How to identify pathogens in cytology specimens: Often times, only one specimen is available

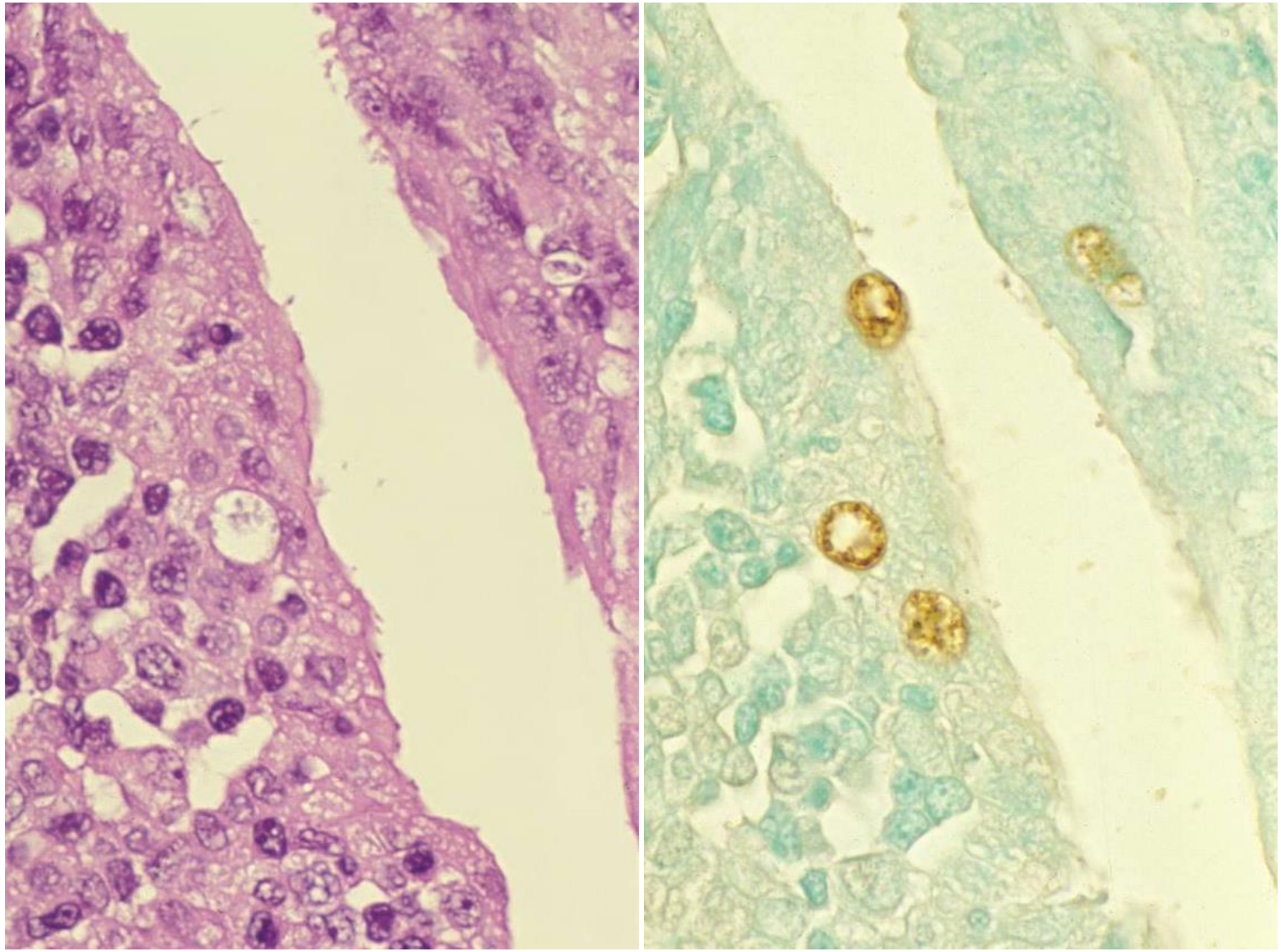
Several methods can be applied to demonstrate pathogens in cytology preparations. The following items are presented. 1) Cell block preparations, 2) restaining method, 3) cell transfer technique (transfer of smears to another glass slide), and 4) applications of the cell transfer technique (repair of broken slides and evaluation with PCR).

Ref.: Tsutsumi Y. Cytological diagnosis of infectious diseases: identification of pathogens and recognition of cellular reactions. IntechOpen 2020. In: Innate Immunity in Health and Disease (eds: Saxena SK, Prakash H). doi: 10.5772/intechopen.95578

How to identify pathogens in single cytology preparations

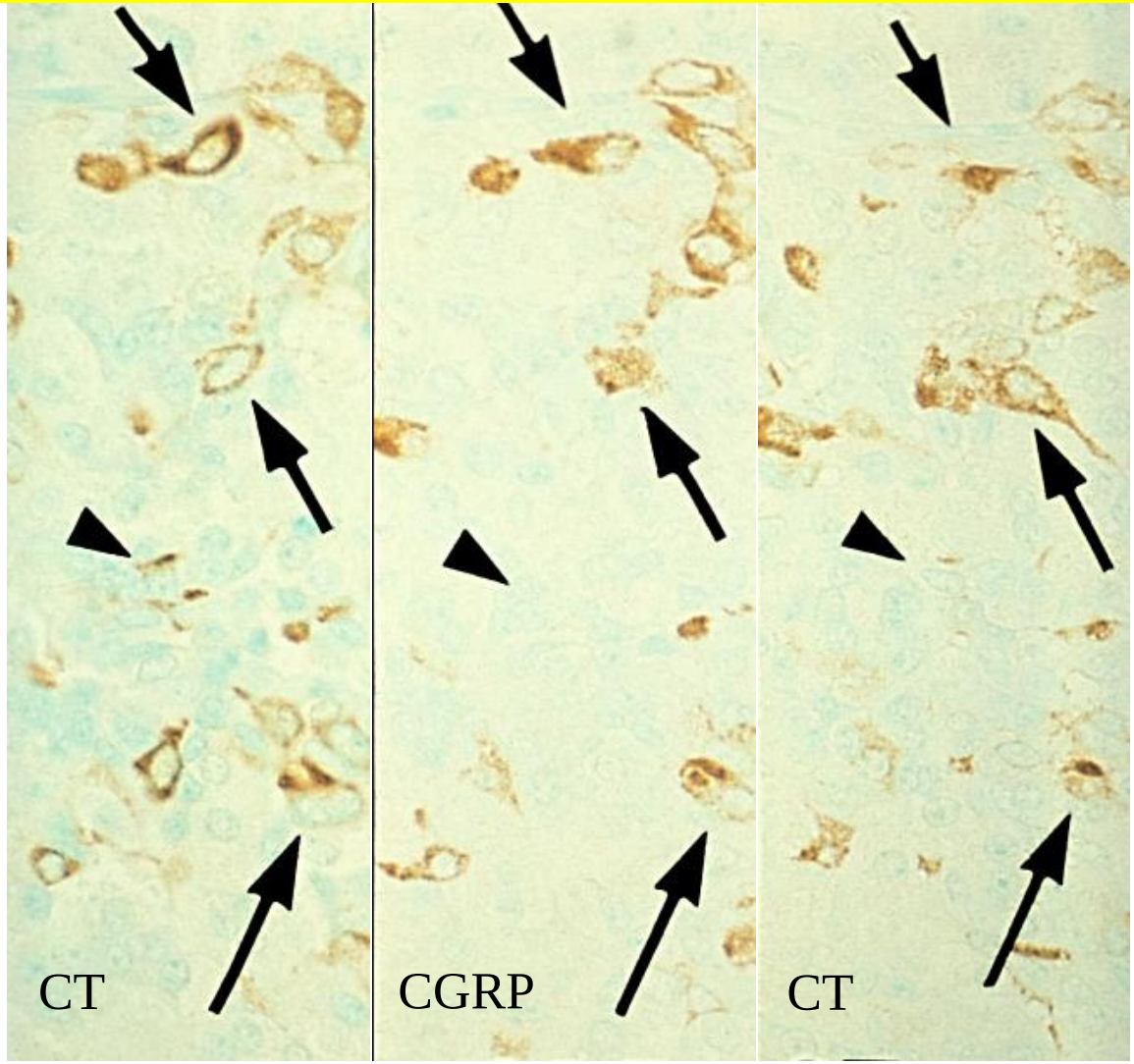
1) Use of cell block preparations
2) Restaining method
3) Cell transfer technique
   (transfer of smears to another glass slide)
4) Applications of the cell transfer technique
   a) Repair of broken slides
   b) Evaluation with PCR

To identify the specific antigen in the target cells in histological sections-1



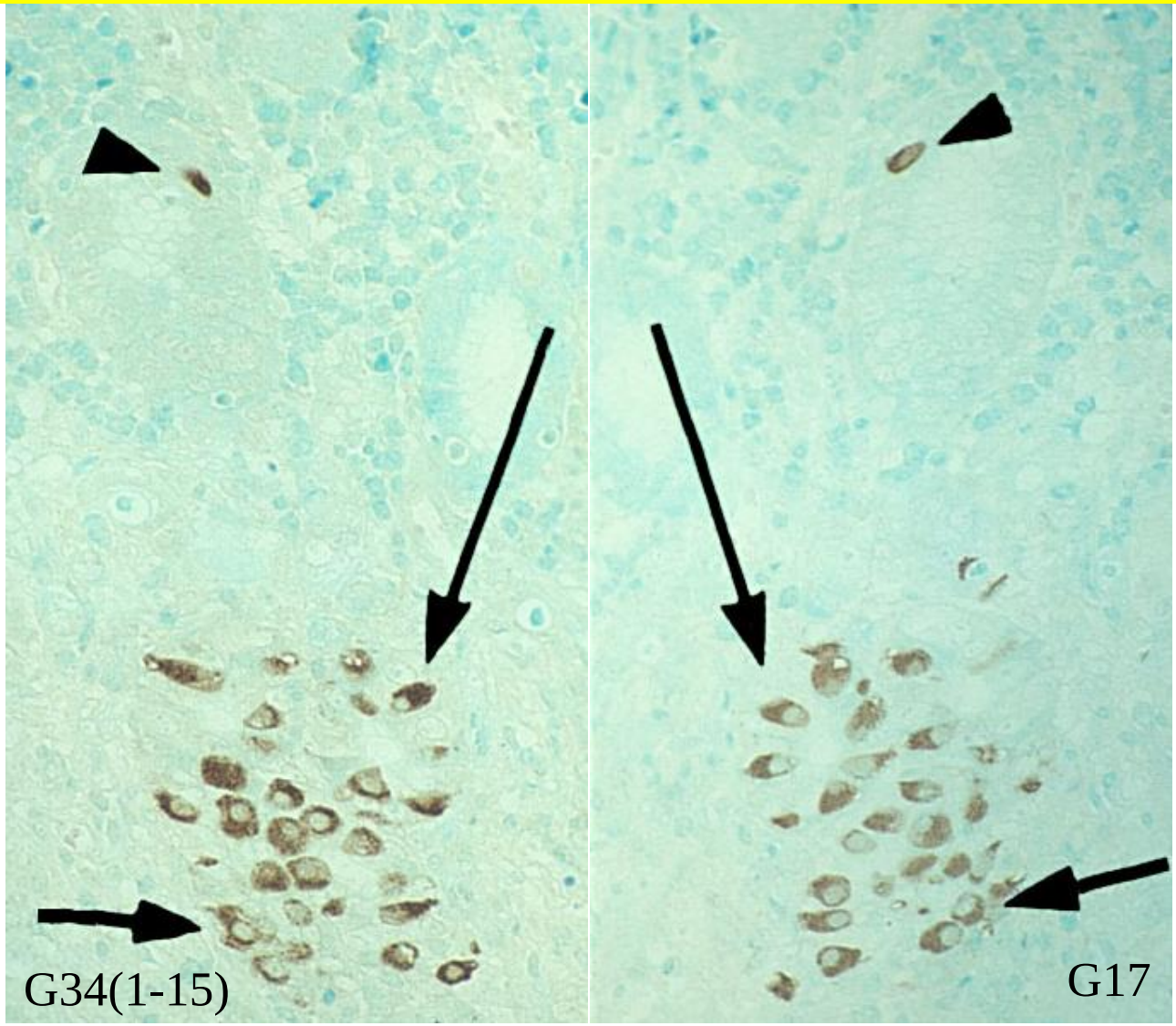
Serial section analysis to demonstrate Chlamydia trachomatis antigen in chlamydial epididymitis. Left: H&E, right: CT antigen

