

# Collecting Optimal Lab Specimens

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P A T H O L O G I S T S · P A T O L O Ë

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**Drs Du Buisson, Kramer, Swart, Bouwer Inc./Ing.**




# Where does the lab come in?

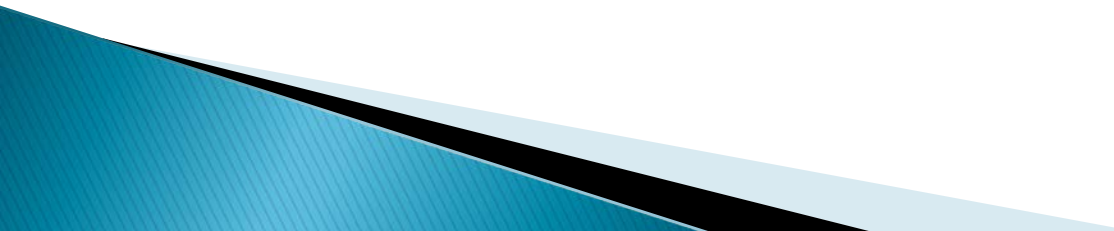
- ▶ Patient
    - Problem
    - Background
  - ▶ First contact
    - History
    - Examination
    - Idea (working diagnosis, differential)
  - ▶ Tests
    - Lab
    - Radiology
    - Referral
  - ▶ Interpretation & final diagnosis
  - ▶ Management
    - Immediate
    - Follow-up
- 
- ```
graph TD; A["Idea (working diagnosis, differential)"] --> B["Lab"]; A --> C["Radiology"]; A --> D["Referral"]; E["Interpretation & final diagnosis"] --> F["Follow-up"];
```

# Levels of interpretation

## Examples

- ▶ X-rays – forearm fracture
  - ▶ Dipstix – ?UTI
  - ▶ Adult with anaemia – FBC
  - ▶ Child anaemia – FBC
  - ▶ ? Leukaemia – Bone marrow biopsy
  - ▶ HIV+ – septicaemia – blood culture
  - ▶ Heart mass – ? cardiac myxoma
  - ▶ Breast lump
- 

# Best interpretation

- ▶ Adequate information
  - ▶ Diagnostic skill levels
  - ▶ Appropriate testing
  - ▶ Good quality specimen
- 
- ▶ All put together → Good diagnosis
- 

# Sampling principles

- ▶ Best possible tissue
  - TB
  - Breast cancer
- ▶ Correct test
- ▶ Correct indication
  - Appendectomy
  - Ovaries
  - Cancer markers
- ▶ Correct referral
  - Biopsies, best person
- ▶ Best test for situation
  - Breast lump – FNA or core Bx
  - Finances

# Sampling principles

- ▶ Know what to expect
- ▶ Interpretation skill level
  - LFT
  - Viral Hepatitis
- ▶ Quality result
  - Correctness
  - Completeness
  - Relevant
  - Up-to-date information
  - Treatable
  - TAT

# HIV patients

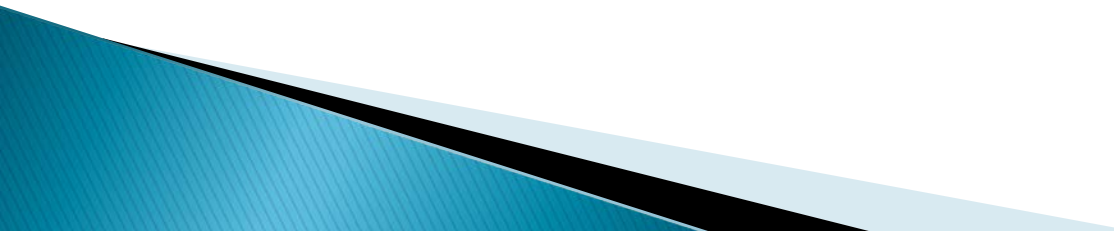
- ▶ Not any different than normal individuals
- ▶ Special emphasis
  - TB, DRTB, XDRTB
  - Other opportunistic infections
    - PCP
    - Cryptococcus
    - Histoplasmosis
  - ESBL
  - MRSA
- ▶ Tumours
  - Lymphoma
  - Kaposi sarcoma

# Fine Needle Biopsies

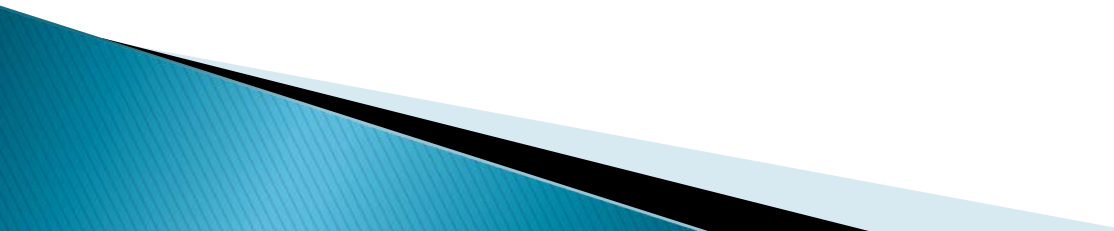




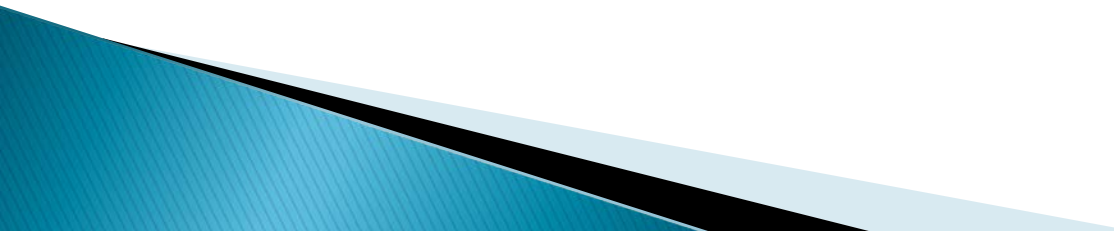
# Biopsy technique

- ▶ Representativeness
  - ▶ Maximize cellularity
  - ▶ Minimize blood (trauma vs. vascularity)
  - ▶ Recognizable patterns → uniformity
  - ▶ Limited material vs. optimal yield
  - ▶ Broad categorization; directing further management
- 

# Biopsy technique

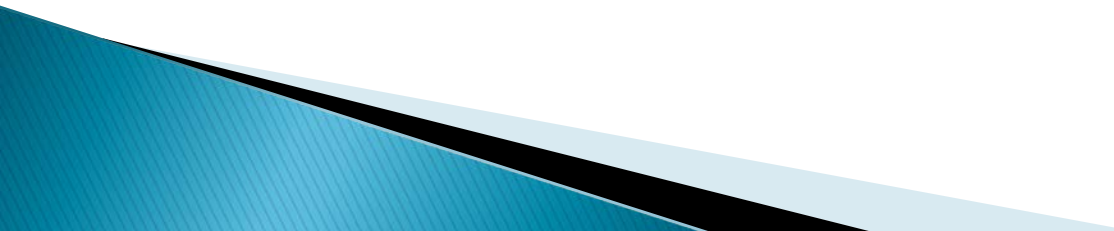
- ▶ Fixation NB
  - ▶ Communication NB
  - ▶ Screening expertise NB
  
  - ▶ Aspiration vs. no aspiration
  - ▶ Material on slide, not in syringe
  - ▶ Rather too many slides
- 

# NB Counselling

- ▶ Procedure
  - ▶ Reasons
  - ▶ Limitations
  - ▶ Expectations
  - ▶ If what, then...
  - ▶ Pain & bruising
  - ▶ Reassurance
  - ▶ Consent
- 

# Sterile technique

(or as sterile as possible)



Open the spray  
fixative!!



