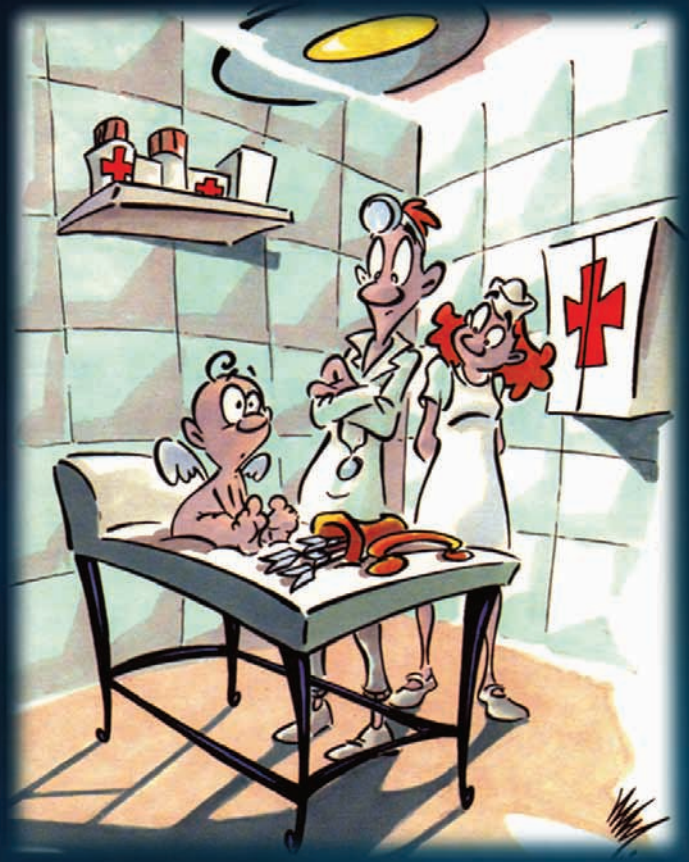
Direct Microscopy in Cervico-Vaginal Infections

G. MINIELLO



Cervico-vaginal infections represent the most common reason of gynecological consultation

Friedrich EG. Am J Obstet Gynec 51:247, 1985

Isolation of the infecting organisms is considered as the clearest method of establishing aetiology and susceptibility to antibiotics

Many organisms can not be identified with the use of culture-based techniques

Franasiak JM et al. Fertil Steril. Dec Vol. 104:1364, 2015

Thus, culture-based data, though still informative, must be interpreted within the limits of the technology

Franasiak JM et al. Fertil Steril. Dec Vol. 104:1364, 2015

Culture is

not the only

way in which

investigators can contribute

to an understanding

of infectious process

Franasiak JM et al. Fertil Steril. Dec Vol. 104:1364, 2015

In our current practice wet mount or direct microscopy is usually performed as an extension of the daily gynecologic checkup, in order to obtain more complete information

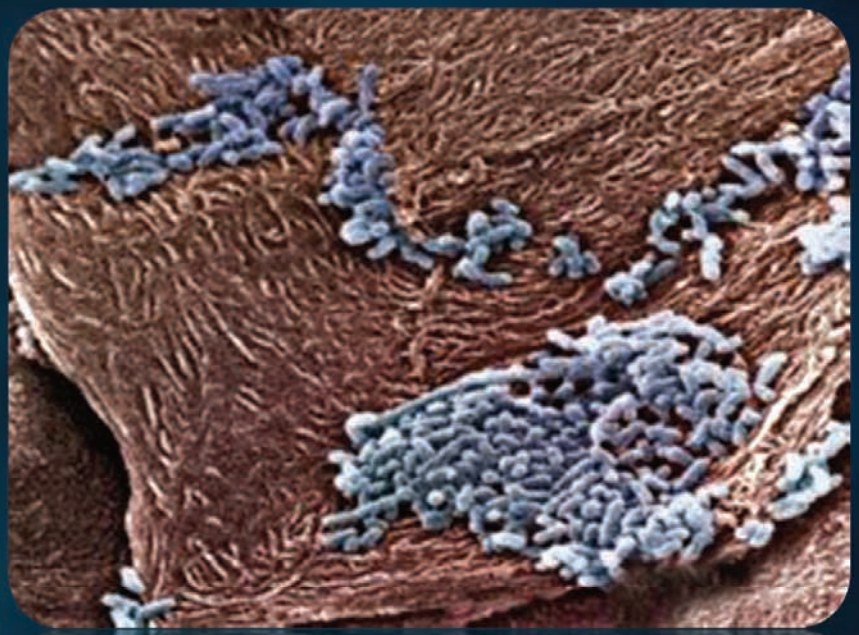
A wet prep can provide immediate information about:

- ✓ hormone status
- ✓ stages of metaplastic process
- ✓ cervico-vaginal microbiology
- ✓ cell changes induced by pathogens
- ✓ cell-mediated immunity
- ✓ presence of atypical cells

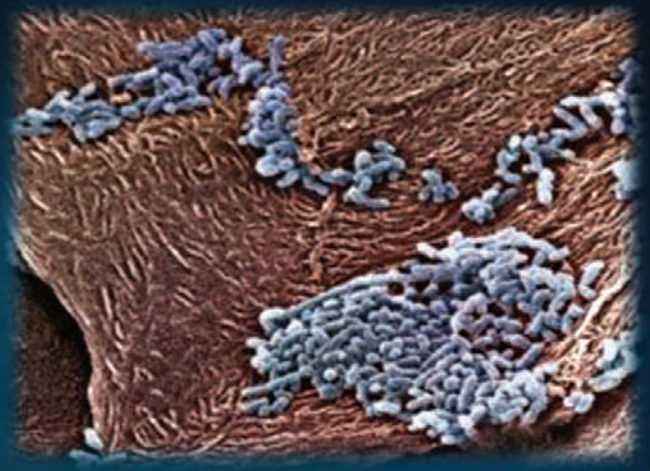
WET MOUNTS

- ✓ Cervico-vaginal wet mount
- ✓ Cutaneous wet mount
- ✓ Urinary wet mount
- ✓ Buccal wet mount
- ✓ Rectal wet mount

Bacterial vaginosis



Bacterial vaginosis is the most frequently found pathology of the female genital tract



Wilson J. SexTransm Infect. Feb;80(1):8, 2004

Bacterial Vaginosis (40%-50%)

Trichomoniasis (15%-20%)

Candidiasis (20%-25%)

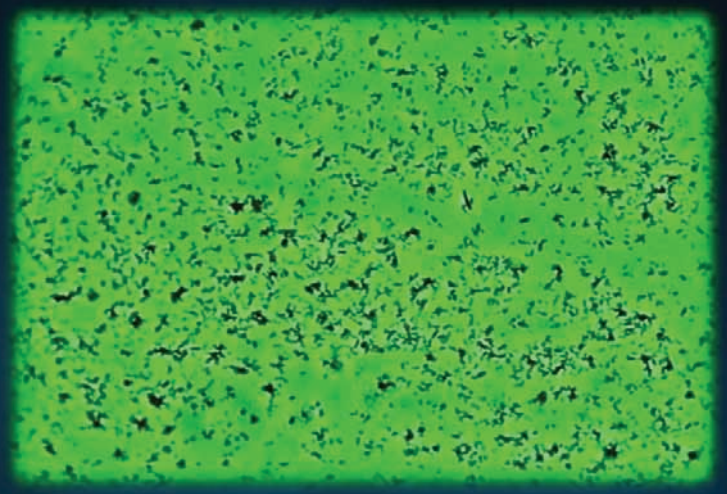
Mylonas I et al. Obstet Gynecol Surv. Jun;66(6):359, 2011

Bacterial vaginosis

is a polymicrobial disorder caused by an imbalance of vaginal microbial flora

Mitra A et al. Microbiome. Nov 1;4(1):58,2016

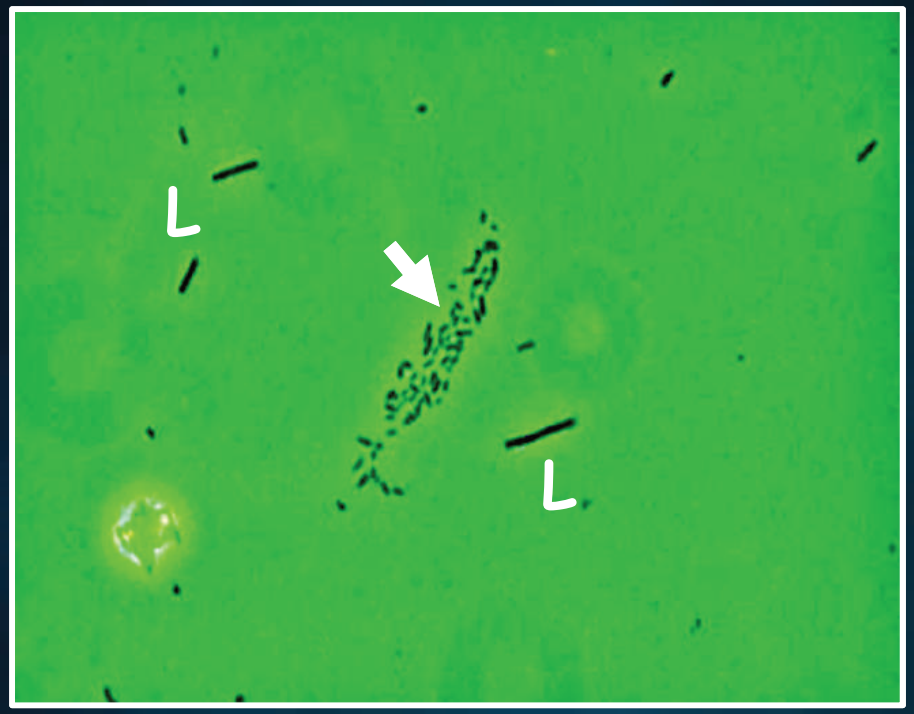
Diminished levels of *Lactobacillus* are associated with overgrowth of anaerobes, particularly *Gardnerella*, *Prevotella* and *Peptostreptococcus species*



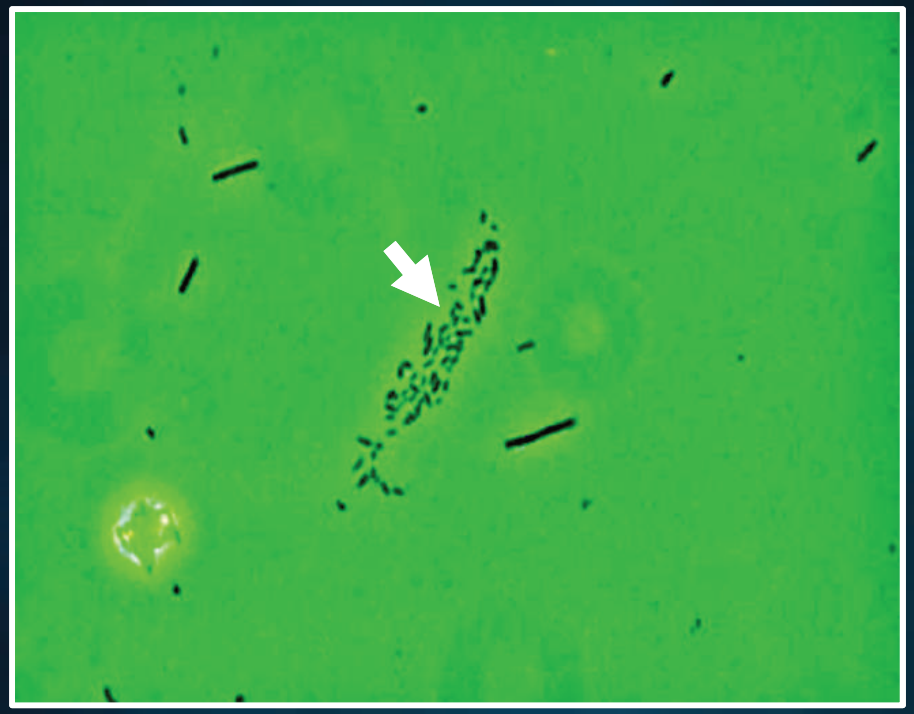
Mitra A et al. Microbiome. Nov 1;4(1):58,2016

Lactobacilli are replaced by other anaerobic organisms, normally found in small amounts in the vagina

Mastromarino P. Clin Microb and Infec.. 15(1):67, 2009



cluster of
pleomorphic bacteria



cluster of
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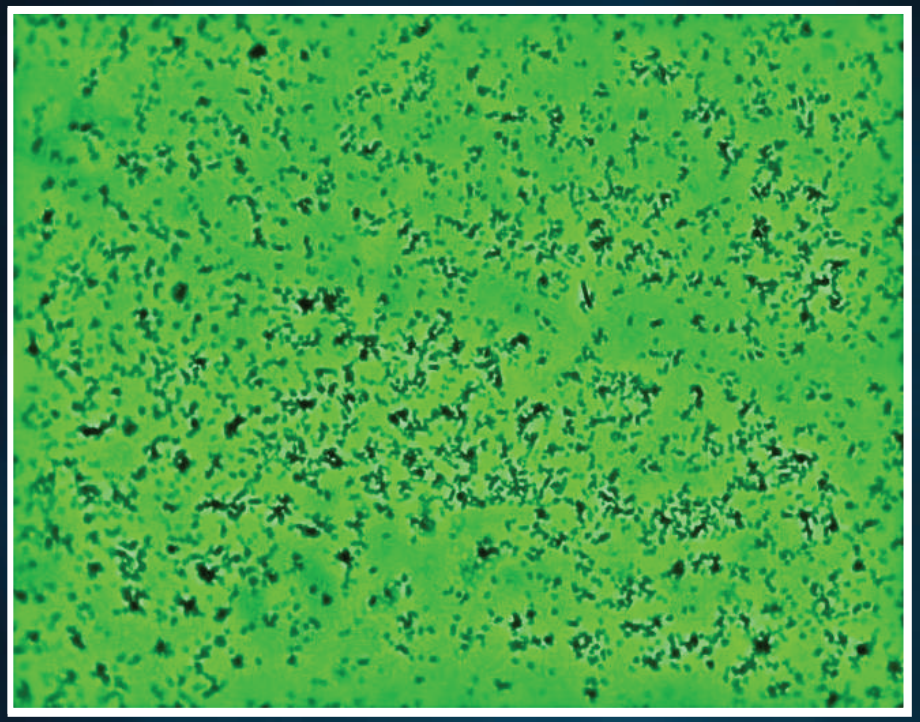


free floating
pleomorphic bacteria

positive colture

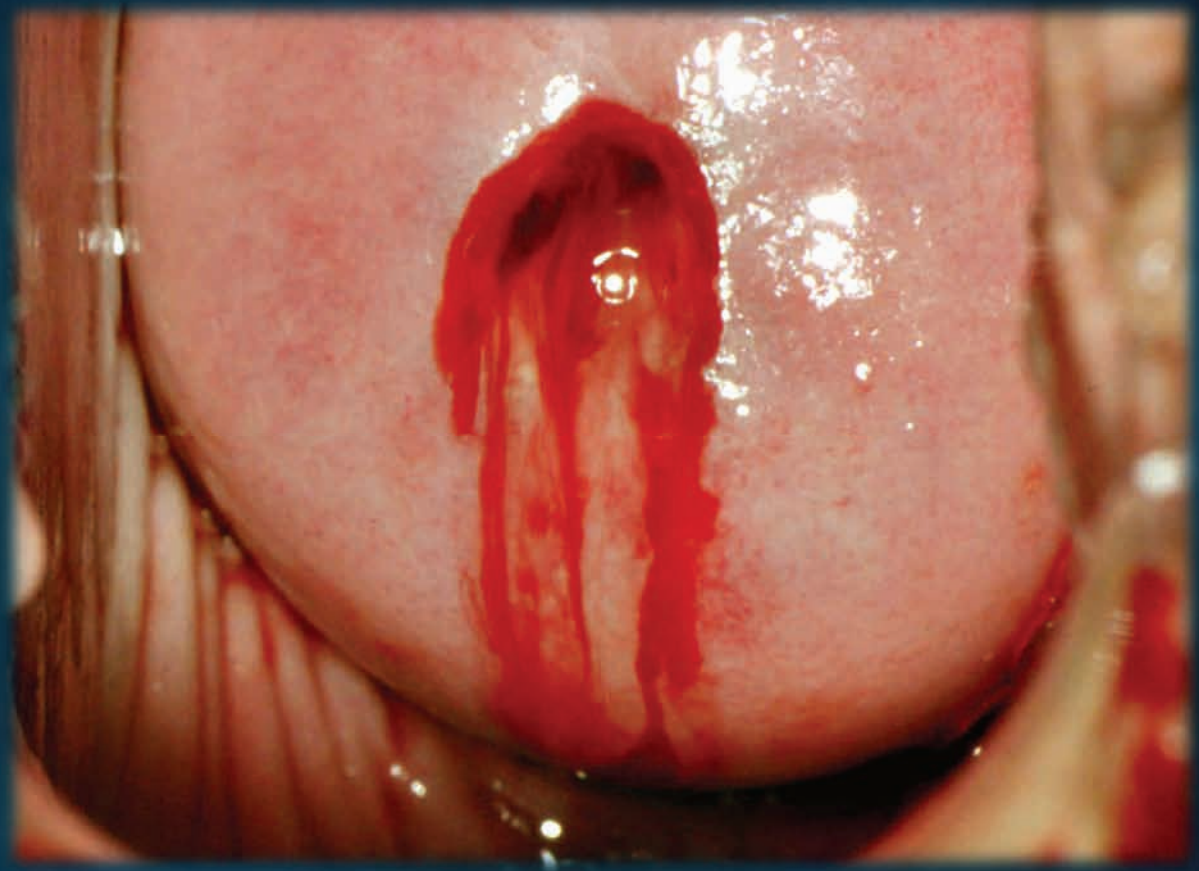
Dominant
lactobacilli
disappear
and anaerobic
bacteria
multiply
1,000-fold

Eun CC et al. J Microbiol Biotechnol. 12(2), 312, 2002

Elevated pH value (>4.5) of vaginal environment has been found in 94,10% of patients affected by BV

Jahic M et al. Med Arch, 67(6): 426, Dec; 2013

Different conditions may modify vaginal pH

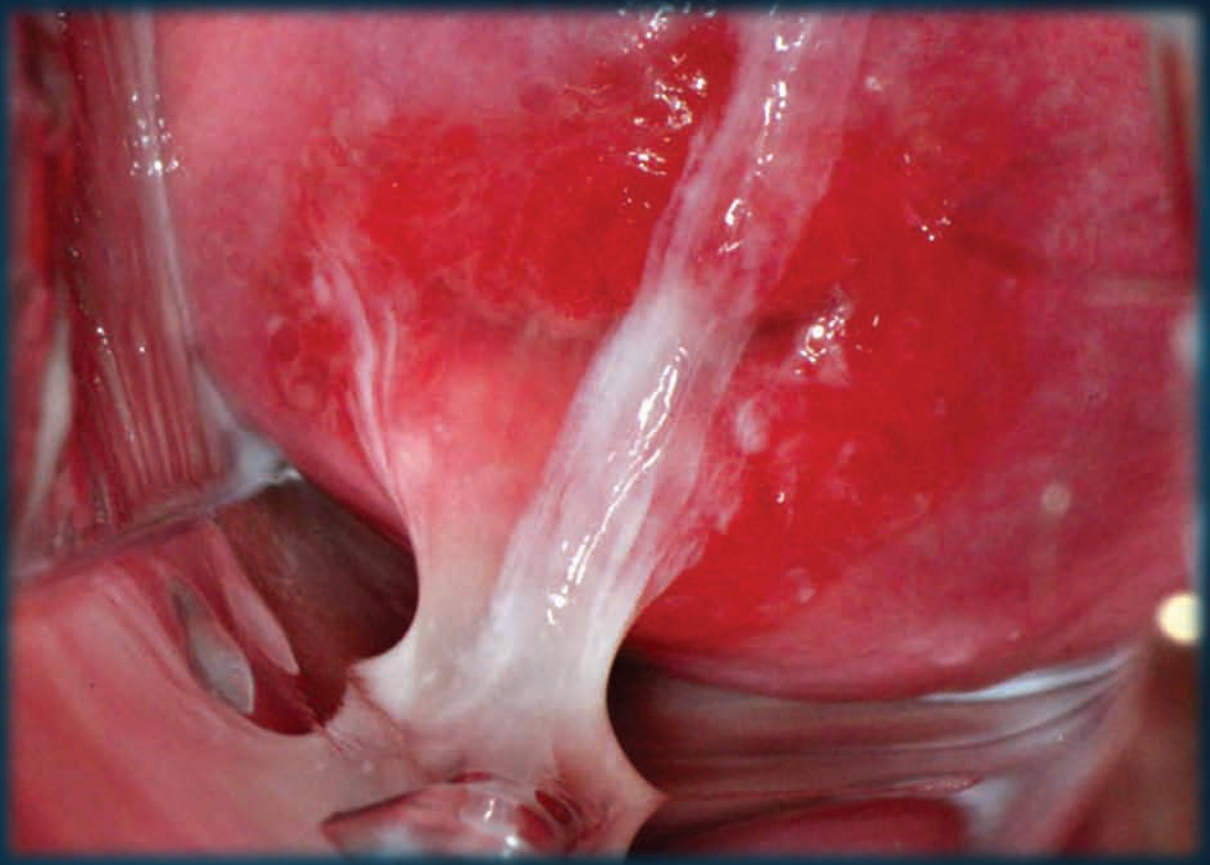


pH

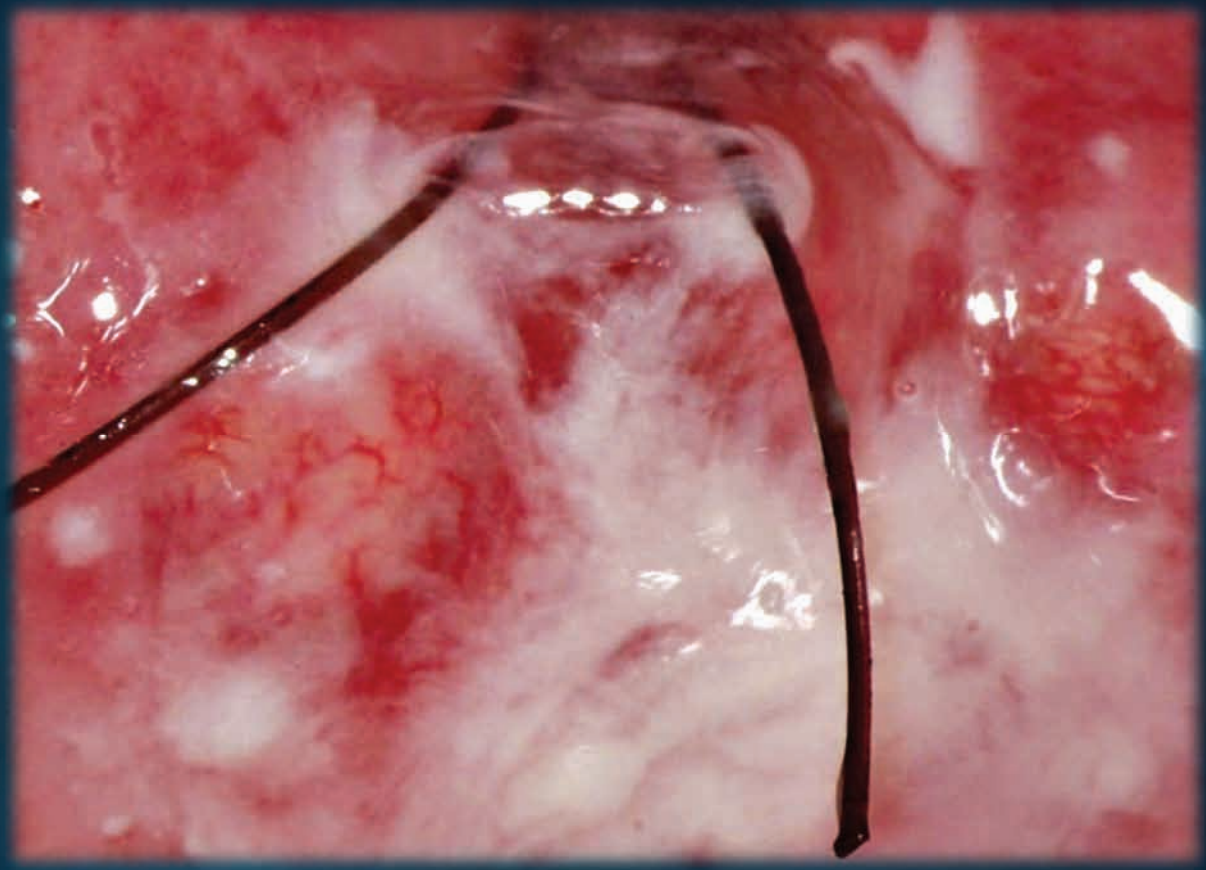
menstruation

pH

abundant alkaline mucus
for hormonal imbalance

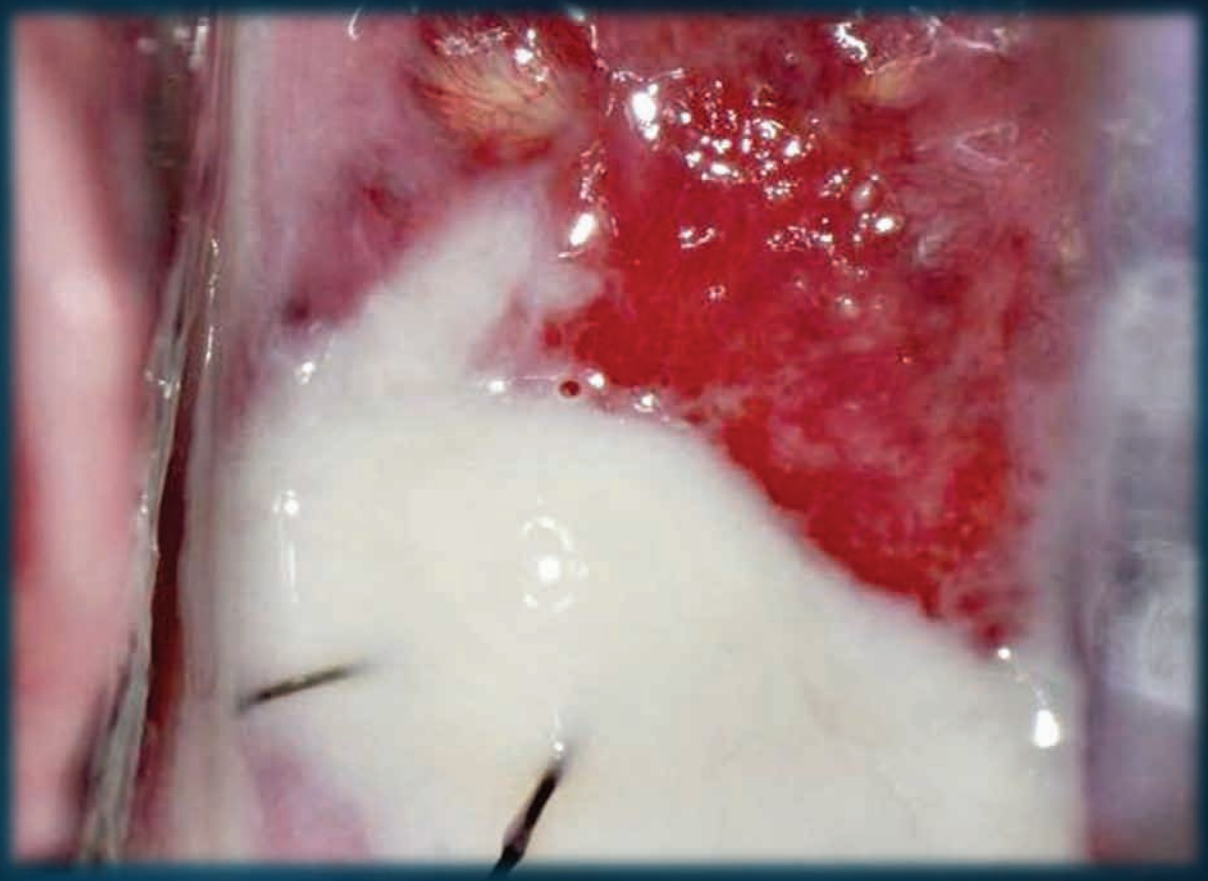


alkaline mucus from columnar
exposed epithelium



pH

reactive alkaline mucus
from IUD



pH

exposed epithelium
and IUD

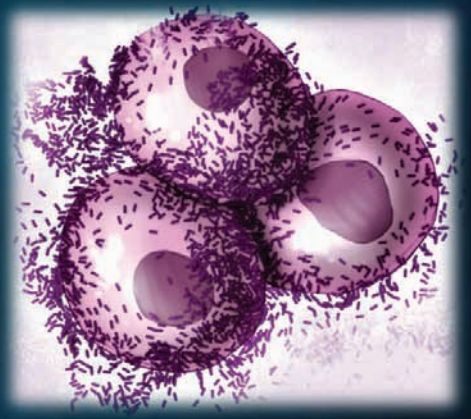
pH

sperm

(pH:7.8-8.2)

It is possible to separate *Gardnerella* into different non-pathogenic and pathogenic species

Non-pathogenic

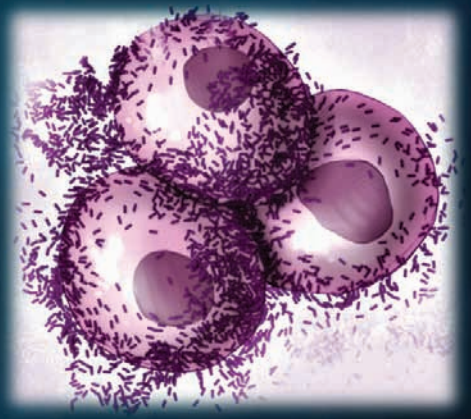


Pathogenic

Hay P. F1000Res. Sep 27, 2017

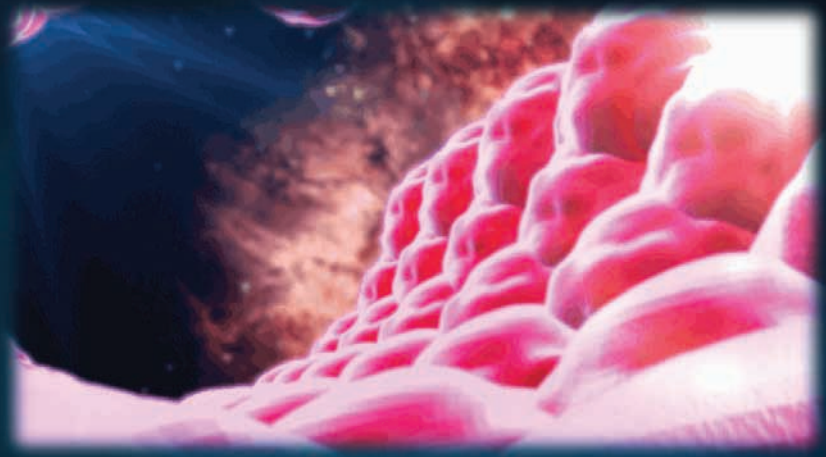
Differences were described in some genes and virulence factors such as adhesion, cytotoxicity and biofilm-forming capability

Non-pathogenic



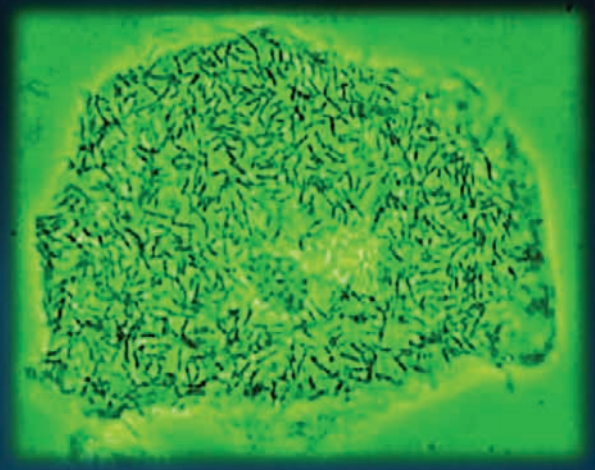
pathogenic

The aggregate microbiome is not a simple accumulation of free-floating bacteria on the surface of a human tissue

