DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Lugol (for colposcopy) technical information
Technical card code 09-251
Product code 09-251
Stability of product properly conserved at 15-25°C in a dark place 24 month.
Pack 500-1000 ml or on request
CND code W0103010301

Produce in Italy by:
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For emergency contact your nearest anti poison unit.

Introduction

Colposcopic examination of the vagina, like cervical colposcopy, is performed with the main focus on cancer prevention. As with cervical examination, the vaginal lesions of interest to the colposcopist share macroscopic and histological features with the premalignant and malignant lesions of the cervix and vulva. Colposcopy of the vagina is of value in the assessment of benign as well as premalignant and malignant vaginal lesions. It permits accurate directed biopsy and precise therapy of intraepithelial neoplasia.

Indications

Colposcopic examination of the vagina is most frequently performed following an abnormal Pap smear or by the presence of concomitant cervical and vulvar HPV lesions. Routine visualization of the vagina is a part of colposcopy technique.

Vaginal colposcopy is of utmost importance when cytology suggests disease in:
- patients with a normal cervix
- in patients in whom the cervix is absent

or when evaluating the possibility of premalignant and malignant disease in:
- immunosuppressed patients with premalignant or malignant vulvar lesions
- suspected or known intrauterine DES exposure

those patients previously treated for premalignant or malignant vaginal lesions.

Premalignant Lesions

Vaginal neoplasia is more common in patients who have previously been treated for cervical or vulvar neoplasia. Therefore acetowhite vaginal lesions may appear in tissue contiguous to previously involved areas, i.e., at the vaginal apex, in the fornices or the lower vagina. It is estimated that 2.5% of women who have CIN develop VaIN (vaginal intraepithelial neoplasia).
The premalignant vaginal lesions appear to be more homogenous than their vulvar counterparts. While it is estimated that 9 of every 10 truly premalignant lesions of the vagina occur in women over 40 years of age, the incidence of such lesions may be rising in younger women and may correspond to a similar rise in vulvar intraepithelial neoplasia, possibly a reflection of the increased incidence of infection with human papilloma virus generally noted in the younger population.
Vaginal premalignant lesions are classified as VaIN 1, 2 or 3, corresponding to mild, moderate or severe dysplasia, respectively. There is now a trend to classify these lesions as low grade or high grade intraepithelial neoplasia based on their histology. This is a spin-off from the change in cervical cytological terminology. The propensity of these lesions to progress to a higher grade is less well established than for CIN, but evokes the same concern.
As with cervical examination, acetic acid (3-5%) is used and may be applied with swabs, sponges or by spraying. Acetowhite lesions will vary in their intensity of “whiteness” as do cervical lesions. VaIN lesions are distinguished as acetowhite surface lesions and by their yellow Lugol’s staining characteristics (1/2 strength Lugol’s iodine - the Shiller test). Such lesions frequently show sharp borders and stand out in relief contrasting with the surrounding darkly staining normal epithelium.
The occasional flat non-staining benign mucosal lesion may reflect differences in metaplastic epithelium, keratin production or glycogen storage or it may signal dysplastic change. Various shades of yellow depend upon the degree of epithelial glycogen content.

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Any non-staining surface erosion that bleeds easily or is easily denuded may harbor inflammatory or neoplastic tissue. High grade acetowhite lesions may appear as flat or somewhat elevated pearly gray-white epithelium. Some lesions may appear smooth and may contain coarse punctation or the occasional atypical vessel. As with cervical lesions, aceotwhite vaginal lesions may or may not be associated with abnormal vascular patterns such as punctation and mosaic. The use of the green filter helps to identify abnormal vascular patterns and gives sharp contrast to non-staining areas. As with the common flat or slightly raised lesions, spiculated, granular or papillary (warty) lesions may be diagnosed as VaIN I or VaIN 2, especially in cases of concomitant benign vulvar warts. The degree of dysplasia is confirmed by biopsy and similar rules of management may be applied as to cervical lesions.

Special cases:
Prior radiotherapy and immunosuppression are also predisposing factors for vagina dysplasia. Diethylstilbestrol (DES) associated changes in the vaginal epithelium of women exposed in utero to DES include adenosis and extension of the transformation zone beyond the cervix into the vaginal fornices. Over time, in many instances there is “healing” or maturation of the adenosis and a return to a more normal appearing transformation zone that has a vaginal extension. Nevertheless, in some women this process is incomplete and the colposcopic appearance of the vagina remains quite abnormal and concerning. Fortunately, occult carcinoma in the absence of abnormal cytology or a palpably suspicious lesion is rare.
Vaginal condyloma may appear similar to VaIN, and may occasionally be confused with vaginal rugae or papillations, though a rugose epithelium and its papillations represent normal tissue and stain darkly with iodine. Condyloma are frequently multifocal and commonly coexist with similar cervical or vulvar lesions. HPV disease can be focal, multifocal or multicentric necessitating complete examination. The discovery of one lesion should therefore precipitate a careful search for others.
Malignant Lesions

