Technical Issue in Body Fluid Analysis

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Preanalytical Variables (1)

- Specimen collection procedures
  - Standardization (SOP)
- Type of collection tubes used to collect
  - Glass tubes--->cellular adherence
    - Artificially change differential cell counts
    - Especially in low protein fluids: BAL or CSF
  - Polypropylene tube is preferred

Preanalytical Variables (2)

- Type of anticoagulant (additive)
  - Additive may not required for CSF
    - Affect the enumeration of WBC and RBC
  - Using the wrong additive (synovial) could introduce artifacts
    - Interfere with the identification of cellular elements or crystals

Preanalytical Variables (3)

- **The proper order of draw**
  - **Reduce the incidence of cellular contamination from tube to tube**
  - **Hemolyzed and clotted specimens are not recommended**

Bronchoalveolar Lavage (BAL)

- **The instillation volume**
  - **Typically is approximately 100-300 mL sterile saline in 20-50 mL aliquots**
  - **The first aliquot should be discarded**
  - **The other aliquots are pooled for further analysis**

Quantitative Assessment (1)

- Mix the specimen
  - Rotation on an automated mixer
    - For a maximum of 2-5 min.
    - Excessive rocking may damage cells
    - Synovial fluid must be mixed for 5-10 min.
      - Due to the viscosity of the fluid
  - Hand mix: inverting the tube 10-15 times

Quantitative Assessment (2)

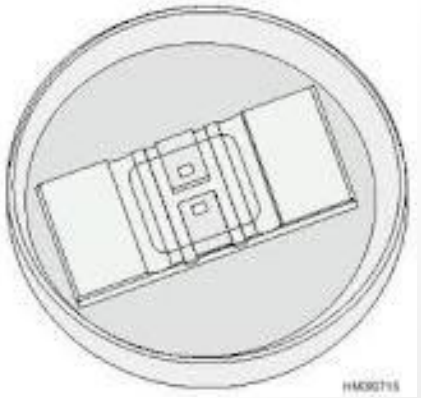
- Specimen dilutions
  - Specimens are usually counted undiluted
    - Unless bloody or cloudy specimens
  - Typical dilutions
    - Range from 1:10-1:200 or higher
    - Depending on the turbidity of the specimen
  - Isotonic saline can be used for both WBC and RBC dilutions
  - Bloody specimens
    - 3% acetic acid may be used to lyse RBCs
    - 0.3% hypotonic saline was used for synovial fluid

Quantitative Assessment (3)

- **Hemocytometer preparation and charging**
  - **Cells must be counted as soon as possible**
    - **If the fluid has drawn back from the sides of the hemocytometer**
      - **The sample has begun to dry out and the counts are invalid**
      - **Re-mix the sample and set the hemocytometer counts up again**

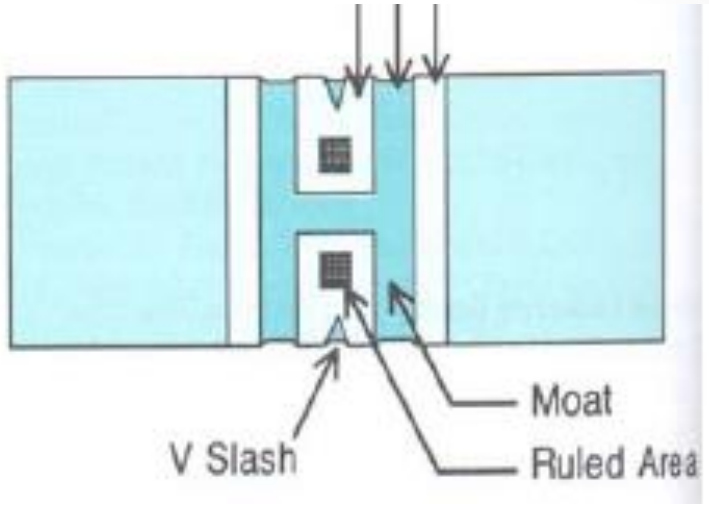
Quantitative Assessment (4)

