The full blood count (FBC) also called as complete blood count (CBC) is usually the first investigation requested by physicians to determine a patient's general health status. It is a group of tests that evaluate the cells that circulate in blood, including red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs). The CBC can evaluate one's overall health and helps to screen a variety of diseases and conditions, such as infections, anemia and leukemia.

Current state-of-the-art hematology analyzers perform these tests within minutes and provide an essential interface for the clinician to assess a patient's condition. They must also meet the demands of today's laboratory, including improved productivity, reduced costs, and excellent service. It goes without saying that the quality of the sample to be analyzed is essential to rapidly obtain a valid result. Poor sample quality results in additional costs, errors, and extra time for clinicians to analyze the result. Homogeneity of sample achieved by adequate mixing of the blood sample is the key to the quality of analytical results.

Preanalytical errors and sample mixing

The majority of errors in laboratory medicine occur in the pre- and postanalytical phases of the testing process. Incorrect phlebotomy techniques due to lack of awareness is the main reason for preanalytical errors (1). In a cross-sectional study on a total of 118,732 samples, the most common error observed is inadequate samples, followed by clotted samples (Figure 1). Other errors are misidentification, incorrect vials, diluted and hemolyzed samples.
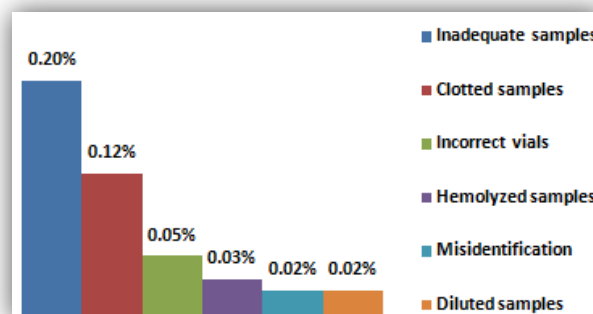

Figure 1: Prevalence and types of preanalytical errors at a tertiary care hospital (2).

Ethylene-diamine-tetra-acetic acid (EDTA) is considered the anticoagulant of choice for CBC. K_2EDTA is recommended by the CLSI (3). K_3EDTA is the liquid form of EDTA and will dilute the sample by 1-2%. Appropriate mixing of the blood tube after the blood has been drawn is an important step which ensures that tube additive is adequately mixed, blood samples are homogenous, and sample quality and integrity are maintained (4).

Manufacturers often provide their specific recommendations on the number of inversions required to achieve sample homogeneity for a particular tube, i.e. gentle inversion of sample tube at least five to ten times. For instance, in erythrocyte sedimentation rate procedure the Clinical & Laboratory Standards Institute (CLSI) recommends a minimum of 12 complete inversions with the air bubble travelling from end-to-end of the tube for standard tubes (10-12 mm x 75 mm, containing 5 mL of blood and with an air bubble comprising at least 20% of the tube volume)(5).Collection directly into a vacutainer is optimal, but difficult to achieve in small or pediatric patients with small veins, that collapse easily. In that case inadequate mixing can result in sample clotting (microclots) (6).

Keywords: Complete Blood Count, Preanalytical errors, EDTA.

Importance of homogenized blood in hematology analyzers

Samples of biological origin demonstrate limited stability in storage over a period of time due to the loss of their in vivo properties as well as degradation of constituent elements. Collection additive and transport conditions do show a significant impact on the blood sample. This necessitates observing quicker and best transport conditions as frequently the place of phlebotomy is separate from that of sample processing (7). As blood cells and coagulation factors are very fragile, special care must be taken during the entire sample handling time. After this preanalytical phase, laboratories want to do their best to obtain the most reliable results. At this precise moment, the specificity of certain samples makes proper mixing before performing the test critically important. If not mixed properly, samples with high viscosity or erythrocyte sedimentary rates (sedimentation is accelerated by an increase in the plasma concentration of acute-phase proteins, which are increased in acute tissue damage, chronic inflammation, chronic infection, and pregnancy) or samples stored in the refrigerator, may give erroneous results.

Mechanical sample mixing

Prior to the analysis, complete sample homogenization is performed in most laboratories using a variety of mechanical mixers. Before their availability, there was a debate among old school hematologists about the number of manual inversions performed by gentle back-and-forth movements of the wrist to achieve homogeneity in blood samples. Some of them considered that about 20 to 25 manual inversions were necessary to achieve complete homogenization of the sample (8, 9). All this process requires time and laboratory personnel. Additionally it significantly lengthens the sample processing time delaying result analysis affecting turn-around-time. Ultimately, it is the patient's precious time that is wasted.

There are two types of mechanical mixers: rocking and rotary-type. Rocking-type mixers are efficient when just enough headspace is present

to effect mixing. However, if the blood collection tube is completely filled, mixing to homogeneity is prevented. Thus in one study the hemoglobin value had doubled compared to the value obtained a week before, and the white blood cell and platelet counts were significantly reduced. However, after the analysis was repeated on the same specimen four times, results on hemoglobin, WBC and platelets were comparable to results obtained on the patient's specimen a week before. Apparently, the removal of aliquots of blood during four repeated analyses created sufficient headspace for the air bubble to move and achieve mixing to homogeneity on the rocking-type mixer (8).

