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Cytology of skin lumps & bumps – tips for improving diagnostic usefulness

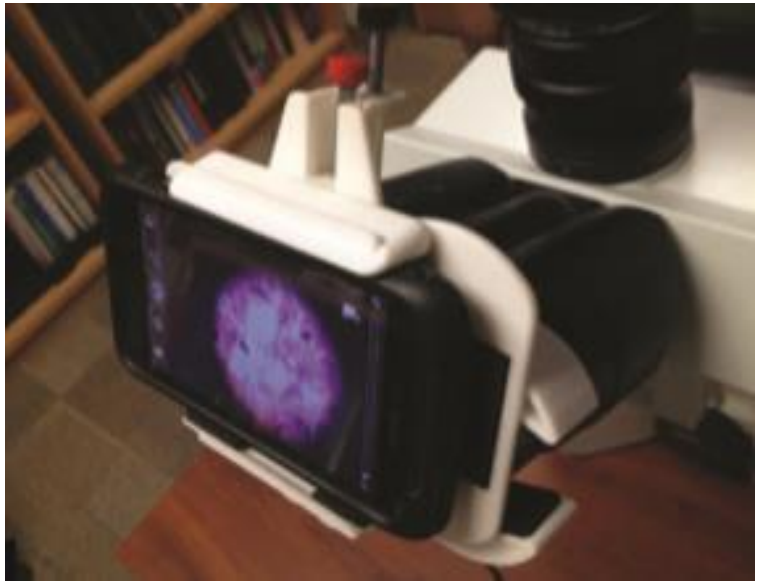
Kostas Papasouliotis

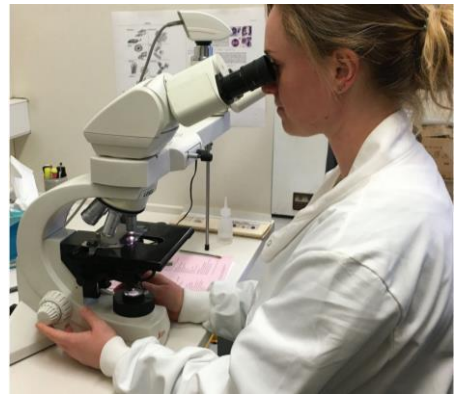
DVM PhD DipECVCP MRCVS

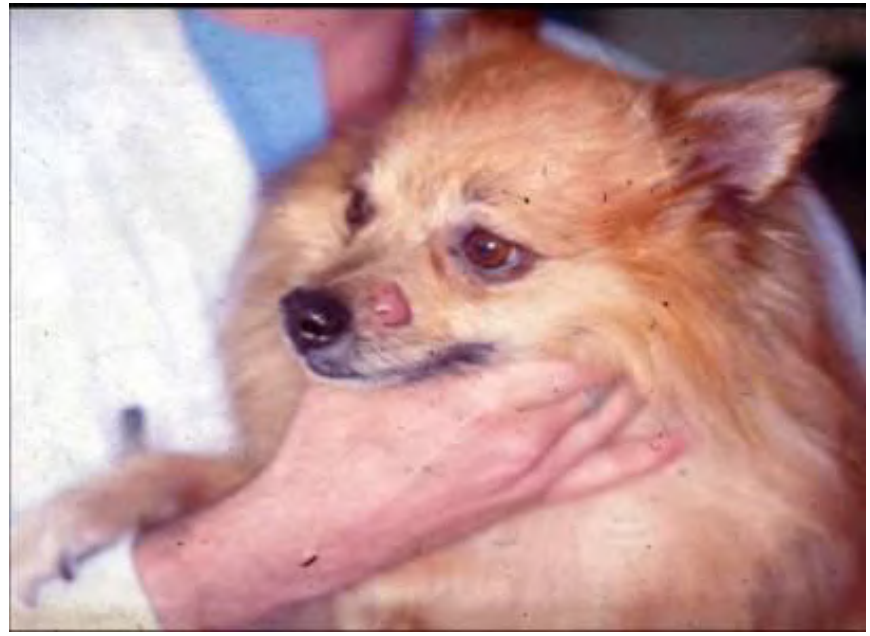
EBVS® European Specialist in Veterinary Clinical Pathology

Diagnostic Laboratories, Langford Vets, Bristol Veterinary School, University of Bristol

kos.papasouliotis@icloud.com

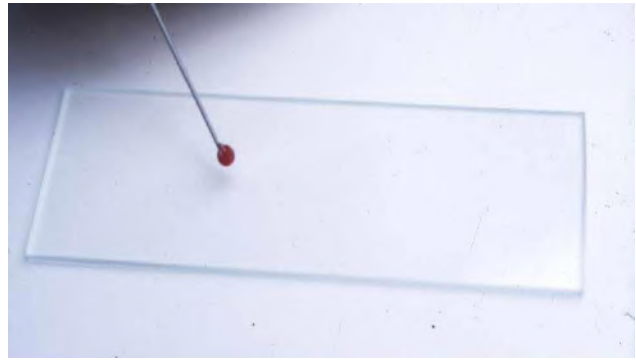


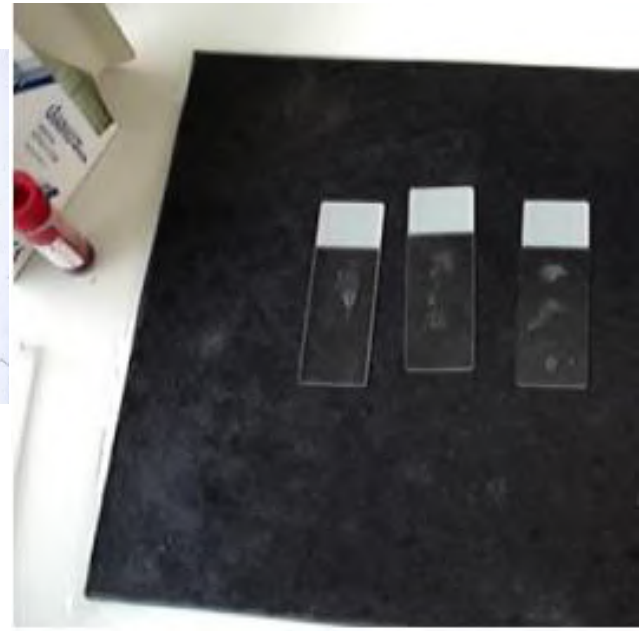




Content

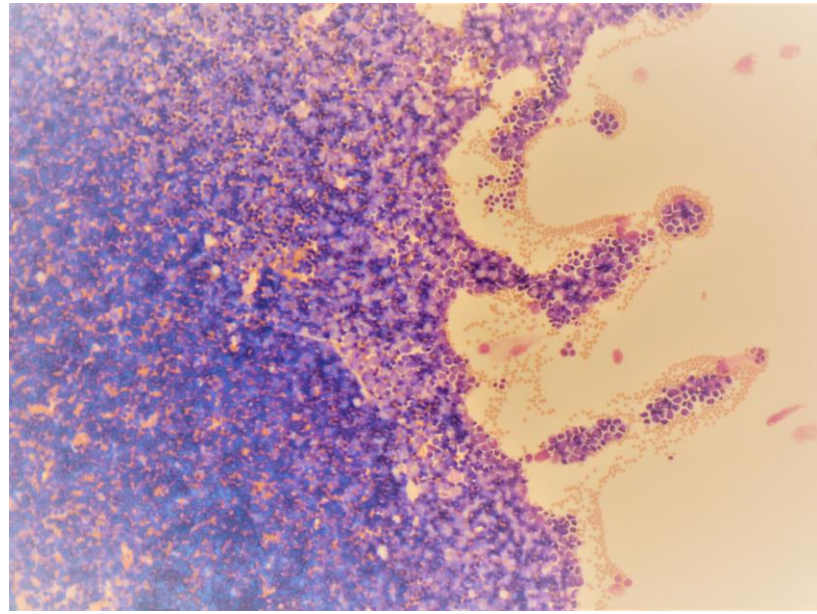
- Advantages & limitations
- Sample collection
- Smear preparation & Staining
- Examination
- Telecytology





Introduction-Cytology of skin lumps&bumps

- **Has been reported that of all cytology samples** (Christoper et al *JAVMA* 2008;232(5):747-54)
  - **~50% reviewed in-house;**
  - **~30% submitted directly to diagnostic laboratory (DL)**
  - **~20% reviewed in-house first and then submitted to DL**
- **Fast and cheap; can increase revenue**
- **Can provide specific diagnosis** (Ghiseni et al *Vet Clin Path* 2006;35:24-30)
  - **Cytology of 292 FNA samples from cutaneous and subcutaneous masses of dogs & cats:**
    - **83% were diagnostic**
  - **Cytology vs Histopathology diagnosis:**
    - **91% agreement**
  - **21 samples with neoplasia on Histopathology, on Cytology were reported as inflammatory/non-neoplastic/necrotic**
  - **Cytology: Sensitivity 89%, Specificity 98%**



Limitations

- **Poor quality of cytology sample**
  - **Collection technique**
  - **Smear quality**
  - **Staining**

Unacceptable quality ~20% of samples submitted to DL (Skeldon & Dewhurst 2009 *JSAP*;50(4):180-5)

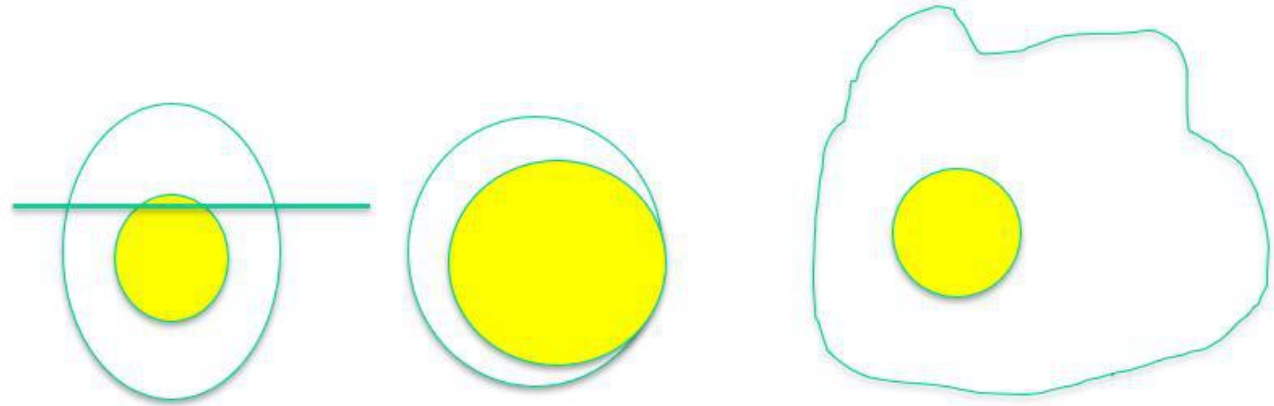
- **Some lesions, require evaluation of tissue architecture and Histopathology**
  - **Cystic or fibrous masses**
  - **Cytology samples with multiple inflammatory and tissue cell types**
  - **Presence of cells without marked morphological criteria of malignancy**

- **Pitfalls**
  - **Limited experience (can cause uncertainty, misinterpretation)**
  - **Incomplete examination of the whole smear(s)**

What do we want?

- **Collect cells from the lesion and spread them in a slide, ideally in a monolayer.**

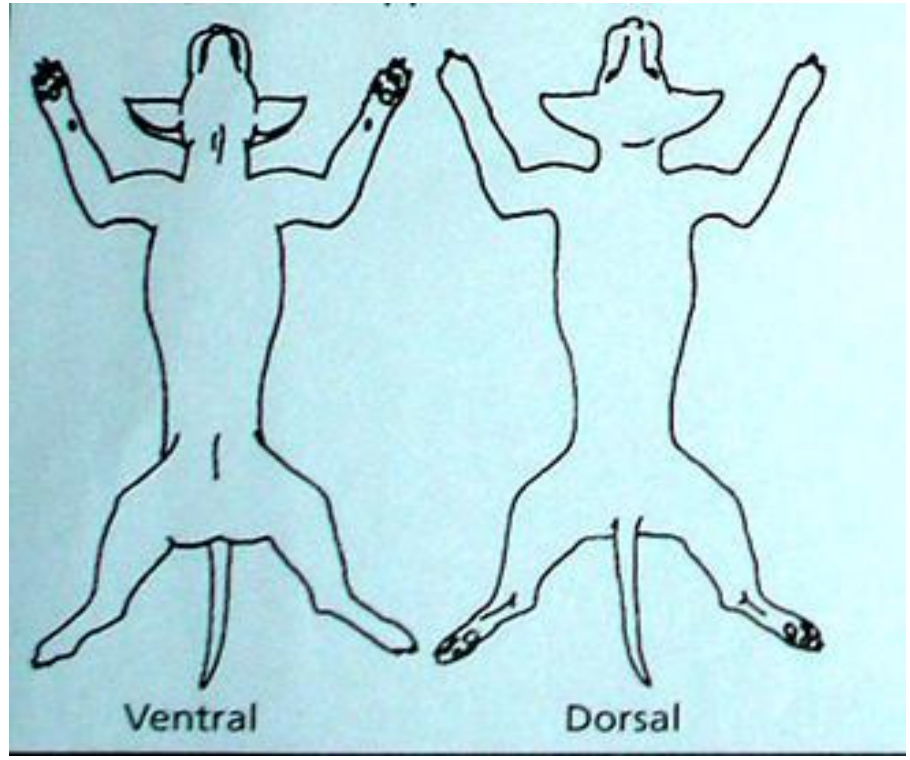
- **We want the cells to be spread enough to be able to see both nuclear and cytoplasmic characteristics.**