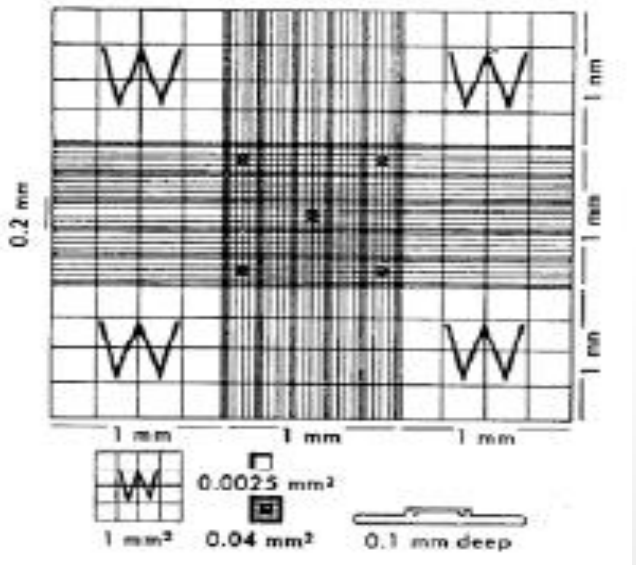
1. Make sure the hemocytometer it is clean and dry
2. Place a coverslip on hemocytometer
3. Place the hemocytometer in a petri dish lined with moist paper



Quantitative Assessment (5)

4. **Fill both sides of the hemocytometer (not to overfill)**
5. **After hemocytometer loaded, allow the cells to settle for 5-10 min.**
6. **Label the petri dish (Specimen identification and the set-up time)**



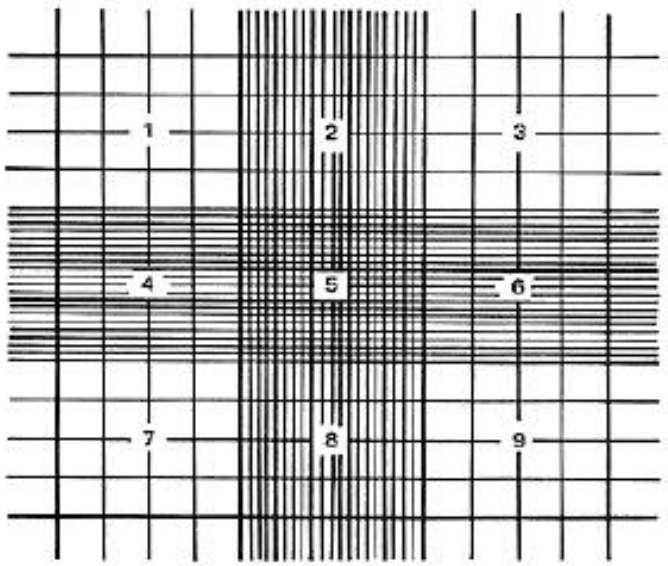


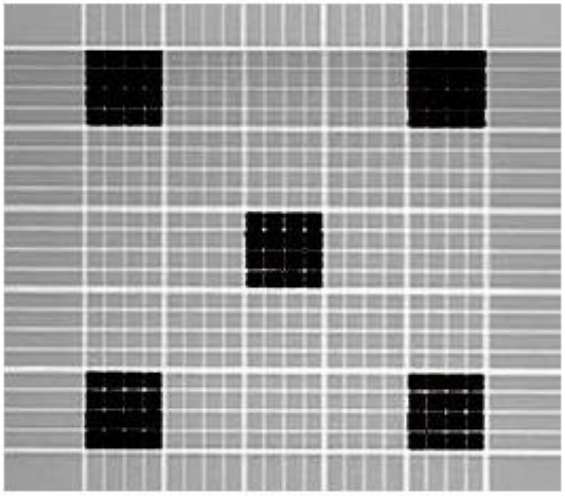
Quantitative Assessment (6)

