Canadian Association of Pathologists
- 2021 Resident Review Course:
CYTOLOGY

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Disclosures:

I have no conflicts of interest to disclose in relation to this educational activity and the contents of my presentation.
OBJECTIVES

- RESOURCES TO PREPARE FOR THE RCPSC EXAM CYTOLOGY SECTION
- DIAGNOSTIC CYTOPATHOLOGY KNOWLEDGE REQUIRED
- QUALITY ASSURANCE AND IMPROVEMENT PRINCIPLES IN CYTOLOGY
RESOURCES

Internet:

- Canadian Guidelines: [http://cytopathology.ca/guidelines/](http://cytopathology.ca/guidelines/)

Reporting Systems:

- Urine: [https://paris.soc.wisc.edu/](https://paris.soc.wisc.edu/)
- GYN: [https://bethesda.soc.wisc.edu/](https://bethesda.soc.wisc.edu/)
  [https://screening.iarc.fr/atlasocyto.php](https://screening.iarc.fr/atlasocyto.php)
- Thyroid: [http://www.papsociety.org/image-atlas/](http://www.papsociety.org/image-atlas/)
- Salivary Gland: [https://milan.soc.wisc.edu/index.htm](https://milan.soc.wisc.edu/index.htm)

Books:
Not (yet) relevant (most likely):

The Papanicolaou Society of Cytopathology System for Reporting Respiratory Cytology
The International Academy of Cytology Yokohama System for Reporting Breast Fine-Needle Aspiration Biopsy Cytopathology
The International System for Reporting Serous Fluid Cytopathology

If you’re planning for extra preparation/fellowship:
Cytopathology Specimen Types

Fluids – Body Cavity Effusions / Natural Body Fluids (CSF, Vitreous, Sputum, Urine)

Exfoliative Sampling – Washing, Brushing, Scraping

Fine Needle Sampling – Palpation-guided / Image-guide (w/ & w/o aspiration)
  ↘ U/S / CT
  ↘ Endoscopic (EUS/EBUS)
  ↘ ProCore, Flexible 19 G

Sampling Force

Fragment size
Cytomorphology is **fundamental**:

- Neoplastic ↔ Non-neoplastic
  - ? neoplastic cell type
  - ? inflammatory cell type
    - Epithelial
    - Hematolymphoid
    - Spindle cell / Melanoma
    - Mesothelial

Ancillary tests are used in specific situations:

- Resolving cases with diagnostic overlap between benign, reactive cells (mesothelial, chronic inflammatory) and malignant cells.
- Tumor subtyping and identification of a primary site of a neoplastic process.
- Assessment of markers of prognostic or predictive value in a given neoplasm.
Be comfortable with the cytomorphology of benign samples in the preparations your lab produces!
Expect variability
Both Sample & Preparation

Find features that serve as reference.
GYN CYTOLOGY
“Hyperchromatic Crowded Group” (ThinPrep, 20x)
HSIL - hypochromatic (ThinPrep, 63xoil)
HSIL - keratinizing (ThinPrep, 63x oil)
Adenocarcinoma in-situ

Remember microarchitectural clues:
“Feathering”
“Palisading”
“Rosettes”
European hedgehog (Erinaceus europaeus)
SMILE (Stratified Mucin-Producing Intraepithelial Lesion): “Jagged” Clusters

Endocervical Adenocarcinoma, HPV-independent, Gastric-Type
NILM (ThinPrep)

- NOT koilocytic change
- Glycogen
Tuboendometrioid Metaplasia (ThinPrep)
Lower Uterine Segment Sampling (ThinPrep)
Trichomonas vaginalis (ThinPrep, 63x oil)
NON-GYN CYTOLOGY
GOSH!
WHAT A MASSIVE PLEURAL EFFUSION!!

www.ribspreader.com
www.rippenspreizer.com
Adenocarcinoma (ThinPrep, 10x)
○ Immunohistochemistry – Solid Tumor (Class 1 Testing):