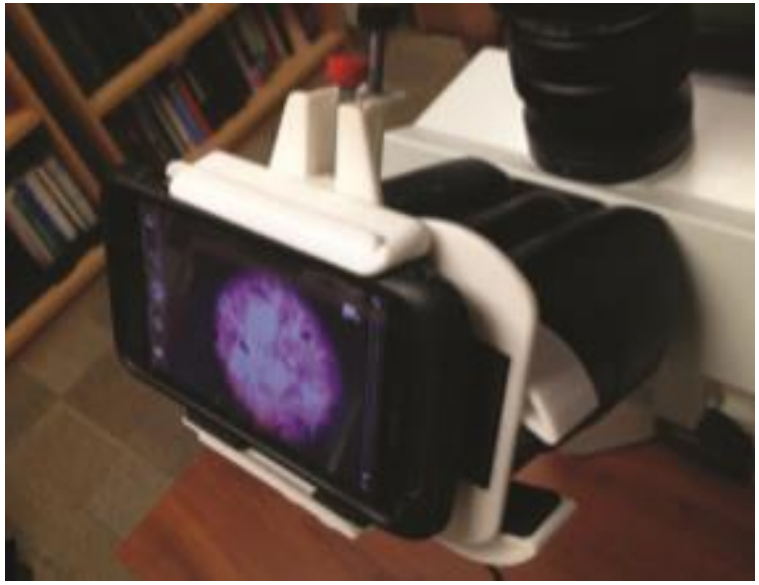


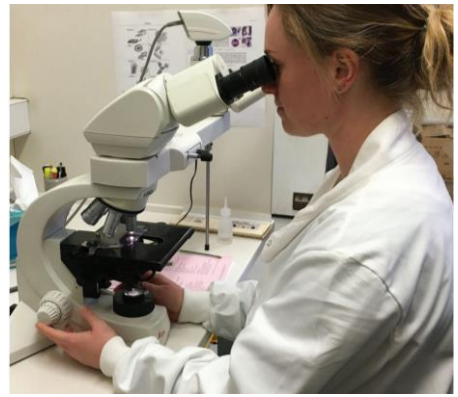
Before you start collecting

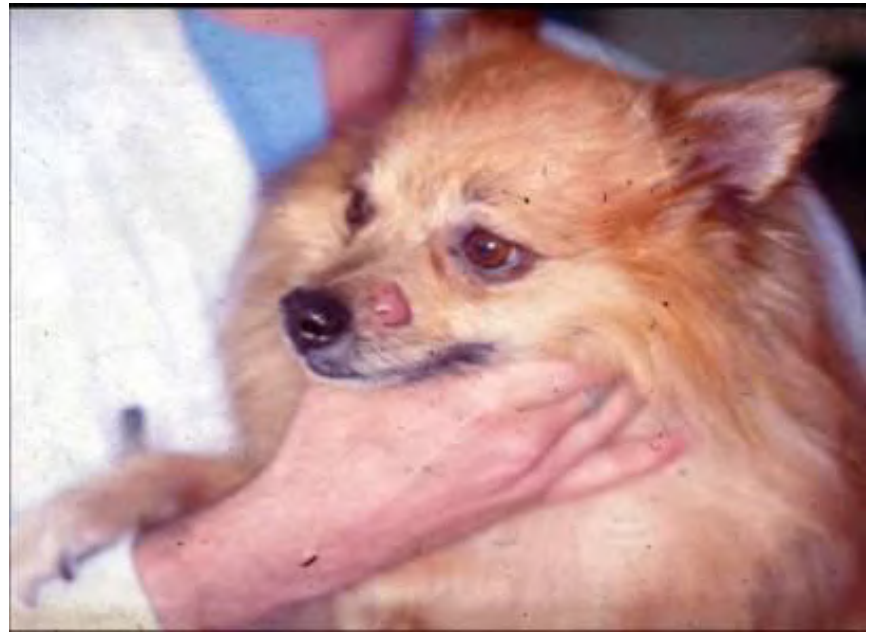
Important clinical information (especially if you are planning to use an external laboratory)

- **Clinical history and lesion evolution**
- **Characteristics of the lesion:**
  - **Localisation**
  - **Firm/soft**
  - **Dimensions**
  - **Painful/nonpainful**
  - **Ulcerated/nonulcerated**
  - **Cutaneous/sub cutaneous**
  - **Adherent/non adherent**
  - **Appearance of aspirated material**









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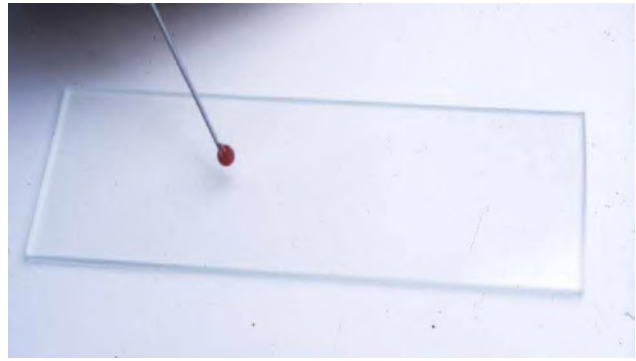
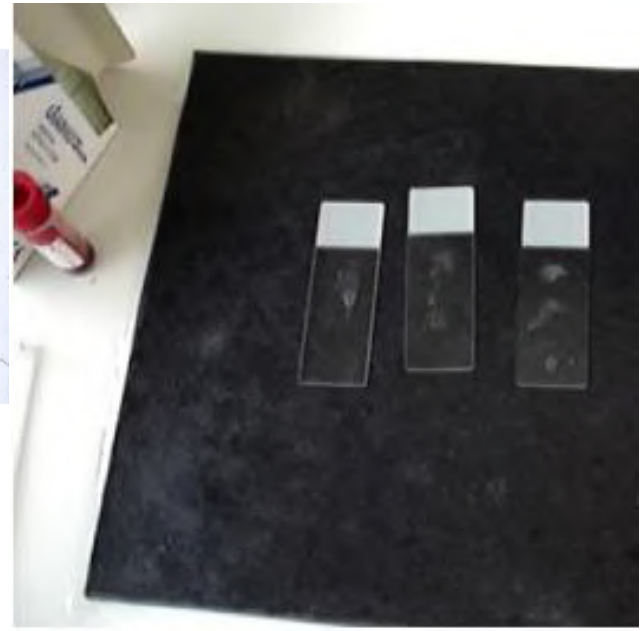
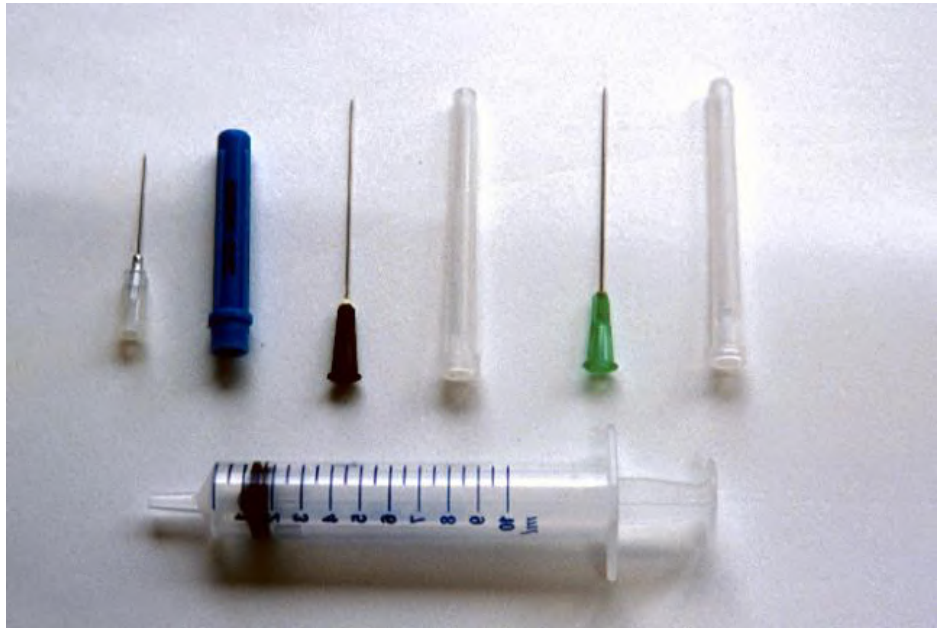
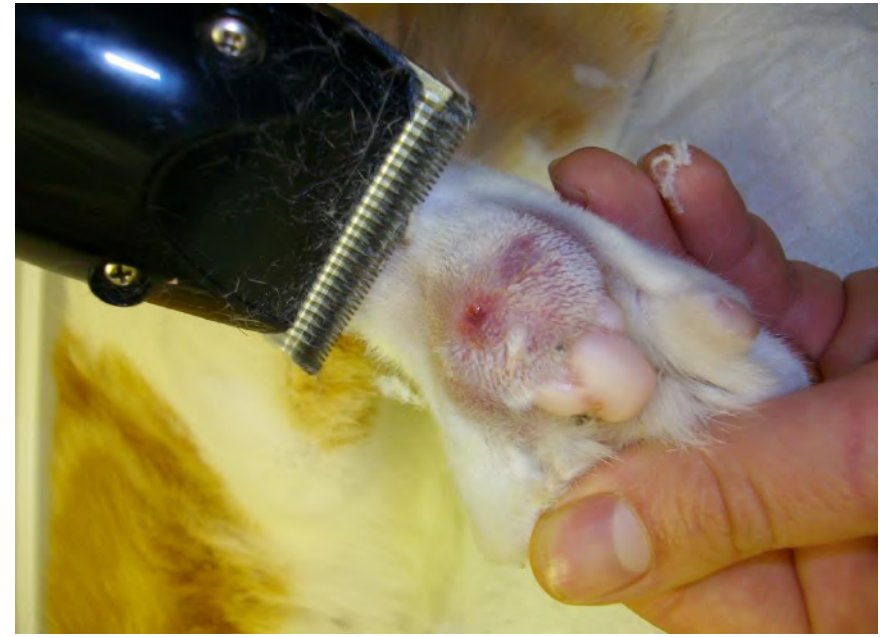
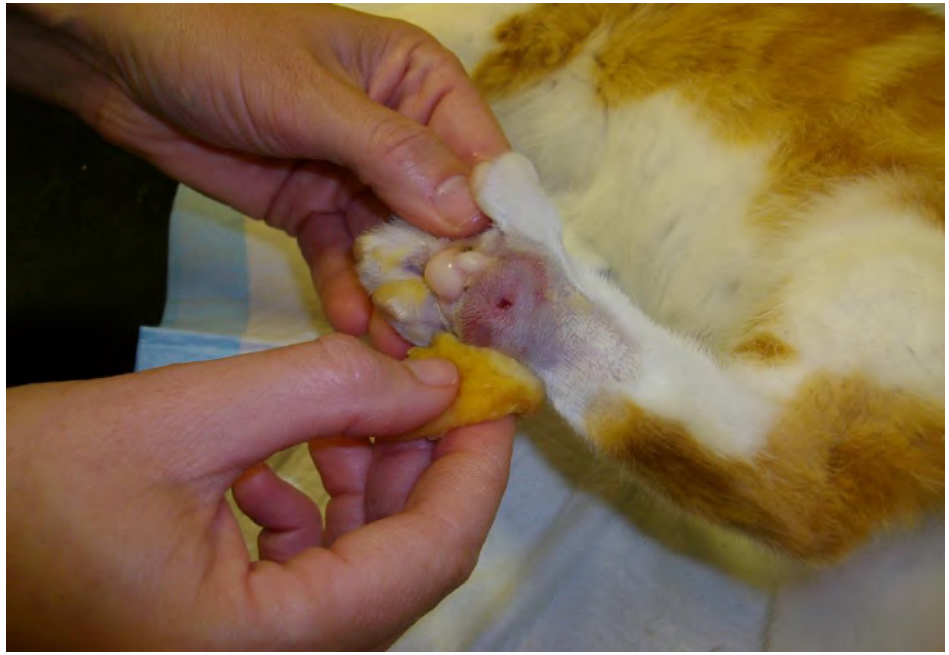
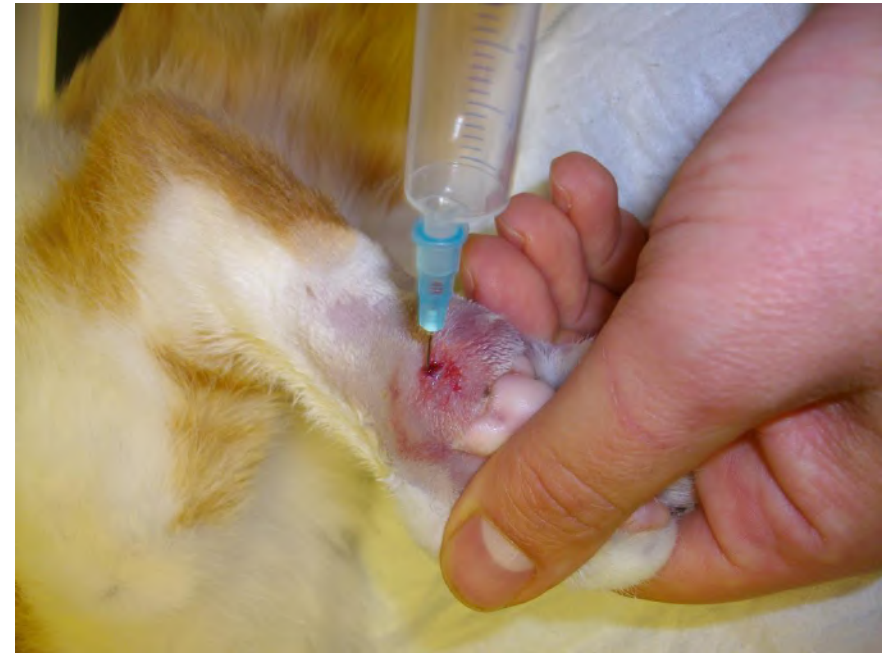






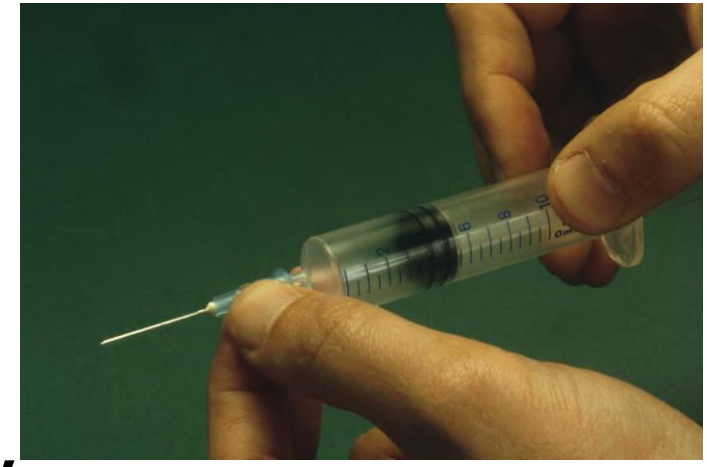




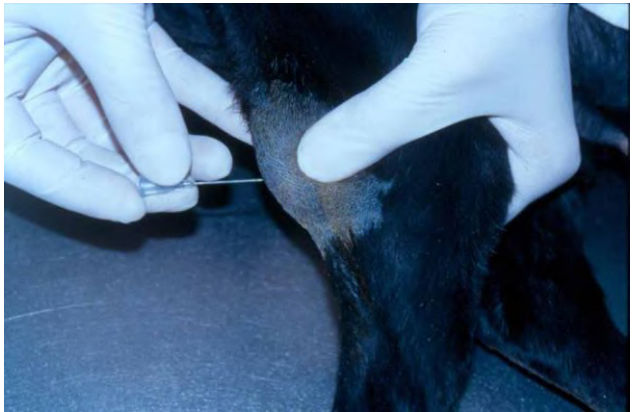


Collection of samples : Fine Needle Aspiration

- Needle & Syringe
  - 21-25 gauge (≤20G)
  - 5/10 ml syringe
  - length of needle is important