- Cell counting procedures
  1. Place hemocytometer under microscope (10X) and adjust to see the cells
  2. Scan the large squares
     - Even distribution of cells
     - Cells should not overlap
  3. For diluted samples, a minimum of 200 cells should be counted
  4. Switch to hpf (40X)

Quantitative Assessment (7)

- Cell areas
  1. All nine squares if no dilution
  2. All nine squares for 1:10 dilution
  3. Four corner squares for 1:20 dilution (1, 3,7,9)
  4. Center square for 1:100 dilution (5)
  5. Red cell counting area for 1:200 dilution





Quantitative Assessment (8)

- **Calculations**

Cells per µL

$$\text{Cells/µL (/microliter)} = \frac{\text{\# of cells counted x dilution factor}}{\text{\# of square mm counted x chamber depth (0.1 mm)}}$$

where 1 mm^3 = 1 µL (microliter)

- **Cell should be counted in duplicated**
- **Laboratory should define the limit of agreement**

Why Automated Body Fluid Cell Counts?

- Limitation of manual cell counts
  - Subjective
  - High interobserver variability
  - Poor reproducibility
  - Difficult to distinguish WBC from other nucleated cells
- Benefit of automation
  - Improvements in accuracy and precision
  - Laboratory efficiency
  - Cost-effectiveness?

Validation of Automated Method for Body Fluid (BF) Cell Counts

- **BF performed in the usual CBC mode of cell counter is inaccurate**
- **Statement of intended use by manufactures**
  - **Indicate the types of BF validated on the analyzer**
  - **Analytical measurement range for each BF**
- **For BF not included in the manufacturer's statements**
  - **Considered as lab-developed method**
  - **Require more extensive validation**

Method Verification/Validation

- Accuracy
- Precision
- Sample carry-over
- Linearity
- Lower limit of quantification (analytical sensitivity)
- Analytical specificity
- Reportable range
- Reference intervals

Accuracy

- Compared with the reference (manual) method
  - A fundamentally flawed approach
  - Pearson correlation is not suitable
  - Spearman correlation and Bland-Altman plot are more appropriate
- No well-defined value for an acceptable correlation
- Another challenge
  - Sample integrity deteriorates over time

Limit of Quantitation (LOQ) and Specificity

- **LOQ：defined as the lowest cell count with C.V.<20%**
  - **10-30 WBCs/uL**
  - **RBC>100/uL**
- **Known substances might interfere with the analysis**
  - **High viscosity**
  - **Crystals**
  - **Microorganisms**

Validation Automated Methods for Leukocyte Differential Counting

- Most validated vs. manual differential count
  - Preferably performed on cytospin smears
    - Cell can be concentrated 20X fold
  - Combined cell categories must be taken into account
- Limitation of manual DC in BF
  - Imprecision & subjectively
  - Time delays
  - Cytospin affect cell recovery & proportion
- Microscopic is still indicated for malignant cell detection

Specific Issues Related to Different Body Fluid Types by Automation (1)

- CSF
  - The greatest challenges for automation
    - Extremely low cell counts
      - Showed a positive bias to manual counting
  - Most published reference ranges were established by manual methods
  - RBC count
    - Intracranial hemorrhage vs. traumatic tap
    - Some pediatric oncologist use 10 RBCs/uL as an indicator of PB contamination

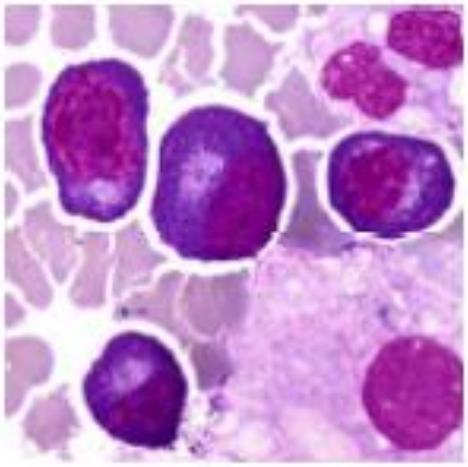
Specific Issues Related to Different Body Fluid Types by Automation (2)

