SPECIMEN PREPARATION AND ADEQUACY OF THE MATERIAL

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STEPS OF THE PREPARATION OF A CYTOLOGIC SLIDE

- Sampling (with or without aspiration)
- Smear
- Fixation
- Staining
- Rapid on-site evaluation
- Division and preservation of the material for special studies
A few important rules for carrying out a satisfactory fine-needle aspiration biopsy:

- The amount of cells is related to the time during which the needle is kept within the nodule.
- Thyroid lesions usually present a rich vascular network which disruption may cause small hematomas responsible of inadequate smears.
- **TAKE HOME MESSAGE:** the thinner the needle the more diagnostic is expected to be the sampling.
The observation method for cytopathologist is mostly based on the cell comparison.

The adequacy of the smear may depend on the size of the surface onto which the material is smeared.

If the cells are smeared on a too large a surface (e.g. the whole slide or many slides) the criteria for adequacy might not be met.

TAKE HOME MESSAGE: the smaller the size of the smear the higher the likelihood of an adequate diagnosis.
## Table II. Performance Characteristics for Unselected Thyroid FNA Series and No. of Passes

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of cases</th>
<th>No. cases with histologic F/U</th>
<th>Sensitivity (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adequacy (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of passes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suen and Quenville&lt;sup&gt;112&lt;/sup&gt;</td>
<td>331</td>
<td>79</td>
<td>94</td>
<td>96</td>
<td>1 or more</td>
</tr>
<tr>
<td>Hamaker et al.&lt;sup&gt;113&lt;/sup&gt;</td>
<td>116</td>
<td>41</td>
<td>95</td>
<td>86</td>
<td>2–3</td>
</tr>
<tr>
<td>Boey et al.&lt;sup&gt;104&lt;/sup&gt;</td>
<td>384</td>
<td>384</td>
<td>96</td>
<td>84</td>
<td>3–4</td>
</tr>
<tr>
<td>Anderson and Webb&lt;sup&gt;114&lt;/sup&gt;</td>
<td>562</td>
<td>373</td>
<td>94</td>
<td>79</td>
<td>1</td>
</tr>
<tr>
<td>Hawkins et al.&lt;sup&gt;88&lt;/sup&gt;</td>
<td>1,399</td>
<td>415</td>
<td>86</td>
<td>98</td>
<td>At least 2</td>
</tr>
<tr>
<td>Goellner et al.&lt;sup&gt;115&lt;/sup&gt;</td>
<td>6,300</td>
<td>382</td>
<td>98</td>
<td>80</td>
<td>1–4</td>
</tr>
<tr>
<td>Hamburger et al.&lt;sup&gt;116&lt;/sup&gt;</td>
<td>1,380</td>
<td>258</td>
<td>100</td>
<td>75%</td>
<td>2–8</td>
</tr>
<tr>
<td>Hamburger and Husain&lt;sup&gt;110&lt;/sup&gt;</td>
<td>888</td>
<td>159</td>
<td>100</td>
<td>75</td>
<td>At least 6</td>
</tr>
<tr>
<td>Caraway et al.&lt;sup&gt;117&lt;/sup&gt;</td>
<td>394</td>
<td>150</td>
<td>94</td>
<td>82</td>
<td>3–5</td>
</tr>
<tr>
<td>Gharib et al.&lt;sup&gt;87&lt;/sup&gt;</td>
<td>10,971</td>
<td>984</td>
<td>94</td>
<td>79</td>
<td>Usually 2 up to 6</td>
</tr>
<tr>
<td>Hanbidge et al.&lt;sup&gt;5&lt;/sup&gt;</td>
<td>123</td>
<td>0</td>
<td>NA</td>
<td>87</td>
<td>4</td>
</tr>
<tr>
<td>Baloch et al.&lt;sup&gt;109&lt;/sup&gt;</td>
<td>662</td>
<td>140</td>
<td>98</td>
<td>89</td>
<td>Average of 2</td>
</tr>
<tr>
<td>Liu et al.&lt;sup&gt;50&lt;/sup&gt;</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99</td>
<td>2</td>
</tr>
<tr>
<td>Poller et al.&lt;sup&gt;118&lt;/sup&gt;</td>
<td>156</td>
<td>75</td>
<td>100</td>
<td>76</td>
<td>3 or more</td>
</tr>
<tr>
<td>Ravetto et al.&lt;sup&gt;119&lt;/sup&gt;</td>
<td>37,895</td>
<td>4,069</td>
<td>92</td>
<td>98</td>
<td>Usually 2</td>
</tr>
<tr>
<td>Baloch et al.&lt;sup&gt;80&lt;/sup&gt;</td>
<td>313</td>
<td>77</td>
<td>100</td>
<td>95</td>
<td>2 or more</td>
</tr>
<tr>
<td>O’Malley et al.&lt;sup&gt;85&lt;/sup&gt;</td>
<td>121</td>
<td>11</td>
<td>NA</td>
<td>78</td>
<td>2–12, mean 4–7</td>
</tr>
<tr>
<td>Baloch et al.&lt;sup&gt;120&lt;/sup&gt;</td>
<td>3,007</td>
<td>101</td>
<td>NA</td>
<td>92%</td>
<td>2–4</td>
</tr>
<tr>
<td>Eedes and Wang&lt;sup&gt;84&lt;/sup&gt;</td>
<td>311</td>
<td>0</td>
<td>NA</td>
<td>86</td>
<td>2–6</td>
</tr>
<tr>
<td>Harvey et al.&lt;sup&gt;38&lt;/sup&gt;</td>
<td>266</td>
<td>22</td>
<td>68</td>
<td>60</td>
<td>1–3</td>
</tr>
<tr>
<td>Redman et al.&lt;sup&gt;32&lt;/sup&gt;</td>
<td>693</td>
<td>0</td>
<td>NA</td>
<td>96</td>
<td>1–11, mean 3.2–5.4</td>
</tr>
</tbody>
</table>

