PROFICIENCY TESTING SERVICE
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PARTICIPANT STATISTICS | **CELL IDENTIFICATION** | **FIRST QUADRIMESTER 2018**

Q1-2018 RBC & WBC Morphological Evaluation: Normal vs. Abnormal

The CBC+ WBC differential is one of the most commonly ordered laboratory tests. It is nearly always one of the initial blood tests ordered when a physician first sees a patient and begins his search for an explanation for the patient's presenting complaint. Since so many of the normal processes in the human body are interconnected, tests that are affected by many disorders can, when normal, rule out a lot of possibilities. But if the test is not normal, the specific abnormality begins to lead the physician in the direction of the ultimate diagnosis.

In addition to the numeric information contained in the automated CBC /Diff results, there can be additional valuable clues as to what a particular diagnosis might be that can be found from the microscopic evaluation of the blood smear. Using information derived from evaluating a blood smear may assist the clinician to more quickly move through a diagnostic algorithm. Modern hematology analyzers can perform an automated differential on every CBC sample. Laboratories use a combination of the flagging capabilities of these analyzers coupled with a variety of numeric thresholds to triage CBC + Diff samples. A well-designed flagging algorithm for automated differentials can, in the average laboratory, generally rapidly categorize 85 percent of automated differentials as essentially normal thus allowing immediate reporting. The remaining 15 percent of automated diffs will require some degree of manual smear review to determine the presence or absence of any clinically significant numeric or morphologic abnormality.
The algorithms that have been designed by laboratories are generally biased to result in a high degree of sensitivity but are only able to do so by sacrificing specificity. In other words, the algorithms generate very few false negatives but the trade off is they may easily generate many false positives. This means that just because an automated differential has been qualified as needing a smear review does not necessarily mean that there will be anything clinically significant found.

From a clinical standpoint, blood smear reviews serves 3 important objectives. First they are used as quality assurance tools by enabling the verification of the results generated by the automated analyzer. Second, reviews increase the probability that, if present, immature and/or abnormal/atypical cells will be identified. Finally, for those samples that have been flagged as suspicious, reviewing their blood smears provides the opportunity to detect and identify clinically significant morphologic abnormalities.

Review of the blood smear may start with a scan, often initiated in order to verify the automated platelet count or to look for an explanation of an abnormal RBCs or WBCs "flag". The scan may be adequate to confirm that the automated results are reportable or the scan may reveal information that necessitates a complete smear review. A complete smear review will usually include a 100-cell differential, a platelet estimate and morphologic evaluation of RBCs, WBCs and platelets. To perform either a scan or a review it is paramount to begin with an appropriately prepared, well-stained blood smear.

There are several technical aspects to preparing an appropriately prepared, well-stained blood specimen and all are subject to significant variability. To insure the least amount of artifact, it is best if blood smears are prepared within 4 hours of sample collection, after 4 hours distortions in WBC morphology may occur. Blood films are usually prepared manually and good technique is required to generate a blood film with a sufficient area of the proper thickness for thorough evaluation. Sufficient drying time and adequate fixation are required to avoid morphologic artifacts. Proper stain to buffer ratios, correct staining times and adequate washing are all necessary to generate blood films where the varying cell types, with their specific staining characteristics and cellular components, have the correct colors. If all of these pre-analytical components are not done correctly, even the most experience morphologist will be unable to correctly evaluate the specimen.

Unlike most laboratory tests, which have fairly well recognized numeric reference ranges, evaluating blood cell morphology has a significant subjective aspect. To identify abnormal morphology, one must first have a solid understanding of what normal looks like. One needs to be aware of the size variability, the nuclear/cytoplasm ratios, the typical nuclear shapes, the typical cytoplasmic pattern and the typical nuclear chromatin pattern between the various cell lines. Although experience and training seem to be most useful tools for evaluating cell morphology, the use of teaching slide sets and atlases are valuable resources.

RBC morphology reporting formats can vary among laboratories. A well-defined, semi-quantitative report format for RBC morphology must be clear, based on clinical significance and useful to the physician. Despite the standardization of many laboratory technologies and test result formats, there are still various protocols in use in the area of red cell morphology reporting. Current methods of reporting and quantifying red cell morphology include descriptive terms such as 'rare,' 'occasional,' 'many,' 'slight,' or 'moderate,' as well as numerical gradings of 1+, 2+, 3+, etc. Laboratories need defined, semi-quantitative schemes that dictates how many cells with a specific morphologic abnormality qualify as "rare", "many," "1+", "3+" and so on. Regardless of the terminology used, consistency between operators is critical. Periodic competency testing is meant to address this issue.

Evaluating WBCs is a combination of correctly categorizing the cell and determining if the cell is normal in appearance. For instance, it may be difficult to determine, by morphology alone, if a mononuclear cell is lymphocytic versus monocytic. A rule of thumb is to identify these kind of borderline cells "by the company that they keep". In other words, if there are a lot of cells that have similar color and size but are more clearly lymphocytic, and only a rare cell that is clearly monocytic, then if one is trying to identify a cell with characteristics of both cell lines, then by identifying it by "the company that it keeps", would characterize the borderline cell as lymphocytic. In this kind of situation, since the cell morphology was abnormal enough to make it difficult to classify the cell it would logically also be considered "variant/atypical/reactive". Conversely if the majority of the lymphs on a slide were small with scant cytoplasm, and a morphologically questionable cell seen on the same slide had a lot of cytoplasm and the monocytes that one saw on the slide had a lot of cytoplasm, then the questionable cell it probably more likely monocytic. When, for diagnostic and/or treatments reasons it is critical to positively identify cell lineage,
flow cytometric immunophenotyping methods are used.

