PERFORMING HEMATOLOGY TESTING AT A CENTRAL LABORATORY: CHALLENGES AND PERSPECTIVE

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One of the greatest challenges facing the hematology department of any central laboratory is time. From the moment blood is collected from a patient, cells begin to deteriorate and platelets begin to aggregate. To slow the deterioration process, anticoagulants such as EDTA, sodium citrate, or heparin are added to blood collection tubes in order to stabilize the sample until analysis. In a hospital setting, analysis often occurs within 1-2 hours post collection; validation and stability assessments often use fresh blood. Central labs, however, must have samples shipped to their location and generally are not able to perform analysis until at least 24 hours post collection. As a result, central labs are often plagued with samples exhibiting hemolysis and platelet clumping due to pre-analytical and in vitro effects. To mitigate these effects, Medpace continually reassesses and optimizes its processes to ensure each precious sample remains viable for hematology testing.

The primary method of ensuring sample stability is proper training of personnel at the local site. Nearly all instances of pre-analytical clotting in whole blood samples is associated with improper mixing of the sample after collection. It is important that whole blood samples be inverted 8-10 times immediately after the draw to insure proper mixing of the anticoagulant.[1] A delay in mixing the sample can result in premature clotting or clumping due to inadequate distribution of the anticoagulant in the sample. To counter in vitro clotting and other pre-analytical issues, MRL has prepared detailed training documents to better educate sites on the importance of sample handling as it relates to a central lab.

The second way to ensure sample stability is temperature control. In a hospital setting, hematology samples are often stored under ambient conditions until analysis. Although MRL internal studies[2] have demonstrated good stability of most hematology parameters up to 96 hours after collection (*Table 1*), it should be emphasized that these samples were stored and evaluated under well-controlled conditions. When samples are shipped under ambient conditions, they may be subject to more drastic temperature fluctuations depending on the geographical location (equatorial vs polar climates), elevation, and the season (hot summers vs cold winters). To better insulate the sample from these potentially confounding temperature fluctuations, MRL recommends samples be shipped under refrigerated conditions as these samples exhibit lower rates of platelet clumping and hemolysis upon analysis in the lab.

Table 1: MRL Stability Data (Time from collection to analysis)

Parameter	Refrigerated	Room temp	Parameter	Refrigerated	Room temp
WBC	96 hrs	96 hrs	**PLT**	96 hrs	96 hrs
RBC	96 hrs	96 hrs	**MPV**	96 hrs	96 hrs
HGB	96 hrs	96 hrs	**Retic**	96 hrs	72 hrs
HCT	96 hrs	96 hrs	**NE%**	96 hrs	96 hrs
MCV	96 hrs	96 hrs	**LY%**	96 hrs	96 hrs
MCH	96 hrs	96 hrs	**MO%**	96 hrs	72 hrs
MCHC	96 hrs	96 hrs	**EO%**	96 hrs	48 hrs
RDW	96 hrs	96 hrs	**BA%**	96 hrs	96 hrs

A critical difference between hematology testing performed at a local hospital vs a central lab is the choice of collection tubes available for valid assessments. EDTA has long been considered the gold standard anticoagulant for hematology assessments due to its ability to preserve the cellular components of whole blood, thus promoting excellent stability and reproducibility of results. In fact, hematology analyzers are often only FDA approved and validated for use with EDTA. Despite these advantages, EDTA does have its limitations. Primarily, it may cause platelet clumping as a result of EDTA induced pseudothrombocytopenia (EDTA-PTCP). Although rare, EDTA-PTCP can result in an artificially low platelet count, which is not valid. If EDTA-PTCP is suspected in a hospital setting, it is common practice for a physician to order a hematology panel using a 3.2% sodium citrate whole blood sample in an effort to obtain a thrombocyte count. However, this approach should be used with caution. Current literature is limited in terms of long term platelet count stability in general, but internal studies performed by MRL (*Figure 1*) support those reports that show platelet counts are significantly decreased in sodium citrate compared to EDTA.[1,3,4] A decrease in thrombocytes over time is not surprising given that 3.2% sodium citrate is the anticoagulant of choice for producing platelet poor plasma used in coagulation testing. Interestingly, the platelet count stabilizes after this initial decrease and remains stable for up to 96 hours under refrigerated conditions. This stabilization translates to a clinically significant, yet consistent, - 48.5 to -36.8% bias when compared to an EDTA whole blood sample (*Figure 1*). Therefore, although sodium citrate whole blood may be used to obtain an informative result in the case of EDTA-PTCP[5], it should be used as a last resort; the result should always be reviewed with caution.