BerEP4, Claudin-4, MOC31, Calretinin, Mesothelin
TTF1, P40, CDX2, GATA3, PAX8, AR, SOX10, AE1/AE3

○ Additional markers#:

Lung: Napsin A
NET: CD56, Synaptophysin, Chromogranin, INSM1
Breast: GCDFP15, Mammoglobin, ER, PR
SqCC: HMWK, CK5/6, P63
Ovary: P53, HNF1b, P16, WT1
GIT: CK7, CK20, CK19, Villin, SATB2
Prostate: PSA, PSAP, NKX3.1
Renal: CA9, CD10, AMACR, RCC
HCC: Arginase1, HepPar1
Melanoma: HMB45, Melan A
GCT: SALL4, OCT3/4
SCST/ACC: SF1, Inhibin
Mesothelial: D2-40, BAP1

#Note: Some markers apply to multiple anatomical sites.
○ Immunohistochemistry – Solid Tumor (Class 1 Testing):

BerEP4, Claudin-4, MOC31, Calretinin, Mesothelin, GATA3, PAX8, AR, SOX10, AE1/AE3

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#Note: Some markers apply to multiple anatomical sites.
BerEP4 (EpCAM)
Biomarker Testing (Class 2 Testing)

1. EGFR mutation analysis (EntroGen® kit):
   - requires 40-80 ng genomic DNA: 7000 cells (~ 6 pg/cell)

2. ALK-1 Immunohistochemistry (5A4, Novocastra Leica):

3. PD-L1 Immunohistochemistry (22C3 pharmDx, Agilent):
DDx? (ThinPrep, 40x)
Synaptophysin

Chromogranin A

MIB1

NGS TST15 (Illumina)
- EGFR Exon 19 deletion
  VAF (%): 70
- TP53 mutation
  VAF (%): 81
Dx:
Small Cell Carcinoma - Transformation of the previously diagnosed EGFR-mut+ Lung Adenocarcinoma

- EGFR Exon 19 deletion
  VAF (%): 70

- TP53 mutation
  VAF (%): 81
Metastatic mammary (ductal) carcinoma (ThinPrep, 10x)
... but they can also come like that ...
... but they can also come like that ...
Class 2 Test

Validation of immunohistochemical tests is important!
“Cannonball”: Metastatic ductal carcinoma of breast origin?
GATA3

Courtesy of Dr. H. Ruff (UHN)
Cytokeratin

Courtesy of Dr. H. Ruff (UHN)
PAX8: diffuse +
WT1: diffuse +
P16: negative - mod/patchy +
ER: focal +
P53: wild-type pattern

Serous neoplasm (DDx: Low-grade serous carcinoma vs. Serous BLT).
PAX8: diffuse +
WT1: diffuse +
P16: diffuse, strong +
ER: focal +
P53: overexpression (>60%)/null expression (<5%)

High-grade Serous Carcinoma of Tubo-Ovarian / Peritoneal Origin.
Peritoneal Fluid

PAX8: diffuse +

WT1: -
P16: -
ER: -
P53: wild-type pattern
HNF1beta: diffuse +

Clear Cell Carcinoma

PAX8: diffuse +
WT1: -
P16: -
ER: -
P53: wild-type

HNF1beta: diffuse +
Peritoneal Fluid: What can make you stumble?