An area that seems not to be addressed in the literature is that of over calling morphology. The goal of evaluating RBC and WBC cell morphology is to convey to the clinician information that cannot be reported numerically. This qualitative information frequently can provide diagnostic clues to the clinician. However if, in an attempt to not miss anything, one ends up reporting everything, the information may no longer be useful but instead confusing or misleading. The value and clinical usefulness of these kinds of subjective laboratory evaluations greatly depends on the knowledge, experience and judgement of the laboratory professional who performs them.

One aspect of proficiency testing that may be under appreciated is testing a laboratory's success at differentiating between normal and abnormal results. Therefore, periodically normal samples are included in the unknowns that are sent to participants. It is good to keep this in mind and when working with a proficiency sample, and assume, just like with patient samples, that the results are just as likely to be normal as abnormal.

There was one image that had two schistocytes arrowed. However, when 10-20 fields were scanned, there were not enough schistocytes seen in those 10-20 fields to warrant a comment. This particular case was an essentially normal blood smear.

Cell Identification

Specimen 1

Result	No.	Flag
Basophil, any stage	166	
PMN with Toxic Granulation/Vacuolization	50	***
PMN with Pelger-Huet Nucleus	26	***
Segmented Neutrophil (PMN, poly)	10	***
Abnormal Granulocyte, would refer	5	
Eosinophil, any stage	5	***
Basophilic Stippling	2	***
Immature Neutrophil	1	***
PMN with Dohle Bodies	1	***

Total Population **266**
Intended result: Basophil, normal
22 of 22 Referee Laboratories correctly identified the intended result of Basophil.

Specimen 2

Result	No.	Flag
Platelet, normal	260	
Platelet, giant	6	***

Total Population **266**
Intended result: Platelet, normal

Specimen 3

Result	No.	Flag
Schistocyte (bite, blister, helmet)	247	
RBC Fragments	10	
Acanthocytes (spur)	4	***
Poikilocytosis	3	***
Abnormal RBC, would refer	1	
Burr Cell (echinocyte, crenated)	1	***

Total Population **266**
Intended result: Schistocyte

Specimen 4

Result	No.	Flag
Monocyte, any stage	259	
Lymphocyte; atypical, Downey, variant	3	***
Lymphocyte, reactive	2	***
Abnormal, would refer	1	***
Blast, undifferentiated	1	***

Total Population **266**
Intended result: Monocyte

Specimen 5

Result	No.	Flag
Lymphocyte, normal	184	
Lymphocyte; atypical, Downey, variant	34	***
Lymphocyte, reactive	22	***
Monocyte, any stage	8	***
Lymphocyte, abnormal/atypical	6	***
Platelet, normal	3	***
Monocyte, normal/any stage	2	***
Abnormal, would refer	2	***
Abnormal Lymphocyte, would refer	2	***
Segmented Neutrophil (PMN, poly)	1	***
Immature WBC, would refer	1	***
Myelocyte	1	***

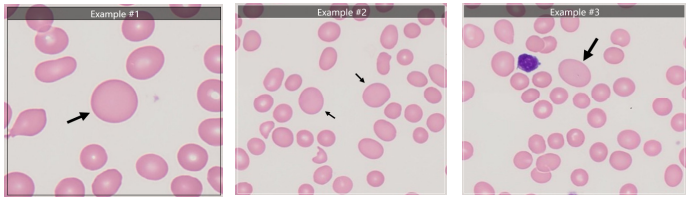
Total Population **266**
Intended result: Lymphocyte, normal
19 of 22 Referee Laboratories correctly identified the intended result of Lymphocyte, normal.

Correct responses are defined as those reflecting agreement among 80% or more of all participants or referees. Unacceptable responses are indicated by "*****" on the Flagging line of each specimen.

Cell Identification - Educational Challenge

Specimen 1	No.
Macrocytic	166
Spherocyte	21
Polychromatophilic RBC	4
Elliptocyte/Ovalocyte	2
Myeloblast	1

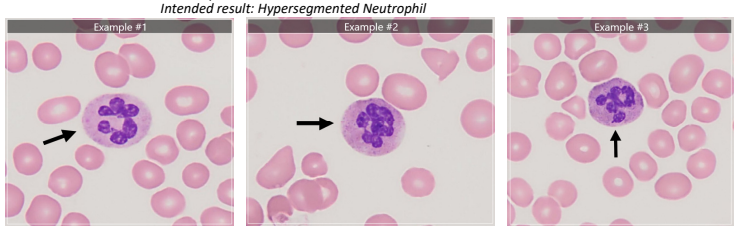
Total Population: **194**
Intended result: Macrocytic



*To see the original fullsized immages, please sign on to your data entry sheet at http://www.aab-pts.org

Specimen 2	No.
Hypersegmentated Neutrophil	181
Segmented Neutrophil (PMN,	6
Segmented Neutrophil (PMN, poly)	2
Abnormal Granulocyte, would refer	2
Macrocytic	2
Erythrocyte, normal RBC	1

Total Population: **194**
Intended result: Hypersegmented Neutrophil



Sample 18Q1 - Clinical Discussion