To identify the specific antigen in the target cells in histological sections-2



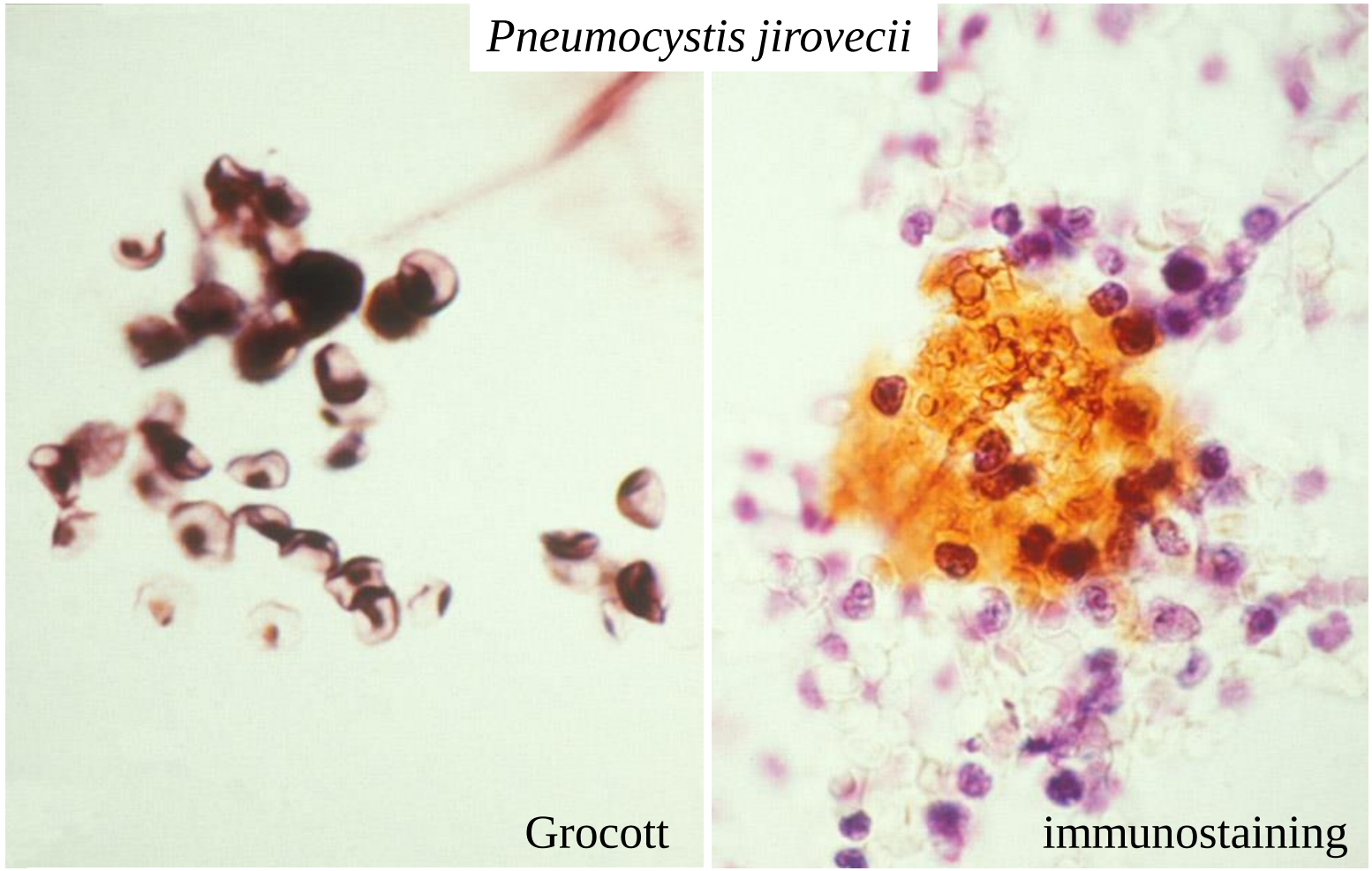
Three serial sections immunostained for calcitonin (CT) and calcitonin gene-related peptide (CGRP) in bronchial carcinoid tumor. The same cells contain both CT and CGRP (arrows). Some cells lack CGRP expression (arrowhead).

To identify the specific antigen in the target cells in histological sections-3

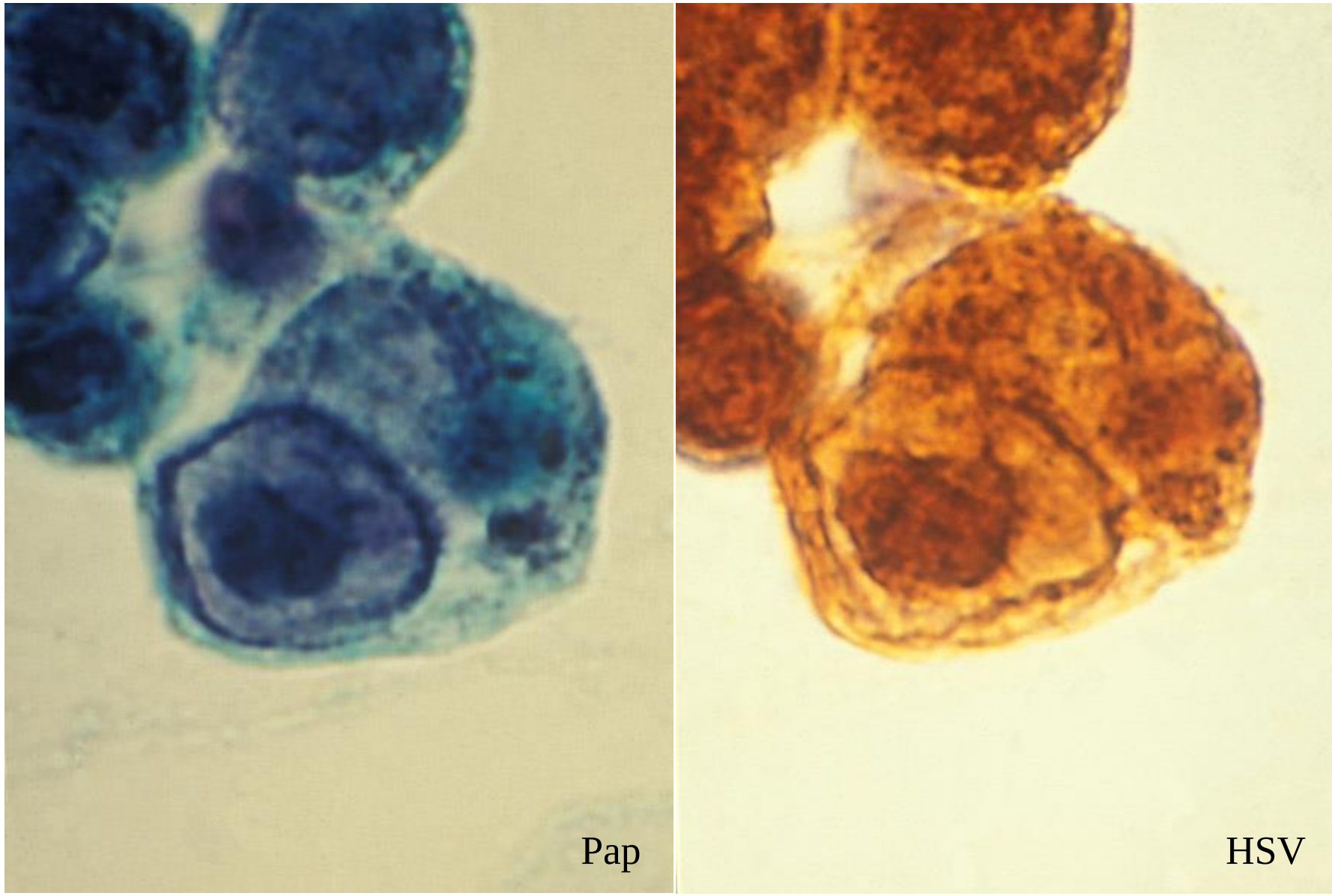


Mirror sections for demonstrating big gastrin G34(1-15) and gastrin (G17) in the sama G-cells in the pyloric gland of the gastric antral mucosa.