Signet Ring Carcinoma (Colon)

Adenocarcinoma (Pancreas)
Peritoneal Fluid (Cytospin, Field stain)
CD10+ B-cells: 86.50%

Ig lambda (CD10+ B-cells): 0.03%

Ig kappa (CD10+ B-cells): 99.90%
Dx:
Large B-cell Lymphoma with Germinal Center Phenotype
Dx:
Large B-cell Lymphoma with Germinal Center Phenotype

FISH:
POSITIVE for MYC rearrangement
POSITIVE for IGH-BCL2 rearrangement
NEGATIVE for BCL6 rearrangement

= High-Grade B-cell Lymphoma with MYC and BCL2 Rearrangements
PMHx: SOB, pleural thickening, effusion (ThinPrep, 10x)
Malignant epithelioid mesothelioma
Malignant epithelioid mesothelioma
Malignant epithelioid mesothelioma
BAP1 (BRCA1–associated protein 1)
### Table 3. Diagnostic Usefulness of MTAP IHC, BAP1 IHC, and 9p21 FISH in Cell Blocks for Distinguishing MPM From Non-Neoplastic RMH

<table>
<thead>
<tr>
<th></th>
<th>MPM N=45</th>
<th>RMH N=21</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>MTAP IHC</td>
<td>19</td>
<td>26</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>BAP1 IHC</td>
<td>27</td>
<td>18</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>9p21 FISH</td>
<td>28</td>
<td>17</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>BAP1/MTAP IHC</td>
<td>35</td>
<td>10</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>BAP1 IHC/9p21 FISH</td>
<td>38</td>
<td>7</td>
<td>0</td>
<td>21</td>
</tr>
</tbody>
</table>

Abbreviations: BAP1, BRCA1-associated protein 1; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; MPM, malignant pleural mesothelioma; MTAP, methylthioadenosine phosphorylase; RMH, reactive mesothelial hyperplasia.

a For IHC, positive indicates expression loss and negative indicates negative expression loss, whereas for FISH, positive indicates positive for homozygous deletion (HD) and negative indicates negative for HD.

Source: Kinoshita et al., Cancer Cytopathol. 2018 Jan;126(1):54-63.
THYROID CYTOLOGY
Benign (thyroid tissue)
Benign (Smear w/ Romanowsky-type, 40x)
Colloid

Skeletal muscle
Benign, Oncocytic (Hürthle cell) metaplasia
Papillary thyroid carcinoma
Papillary thyroid carcinoma

I’m NOT a psammoma body!
Papillary thyroid carcinoma
Compare nuclear SIZE and CONTOURS!

- **Benign follicular epithelium**
- **Papillary thyroid carcinoma**
Suspicious for follicular neoplasm (1)
Suspicious for follicular neoplasm (2)
Anaplastic thyroid carcinoma (DDx Metastasis/Direct Invasion)
Medullary thyroid carcinoma
Adenocarcinoma, metastatic colorectal
The NIFTP problem ...

<table>
<thead>
<tr>
<th></th>
<th>Original Diagnostic Criteria</th>
<th>Revised Diagnostic Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encapsulation or clear demarcation</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Follicular growth pattern</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>1&lt; papillary structure</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>No well-form papillae</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Nuclear features of PTC</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>No psammoma bodies</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Nuclear features</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Score of 2-3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Nuclear elongation/ grooves/ chromatin clearing</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Submit the entire nodule for nuclear score of 3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Molecular testing for nuclear score of 3</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>0&lt;30% solid/ trabecular/ insular growth pattern</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>No vascular or capsular invasion</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>No tumor necrosis</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>No high mitotic activity</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Lack of BRAF^{V600E} mutation with IHC or molecular testing</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Lack of BRAF-like mutations or other high-risk mutations (TERT and TP53)</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Abbreviations: FNA, fine-needle aspiration; IHC, immunohistochemistry; NIFTP, noninvasive follicular thyroid neoplasm with papillary features of PTC, papillary thyroid carcinoma.

aBased on Nikiforov et al.²
bBased on Nikiforov et al.³

... may not be a problem after all.

Since

“...most cases of NIFTP show a nuclear score of 2 rather than 3.”

