Basic Professional Training Program for Associate Medical Technologist

Basic Cytology – Part 1 (Preparation and normal morphology)

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Contents

- Different types of cytology specimen and how they are handled.
- General cytology preparation technique, associated reagents and equipment.
- Quality assurance in cytopreparations.
- Laboratory safety in cytology setting.

Cytology specimens

- Gynaecological specimens
- Non-Gynaecological specimens
  - sputum
  - urine
  - Fluid
  - C & F
- FNA specimens

Gynaecological specimen
(Liquid based preparation)

- BD Diagnostic/TriPath Imaging
  PrepStain system / SurePath test pack (consumables)
- HOLOGIC:
  ThinPrep 2000 System / ThinPrep 5000 processor
Specimen Collection

- **SurePath preservative fluid** (10 ml of buffered 24% ethanol solution)
- **ThinPrep PreservCyte Solution** (20 ml of methanol-based, buffered solution)

Collection of cervical scrap with boom shaped brush

- **SurePath**: Immediately disconnect the entire broom head from the stem and add the broom head into a vial of SurePath Preservative Fluid
- **ThinPrep**: Rinse the broom as soon as possible into the PreservCyt Solution vial by ... Discard the collection device. Do NOT leave the broom head in the vial

PrepStain slide processor
SurePath specimen handling and cell enrichment

- Sample randomization by multi-vial vortexer

Add Density Reagent to a labeled centrifuge tube

Assemble specimen rack with:
- Syringing pipette
- Specimen bottle
- Centrifuge tube with Density Reagent

How to maintain specimen integrity?

Mixing and layering by PrepMate
SurePath specimen handling and cell enrichment

- Centrifugation: Soft spin / Tube Vac/ Hard spin
  - How to maintain specimen integrity?

Specimen processing by the PrepStain system

- PreCoat slide: coated with high mw cationic solution resulted in positive charge
- Settling chamber

Slide preparation

- Assemble slide rack
  - How to maintain specimen integrity?

PrepStain slide processor
SurePath slide preparation and staining by PrepStain processor

- Connect reagent tubings
- Buffer water (pH 8)
- EA/Orange –G Combo Stain
- Hematoxylin Stain
- Alcohol Blend (isopropanol + ethanol)

SurePath slide preparation and staining by PrepStain processor

- Resuspends the pelleted cell samples in buffered deionized water
- Transfers aliquots of cell suspensions to settling chambers mounted on SurePath PreCoat slides
- Incubation period allows cells to settle onto slide surface
- Sequences of washes and staining steps to stain slide by Papanicolaou method

Screening of SurePath slide

- Serpentine, double screening method

SurePath slide

- LP
- HP
Gynaecological Sample Preparation For ThinPrep System

- Collection: Deposit specimen into a PreservCyt Solution Vial.
  - Allow to Stand in PreservCyt Solution for at least 15 minutes. Why?
- Run on ThinPrep 2000/5000 Processor

Loading/unloading the ThinPrep 2000 Processor

- PreservCyt Sample vial
- ThinPrep Pap Test Filter
- ThinPrep Microscope slide
- Fixative Vial
  - How to maintain specimen integrity?

ThinPrep Sample Preparation Process

- Closing the door
- Selecting and starting the Gyn programme
  - Dispersion of sample by rotation of the ThinPrep Pap Test Filter
  - Cell Collection by vacuum
  - Cell Transfer by positive pressure and natural attraction
Screening of ThinPrep Slide

• Normal overlapped screening

Common non-gynaecological specimens

• Body Fluids: pericardial, pleural, peritoneal etc.
• Bronchial aspirate, washing and brushing
• Bile aspiration/bile duct brushing
• Cerebrospinal fluid (CSF)
• Joint fluid
• Sputum
• Urine: catheterized or voided
• Fine needle aspiration (FNA)
Volume of specimen required

- 20-50 ml of specimen should be sufficient
- CSF: prefer at least 3 ml
- Rinsing fluid: around 10ml should be enough for rinsing syringe
- Sputum: not specify

Fixatives for cytology specimens

- 50% ethanol: pre-fixative for fluid specimens, liquefying sputum
- 95% ethanol: fixative for smears
- Absolute ethanol: fixative for joint fluid
- 2% acetic acid in 50% alcohol: fixative for on-site staining
- 10% buffered formalin: for cell block

Preservatives for cytology specimens

- 25% saline alcohol: general purpose preservative
- SurePath Preservative fluid
- PreservCyt Solution: gyn & non-gyn

Specimen fixation

- For specimen with a large amount of fluid, eg. Urine, body fluid, washings, add equal volume of 50% ethanol
- For specimen with small amount of fluid, eg. FNA, brushings; rinse collection device with 25% saline alcohol
- CSF / Joint fluid: prefer fresh unfixed specimen
- Sputum: fresh specimen acceptable
General procedures in handling specimen

- Wear appropriate PPE: gloves and mask
- Keep specimen at 4°C if not attended immediately
- Accept or reject specimen according to lab policies
- Assign lab number to specimen

❯ How to maintain specimen integrity during accessioning?
- Macroscopic description: volume, turbidity and colour of specimen
- Add pre-fixative if require

Cytopreparation technique

- Direct smear e.g. conventional Pap smear, sputum.
- Centrifuged smear e.g. body fluid

Cytopreparation technique

- Cytospin e.g. CSF, FNA.