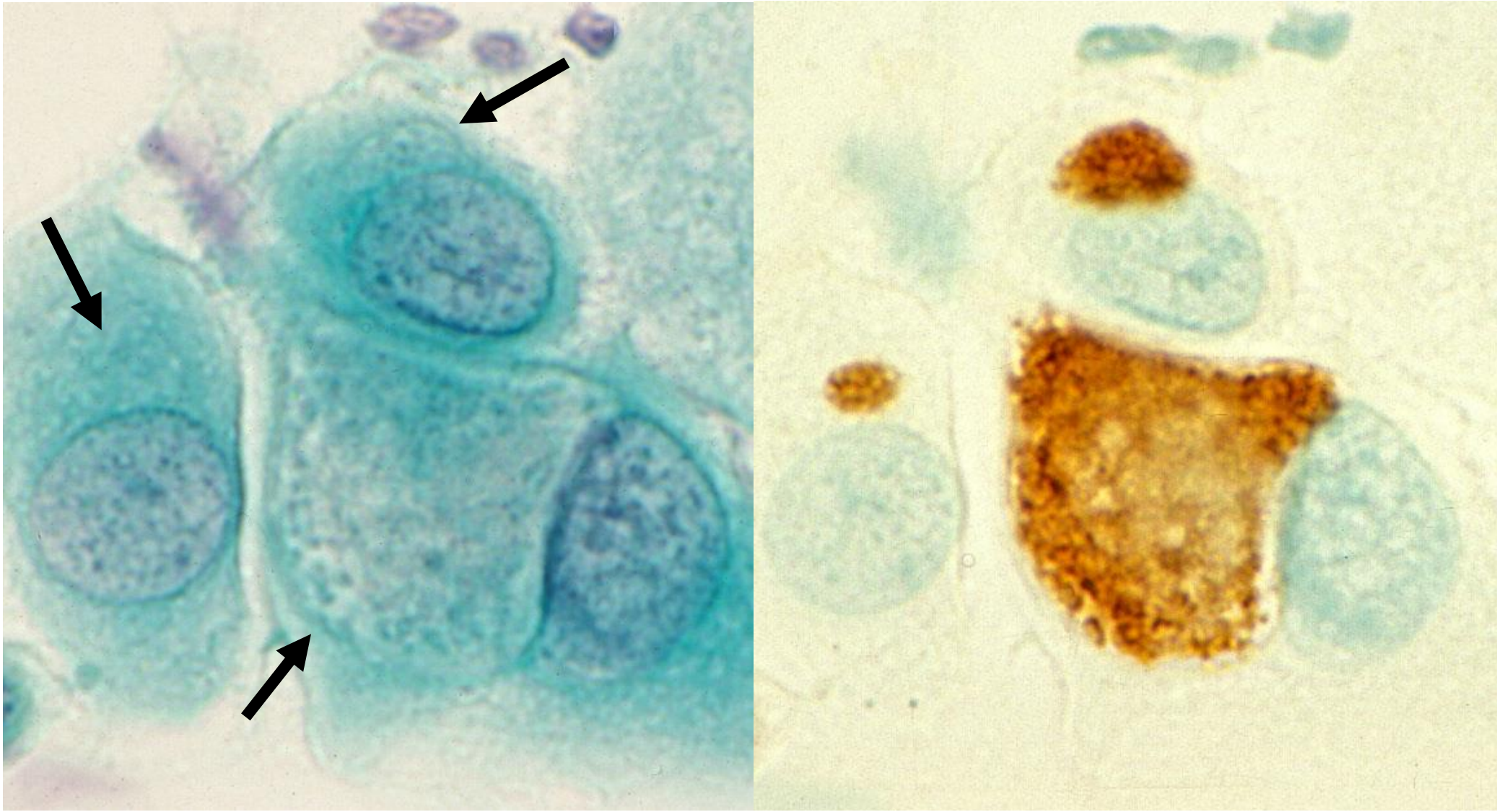
When plural cytology specimens can be prepared:



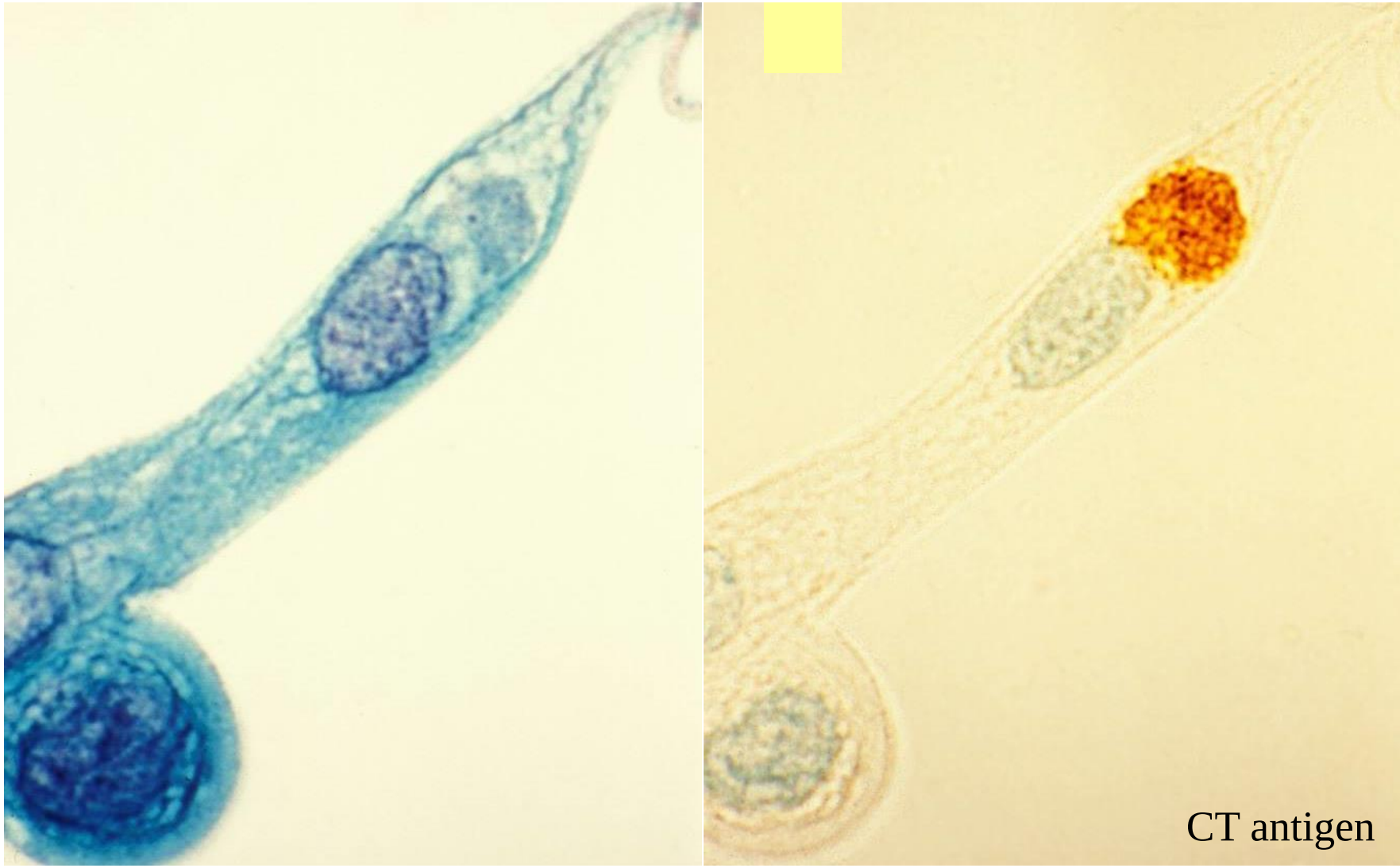
Pneumocystis jirovecii pneumonia. Grocott staining in broncho-alveolar lavage (BAL) material (left). Dot-like structure is observed in the cysts. In an additional cytology material, immunostaining for *P. jirovecii* using a monoclonal antibody can be performed (right). Note that multiple specimens can be obtained in liquid-based cytology preparations.



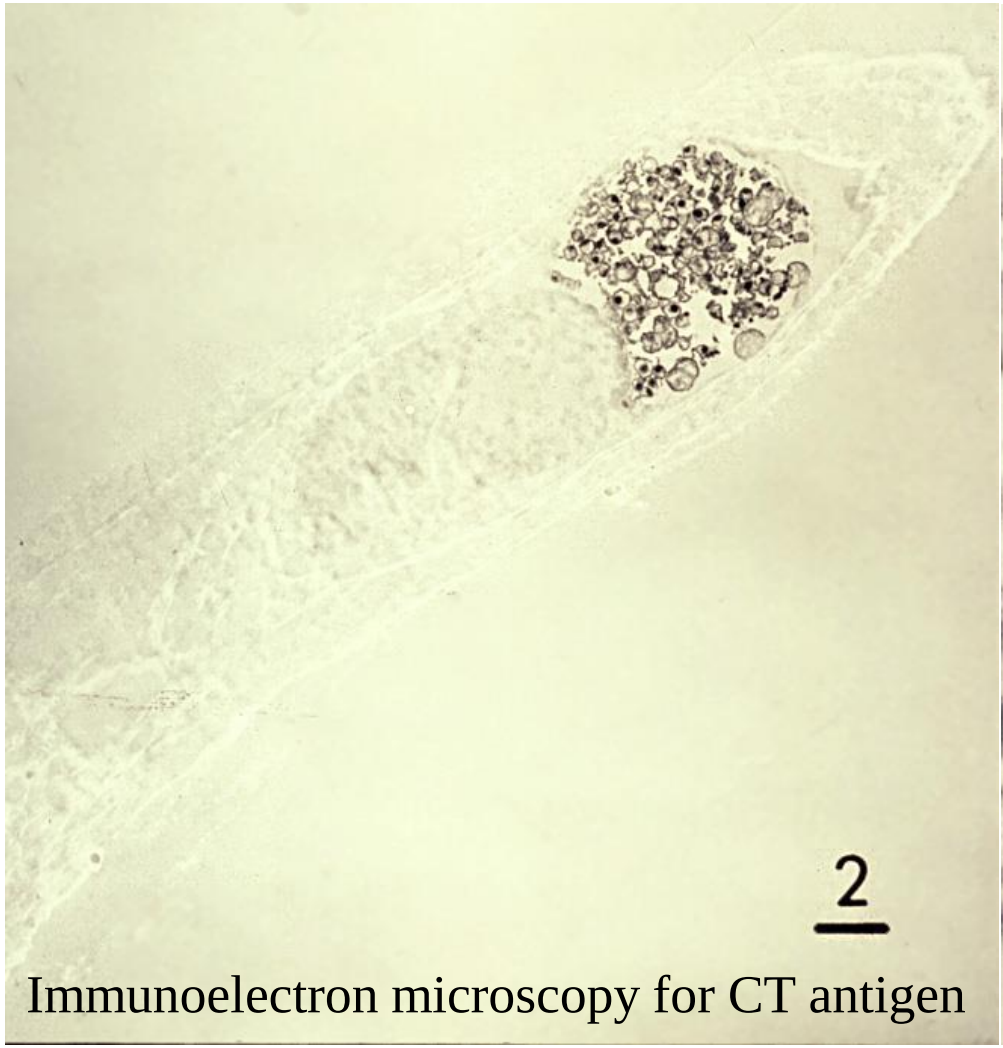
Re-staining method: herpes simplex viral antigen was restained using a single slide of the cervical smear. The HSV antigen is localized both in the nuclei and cytoplasm.