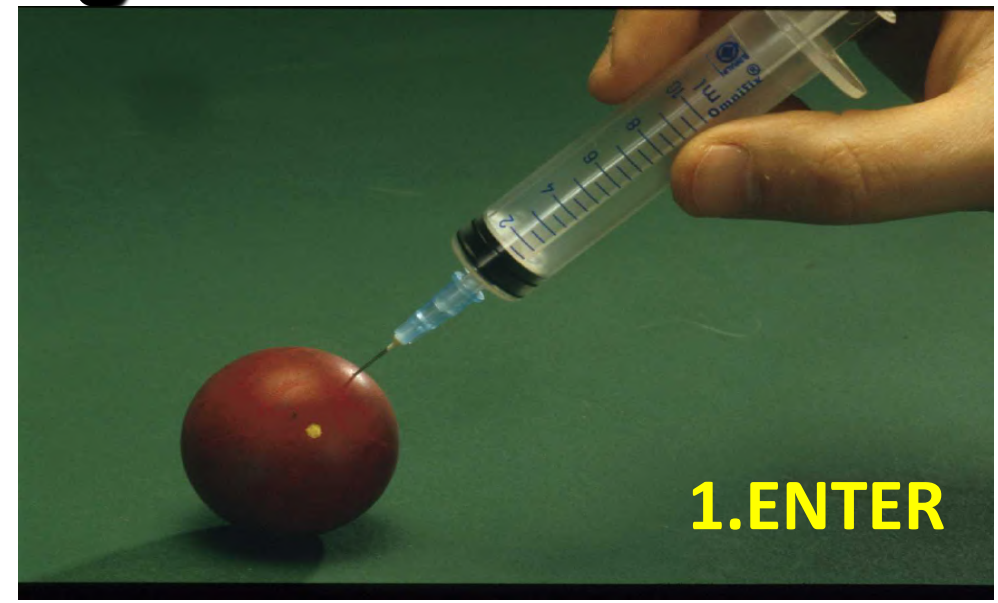
- Needle only

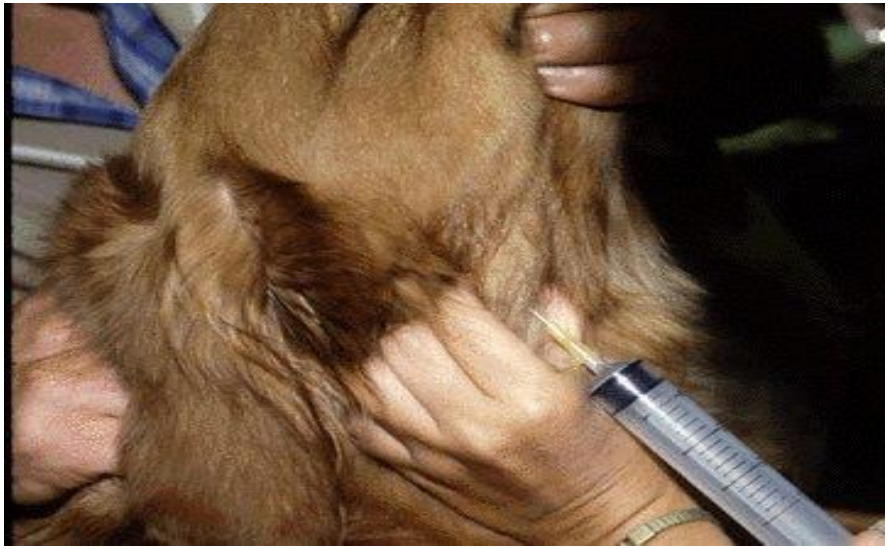


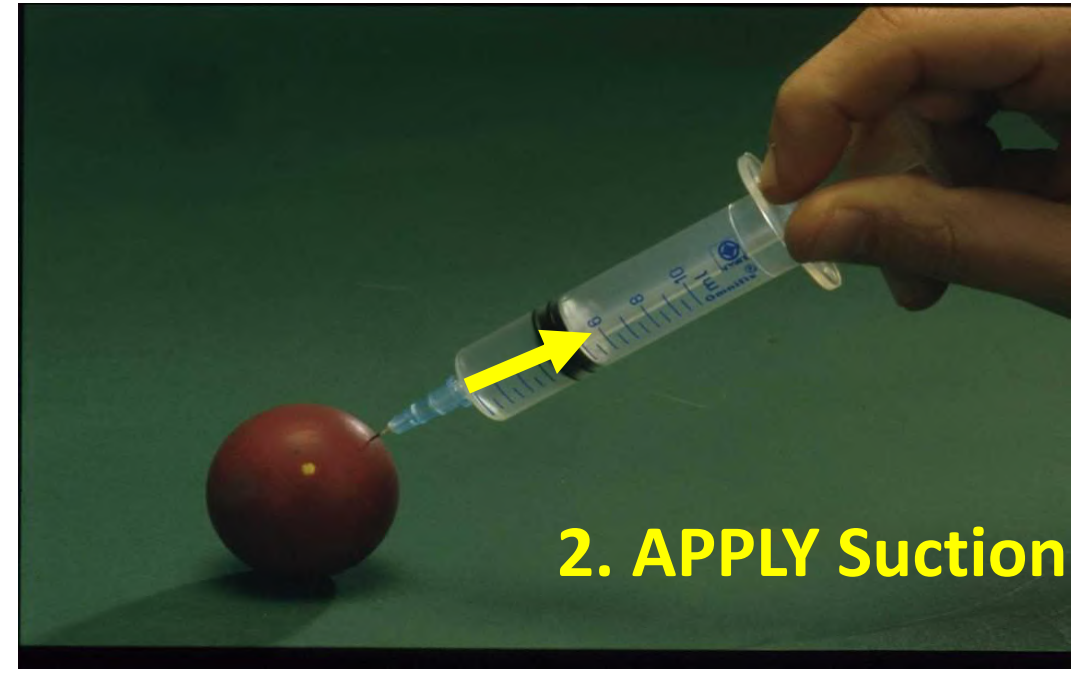
- Needle & Syringe
- Collection method
  - ENTER
  - APPLY Suction (5-6 ml)
  - REDIRECT needle without releasing suction
  - RELEASE suction
  - WITHDRAW needle
  - EXPEL material on slides

  - Do not stay in the lesion long
    - 5 seconds

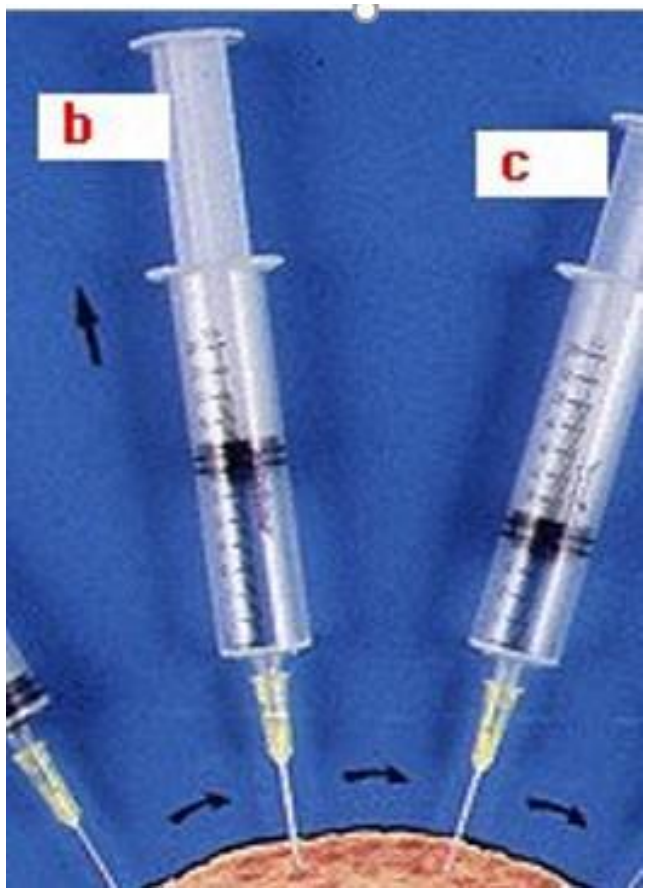
Needle & syringe- 1



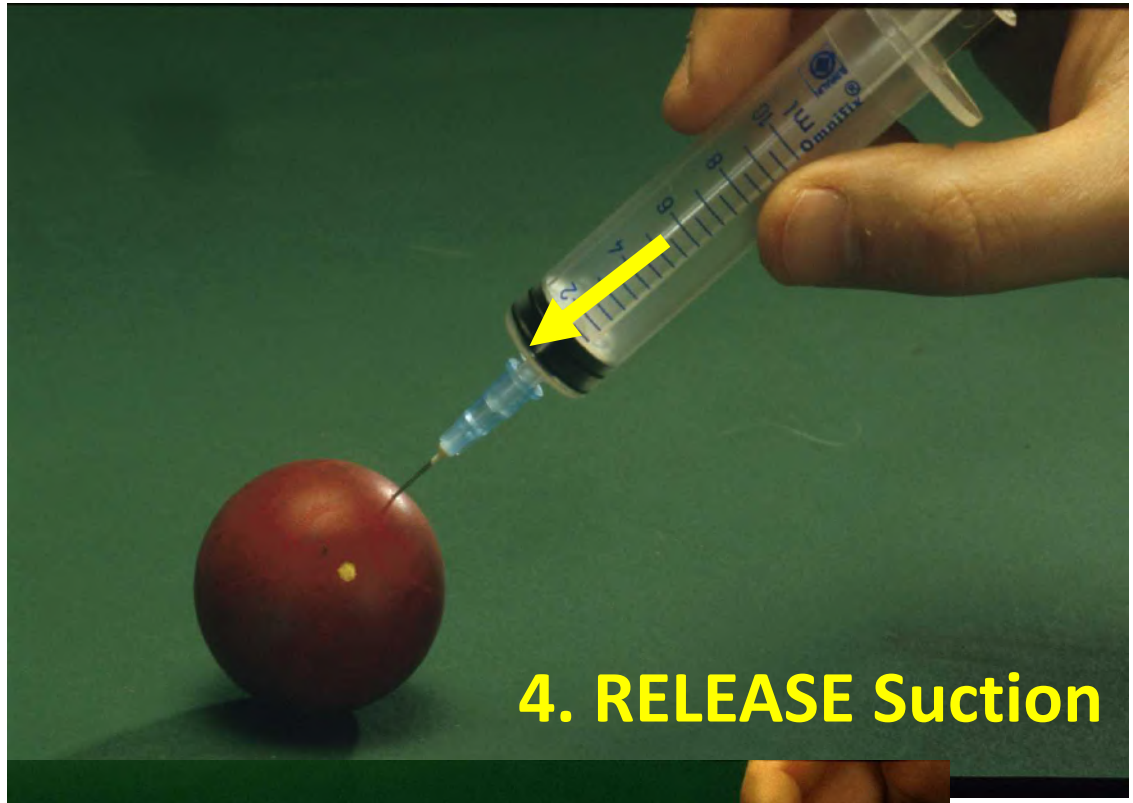


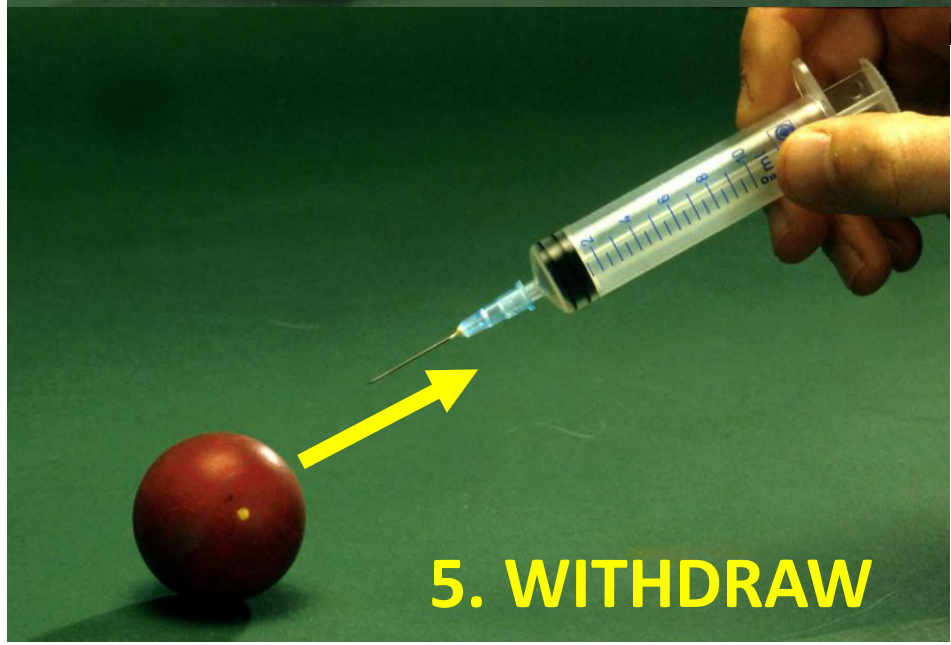


FNA - 2

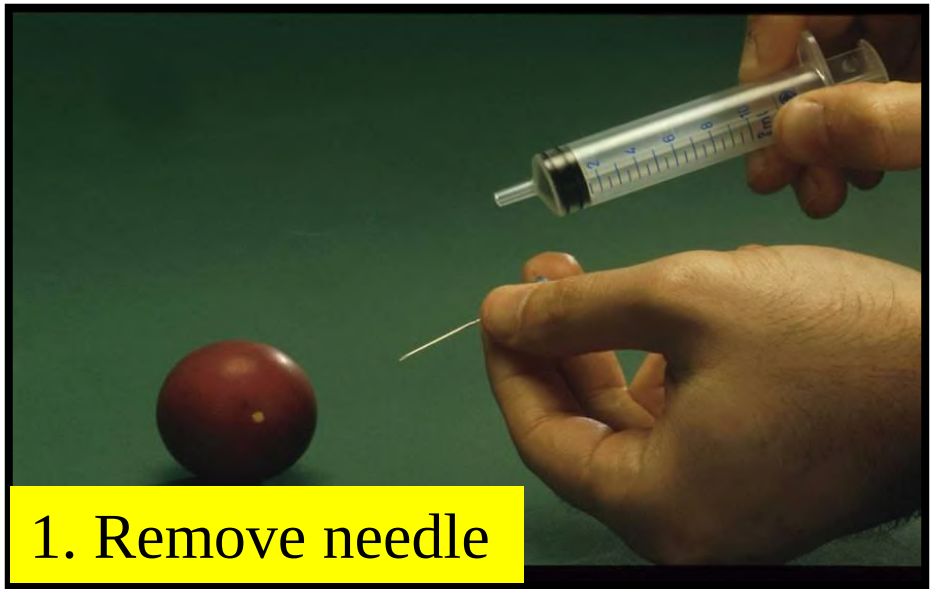


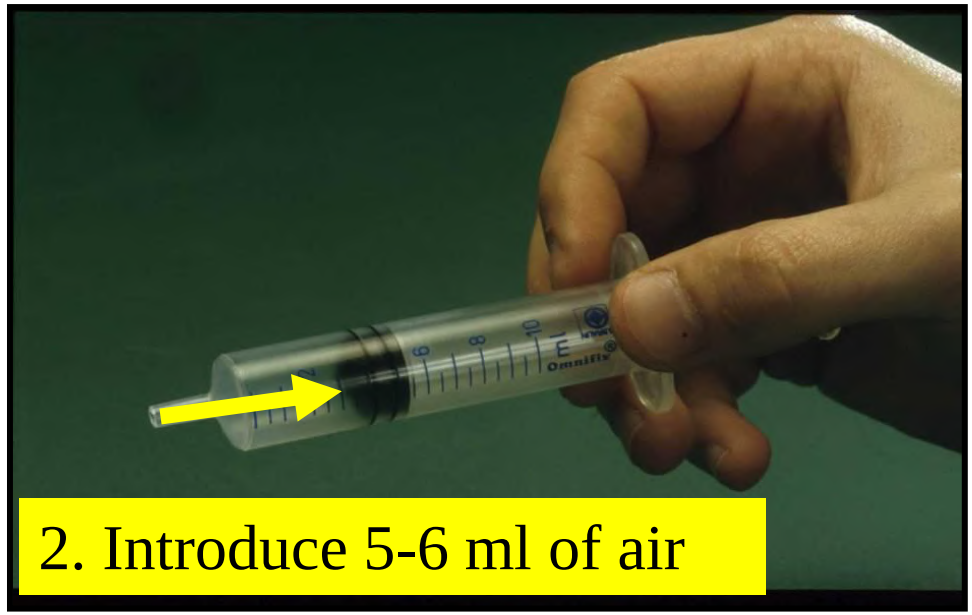
3. REDIRECT needle without releasing suction