# Indications

- ▶ NB → PALPABLE LESIONS \*
- ▶ Lymphadenopathy – neck, axillae, groin
- ▶ Neck masses / cysts
- ▶ Thyroid nodules
- ▶ Breast lumps / cysts / abscesses
- ▶ Soft tissue lumps / cysts / abscesses

*\* Exception – deep lesions FNABx under guidance*

# Contra-indications

- ▶ Not many
  - ▶ Vascular tumours
  - ▶ Aneurisms
  - ▶ Dangerous locations (close to eye, carotid, etc.)
  - ▶ Ulcerated / infected skin
  - ▶ Better diagnostic modalities available
  - ▶ Sedation (?)
  - ▶ Intestines (hernias)
- } Risk for haemorrhage

# Axillary, cervical, inguinal lymph nodes / lymph node abscesses

- ▶ TB, TB, TB, TB, TB ... and more TB
  - Granulomatous inflammation
  - Suppurative inflammation
  - Caseous necrosis
  - Combination of above
  - Acid-fast bacilli on ZN
- Negative ZN?
  - TB culture
  - TB PCR



# Axillary, cervical, inguinal lymph nodes

- ▶ Abscess (pyogenic)
  - Suppurative inflammation
  - ZN negative
  - No caseous necrosis
  - No malignant cells
  - MCS

# Axillary, cervical, inguinal lymph nodes

- ▶ **Fungal infection**
  - Suppurative inflammation
  - **Granulomata**
  - Necrosis +/-
  - **Fungi identifiable** – hyphae or spores, yeasts (Candida, Aspergillus, Cryptococcus, Histoplasma)
  - Microscopy
  - Fungal culture

# Axillary, cervical, inguinal lymph nodes

## ▶ Malignancies

### ◦ Lymphoma

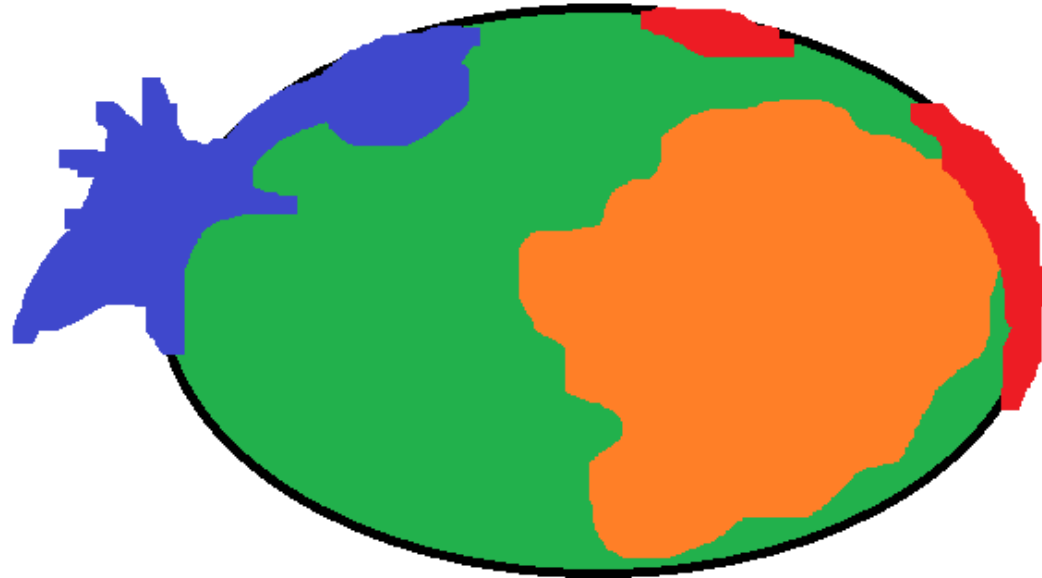
- Large cell
  - Small cell
  - Hodgkin
- } Morphology alone difficult
- Immunophenotyping
  - Flow cytometry

### ◦ Metastatic

- Carcinoma
  - Adenocarcinoma
  - Squamous cell carcinoma
- Melanoma

# Axillary, cervical, inguinal lymph nodes

- ▶ Kaposi sarcoma
- ▶ Combination with infection
- ▶ “Reactive” lymph node – follicular hyperplasia



# Neck masses / cysts

- ▶ Abscesses
- ▶ Lymphoepithelial cysts in HIV
- ▶ True cysts
  - Branchial cleft
  - Thyroglossal
  - Epidermal
  - Salivary
- ▶ Cystic tumours → Necrotic squamous cell carcinoma
- ▶ Solid salivary gland tumours
  - Pleomorphic adenoma
  - Warthin tumour

# Breast lumps

- ▶ Cyst
  - With apocrine cells
- ▶ Abscess
  - Ductal ectasia
- ▶ Papillary lesions
  - Benign
  - Malignant LG
  - Malignant HG
- ▶ Fibroadenoma
- ▶ Phylloides tumour

# Breast lumps

- ▶ Carcinoma
  - Ductal HG
  - Ductal LG
  - Mucinous / colloid
  - Metaplastic
  - Lobular
  - Medullary
- ▶ Fat necrosis

Primary diagnosis? Depends on the case  
Metastatic or recurrent tumour? Definitely

# Breast lumps

- ▶ Good indications
  - Cystic lesions
  - Recurrent / metastatic breast cancer
  - Confirmation of locally advanced disease
  - Axillary staging
  - Primary screening in low resource settings
  
- ▶ Majority of false negatives
  - Sampling error
  - Poor fixation



# Thyroid nodules

- ▶ Abscess
- ▶ Colloid cyst
- ▶ Papillary carcinoma
- ▶ Follicular neoplasms
  - Adenoma
  - Carcinoma
- ▶ Subacute thyroiditis
- ▶ Anaplastic carcinoma

