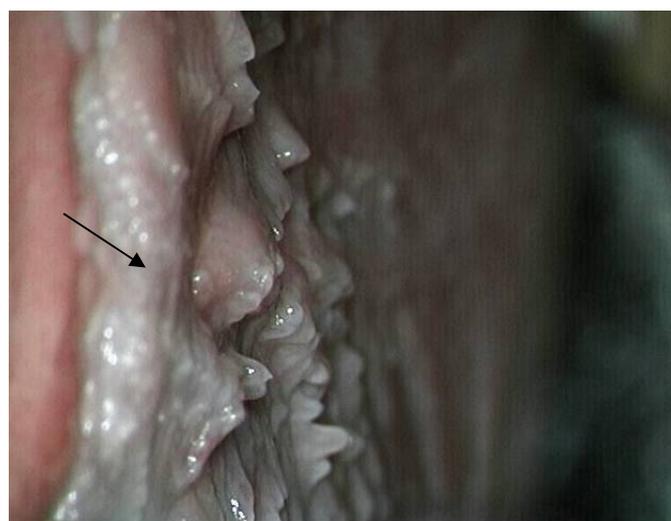
Normal



Erythema

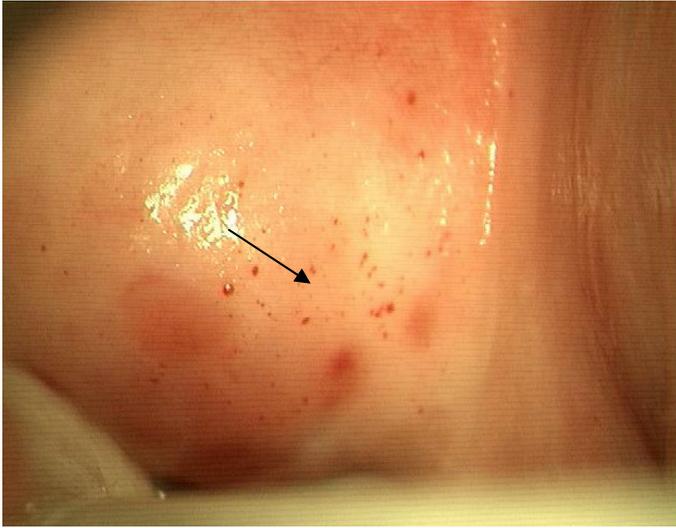


Edema

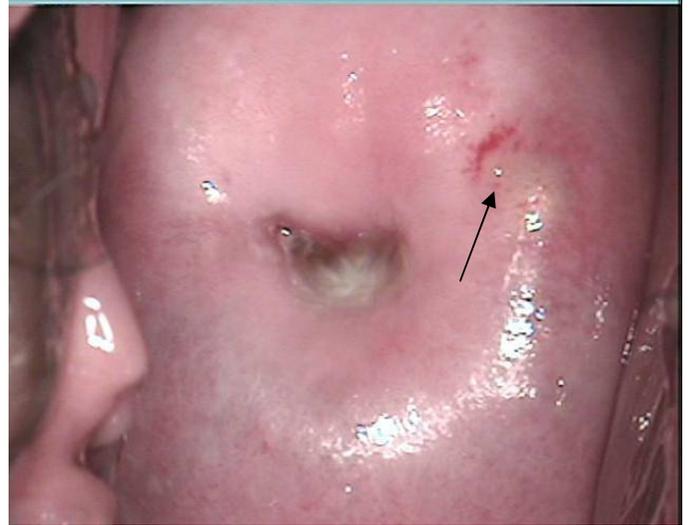


Grossly White Finding

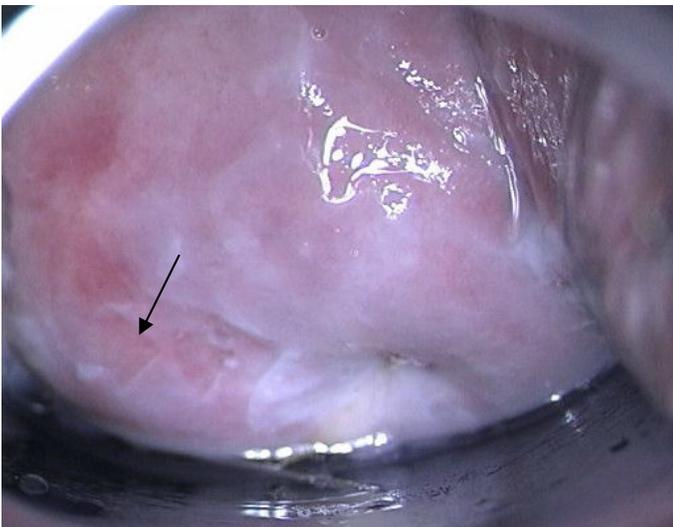
PHOTOGRAPHS – 2



Petechiae



Ecchymosis

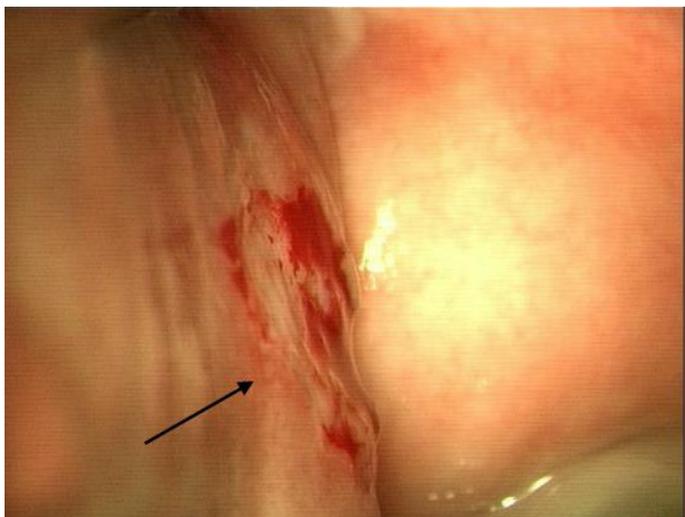


Peeling

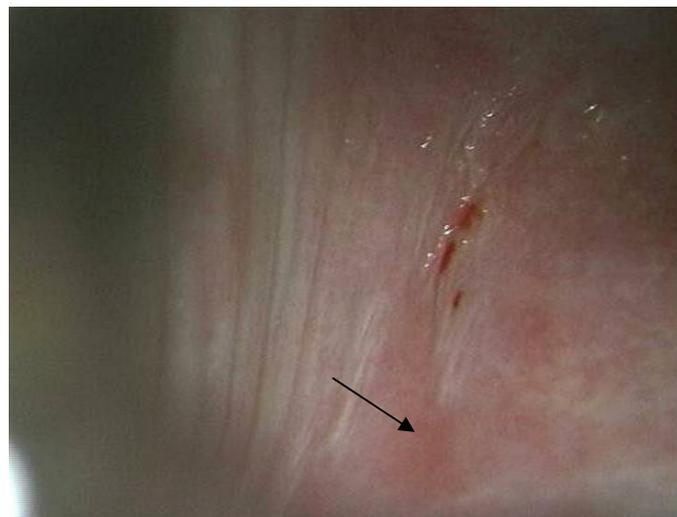


Ulcer

PHOTOGRAPHS – 3