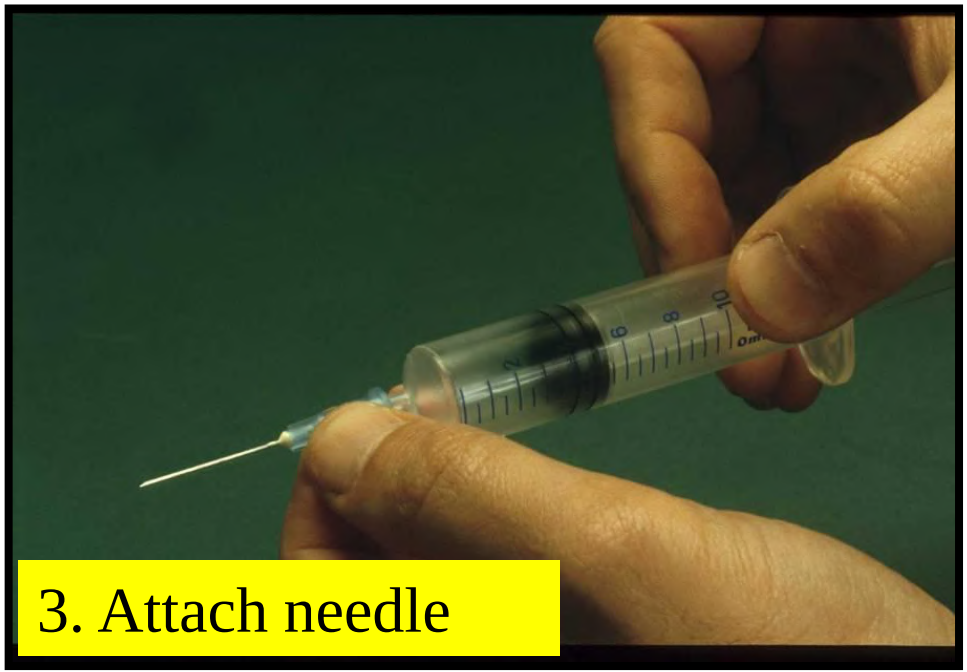


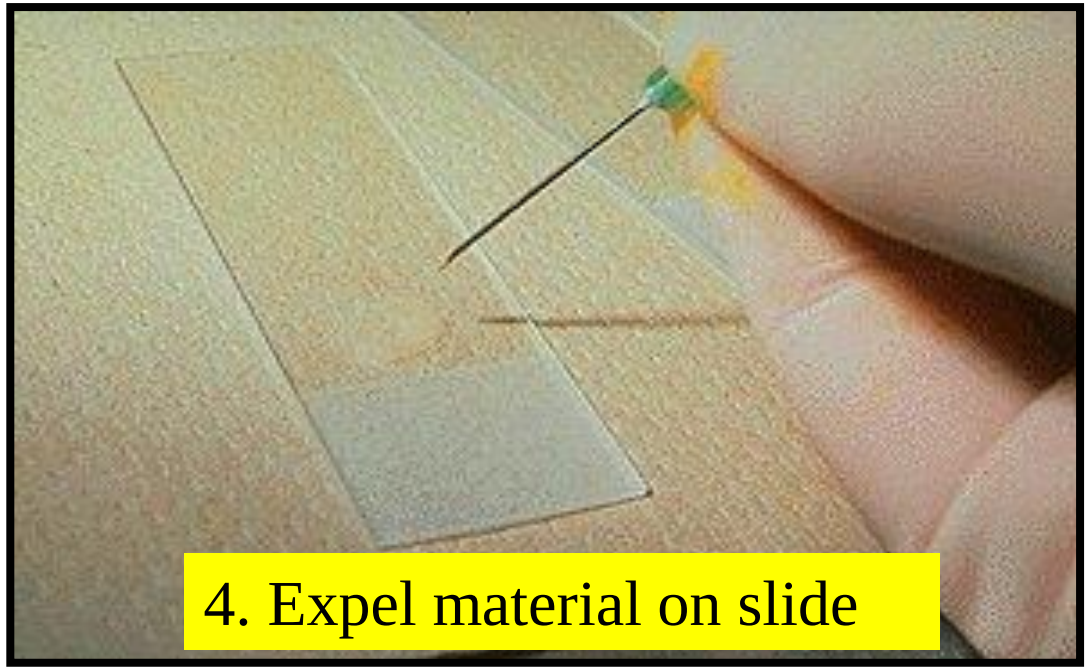


FNA – 3 (Expulsion of aspirated material)







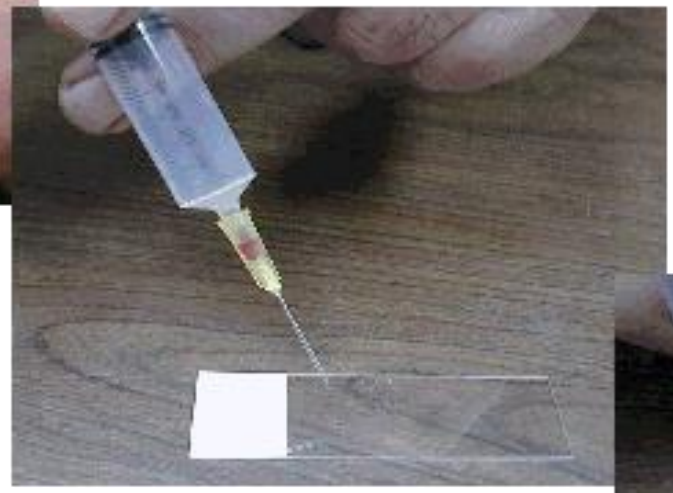


Needle only

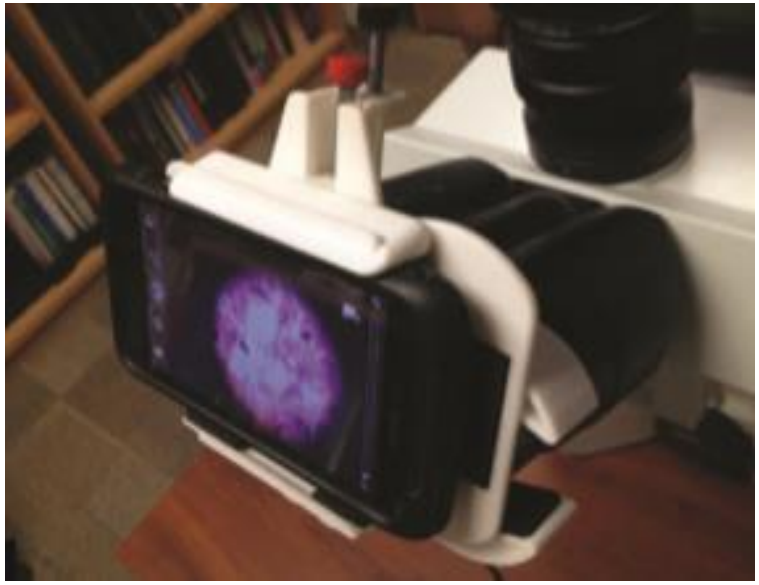
-Vascular lesions/tissue

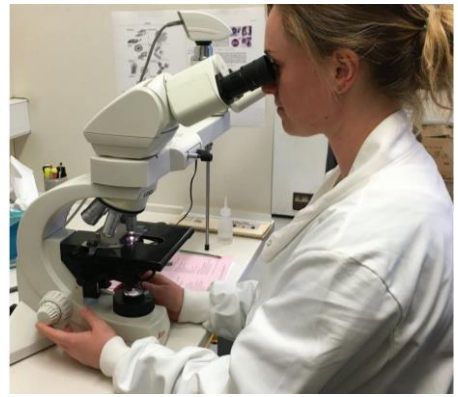
-Masses that contain fragile cells (very good for lymph node)

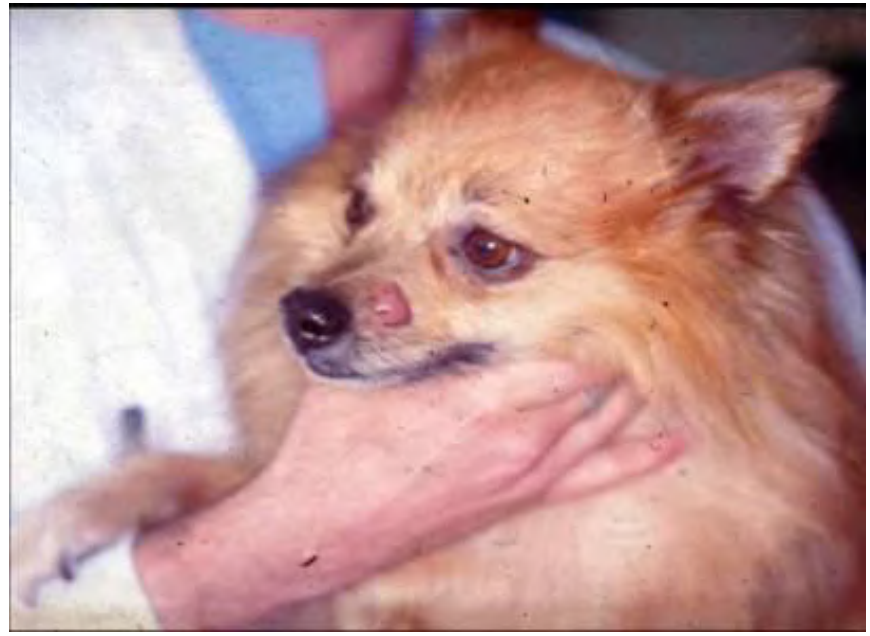
-If lesion appears cystic/contains fluid, aspirate solid parts/wall of lesion (may collect more tissue cells)



Gently inserted in and out several times-different areas







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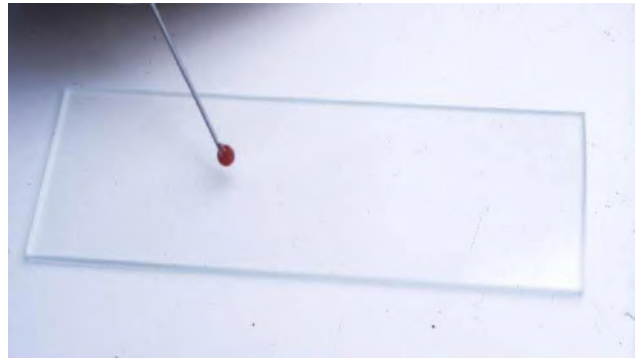
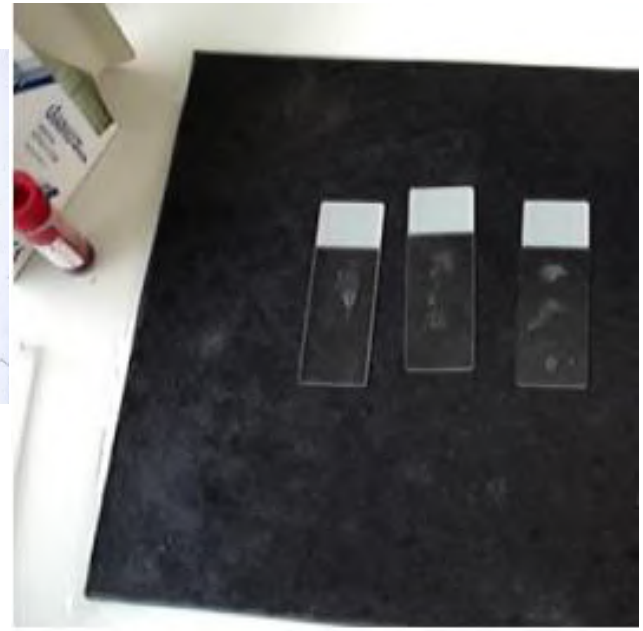
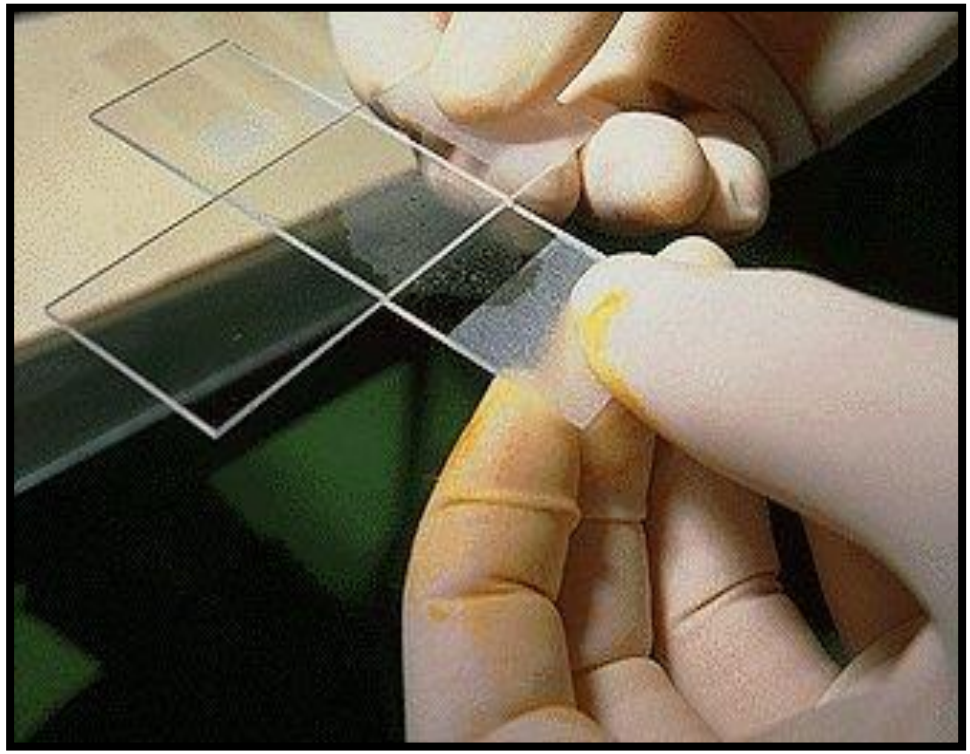
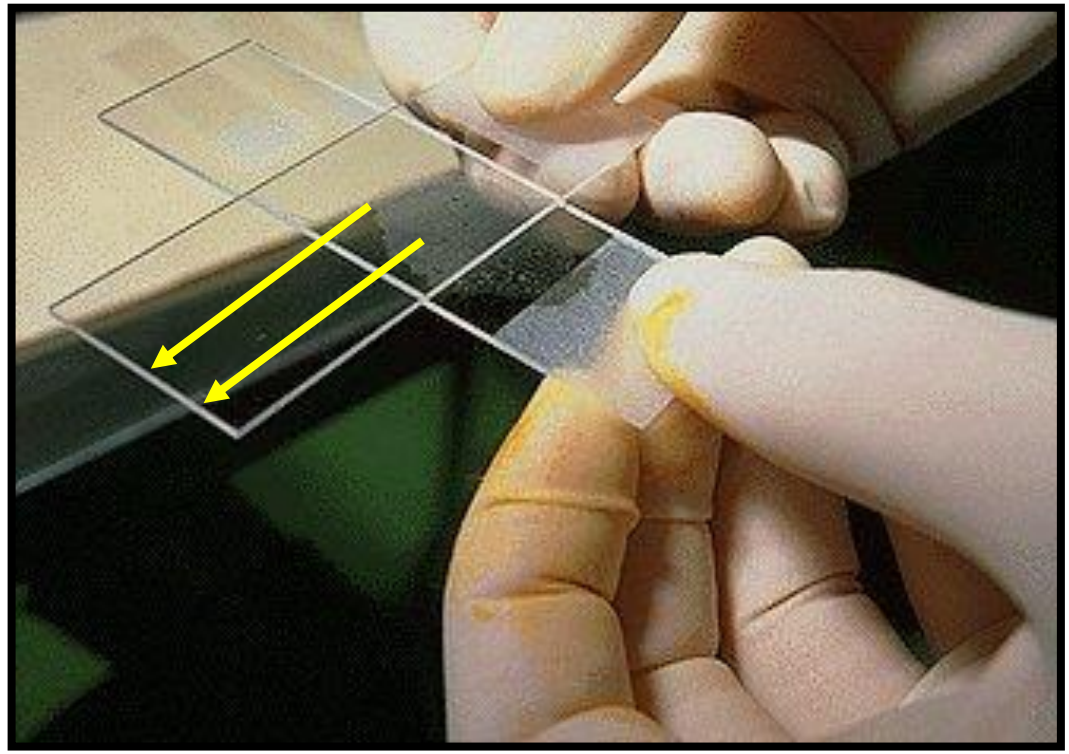




