Issues in Cytology On-site Diagnoses

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On-Site Evaluation
Cytology’s Frozen Section
- Objectives of on-site evaluation
  - Obtain lesional sampling
  - Acquire sufficient sample for definitive diagnosis
  - Triage sample for ancillary studies
  - Diagnosis for immediate management
    - Sample acquisition
    - Intra-operative

Can’t the Cytotech do it?

On-Site Evaluation
Cytology’s Frozen Section
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Cytotech might be able to perform - if they know what is required!

Cytotech cannot perform
Is On-site Evaluation Necessary?

The Value of On-site Evaluation

- Necessity is contextually defined
- Looking at ONLY recovery of diagnostic material, on-site assessment will result in 10-15% fewer unsatisfactory samplings¹
  - Is that necessary? Is that worth it?
  - To the individual patient – yes
  - To the healthcare payer and to society - the few studies of cost effectiveness have concluded²,³ - yes


Ensuring Lesional Sampling

Ensuring Lesional Sampling
I’m in the lesion! (The Battle Hymn of the Radiologists)

- No – surprisingly, they often are not
- If they were in the lesion
  - Each procedure would need only 1 pass
  - They average 3.6 passes
- You see the same thing with core biopsies

Ensuring Lesional Sampling
I’m in the lesion! (The Battle Hymn of the Radiologists)

- Accuracy of needle placement depends on
  - Target organ sampled
  - Anatomic location of targeted organ
  - Obstructing anatomic structures
  - Location and size of lesion within targeted organ
  - Imaging modality used and modality’s parameters
  - Experience/skill of user
- These statements apply to cores and FNAs

Ensuring Lesional Sampling
I’m in the lesion! (The Battle Hymn of the Radiologists)

- Needle placement is only part of the job
  - Extracting sample is another
  - Obtaining sufficient sample for definitive diagnosis is still another
- Many more – but not our concern
Acquiring Sufficient Sample

Sample Sufficiency
- The molecular era has ushered in greater demands for comprehensive testing
  - It is NOT just a diagnosis any more!
- A multitude of pressures drive clinical practice to techniques employing smaller tissue collection processes
  - To ensure sufficient tissue for testing evaluation of the sample during collection is critical

Triage for Ancillary Testing

Ancillary studies
- Histochemical staining
- Immunohistochemical staining
- Microbiology studies
- Flow cytometry
- Molecular studies
- Cytogenetic studies

Fresh vs. Cell Block
- Histochemical staining
- Immunohistochemical staining
- Microbiology studies
- Flow cytometry
- Molecular studies
- Cytogenetic studies

Require fresh sample
Fresh vs. Cell Block
- Histochemical staining
- Immunohistochemical staining
- Microbiology studies
- Flow cytometry
- Molecular studies
- Cytogenetic studies

DNA Recovery (Fresh vs. CytoLyt vs. Formalin)

Direct Comparison of DNA Yield FNA vs Core (UHN MATCH Study)

<table>
<thead>
<tr>
<th></th>
<th>Core Biopsies</th>
<th>FNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Number of Cores</td>
<td>3.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Minimum DNA Recovered (ng)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maximum DNA Recovered (ng)</td>
<td>11,340</td>
<td>5,460</td>
</tr>
<tr>
<td>Mean DNA Recovered (ng)</td>
<td>2243</td>
<td>1713</td>
</tr>
<tr>
<td>Mean DNA Recovered per Core/Pass (ng)</td>
<td>664</td>
<td>593</td>
</tr>
</tbody>
</table>

Fresh vs. Cell Block
- Histochemical staining
- Immunohistochemical staining
- Microbiology studies
- Flow cytometry
- Molecular studies
- Cytogenetic studies

↑ DNA recovery fresh sample

↑ Truncation artefact with intact nuclei – cyto preps
Fresh vs. Cell Block
- Histochemical staining
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↑ DNA recovery fresh sample
↓ Truncation artefact with intact nuclei – cyto preps

What’s Boerner’s problem with cell blocks?