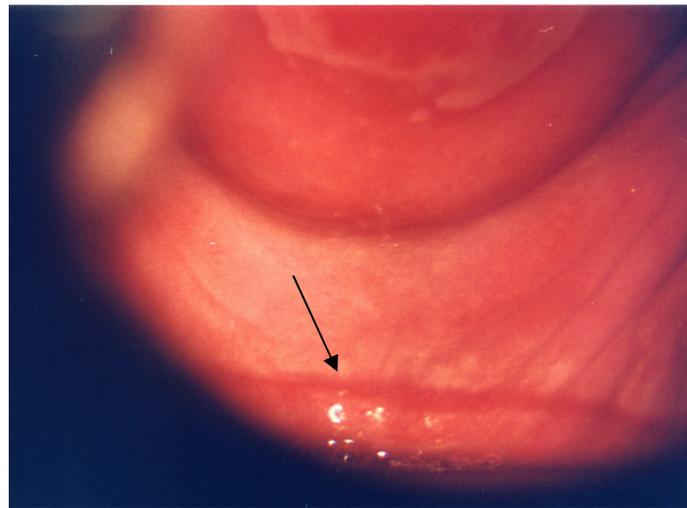
Abrasion



Laceration



Ectopy



Speculum Trauma

SAMPLE DATA COLLECTION FORM

The following two pages show a sample Data Collection Form
which may be used to record colposcopic findings.

It may be modified as necessary to accommodate the needs of individual study protocols.

(See Excel file [Sample DCF.xls](#))

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