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Risk of malignancy with NIFTP (%)</th>
<th>Optional note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic or Unsatisfactory</td>
<td>No significant change</td>
<td>None</td>
</tr>
<tr>
<td>Benign</td>
<td>No significant change</td>
<td>None</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance</td>
<td>6–18</td>
<td>None</td>
</tr>
<tr>
<td>Follicular Neoplasm or Suspicious for a Follicular Neoplasm</td>
<td>10–40</td>
<td>The histopathologic follow-up of cases diagnosed as such includes follicular adenoma, follicular carcinoma, and follicular variant of papillary thyroid carcinoma, including its recently described indolent counterpart NIFTP.</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>45–60</td>
<td>The cytomorphologic features are suspicious for a follicular variant of papillary thyroid carcinoma and its recently described indolent counterpart NIFTP.</td>
</tr>
<tr>
<td>Malignant</td>
<td>94–96</td>
<td>A small proportion of cases (~3–4%) diagnosed as malignant – compatible with papillary thyroid carcinoma – may prove to be NIFTP on histopathologic examination.</td>
</tr>
</tbody>
</table>

Sources: Nikiforov et al., JAMA Oncol. 2018 Aug 1;4(8):1125-1126.
Baloch et al., Chapter 1, The Bethesda System for reporting Thyroid Cytopathology, 2nd Edition (2018)
RESPIRATORY TRACT CYTOLOGY
Normal tissue: Bronchial epithelium
Lymphoid tissue; germinal center fragment (Romanowsky-type)
Lymphoid tissue; germinal center fragment (Papanicolaou)
Rapid on-site assessment: “Lesional tissue present.”

- Bronchial epithelium
- Lymphoid tissue
- Adenocarcinoma
Adenocarcinoma
Adenocarcinoma
Granulomatous inflammation (Romanowsky-type)
Granulomatous inflammation (Papanicolaou)
Granulomatous inflammation: Infectious causes
Granulomatous Inflammation: Neoplastic cause - Hodgkin Lymphoma

Background cells are important!
Granulomatous Inflammation: Neoplastic cause - Germ Cell Tumor (Seminoma)

**Background** extracellular (tigroid) material is important!
BAL (ThinPrep, 20x)
PMHx: Lung Tx
Dx: Non-pigmented (hyaline) mold with septate hyphae and acute angle branching, favor Aspergillus spp. (CRITICAL VALUE!).
Bronchial Wash (ThinPrep, 40x)
PMHx: pneumonia, lung opacities
Dx: Asbestos (ferruginous) body
! Beware of anchoring / expectation bias !
Pleomorphic adenoma (Romanowsky-type)
Pleomorphic adenoma (Papanicolaou)
Mucoepidermoid carcinoma (Romanowsky-type)

- Extracellular mucin
- Epidermoid/intermediate cells
- “Pseudohistiocytic” mucin-producing cells
Mucoepidermoid carcinoma (Papanicolaou)

- Extracellular mucin
- Epidermoid/intermediate cells
- “Pseudohistiocytic” mucin-producing cells
Salivary gland mass (ThinPrep, 40x)
Dx: ???
Salivary gland mass (Cell Block, 40x)
Dx: ???
### Table 4. SOX10 Expression in Salivary Gland Tumors

<table>
<thead>
<tr>
<th>SOX10-positive tumors</th>
</tr>
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<tbody>
<tr>
<td>Acinic cell carcinomas</td>
</tr>
<tr>
<td>Adenoid cystic carcinomas</td>
</tr>
<tr>
<td>Epithelial-myoepithelial carcinomas</td>
</tr>
<tr>
<td>Myoepithelial carcinomas</td>
</tr>
<tr>
<td>Pleomorphic adenomas</td>
</tr>
<tr>
<td>SOX10-negative tumors</td>
</tr>
<tr>
<td>Salivary duct carcinomas</td>
</tr>
<tr>
<td>Mucoepidermoid carcinomas</td>
</tr>
<tr>
<td>Squamous cell carcinomas</td>
</tr>
<tr>
<td>Oncocytic carcinomas/oncocytomas</td>
</tr>
<tr>
<td>Warthin tumors</td>
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Abbreviation: SOX10, SRY-related HMG-box 10.