Cell block production is a very ineffective process
- Best to assume you lose 50 to 85% of the sample
  - Cell suspension – 90+% loss
  - Tissue fragments – 50% loss, size dependent

Requirements for On-site Evaluation
Making the On-site Slides
Dry Fixation versus Wet

- Air-drying is a form of smear preparation
  - It is not a form of fixation
  - Suitable for Romanowsky stains
  - Can be “re-hydrated” for Papanicolaou staining
    - Never as good as direct alcohol fixation
  - Air-drying – cytoplasmic and nuclear enlargement

Field’s Stain (Modified)*

- Variant Romanowsky stain
  - Inexpensive alternative to Diff Quik
  - ~ 1/10 the cost of Diff Quik
  - Four steps
    - Methanol fixation – 10 dips
    - Solution 1 – (Eosin) – 5 dips
    - Solution 2 – (Azure) – 5 to 10 dips
    - Distilled water – rinse excess dye

Field’s Stain (Modified)*

Field’s Solution 1

<table>
<thead>
<tr>
<th>Eosin Yellow</th>
<th>4.0 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>25.0 g</td>
</tr>
<tr>
<td>Deionized (type 1) water</td>
<td>2000 mL</td>
</tr>
</tbody>
</table>

Field’s Solution 2

<table>
<thead>
<tr>
<th>Methylene Blue</th>
<th>1.2 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azure B</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>25.0 g</td>
</tr>
<tr>
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* In this modified version of Field’s stain, the order of solutions is reversed from the original description

Dry Fixation versus Wet

- Wet fixation – immediate immersion of smear into alcohol
  - We use modified Carnoy’s solution (ethanol + acetic acid to lyse the blood) [1:9 ratio]
    - 300 mL glacial acetic acid
    - 2,700 mL 95% ethanol
  - Smears Papanicolaou stained in the lab post on-site evaluation

Needle Rinse

- After a drop of sample is placed on a slide,
  - Remainder is expelled into a solution
  - Needle is rinsed in that solution
- Sterile centrifuge tube with 10 mL of sterile saline
Why Sterile Saline Needle Rinse

- Allows reuse of needle
- Rinse can be used for:
  - Flow cytometry
  - Microbiology
  - Cyto preps (Cytospin/ThinPrep/etc.)
  - Cell block
    - Immediately upon return to the lab and equal volume of 20% NBF is added to the portion for cell block

Sample Handling (Adequacy)

- For adequacy – not definitive diagnosis
  - First pass – from a minute quantity of the sample
    - 1 drop (<1 mm) on 1 slide, use a touch-off to make 2 slides, each with one drop
    - Produce 2 good quality, representative smears
      - 1 air-dried / Field stained
      - 1 in Carnoy’s
      - Reminder (90% +) of sample into needle rinse for ancillary studies

Sample Handling (Adequacy)

- Microscopic Review
  - Look over whole slide on 5x
    - Selective 10x or rarely 20x on cells/tissue of interest
  - DO NOT SCREEN
  - If it ain’t obvious, it ain’t good enough

Sample Handling (Adequacy)

- Repeat FNA for adequacy
  - Second pass – from a minute quantity of the sample
    - 1 drop (<1 mm) on 1 slide
    - Produce 1 good quality, representative smear, air-dried / Field stained
    - Reminder of sample into needle rinse for ancillary studies

Sample Handling (Diagnosis)

- Priority is given to air-dried/Field stained slides
  - Reduced number / no alcohol fixed slides produced
No Lesion
- If using a co-axial technique
  - After 2 FNA passes with no lesional material, needle positioning should be re-confirmed
- If needle placement/positioning is deemed good, repositioning within the target should be considered
  - May be in a sclerotic area

Normal Tissues
- Recovery of normal organ tissues
  - Needle is not properly positioned
  - Normal tissues should not be causing a clinical lesion
- Luminal contaminants
  - Respiratory epithelium – EBUS
  - Gastric/duodenal epithelial – EUS
  - Normal keratinizing squames – EUS / transvaginal

Blood (Only or Mainly)
- Do not apply suction
  - Use a non-aspiration FNA technique

QA Requirements
- CAP Regulations  CYP.03333
  - FNA Specimen Labeling
    - If the pathologist performs FNA procedures or if laboratory personnel participate in FNA procedures, at least two patient identifiers are placed on the prepared slides and any specimen container at the time of the procedure.
    - NOTE: All specimens must be labeled at the time of collection to provide unique identification. Each prepared slide must be labeled separately and any specimen container with collected material (e.g. fluid from aspiration) must also be labeled.
- QA Requirements
  - CAP Regulations  CYP.03850
    - Cytology Assessment Record
      - If a statement of adequacy, preliminary diagnosis, or recommendations for additional studies is provided at the time of cytology sample collection, documentation of that statement is maintained.
      - NOTE: Documentation might include a note in the medical record or in the final report.