Collecting Optimal Lab Specimens

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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
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, XDR-TB
  - 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 $\rightarrow$ 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)
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
      - Immunophenotyping
      - Flow cytometry
  - Metastatic
    - Carcinoma
      - Adenocarcinoma
      - Squamous cell carcinoma
    - Melanoma

Morphology alone difficult
Axillary, cervical, inguinal lymph nodes

- Kaposi sarcoma
- Combination with infection

- “Reactive” lymph node – follicular hyperplasia
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
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
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
Light Blue
Lavender
Gray
Green
Gold
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)
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 potassiuims – 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…”
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**
  - >1ml / kg
  - <15kg – paediatric bottles
  - >15kg – adult bottles (5ml each)
  - >25ml – 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°
- 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*
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