Smear preparation-1



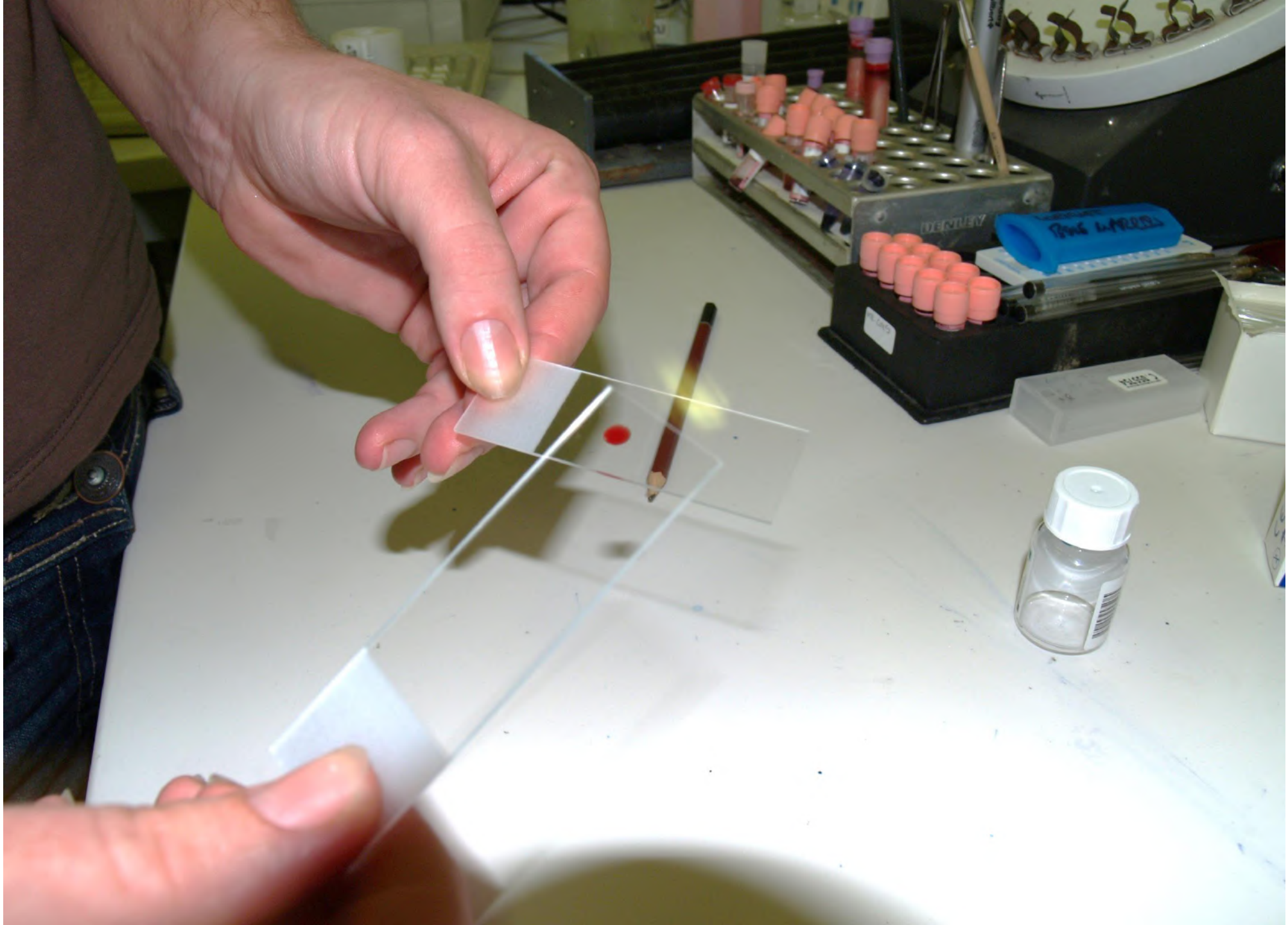


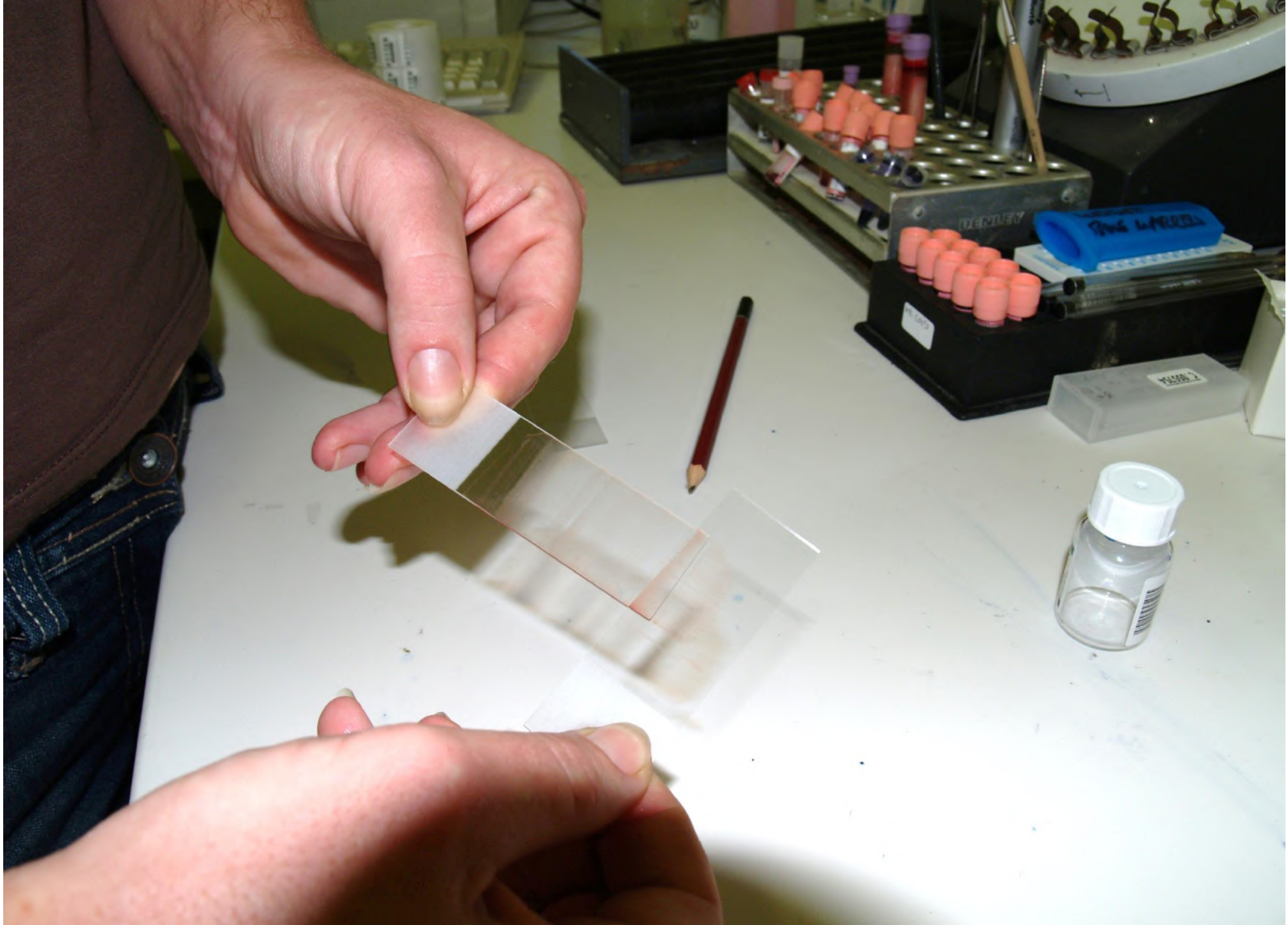
SLIDE & SPREADER: CROSS

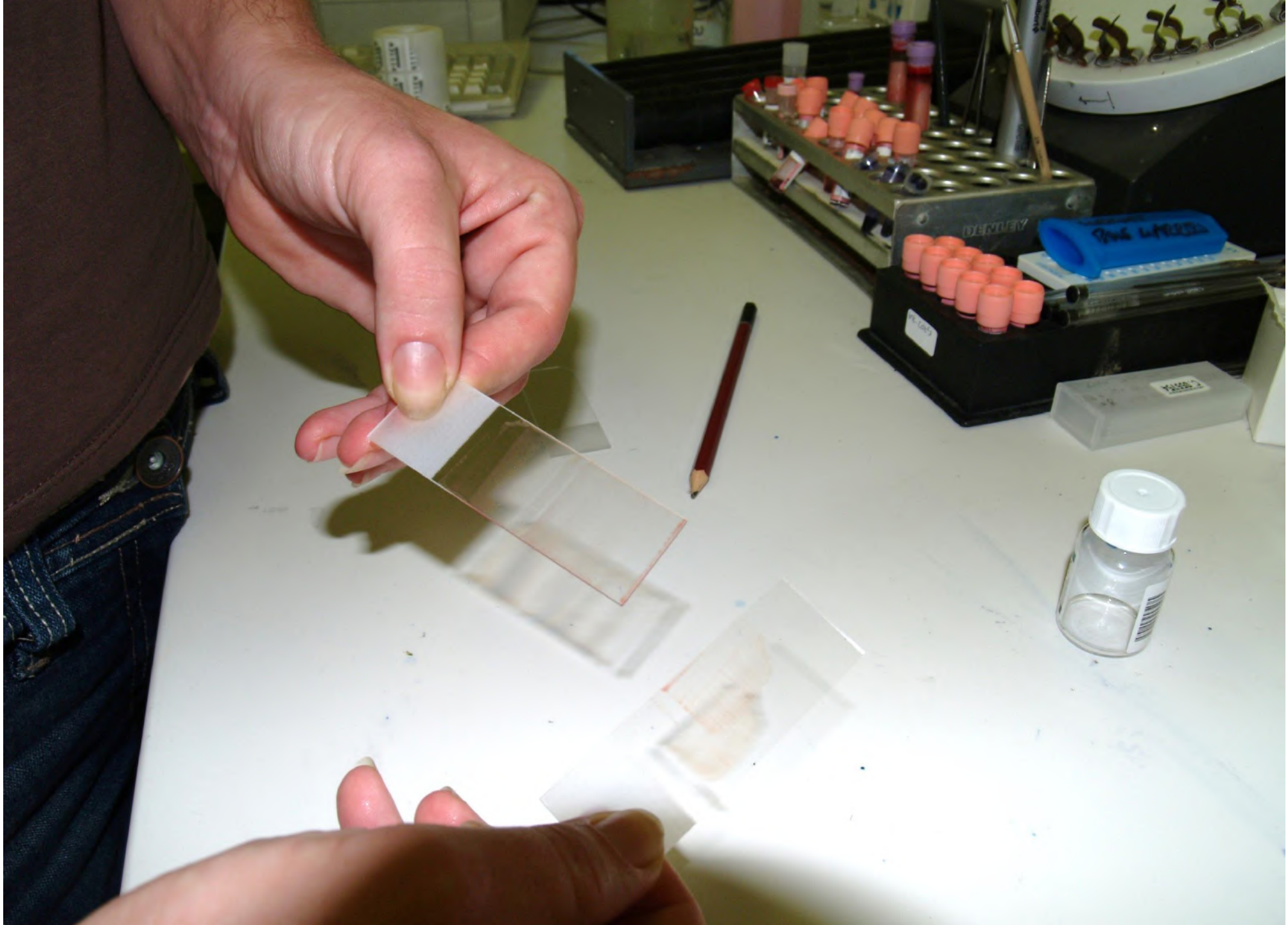
Do not apply excessive downward pressure

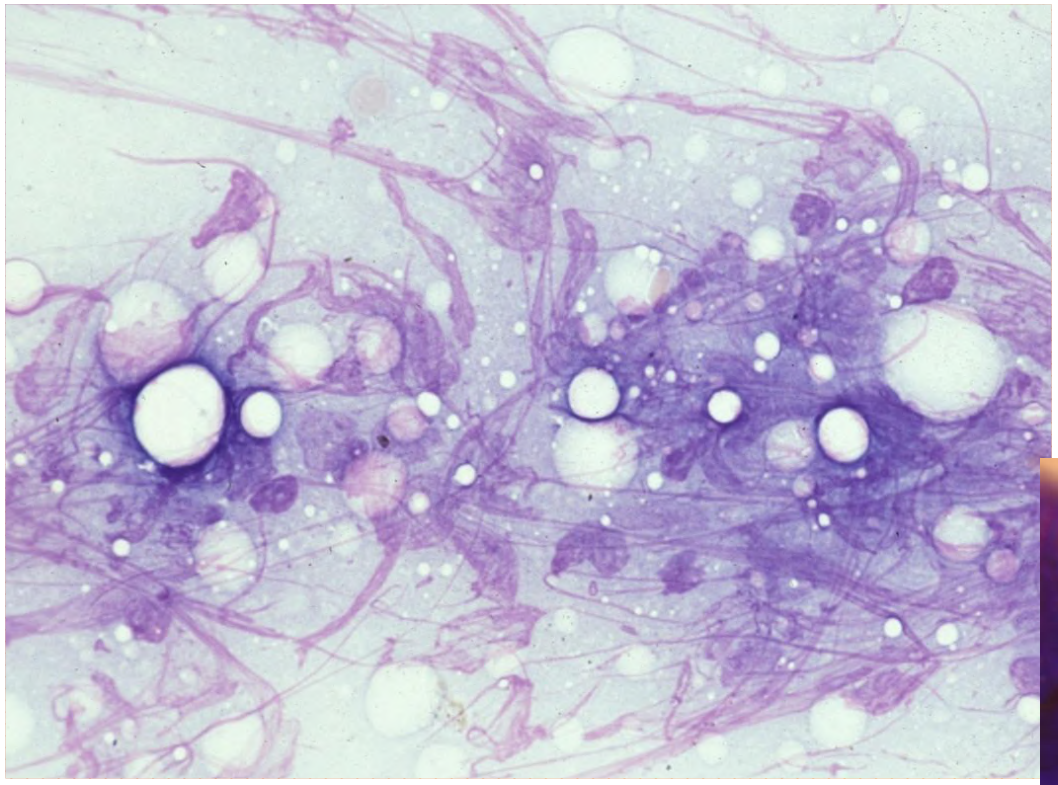
Stop when material starts spreading

Two smears for evaluation (material under the spreader slide)