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Abbreviation: SOX10, SRY-related HMG-box 10.
Acinic cell carcinoma (different case)
Warthin Tumor (Papanicolaou)
DDx: Oncocytoma/oncocytic-other
Compare the cytoplasm!

Acinic cell carcinoma

Oncocytoma
PANCREATOBILIARY TRACT CYTOLOGY
Adenocarcinoma (CBD Brush)
Adenocarcinoma, pancreatic ductal (Cytospin Prep)
Anisonucleosis
Pancreas Cyst Aspirate (Cytospin Prep)
Adequacy - Satisfactory for evaluation
Interpretation - Neoplastic: Other
Diagnosis - Extracellular mucin consistent with a neoplastic mucinous cyst.
Comment - The specimen shows cyst fluid with thick colloid-like extracellular mucin containing cyst debris consistent with a neoplastic mucinous cyst. A MCN is favored given the clinical and imaging findings of a 45-year-old female with a multiloculated cyst in the pancreatic tail. Scant benign appearing mucinous epithelium is present of uncertain origin, favor gastric contamination. No high-grade epithelial atypia is present.
Pancreas Cyst Aspirate (Cytospin Prep)
Lesional or not???
Neuroendocrine tumor, well differentiated
Neuroendocrine tumor, well differentiated
Solid – cellular pattern

NOT site-specific → Carcinoid (lung)
Solid pseudopapillary neoplasm
Solid pseudopapillary neoplasm (Eosinophilic Globules)
GENITOURINARY / URINE CYTOLOGY
Urine (ThinPrep, 40x)
Umbrella Cells
Benign urothelial tissue fragment
High-Grade Urothelial Carcinoma

- High N:C Ratio (>0.7)
- Hyperchromasia
- Irregular Nuclear Membrane
- Coarse (Irregular) Chromatin
High-Grade Urothelial Carcinoma
Polyoma Virus Cytopathic Effect
Bladder Wash (ThinPrep, 63xoil)
PMHx: Urothelial carcinoma in-situ
Dx: ???
Dx: Negative for high-grade urothelial carcinoma, Granulomatous inflammation (likely BCG-related)
MISCELLANEOUS LUMPS AND BUMPS
Mammary carcinoma (ductal):
Cell-in-cell arrangement
Malignant Melanoma: “Bug-eyed” cells
Leiomyosarcoma: “Cigar-shaped” nuclei

Cell Block
Strongyloides (BAL)
CANADIAN SOCIETY OF CYTOPATHOLOGY
GUIDELINES FOR PRACTICE & QUALITY ASSURANCE IN CYTOPATHOLOGY

http://cytopathology.ca/guidelines/
Quality Assurance in GYN cytology:

http://cytopathology.ca/guidelines/

Re-(pre)screening of negative cytology

→ Prospective
  → Targeted* \[10\%\]
  → Random
  → Rapid (ALL negative GYN cases)
  → Prescreening (ALL GYN cases + Full screen)

* Hx of vaginal bleeding/spotting
  Hx of cervical/vaginal/vulvar ca
  Hx of DES exposure
  Hx of >/=ASCUS/AGC within prior 2y
  Abnormal cervix on exam

→ Retrospective

“All negative GYN cytology from at least the previous 3 years (or up to six years if the screening interval is 3 years) in a woman with current cytology showing >/= HSIL or AIS should be rescreened by a cyto-technologist and then referred to a pathologist.”
Screening Practices/Referral to the Pathologist:

http://cytopathology.ca/guidelines/

- GYN cytology screened as UNSAT or NILM (excluding repair) may be finalized by a cytotechnologist. ALL other GYN cytology must be referred to a pathologist for reporting.