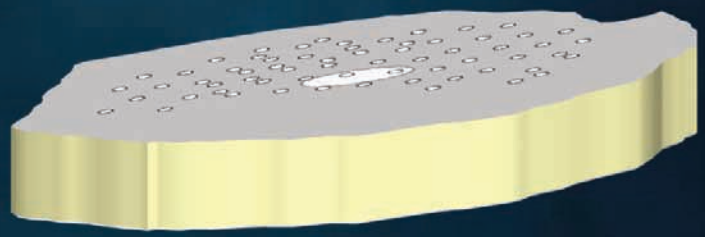


Franasiak JM et al. Fertil Steril. Dec Vol. 104:1364, 2015

In many cases, complex three--dimensional lattices, called biofilms, are formed

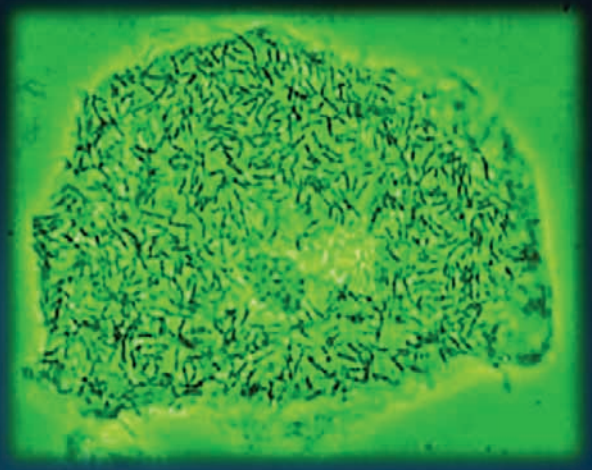


bacilli

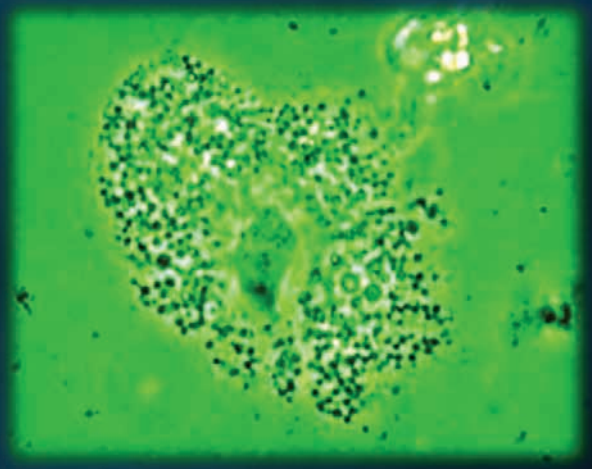


Trinidad et al. Formatex Research Center:204, 2010

At times, these biofilms may inhibit immune detection and reduce the effectiveness of antimicrobial treatment



bacilli

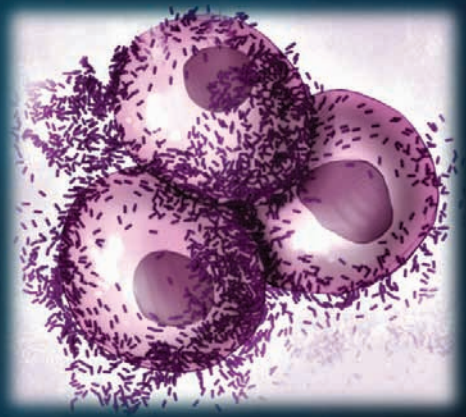


cocci

Trinidad et al. Formatex Research Center:204, 2010

Not detectable by cultures

adhesion, and biofilm-forming capacity

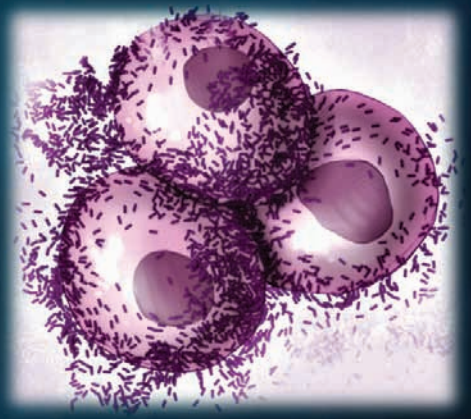


pathogenic

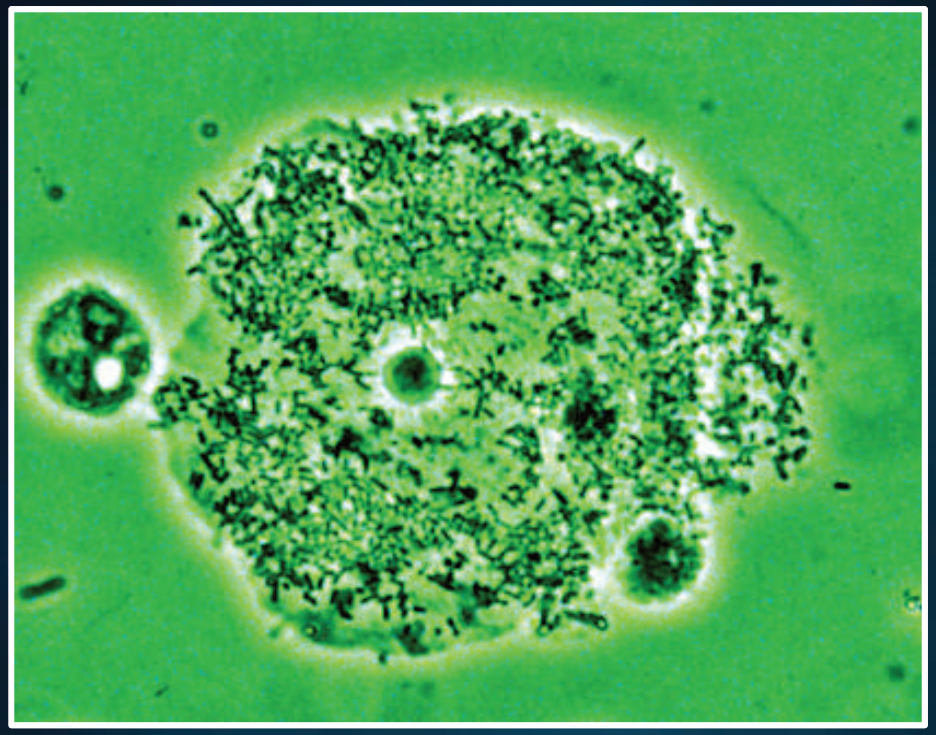
Hay P. F1000Res. Sep 27, 2017

Detectable only by microscopy

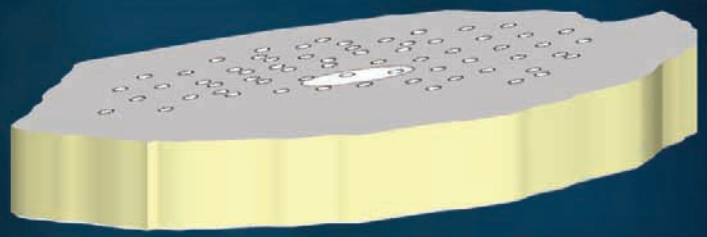
adhesion, and biofilm-forming capacity



pathogenic



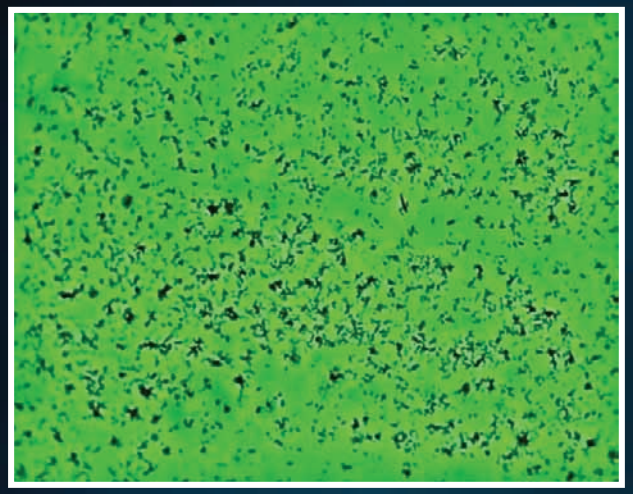
adhered
pleomorphic bacteria:
clue cell



Pathogenic
species

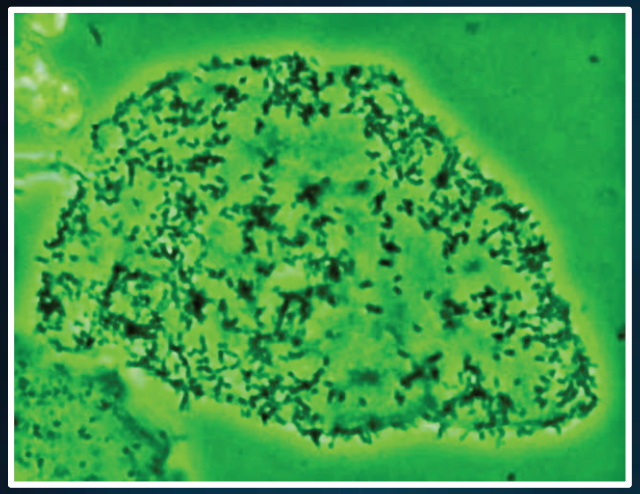
Laboratory

Traps



Vaginal **cultures** have excellent sensitivity for the presence of BV-associated bacteria

Hillier SL. Am J Obstet Gynecol; 169:455, 1993



But because the predictive value of a positive G. vaginalis culture is less than 50%

cultures are not recommended

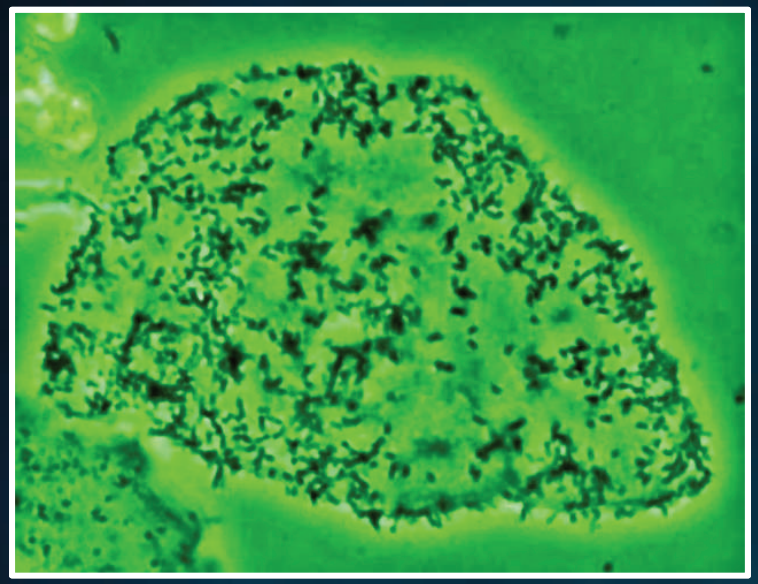
Hillier SL. Am J Obstet Gynecol; 169:455, 1993

The microscope is the most valuable diagnostic aid, the clue cell being a high characteristic feature (specificity 98%)

van der Meijden WI. Scand J Infect Dis 40(suppl):85, 1983

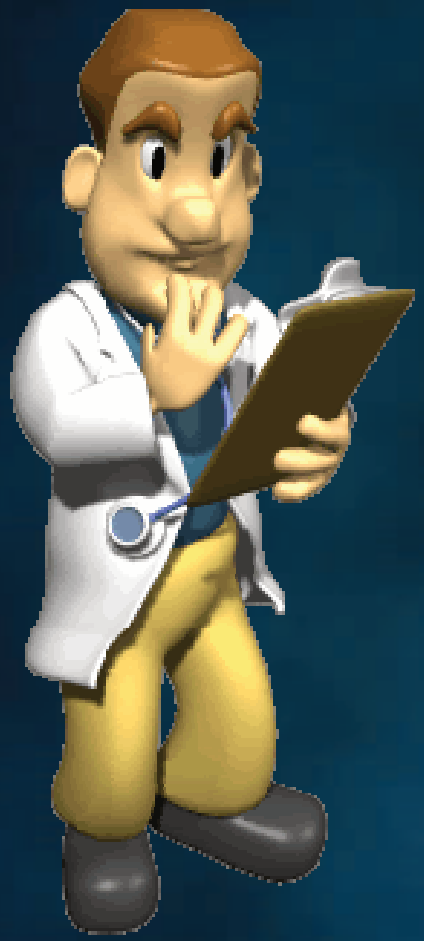
BV is worldwide the most common cause of vaginal discharge, but the condition remains asymptomatic in, at least, half of the cases

Noortje van Oostrum et al. Hum Reprod. Vol. 28(7):1809, 2013



Bacterial Vaginosis is often sub-clinical

Haahr T et al. Hum Reprod. 31(4):795, 2016



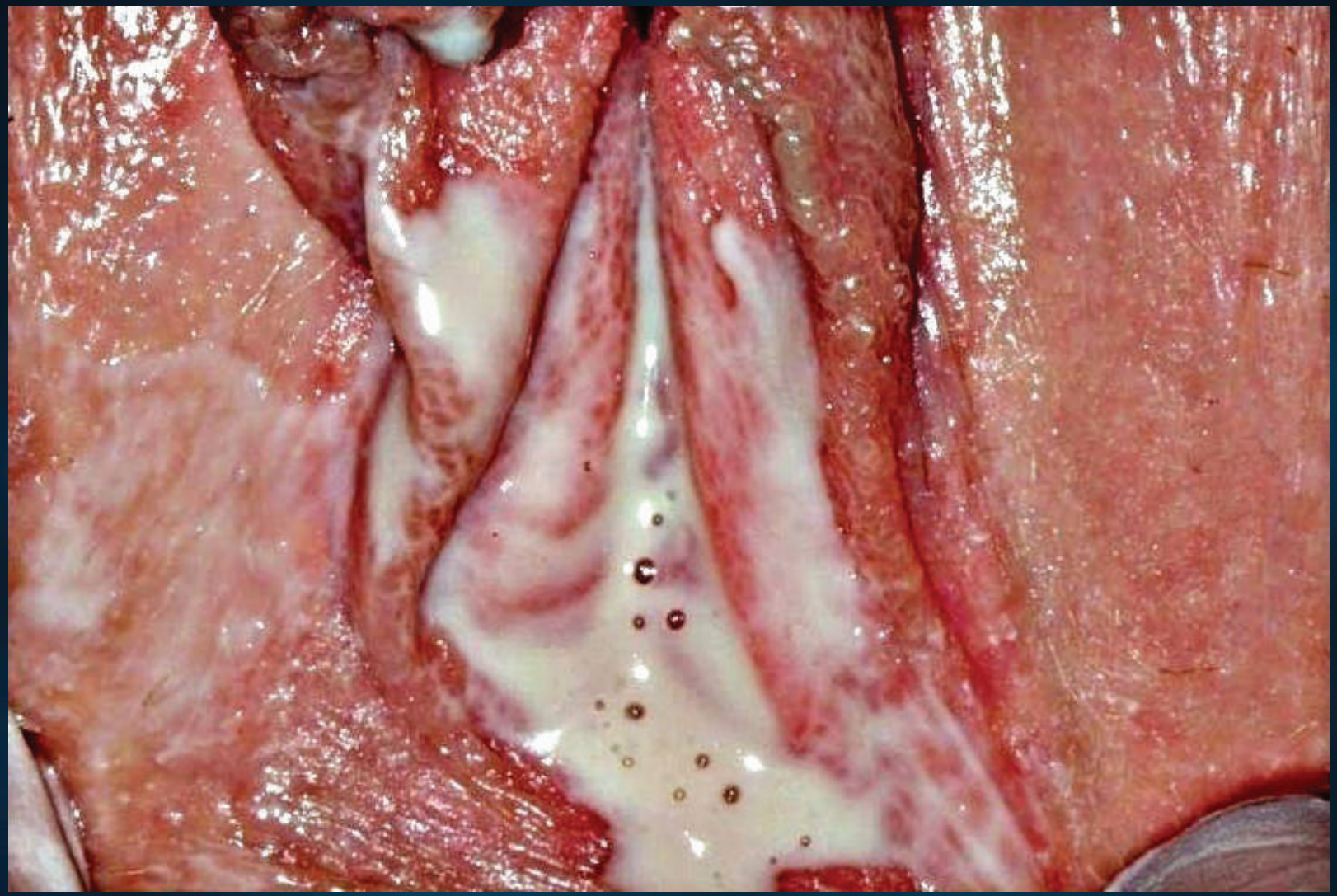
How can we diagnose Bacterial Vaginosis?

DIAGNOSTIC CRITERIA

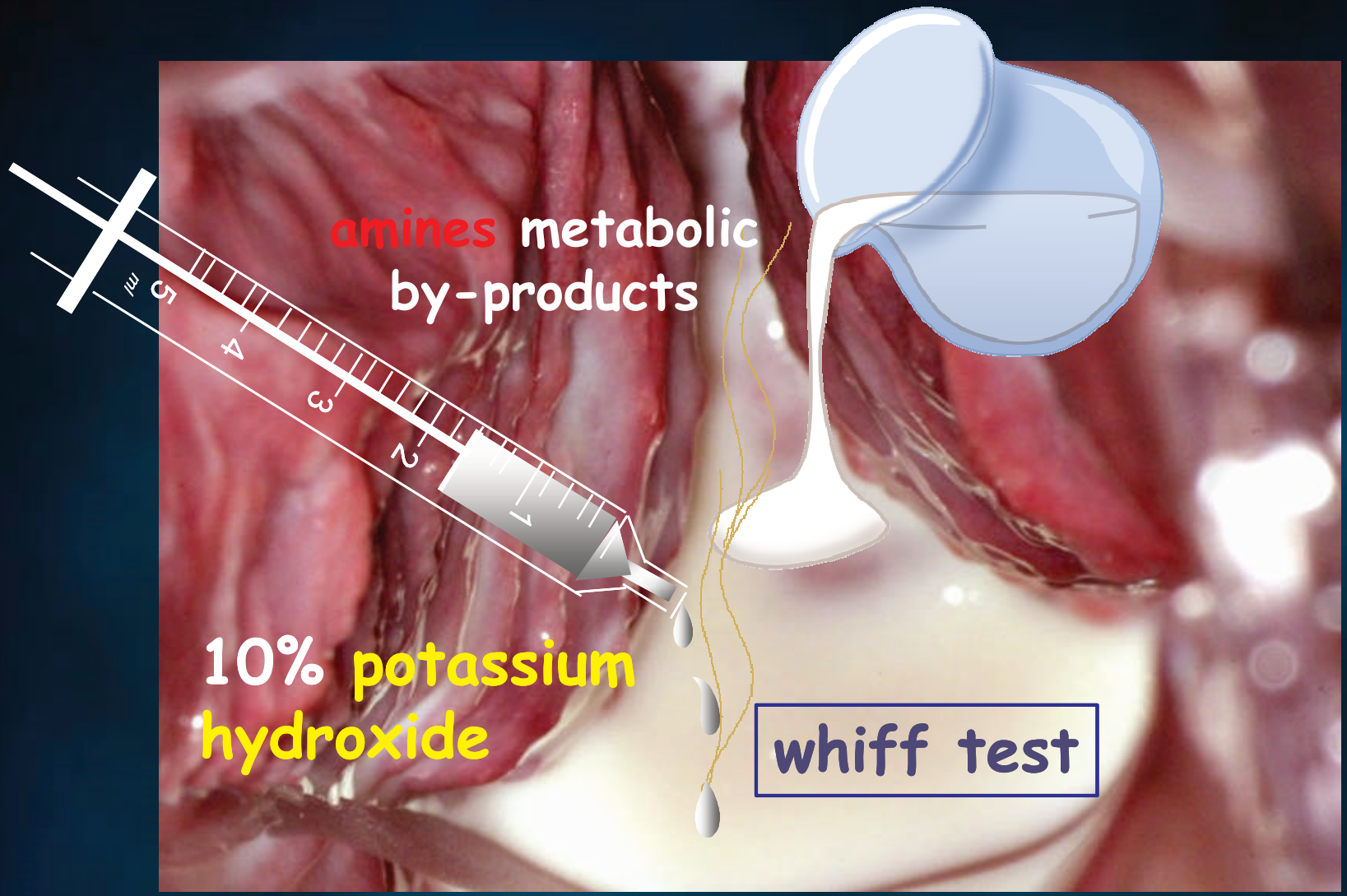
- **fishy** smell (sniff test)
- white, omogeneous, **frothy** and **malodorous** discharge
- vaginal pH **> 4.5**
- positive **amine-test** (whiff test)
- **clue cells** at microscopy

fishy smell

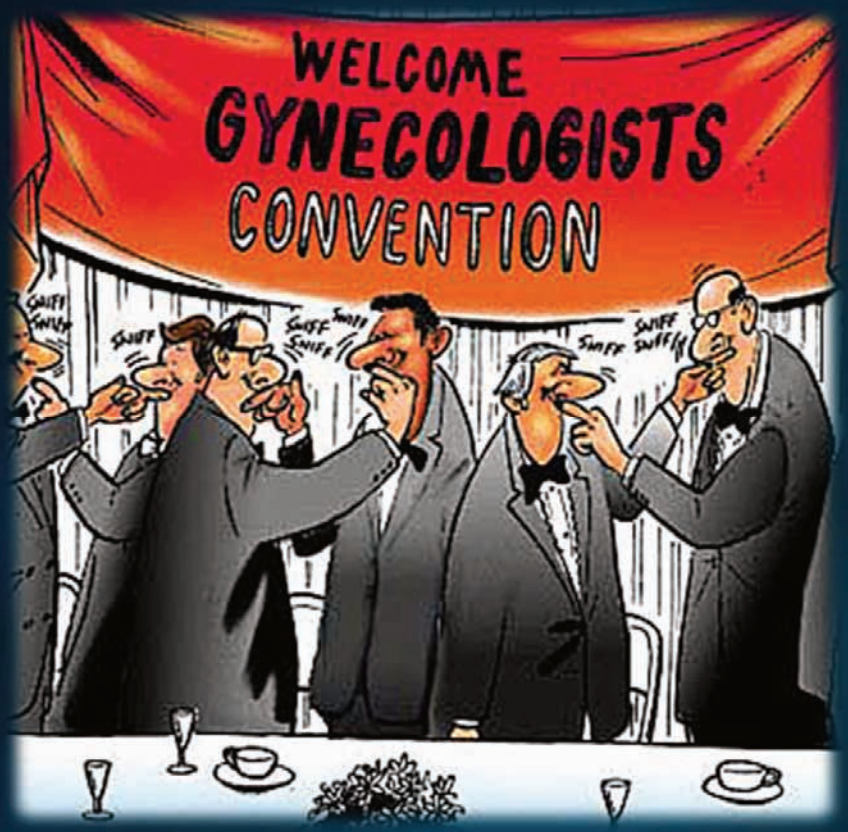
sniff test



white, omogeneous and frothy discharge



abundant white discharge as if
milk was poured into the vagina



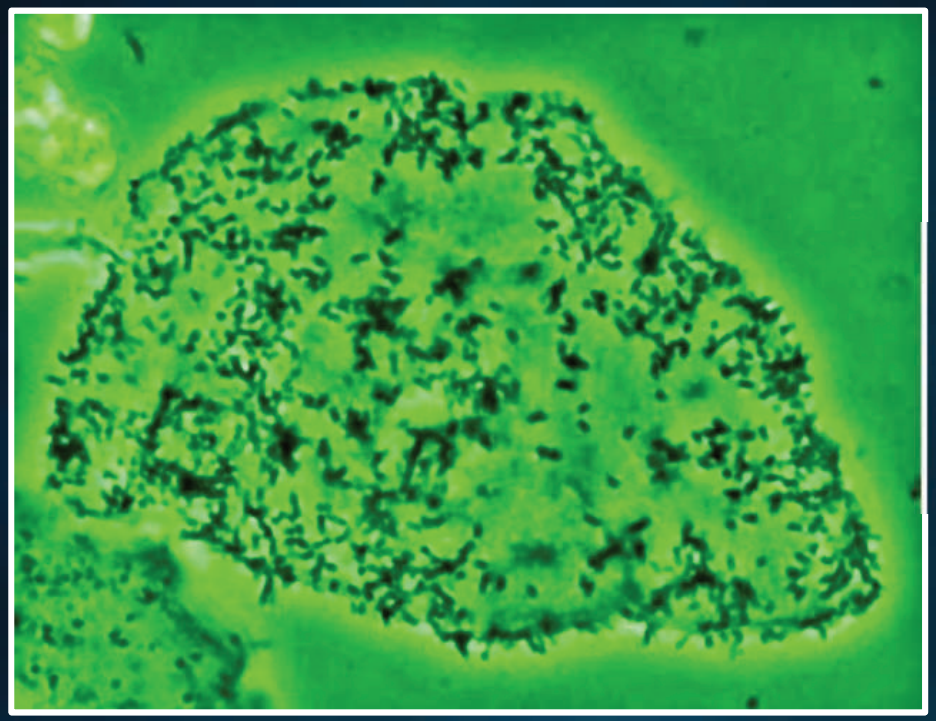
Sometimes it is not enough to be good somellier...

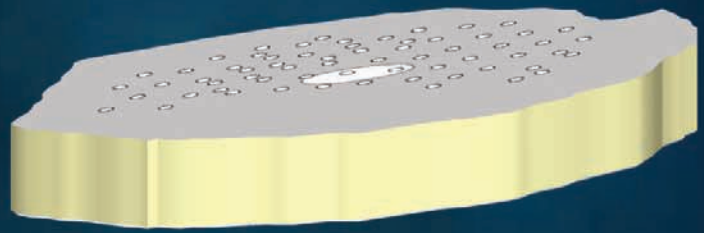
DIAGNOSTIC CRITERIA

- fis[illegible] smell (sniff test)
- white, omogeneous, frothy and malodorous discharge
- vaginal pH > 4.5
- positive amine-test (whiff test)


Trichomonas v.

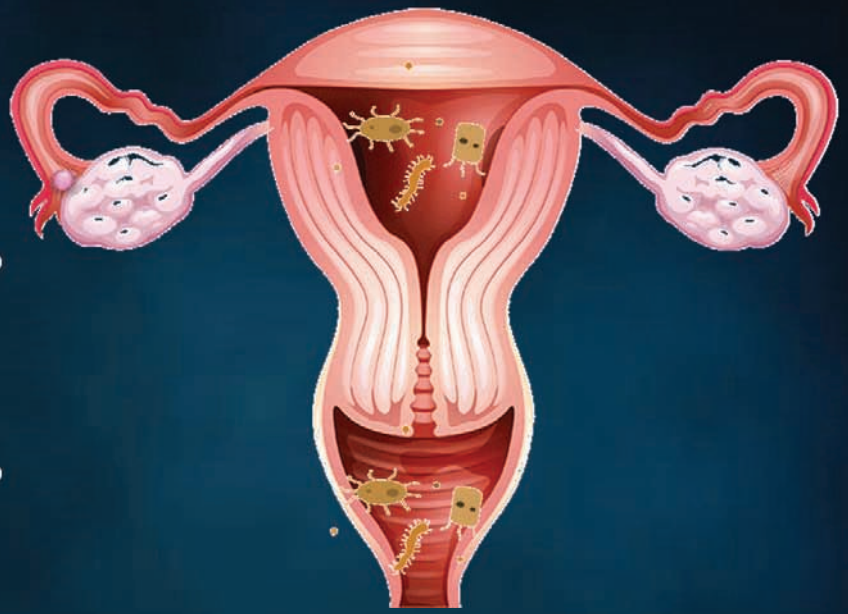
- clue cells at microscopy BV





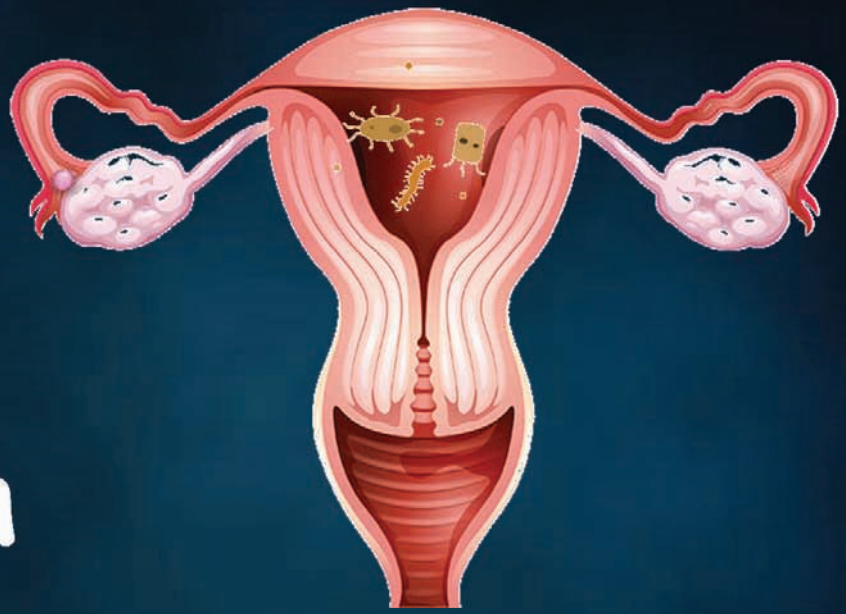
adhered
pleomorphic bacteria:
clue cell

Biofilms are routinely present in the **vagina** but commonly extend into the **endometrial** cavity and even up into the **fallopian** tubes



Swidsinski A et al. Plos One: 8 e53997, 2013

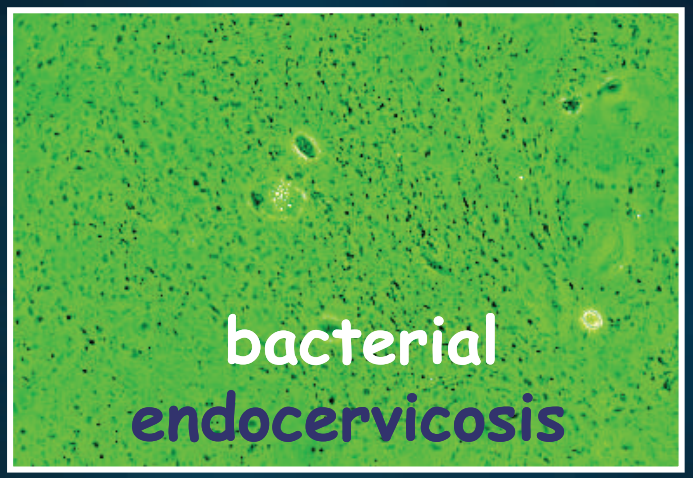
Half of the women presenting with BV had a polymicrobial biofilm adhered to the endometrium

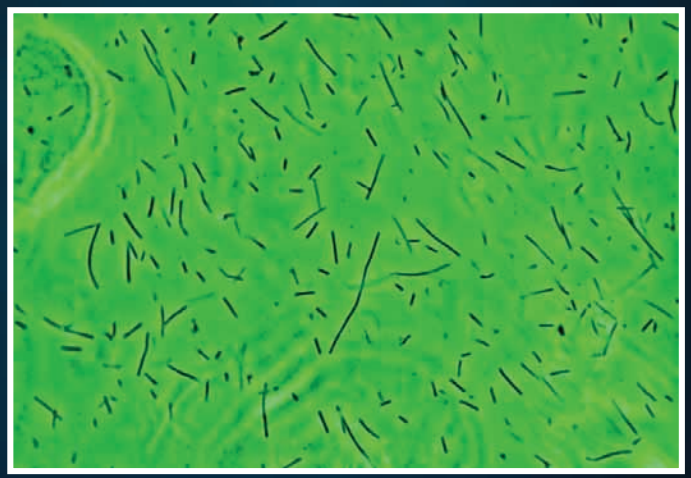


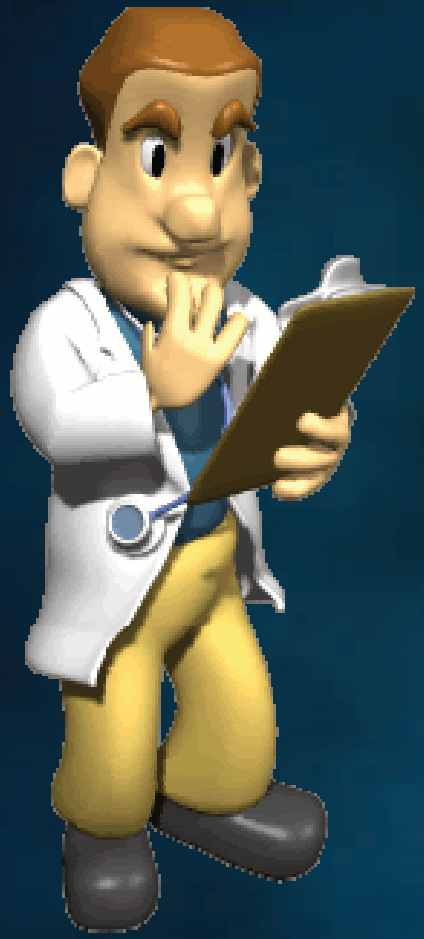
Swidsinski A et al. Plos One: 8 e53997, 2013

BV- associated bacteria

ENDOCERVICAL wet mount







Which

Consequences?