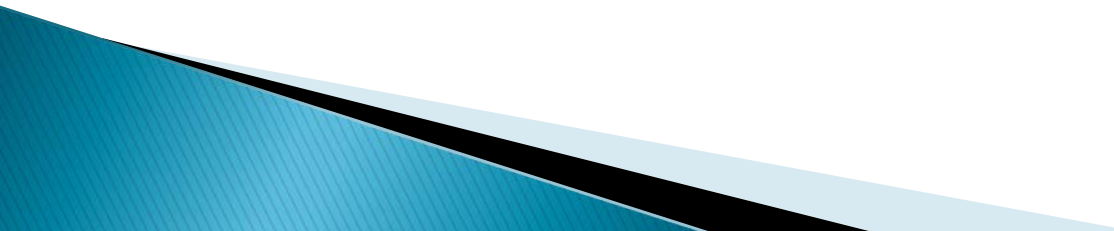
# Soft tissue lumps

- ▶ Abscess
- ▶ Lipoma
- ▶ Spindled cell tumour
  - LG
    - Benign
    - Malignant
  - HG
- ▶ Reactive (nodular fasciitis)
- ▶ Fat necrosis

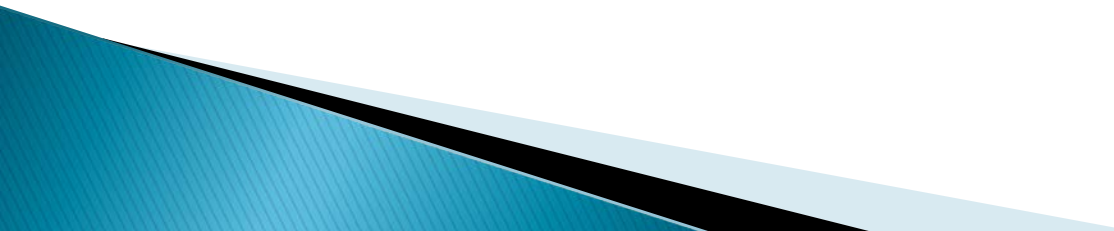
# Skin lesions

- ▶ Abscess
- ▶ Epidermal cysts
- ▶ Solid tumours (pilomatrixoma)
- ▶ Melanoma

# FNABx – Conclusion

- ▶ Limited indications
  - ▶ Importance of good technique
  - ▶ Correct processing
  - ▶ Realistic expectations
  - ▶ Good communication
  - ▶ Cost effectiveness
- 

# Cervical cytology

- ▶ South Africa has one of the highest incidences of human immunodeficiency virus (HIV) infection in the world
  - ▶ 2010 mid-year population – 10.5%
  - ▶ Total 5.2 million
  - ▶ 17% of the population between 15 and 49 years of age is HIV-positive
- 

# Cervical cytology

## ▶ HIV infection

- increase in genital infections by HPV family
- Persistent HPV infection → incorporation of the viral genome → high-grade dysplastic lesions and invasive carcinoma

## ▶ Integration of HPV viral genome

- disruption or deletion of the viral E2 gene which normally down-regulates E6 and E7
- Over-expression of E6 and E7 gene products → deregulation of the host cell growth cycle
  - binding to the tumour suppression molecule p53
  - inactivating the tumour suppression retinoblastoma gene product

# Cervical cytology


- ▶ The Bethesda System 2001
  - HPV infection & CIN1 → LSIL
  - CIN2 and CIN3 → HSIL
- ▶ Atypical cells
  - ASC-US
  - ASC-H
- ▶ Squamous Ca and others

# Cervical cytology

- ▶ Management approach
  - conservative approach in LSIL and ASC-US
    - repeating the smears in 6 to 12 month intervals
  - pro-active “see-and-treat” approach in HSIL, ASC-H, and persistent LSIL and ASC-US
    - colposcopic evaluation and LEEP / LLETZ
    - local anaesthetic
    - money- and time-saving benefit



# Cervical cytology

- ▶ LSIL 22.1%, HSIL 30.9% and squamous cell carcinoma 0.6%
  - ▶ Figures in line with similar rural African populations
  - ▶ Significantly worse than in other South African studies in urban centers
  - ▶ Highlights the disproportionate number of HIV-positive women with progressive premalignant HPV-induced cervical disease in a rural area with scarce resources
  - ▶ Younger patients vs. general population
- 

# Cervical cytology

- ▶ HIV-positive women should be screened more often (perhaps annually)
- ▶ Screening must be initiated earlier (age 25)
- ▶ Majority diagnosed histologically with CIN2 & CIN3
- ▶ High incidence of Bilharzia & Bacterial vaginosis
  - possible relationship between cervical Bilharzia infections with progressive HPV disease and cervical carcinogenesis in regions where Bilharzia is endemic
  - Bacterial vaginosis (perhaps in combination with Bilharzia) may compromise the normal barriers against HPV and HIV infection

# Cervical cytology

- ▶ Failure of the system to retain patients
  - 152 with HSIL
  - 2 with squamous carcinoma
  - 192 patients with LSIL without follow-up for possible persistence or progression
- ▶ Much bigger socio-political problem, infrastructure, etc.
- ▶ Logistical challenges of a cytological screening system in a region with scarce resources

▶ **Fixation!!!**                      **ASC-US!!!!**



# Pitfalls of the Conventional Pap Smear Technology

## 1. Sampling Errors


- Cells are not collected during sample taking
- Cells are not transferred on the slide
- Cells are not well preserved/fixed on the slides

## 2. Preparation Errors

- Obscuring elements such as blood and lubricants
- Not enough cervical cells on the slide
- Poor and not standardized staining

## 3. Interpretation Errors

- Abnormal cells not seen by the cytologist
- Abnormal cells are seen but wrongly interpreted