- ALL NON-GYN cytology should be referred to a pathologist for reporting.
Quality Assurance in GYN/NON-GYN cytology:

http://cytopathology.ca/guidelines/

Examples of cytology critical/alert values/diagnoses (Appendix)

1) Any unusual or unexpected cytology result, which may include an unexpected malignancy in a GYN, NON-GYN or FNA specimen.
2) A malignancy involving a critical anatomic site in a NON-GYN or FNA specimen (e.g. malignancy causing superior vena caval syndrome or paralysis.
3) Identification of possible pathogenic organisms in a NON-GYN or FNA specimen from an immunosuppressed patient or in any orbital or CSF sample (e.g. finding bacteria, pneumocystis, fungi, mycobacteria or viral (CMV, Herpes) cytopathic effect).
4) Identification of Herpes Simplex viral changes in a cervical/vaginal sample of near-term pregnant patient.
5) Any corrected report, where the diagnosis is significantly changed and will result in a significantly different patient management.
Thank you!

Good luck with the exam!
Multiple Choice Questions

CHANGING EXAM PATTERN
Year 1995: Answer all questions.
Year 2000: Answer any 5 questions.
Year 2005: Select the correct answer (A, B or C).
Year 2010: Write either A or B.
Year 2015: Please only read the questions.
Year 2020: Thanks for coming!!! :)

[Image of cartoon characters with the text “ZZZZZ” over one of them]
Question 1:

What reporting system is used in urine cytology:

(A) The Bethesda System.

(B) The Milan System.

(C) The Yokohama System.

(D) The Papanicolaou Society for Cytopathology System.

(E) The Paris System.
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Question 2:

What feature is NOT part of the main criteria for the diagnosis of high-grade urothelial carcinoma according to the 2016 consensus system:

(A) N/C ratio ≥0.7.

(B) Coarse/clumped chromatin.

(C) Prominent nucleoli.

(D) Markedly irregular nuclear membrane.

(E) Moderate to severe nuclear hyperchromasia.
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(C) Prominent nucleoli.

(D) Markedly irregular nuclear membrane.

(E) Moderate to severe nuclear hyperchromasia.
Question 3:

What is NOT an example of a critical diagnosis in cytology requiring expedited notification of the most responsible physician or delegate:

(A) Bacteria identified in fluid from an Ommaya reservoir.

(B) Actinomyces identified in the Pap smear of a patient with IUD.

(C) CMV cytopathic effect identified in a routine BAL of a lung transplant patient.

(D) Strongyloides identified in the sputum of a patient with the clinical diagnosis of asthma.

(E) Lymphoblasts in an FNA from an anterior mediastinal mass of a 21 year old male patient.
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Question 4:

Which one of the following lesions of the uterine cervix is unlikely to be detected by HPV testing for primary cancer screening:

(A) Intestinal variant of mucinous adenocarcinoma.

(B) Stratified Mucin-Producing Intraepithelial Lesion.

(C) Villoglandular variant of usual-type adenocarcinoma.

(D) Signet-ring cell variant of mucinous adenocarcinoma.

(E) Gastric-type adenocarcinoma.
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Question 5:

Which of the following statement(s) is/are most accurate with respect to rapid on-site assessment in cytology:

(A) It is frequently requested for the pre-operative mediastinal staging in patients with lung cancer.

(B) Close collaboration between cytopathologist or -technologist and other physicians is required for optimal resource utilization.

(C) It’s main task is to render a precise and expedited diagnosis.

(D) It is often performed with liquid-based cytology slide preparations.

(E) Statements A and B are most accurate.
Question 5:

Which of the following statement(s) is/are most accurate with respect to rapid on-site assessment in cytology:

(A) It is frequently requested for the pre-operative mediastinal staging in patients with lung cancer.

(B) Close collaboration between cytopathologist or -technologist and other physicians is required for optimal resource utilization.

(C) It’s main task is to render a precise and expedited diagnosis.

(D) It is often performed with liquid-based cytology slide preparations.

(E) Statements A and B are most accurate.
“Hungry” for more?

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