



### When do you have to consider a pseudothrombocytopenia?

If during analysis, for a patient, a low thrombocyte count is found without according clinical explanation for it, i.e., signs of a hemorrhagic diathesis exist, a pseudothrombocytopenia through EDTA must be considered.

Additional information on large thrombocyte aggregates can be found in the histograms and flags of the hematology device.

### Influence of the sample temperature on the EDTA induced pseudothrombocytopenia

The antibodies triggering the phenomenon are often of the cold antibody type. Due to their reaction optimum of 0-4 degrees Celsius, their impact is stronger, the more a blood sample is cooled down during analysis.

This influencing factor must be weighed stronger depending on the laboratory type. In medical practices and outpatient clinics, where the blood samples are in general analyzed within the shortest time after collection, the effect is less, which may be the reason, among others, why these pseudothrombocytopenia are observed less frequently in the medical practice area. In a hospital or private laboratories with usually longer transport routes, the effect of the sample cooling prior to analysis is stronger.

Due to this fact, with further clarification of the pseudothrombocytopenia, next to testing with alternative anticoagulants, it should be taken care that there is as little cooling of the blood sample as possible.

## Pseudothrombocytopenia

### Introduction

A pseudothrombocytopenia is present if the thrombocytes measurement in vitro results in thrombocytopenia, which is not given in vivo. Possible causes for such mismeasurements are abnormally large thrombocytes, mostly occurring due to illness, but also platelet plugs or accumulation of thrombocytes on leukocytes (thrombocyte satellitism). The latter two phenomena have no pathological impact. The risk in everyday clinical practice is that the misinterpretation of the thrombocyte value may lead to unnecessary further examinations or treatments.

Our round robin test specimen 2019-02 H3B originates from a 77-year-old female patient with, as collateral diagnosis, EDTA induced pseudothrombocytopenia.

### Causes for pseudothrombocytopenia

#### Giant thrombocytes

They have pathological value and often occur with congenital disorders (e.g., May-Hegglin-Anomaly), but also as part of myelodysplastic syndromes and myeloproliferative neoplasms.

#### Platelet plugs and thrombocyte satellitism

The use of anticoagulants, mostly EDTA, can lead to the formation of platelet plugs or rarely to the accumulation of thrombocytes on leukocytes in the shape of rosettes (satellitism, rare).

### What causes platelet plugs despite anticoagulants??

For the measurement of the hematological parameters, such as the thrombocytes, usually the anticoagulant EDTA (ethylenediaminetetraacetate) is used. EDTA forms, with calcium ions as the central atoms, irreversible complexes. This way, the function of the calcium in the blood coagulation is eliminated, and the coagulation of the blood sample is inhibited. Nevertheless, for around 1 in 1000 persons, a platelet plug occurs in EDTA anti-coagulated blood samples and, therefore, a mismeasurement of the thrombocyte count results in the impedance measurement.

The effect is observed most frequently with the use of EDTA, but also, rarely, with other in vitro anticoagulants such as citrate and heparin. This may complicate further clarification via these two alternative anticoagulants.

Cause for the EDTA induced pseudothrombocytopenia should be the exposure of proteins at the thrombocyte surface through EDTA. Subsequently, antibodies may bind to those proteins (GPIIb/IIIa-complex, receptors for fibrinogen and Von Willebrand Factor) and, therefore, trigger a thrombocyte activation and aggregation. Often these antibodies derive from the spectrum of the cold antibodies.

### How to detect an EDTA induced pseudothrombocytopenia and measure a correct thrombocyte value?

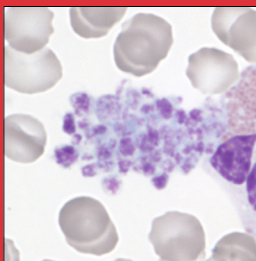
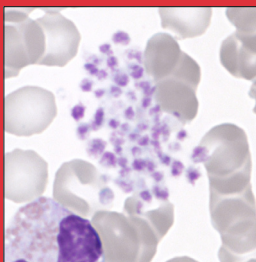
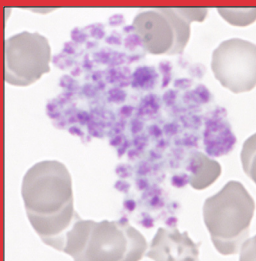
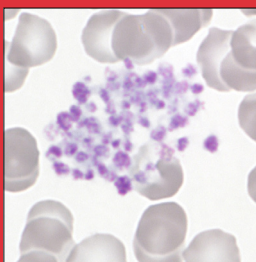
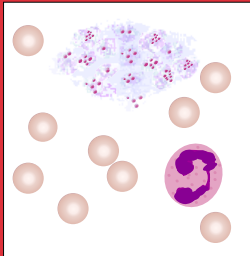
Indications of an EDTA induced pseudothrombocytopenia are:

1. Detecting thrombocytopenia when measuring EDTA blood without according clinical correlation (e.g., signs of a hemorrhagic diathesis) and after exclusion of pre-analytic errors such as slow capillary blood collection, test tube overfilling or insufficient mixing of the tube after blood collection.
2. Microscopic evidence of large thrombocyte aggregates in the EDTA blood smears (often at the top and bottom edge of the smear).
3. Measuring higher thrombocyte values in heparin or citrate anticoagulated samples.
4. Measuring higher thrombocyte values in special tubes with alternative anticoagulant „Thromboexact®“ by Sarstedt).
5. Progressive decrease of the thrombocyte count with increasing cooling of the blood sample. This effect can often be observed, as the related antibodies are frequently of the cold antibody type and therefore have a reaction optimum of 0-4 degrees Celsius.



**Thrombocyte aggregates with EDTA induced thrombocytopenia**

Notably large aggregates, often at the top and bottom edge of the smear, as well as in the flag.



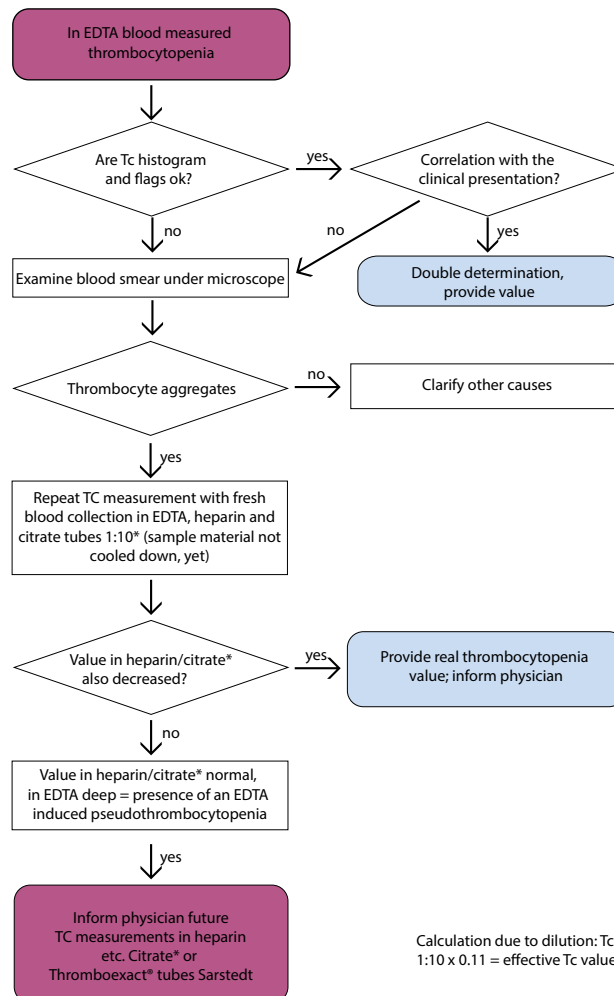
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**Flow diagram for clarification**



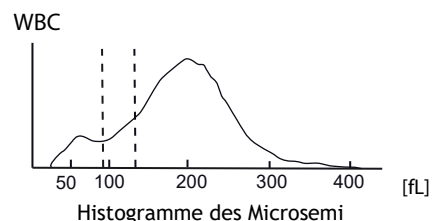
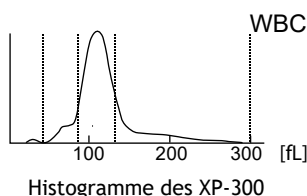
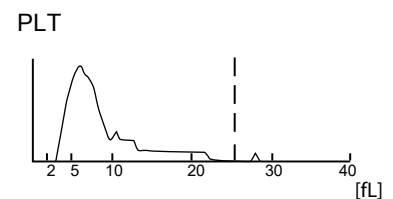
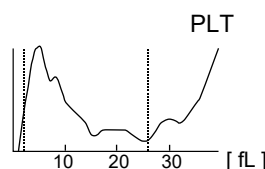
Calculation due to dilution: Tc value from citrate 1:10 x 0.11 = effective Tc value

**Histograms MQ 2019-2 H3B**

In the current sample, we measured a thrombocyte concentration of 22 G/l in the EDTA blood, in the heparin blood, it was 242 G/l.

With the 3-part hematology automates, such thrombocyte aggregates can lead to an increase of the thrombocyte curves on the right side and/or to an increase of the WBC curve on the left side. With the devices that we used, there was only a change in the thrombocyte histogram with the Sysmex XP-300. The device provided the PL flag, which indicates cryoglobulins or erythrocyte fragments. For the distributonal width of the thrombocytes, the DW flag appears, which indicates a significant anisocytosis. The MP flag, which actually should indicate thrombocyte aggregates, does not appear.

For Mythic and Microsemi, we did not observe anything special. The example shows that you cannot always rely on the warnings. The medical validation of the results is paramount.



Histogramme des XP-300

Histogramme des Microsemi