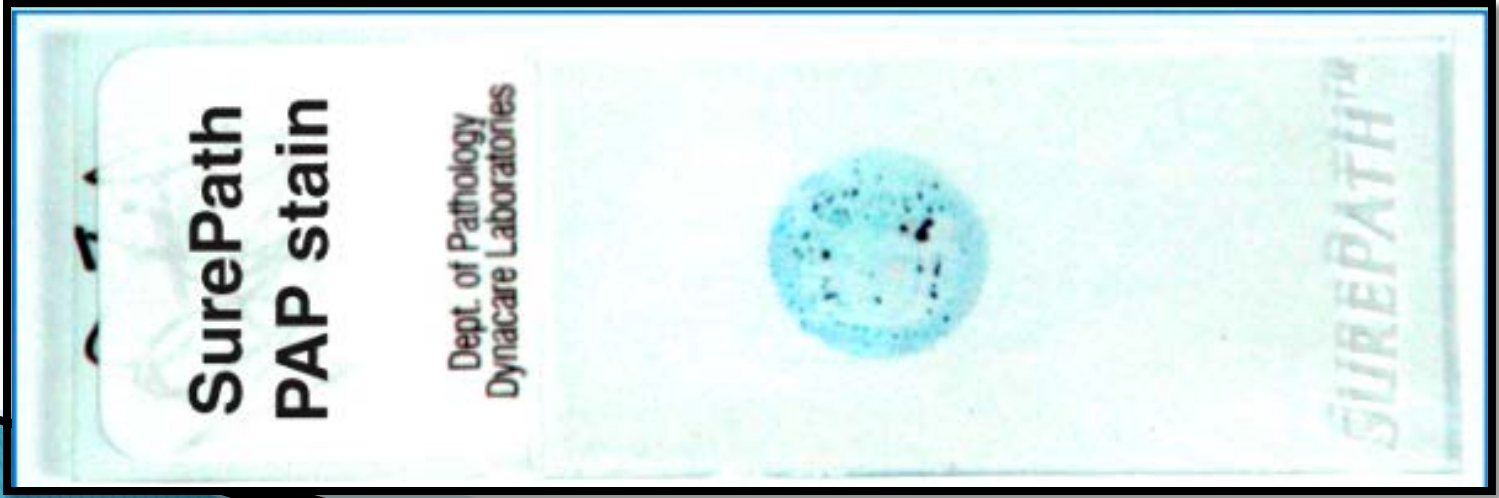
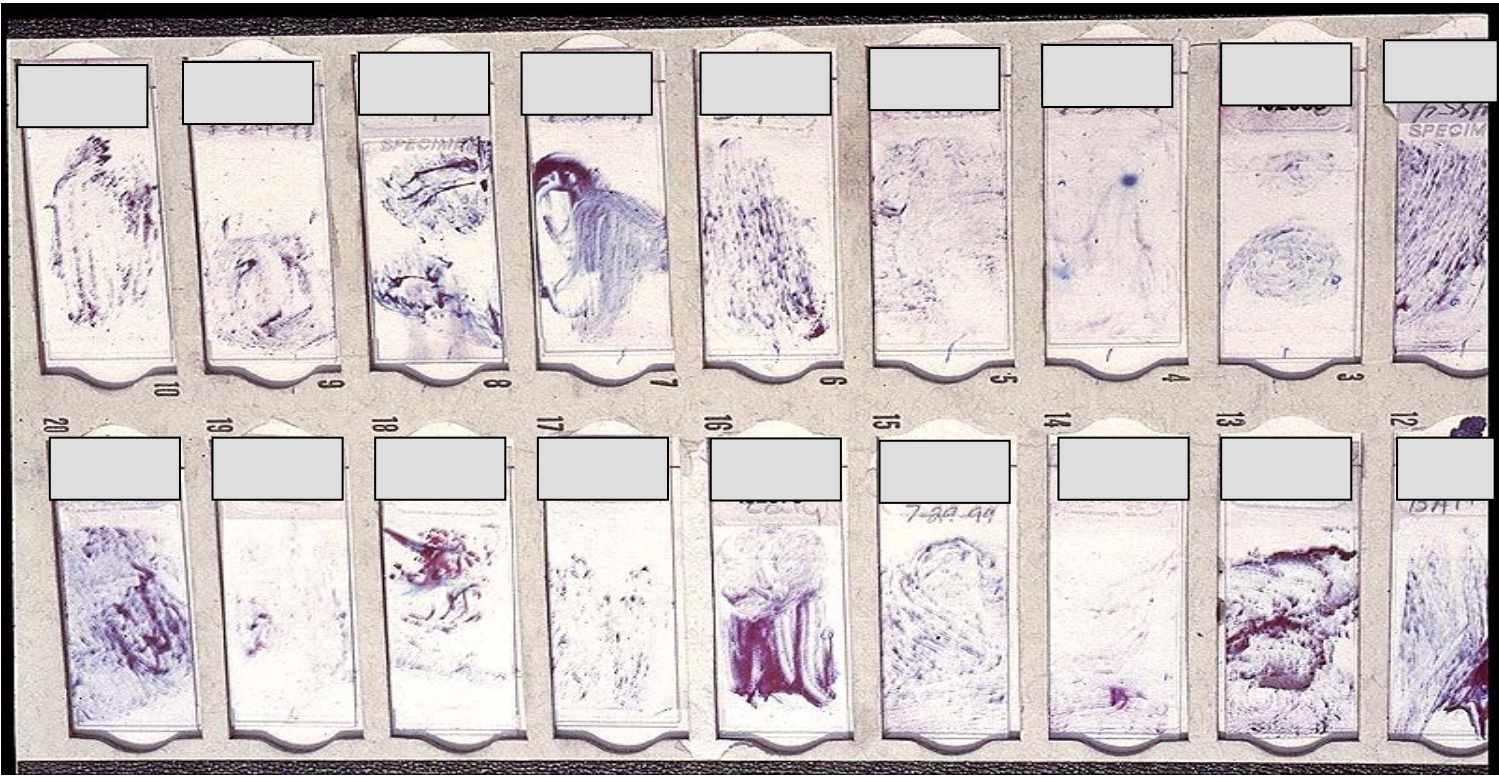
60% of False  
Negatives

# Liquid-based cytology

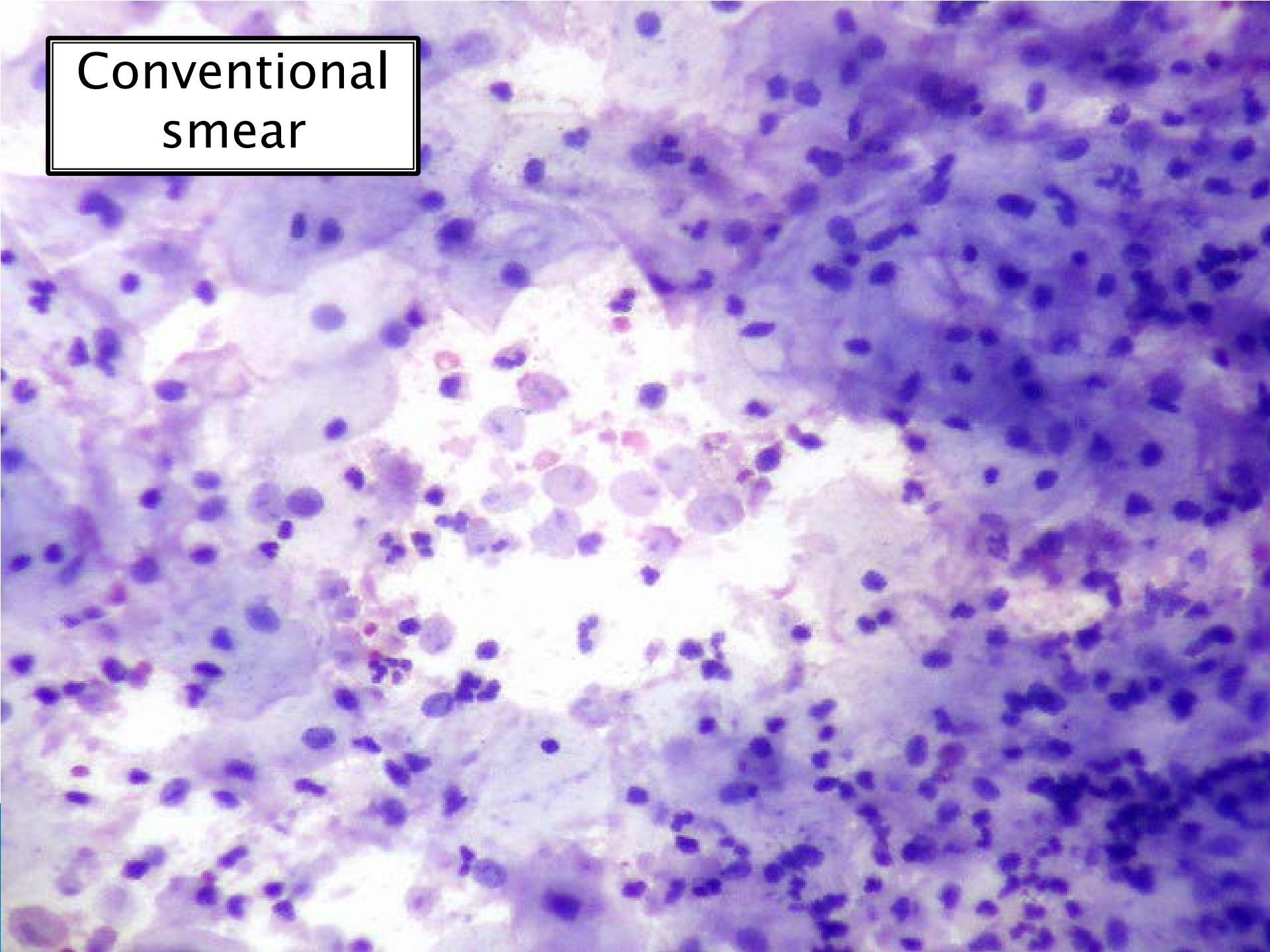
- ▶ Relatively new technique
- ▶ Ideal fluid fixative / transport medium
- ▶ Automated / semi-automated
- ▶ Advantages
  - Rapid screening
  - Easier screening
  - Clean smears
  - Well-preserved cells
  - Monolayer
  - Less blood
  - Fewer inflammatory cells
  - More than enough diagnostic material
  - Less ASCUS
  - Greater diagnostic yield
  - Fluid reserve for HPV testing
  - Cost