Uterine colonization with BV-associated bacteria has been hypothesized to promote carcinogenesis

Baker JM et al. Front Immunol. 9:208, Mar 2018

BV is a <u>common</u> genital disorder with a prevalence of approximately **19%** in the **infertile** population

Haahr T et al. Hum Reprod. 31(4):795, 2016

There is a consistent association between dysbiosis of the vaginal microbiome and unfavourable reproductive outcomes, such as subfertility and ART failure

Sirota et al. Seminars in Reproductive Medicine. 32:35, 2014

HPV infection

with BV infection

may increase the

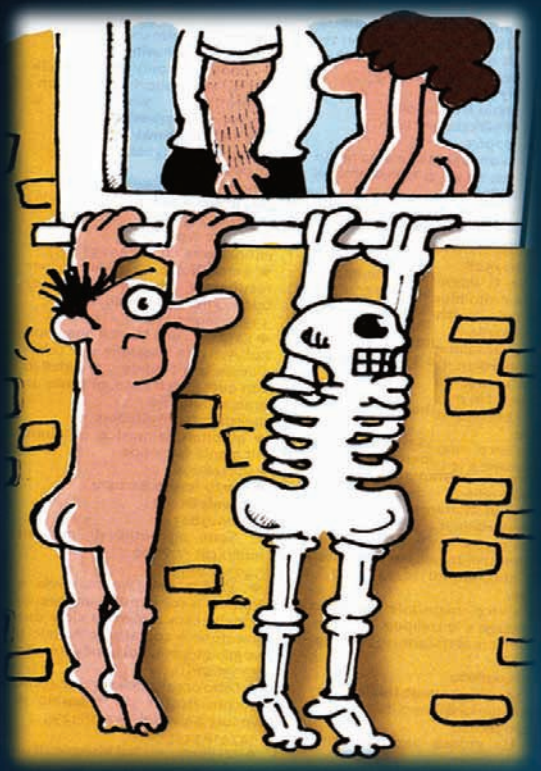
incidence of CIN

and cervical cancer

Huan L et al. Int J Clin Exp Med. 8(11): 21080, 2015

Therapy with only recommended **antibiotics** results in low cure rates and unacceptably **high recurrence** rates

Ma L et al. BMJ Open 15,7(10), 2017



Several studies in the last decade support the concept of BV as a sexually transmitted infection

Hay P. F1000Res. Sep 27, 2017

Infective

male partner

usually presents

no penile signs

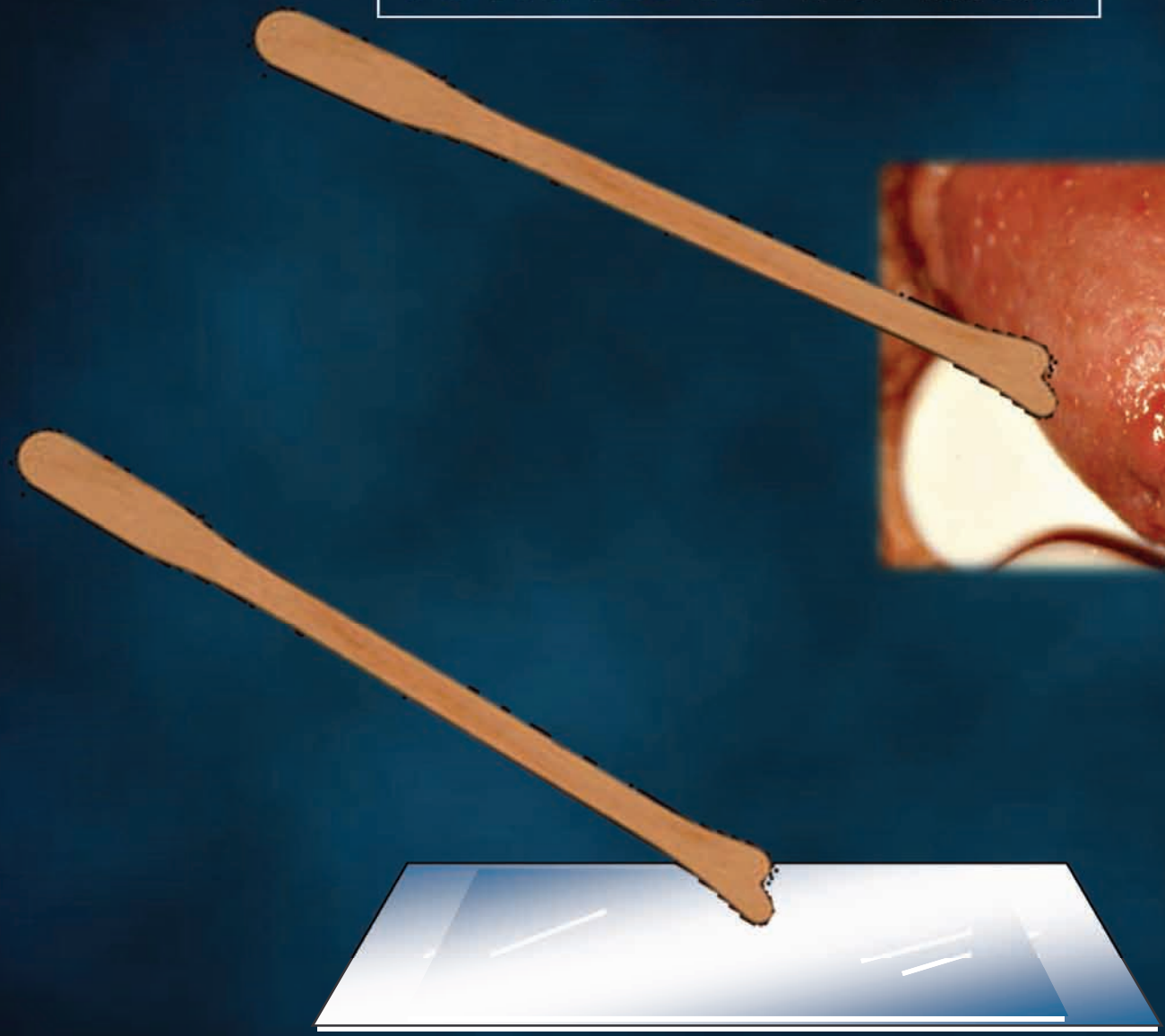
or symptoms

Is it possible to investigate the recalcitrant male partner?

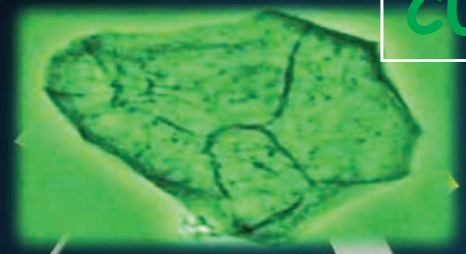
WET MOUNTS

- ✓ Cervico-vaginal wet mount
- ✓ Cutaneous wet mount
- ✓ Urinary wet mount
- ✓ Buccal wet mount
- ✓ Rectal wet mount

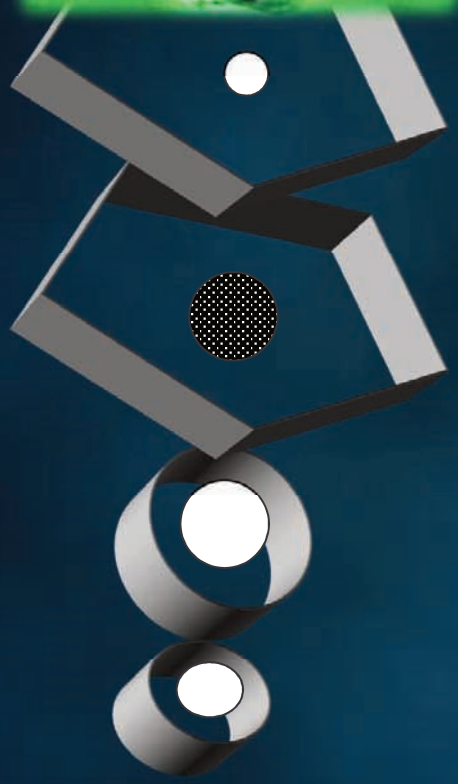
CUTANEOUS wet mount



CUTANEOUS wet mount

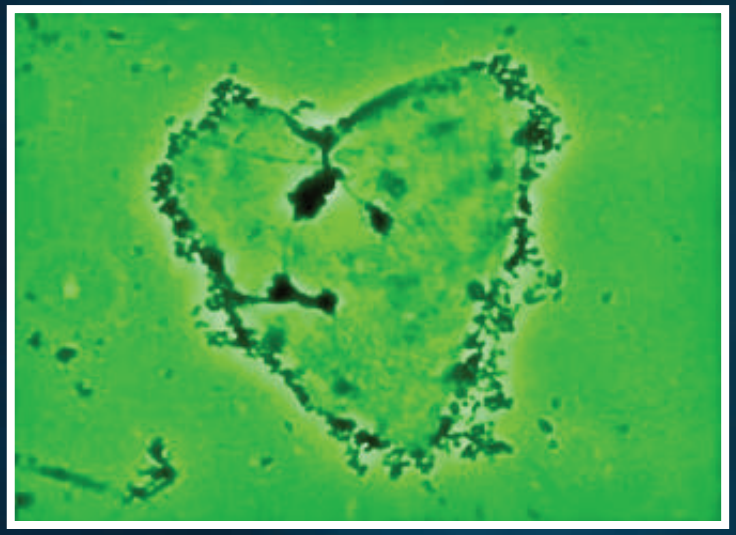


horny cell



keratinized stratified
SQUAMOUS
epithelium

CUTANEOUS wet mount



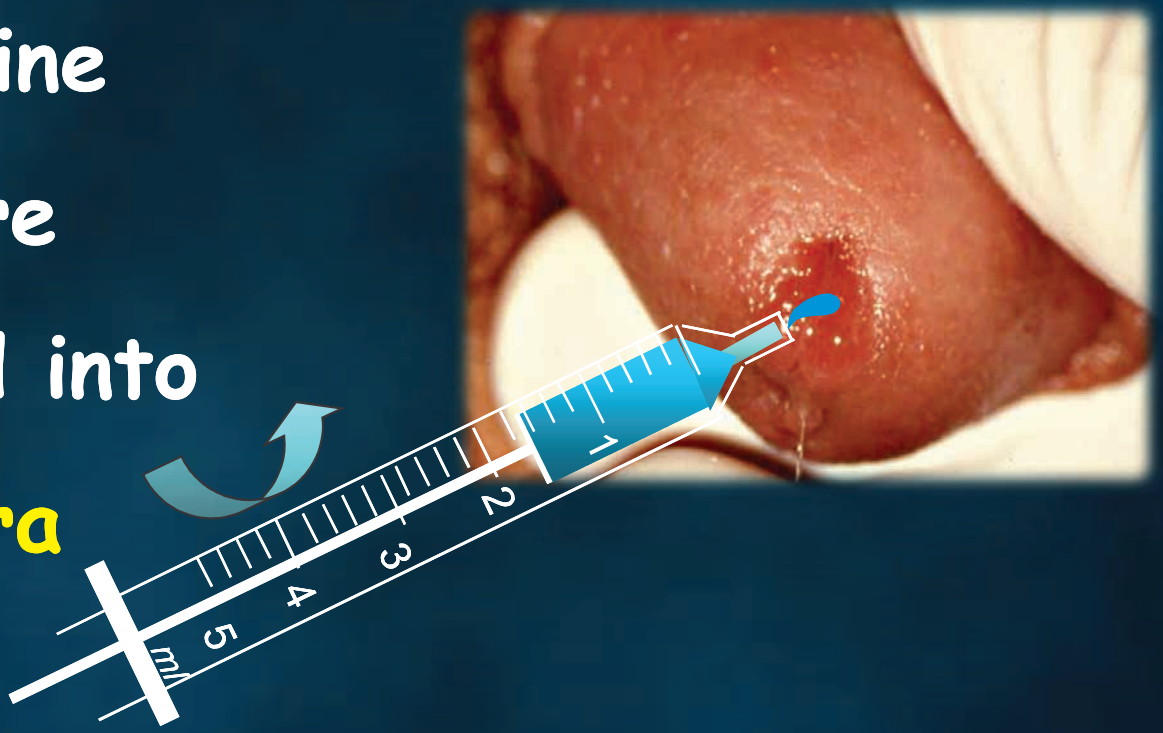
clue cell (horny cell)

WET MOUNTS

- ✓ Cervico-vaginal wet mount
- ✓ Cutaneous wet mount
- ✓ Urinary wet mount
- ✓ Buccal wet mount
- ✓ Rectal wet mount

URINARY wet mount

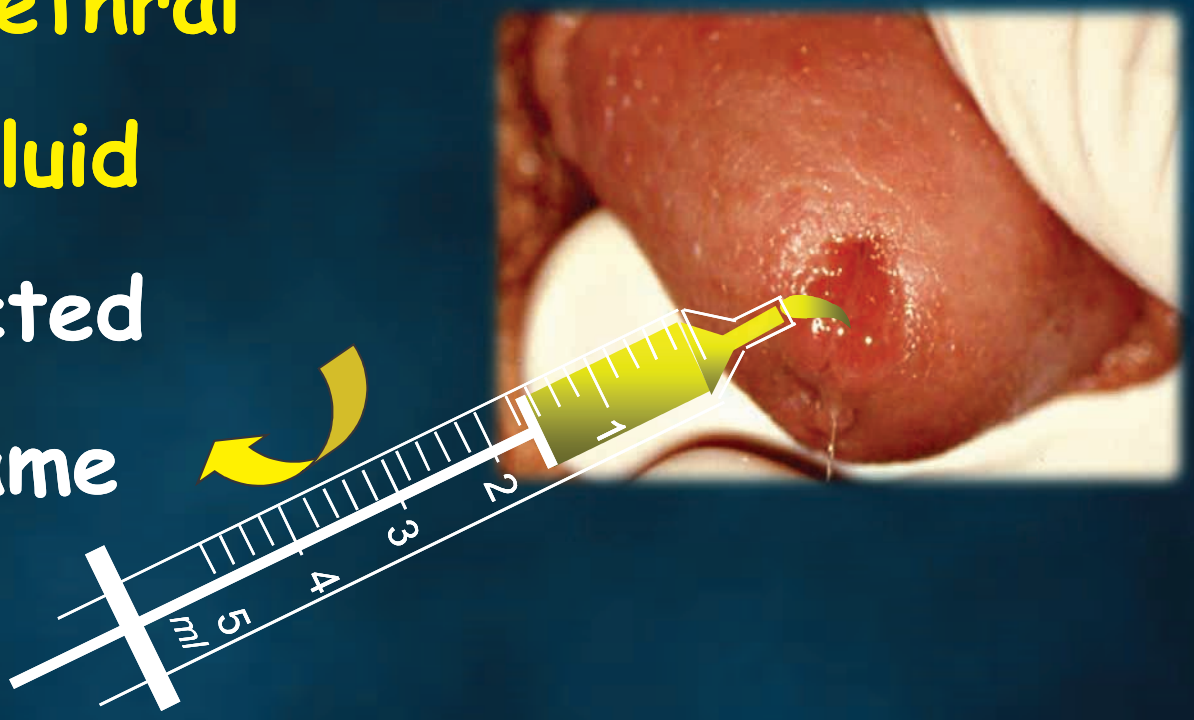
5-10 ml of sterile saline solution are introduced into the urethra



Miniello G. Gynecological Urology; p.52, Orient Longman Eds., 2001

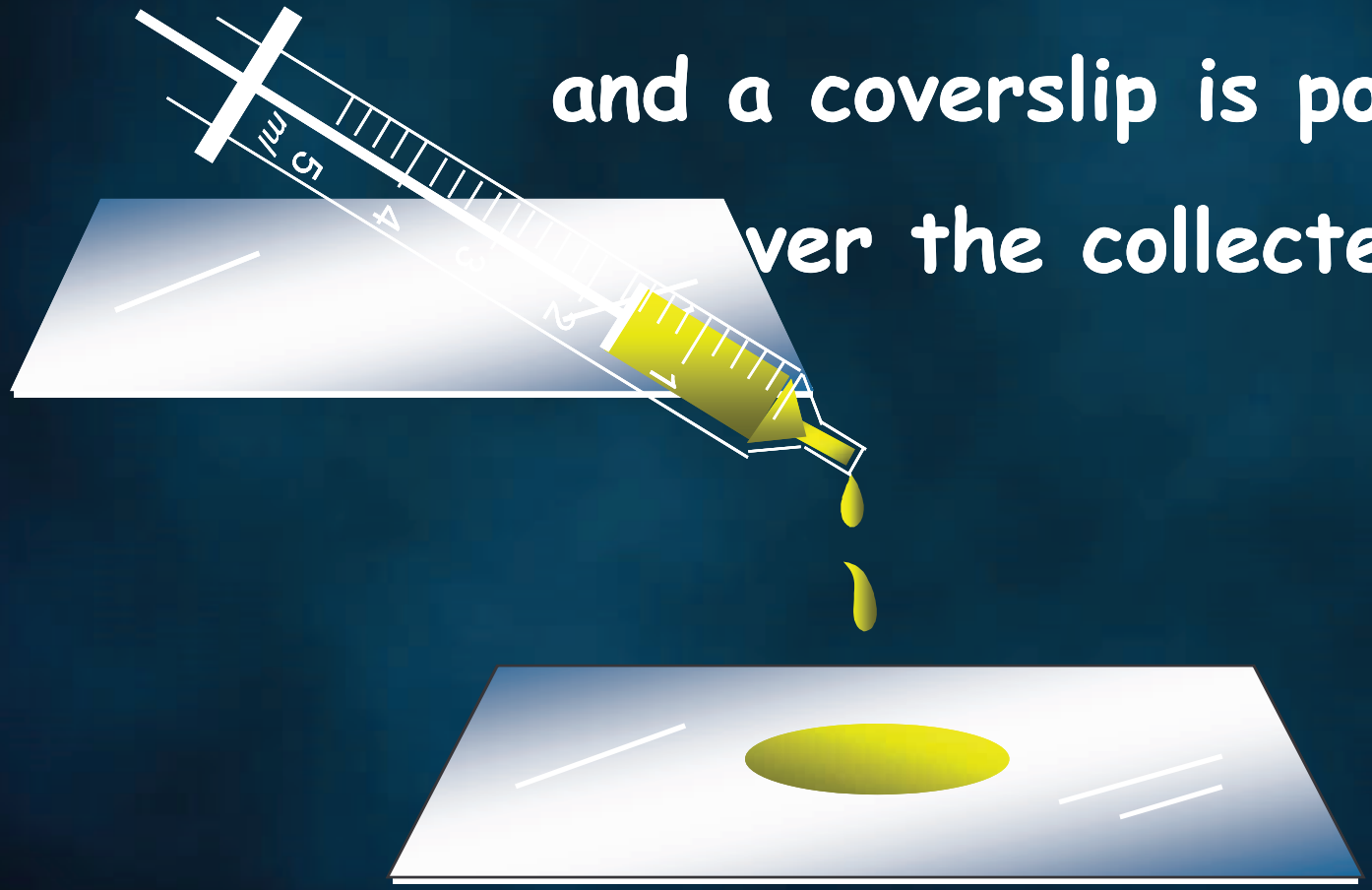
URINARY wet mount

Some drops
of the urethral
washing fluid
are collected
by the same
syringe



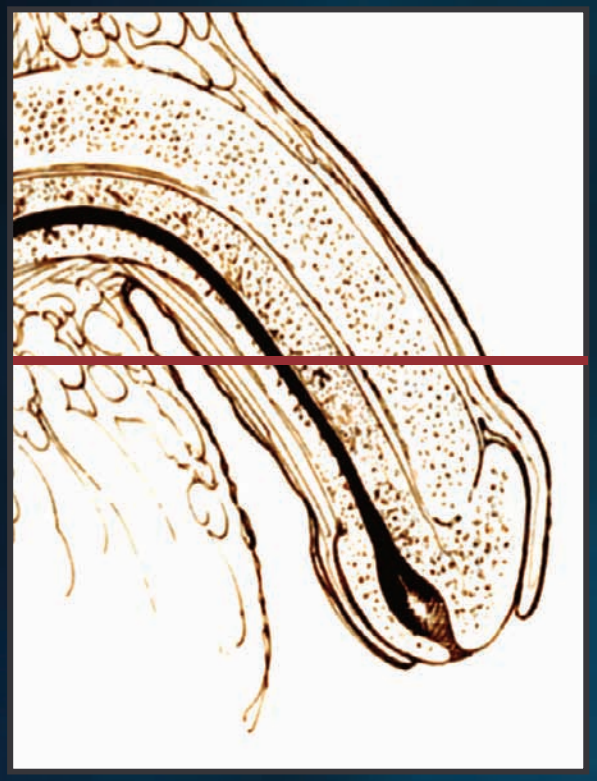
Miniello G. Gynecological Urology; p.52, Orient Longman Eds., 2001

A few drops of the **urethral washing** fluid are applied to a microscope slide and a coverslip is positioned over the collected drops



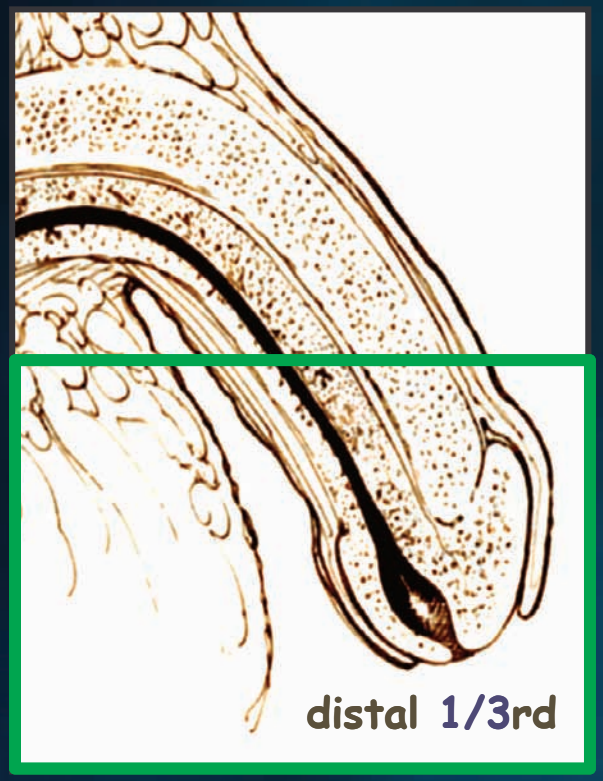
Miniello G. Gynecological Urology; p.52, Orient Longman Eds., 2001

URINARY EPITHELIA



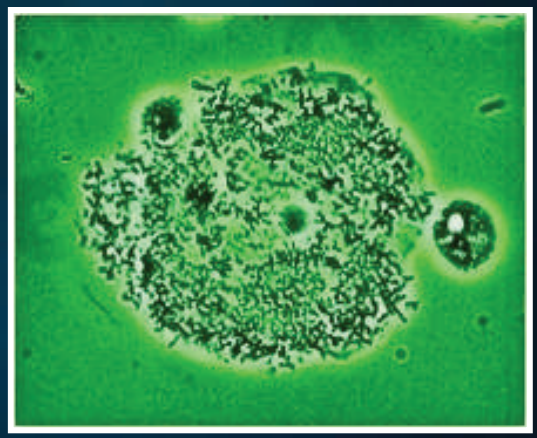
Colonization Level

URINARY EPITHELIA

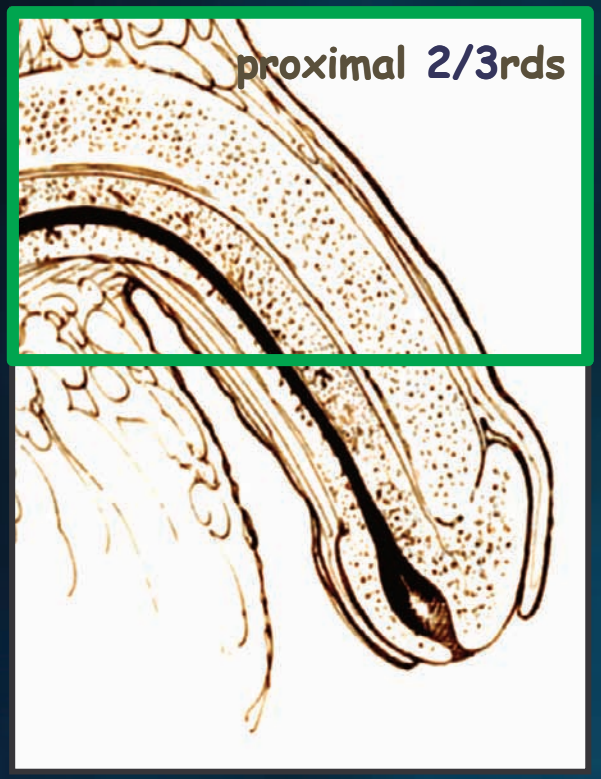


squamous

URO-wet mount



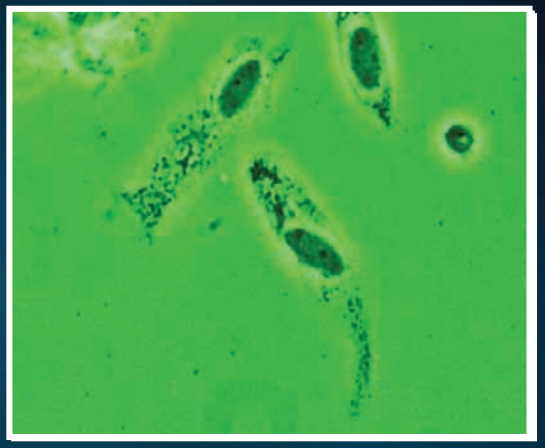
URINARY EPITHELIA



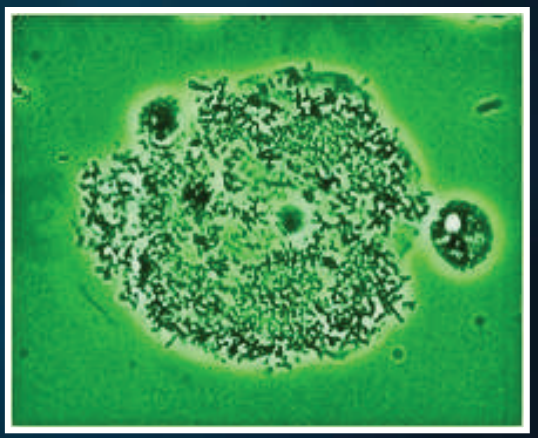
transitional

squamous

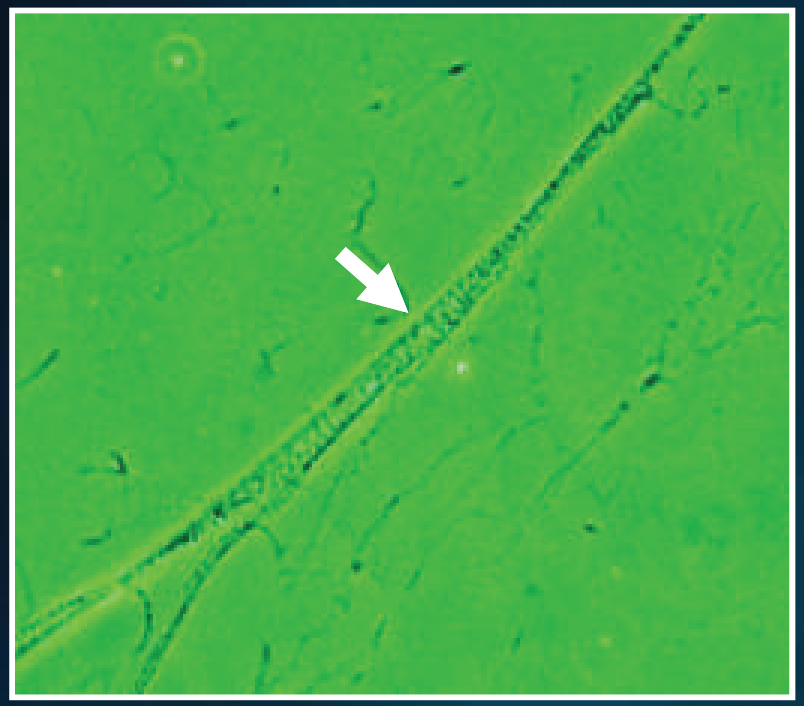
URO-wet mount



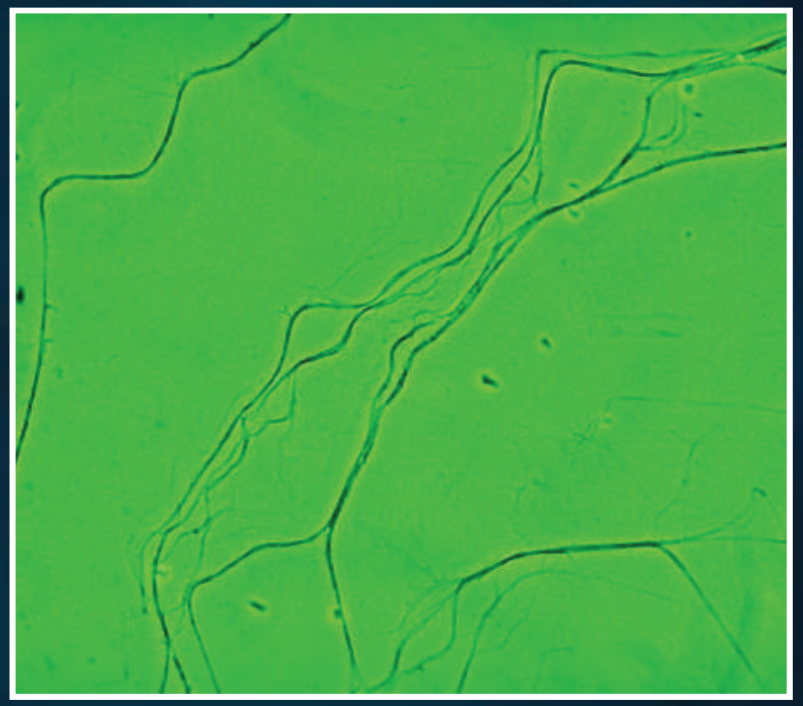
bacterial urethrosis



URINARY wet mount

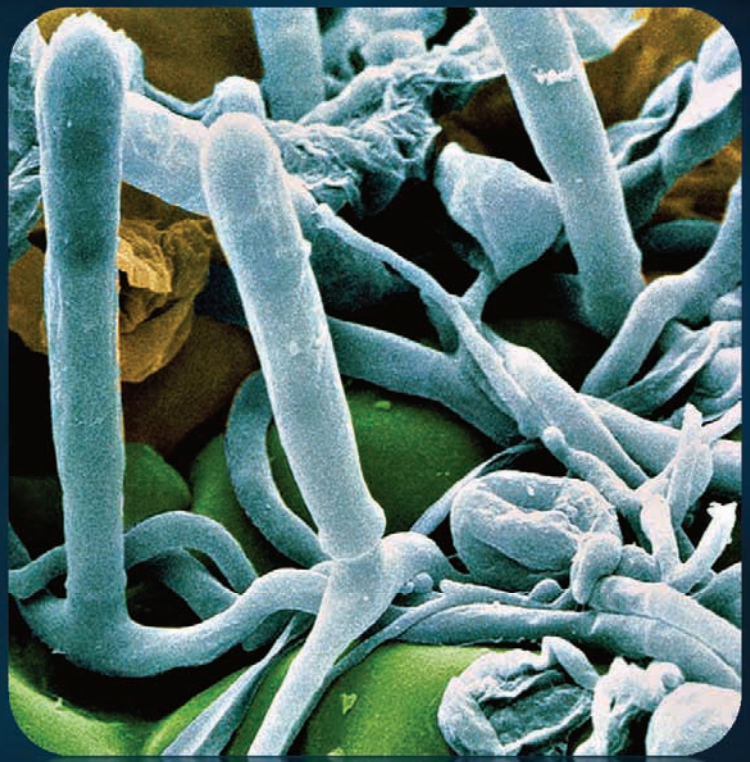


Adhered bacteria
to mucus filaments



Clean mucus filaments
<u>after treatment</u>

Candida albicans



Bacterial Vaginosis
(40%-50%)

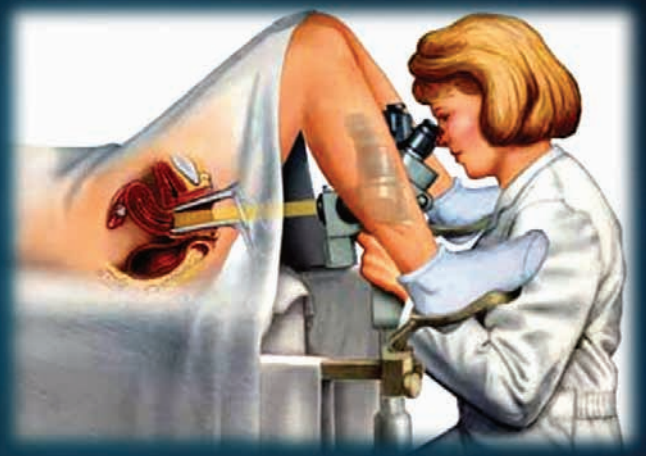
Trichomoniasis
(15%-20%)

Candidiasis
(20%-25%)

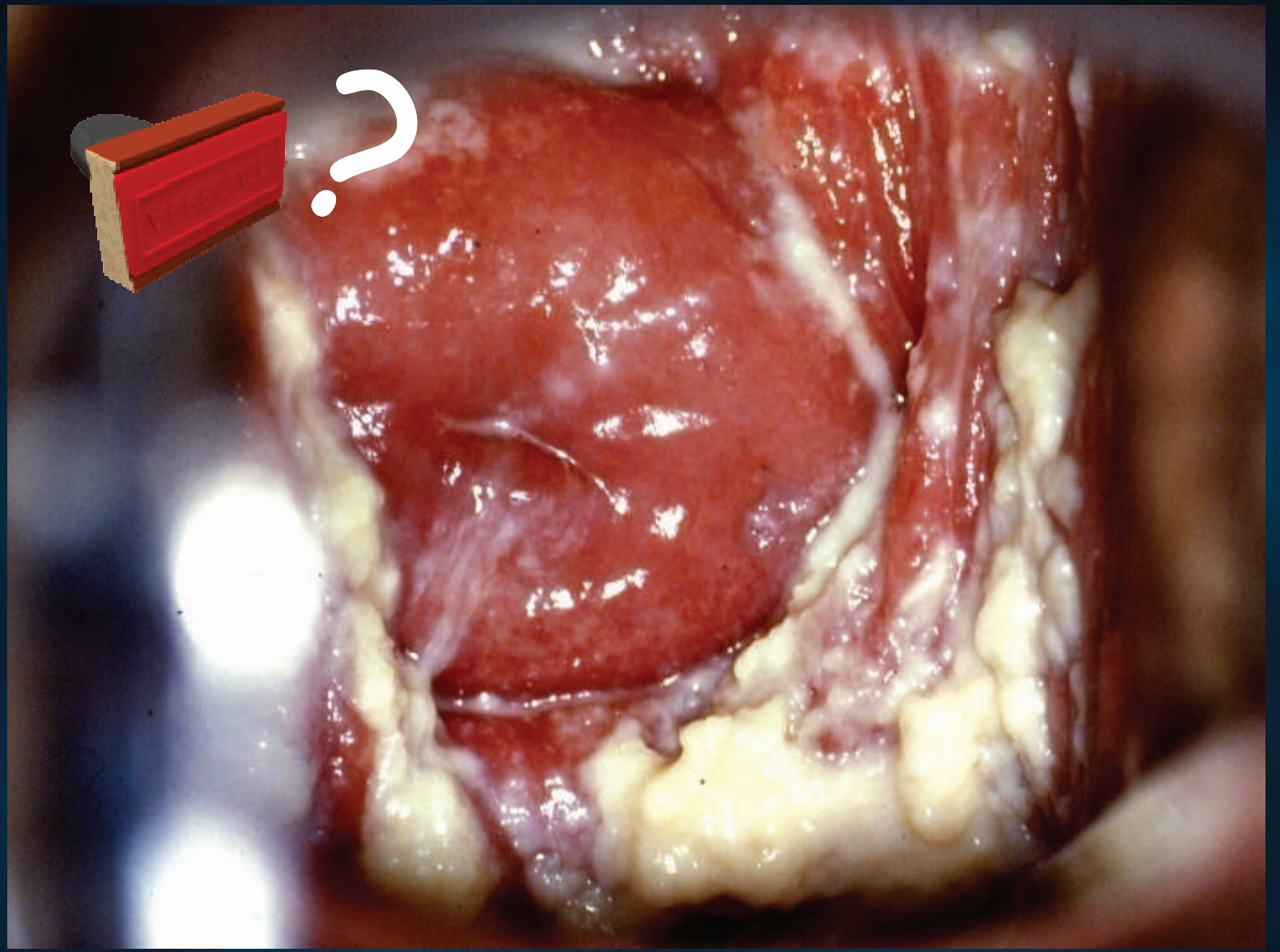
Mylonas I et al. Obstet Gynecol Surv. Jun;66(6):359, 2011

