

Platelet Counting and Morphology

Implications for Hemocompatibility Testing of Biomaterials



CASY's capabilities for accurate cell counting, and importantly for straightforward cell volume and aggregate measurement supports standardization of hemocompatibility experiments

Standardized preparation of platelet rich plasma (PRP) is essential for in vitro studies of the interaction of platelets with implant materials. Here, aging of platelets and loss of their activation response is a critical factor of thrombogenicity, if preparation or storage time is prolonged.

- **Application**

In a recent study by Braune et.al. (1), the authors quantified circulating platelets from blood immediately after collection as well as after 4 and 24 hours after collection. As indicator for platelet thrombogenic function, also morphology of platelets and platelet aggregates were measured using CASY and visualized microscopically.

CASY was also employed to measure platelet volume, due to its pulse area analysis capabilities, allowing to accurately determine cell volumes (2).

- **Results**

Platelet count in PRP decreases significantly from 0 hours to 4 hours, but not further after 24 hours. This was in accordance with measurements performed with a Sysmex-XS800i hematology analyzer.

Platelet volume and aggregates: Aggregates were defined as percentage of events larger 5.04µm in size and 12fL in volume. CASY revealed that both parameters increased significantly after 4h and 24h. Platelet aggregates of up to 90µm diameter were observed after 24h. A finding confirmed by phase contrast microscopy. Since the Sysmex system is restricted by an upper volume limit of 40fL, larger platelets cannot be determined directly.

- **Conclusion**

From the experiments, the authors conclude that even after 4h, platelet function was affected. Therefore, they recommend to perform hemocompatibility studies to be completed within 4h after blood taking, enabling a more reliable and reproducible testing and helping to avoid underestimation of the thrombogenicity of biomaterial.

Title micrograph: 1000x giemsa stained blood smear with clump of platelets. By CDC, Dr. Mae Melvin.

● Examples

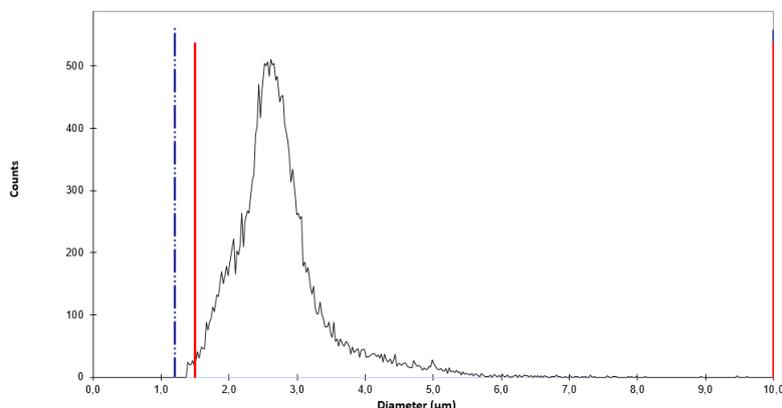


Fig. 1 Measurement of human Platelets using CASY TT Analyzer. Platelets show a homogenous size of about 2.6 µm diameter and a volume of 5.5 fL.
Data from CASY analysis database, analyzed using CASYworX 2.4.

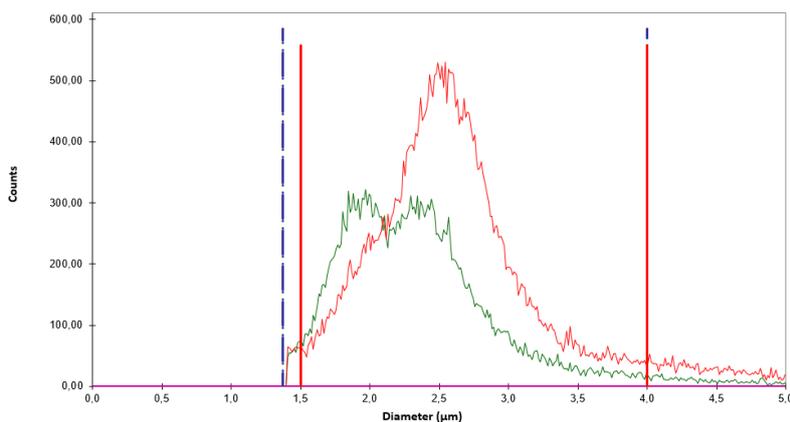


Fig. 2 Platelet-Activity, measured with CASY TTC. Red: Patient with normal, active platelets. Green: Patient with mostly inactive platelets (small volume) after being transfused with active platelets (bigger volume). Data from CASY analysis database, analyzed using CASYworX 2.4.

Parameters

Capillary	60 µm
Size scale	10 µm
L.Norm.Cursor	1,30 µm
R.Norm.Cursor	10,00 µm
L.Eval.Cursor	1,50 µm
R.Eval.Cursor	10,00 µm
Sample vol.	1 x 400 µl
Dilution	2,500E+04
Aggr.mode	Off

Results *modified*

Conc. check	Ok
Counts	21517
Counts/ml > 10 µm	1,035E+06
Counts/ml	1,392E+09
Tot.counts/ml	1,399E+09
%Counts	99,5 %
Volume/ml	1,873E+10 fl
Mean volume	1,346E+01 fl
Peak volume	9,451E+00 fl
Mean diameter	2,76 µm
Peak diameter	2,62 µm

● References

1. Braune S, Walter M, Schulze F, Lendlein A, Jung F. Changes in platelet morphology and function during 24 hours of storage. Clin Hemorheol Microcirc. 2014;58(1):159-70. doi: 10.3233/CH-141876.
2. T. Lindl, B. Lewandowski, S. Schreyögg and A. Stäudte, An evaluation of the in vitro cytotoxicities of 50 chemicals by using an electrical current exclusion method versus the neutral red uptake and MTT assays, Altern Lab Anim 33 (2005), 591–601.

Would you like to evaluate the software tool? CASYworX is available for free, simply order the example files and run the analysis on your Windows-based PC or tablet: casyworx@ols-bio.de