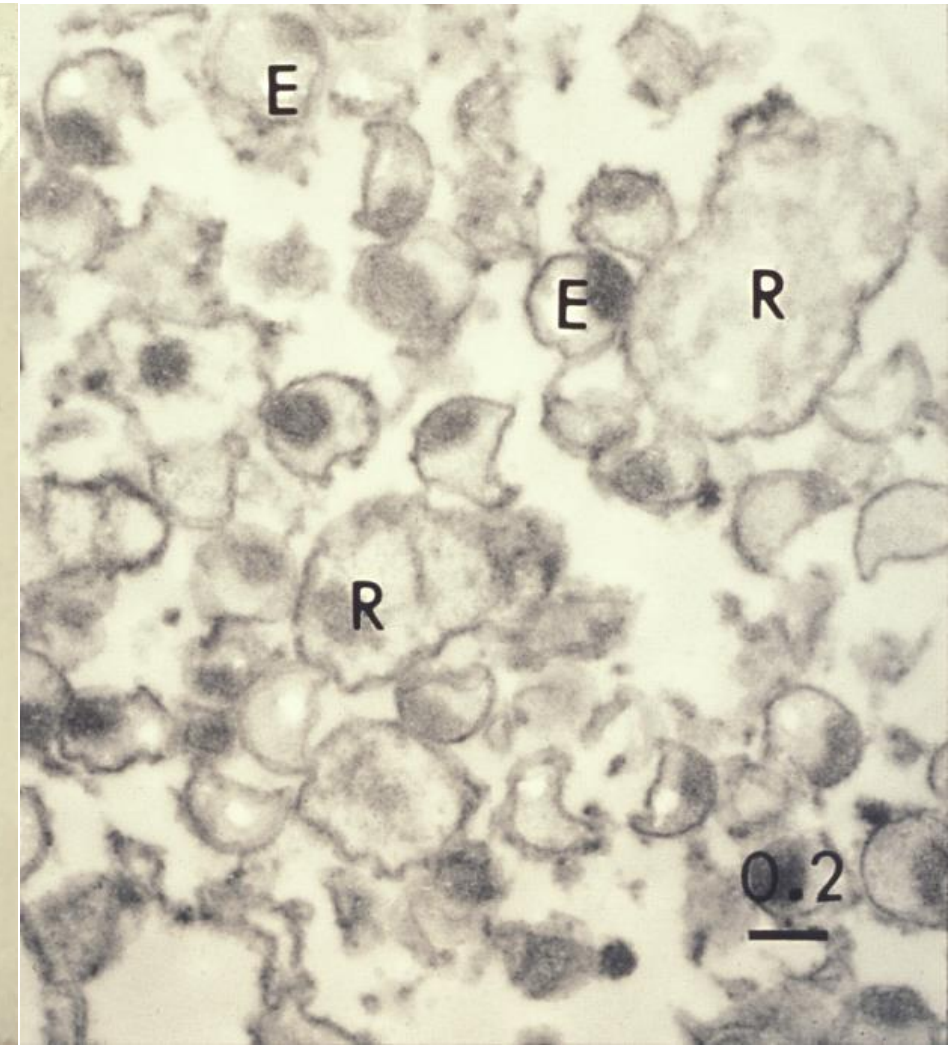


Restaining method. Demonstration of *Chlamydia trachomatis* antigen in the nebular inclusion bodies in the cervical smear preparation. A 20 y-o female patient complained of increased fluor. Pap smear reveals nebular inclusion bodies (arrows, left). The restaining method demonstrates chlamydial antigen in the inclusions (right).

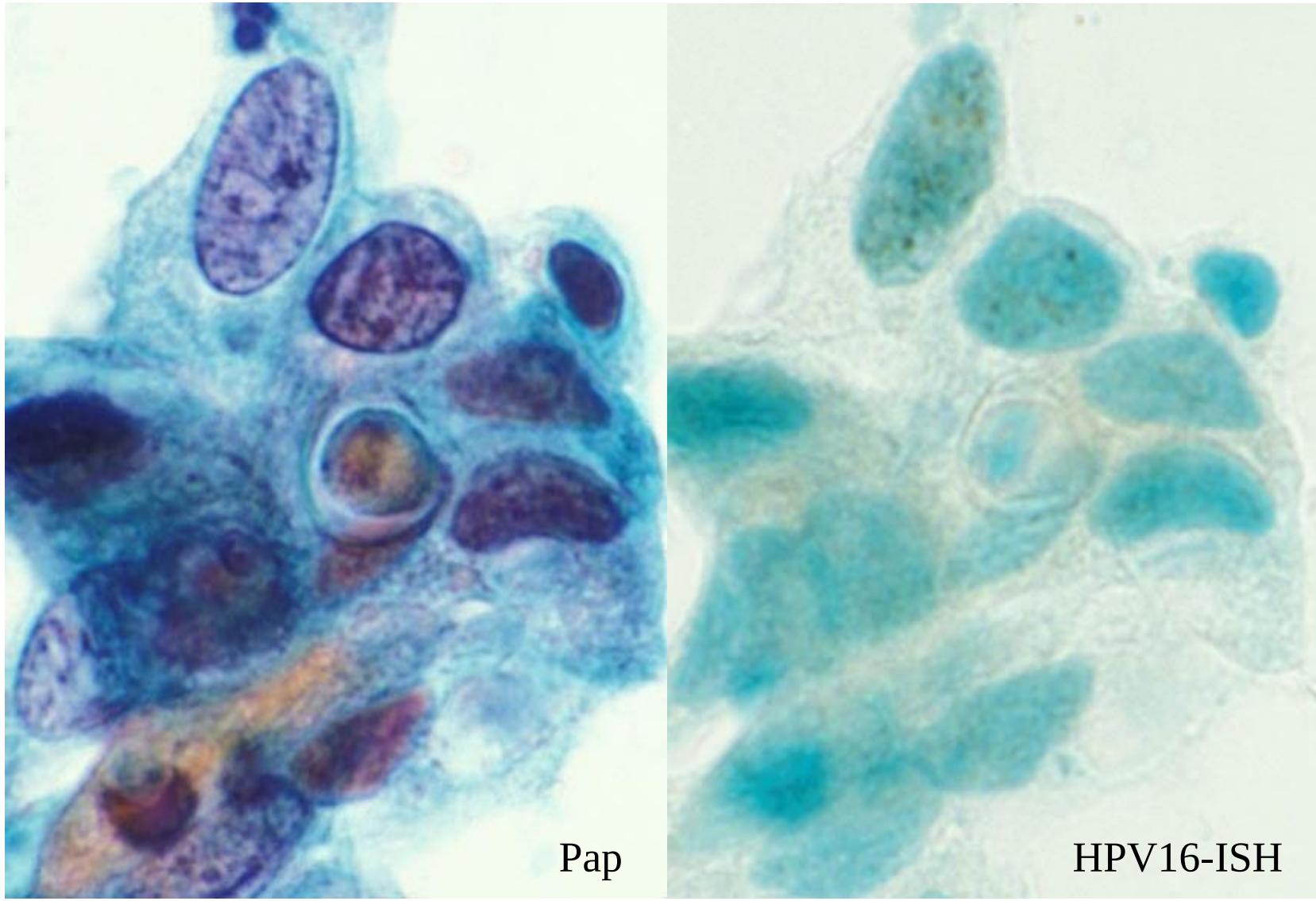


Restaining method: *Chlamydia trachomatis* antigen is visualized in the nevular inclusion body in the cytoplasm of a metaplastic cell seen in the cervical smear.

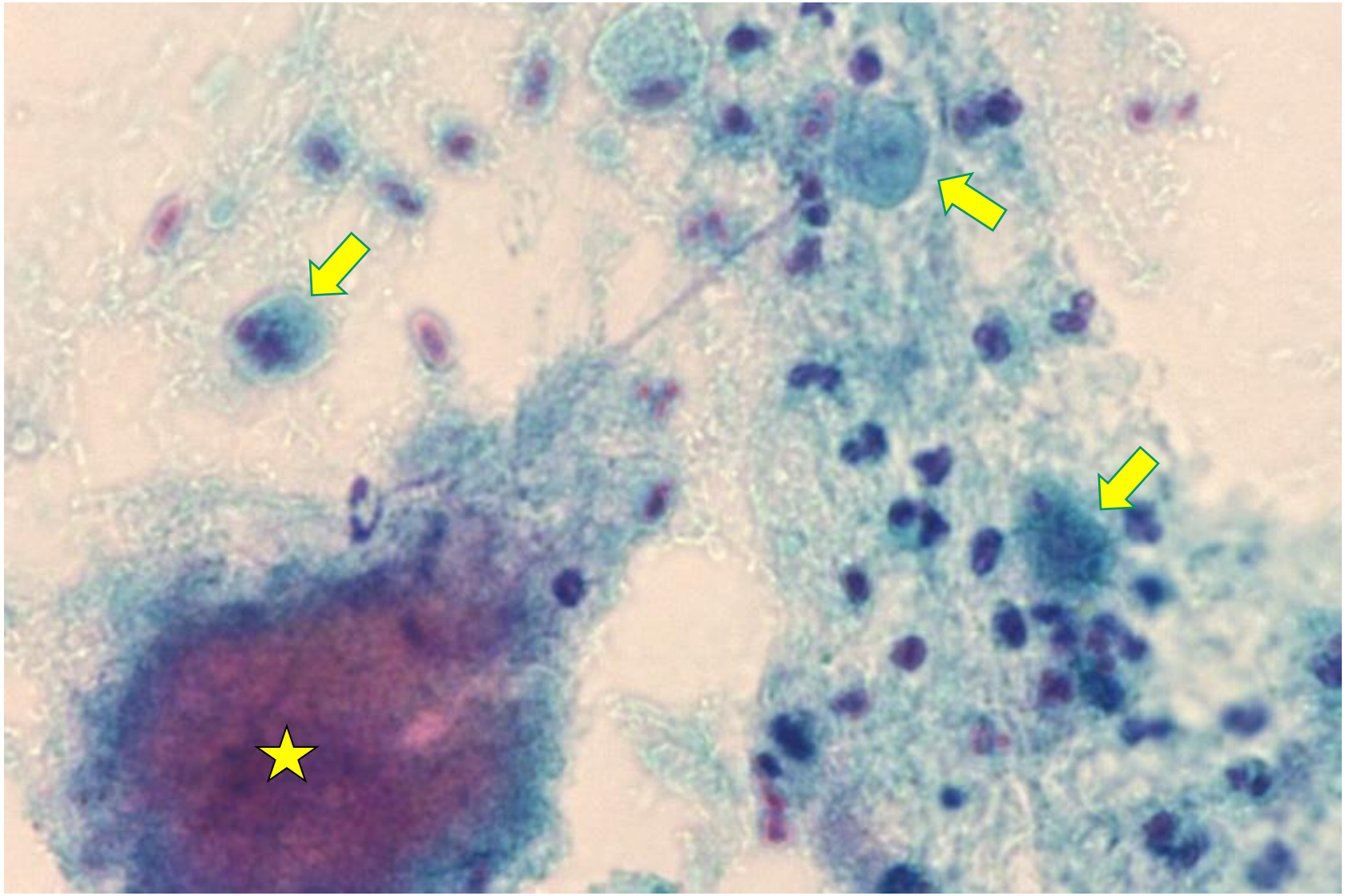




Immunoelectron microscopy, pre-embedding method, using an ethanol-fixed cervical smear of chlamydial cervicitis (see also the previous slide). The cell membrane of *Chlamydia trachomatis* is clearly labeled even after routine alcohol fixation. E : elementary bodies (infectious form), R: reticulate bodies (proliferative form). **Ref.**: Hori S, et al. Immunoelectron microscopic detection of chlamydial antigens in papanicolaou-stained routine vaginal smears. Acta Cytol 1995; 39(4): 835-837. PMID: 7631568

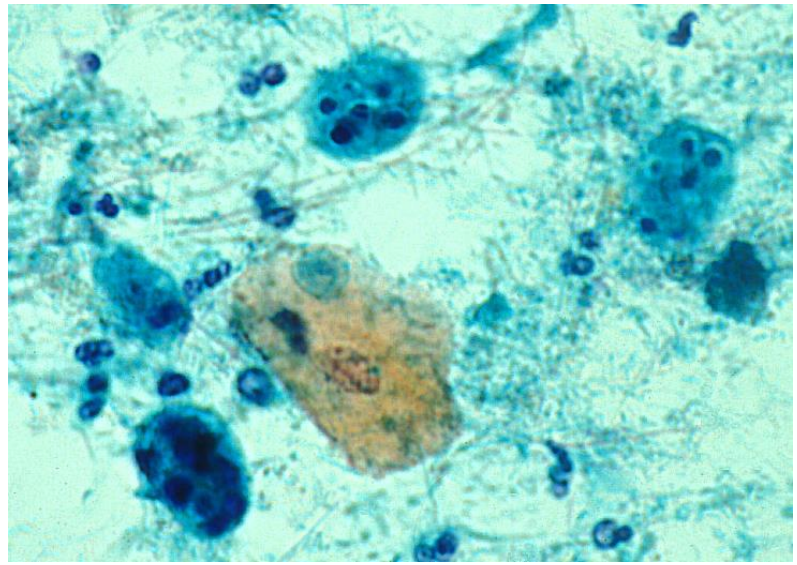


Re-staining method after cell transfer to silane-coated glass slide. Dot-like intranuclear signals of HPV16 genome are observed in the same cells of severe dysplasia of the cervix. The cell transfer technique is indispensable for performing ISH, because a heating step for making double-stranded DNA into single-stranded is included in ISH.