CANDIDA

colposcopy:

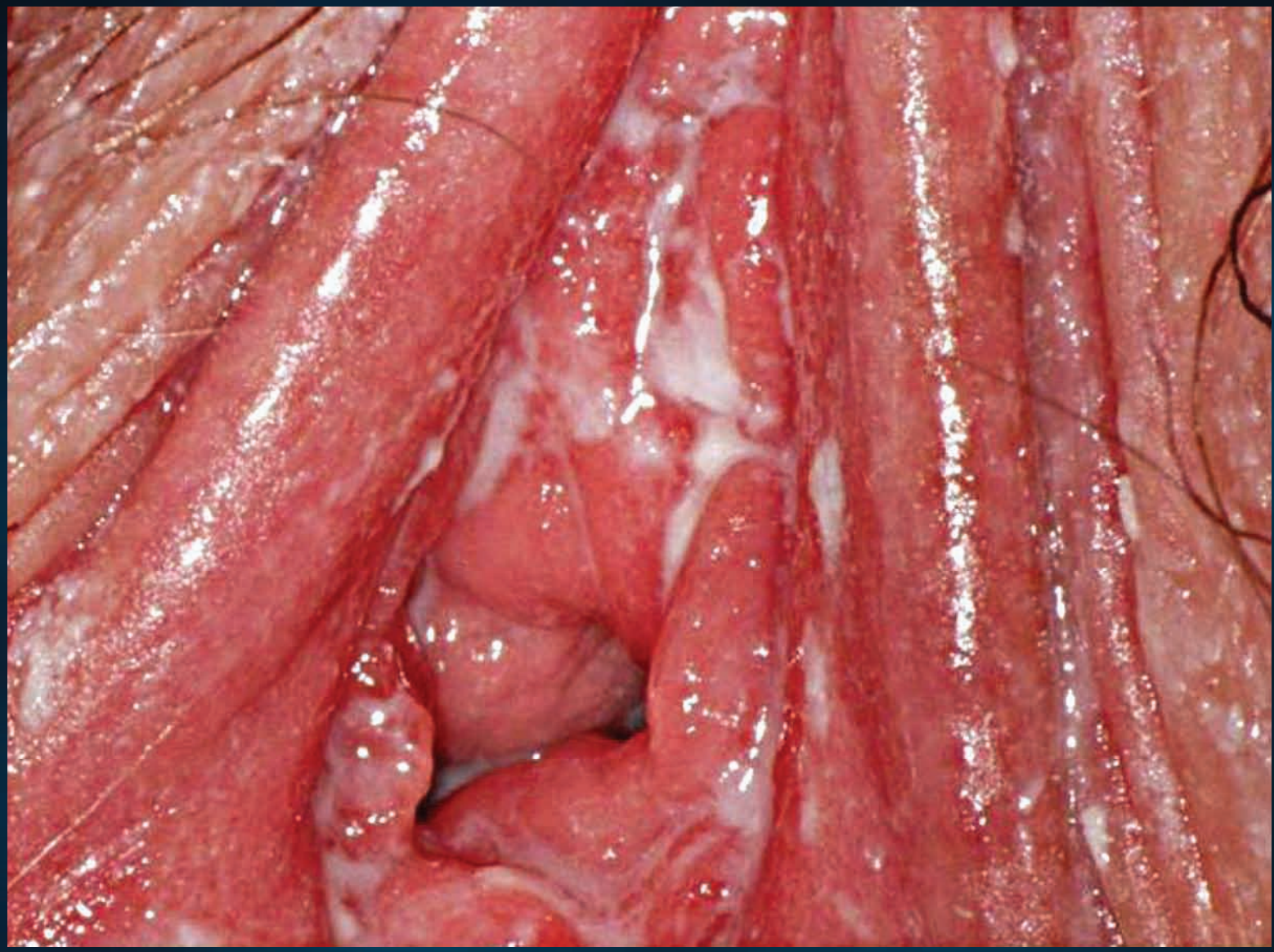


- cervical congestion and, white and **clumpy** discharge
- **erythematous maculae**
- **erythematous papulae**
- **white punctation**

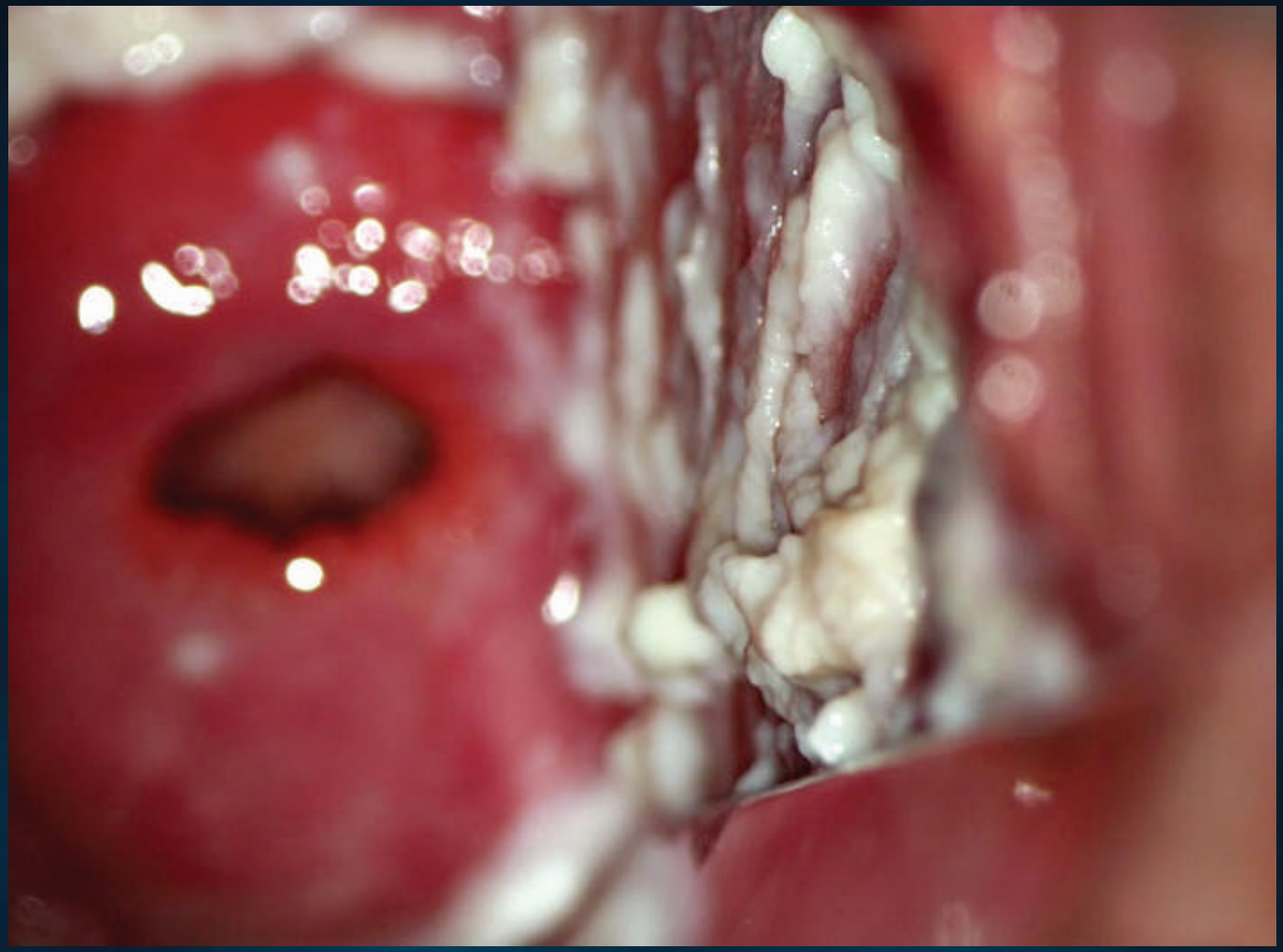


white and clumpy discharge

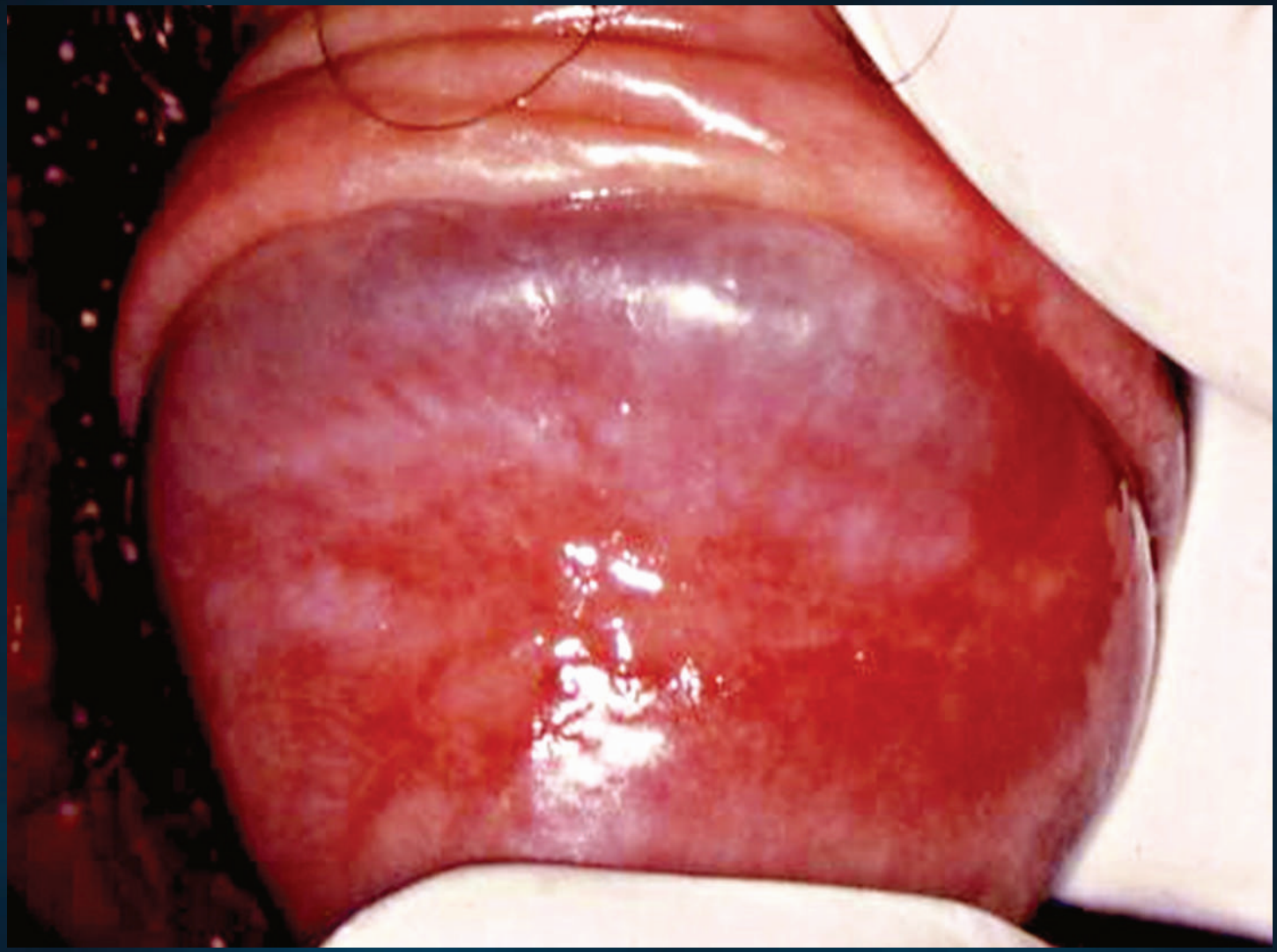
Clinical Traps



vulvar erythema and creamy discharge



white and clumpy discharge



post-coital erythema

In some of the patients who have symptoms and signs of vaginal candidiasis, which is unresponsive to antifungal drugs, a diagnosis of cytolytic vaginosis may have to be suspected

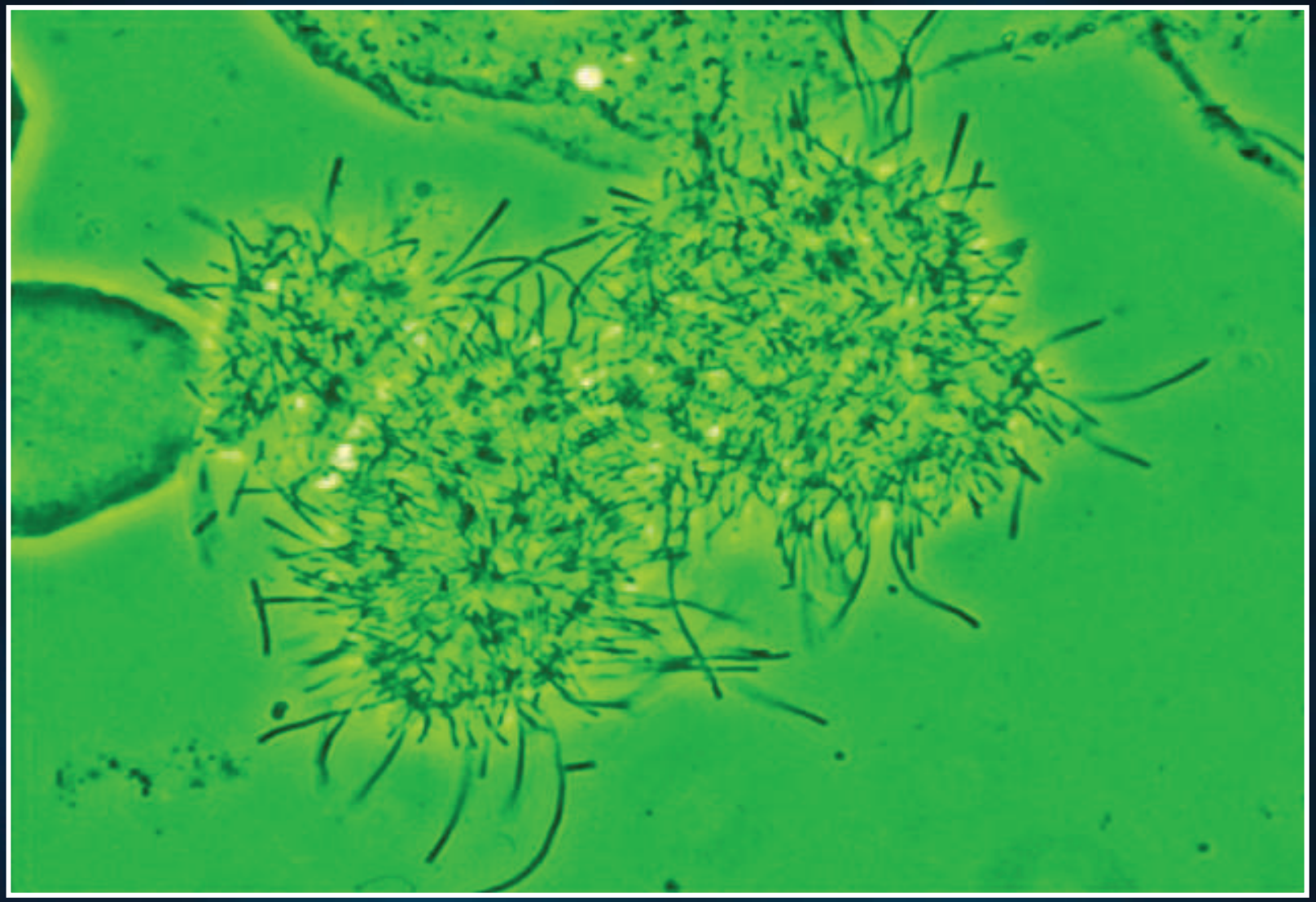
Suresh A et al. Indian J Sex Transm Dis. Jan-Jun; 30(1): 48, 2009

Cytolytic vaginosis is also known as vaginal lactobacillosis

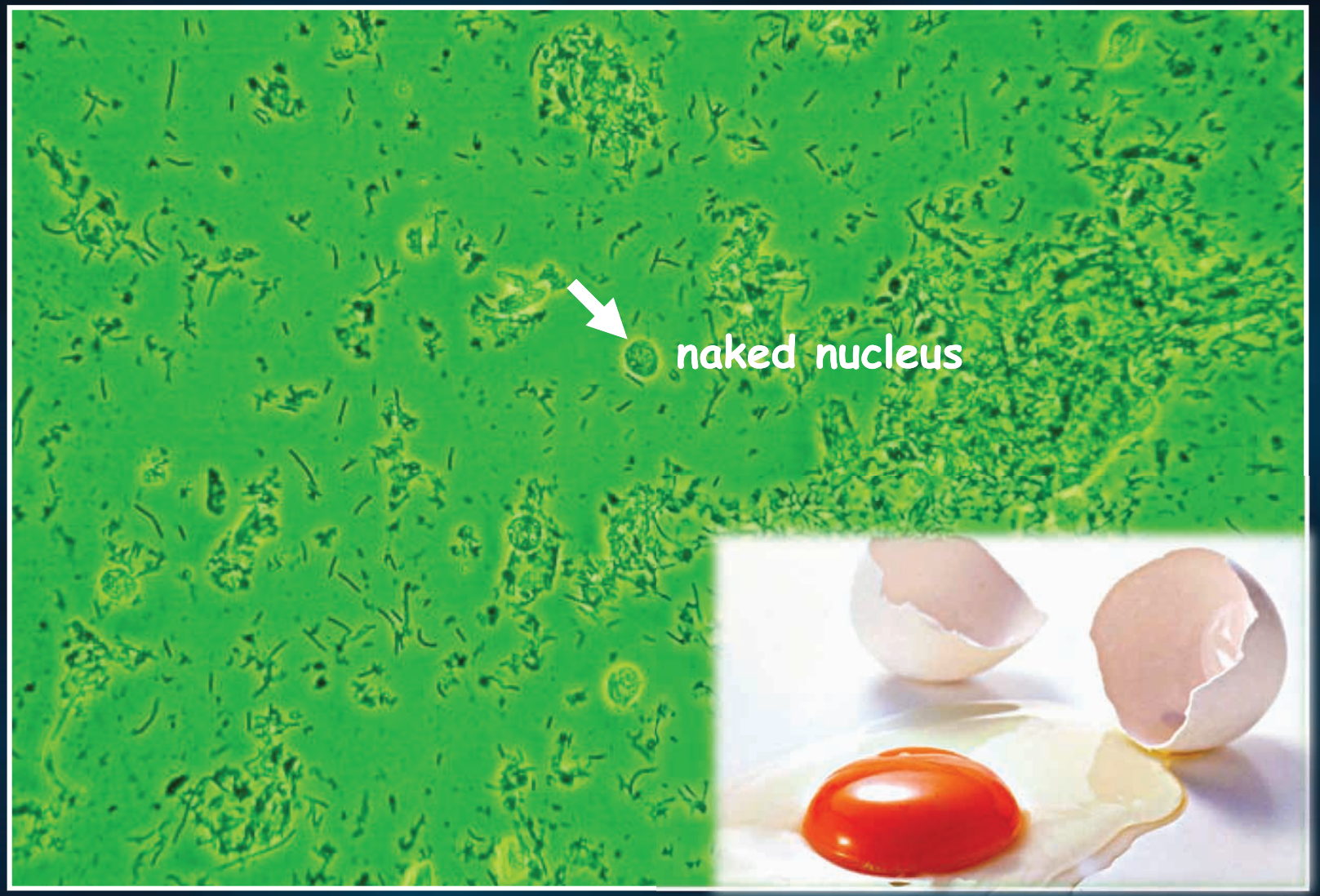
Miniello G. Direct Microscopy in Gynecologic Practice. Edra Ed. 2017

It is characterized by abundant growth of Lactobacilli resulting in lysis of vaginal epithelial cells

Suresh A et al. Indian J Sex Transm Dis. Jan-Jun; 30(1): 48, 2009



adhered filamentous Lactobacilli



Doderlein's cytolysis

Very annoying, profuse vaginal discharge, often associated with vulvar and vaginal itching

Miniello G. Direct Microscopy in Gynecologic Practice, Edra Ed. 2017

The etiology is unknown and the prevalence is approximately 15%

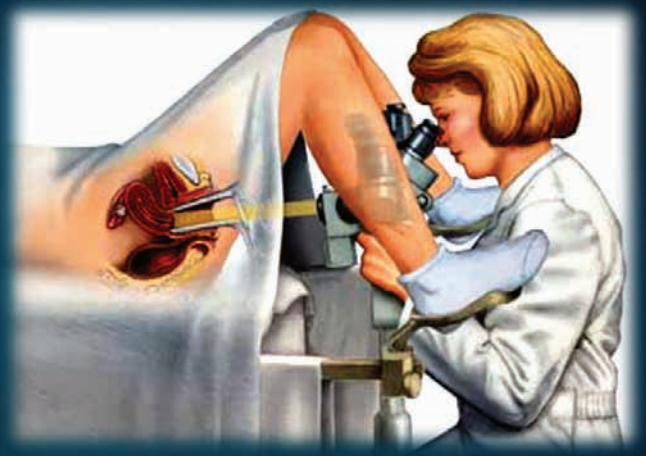
Ventolini G et al. J Clin Gynecol Obstet. 3(3):81, 2014

If vaginal lactobacillosis is misinterpreted as a fungal infection, allergic reactions to antimycotic therapy may result in perpetuation of symptoms that are incorrectly thought to be caused by yeast

Witkin SS et al. It J Gynaecol Obstet; 3:114, 2000

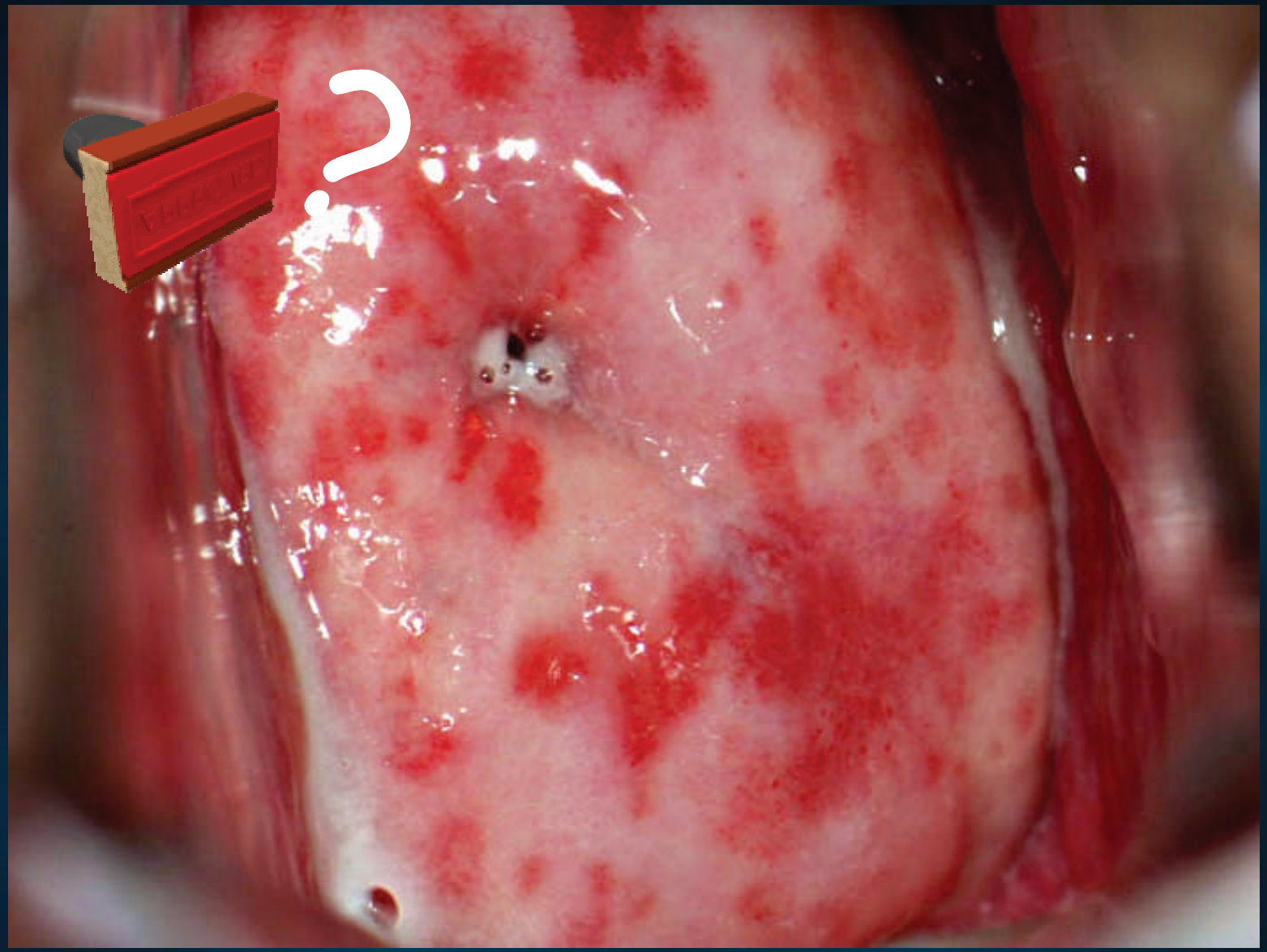
CANDIDA

colposcopy:

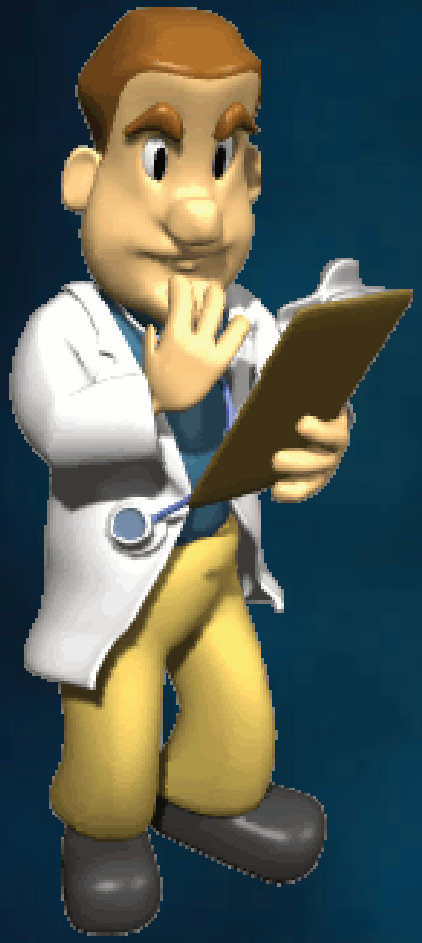


- cervical congestion and, white and **clumpy** discharge
- **erythematous maculae**
- **erythematous papulae**
- **white punctation**

fungal erythematous maculae



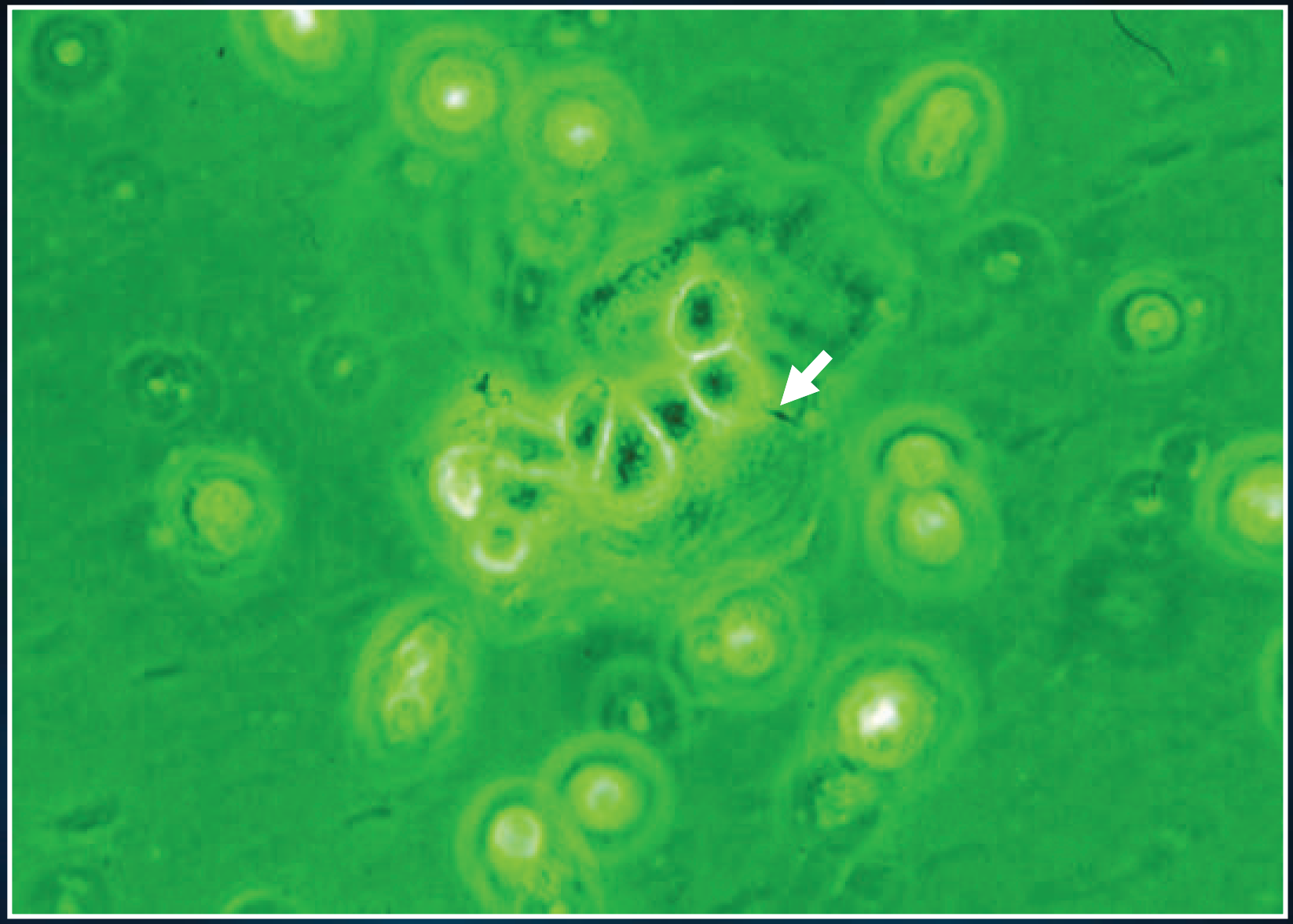
fungal erythematous maculae



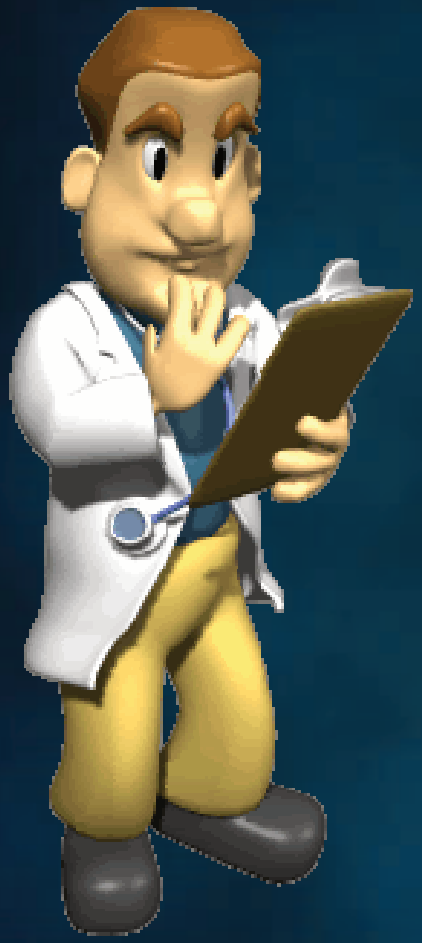
Differential diagnosis

vs Trichomonas petechiae

Trichomonas strawberry appearance



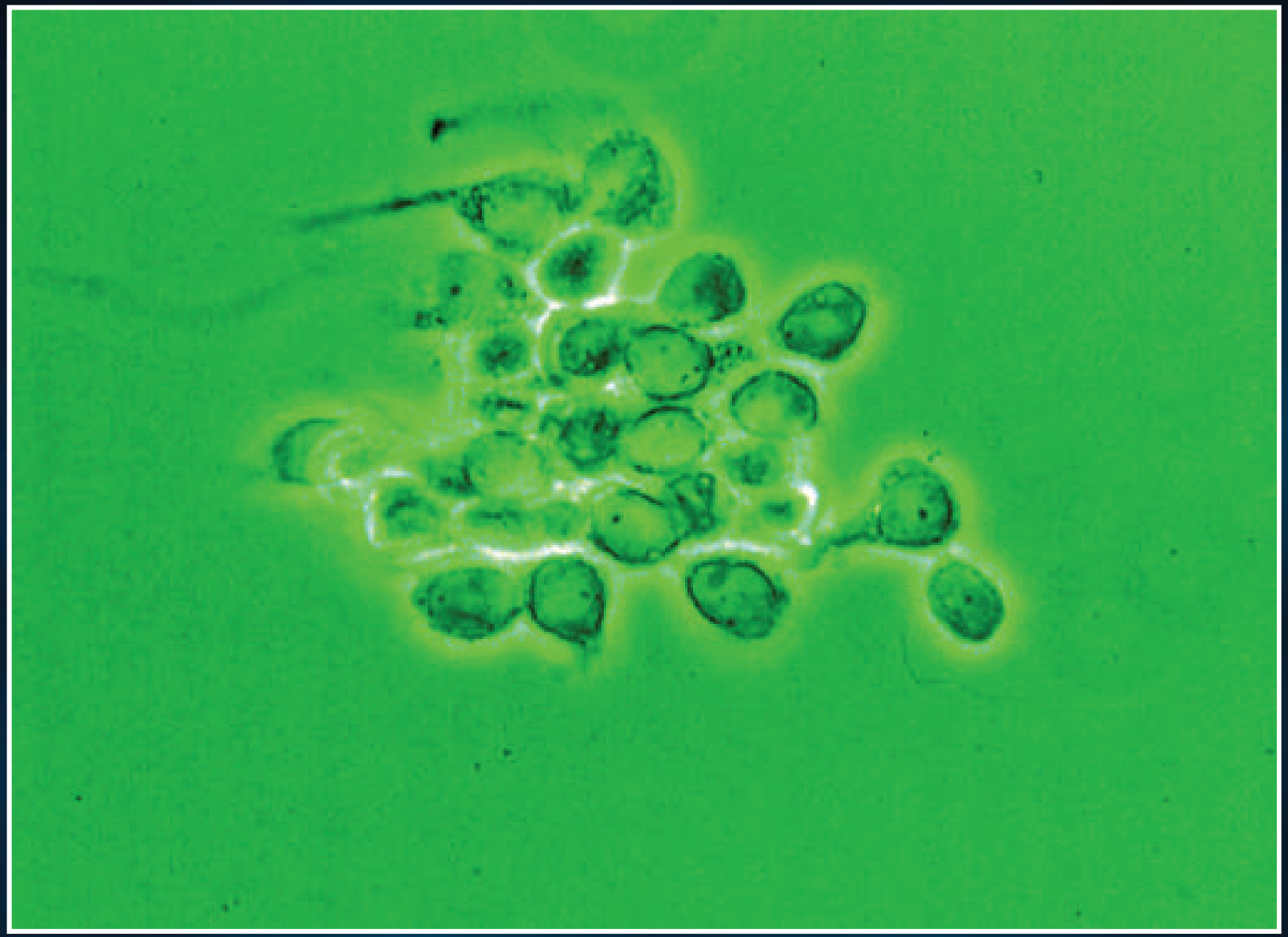
cluster of Trichomonads



Differential diagnosis

vs dystrophic petechiae

dystrophic petechiae



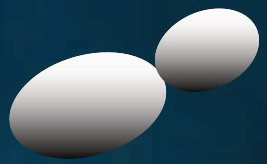
atrophic smear

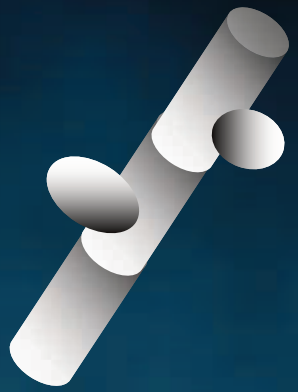
CANDIDA

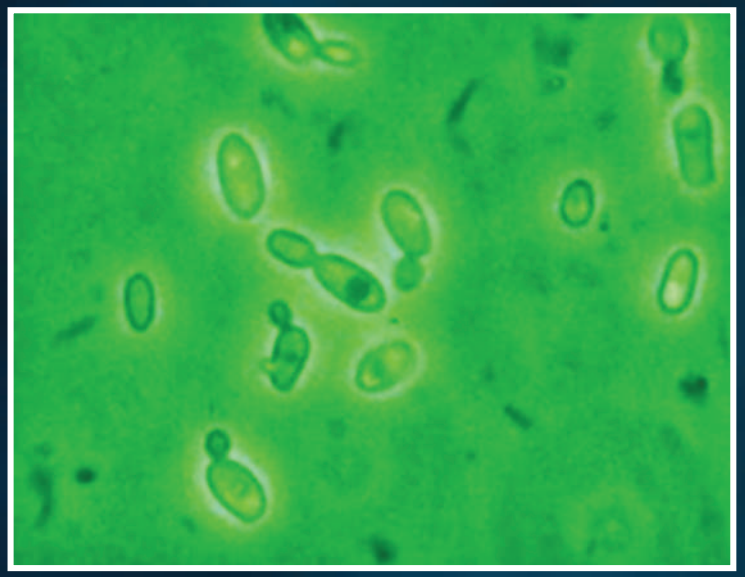
Direct microscopy:

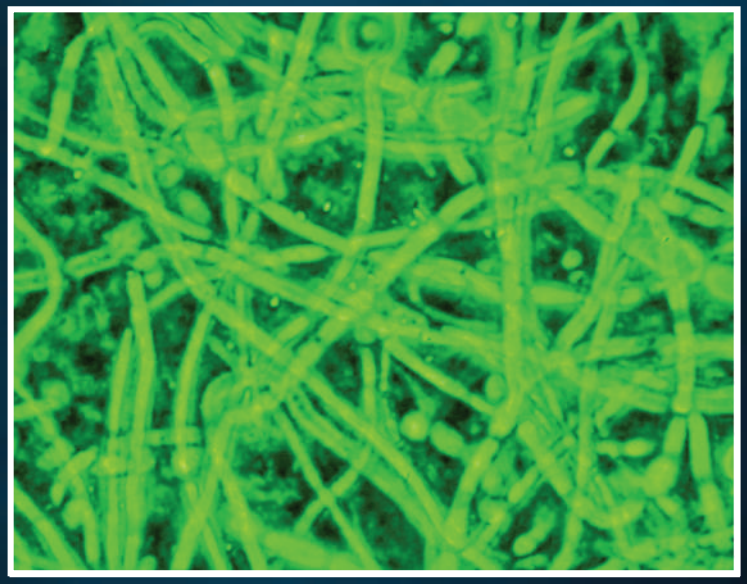
- blastospores
- hyphae
- Candida cytopathy

CANDIDA



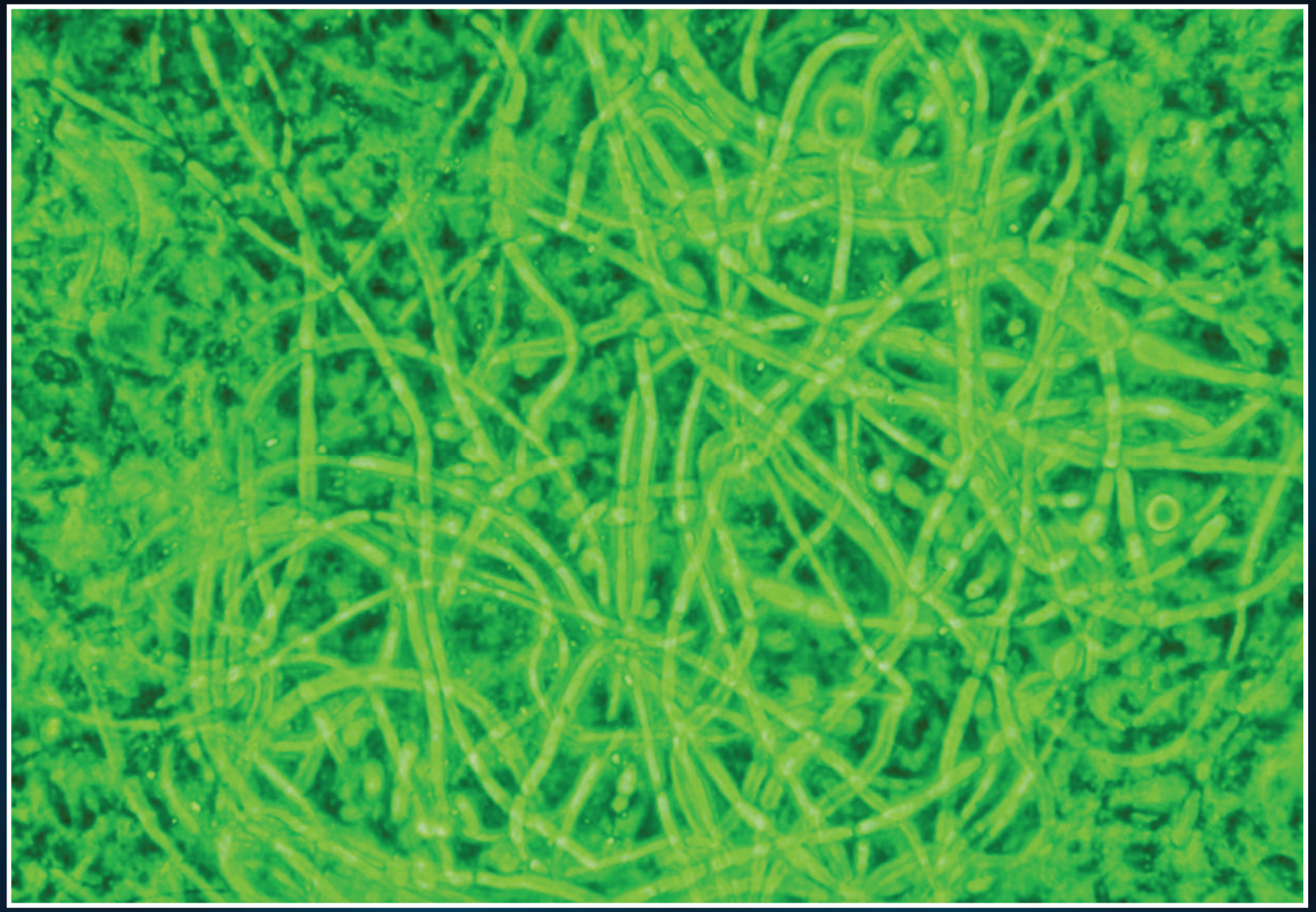






budding blastospores

branching Hyphae

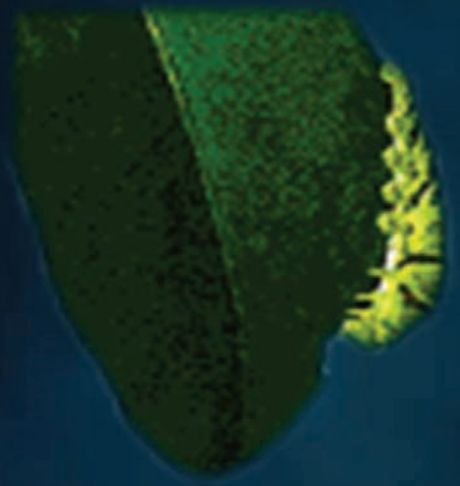


branching and budding hyphae

Laboratory

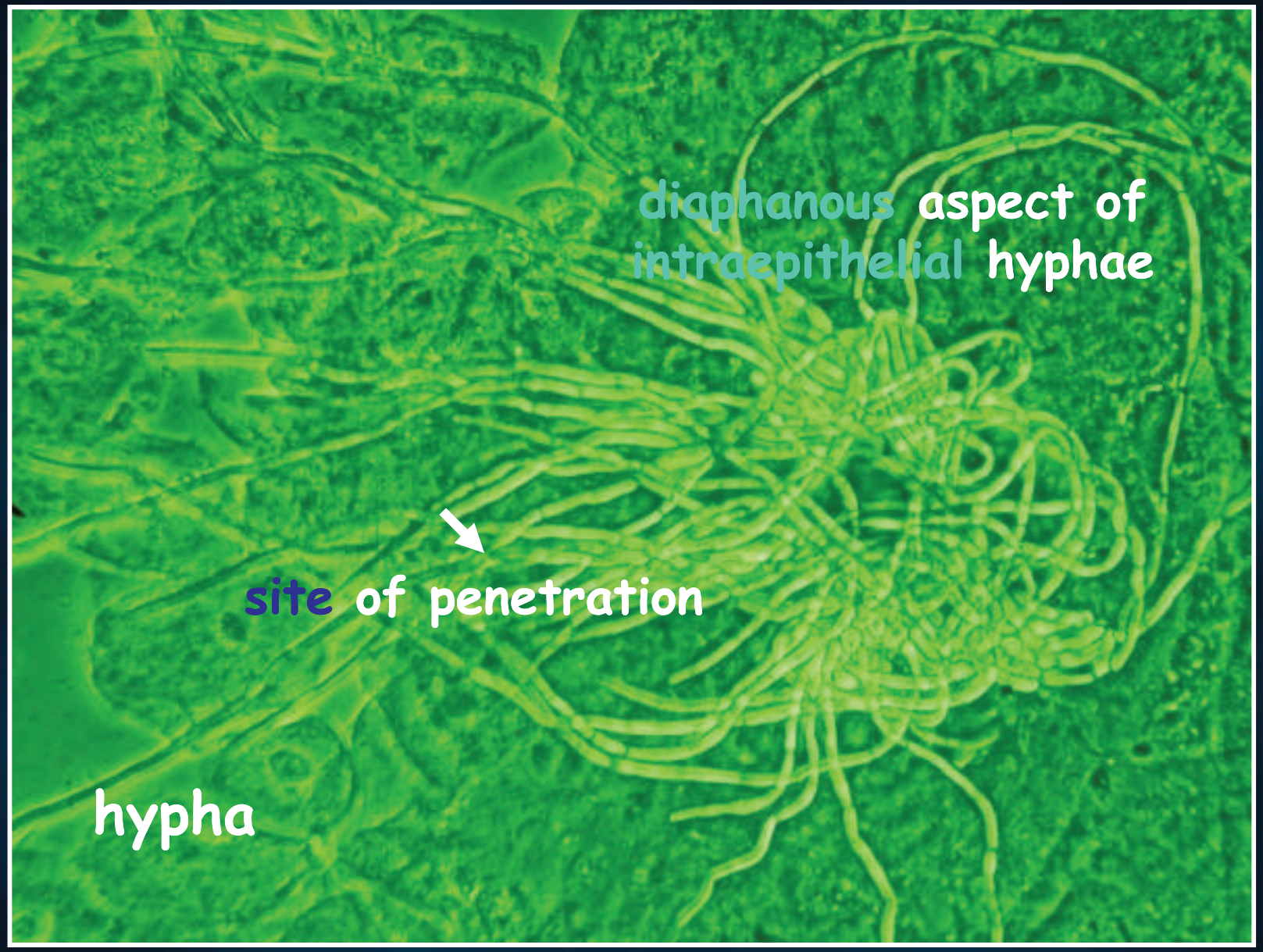
Traps

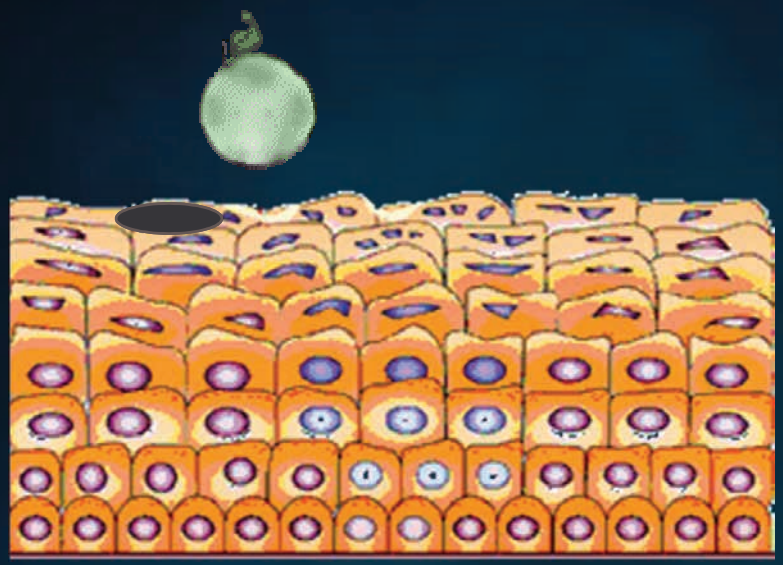
Frequently, despite the presence of irritative symptoms, fungal blastospores and hyphae are not visible under direct microscopic examination and cultures yeld negative results



Candida uses proteases to penetrate through the vaginal epithelium

Miniello G. Vaginal Cytogram CIC Ed, 1994

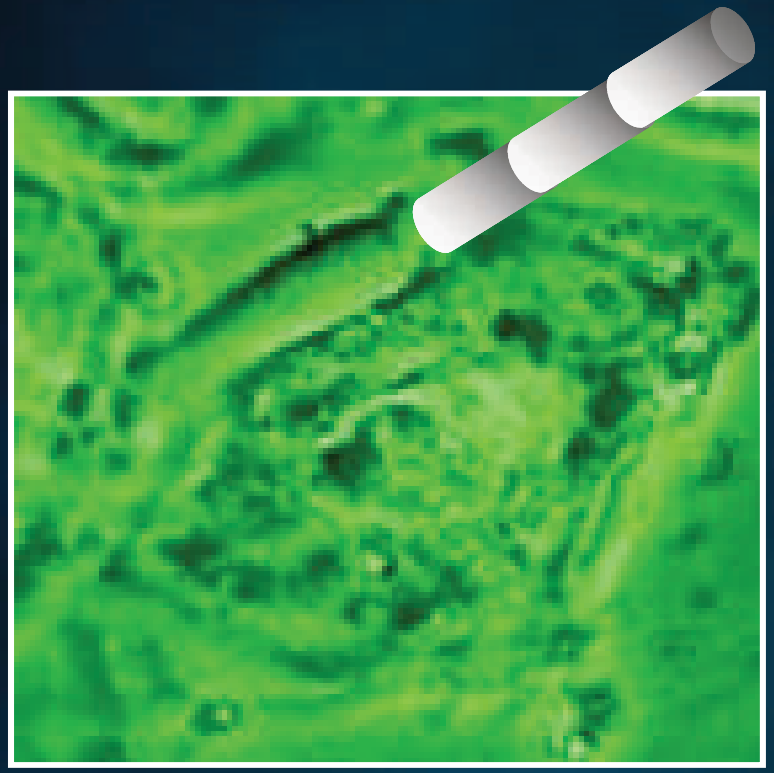

diaphanous aspect of
intraepithelial hyphae
site of penetration
hypha

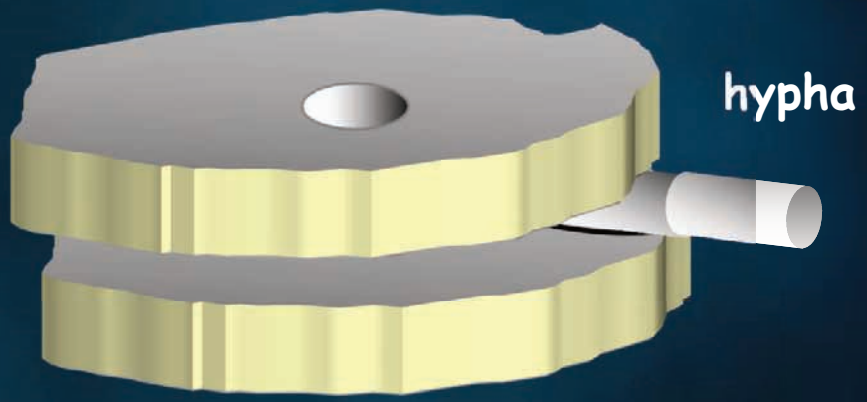


Passing through the vaginal epithelium, Candida causes a specific cytopathy, that can be easily detected by direct microscopy

Miniello G. Vaginal Fungal Infections by Phase Contrast Microscopy, CIC Ed., 2001

CANDIDA EPITHELIAL INVASION

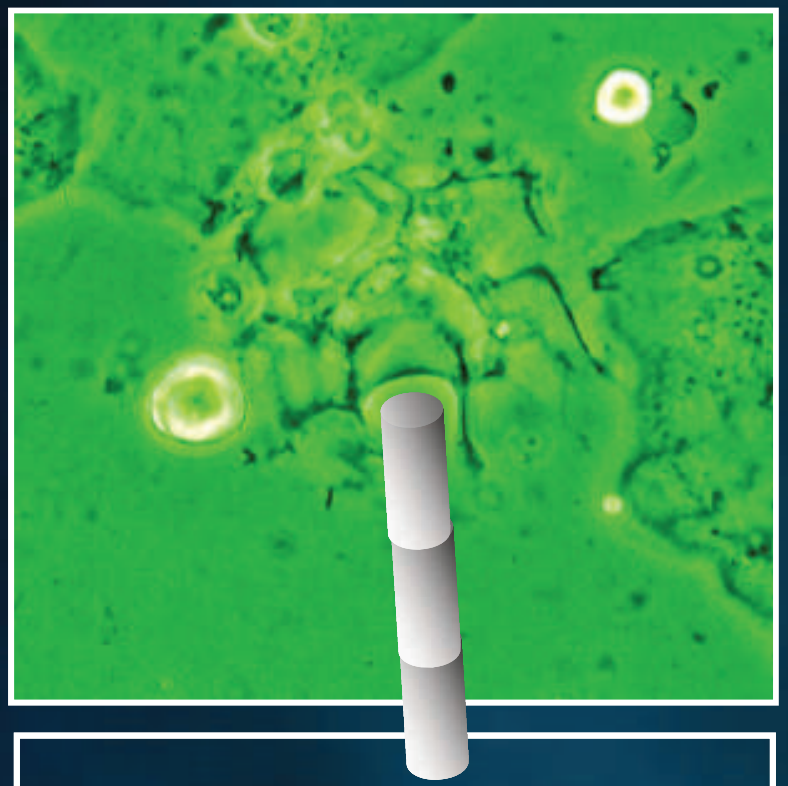


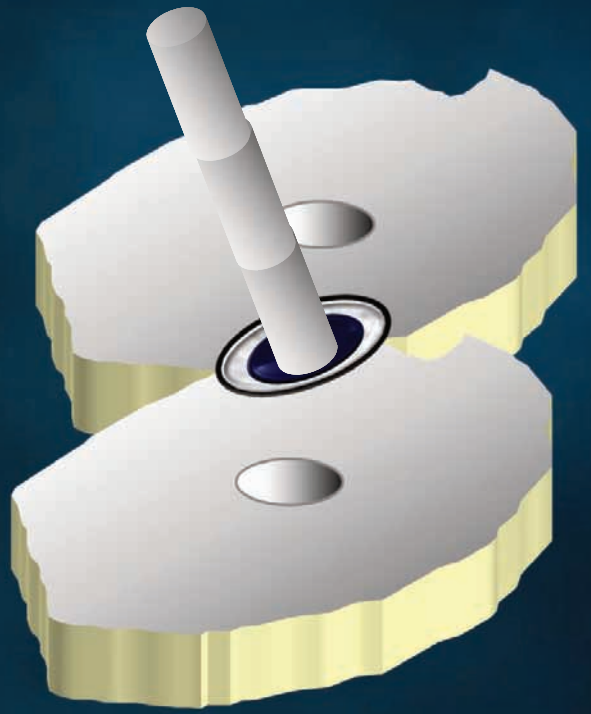


Candida cytopathy:
cytoplasmic GROOVE

Miniello G. Vaginal Cytogram CIC Ed, 1994

CANDIDA EPITHELIAL INVASION

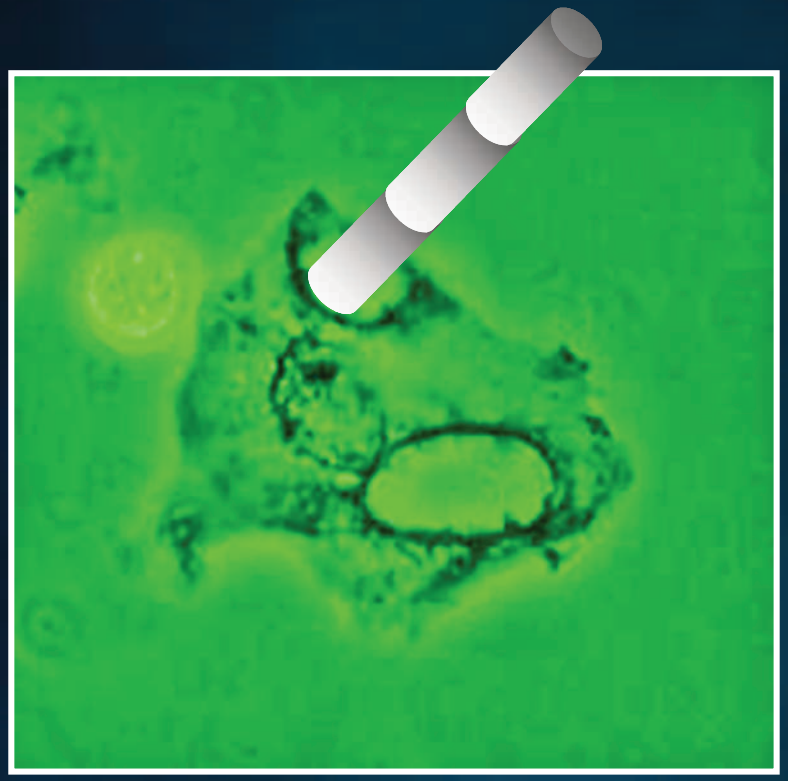


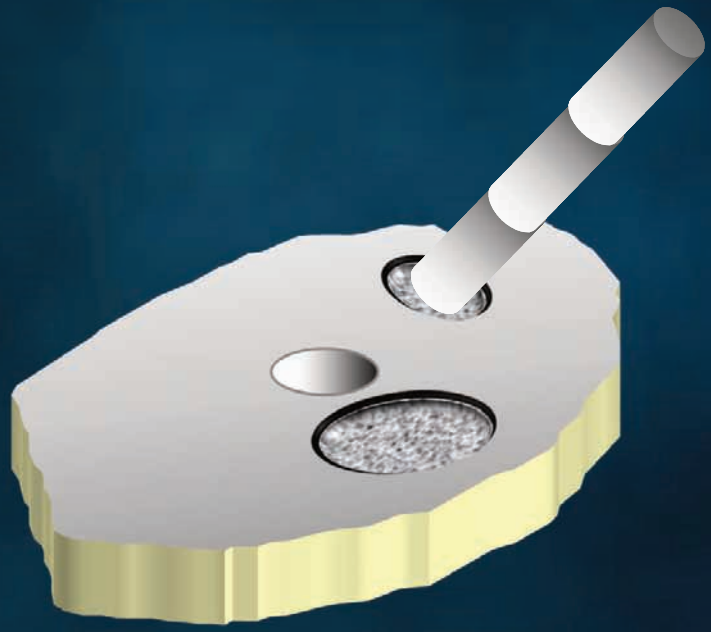


Candida cytopathy:
marginal EROSION

Miniello G. Vaginal Cytogram CIC Ed, 1994

CANDIDA EPITHELIAL INVASION

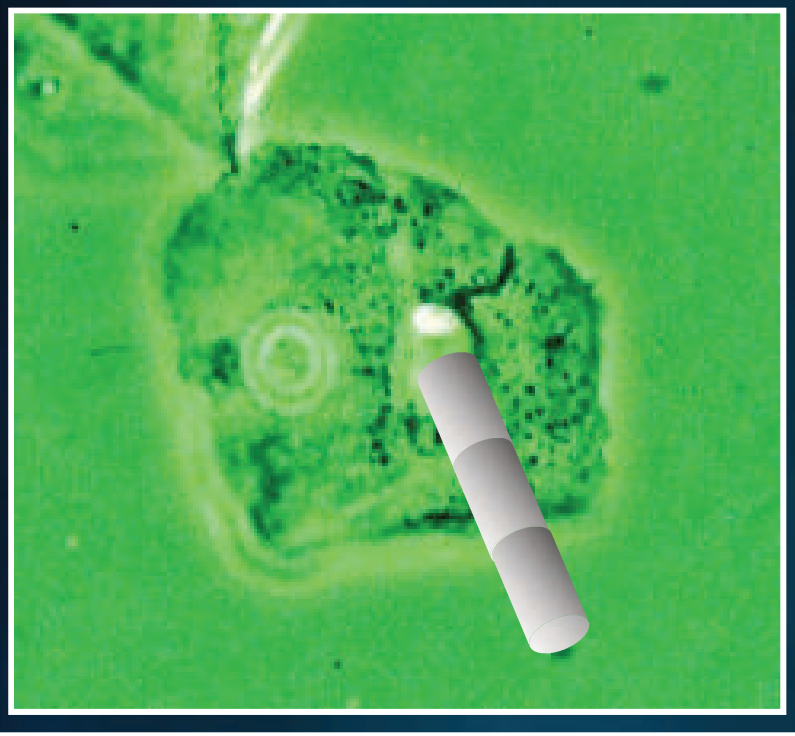


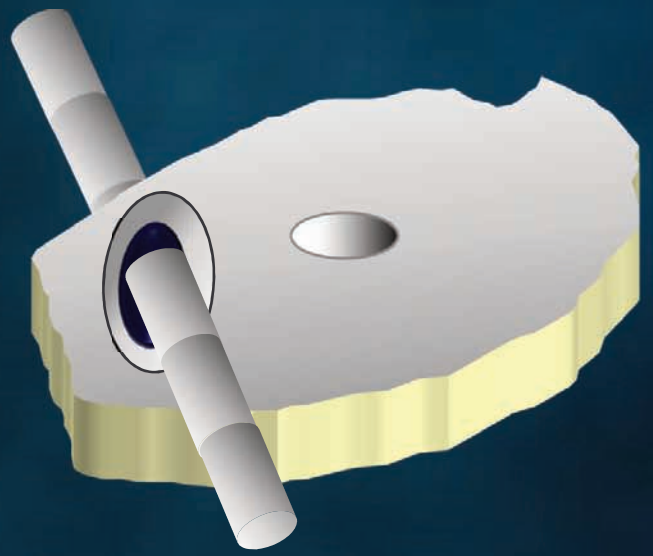


Candida cytopathy:
cytoplasmic HOLES

Miniello G. Vaginal Cytogram CIC Ed, 1994

CANDIDA EPITHELIAL INVASION





Candida cytopathy:
cytoplasmic TUNNEL

Miniello G. Vaginal Cytogram CIC Ed, 1994

CANDIDA EPITHELIAL INVASION

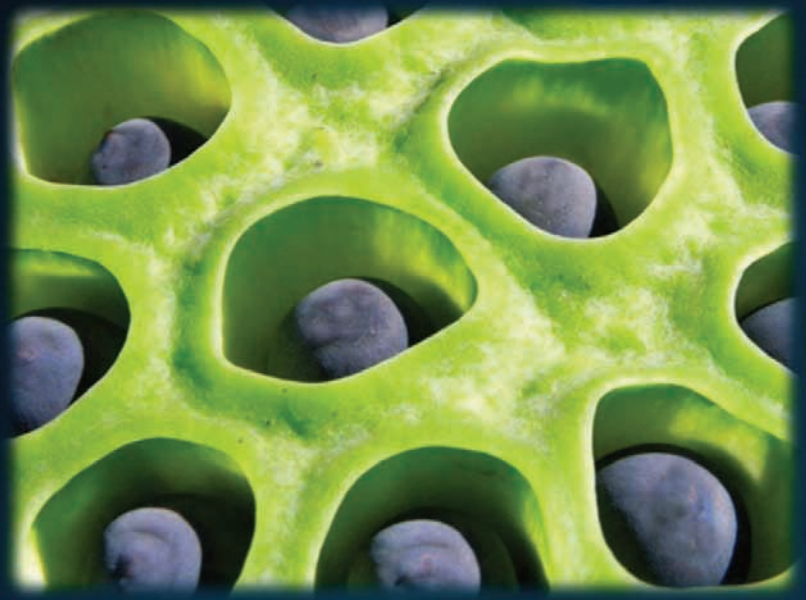
Candida cytopathy

can be recognized

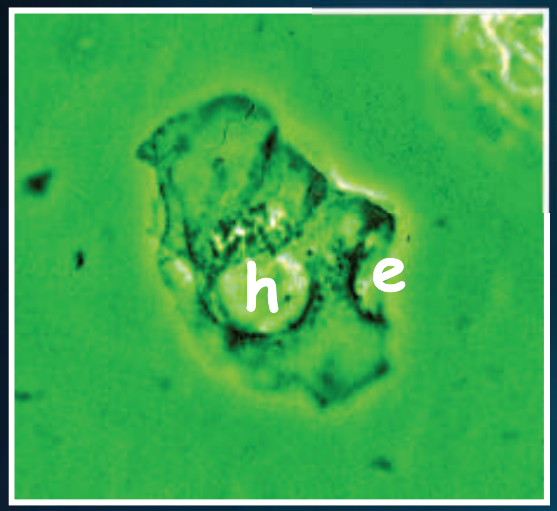
only by the use of

direct microscopy

Miniello G. Vaginal Cytogram CIC Ed, 1994



Candida cytopathy detection may be usefull to diagnose hidden fungal infections in different districts



Candida cytopathy
(cytoplasmic hole
and marginal erosion)

10 ids after local treatment

Infective

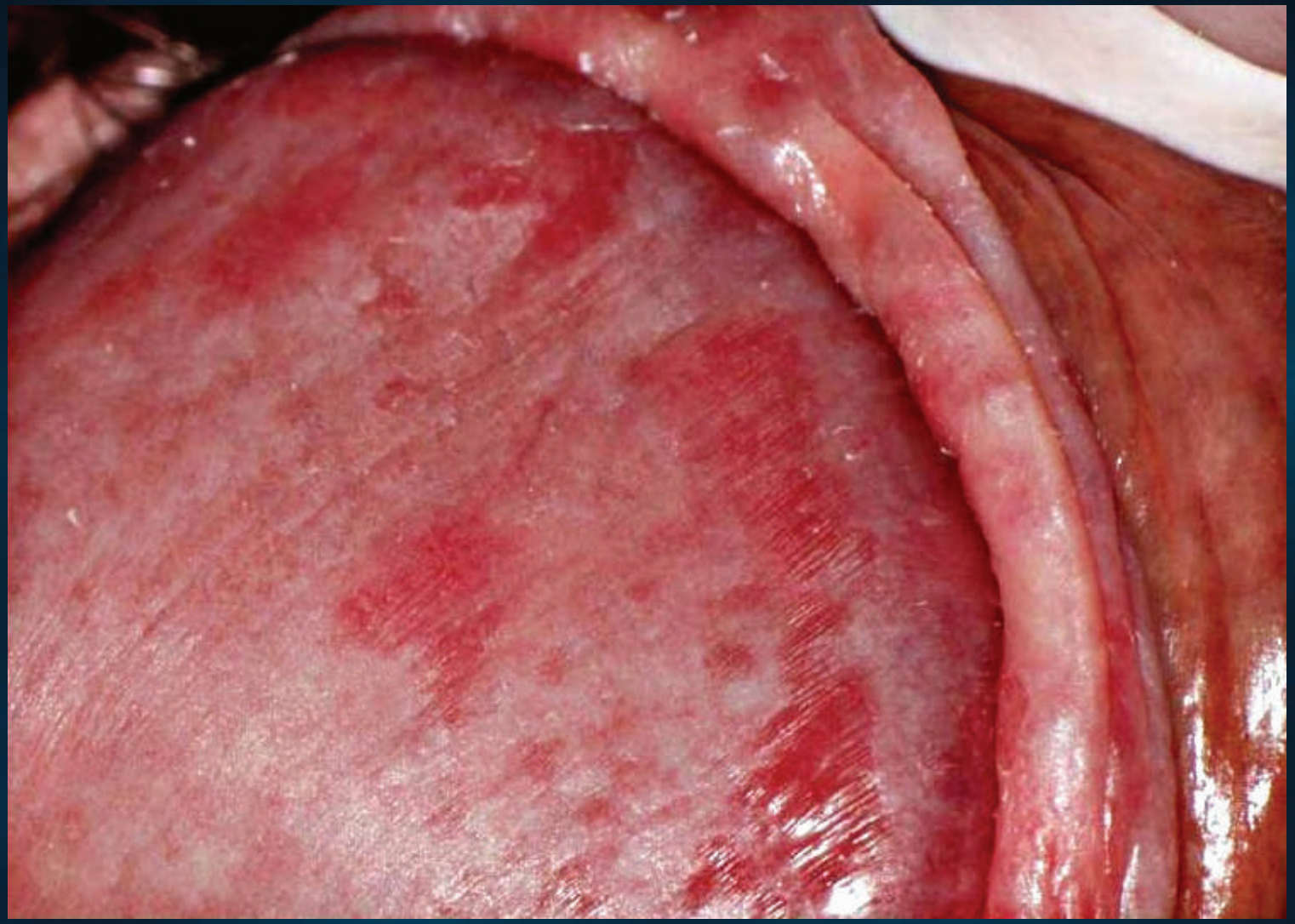
male partner

usually presents

no penile signs

or symptoms

erythematous balanoposthitis



erythematous patches

erythema and fissure

maculae

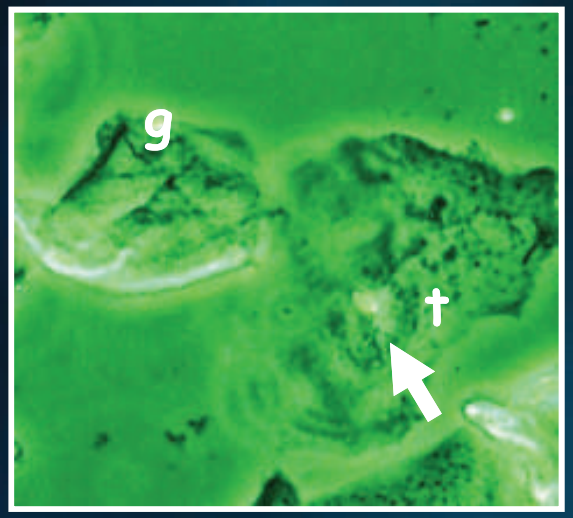
micro blisters

exfoliating balanoposthitis

exudative balanoposthitis

Is it possible to investigate the recalcitrant male partner?