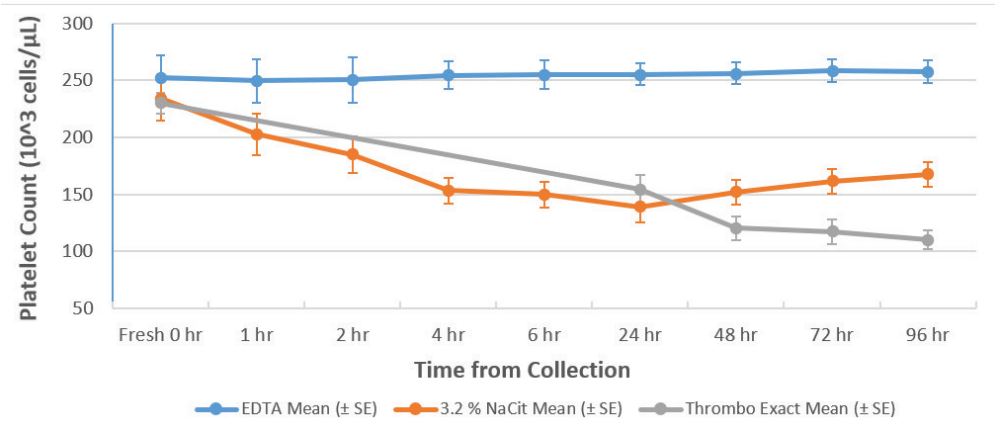

Figure 1. Time dependent stability of platelet counts in whole blood after collection in EDTA, 3.2 % sodium citrate, and MgSO4 (ThromboExact) collection tubes. Whole blood samples from 20 presumably healthy laboratory volunteers were collected in each type of collection tube. EDTA and 3.2 % Sodium Citrate samples were analyzed immediately after collection (Fresh 0 hr) and at 1, 2, 4, 6, 24, 48, 72, and 96 hr. MgSO4 (ThromboExact) samples were analyzed at baseline, 24, 48, 72, and 96 hr. Error bars represent Standard Error (± SE).

As more clinical trials begin to move into the hematology/oncology disease space, MRL continues to research, develop, and optimize new and better methods for supporting such studies. Even when analyzed fresh, whole blood collected from patients with oncologic or hematologic disorders, such as hemolytic anemia or thrombocytopenia, is inherently prone to hemolysis and platelet clumping.[5,6,7] In these instances, alternative collection tubes (e.g. the ThromboExact tube by Sarstedt) have shown some promise but have not been fully accepted into practice.[6,7,8] Additionally, internal studies performed by MRL *(Figure 1)* have shown poor stability when using these alternative collection tube types.[9] Ultimately, rapid receipt of EDTA whole blood in combination with a well-executed blood smear from the local site continues to be best practice for obtaining reliable hematology results.

Despite all the challenges a central lab faces, the use of EDTA whole blood is not a significant contributing factor to non-reportable hematologic parameters. Since 2004, the MRL global rate of non-reportable results due to hemolysis or clotting has ranged from 1.13 to 1.97% of total samples received (*Figure 2*). For non-reportable results due platelet clumps, the global rate ranges from 0.56 to 0.94% total samples received. Starting in 2017, analyses specific to the hematology/oncology disease space showed nearly 6.00% of samples relating to these studies were deemed unreportable due to hemolysis, clotting, or platelet clumping. Since that time, MRL has made concerted and successful efforts to minimize the rate of samples generating non-reportable results due these phenomena (*Figure 2*). Overall, these rates remain exceptionally low and underscore the value a central lab offers in terms of consistent and reliable analysis. By combining experience with continued optimization of sample handling and processing during the pre-analytical phase, MRL will continue to observe improvements in these rates every year.