A 50 y-o female patient with prolonged insertion of contraceptive device into the uterine cavity complained of abnormal fluor. The device was removed, and the cytology specimen of exudates around the device shows actinomycotic grains (asterisk) and infection of amebic trophozoites phagocytizing neutrophils (arrows)（pap）

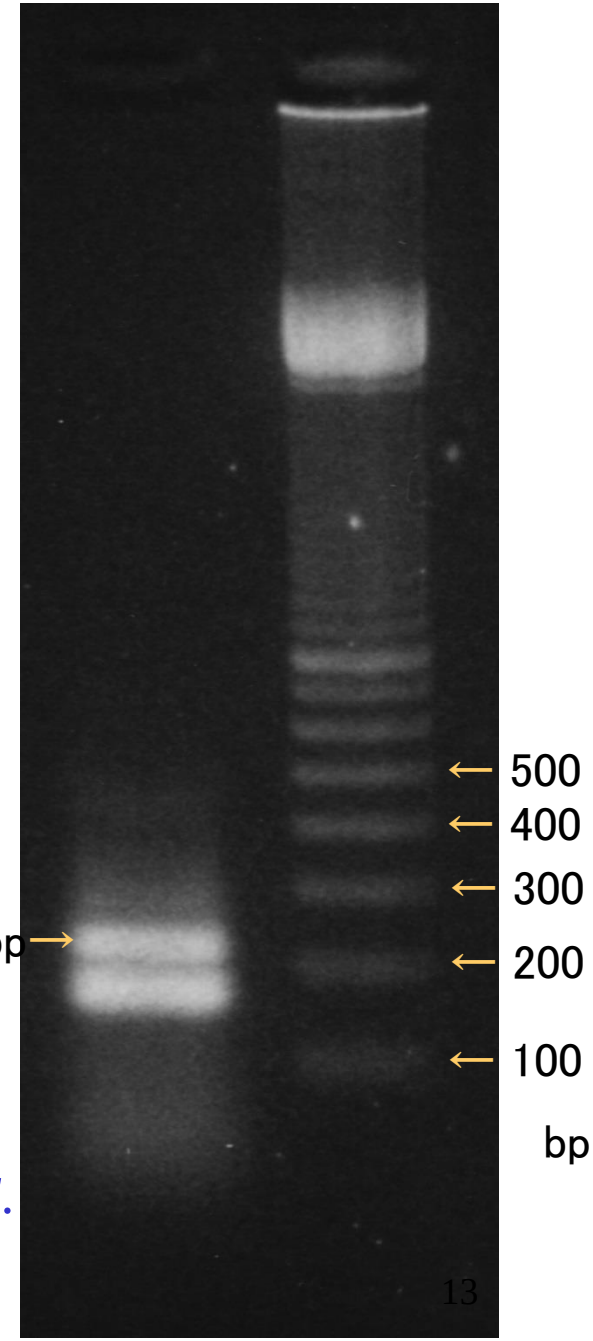
PCR for *Entamoeba* species



*Entamoeba gingival*is seen in the dental plaque, phagocytizing neutrophils

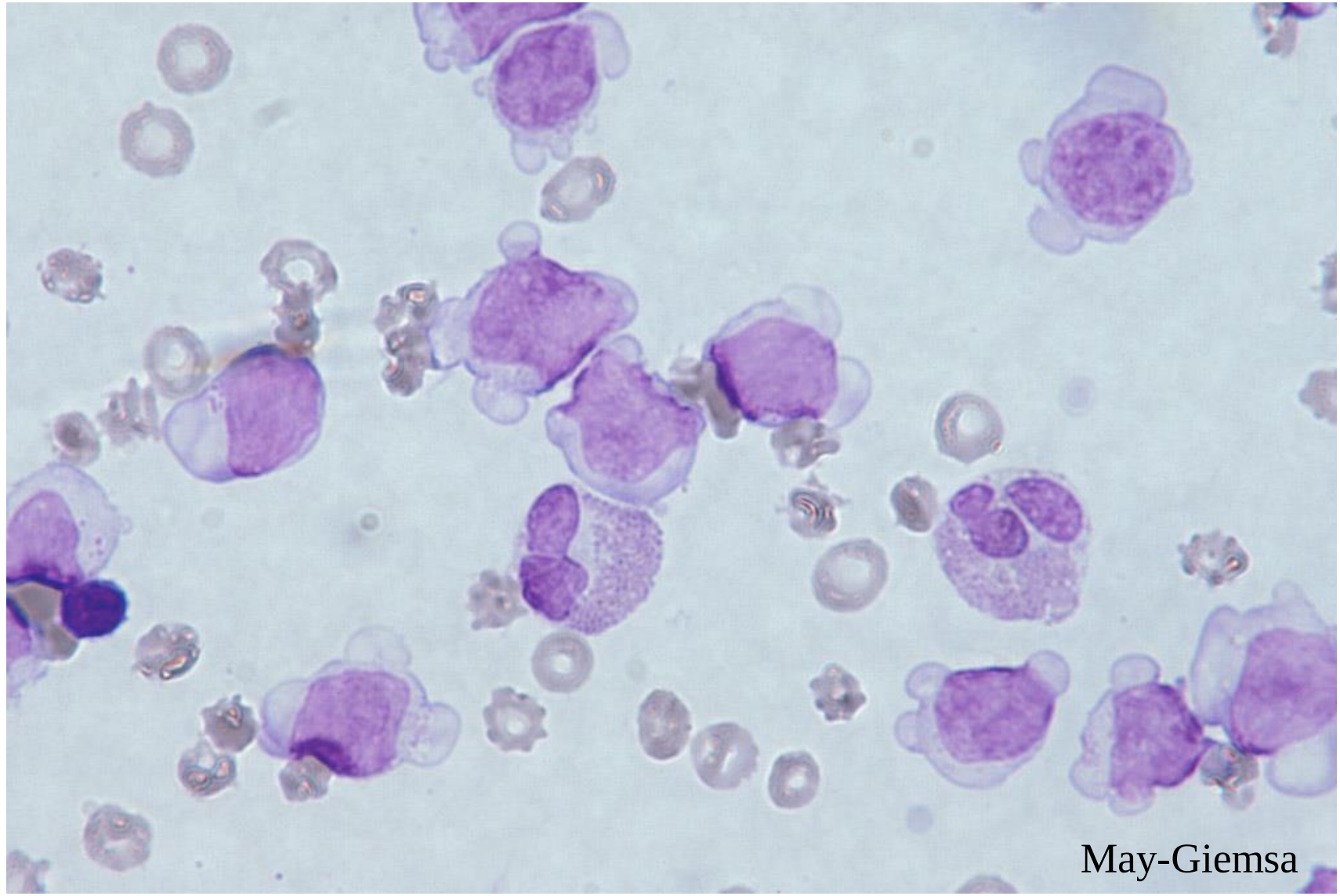
Primers sense 5'– tcagataccgtcgtagtcct – 3'
antisense 5'– cctggtgtgcccttccgt – 3'

PCR conditions: 94℃15 sec、55℃30 sec、72℃30 sec : 35 cycles

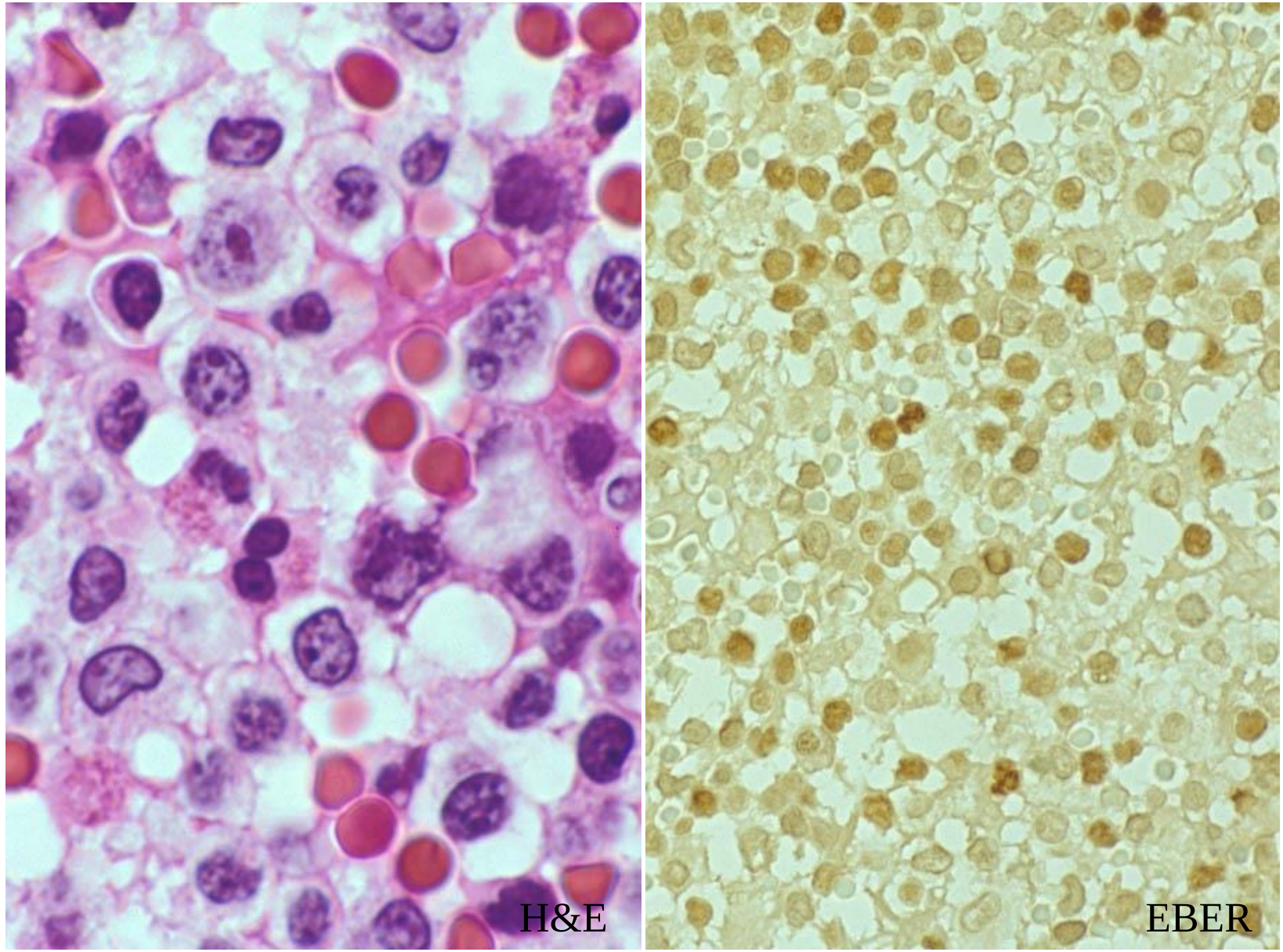


The 221bp fragment: High homology with *E. gingivalis* genome

Colonization of *E. gingivalis* in the uterine cavity was confirmed. The colonization was mediated by oral sex. Both *Actinomyces* and *E. gingivalis* are normal flora of the oral cavity. The presence of the foreign material (IUD) accelerated the sexually transmitted infection.