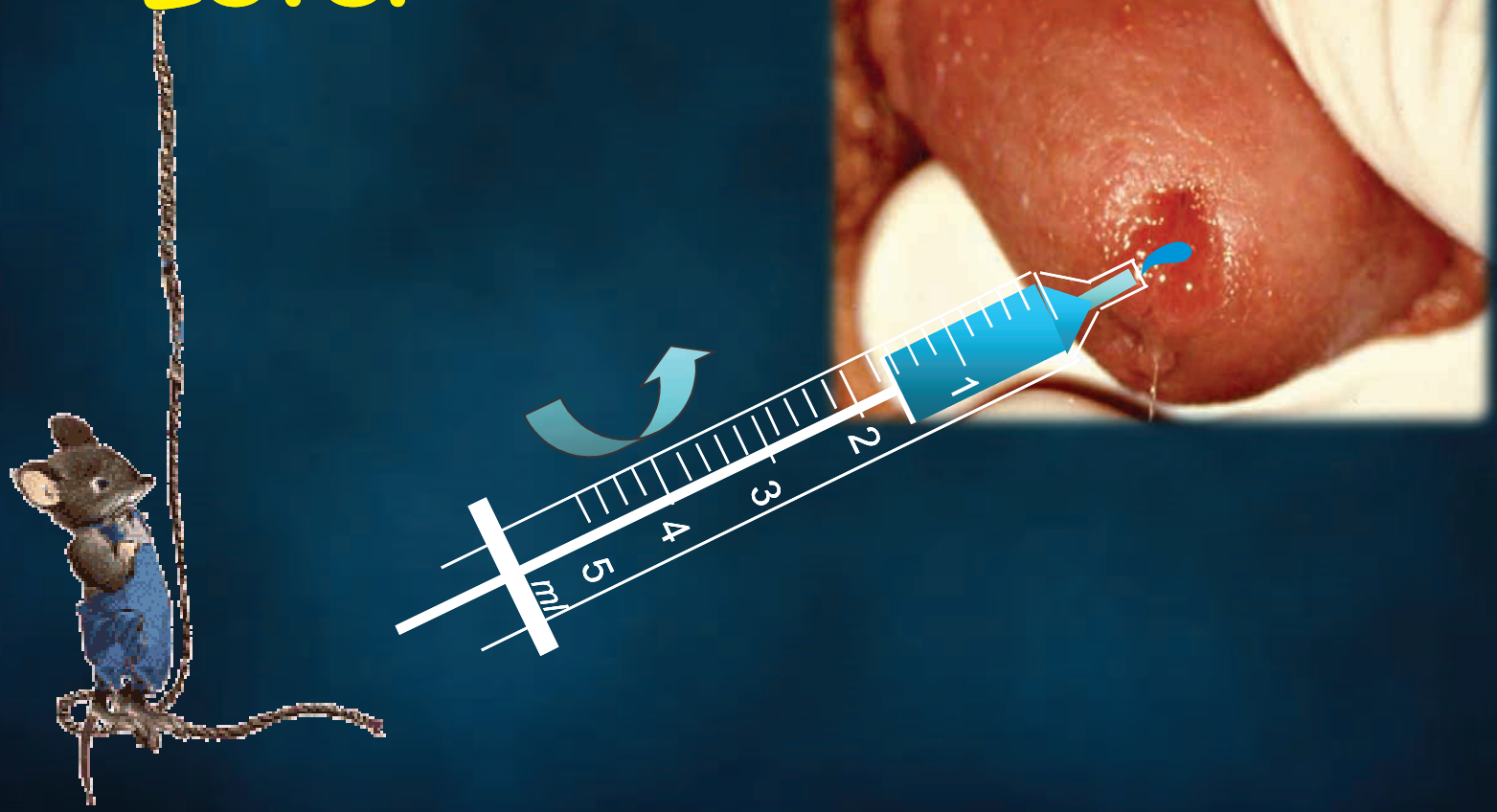
CUTANEOUS wet mount



Candida cytopathy:
(cytoplasmic groove and
and tunnel in horny cell)

URINARY wet mount

Colonization Level

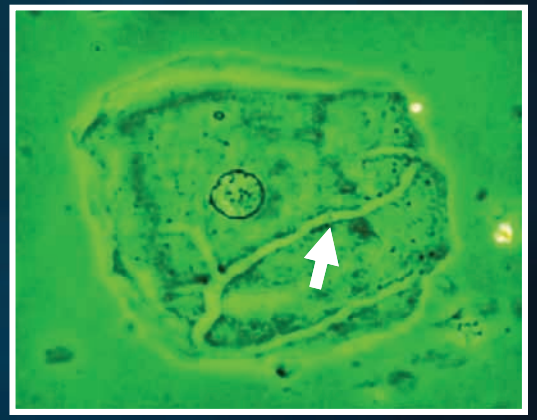


Miniello G. Gynecological Urology; p.52, Orient Longman Eds., 2001

URINARY EPITHELIA

squamous

URO-wet mount

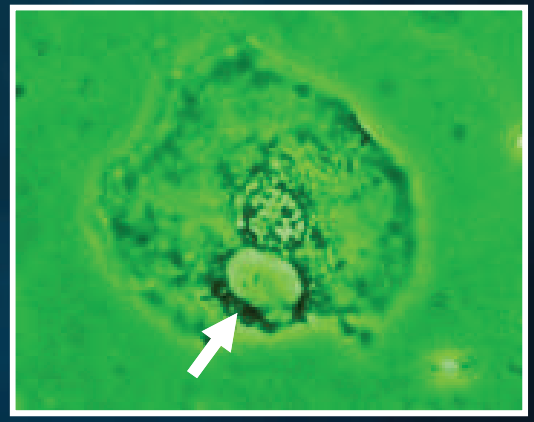


grooves

URINARY EPITHELIA

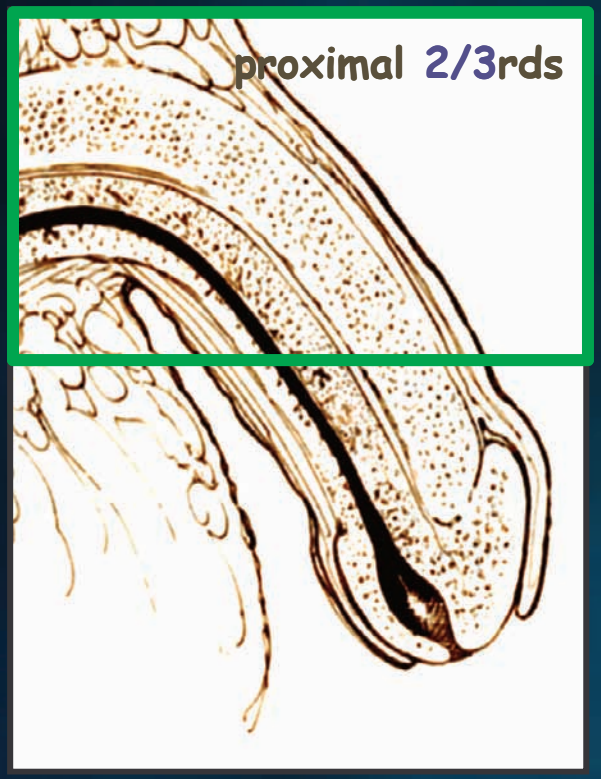
squamous

URO-wet mount



hole

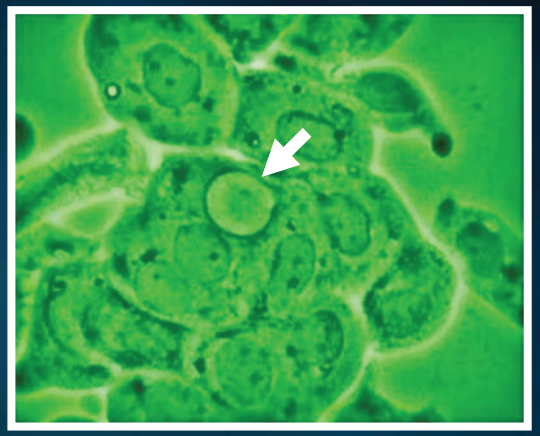
URINARY EPITHELIA



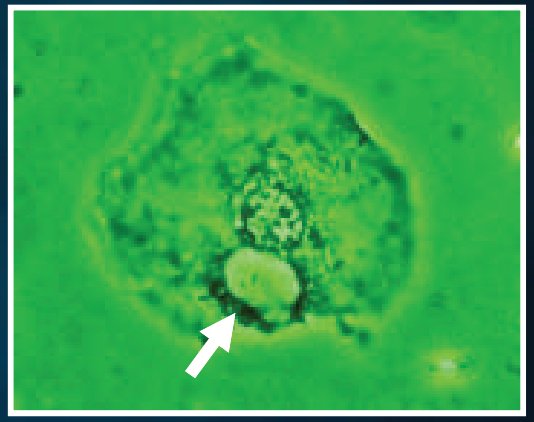
transitional

squamous

URO-wet mount

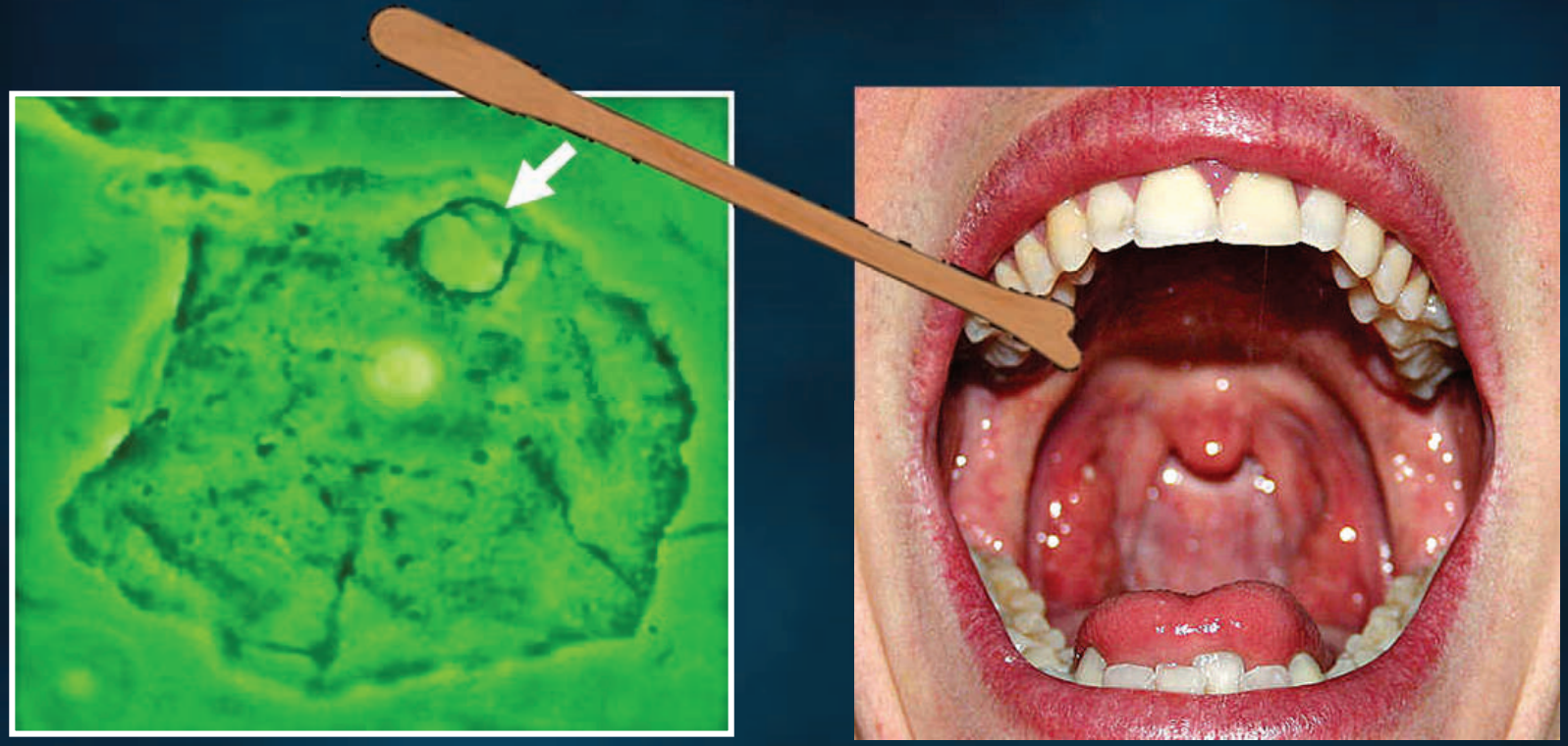


hole



hole

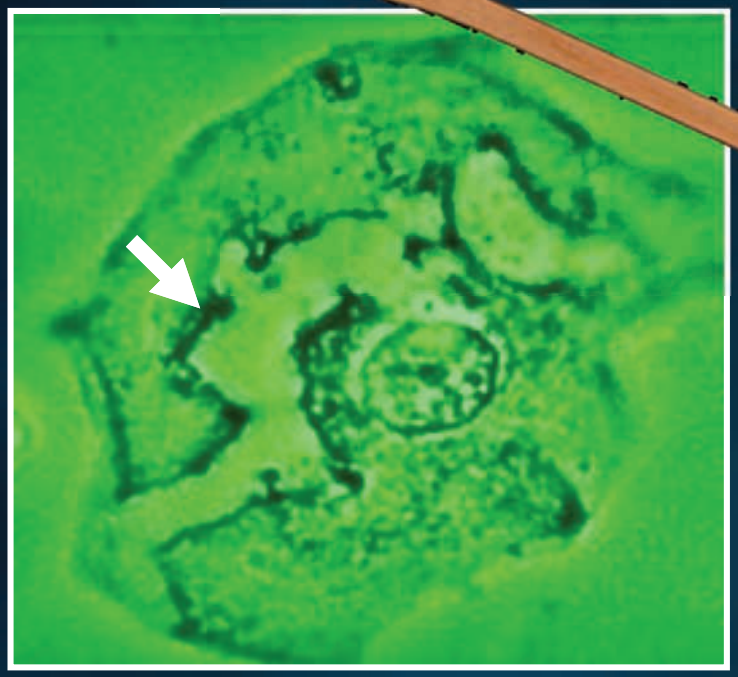
BUCCAL wet mount



Candida cytopathy:
cytoplasmic tunnel

Miniello G. Direct Microscopy in Gynecologic Practice, Edra Ed. 2017

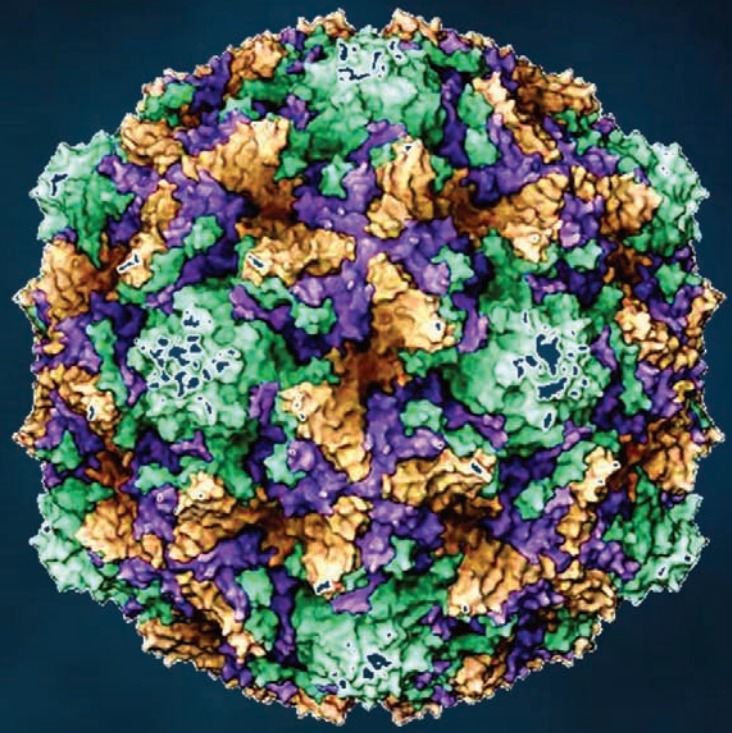
RECTAL wet mount



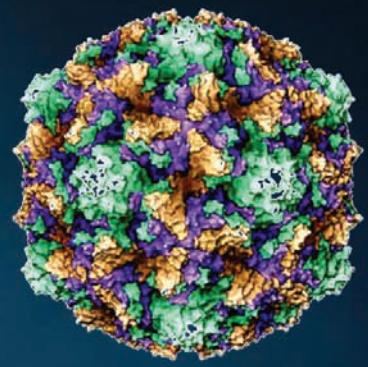
Candida cytopathy:
cytoplasmic holes

Miniello G. Direct Microscopy in Gynecologic Practice, Edra Ed. 2017

Human Papilloma Virus

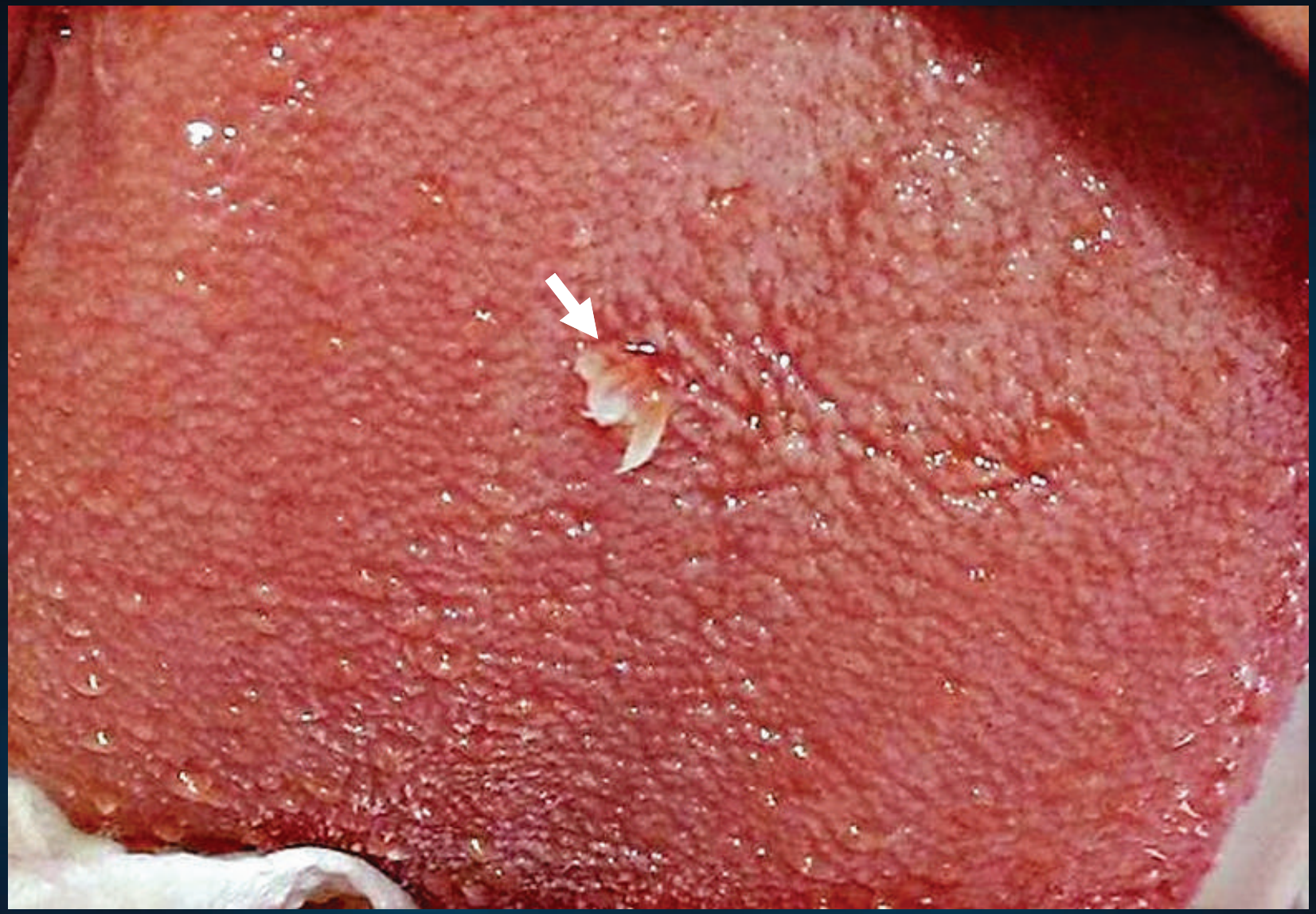


Human Papilloma Virus is the most common sexual transmitted infection



Smelov V et al.Sex Health. Nov;10(6):591, 2013

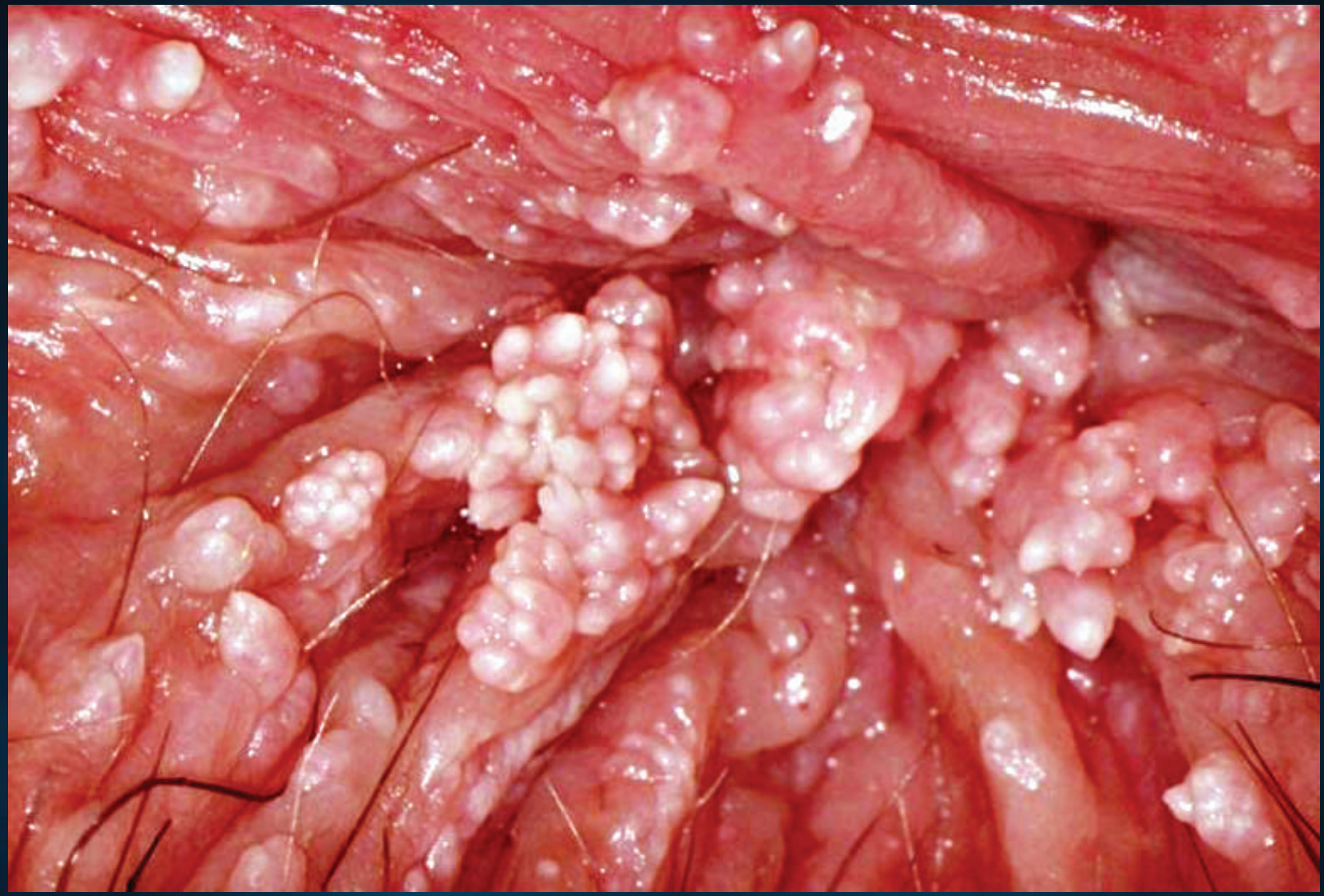
condyloma acuminatum of the palate



condyloma acuminatum of the tongue

hymenal flat condyloma

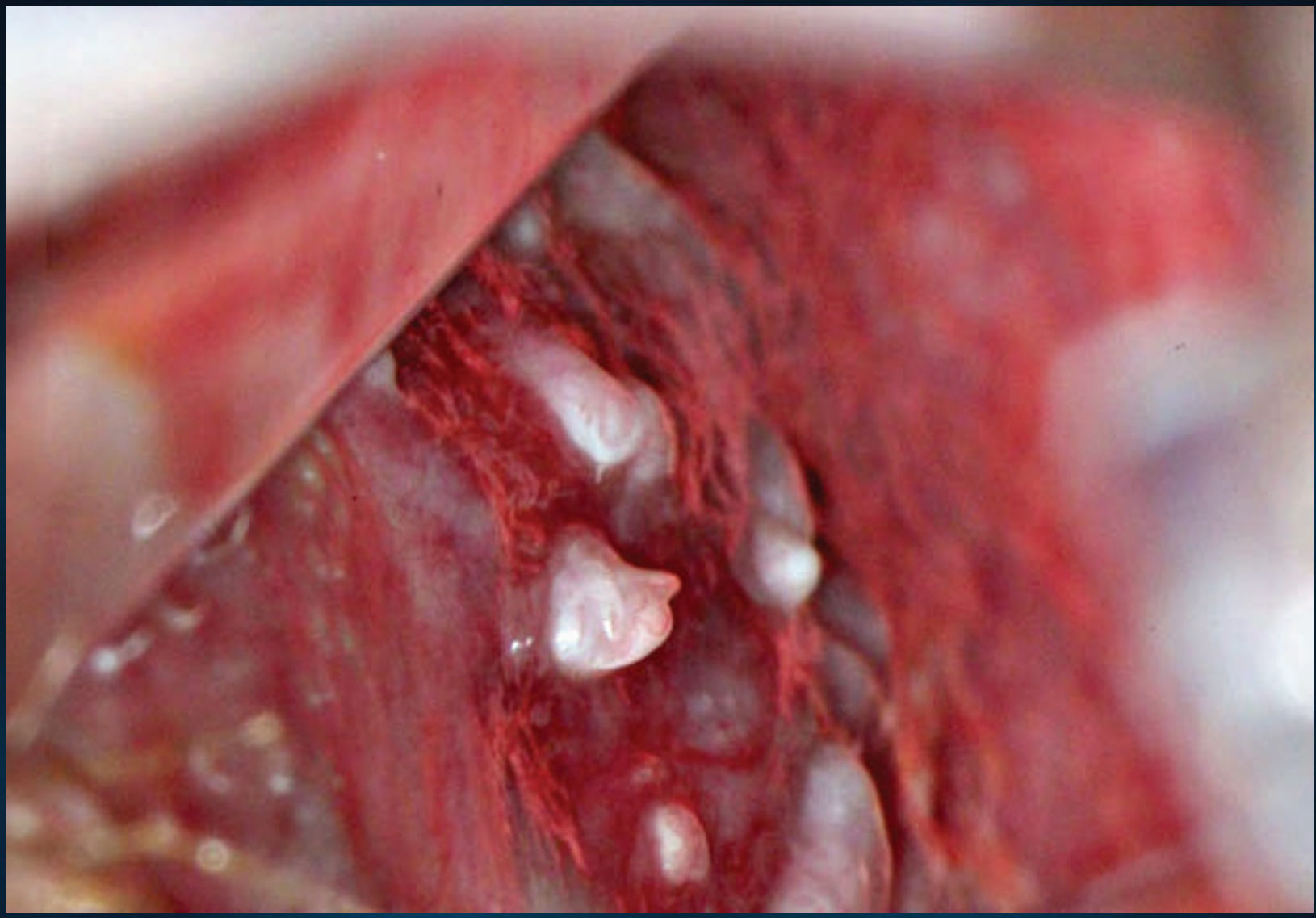
perianal flat condyloma



anal florid condyloma

In presence of anal condyloma is advisable to inspect the lower rectal mucosa

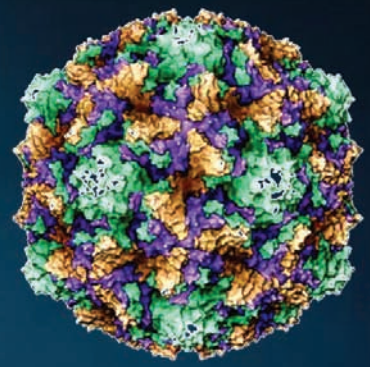
Saraya UB, Miniello G. Atlas of Cytology and Colposcopy. Jaypee Brothers Medical Pbs, 2009



rectal florid condyloma

penile florid condyloma

HPV is the major infectious aetiological agent associated with the development of pre-cancerous lesions of cervix



HPV infection may have no abnormal colposcopic finding

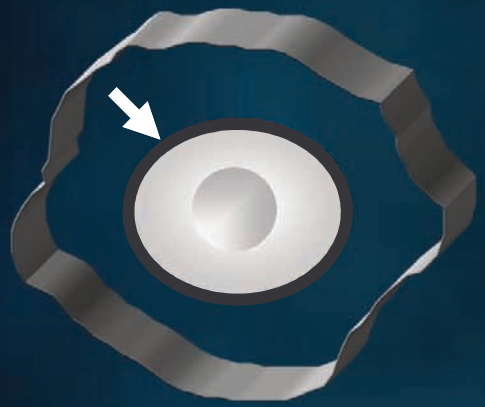
Saraya UB, Miniello G. Atlas of Cytology and Colposcopy. Jaypee Brs Pbs, 2009