- **Serous fluids**
  - **Mesothelial cells are normally present and can be numerous**
    - **Total nucleated cell (TNC) vs. Leukocyte**
    - **Differential counts (DC)**
      - **Include mesothelial cells in 2-part DC as MNs**
      - **Classification mesothelial cell as a category**
      - **Combination of mesothelial cell and histiocyte (M+H)**
  - **Reference ranges are generally not reported**

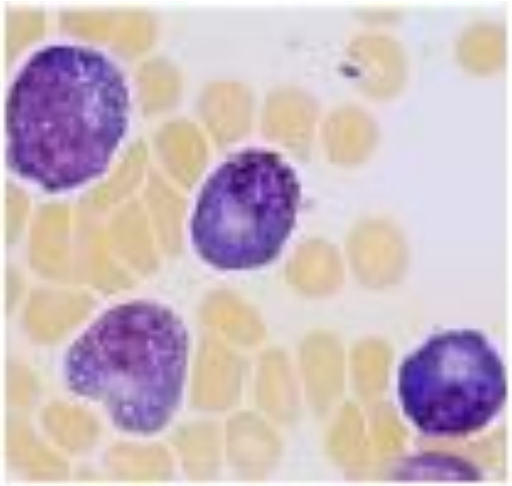
Specific Issues Related to Different Body Fluid Types by Automation (3)

- Synovial fluids
  - Cell counts are generally higher
  - Pretreatment with hyaluronidase
    - Prevent clogging of the flow cell in analyzer
  - Potential interference with automation
    - Crystal
    - Fat globules
    - Microorganisms

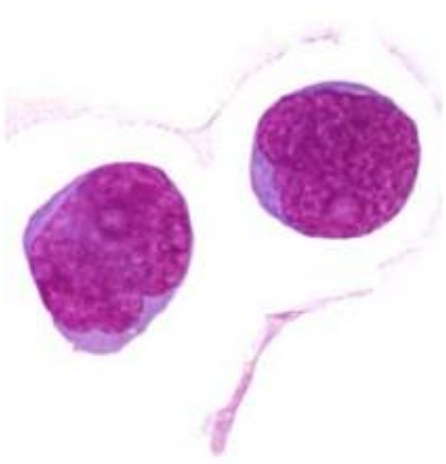
Evaluation of Nucleated Cell Subtypes (Hematopoietic Cells)



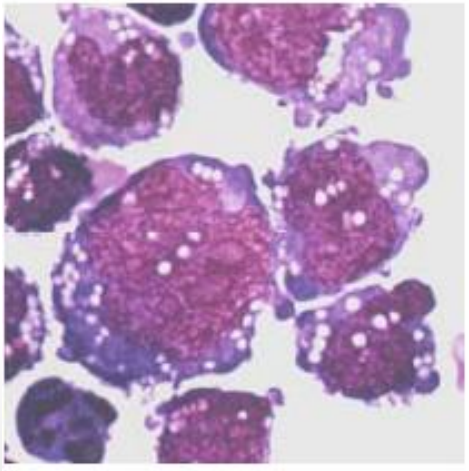
Reactive Lymphocytes



Plasma Cells



Blast Cells



Lymphoma Cells

Sample Processing/Techniques to Enhance Slide Quality

- Prepare slide as soon as possible (<4 hrs)
  - Especially for BF with low protein
- Washing cell before cytocentrifugation
  - Especially serous fluid with fibrin
- Viscous synovial fluids can be liquefied
  - Adding 400 units hyaluronidase to 1 mL of fluid
  - Incubating at 37˚C for 10 min.
- Cellular or bloody samples need to be diluted before cytocentrifugation
- 22% albumin can enhance cell adherence and reduce cell smudging in CSF

Postanalytical Variables

- Specimen & smear storage
- Physician or supervisor review
- Critical value notification
  - Blast in CSF
  - Presence of malignant cell
  - Microorganism found in aseptic fluids
- Morphologic observation assessment
- Microscopic result comparison
  - Especially when a diagnosis of malignancy is suspected

Thanks for Your Attention !