Large granular lymphocytes and eosinophils in hemorrhagic ascitic fluid in a young male case of chronic active EBV infection (May-Giemsa). Clinically, autoimmune disorder was suspected in the present case.



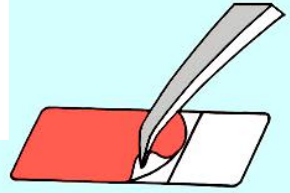
Cell block prepared from the ascites, demonstrating EBER reactivity in the nuclei. The diagnosis of chronic active EBV infection was made.

Techniques for cell transfer

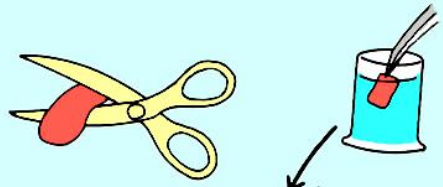
Note that this technique is not applicable to Giemsa-stained preparations or Pap-stained preparations mounted on Silane-coated glass slides.

The multistep procedures are time-consuming, but valuable for extended cytological evaluations.

Detach the cover slip in xylene

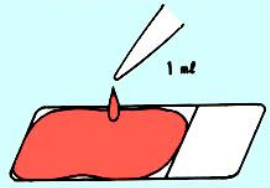


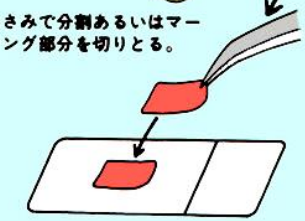
Peel off the membrane



Cut with scissor

Cover the slide with Malinol mounting solution

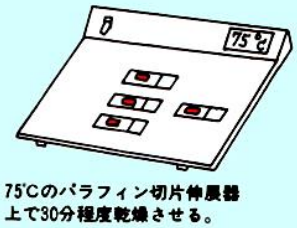




Solidification of the Malinol membrane on a hot plate

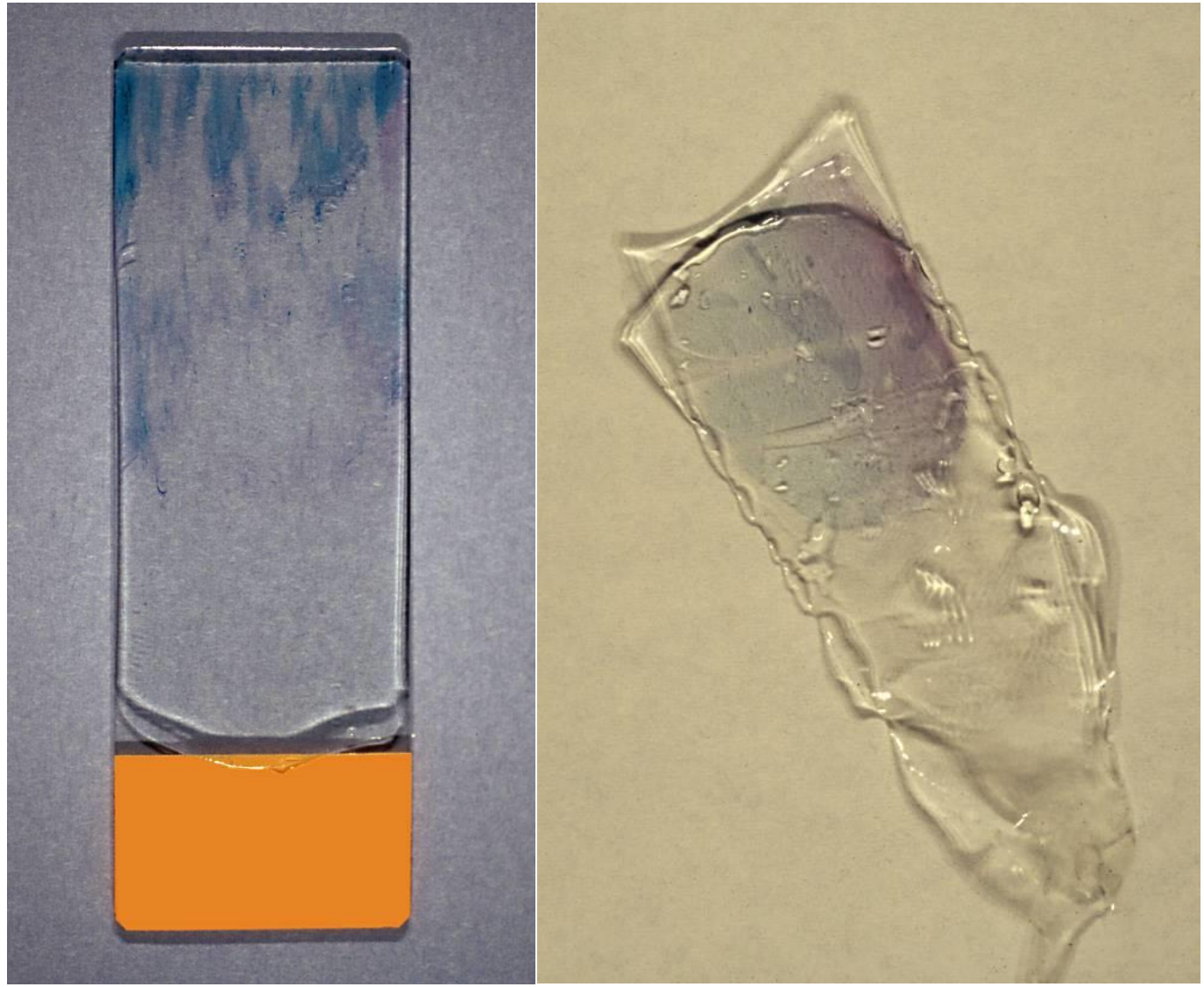
Wipe off excessive water with paper

Soak the section in warm water



Place onto silane-coated glass slide and dry

Soak in xylene, then in ethanol and finally in water to be ready for the next step

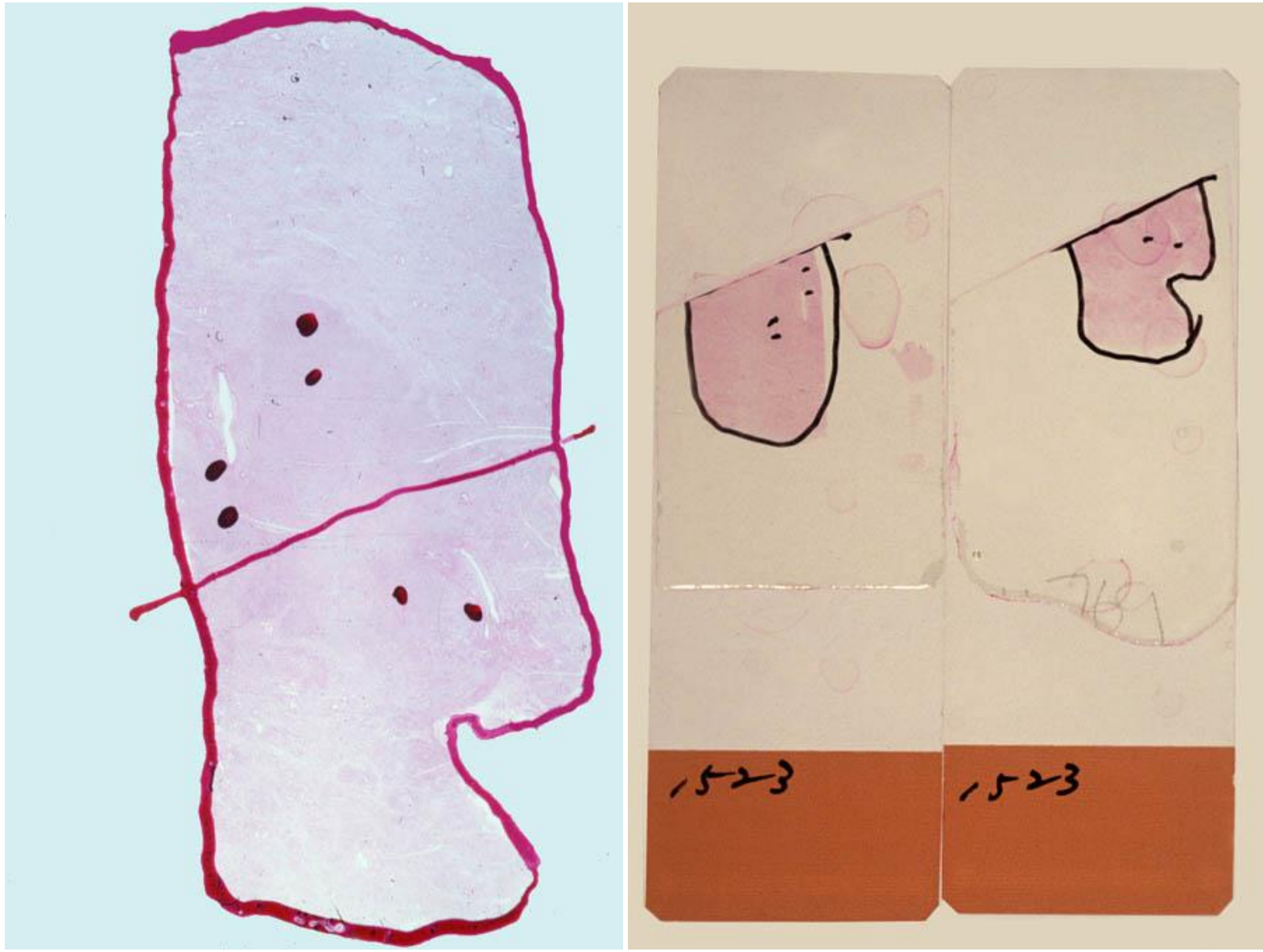


After detaching the cover slip, Malinol mounting solution is covered and solidified to form a Malinol membrane (left). The specimen is dipped in warm water, and then the solidified membrane can be peeled off. All the cells are transferred on the Malinol membrane (right).