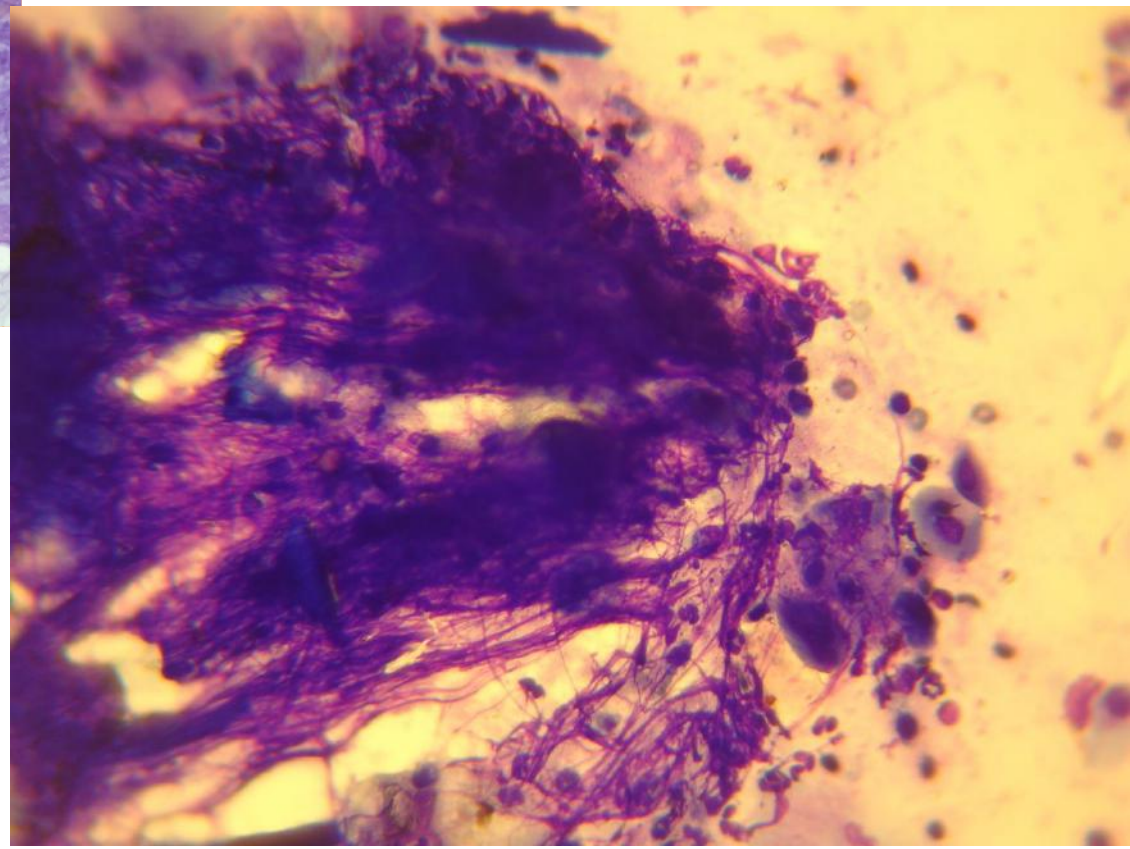


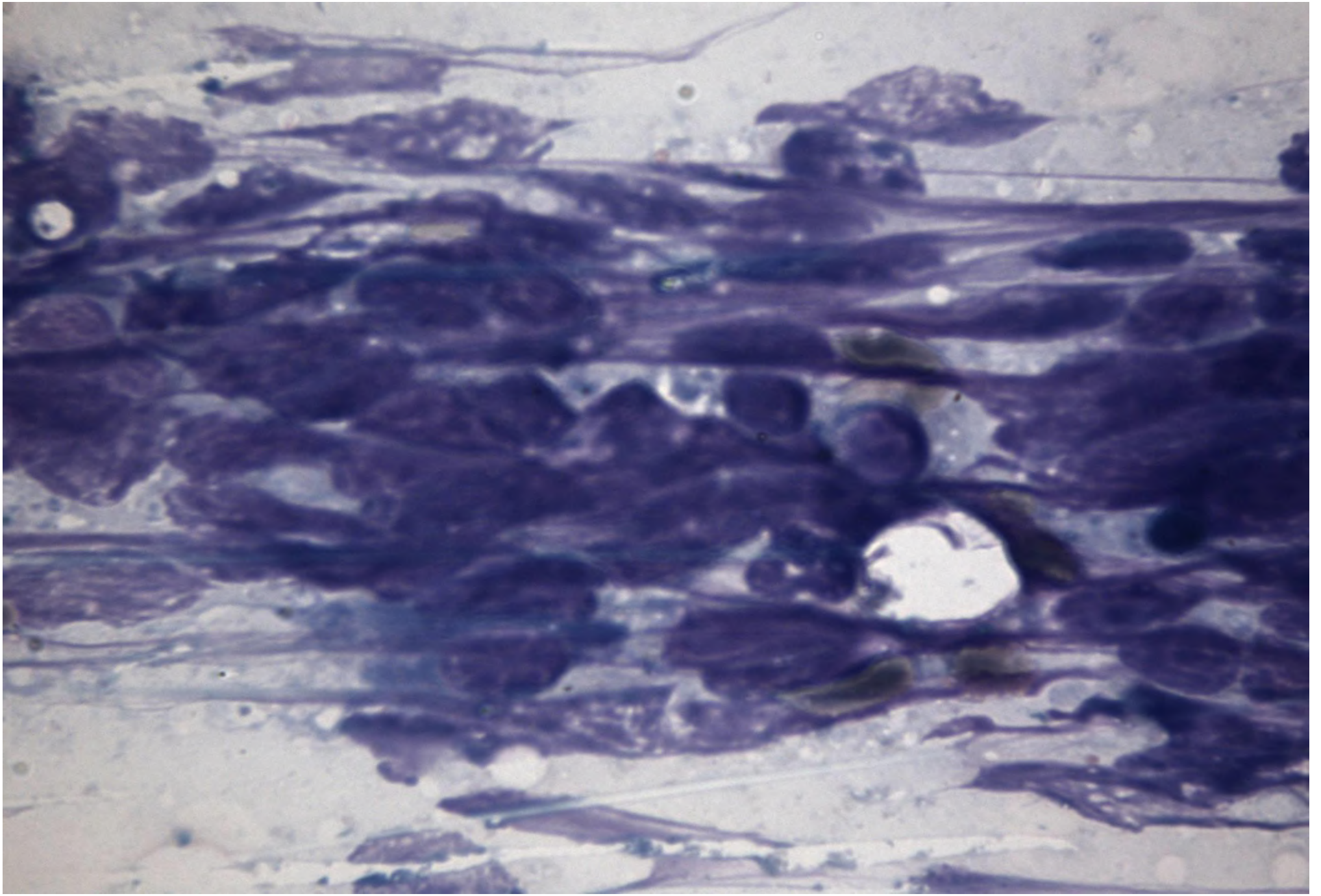






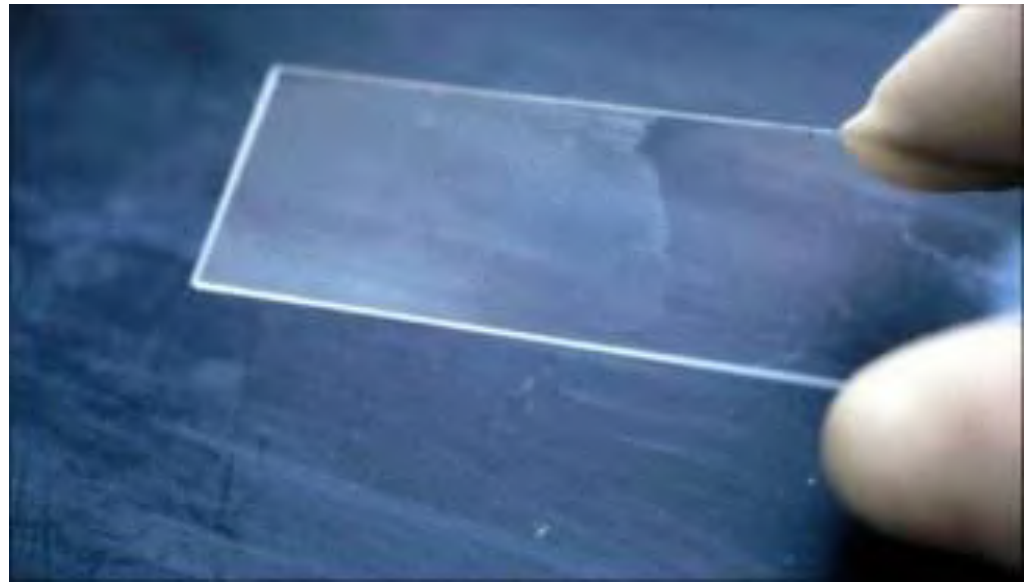
Cell preservation
(do not apply excessive
pressure during
spreading)





Smear Preparation

- **Prepare multiple smears**
- **Air-dry slides at room temperature**



Stain: Rapid stains (Diff-Quik)

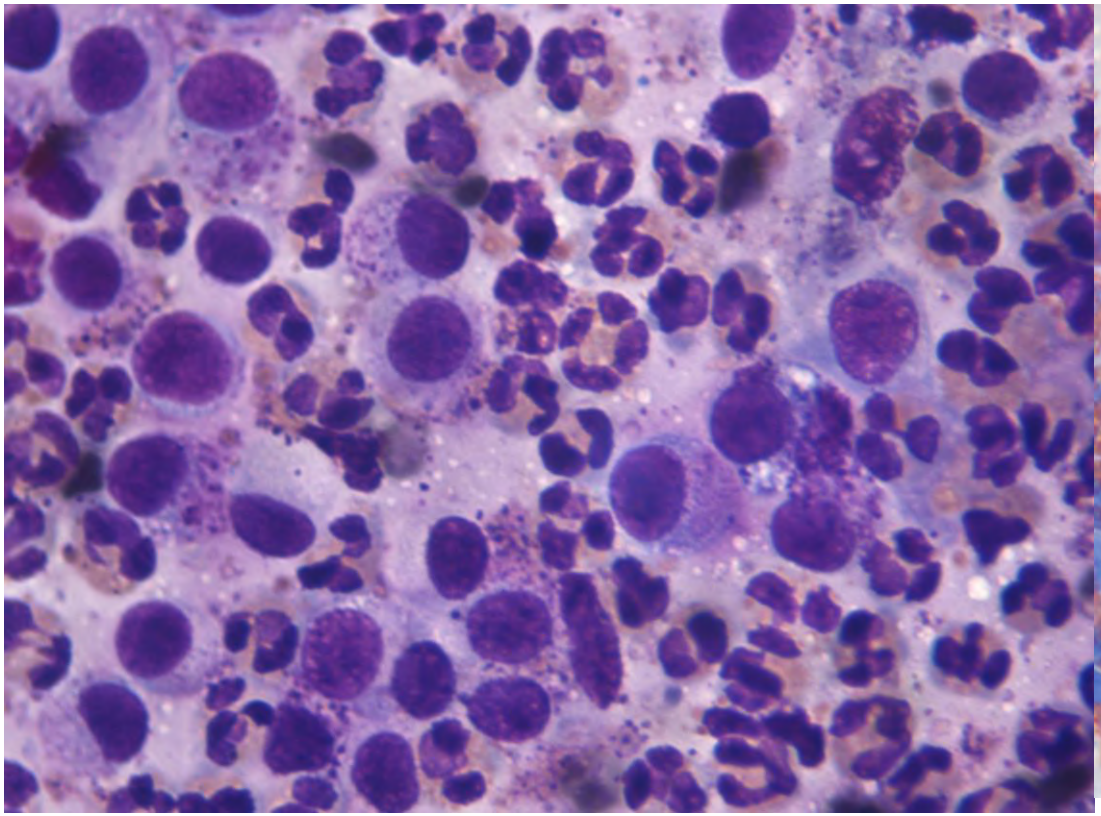
- 1. the fixative solution
- 2. stain solution No1 (red)
- 3. stain solution No2 (blue).

- Water based (aqueous stains)
- Although these stains are relatively expensive, are convenient and come with easy to use instructions
- When appropriately maintained provide adequate quality staining (blood smears and Cytology smears)

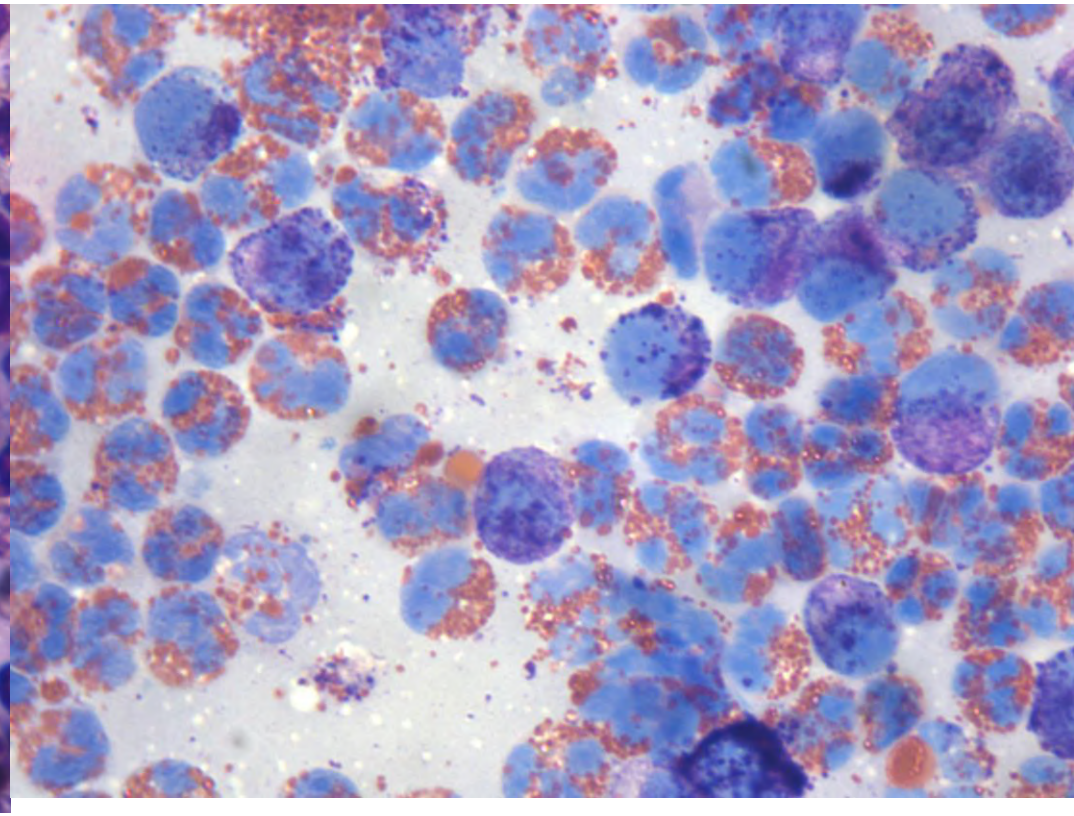
Stain: Diff-Quik –Points to remember

- Tap off excess red stain before moving into blue
- Do not dry slide using blotting paper
- Longer staining time is needed when smears are thick
- Dip slide back to blue if cells appear unstained
- Replace blue stain regularly - red stain doesn't deteriorate as rapidly as blue
- Check the quality of the Diff-Quik stains by staining a blood smear
- Create a protocol for routine filtering and complete replacement of stains & fixative
  - Based on usage & stain quality check
  - Stain & fixative jars should not be topped-off but completely replaced
- Keep a second set of Diff-Quik for "Dermatological" cytology
  - Bacteria, yeasts, cellular debris from skin (& ear) may end-up in stain
  - If deposited on tissue FNA slides can create artefacts and diagnostic confusion.