Primary invasive cancer is rare and most (80-90%) of vaginal carcinoma is secondary to an occurrence elsewhere. The majority of invasive lesions present with a clinically apparent lesion. The colposcopist may observe granular red lesions, white epithelium with or without abnormal vessels, ulceration, tumor formation and intraepithelial or sub-epithelial hemorrhage in invasive lesions. Most of these are cancers are squamous, although adenocarcinoma and others (melanoma) are reported. Metastatic tumors to the vagina most commonly occur among women with concurrent or previously treated cervical cancer. Ovarian and rectal cancers, as well as choriocarcinoma, may metastasize to the vagina. In these instances cancer is generally suspected after gross visual inspection and palpation, thus colposcopy is not likely to be clinically useful.

Benign Lesions
Many benign conditions may masquerade as neoplastic lesions and only through colposcopy and biopsy will the precise nature be ascertained. Atrophic changes are great mimics for colposcopists and pathologists. It is often difficult for a cytologist to distinguish basal cells associated with severe atrophy from glandular cells or severely dysplastic squamous cells. Prior radiation therapy to the vaginal vault may lead to bizarre, atypical vessels and distinguishing cytological benign radiation changes from more serious lesions is usually only settled after colposcopy and biopsy. Vaginal endometriosis and granulation tissue are usually apical vaginal findings whereas chronic ulcers or vaginal adenosis (columnar metaplasia) that may follow treatment with thermal energy sources or 5-fluorouracil are found anywhere in the vaginal canal. Such changes are associated with abnormal colposcopic findings and require biopsy for definitive diagnosis.

Technical Aspects
Use 3 -5 % acetic acid and 1/2 strength Lugol’s solution (the Schiller test) in separate steps. Check for iodine allergies.
If present, do not use Lugol's solution.
Colposcopic examination of the vagina is performed with a typical colposcope at the usual (13 - 15 X) or lower magnification (7-10X), and is similar to cervical examination. Three to five percent acetic acid is applied and a complete vault examination is performed. Subsequently 1/2 strength Lugol’s solution (the Schiller test) is applied to the mucosa and the examination is repeated.

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Atrophy and vaginitis must first be cleared as noted later. Because of the geometry involved, the examination of the vagina may be a challenge. Viewing the lateral vaginal walls with their folds and rugae, is more time consuming than cervical colposcopy.
Most vaginal neoplastic lesions occur in the upper one third of the vagina, with about 10% being found in the lower one third. The mid vagina is least likely to be the site of primary neoplastic change but has been found to be a site for extension of bladder or rectal carcinoma. Vaginal lesions may be multifocal. This requires that the lateral vaginal wall and fornices be examined carefully.
Application of 50-60% aqueous Lugol's iodine (the Shiller test) will "light up" acetowhite epithelial lesions and their margins.
The Schiller test (1/2 strength Lugol's stain) is an important aid to vaginoscopy and should not be omitted, unless the patient is allergic to iodine. It is important that the entire vaginal wall be stained, which necessitates rotating the speculum 180 degrees to ensure the iodine reaches all of the surface of the vaginal canal and that all of the vagina is seen. One of the technical challenges in performing the colposcopic examination of the vagina on women who have undergone hysterectomy is that the mucosa at the lateral margins of the vaginal apex, referred to as "dog ears", may be somewhat elevated and variably invaginated.
Such invaginations, created by hysterectomy (angle sutures) prevent full examination of the entire mucosal surface of the vaginal cuff. This results in occult or "buried" lesional tissue that may result in invasive disease later on. Small tissue hooks and or grasping forceps may be used to evert such areas. Such lesions may require examination under anesthesia for full evaluation.
At that time local excision, lesion ablation with laser or apical vaginectomy may be performed.
Postmenopausal women often present with atrophy, which hinders the identification of abnormal epithelial areas. Lack of glycogen in the epithelium, especially when associated with inflammation, decreases the reliability of vaginal colposcopy. The transition from lesional to normal tissue that is noted with Lugol's solution (the Shiller test) is attenuated and the very atrophic vagina may appear diffusely "dirty brown". A useful strategy for the atrophic vagina is to prescribe several weeks (4 - 6 weeks) of topical vaginal estrogen, which should correct atrophy and lessen patient discomfort. Estrogen therapy "brings out" or helps one identify the source of abnormal vaginal cytology by thickening the mucosa and diminishing tissue inflammation. Further, dilute acetic acid or iodine used for staining, may produce significant burning and be generally irritating to the atrophic vagina. The prevention of severe discomfort at examination is another reason for pre-colposcopy estrogen therapy. Also consider that vaginal stenosis may add to examination difficulty, discomfort and anxiety at colposcopy. Unfortunately, estrogen does little for stenosis but is used as an adjunct to vaginal dilation prior to colposcopy of the stenotic vagina.