Therefore, the blood collection tube should be examined to see if there is sufficient headspace available for mixing, especially when rocking-type mixers are used.
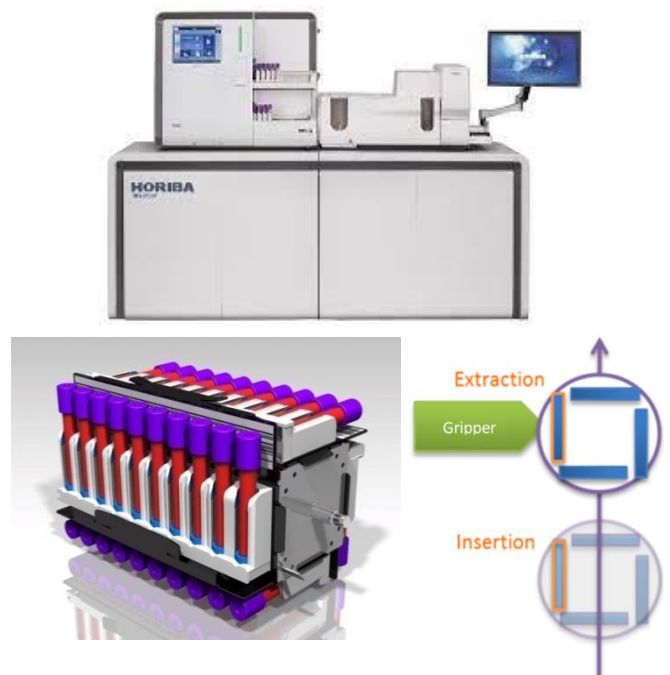

Figure 2: Four sided mixing and barcode reading module on Yumizen H1500/H2500 analyzers

Yumizen H1500 & H2500 sample mixing process

In order to ensure perfect homogeneity of the samples to be analyzed and achieve optimal conditions for obtaining precise results, Yumizen H2500 / H1500 analyzers are equipped with a rack rotation module (rotary-type). This unique feature consists of an automatic 360 degree rotating mixing for perfect homogeneity of the samples to be analyzed.

Keywords: Homogenous sample, rocking and rotary mixing.

Once the racks have been loaded into the analyzer, they are transferred into a four sides mixing module (Figure 2). The rotating tray can receive four racks (40 sample tubes), one on each side. The system performs minimum 40 inversions of the tube before sampling. This ensures a homogeneous mixture for measurement.

Thus, for the rack mode, manual mixing of the sample is not necessary because the instrument performs an automatic pre-mixing cycle by rotation which lasts 1 minute. This mixing method implies a decrease of workload for laboratory personnel and provides a standardized sample preparation that improves quality and reliability of measurement procedures.

High-throughput labs require automation of their hematology workflow and the capability to integrate with other systems. The 360 degree rotating mixing module was designed to optimize this workflow, saving time and adding precision.

In this context, the HELO* solution is an automation solution designed to cover the needs of a high throughput hematology platform with data and tube management, waste, blood film and digitalization management capability.

*HORIBA Evolutive Laboratory Organisation

Conclusion

Mixing is a crucial step while collecting a blood sample, but also just before performing a test. While manual mixing of the sample before loading it into the analyzer is common, automated blood sample mixing is a standardized and time saving approach. Rocking and rotary type mixers are commonly used for this purpose.

The unique rotating mixing module integrated to the Yumizen H1500/H2500 analyzers performs 40 reversals of blood sample tubes within one minute. It was designed to meet the demand of high throughput laboratories and focus on obtaining the best possible results for the benefit of the patient.

Keywords: HELO solution, 360 degree rotating mixing.

References

1. *A. Simundic, K. Bolenius, J.Cadamuro et al., EFLM Recommendation for venous blood sampling, v 1.1, October 2017*
2. *P. Arul, M. Pushparaj et al. Prevalence and types of preanalytical error in hematology laboratory of a tertiary care hospital in South India, Journal of Laboratory Physicians, Apr-Jun 2018;10(2):237-240.*
3. *Clinical & Laboratory Standards Institute(CLSI Guideline): Tubes and Additives for Venous Blood Specimen Collection; Approved Standard —Fifth Edition H1-A5,Vol. 23 No. 33.*
4. *Clinical & Laboratory Standards Institute(CLSI Guideline): Validation, Verification, and Quality Assurance of Automated HematologyAnalyzers, Approved Standard - Second Edition, CLSI document H26-A2 (ISBN 1-56238-728-6), 2010*
5. *Clinical & Laboratory Standards Institute (CLSI Guideline): Reference and Selected Procedure for the Erythrocyte Sedimentation Rate (ESR) Test; Approved Standard—Fourth Edition, H2-A4, Vol. 20 No. 27*
6. *B. De la Salle, Pre- and postanalytical errors in haematology, 19 February 2019*
7. *D. Wu, Y. Li, and F. Wang. How Long can we Store Blood Samples: A Systematic Review and Meta-Analysis.ElsevierBioMedicine. 2017 Oct; 24: 277–285.*
8. *S. Narayanan, Preanalytical issues related to blood sample mixing, Oct 2005.*
9. *S. Narayanan. Preanalytical issues in hematology. Journal of Laboratory Medicine, 2003; 27: 243-48*