Conflicting literature

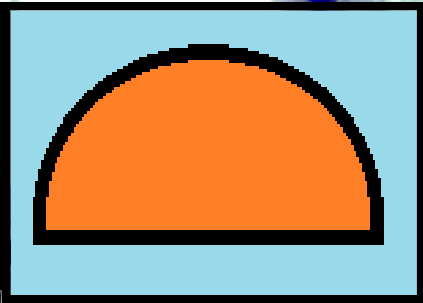




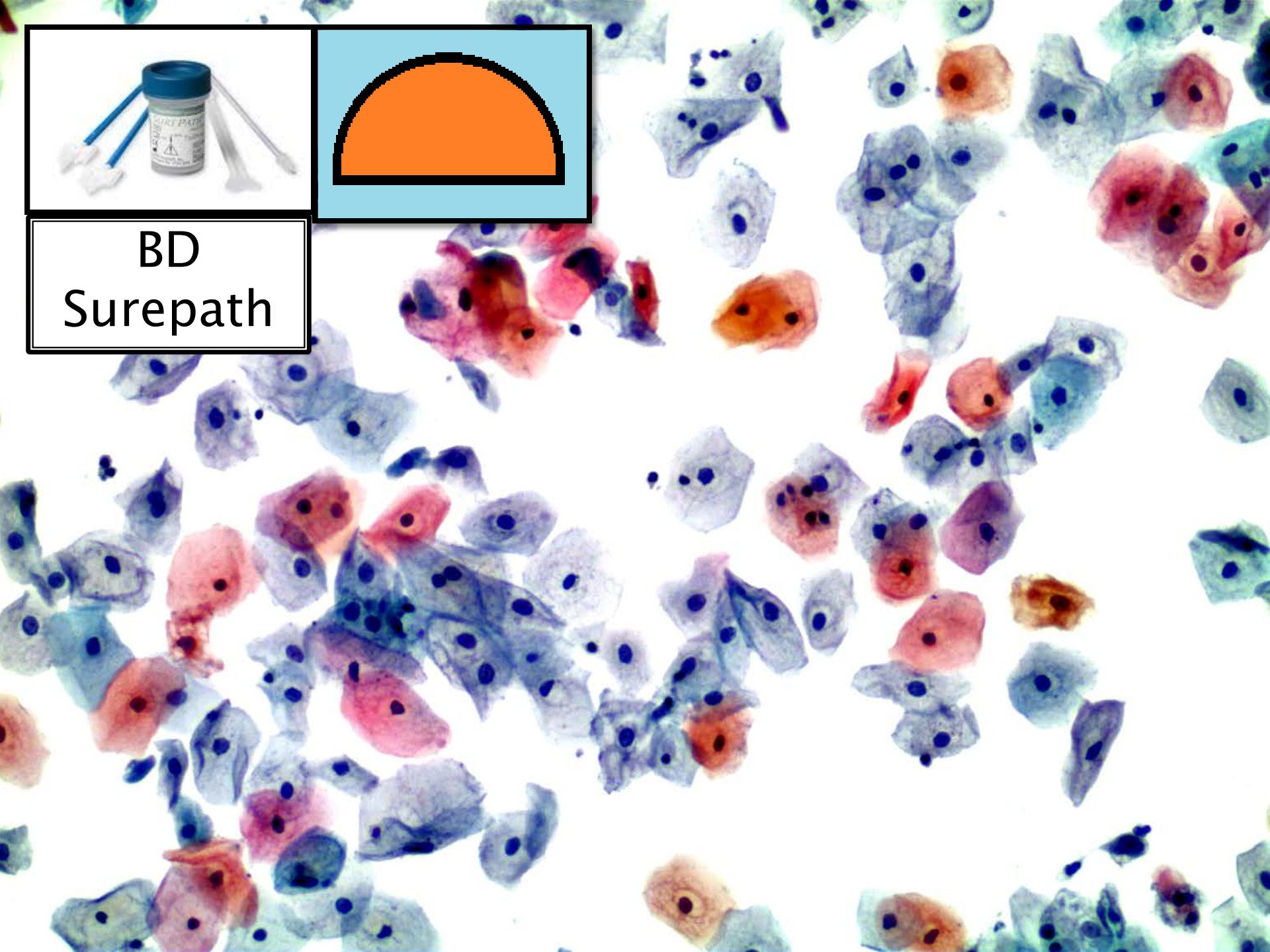
Conventional  
smear





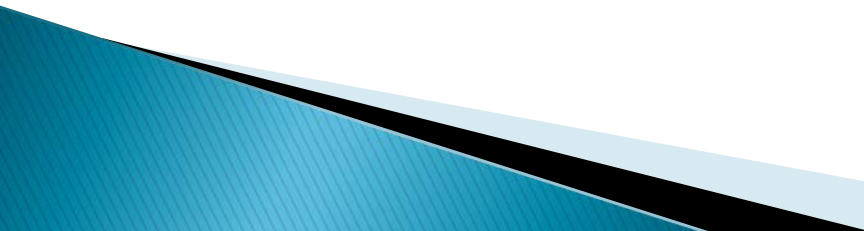


BD  
Surepath

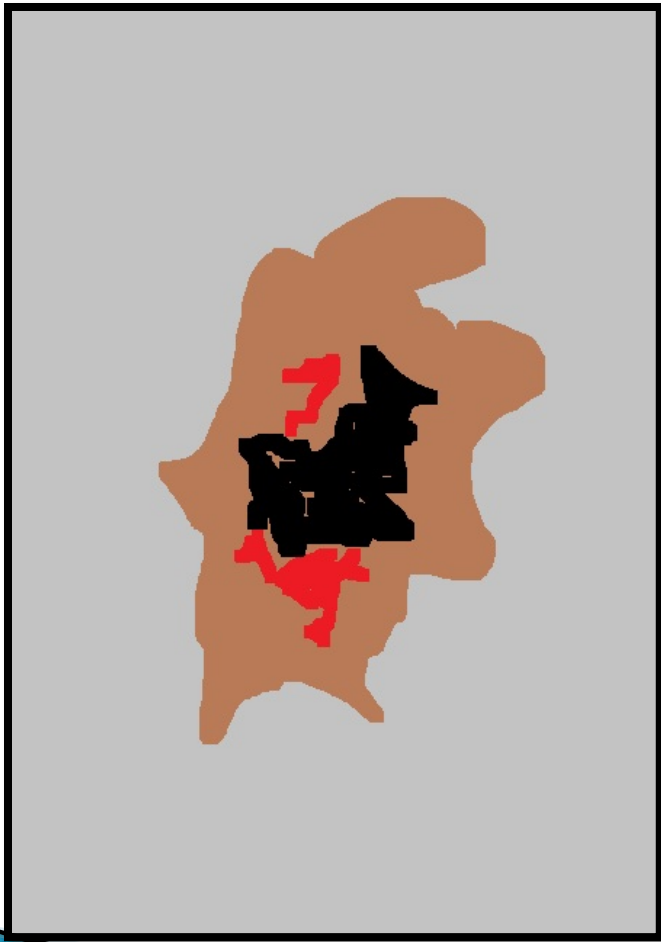




# HPV PCR


- ▶ High risk types 16 & 18 + other
  - ▶ Indications
    - Primary screening?
    - Follow-up
    - ASC-US
    - Persistent ASC-US, LSIL
    - ASC-H
    - High risk patient
  - ▶ DNA (dead or alive)
  - ▶ Dry swab
- 

# Tru-cut needle biopsies

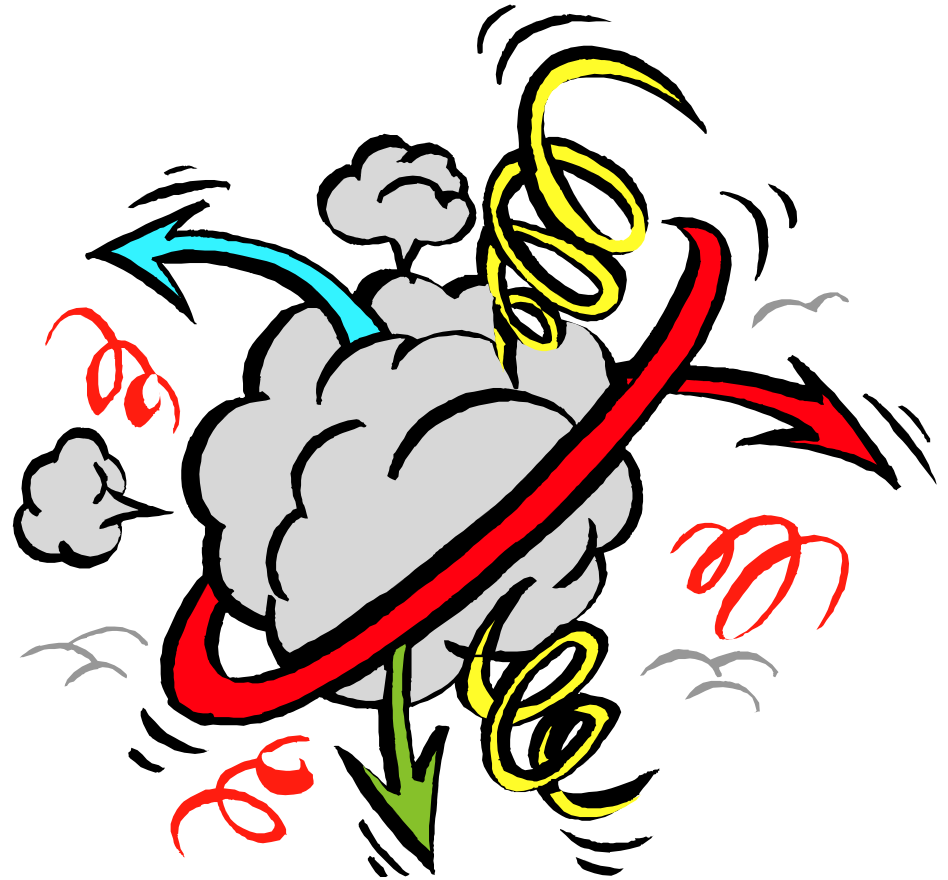


- Biopsy needle placement
- Multiple biopsies
- The bigger the better
- Formalin!!

# Skin biopsies

- ▶ Nodules
  - ▶ Ulcerated tumours
  - ▶ Annular lesions
  - ▶ Suspect pigmented skin lesions
  - ▶ Neoplastic vs. inflammatory vs. infective
  - ▶ Inflammatory skins – bigger biopsies, PLEASE
  - ▶ Special fixatives
    - Histology → Formalin
    - Immunofluorescence → Gluteraldehyde
    - MCS → No fixatives
    - Fungal cultures
- 

# Tubes



# Red Top



- ▶ No anticoagulants or preservatives
- ▶ Used for collecting serum
  - Needs to clot

# Gold or “Tiger” top



- ▶ Same except Gel separator
- ▶ Gel may (rarely) affect some tests

# Gray Top



- Fluoride Oxalate
- Stabilize glucose (and some other things)

# Green Top



- ▶ Lithium heparin
- ▶ Heparin inhibits thrombin (anticoagulant)
- ▶ Plasma rather than serum
- ▶ One advantage – less time wasted waiting for the specimen to clot



# Purple top



- ▶ Contain EDTA
- ▶ Chelates calcium and inhibits coagulation
- ▶ Used for haematology, and some chemistries

# EDTA Contamination

- ▶ Low calcium
- ▶ Low magnesium
- ▶ Low alkaline phosphatase



# Light blue top



- ▶ Contain sodium citrate
- ▶ Chelates calcium and inhibits coagulation
- ▶ Used for coagulation studies.