Finally, obtaining a good biopsy specimen of flat lesions from an atrophic epithelium (or stenotic canal) may be technically difficult because of the limited space and the thin friable mucosa. In these and other uncommon situations (i.e., children), the vaginal colposcopic examination may be performed under anesthesia.

* Notes. Distilled water or tap water can be used for rinsing and moisturizing. Always check the pH of your tap water and chlorine levels before proceeding with any type of biological tissue and stain.

* Technical's note: staining time vary according to age, types of solutions, thickness of sections, et. When Gill (code 09-178) modified solution is used, get the best result, staining time (maximum 1-5 minutes), for best change in color, wash quickly in tap water, and then in Scott acidulated solution, (code 00-136). For sections fixed in Bouin, we recommend the use of haematoxylin modified acid AB (code 09-183).
Please note the alcoholic loses eosin stain with the use, of the days are stretched over time colouring. If you are using purified eosin, check the time, and possibly diluted in ethyl alcohol 96°C, if the cytoplasmic staining was too strong.

* Before use, filter the following solutions; alcoholic eosin, eosin phloxine; Harris haematoxylin, Gill's haematoxylin. The acidified aqueous solution of eosin is prepared by slowly adding glacial acetic acid. Follow normal precautions for laboratory reagents. Dispose of waste according to regulations at the local, regional or national level. Refer to Data Sheet Material Safety Data for updated information on risks, hazards and safety associated with the use of these products.

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* Risk and Safety Statements outside the EU. The eosin solution in alcohol is flammable and harmful. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

* Target organs: eyes and nerves. Eosin in aqueous solution. Caution: substance not yet fully tested. Avoid contact and inhalation of the solution of Harris haematoxylin. Organs: heart and nerves. Solutions based hemallum are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing. Organs affected: liver and kidneys. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

* Risk and Safety Statements (U.E.).
The eosin solution in alcohol is highly flammable and harmful.
Highly flammable.
Harmful by inhalation, in contact with skin or if swallowed.
Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Keep away from sources of ignition - No smoking.
Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).
Eosin in aqueous solution.
Caution: Substance not yet fully tested. Solution of hemallum.
Do not breathe vapours.
Avoid contact with skin and eyes. Gill haematoxylin Solutions are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing.
* The microscope used should meet the requirements of a medical diagnostic laboratory. Carefully follow the instructions for the fixative. If an automated tool was used for staining, follow the instructions of the equipment and software. Remove surplus immersion oil before storing.

* Sample preparation
All samples must be treated according to the technology.
All samples must be marked so as to be easily identified. Tools should be used for sampling and sample preparation, which must be observed strictly to manufacturer's instructions about the application and instructions.

* Diagnostics
The diagnosis should be performed only by authorized and trained persons. Valid nomenclatures must be used. Further tests must be selected and implemented according to recognized methods.

* Conservation. The staining solution should be stored at a temperature between +15°C to 20°C, the dye at +5°C to 30° C. Store at 4-6 °C all kit containing silver solutions and Schiff reagents. The solution and dyes must be used before the expiration date. Stability. After first opening the bottle, the dye solution and the dyes are stable until the expiration date when stored at the temperature requested. Always keep the bottles tightly closed.

* Instructions for use
To avoid errors, the staining process must be performed by qualified personnel. For professional use only. Must observe the National guidelines for work safety and quality assurance. Microscopes are used according to the standard. Protection against infection. Must be taken with laboratory guidelines for the protection against infection.

* Instructions for disposal
The solutions used and those have expired must be disposed of as special waste according to local regulations regarding disposal of waste.

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Endnotes
1 The timing suggested in the leaflet are approximate and may vary according to your specific needs. If they are used intensively, for staining solutions may lose their dyes, so it is necessary to extend the time of staining solutions, or replace with new products.
2. Include positive control slides in each session.
3. Some hydraulic systems deliver acidic water, unsuitable for use for the part of the procedure for the blue coloration. If tap water is acidic, instead using a dilute alkaline solution, for example, water buffered by Scott.
4. The presence of purple or red-brown nuclei a blue color indicates unsatisfactory.
5. If you over-eosin staining, nuclear staining may be masked. If done correctly, with eosin staining shows a three-tone effect. To increase the differentiation of eosin, extend the time of immersion in alcohol, or use a first alcohol with a higher water content. You can adjust the times of immersion in alcohol to obtain an adequate eosin staining.
6. We do not recommend the addition of stock solution in the working solutions of haematoxylin and eosin.
7. Avoid excessive drag (carryover) of water solutions in alcoholic eosin.
8. The data generated by this procedure are to be used only to support the diagnosis and should be evaluated in conjunction with other tests and diagnostic data