Direct microscopy may represent the only warning signal in patients not referred for Pap smear

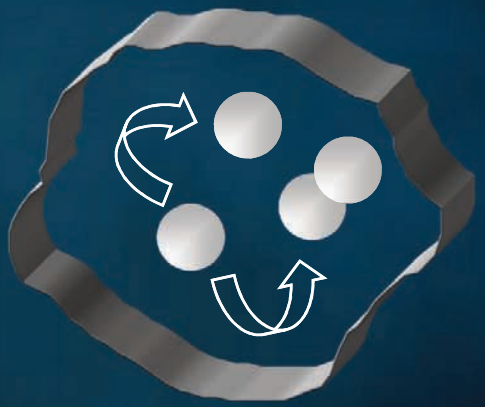
Saraya UB, Miniello G. Atlas of Cytology and Colposcopy. Jaypee Brs Pbs, 2009

HPV-related CELL findings

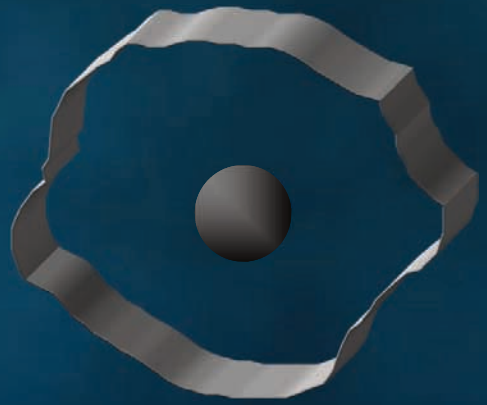
Pap smear



koilocyte



multinucleation

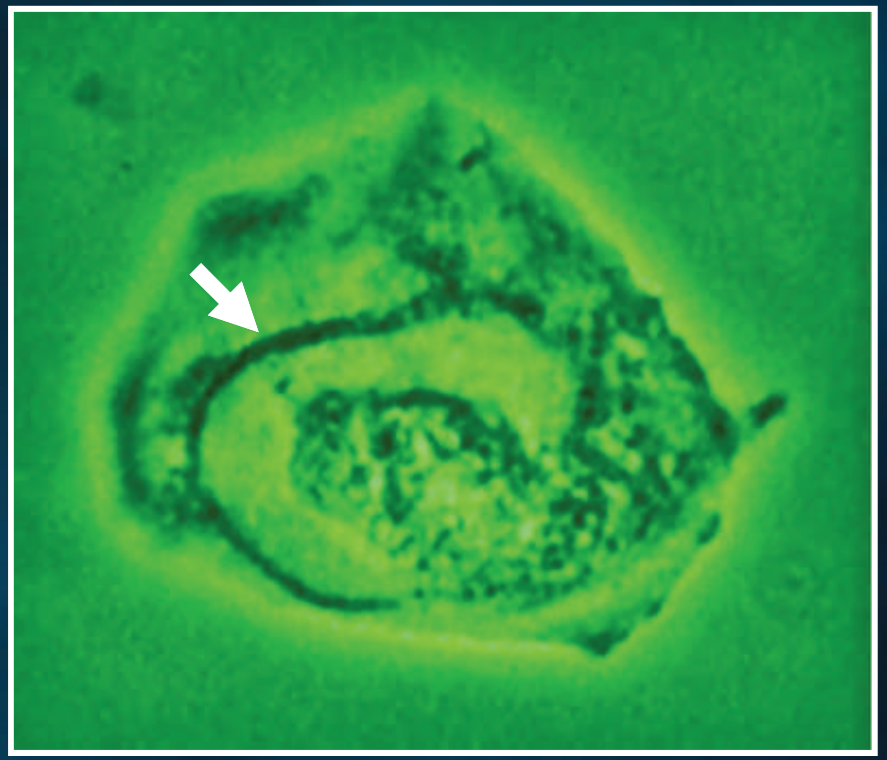


dark nucleus

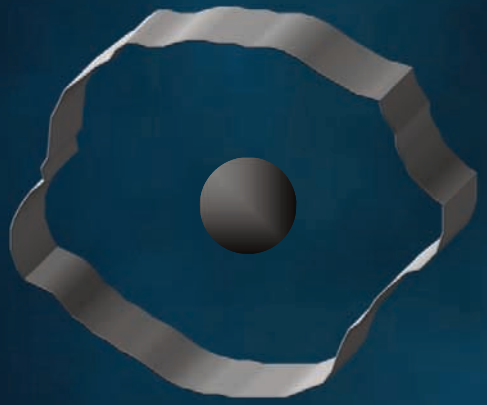
Miniello G. Integrated Colposcopy and Phase Contrast Microscopy, 1998

WET mount

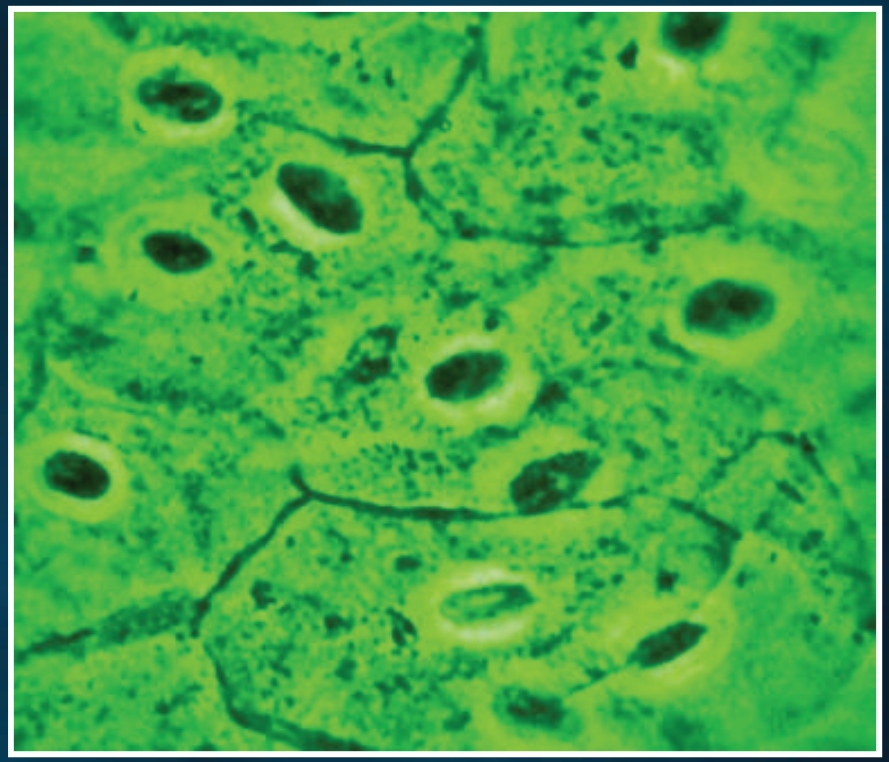
koilocyte



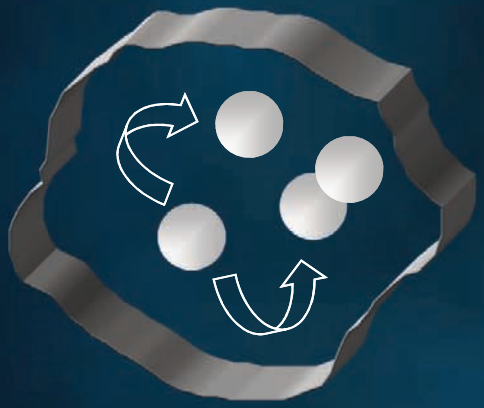
WET mount



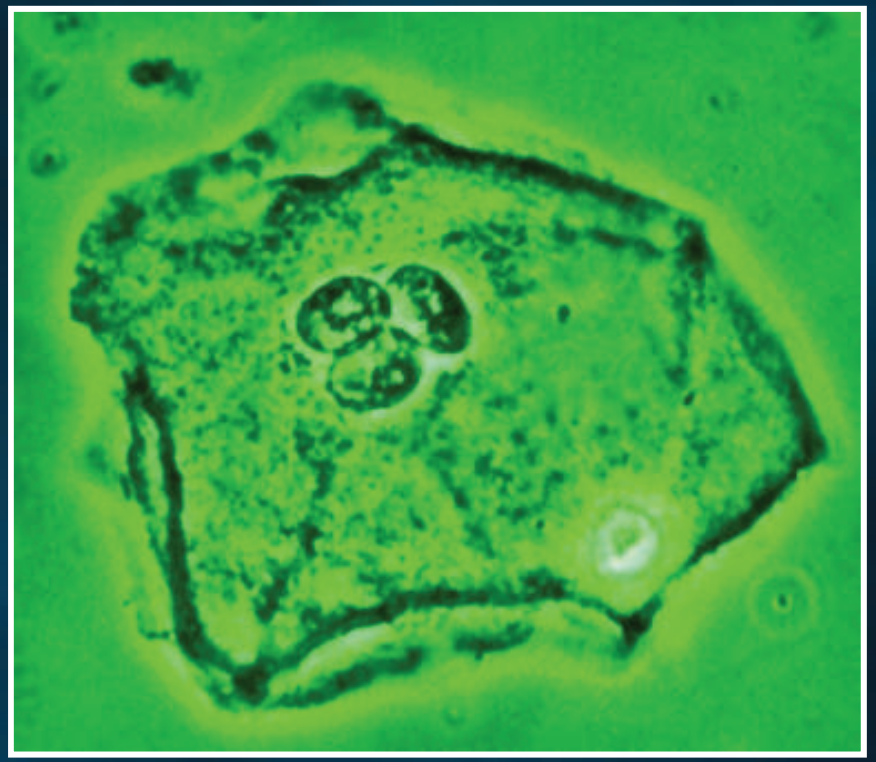
dark nuclei



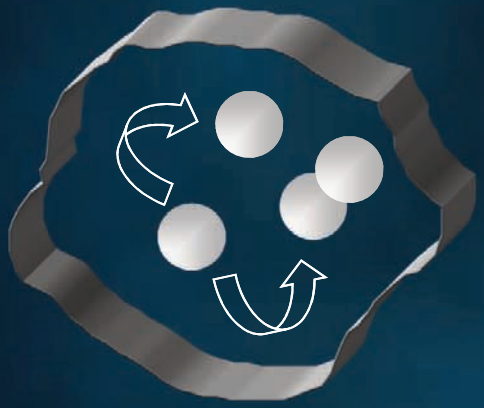
WET mount



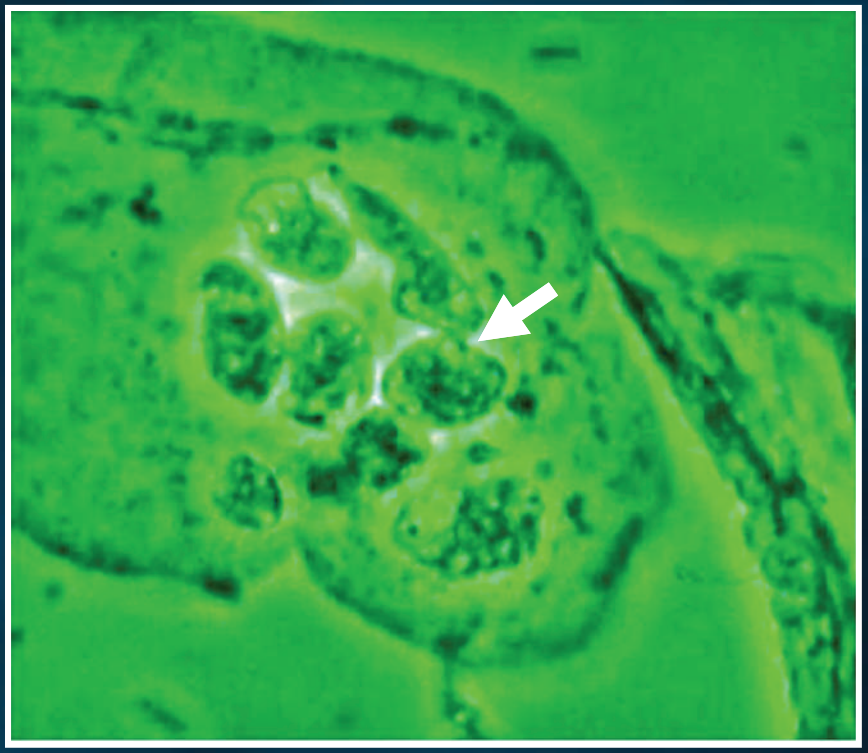
multinucleation



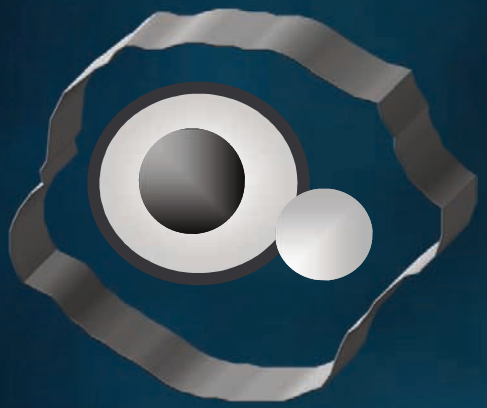
WET mount



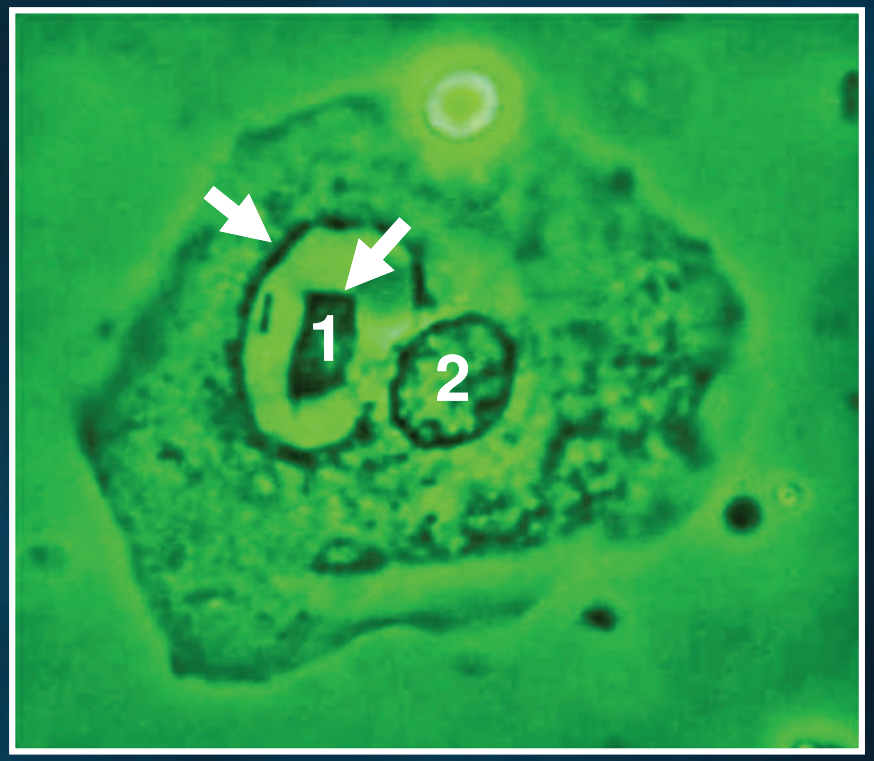
multinucleation



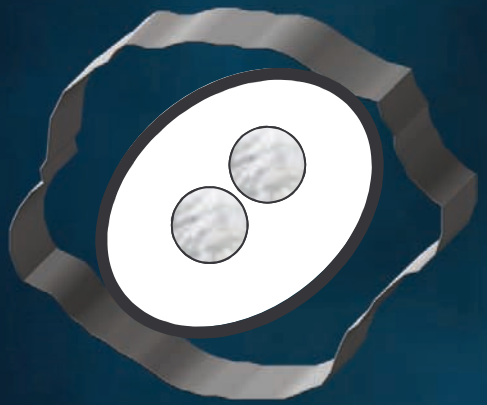
combined findings



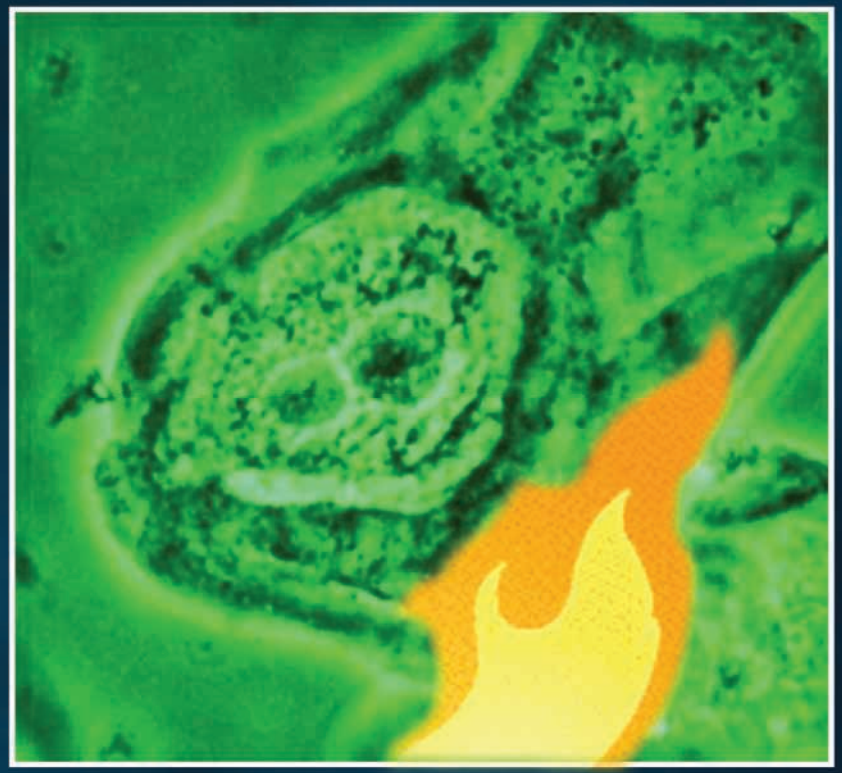
koilocyte
&
dark nucleus
&
binucleation



dyskaryotic cells

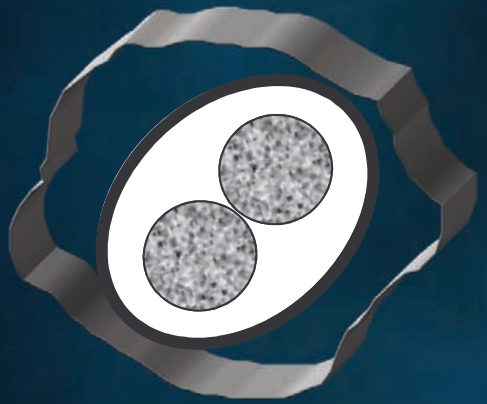


koilocyte
&
binucleation

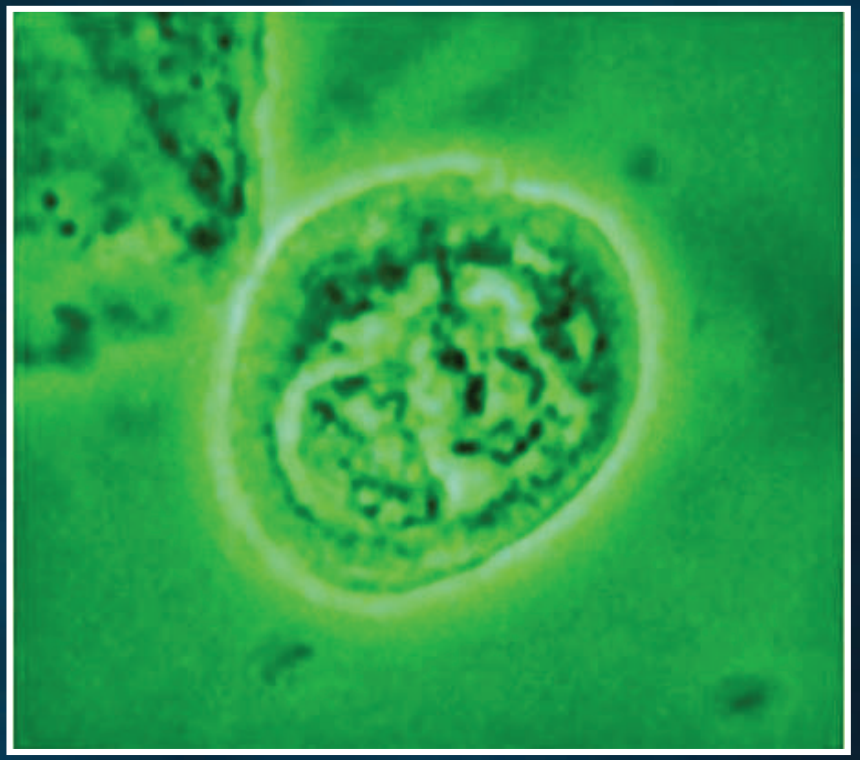


CIN 1/HPV

dyskaryotic cells

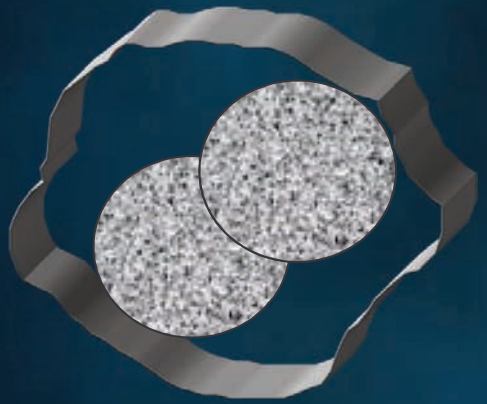


koilocyte
&
binucleation
with altered N/C R



CIN 2

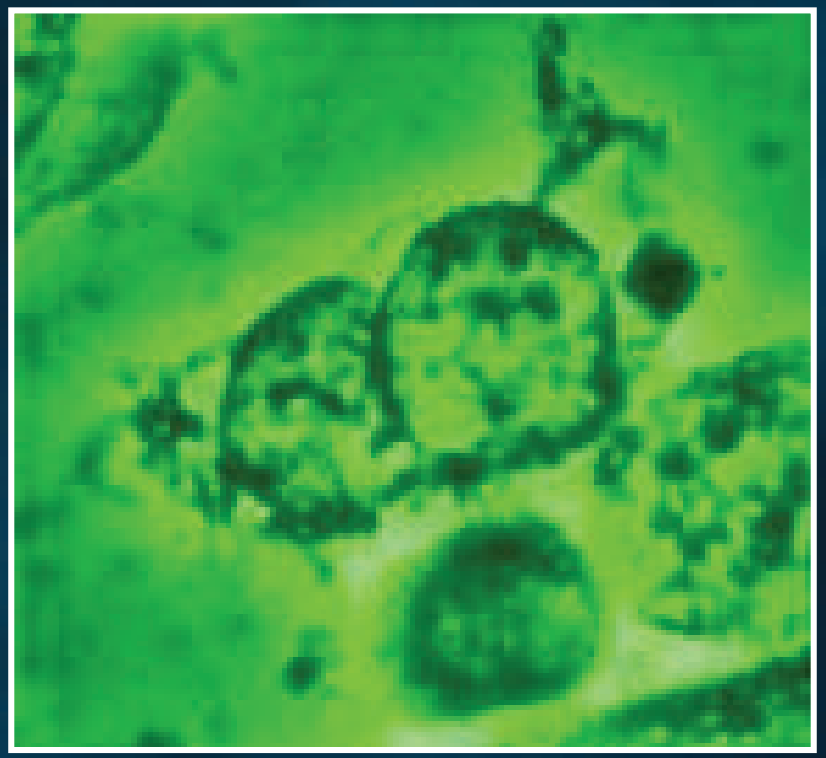
dyskaryotic cells



binucleation

&

inverted N/C R



CIN 3

HPV test

cytology

Which is the use of cytology if HPV test is available?

Positive

HPV test

indicates infection

NOT disease!

+HPV test

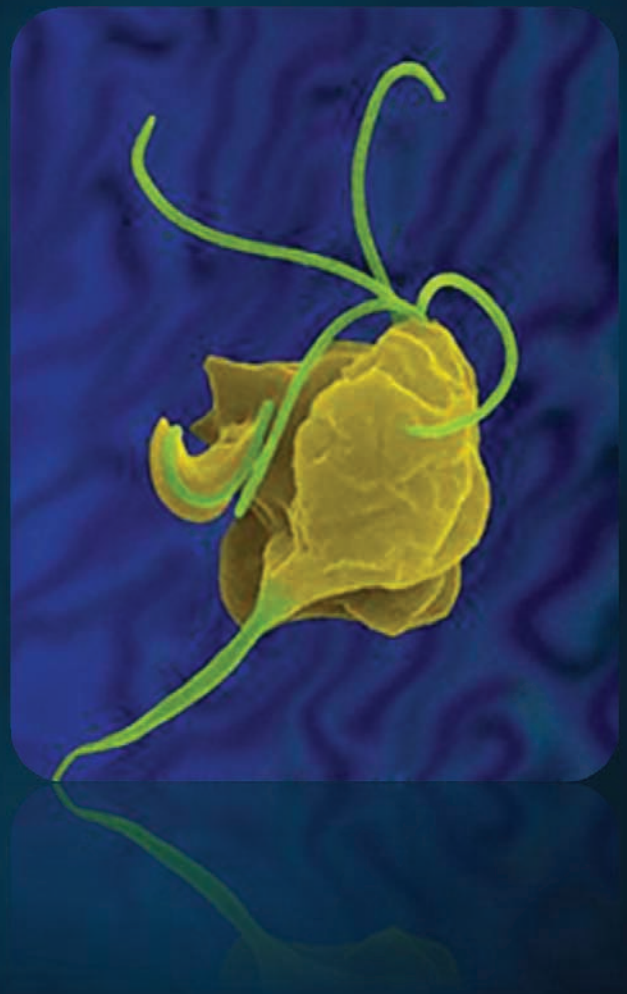
+cytology

LATENT infection

PRODUCTIVE infection (LSIL)

TRANSFORMING infection (HSIL)

Trichomonas vaginalis

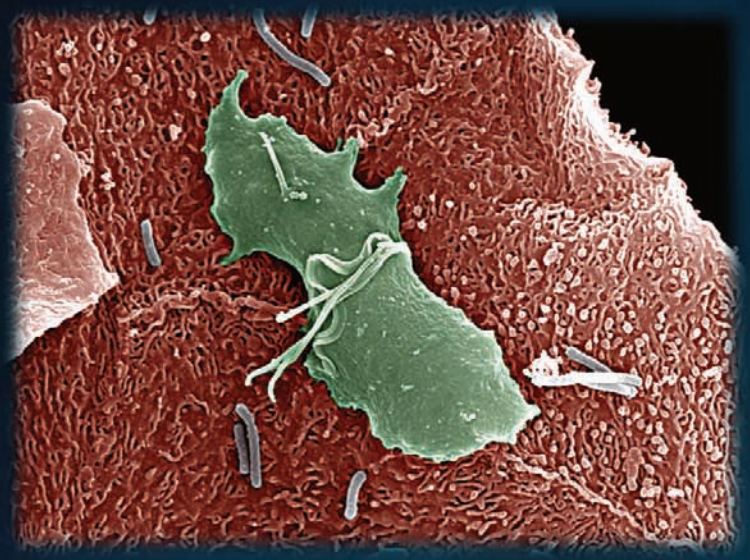


Bacterial Vaginosis (40%-50%)

Trichomoniasis (15%-20%)

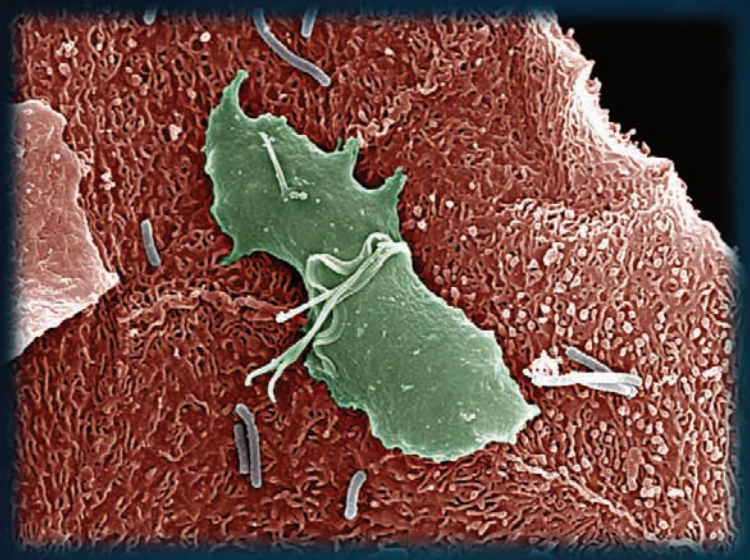
Candidiasis (20%-25%)

Mylonas I et al. Obstet Gynecol Surv. Jun;66(6):359, 2011



Trichomoniasis is the most common non-viral sexually transmitted pathogen

Fichorova RN. J Immunol Reprod. Dec;83(1-2):185, 2009



The WHO has estimated that more than 160 million people worldwide are annually infected

Harp DF, Chowdhury I. Eur J Obstet Gynecol Reprod Biol. Jul;157(1):3, 2011

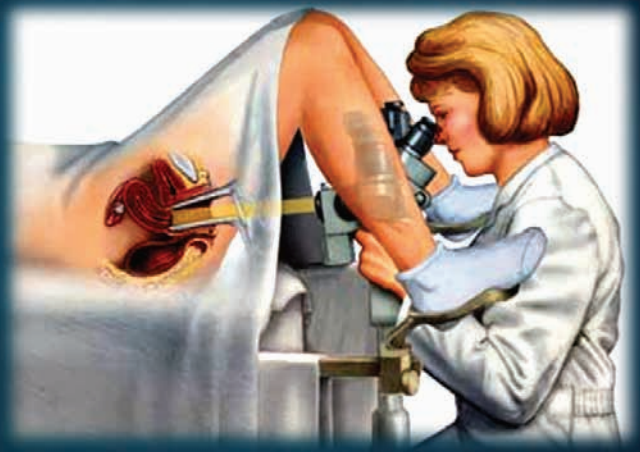
TV has been associated with other STDs such as HIV, and may also be a cause of PID

Lo BM et al. J Emerg Med. Dec;43(6):964, 2012

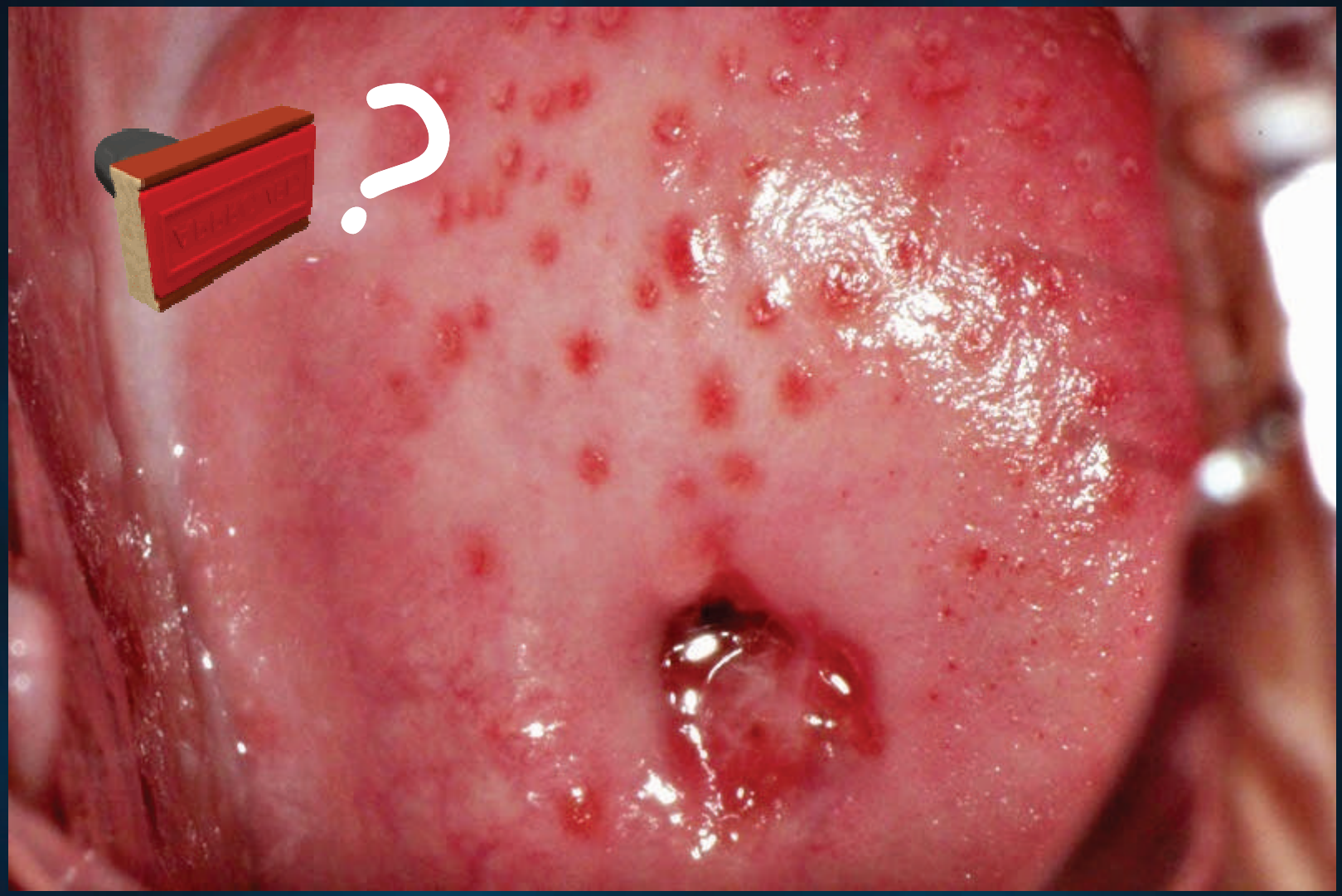
TRICHOMONAS v.