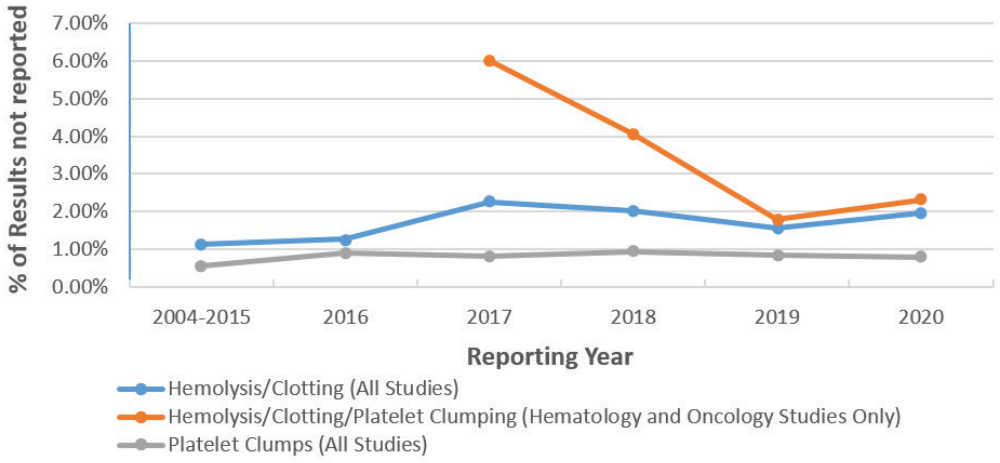

Figure 2. Annual global percentages of complete blood count (CBC) results not reported by MRL due to hemolysis, clotting, or platelet clumping since 2004. Blue: Percentage of CBC not reported due to hemolysis or clotting (all studies). Gray: Percentage of CBC not reported due to platelet clumping (all studies). Orange: Percentage of CBC not reported due to hemolysis or clotting (hematology/oncology studies only).

REFERENCES

1. Baccini, V., Geneviève, F., Jacqmin, H., Chatelain, B., Girard, S., Wuilleme, S., Vedrenne, A., Guiheneuf, E., Toussaint-Hacquard, M., Everaere, F., Soulard, M., Lesesve, J. F., & Bardet, V. (2020). Platelet Counting: Ugly Traps and Good Advice. Proposals from the French-Speaking Cellular Hematology Group (GFHC). Journal of clinical medicine, 9(3), 808. DOI: 10.3390/jcm903080838

2. Complete Blood Count and Leukocyte Differential stability – Room temperature (15-25 °C) and refrigerated temperature (2-8 °C). Stability Study Report. MRL-US; June 2019

3. Weber, D., Nakashima, M., (2018) Platelet Count Stability in Sodium Citrate-Anticoagulated Whole Blood Samples. Am J Clin Pathol 149, S84-S89. DOI: 10.1093/AJCP/AQX121

4. Vedy S., Boom B., Perez P., Schillinger S., Ragot C., Bakkouch S., Puyhardy J.M. (2011) Automatic platelets numbering with citrate as anticoagulant: Is the result valid? Ann. Biol. Clin. (Paris) 69:453–458. DOI: 10.1684/abc.2011.0596.

5. Podda, G.M., Pugliano, M., Femia, E.A., Mezzasoma, A.M., Gresele, P., Carpani, G., and Cattaneo, M. (2012) The Platelet Count in EDTA-anticoagulated blood from patients with thrombocytopenia may be underestimated when measured in routine laboratories. Am. J. Hematology 87(7), 727-728. DOI: 10.1002/ajh.23216

6. Mannub, S., Schuff-Werner, P., Dreibiger, K., and Kohlschein, P., (2016) Magnesium Sulfate as an Alternative In-Vitro Anticoagulant for the Measurement of Platelet Parameters? AM J Clin Pathol 145, 806-814. DOI: 10.1093/ajcp/aqw066

7. https://labmedicineblog.com/2019/10/29/hematology-case-study-the-story-of-the-platelet-clump-edta-induced-thrombocytopenia/

8. Schuff-Werner, P., Steiner, M., Fenger, S., Gross, H-J., Bierlich, A., Dreissiger, K., Mannub, S., Siegert, G., Bachem, M., and Kohlschein, P., (2013) Effective estimation of correct platelet counts in pseudothrombocytopenia using an alternative anticoagulant based on magnesium salt. Brit. J. Haem. 162. 684-692.

9. Automated Platelet Count in Different Whole Blood Types on Beckman Coulter DxH 900 Analyzers. Analytical Specificity/Collection Tube Assessment Report. MRL-US; July 2020

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