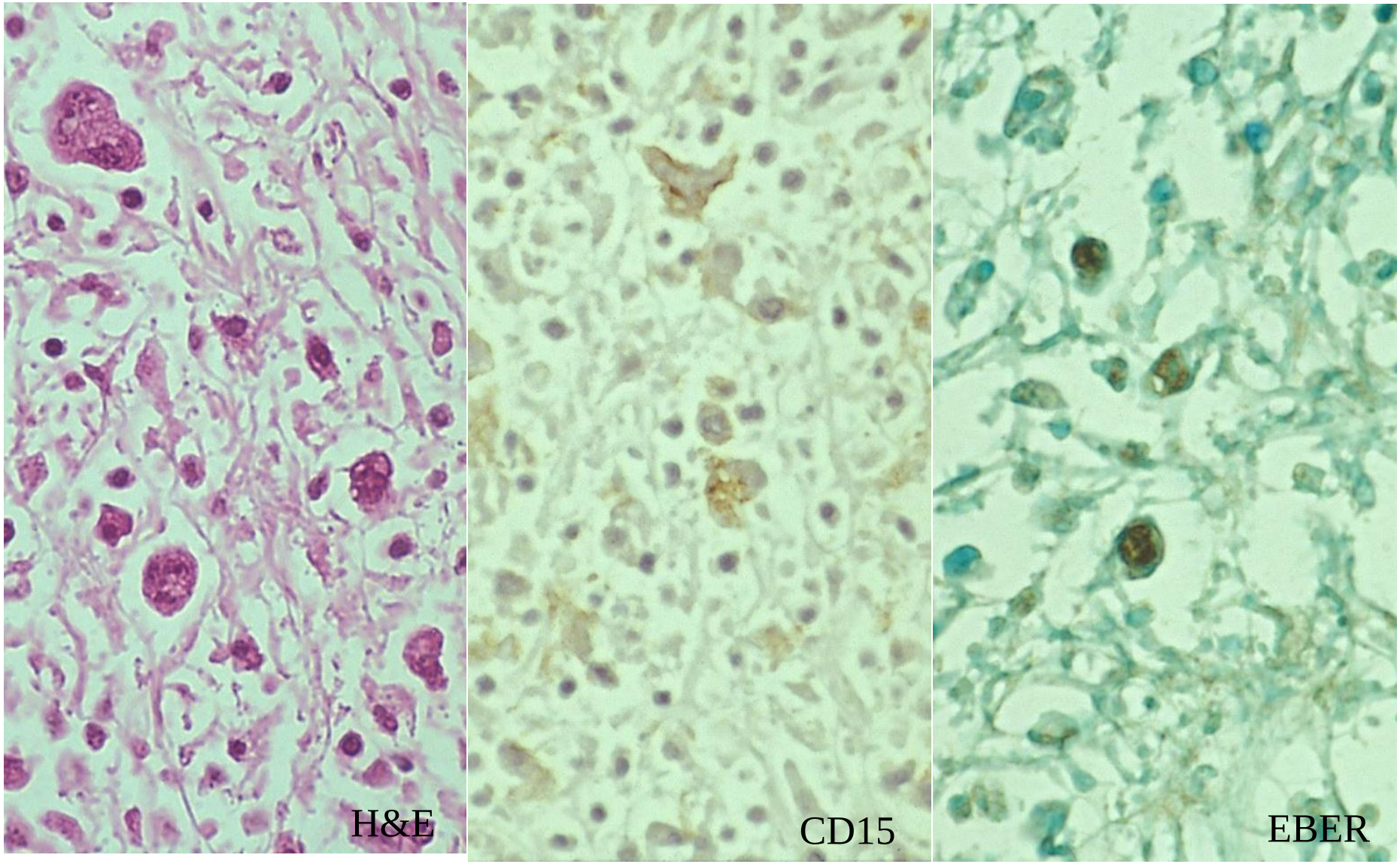
Demonstration of CD15 and EBER in the archival pathology specimens of Hodgkin's lymphoma autopsied by Thomas Hodgkin himself in 1820's. Use of the cell transfer technique.

Dr. Thomas Hodgkin
(1797-1866)
Guys Hospital, London

Presentation of samples of 3 cases autopsied by Thomas Hodgkin. Gordon museum, Guys Hospital, London. Single unstained glass slides (a total of 6 slides) were the precious gifts to the author from the President of the Museum. The specimens were fixed in ethanol for 80 years and thereafter in formalin for 90 years.



The precious single slide of the liver was stained with H&E, and then cell transferred onto the Silane-coated glass slides to get two slides for staining. Hodgkin' cells and Reed-Sternberg cells were identified in the dotted areas.



Hodgkin's cells and Reed-Sternberg cells in the lymph node autopsied by Dr. Thomas Hodgkin. The plasma membrane expression of CD15 (Leu M1) and nuclear expression of EBER can be confirmed after long fixation in ethanol and formalin. **Ref.**: Tsutsumi Y. Demonstration of Epstein-Barr virus genome in archival paraffin sections of Hodgkin's lymphoma autopsied by Dr. Thomas Hodgkin nearly 170 years ago. Acta Histochem Cytochem 2003; 36: 511-514. doi: 10.1267/AHC.36.511

Rapid transfer of sections or smears to other Silane-coated glass slides

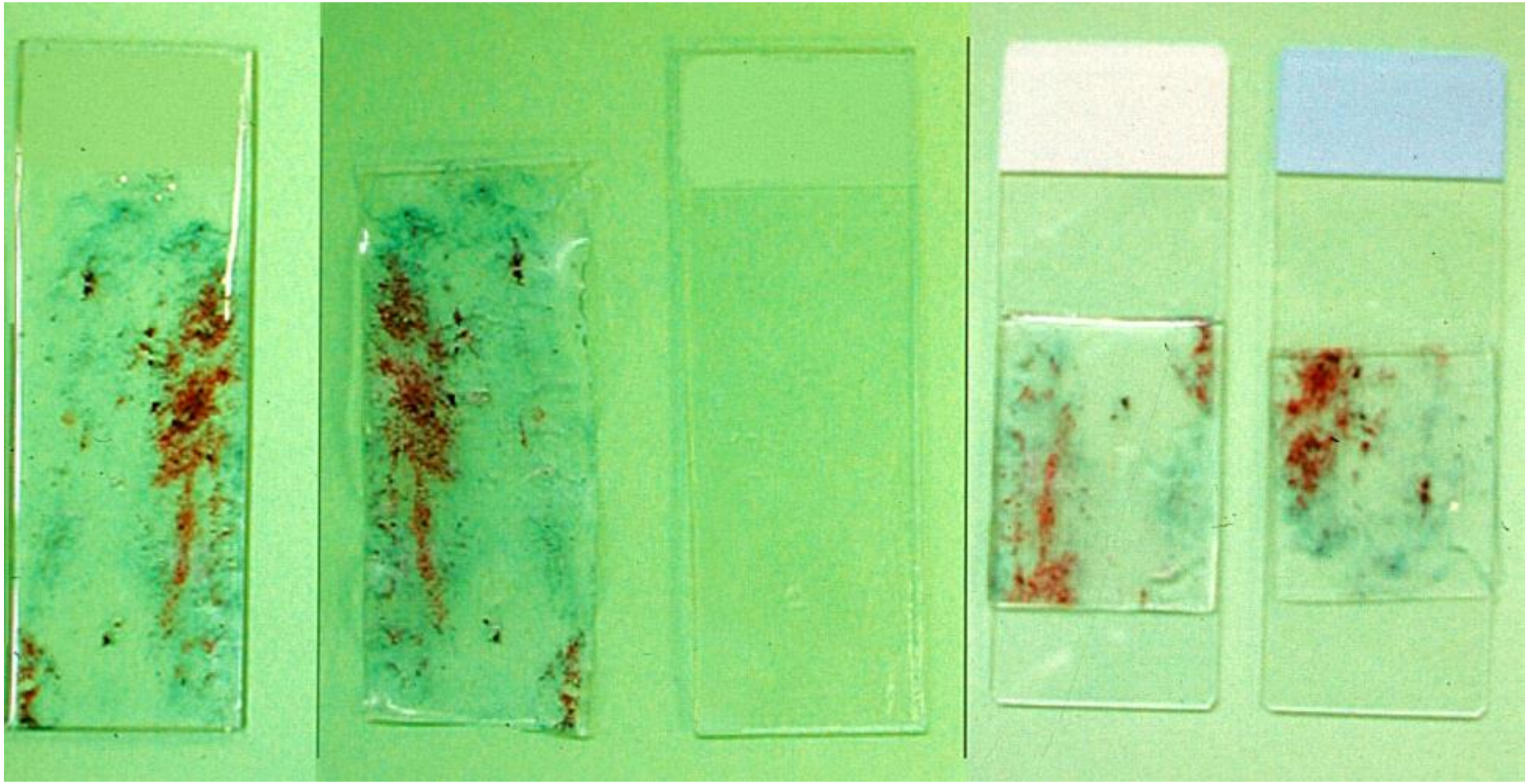
. Separation from a single slide into several specimens

2. Transfer of smeared cells located in the shoulder uncovered by cover slip
3. Repair of broken slides
4. Transfer of attached cells cultured on Plastic slides