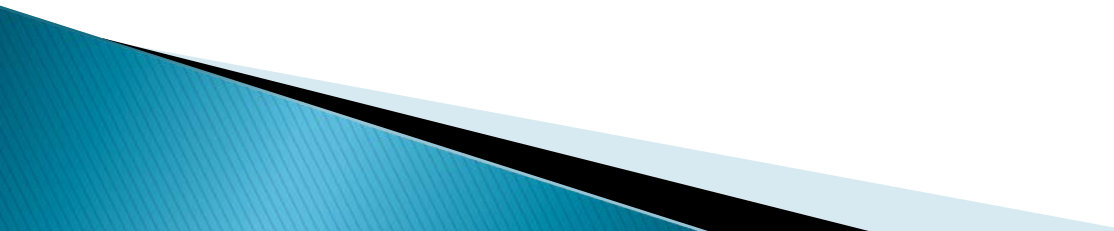
# Troubleshooting erroneous potassiums – Collection issues

- ▶ Leaving tourniquet on for too long
  - Can get high result
  - Red cell rupture
- ▶ Excessive fist clenching
  - Some release from muscle
- ▶ Betadine antiseptic contamination
  - Contains potassium
- ▶ Order of draw (EDTA contamination)
  - Recommended 1) Culture 2) non additive 3) additive
  - ? Any real problem
- ▶ Vigorous mixing of tubes
  - High results
- ▶ Tiny needles

# Troubleshooting erroneous potassiums – Sample delivery

- ▶ Pneumatic tube systems
- ▶ Delay in transport
- ▶ Centrifuges
  - Too fast
  - Too wobbly
  - Recentrifugation (mix serum below/above gel)
- ▶ Chilling samples
  - K Leaks

# Microbiology ..... \$^%&!!!

- ▶ Very complex topic
  - ▶ NB. Clean surfaces
  - ▶ NB. Clean wounds sterile Saline
  - ▶ NB. Biopsies much better than swabs
  - ▶ NB. Multiple specimens
  - ▶ NB. Multiple sites
  - ▶ NB. Specimen prior to AB's
- 

# Skin / soft tissues

- ▶ Tissue biopsies, not swabs
  - Steel
    - Needles
    - Blades
  - No plastic or cotton wool swabs
  - Deep much better than surface
    - Invasive infections

# Sputums

- ▶ Early morning
- ▶ Mouth cleaning
- ▶ Deep breath
- ▶ Sputum, not saliva
- ▶ TB (ZN) – Multiple specimens (at least 3)
  - “If positive, then...”



# Pus

- ▶ Clear the air from the syringe
- ▶ Take off the needle
  - Cap
  - Secure the needle & cap
- ▶ “Empty” tubes
- ▶ NB state the site
- ▶ NB. say if you suspect TB
  - Ask for
    - MCS
    - TB direct microscopy (ZN)
    - Other TB tests

# Not routine cultures

- ▶ TB
- ▶ Virusses
- ▶ Atypical bacteria (weird & wonderful)
  
- ▶ Stipulate
- ▶ Communicate
  
- ▶ PCR's
- ▶ Serology
  - NB to repeat and look for raise or fall of titers

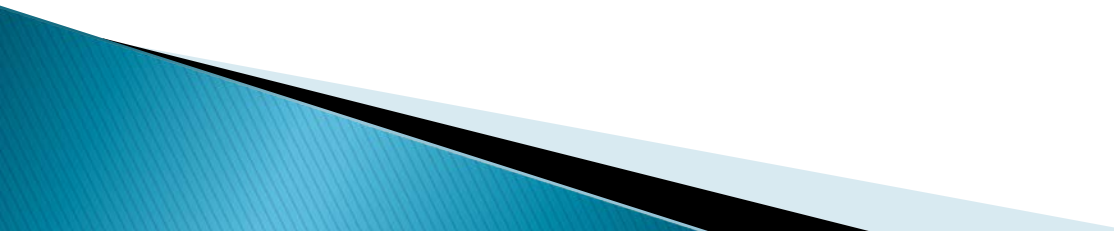
# Colonization

- ▶ Read reports
- ▶ Don't treat colonization
  - Doesn't fit clinically
  - Surfaces
  - Old specimens (urines)
- ▶ Exceptions – growth in “sterile” specimens

# Blood cultures

- ▶ Never only one
- ▶ Different sites
- ▶ Different times
  - Fever peak? NO!!
  - Strong suspicion
    - Take 2
    - Take 2 later
- ▶ Not from IV lines
- ▶ Correlate with symptoms
- ▶ Before AB's
- ▶ If AB's – special bottles with resin

# Blood cultures

- ▶ Clean the skin thoroughly
  - ▶ Two needles
    - One for the take
    - One for the bottle
  - ▶ Clean the top of the bottle
  - ▶ No delay!!
  - ▶ NB. Clinically relevant result
- 

# Blood cultures

- ▶ Adults
  - 10ml aerobic (more NB)
  - 10ml anaerobic
- ▶ Sensitivity = volume
- ▶ Kids
  - $> 1 \text{ ml / kg}$
  - $< 15 \text{ kg}$  - paediatric bottles
  - $> 15 \text{ kg}$  - adult bottles (5ml each)
  - $> 25 \text{ ml}$  - as for adults

# Blood cultures

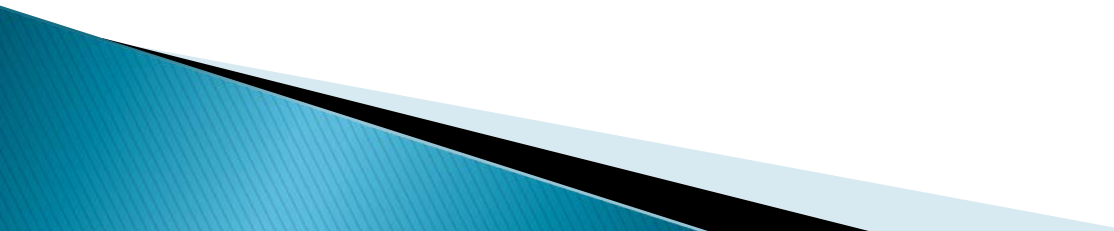
- ▶ 1 specimen – 73% yield
- ▶ 2 specimens – 89%
- ▶ 3 specimens 99%

# Catheter tips

- ▶ Remove and submit if suspicious
  - Take peripheral blood culture first
  - Then remove tip and submit



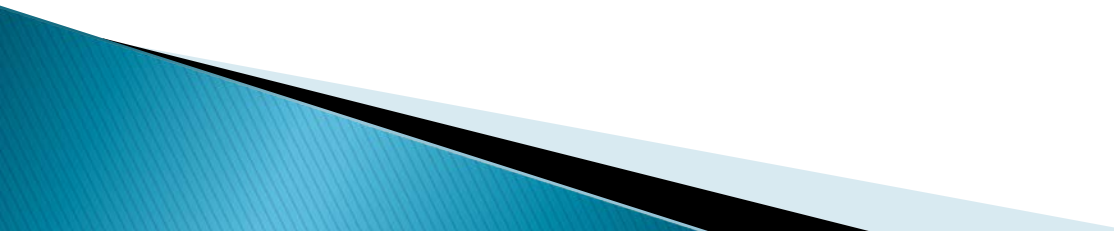
# Blood parasites (Malaria)

