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A guide for standardizing colposcopic observations for the evaluation of topical microbicide products in the pigtailed macaque model

Dorothy L. Patton

Department of Obstetrics & Gynecology, University of Washington, Seattle, WA, USA
dpatton@u.washington.edu

Introduction

This laboratory has been conducting colposcopy on *Macaca nemestrina* research animals since the mid-1990s. Our observations are always based on those recorded in human studies, and we have developed a standardized colposcopic observation form specifically for pigtailed macaque studies. All findings are based on the 'Manual for the Standardization of Colposcopy for the Evaluation of Vaginal Products: Update 2004', developed by WHO and CONRAD for use with humans [1]. Our studies have been conducted exclusively at the Washington National Primate Research Center (WaNPRC) in Seattle, Washington. Approval for the use of monkeys was obtained from the Institutional Animal Care and Use Committee at the University of Washington. Animals were handled humanely, and the experiments were performed within the National Institutes of Health's animal-use guidelines, in compliance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

Our earliest studies were conducted with a modified endoscope, which was hand held through the speculum-opened vagina. It was attached to a video camera for video documentation of mucosal tissue assessments. We have since upgraded to a Leisegang swing arm colposcope, fitted with a digital camera and a video recording unit (CooperSurgical, Inc., Trumbull, CT, USA). Standardized studies conducted in this laboratory assess cervicovaginal tissues prior to and after single and repeated vaginal exposures to test products. One or two members of a team of cross-trained colposcopists conduct the vaginal examinations. The findings are recorded on standardized observation sheets and documented by still and video photography.

Our primary use of colposcopy in the macaques is to evaluate the safety of topical microbicide products being developed for eventual use by humans [4–12]. Similar use of colposcopy in various rhesus and cynomolgus macaque models has been reported by other laboratories [2, 3, 13–16].

It is our hope that the terminology and photo atlas presented here will provide a reference for documenting colposcopic findings across multiple macaque models.

Guidelines for documenting colposcopic findings in the pigtailed macaque model

For colposcopic examination, a ketamine-sedated (10–15 mg/kg) macaque is placed on the examination table positioned in sternal recumbency (on belly, knees bent). A water-dampened, sterilized pediatric speculum is gently inserted to the vagina and opened to allow for visualization of the cervix and lateral vaginal walls. Cervical exudate is noted (Fig. 1. Colposcopy assessment guide) and then gently removed with polyester-tipped swabs, if necessary for tissue visualization.

Vaginal findings

With a pediatric speculum in place, the colposcope is used (7.5–15× magnification) to examine the right- and left lateral walls of the vagina, followed by the posterior and anterior vaginal fornices (Fig. 1). The vaginal walls may appear smooth or, more frequently, rugated. All the colposcopic observations are documented (Table I, Terminology for colposcopic findings) in each of these four vaginal areas. For each site, every observed finding, its color, the status of the epithelium, and the size of the finding is noted. If the area under observation is normal, it is noted as such. If an area of the vagina is obscured or otherwise cannot be viewed through the colposcope, a notation is made that the area was not evaluated.

Cervical findings

After evaluating the vaginal tissues, the face of the cervix is examined. If present, cervical ectopy is noted. Ectopy refers to the columnar epithelial cells (hall-mark of endocervical mucosal surface) present on the ectocervix or protruding through a lax cervical os. To document the location of colposcopic findings, the cervix is divided into four quadrants for individual assessment. Imaginary crosshairs are positioned over the face of the cervix, with the vertical and horizontal axes resting on the cervical os (see Fig. 1). For each quadrant of the cervix, every finding, its color, the status of the epithelium, and the size of the finding, is documented (Fig. 1). Every observed finding in each cervical quadrant should be documented. Notation is made if the finding extends to the cervical os or into the area of ectopy. If the quadrant under observation is normal, it is noted as such. If an area of the cervix is obscured by the vaginal wall or otherwise cannot be viewed through the colposcope, a notation is made that the area was not evaluated.

Reporting findings

Our extensive use of colposcopy has provided important information regarding fluctuations in vaginal and cervical tissues that are frequently noted in the absence of test product exposure. Variations in the appearance of subepithelial blood vessels, tissue color ranging from pale to dark pink, grossly white findings on vaginal walls, and the appearance of petechiae are all noted frequently in control animals and thus considered findings within normal variation in our studies. The term ‘grossly white findings’ is borrowed directly from the clinical colposcopy manual and is descriptive of sharply demarcated, blanched tissues raised in peaks, most often noted on vaginal walls. Edema and ecchymosis are also noted,

though less frequently, in control animals, so are considered moderate findings which might be associated with product use. Adverse findings include any break in the integrity of the epithelial layer, deeply reddened erythema, and friable tissues.

We have rarely noted laceration and to date have never noted peeling of the epithelium at colposcopy. The finding of blisters on the cervix is strongly associated with chlamydial infection, but rarely noted during topical product safety evaluations.

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Fig. 1.
Colposcopy assessment guide.

Table 1

Terminology for macaque colposcopy findings

Term	Status of epithelium	Size measure	Comments
Normal	Intact	Not applicable	Color could be pink, slightly red or pale. All other colors indicate the observation is not normal.
Erythema	Intact	%	Redder than normal color, may be sharply or diffusely demarcated (congested vessels).
Edema	Intact	%	Color may be normal or pale. Tissue surface appears swollen with excessive fluid retention. May be sharply or diffusely demarcated.
Grossly white finding	Intact	%	White and sharply demarcated, most frequently noted on vaginal walls as white peaks.
Petechiae	Intact	Not applicable	Punctate Color of finding is red or purple (subcutaneous ruptured vessels).
Ecchymosis	Intact	mm	>2 mm
Peeling	Disrupted, superficial		Fragment of disrupted epithelium may remain attached to the area from which it has peeled off. Underlying epithelium looks normal.
Ulcer	Disrupted, superficial or deep	mm	Pitted appearance on (disrupted) tissue surface. Generally round or oval with sharply demarcated outline.
Abrasion	Disrupted, superficial or deep	mm	Distinguished from other findings in this class by diffuse or poorly demarcated outline.
Laceration	Disrupted, superficial or deep	mm	Sharply demarcated linear finding. Includes fissures.
Blisters	Intact	mm	Raised area on surface of tissue that appears to be filled with fluid.
Friable	Intact	Not applicable	Bleeding from the epithelial surface after it has been touched with a swab. The epithelium appears to be intact.
Ectopy	Intact	Not applicable	Columnar epithelial cells (hallmark of endocervical mucosal surface) are present on the ectocervix, or protruding through a lax cervical os. Distinguished by reddened color and appearance of columnar rather than squamous epithelium.

Definitions are adapted from the WHO/CONRAD Manual for the standardization of colposcopy for the evaluation of vaginal products. (2004).