Diff-Quik stain: Inconsistent in staining granules of mast cells, eosinophils & basophils



Diff-Quik

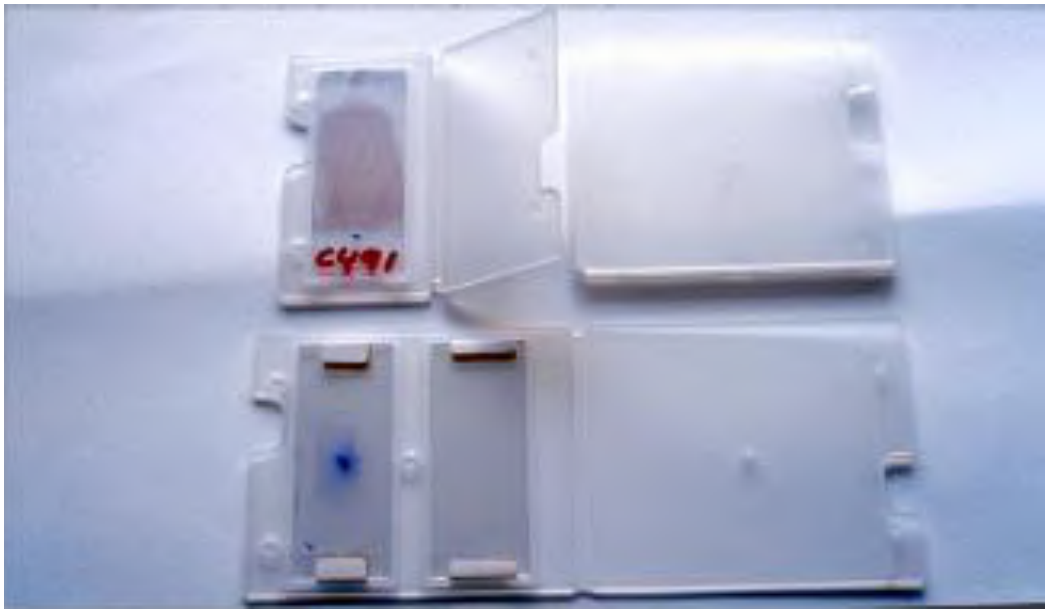


Modified Wright-Giemsa
(Reference Lab)

Mast cells - Eosinophils

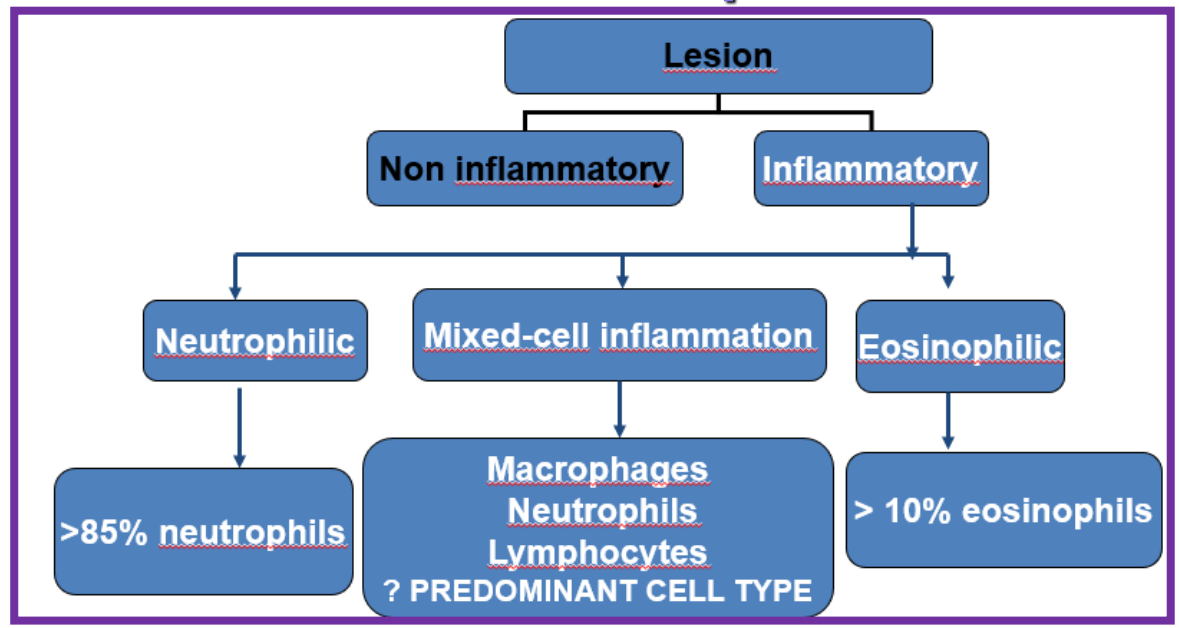
Submission of smears to an External Diagnostic Lab

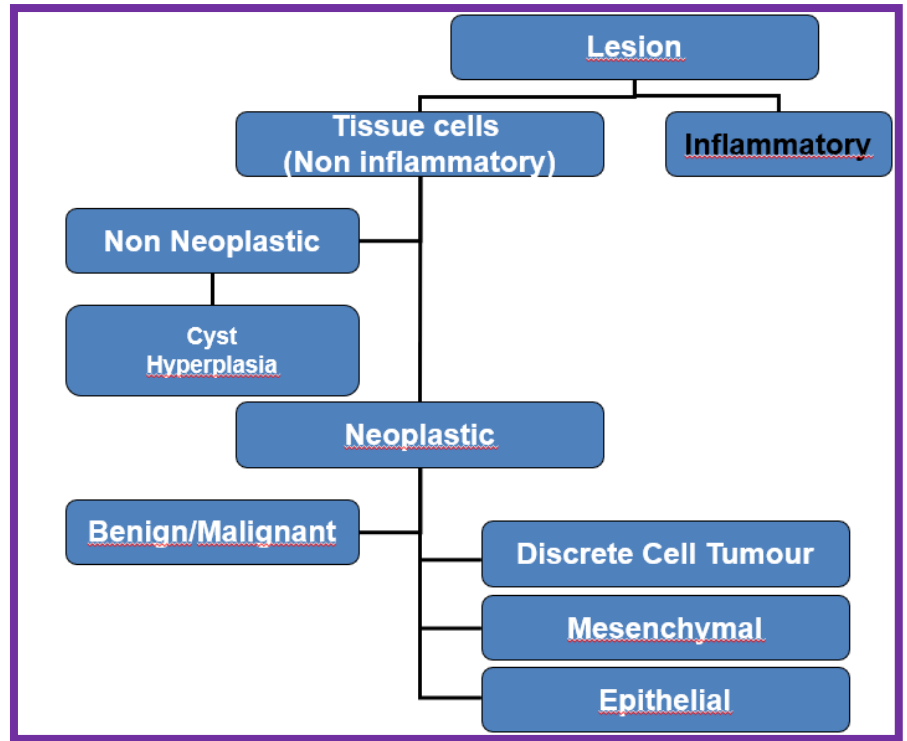
- Good communication with the clinical pathologist is essential
  - Clinical history
  - Physical exam findings
  - Duration of lesion is known
  - Anatomical position and description of lesion
- Smears should be packaged separately from biopsy samples in formalin pots
  - fumes from sealed formalin-filled containers will damage the cytology samples
- Smears should be completely air-dried before packaging
- Slides should be kept out of the fridge.

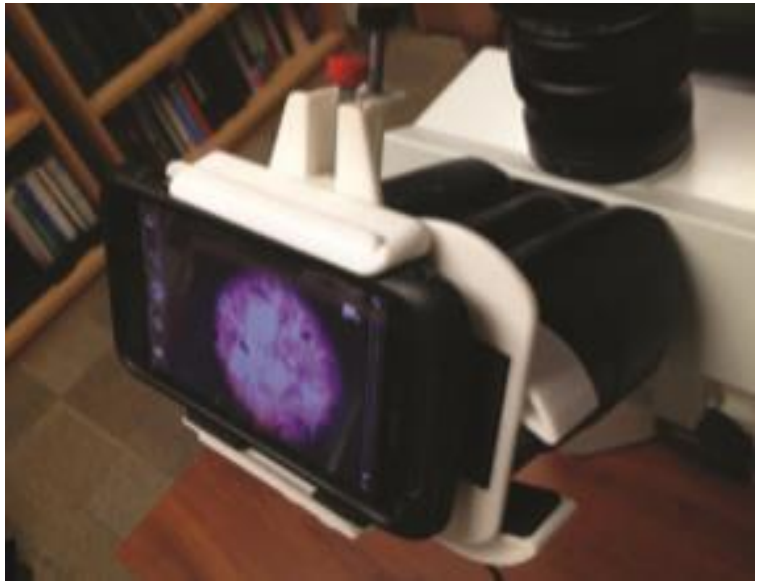


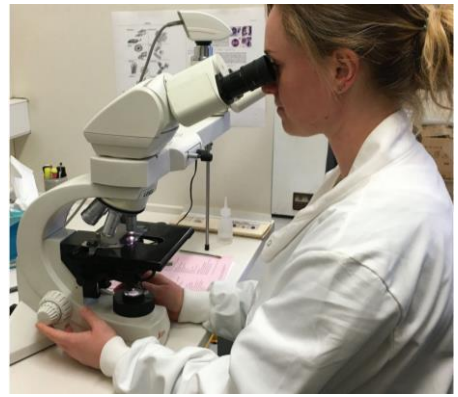
In-clinic microscopic examination - Principles

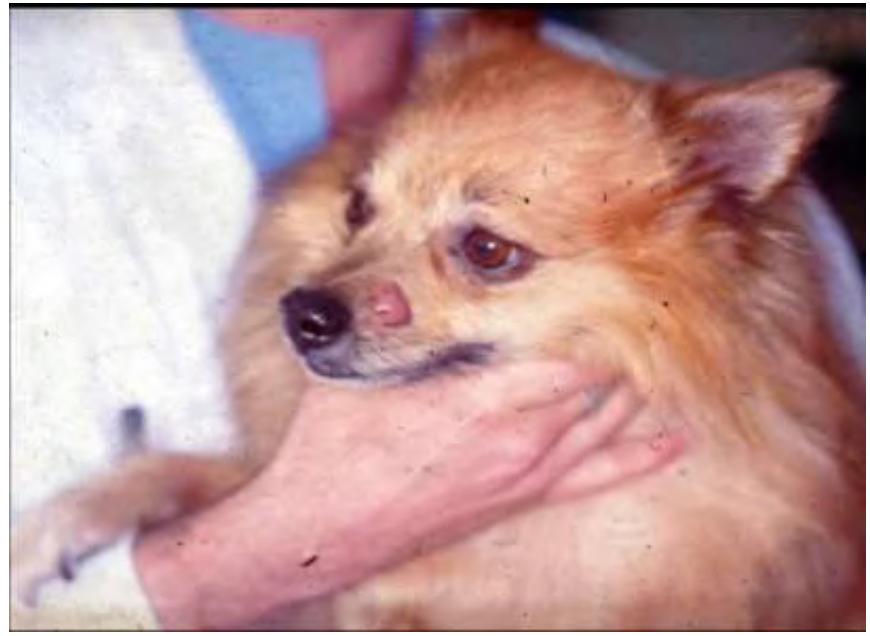
- Examine the entire smear(s) spending most time using a low magnification lens (x10/x20)
- Evaluate only intact cells
- Recognise artefacts, stain precipitates
- Establish a methodical approach
- Train in order to be able to answer clinically relevant questions
- PRACTICE – PRACTICE-PRACTICE
  - Plan your day so you can have time for Cytology
  - Comfortable area
  - Invest in a good microscope
  - Take good care of the microscope





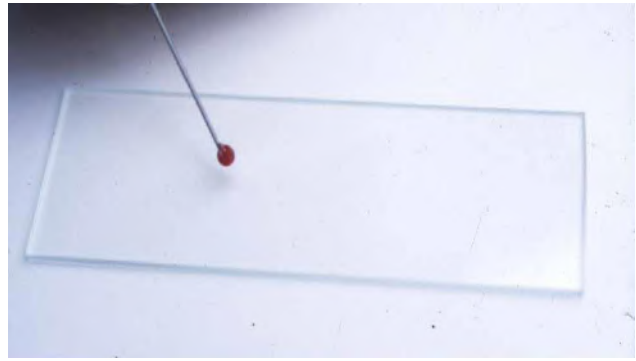


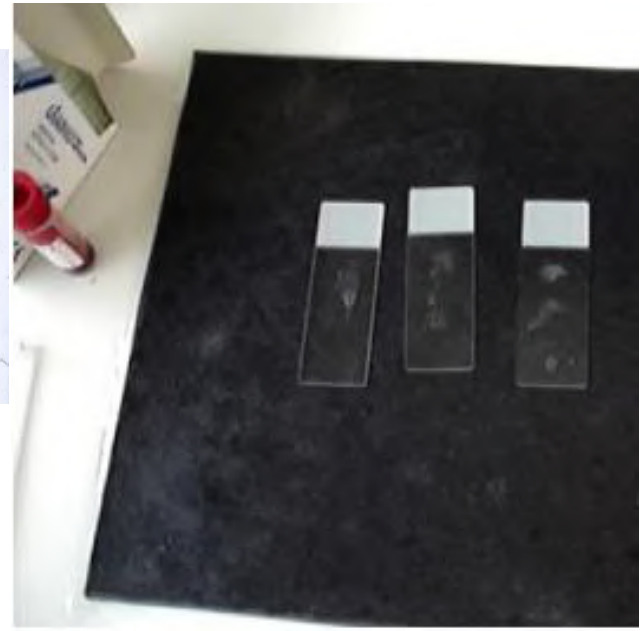


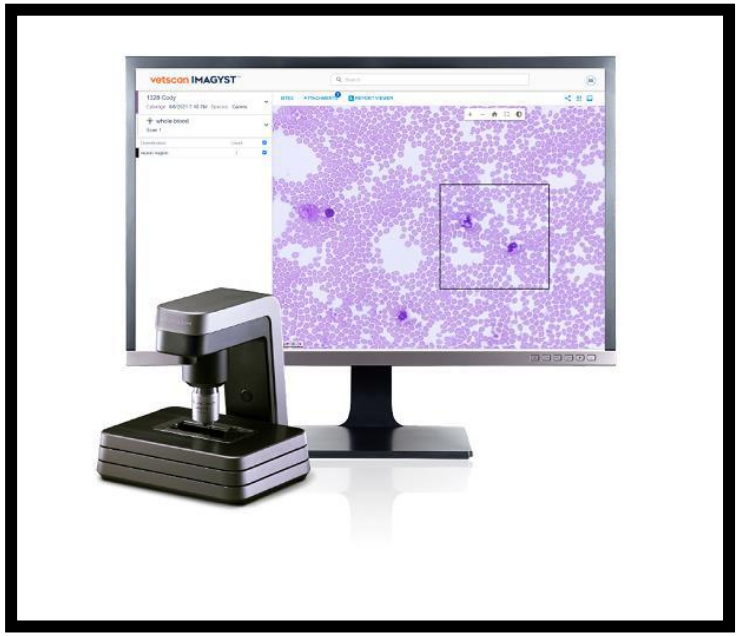


Content

- Advantages & limitations
- Sample collection
- Smear preparation & Staining
- Examination
- **Telecytology**







In-Clinic Automatic slide scanning & image acquisition

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Technical Note

Smartphone adapters for digital photomicrography

Somak Roy, Liron Pantanowitz, Milon Amin, Raja R. Seethala, Ahmed Ishtiaque, Samuel A. Yousem, Anil V. Parwani, Ioan Cucoranu, Douglas J. Hartman

Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

E-mail: *Dr. Douglas J. Hartman - hartmandj@upmc.edu
*Corresponding Author

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DOI: 10.1002/dc.23851

ORIGINAL ARTICLE

Diagnostic Cytopathology. 2018;46:40–46. WILEY

Telecytology: Is it possible with smartphone images?