- ▶ Take during febrile episodes
  - ▶ Multiple
  - ▶ 6-hourly for 24h
  - ▶ Test until
    - POSITIVE
    - WELL
  - ▶ Inform, phone ahead if you must
- 

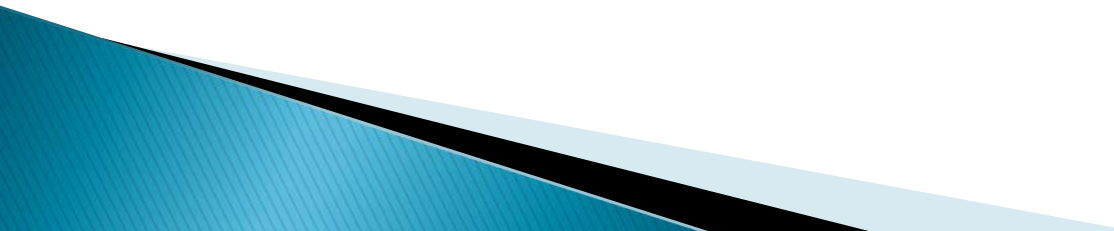
# Stool cultures

- ▶ Only in immunocompromised patients
  - Those that you will treat
  - Serious infections
- ▶ 2 or 3 consecutive days
- ▶ Symptomatic specimens (hard stool is not diarrhoea)
- ▶ Preferably within 3 days post admission (hospital acquired infections / colonization)
- ▶ *C. difficile* (?)
  - Ask for toxin PCR
  - Routine testing is for Salmonella, Shigella, Yersinia, Vibrio, Campylobacter, E. coli 0157H7, Aeromonas, Rota, Adeno and Cryptosporidium
- ▶ Remember non-infectious causes for diarrhoea

# MRSA screening

- ▶ Moisten swab
  - ▶ Bilateral anterior nares
  - ▶ Just hide the tip of the swab
  - ▶ Only need one swab for both nares
- 

# Viral cultures

- ▶ NB best site
  - ▶ Nasopharynx > Nose > Pharynx (Influenza virus)
  - ▶ ASAP
  - ▶ Within first 3 days of symptoms
  - ▶ Differs from case to case
- 

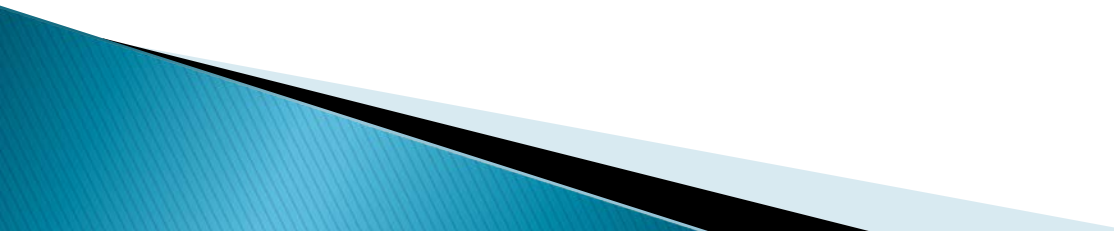
# TB

- ▶ Culture = gold standard
  - Slow (3–6 weeks)
- ▶ PCR – 48 – 72 hours
  - ID
  - Sens PCR
  - Second line sens PCR
  - ZN negative → PCR
- ▶ ZN? Still good, depending on the person behind the microscope
- ▶ PCR on histology?
- ▶ TB spot test / Quantiferon gold?
  - Similar to Mantoux (not quite, slower to react)
  - Only to confirm clinical suspicion

# Urine MCS

- ▶ NB contamination issues
- ▶ Time and technique is NB
- ▶ Overnight –  $<4^{\circ}$
- ▶ First morning urine
- ▶ Volume
- ▶ Mid-stream
  
- ▶ STD's
  - First voided specimen
  - First stream
  - Multiplex PCR

# Sputums / bronchial aspirates

- ▶ NB contamination from mouth
  - ▶ Inform patient
  - ▶ Mouth hygiene
  - ▶ ICU patients
  - ▶ Correlate with symptoms and other parameters
- 

# CSF MCS

- ▶ 2ml / tube
- ▶ 2–3 tubes
- ▶ To avoid contamination
  - One for Culture
  - One for Microscopy
  - One for Chemistry
  - Most turbid tube – culture
- ▶ NB state if for Cryptococcus
  - India ink preparations
  - Antigen test



# General

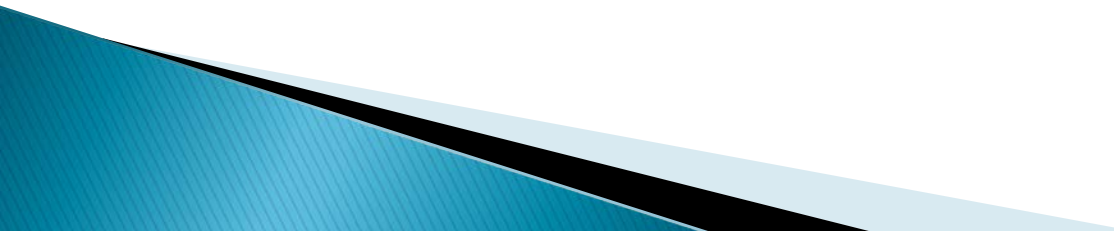
- ▶ Poor outcomes
  - = Young patients
  - = Young nurses
  - = Young doctors
  - = Young lab personnel

*Ped Infect Dis 2006; 25:611-614*

# General

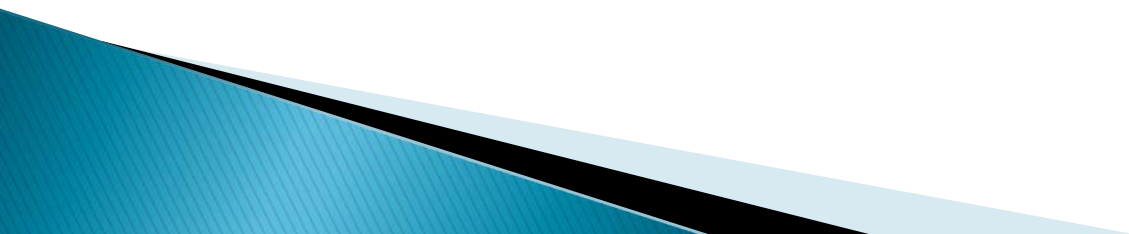
- ▶ Proper labelling
  - Proper identification
    - Two identifiers
      - ID
      - Name
  - Specimen type
  - Test required

# General

- ▶ Appreciate what it takes to generate a report
  - ▶ Be aware of the pitfalls – clinically and in the lab
  - ▶ Lab tests take time (for various reasons)
    - Histology
    - Goggas moet groei
  - ▶ Keep it real... (relevant)
- 

General

If in  
doubt...



General

**ASK!!!**

Take-home message

Please give as  
much as possible  
relevant  
information