colposcopy:

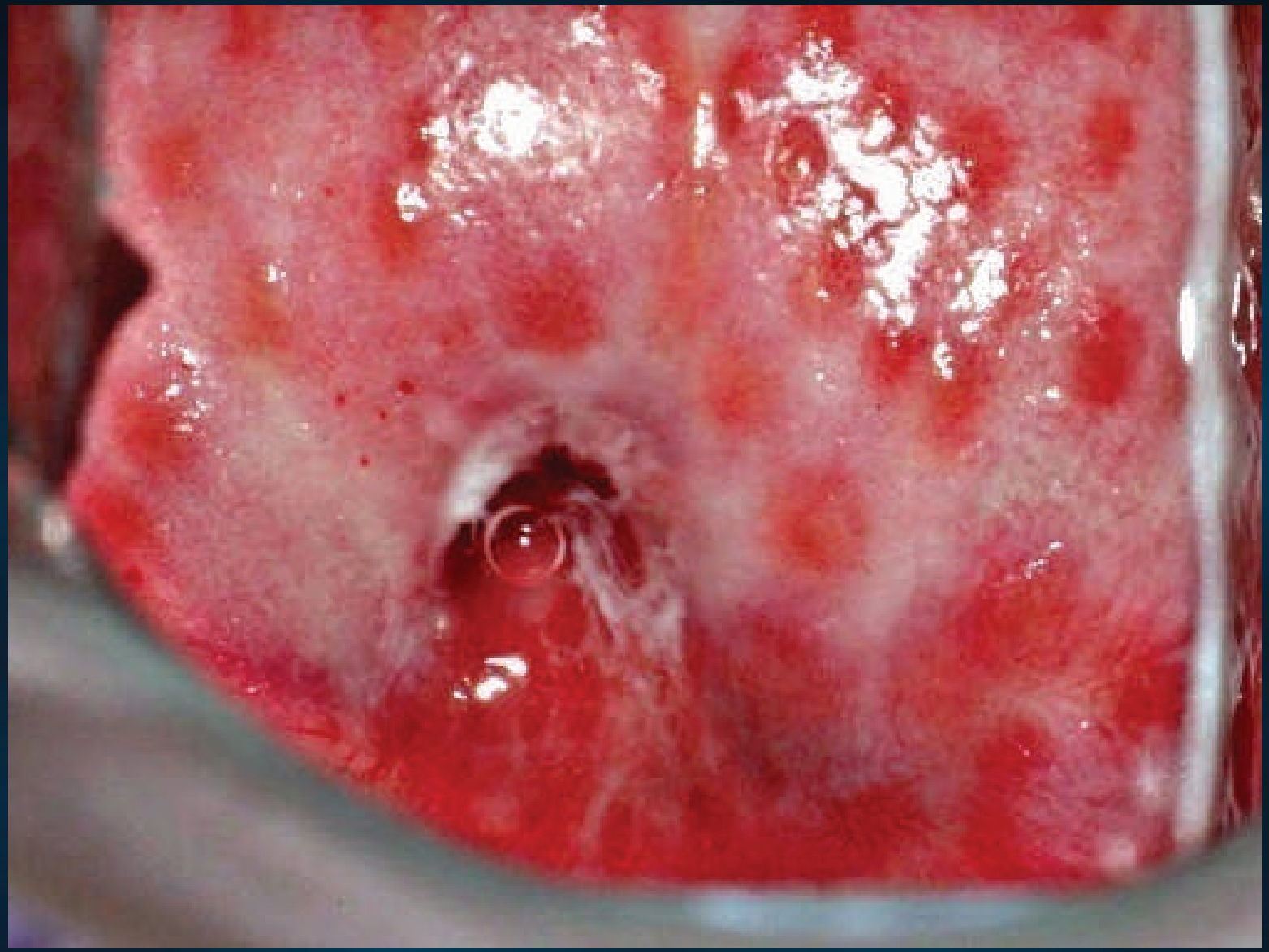
- subepithelial punctate **petechiae**



strawberry appearance



Trichomonas strawberry appearance



fungal erythematous maculae

Currently, wet prep is a quick and easy test that can be done in real time and is commonly used to diagnose TV

Lo BM et al. J Emerg Med. Dec;43(6):964, 2012

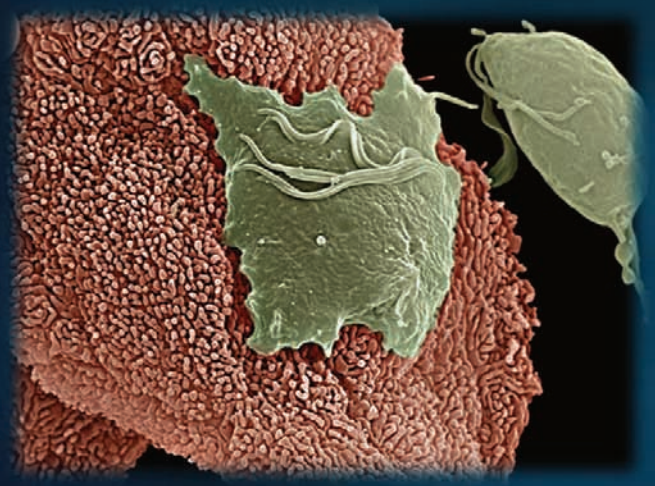
TRICHOMONAS vaginalis

anterior flagella: **motion**

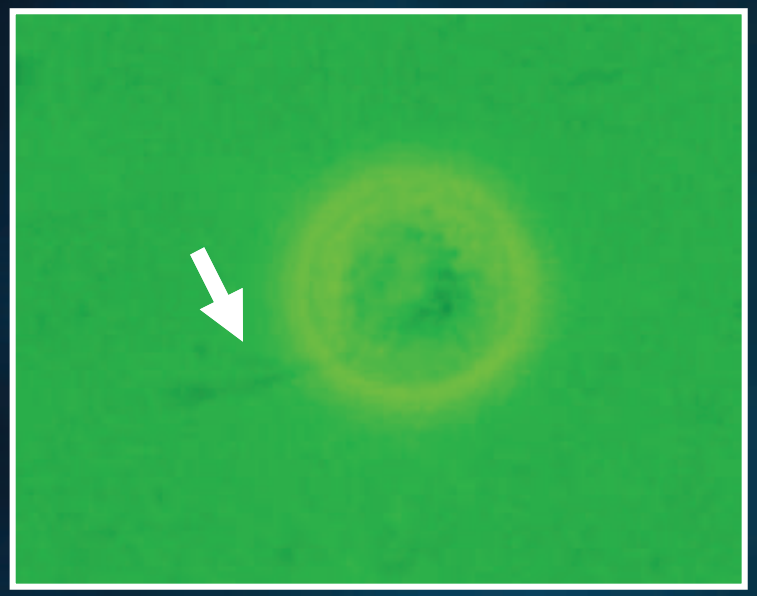
undulating membrane: **motion**

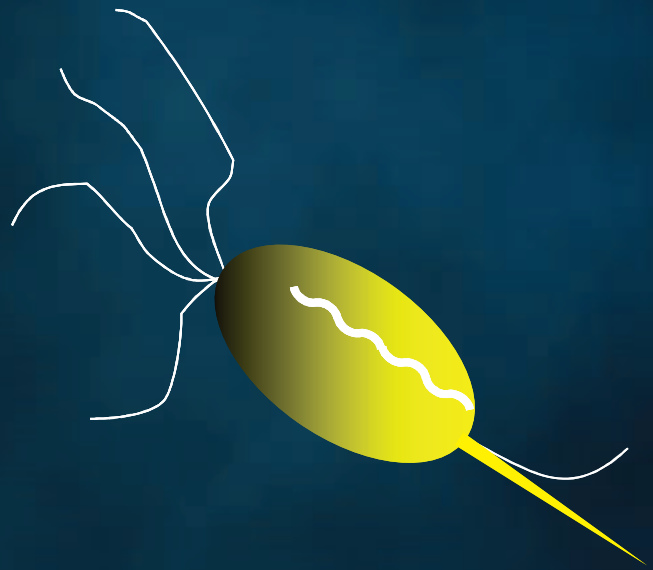
posterior flagellum: **?**

axostyle: **cytadherence** and **tissue damage**



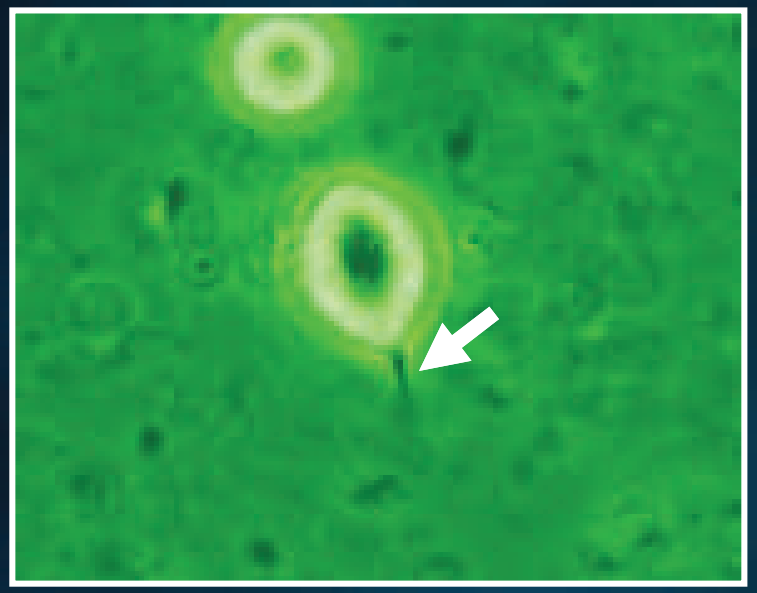
TRICHOMONAS vaginalis

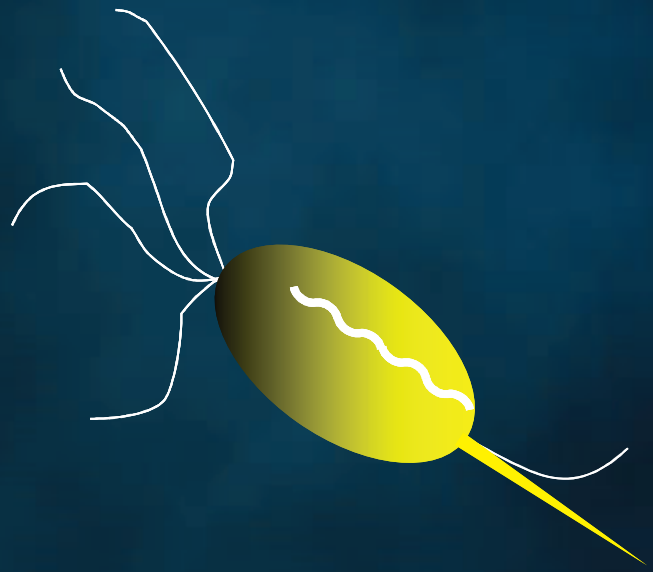




anterior **flagella**

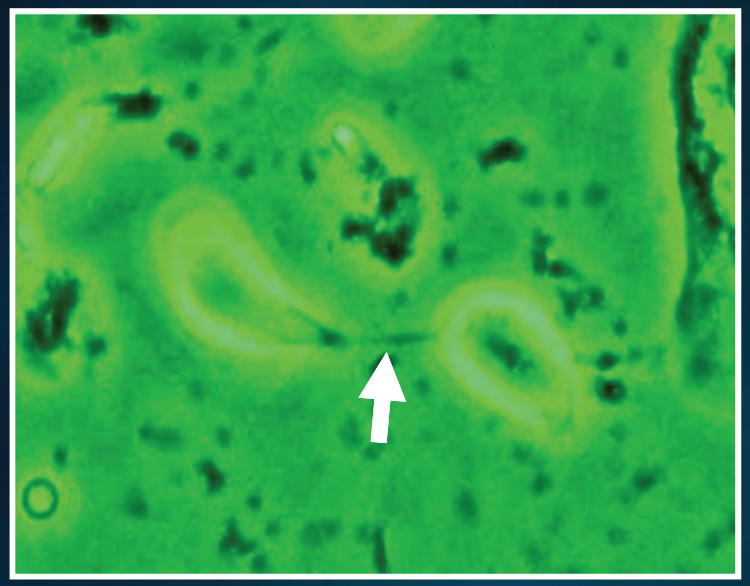
TRICHOMONAS vaginalis

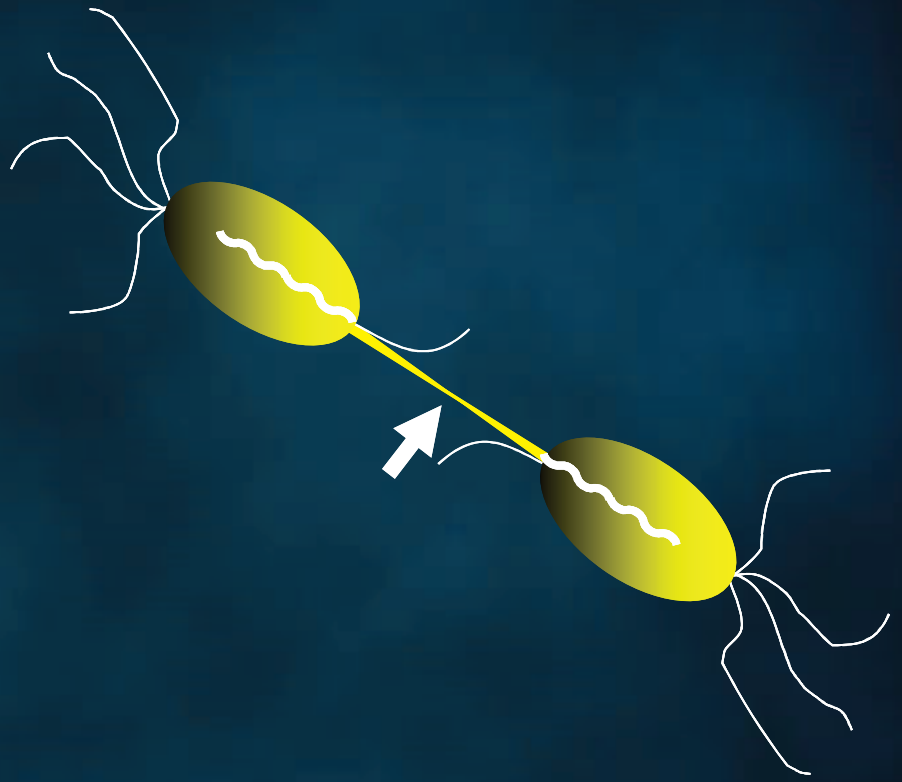




axostyle

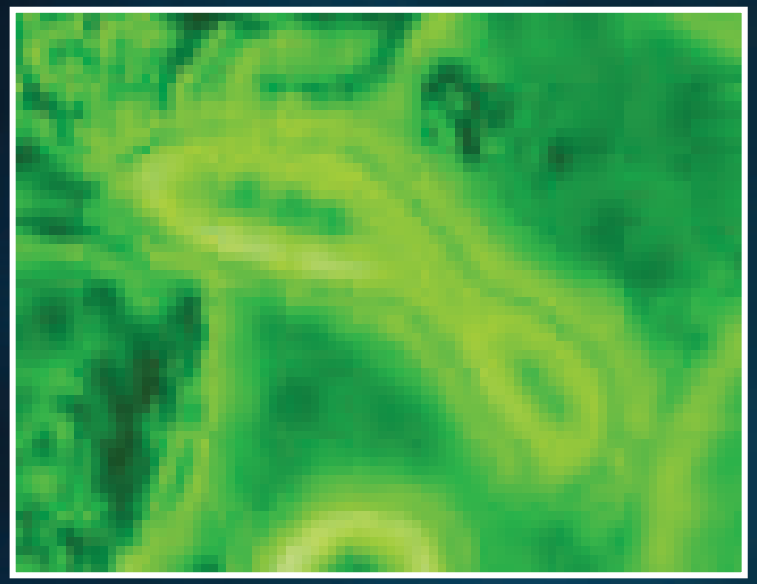
TRICHOMONAS vaginalis

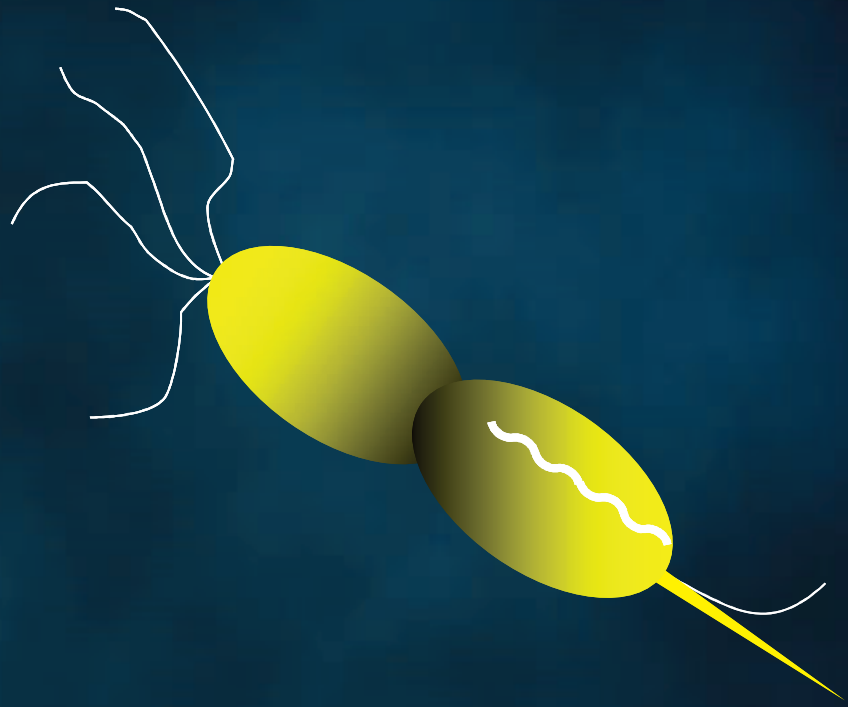




connecting axostyles

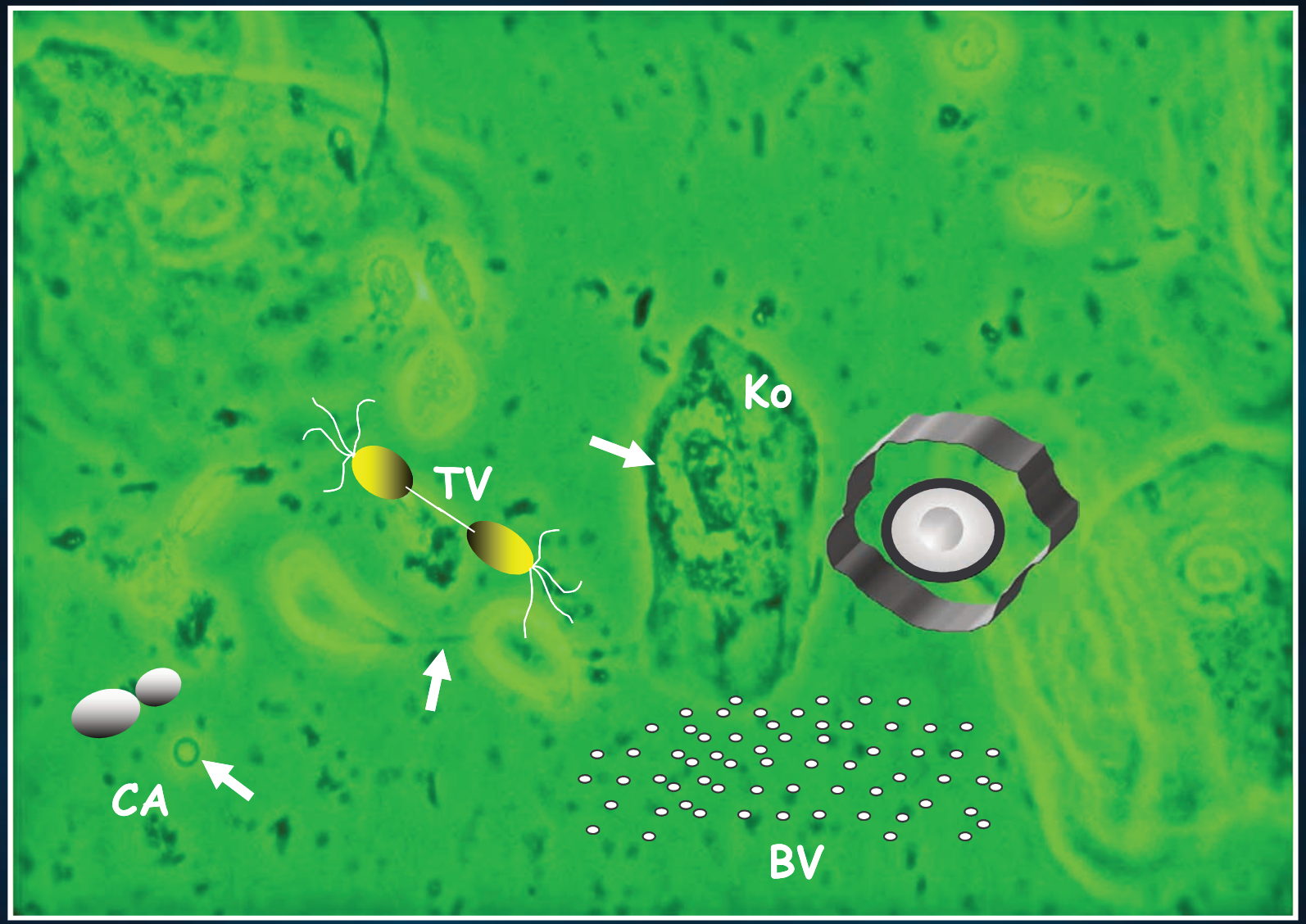
TRICHOMONAS vaginalis

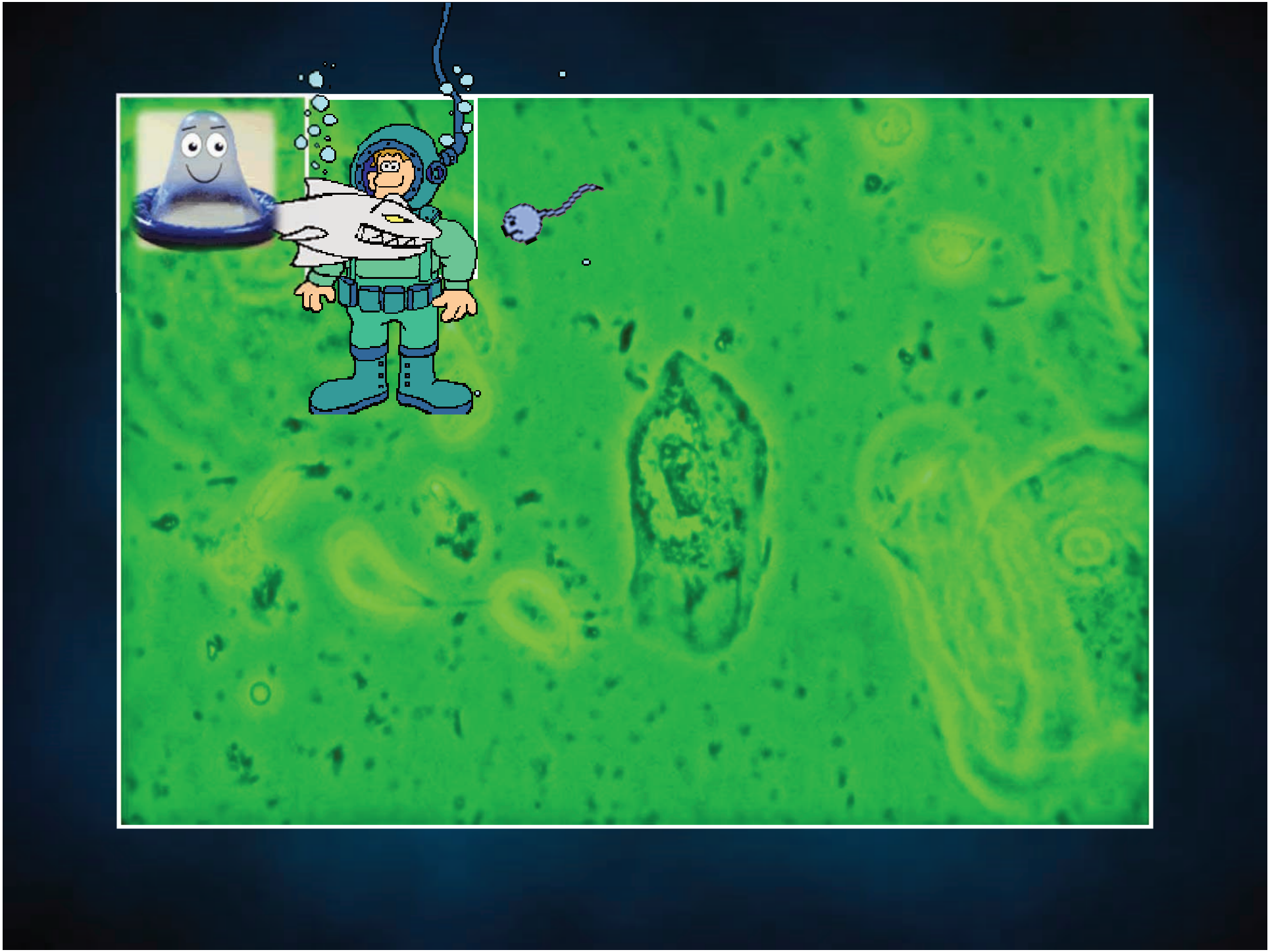




duplication

strawberry appearance


Ko
TV
CA
BV



U.S. Department of Health and Human Ser
Centers for Disease Control and Prevention

Sexually Transmitted Diseases Treatment Guidelines, 2015

Prepared by
Kimberly A. Workowski, MD[1,2]
Gail A. Bolan, MD[1]
[1]*Division of STD Prevention*
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
[2]*Emory University, Atlanta, Georgia*

If infection is suspected as the primary cause, a sample of the vaginal discharge should be taken and examined microscopically

Mylonas I et al. Obstet Gynecol Surv. Jun;66(6):359, 2011

Physicians should assess the clinical practicality and usefulness of wet mount microscopy and use wet mount microscopy to diagnose common vaginal infections

Mylonas I et al. Obstet Gynecol Surv. Jun;66(6):359, 2011

Wonderful Atlas. I have made reference to it many times in my lectures

Albert Singer

Whittington Hospital, London

The quality of the images is excellent and accompanying explanatory text illuminating

Charles Redman

President European Federation of Colposcopy

This text will be a reference work for Gynecologists for many years to come

Walter Prendiville

Past President International Federation of Colposcopy and Cervical Pathology

The Atlas is superb. This publication has a great historic value, as a gift for future generations

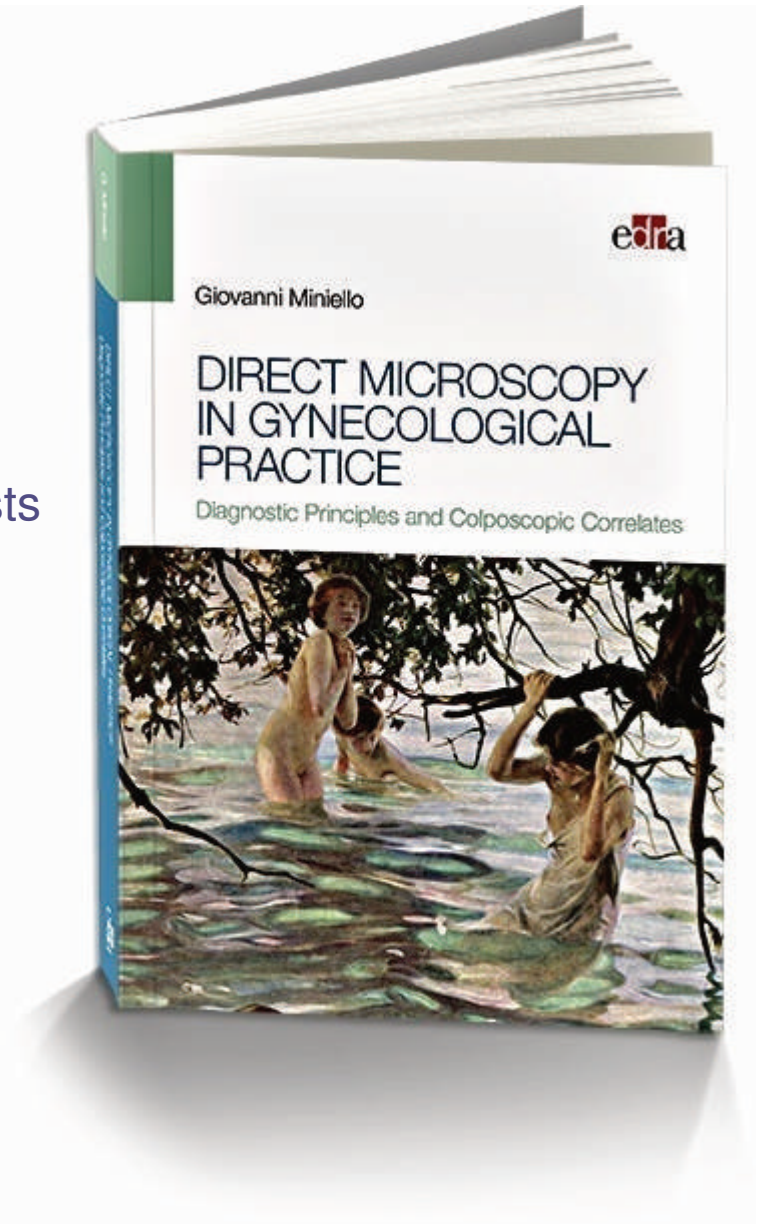
Usha Saraiya

Founder Member and President Indian Academy of Cytologists

This Atlas, beautifully illustrated, is a ‘master piece’ of work

Sabaratnam Arulkumaran

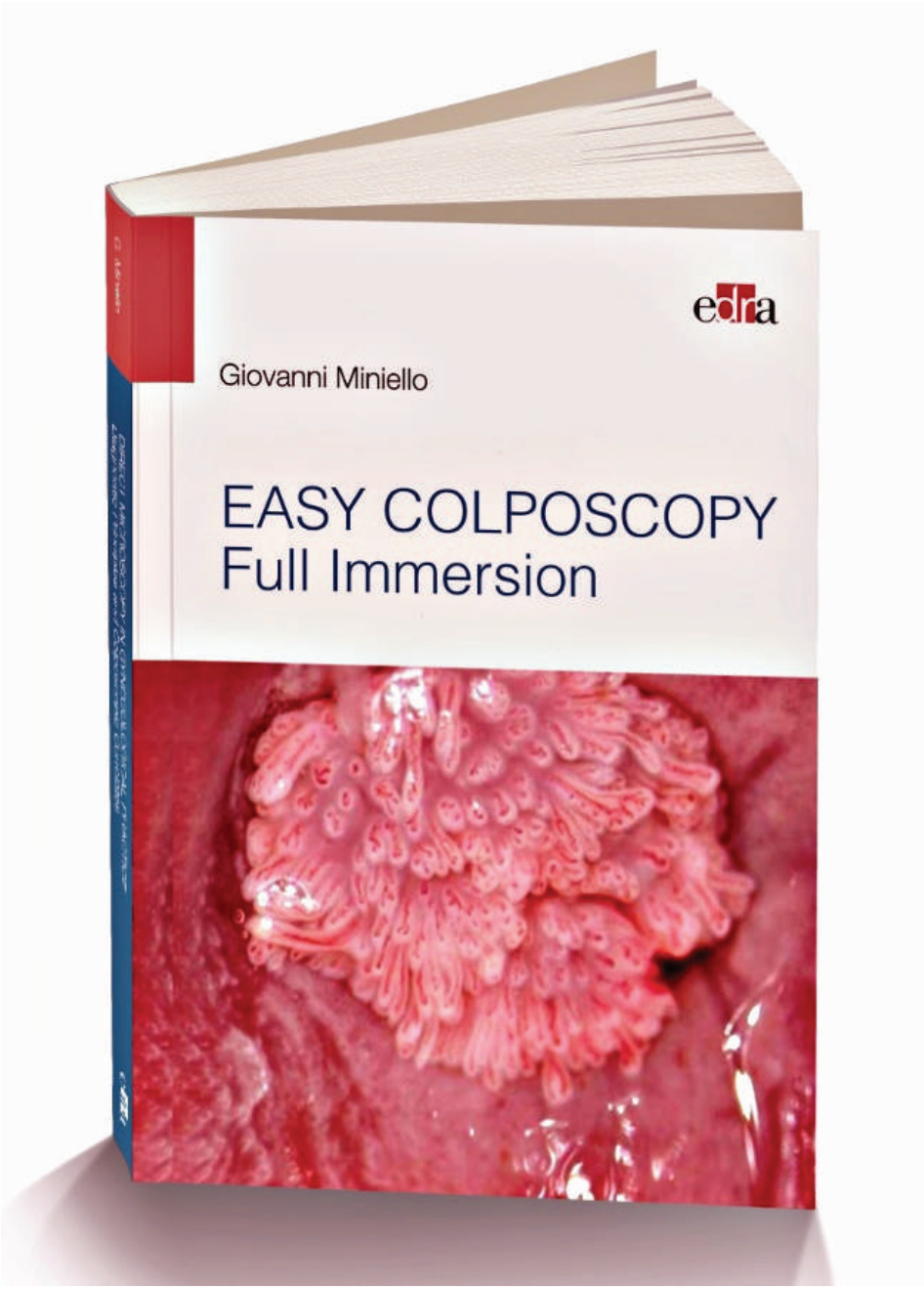
Past President FIGO



I had not seen such high standard of colposcopy photographs

Albert Singer

Whittington Hospital, London




GOOD JOB