<sup>a</sup>Sensitivity for malignancy, atypical and above as the diagnostic threshold, histologic f/u cases only.

<sup>b</sup>Includes both nondiagnostic and nonspecific, and cases b core biopsy F/U (follow-up).

**NUMBER OF PASSES: FROM 1 to 12!**
METHODS OF FIXATION AND PRESERVATION OF THE MATERIAL

- Air drying (only for Romanowsky May-Grunwald Giemsa)
- Submersion of the smear in ethanol
- Spraying of the smear with alcohol-based fixatives (Cytofix)
- Dilution in isotonic solution (saline)
- Submersion in fixative solutions (liquid-based cytology)
STAINING METHODS

- Romanowsky May-Grunwald Giemsa (air dried smears)
- Hematoxylin-eosin (alcohol fixed)
- Papanicolaou (alcohol fixed)
The technique of FNAB is largely dependent on the skill of the operator

The amount of the aspirated material cannot be predicted without an immediate control of the adequacy

The remaining material cannot be stored safely

Thus:

Ancillary techniques cannot be easily applied to the smeared cells
Collect all the material in the same vial
Rinse the needle and the syringe in a hemolytic and preservative solution
Obtain a slide with a single uniform layer of the cells present in the vial
Make a portion of the material available for special techniques
RINSE IN THE HEMOLYTIC SOLUTION

THE NEEDLE IS LEFT IN THE SOLUTION

THE THIN PREP 5000™ DEVICE

COMPARISON BETWEEN LBC AND CONV.
• It is not cost-effective in the short term
• Increases the technical workload
• Some morphologic features are different in conventional smears compared to LBC
• The rapid on-site evaluation cannot be done
Review

Acta Cytologica 2011;55:389–400
DOI: 10.1159/000329029

Liquid-Based Cytology in Fine-Needle Aspiration Biopsies of the Thyroid Gland

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MOST DIFFUSED METHODS FOR LIQUID-BASED CYTOLOGY

• THIN PREP 2000 AND 5000 (RELEASED BY HOLOGIC CO., MARLBOROUGH, MA)
• SUREPATH (RELEASED BY TRIPATH INC., BURLINGTON, VA)
TECHNIQUE OF FNA: RAPID ON-SITE-EVALUATION (ROSE)