Cytospin preparation

- For specimen of low cellularity
- Decant supernatant
Cytospin preparation

• Resuspend sediment

• Adjust turbidity by adding 25% saline alcohol

Cytospin preparation

• Assemble the Cytoclip, labeled glass slide, filter paper and Cytofunnel

Cytospin preparation

• Before operation the Cytoclip is held tilted so that the specimen cannot trickle forward and be absorbed by the filter card

• Add 100 μL of cell suspension to each cytofunnel

Cytospin

• Spin at 600 rpm for 5 minutes at high acceleration

• During centrifugation the sample holders tilt forward and the suspension is forcefully sedimented out onto a microscope slide

• Excess fluid is absorbed by the filter card

➢ How to maintain specimen integrity?
“Pick & Smear” technique of sputum (Koss, 2006)

Centrifuged smear

• Centrifuge specimen at 1500 rpm for 15 minutes and for specimen with sufficient amount of the sediment
• Pour off the supernatant and invert the centrifuge tube on absorbent towel for a few seconds until the tube is well drained
• Agitate and homogenize the sediment
• Using a Pasteur pipette, transfer 1 to 2 drops of sediment onto a albuminised slide

Reagents and Stains preparation

• Egg albumin glycerine mixture
• Mayer’s Haematoxylin
• Harris Haematoxylin
• Eosin (1%)
• Scott’s Tap Water
• OG 6
• EA 50
• May Grunwald
• Giemsa
Routine staining procedures

• Haematoxylin & Eosin
• Papanicolaou stain
• May Grunwald Giemsa

Papanicolaou stain

Principle:

• Papanicolaou method is a polychrome staining procedure. It is designed to display the variation of cellular morphology due to different cellular maturity and metabolic activity.
• OG 6 and EA are the two principal components in the cytoplasmic stain. They are synthetic dyes. OG 6 is monochromatic stain that stains keratin in brilliant orange. OG 6 has relatively small molecules so that it can penetrate the cytoplasm.

Principle (continue):

• Keratin, not normally present in vaginal epithelium, is found in keratinizing carcinoma. Hence, bright orangephilic cytoplasm is significant in Pap stain.
• EA is a polychrome stain composed of eosin, light green and in original formula, Bismarck Brown. Eosin stains the cytoplasm of mature squamous cells, nucleoli, and cilia. Light green stains the cells that are metabolically active, such as parabasal cell and intermediate cells.
### Incubators and refrigerators

- Monitor and record temperature daily by usually a glass thermometer put inside the equipment

### Centrifuge / Cytospin

- Daily cleaning and disinfection
- Regular inspection by contractor: Qualitative tests
- Quantitative tests: electrical safety test, speed test etc.

### Autostainer

- Renew or filter reagent
- Wash containers
- Wash slide racks
- Clean interior surface
- Renew carbon filter (every 3 months)
Automatic Coverslipping machine

- Clean nozzle with xylene
- Remove excess mountant and coverglass fragments
- Empty waste bucket
- Refill mountant and coverglass

Biological safety cabinet

- UV illumination
- Audible alarm check
- Clean interior and work surface with 70% alcohol
- Clean underside of work tray with 70% alcohol (weekly)
- Visual inspection of seal on the front of cabinet
- Regular inspection by contractor

ThinPrep 2000 System

Daily:
- Empty waste bottle
- Clean cap seal
- Clean filter cap
- Filter seal O-ring lube

Weekly:
- Filter cap seal O-ring lube
- Pneumatic test
- Door cleaning

Monthly:
- General cleaning

Laboratory Safety

- Chemical safety
- Biological safety
Chemical Safety

- Store volatile, flammable solutions such as absolute alcohol, methanol, EA50, and OG 6 in the metal cabinet (yellow cabinet).
- No mouth pipetting of any chemical reagents.
- Volatile, toxic, or irritating chemicals such as glacial acetic acid and hydrochloric acid should be transferred under fume hood. Be sure to turn on and check normal functioning of the fume hood before commencing procedure.
- All reagents should be labelled with the date and the name of the person preparing the solution. Expiry dates should be indicated.
- Chemicals should not be stored above shoulder height.