Davut Sahin MD[1] | Uguray Payam Hacisalihoglu MD[2] | Saime Hale Kirimlioglu MD[3]

Diagnostic Pathology

BioMed Central

Diagnostic Pathology 2009, **4**:19 doi:10.1186/1746-1596-4-19

Open Access

Methodology

Mobile cell-phones (M-phones) in telemicroscopy: increasing connectivity of isolated laboratories

Livia Bellina[†1] and Eduardo Missoni*[†2]

Received: 8 October 2018 | Revised: 29 January 2019 | Accepted: 13 February 2019

DOI: 10.1111/vcp.12768

ORIGINAL ARTICLE

Veterinary Clinical Pathology
An International Journal of Laboratory Medicine
WILEY

Impact of photographer experience and number of images on telecytology accuracy

Alyssa J. Brooker[1] | Paula M. Krimer[2] | Kristina Meichner[1] | Bridget C. Garner[1]

(*Vet Clin Pathol.* 2006;35:303–306)

Evaluation of static telepathology in veterinary diagnostic cytology

Paola Maiolino, Brunella Restucci, Serenella Papparella, Gionata De Vico

Telecytology: In-Clinic *MANUAL acquisition*

- **Mobile phone (smartphone) & Microscope**
  - **(static photos, videos)**

- **Most common type of samples: Blood smear, FNA, Urine**
- **Not suitable for all types of samples**
- **Diagnostic usefulness depends on microscopist's experience**
  - **Very good agreement in diagnosis when is used by Clinical Pathologists**

Smartphone – Image acquisition

Scan slides

a. Identify areas with more dense cellularity
b. Identify areas of interests

Take photos

a. 2 images (x 10 objective)
b. 5 images of different microscopic fields (x40 and/or x100 oil lens)

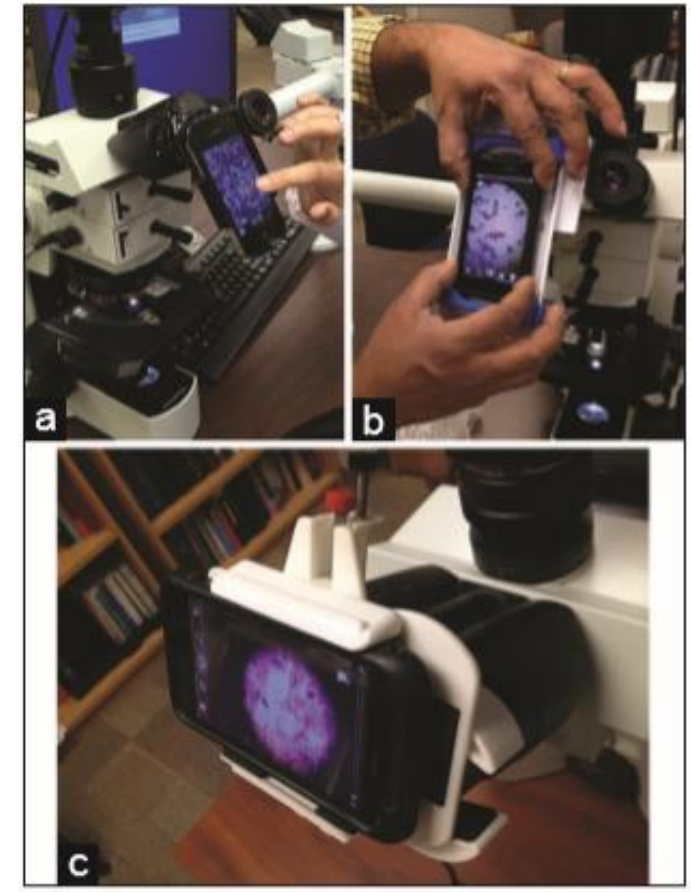
Make videos

i. 3-6 videos of 30-60 seconds each
ii. Moving smoothly around slide as with traditional assessment
iii. Stopping for few seconds in areas with clusters of cells

Capture images and make videos from different microscopic fields

i. in cellular areas with good staining,
ii. avoid areas with mostly ruptured cells
iii. avoid areas where the cells are too thick

Ensure image is in focus



If buying an adapter

1. Measure the eye piece diameter of your microscope (internal and outer diameter as different products based on different measurements)
2. Check if your eye piece is removable.
3. Many options for adaptors ranging from ~400-600 kr
4. Review the specifications to ensure compatible with your microscope and smartphone

Finally: a good smartphone adapter for a microscope

August 8, 2019

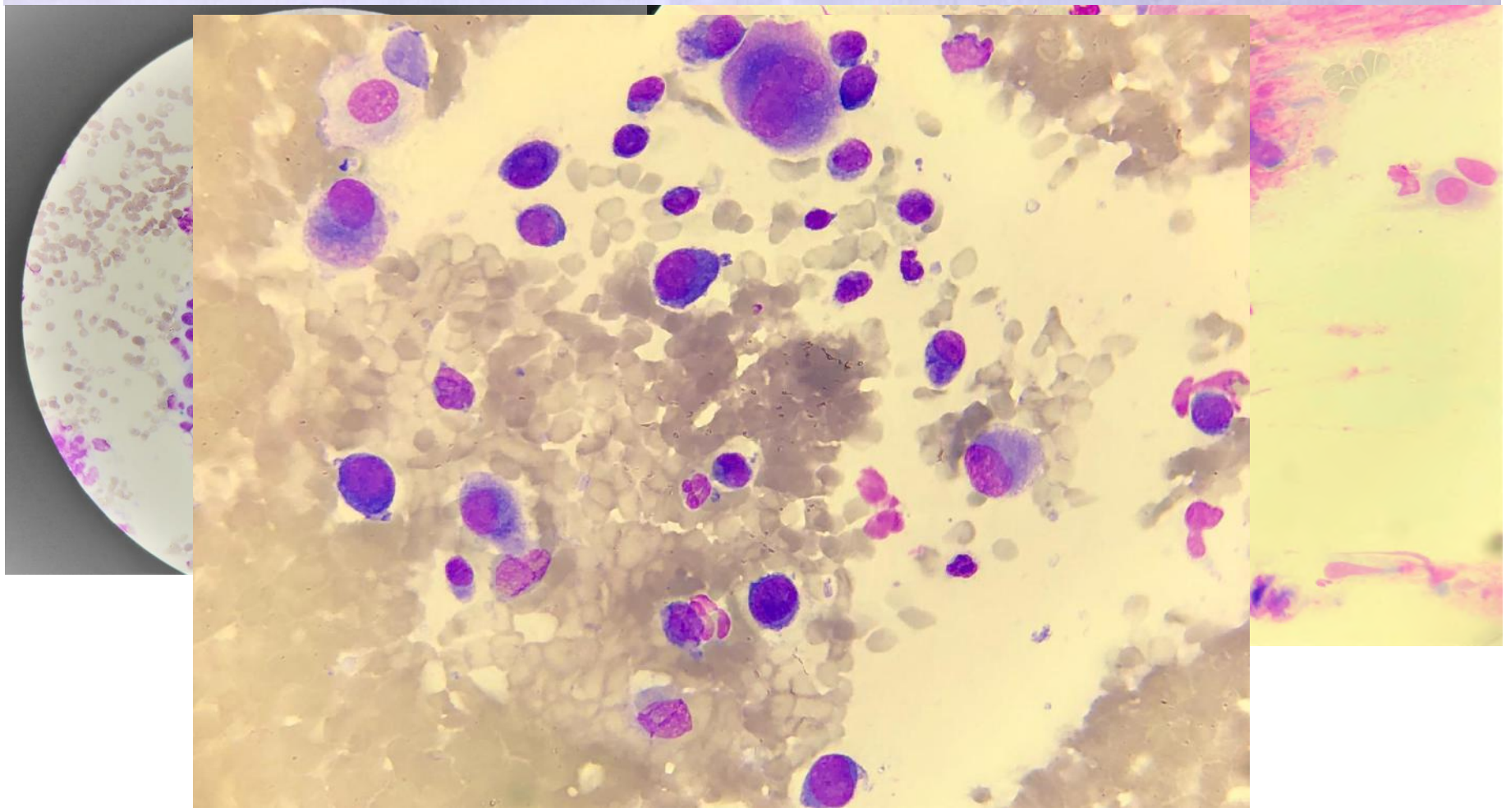
https://www.youtube.com/watch?v=ZQUxmHl4xf0&

Article overview: First I give some theoretical background information, and later on in the article I will talk specifically about the smartphone adapter that I presented in the video.

Clinical Notes:

Malignant mesenchymal neoplasm - SARCOMA

Lump 2x2cm on Dorsum to Right of thoracic spinal processes , 1st noticed 2-3d ago, hard mass below epidermis, no palpeable roots to underlying tissues. V bloody on FNA - 2 sites from same mass taken.



Another idea – Manual slide scanning

manual Whole Slide Imaging | M

microvisioneer.com/manualwsi

Camera on Microscope + Software

MICRO VISIONEER

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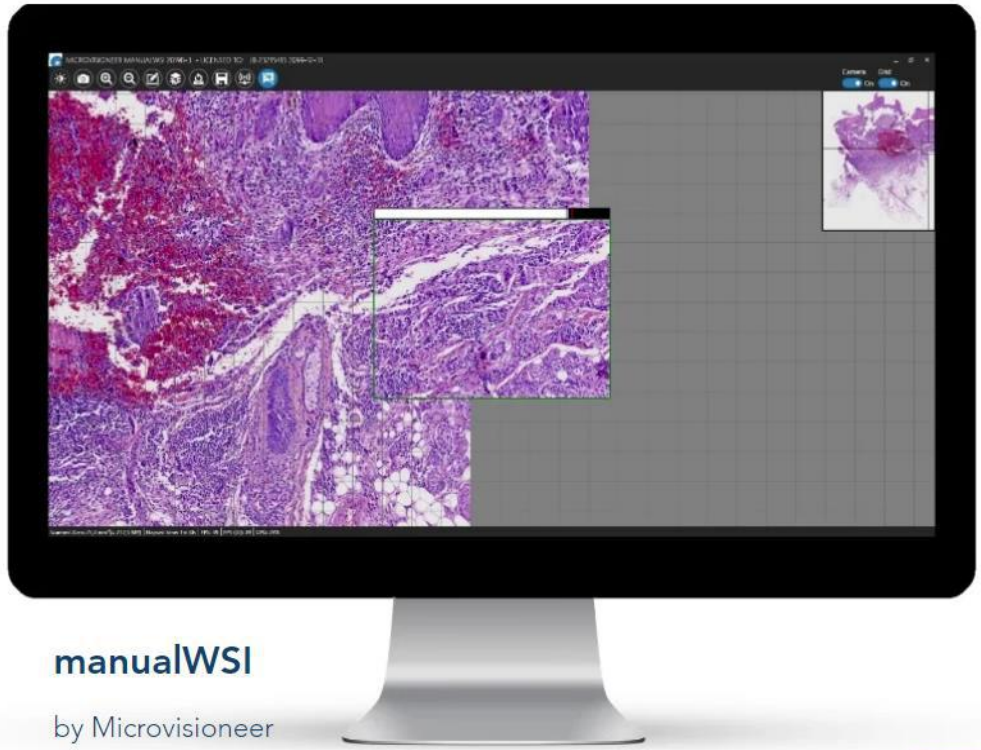
MANUAL WHOLE SLIDE IMAGING

Save time. Save money. Save images.

manualWSI software

Upgrade your microscope to a manual slide scanner.
Start creating virtual microscope slides now for any digital pathology application.

Our software solutions are affordable and immediately available.
Unlimited panoramic image stitching used in 55+ countries worldwide.



Now also available as **Fluorescence-Edition** for fluorescence slide scanning!

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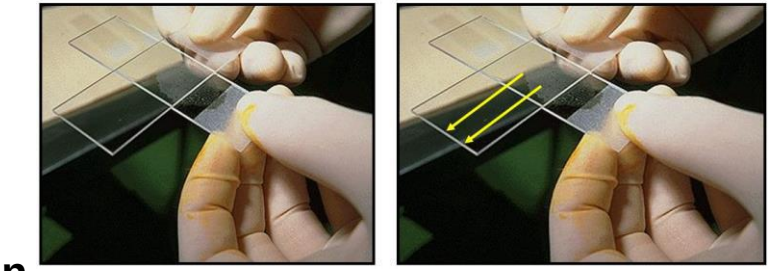
Camera on Microscope + Software

Example of manual scanning using

- **FNA**
  - Use “Needle” only technique when appropriate
  - When use “Syringe+Needle” technique
    - Apply 2-3 ml suction
    - Release the plunger before removing the needle
    - Do not spend more than 5-6 seconds sampling the lesion

Smear preparation



- **Smear preparation**
  - Expel the material on one end of the slide
  - Always spread the material
  - Avoid applying excessive pressure with spreader slide on the aspirated material
  - Remember to examine the side of the spreader slide which came in contact with the material
- Look after the **rapid stain** you are using – always keep the lids of the jars on
  - Use a blood smear to decide whether the stains need to be replaced with a fresh batch
- **Submission to an external veterinary diagnostic laboratory – Provide information related to the lesion**
  - Use appropriate slide holders – Do not put the slides/slide holders in the fridge
  - Do not pack the Cytology/Blood smears in the same bag with the tissue formalin pots for Histopathology
- **In-clinic Cytology**
  - Invest in a good microscope; look after the microscope; be in a comfortable space
  - Take your time; Be methodical during the microscopic examination
  - Do Microscopy every day, if you can; Attend courses; Know your limitations
  - Consider Telecytology

Tusen takk!

Har du noen spørsmål?