- The majority of authors report a significant decrease of inadequate smears when ROSE is performed.
- The evaluation refers exclusively to the amount of material not to its diagnostic quality.
- Usually ROSE cannot be done with cystic lesions and when the material is processed by LBC.
- The number of passes is related to the characteristics of the lesion and to the skill of the operator.
At least 6 groups of benign follicular cells, each group composed of at least 10 cells

ADEQUACY CRITERIA 2

- Hamburger and Husain. *Diagn Cytopathol* 1988;4:14-17: 6 groups (no amount of cells) in 2 slides
- Kini. *Guides to Clinical Aspiration Biopsy Thyroid* 2nd ed. 1996: 2 smears with 8-10 clusters
- Sidawy et. al. *Cancer* 1997;81:253–9: 10 groups with 10-20 cells
- Renshaw. *Diagn Cytopathol* 2010: 30 epithelial cells lacking atypia with no Hürthle cells
Can a smear containing only colloid be defined as adequate?

- **Goellner et al. Acta Cytol 1987;31:587-590**
  - Colloid without cells is “non-diagnostic”

- **The Thyroid Bethesda System (2008) and Italian SIAPEC-AIT (2013, in press)**
  - “Abundant colloid” lacking epithelial cells is benign
HEMATOXYLIN FOR 1 MIN.
DIVIDING THE MATERIAL FOR SPECIAL TECHNIQUES

Split-sample

- The sample is split in two equal parts by the operator
- Pro: one single pass may get a satisfactory amount of cells for diagnosis and additional studies
- Con: if the operator is not skilled the material for additional studies may be too scanty
DIVIDING THE MATERIAL FOR SPECIAL TECHNIQUES

Separate passes

- Each single pass is alternately used for making a smear and for additional techniques
- Pro: The likelihood of getting adequate cellularity is independent on the smearing skill of the operator
- Con: The patient may receive too many unnecessary punctions
## Efficacy of a Second LBC Slide in Decreasing the Inadequacy Rate

<table>
<thead>
<tr>
<th></th>
<th>NON DIAGN</th>
<th>CYST</th>
<th>BENIGN</th>
<th>FN/SFN</th>
<th>SUSP</th>
<th>MAL</th>
<th>OVER ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>After I TP</td>
<td>46</td>
<td>10</td>
<td>90</td>
<td>16</td>
<td>1</td>
<td>4</td>
<td>166</td>
</tr>
<tr>
<td>Rate %</td>
<td>27.1</td>
<td>6</td>
<td>54.2</td>
<td>9.6</td>
<td>0.6</td>
<td>1.4</td>
<td>100</td>
</tr>
<tr>
<td>After II TP</td>
<td>16</td>
<td>10</td>
<td>108</td>
<td>27</td>
<td>0</td>
<td>5</td>
<td>166</td>
</tr>
<tr>
<td>Rate %</td>
<td>9.6</td>
<td>6</td>
<td>65.1</td>
<td>16.3</td>
<td>0</td>
<td>3</td>
<td>100</td>
</tr>
</tbody>
</table>

Rossi ED et al.: Cytopathology 2010; 21: 97-102
PROCESSING OF THE MATERIAL FOR SPECIAL TECHNIQUES

- Immunocytochemistry
- Flow cytometry
- Molecular analysis
PROCESSING OF THE MATERIAL FOR SPECIAL TECHNIQUES

- **Immunocytochemistry**
  - Cell blocks (formalin fixed)
  - LBC (CytoLit)

- **Flow cytometry**
  - Cytospin (balanced salt solutions)
  - LBC (CytoLit)

- **Molecular analysis**
  - Cell blocks (formalin)
  - LBC (CytoLit)
  - RPMI (transport medium)
CONCLUSIONS

- In thyroid FNA a correct technique is more important than the instruments in providing adequate cellularity.
- A smear correctly made may obviate many diagnostic problems (not only inadequacy!)
- For a small though increasing proportion of cases the availability of non-smeared material for special technique may be required.
- LBC and cell block are effective methods for processing the cells for these special techniques.
REFERENCES