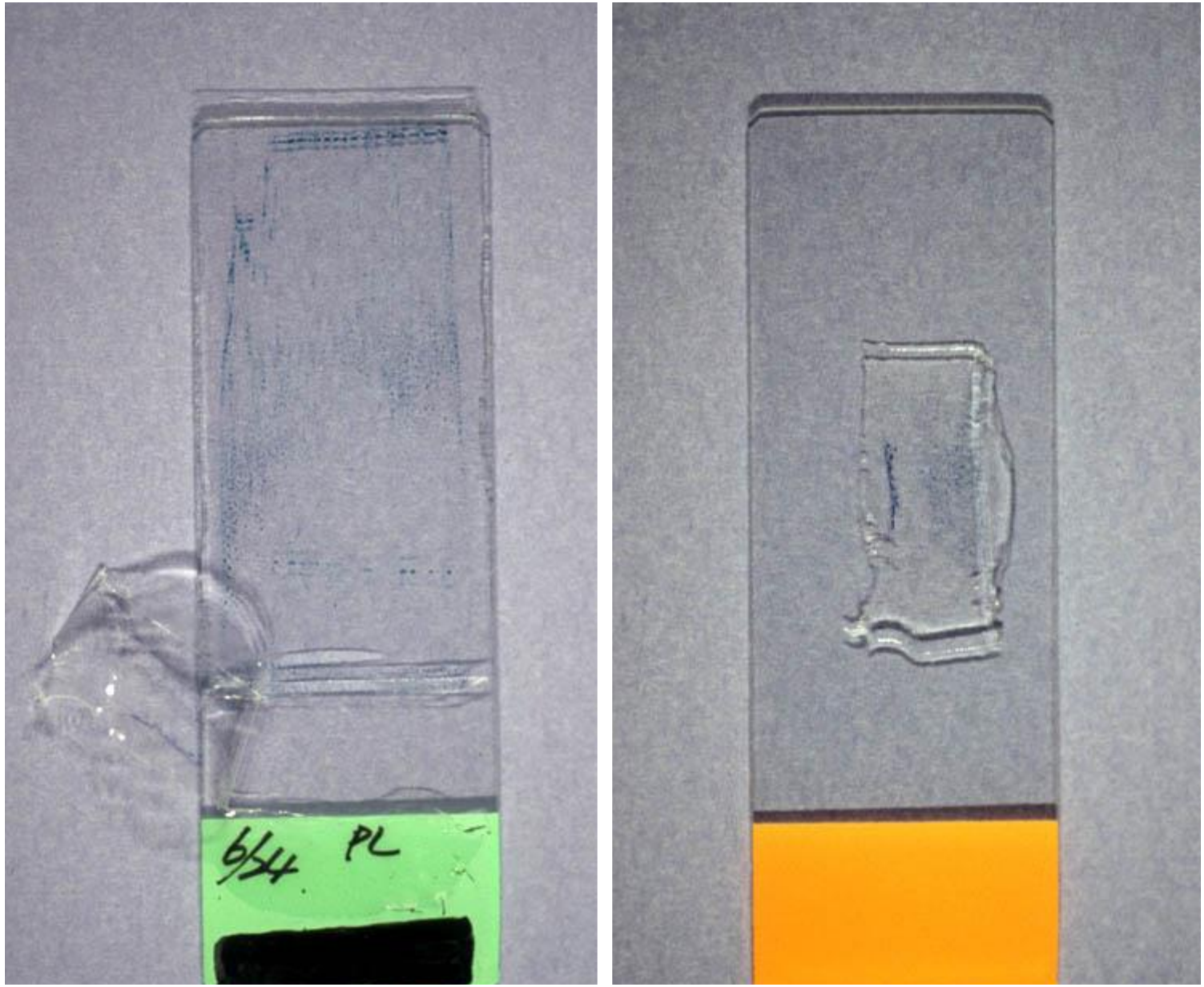
Quick cell transfer technique (Ito's method)

1. Detach cover slip by dipping in warm xylene
   (for rapid detachment, injure cover slip by a diamond pen)
2. Drop 1 ml diluted Malinol (mounting solvent) onto specimen
   (Dilution: Mix Malinol with the same volume of xylene)
3. Dry and solidify Malinol on a 70°C hot plate for 30 min
4. Dip slide in warm water for 15 min for softening
5. Detach sheet of Malinol by forceps
   (If necessary, cut specimen into several pieces by scissors)
6. Place sheet of Malinol on Silane-coated glass slides
   (Check carefully the side of the sheet)
7. Dry slides on a 70°C hot plate
8. Clear in xylene to be ready for immunostaining

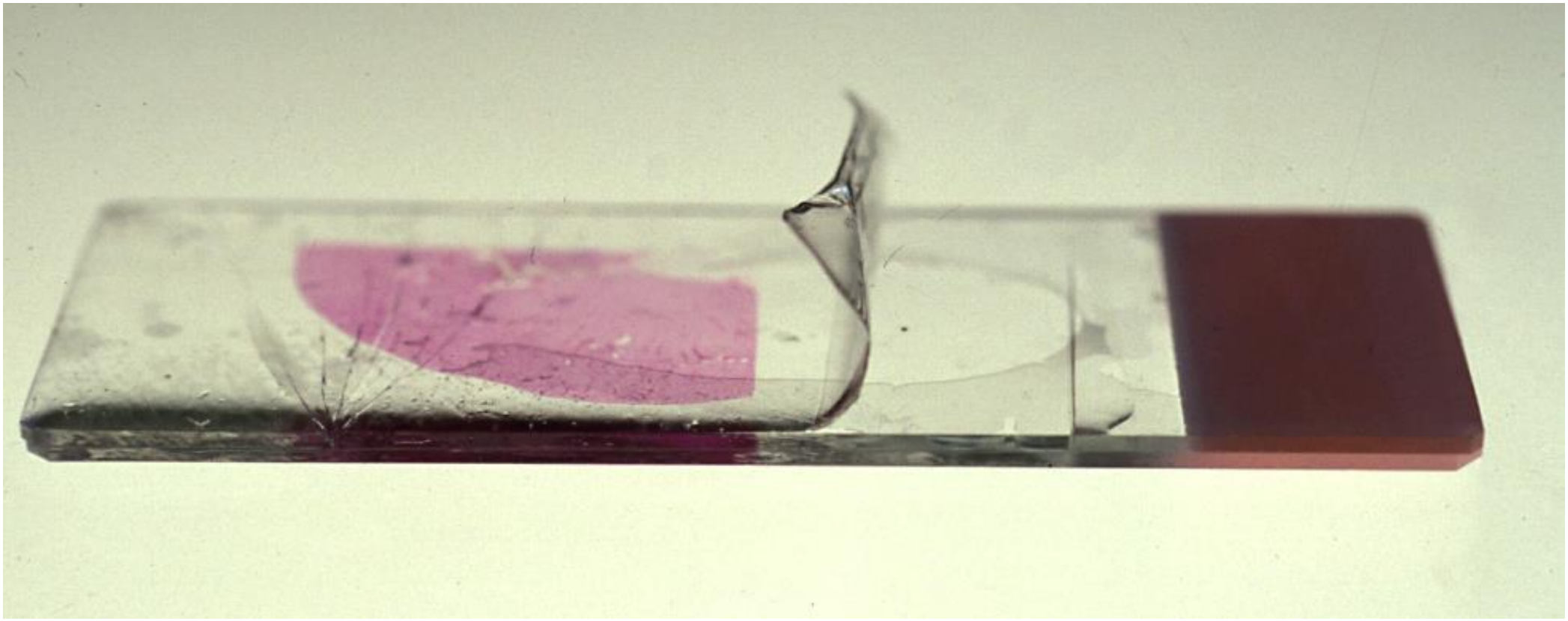
Ref.: Itoh H, et al. Development of the rapid cell transfer technique. Nihon Rinsho Saibo Gakkai Zasshi 2002; 41: 302-303 (in Japanese with English abstract).



Cell transfer technique. Detached Malinol membrane is cut into plural pieces by knife and attached onto the silane-coated glass slides. The Pap-stained cytology specimens on uncoated glass slides can be transferred. It should be noted that Giemsa-stained glass slides or Pap-stained preparations on coated glass slides can not be transferred.



The technique without detaching the cover slip: the shoulder portion of the cervical smear preparation can be used for the cell transfer.



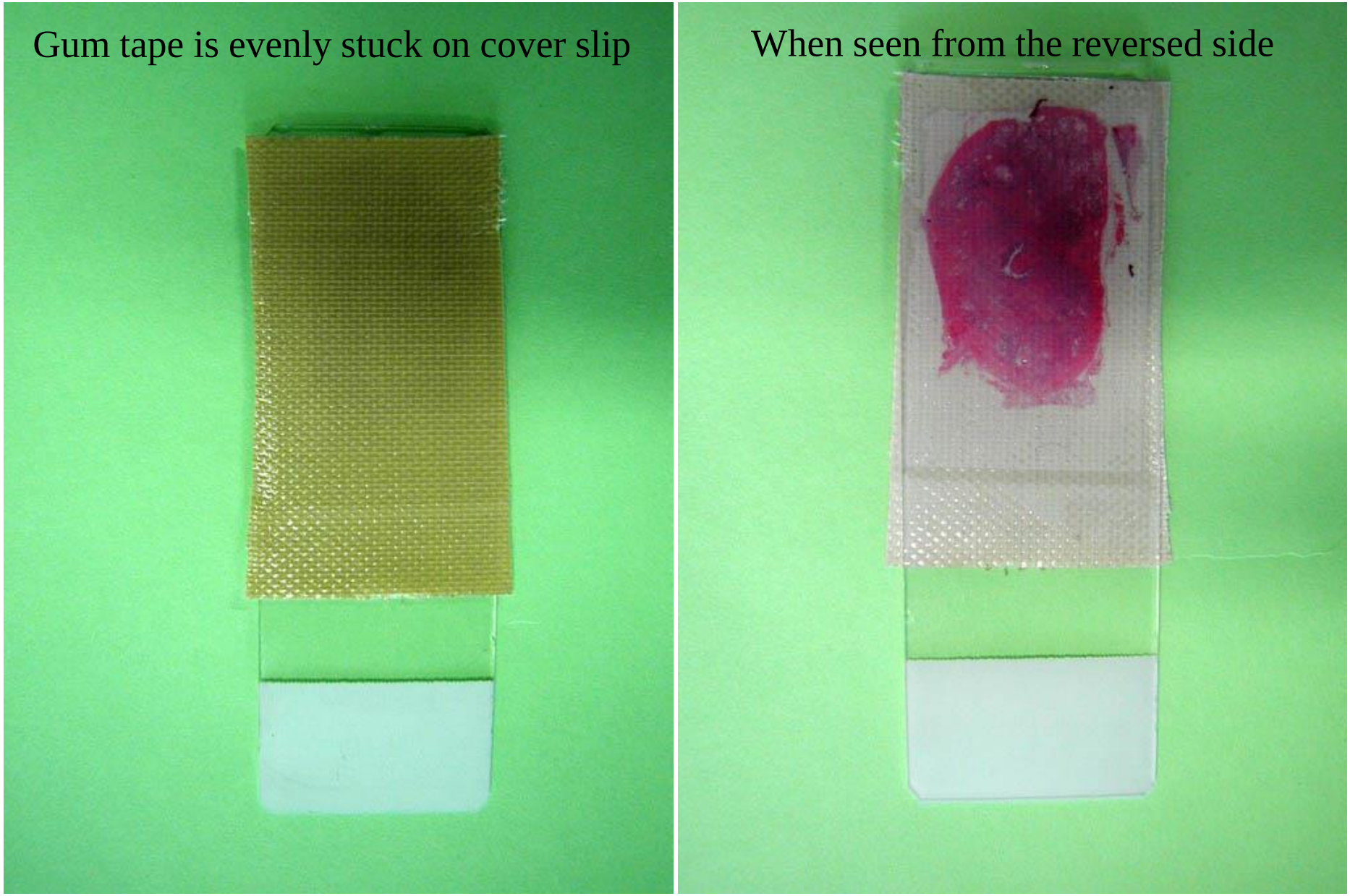
The cell transfer technique can be applied to repairing a broken glass slide. Here, a broken H&E preparation of the kidney was repaired by supporting the broken glass slide with another glass slide attached.

Rapid detaching of cover slip (Harada's method)

1. Dip glass slide in 65℃ warmed xylene for 30 min
2. Wipe off excessive xylene by Kim Wipe
3. Stick tightly size-adjusted Gum Tape on cover slip
4. Detach cover slip in a breath by peeling Gum Tape
5. Dip glass slide in xylene to be ready for transfer

Ref.: Harada E, et al. Rapid detachment of cover slips. Byori-to-Rinsho 2003; 21(11): 1306-1307 (in Japanese).

Rapid detaching of cover slip 1



Rapid detaching of cover slip 2

Rapid detaching of cover slip 3