Chemical Safety

- When diluting acid solutions such as preparing 0.5% aqueous HCL, always add the concentrated acid to water slowly and never vice versa.
- Wash hands after handling any chemical.
- Both hands should be used when carrying large bottles containing chemicals, with one hand underneath the container to give support.
- For long distance travel, carry chemical bottles in basket or trolley.
- If your are handling chemicals you are not familiar, consult the relevant Material Safety Data Sheet.

Biological Safety

- Treat all clinical specimens as potential infectious and standard precautions should be taken.
- All wounds and lesions should be covered with waterproof dressing before handling specimens.
- Staff should wear gloves, isolation gown, cap, goggles, and N95 masks when processing specimens.
- To avoid spreading infectious agents around the laboratory and contaminating other objects with “dirty” gloves, remove gloves before undertaking any further activity. In particular, precaution should be exercised when using the telephone and computers. Staff processing specimens in the biological safety cabinet should finish their work first before undertaking other activities.
Biological Safety

• Preparation of specimens should be performed in biological safety cabinet.
• Wash hands properly after completion of specimen processing.
• The specimen reception benches should be disinfected by wiping with 2% Clorox after daily preparation. Specimen should be decontaminated by adding 10% formalin before for disposal.
• Any contaminated pipettes and filter cards should be put into 2% printol/Hycolin or 5% Virosolve for disinfection before disposal.
• If the outside of the specimen container become contaminated, it should be wiped with 2% Clorox.

Qualities of Cytopreparation

• Correct labeling (Specimen integrity)
• Correct cytopreparation type(s)
• Optimal cellularity *
• Optimal cellular morphology *
• Optimal staining results
• Complete dehydration & clearing
• Optimal coverslapping
• No contamination (floater)

Optimal Cellularity

• Sufficient cells for diagnosis (according to different specimen types)
• Even distribution of cells
• Minimal overlapping (Optimal thickness)
• Monolayer

Optimal Cellular Morphology

• Fresh sample
• Transportation: 4°C, no delay, CSF
• Prompt fixation/pre-fixation
• Correct fixative
• Sufficient fixation esp. blood stained specimen
• Proper processing:
  • Drying artefact
  • Smearing artefact
  • Cornflake artefact
Examples

• Cytopreparation Problems
FNA thyroid (microscopy view)

What are the problems?

- Too thick cytopreparations
- Blood obscuration of diagnostic cells

Solutions for Blood Obscuration

- Pre-fixation with 2% acetic acid in 50 % alcohol
- Use centrifuged smear instead of cytospin

Conduit Urine
What are the problems?

- Overlapping of cells (Thick)
- Uneven distribution of cells
  - Slough-off of cells

Solutions

- Thick:
  - Lesser cellular density for cytospin, but...
  - Use centrifuged smear
- Uneven: Apply more homogenization
**Cause**

- Concentric distribution pattern in cytospin
- **Cause**: Slough-off of cells
  - **Urine**: presence of amorphous material in centrifuged deposit: wet and thick

**Cause & Solution for Slough-Off**

- **Cause**: layer of cells too thick
- **Solution**:
  - **For cytospin**: Dilute cell suspension
  - **For centrifuged smear**: Apply lesser...

**Cause & Solution for Slough-off**

- **Cause**: Too wet
- **Solution**:
  - **For cytospin**: use lesser volume
  - **For CS**: totally decant the supernatant + dry the cell pellet
- **Cause**: Insufficient Albumin adhesive
- **Solution**: more Albumin

**Body Fluid (cytospin)**
Problem

- Localized in the periphery area of cytospin
- Pale nuclear and cytoplasmic staining
- Cells appear thinner and wider
- Blur/Net chromatin pattern
- Cause: Drying Artefact

Causes and Solutions for Drying Artefact

- Insufficient cytocentrifugation sample volume
- Low cellularity sample e.g. FNA dry faster
- Prolonged cytocentrifugation
- Delay processing after cytocentrifugation

Findings

- Nuclear material spread out of cytoplasm
- Cell lysis
Causes and Solutions for Cell Lysis

- Increase cellularity fragility
  - adverse conditions during transportation
- Over centrifugation
- Water contaminated fixative (abs alcohol)
- Delay fixation/prolonged air drying

Problem(s)

- Scratched smear
- Streaking smear

Urine

Sputum (salivary)
Insufficient Cells for Diagnosis

- Apply more albumin adhesive for salivary sputum

Finding & Cause

- Finding: U shape smear
- Cause: Poor smearing technique?
- Cause: Probably alcohol mark on smear

Body fluid

What had gone wrong?
Cause(s)

- Forgot to charge cytofunnel?
- Charge a wrong cytofunnel!!!
- Probably marked slough-off of cells

End