

# Different WBC count in EDTA caused neutrophil aggregation by hematology analyser

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Aggregation of neutrophils in peripheral blood smears is a very rare, mostly self-limiting phenomenon and may result in pseudo leukopenia. The exact reason for neutrophil aggregation *in vitro* has not been clarified, its relation to the use of ethylene diamine tetra acetate acid as an anticoagulant has been described in adults

A 3-year-old boy was referred to Valli e Asr hospital Arak, Iran. The EDTA blood sample was first loaded with the Impedance-based KX-11 Sysmex,

which showed a decrease in neutrophils and was observed in the peripheral blood of the Neutrophil aggregation. The blood sample was re-read with the XN-L350 Sysmex, which is based on the optic method, which showed the number of neutrophils in Norman. Although neutrophil aggregation is extremely rare in daily haematological practice, physicians should be aware of this phenomenon as it may be the cause of leukocytopenia. Therefore, manual differentials should be performed in cases flagged for low leukocyte or neutrophil counts.

**Key Words:** Neutrophil aggregation, EDTA (Ethylene Diamine Tetra Acetate), Sysmex KX-21, Sysmex XN-L350

## INTRODUCTION

Due to the wide spread application of automated analyzers for hematological routine tests, spurious results are very likely to occur which should be taken into careful consideration. These out comes might show up as a result of blood cells with serum antibodies interacting as well as reacting with drug or additives in reagents. The effects that repeatedly mentioned are: neutrophil-platelet clumping (satellitism), platelet aggregation and aggregation of nucleated red cells, resulting in pseudo leukocytosis or pseudo thrombocytopenia [1]. A phenomenon less widely spread and more self-constraining in patients who are com of age, is Aggregation of neutrophils in peripheral blood smears, also known as leuko agglutination, neutrophil agglutination, granulocyte aggregation, or leukocyte clumping.

The exact mechanism for neutrophil aggregation *in vitro* has not been clarified. Its relation to the use of Ethylene Diamine Tetra Acetate acid (EDTA) as an anticoagulant has been described in all reports. Aggregation of neutrophils may result in pseudo leukopenia, thus eliciting further laboratory investigations [2].

## CASE PRESENTATION

A 3-year-old boy was referred to Valli e Asr hospital Arak, Iran. He was admitted to hospital because of burning in his left arm. He had had no previous illness and was not taking any drugs. A Full Blood Count (FBC) performed on K2EDTA-anticoagulated blood on a Sysmex KX-21 (Sysmex Corporation, Kobe, Japan) showed Red Blood Cell (RBC) count  $3.36 \times 10^{12}/L$ , haemoglobin 98/L, White Blood Cell (WBC) count  $3.2 \times 10^9/L$  and platelet count  $457 \times 10^9/L$ . A blood film showed neutrophil aggregation (Figure 1). The anticoagulant EDTA is recommended to be 1.4 mg per every blood ml by World Health Organization

A repeat FBC performed on citrate anticoagulated blood showed a WBC of  $10.6 \times 10^9/L$ . the sodium citrate is recommended 3.2% (109 mmol/L) by WHO [3]. Further investigation of a K2EDTA-anticoagulated sample with a Sysmex XN-L 350, which uses fluorescence flow cytometry, showed a WBC of  $10.6 \times 10^9/L$  (Figure 2).

The print out of Sysmex XN-L350 showed no aggregation on sample with EDTA-anticoagulated blood (Figure 3A) but the same sample analysed with the Sysmex KX-21 showed an incorrect WBC (Figures 3A and 3B).

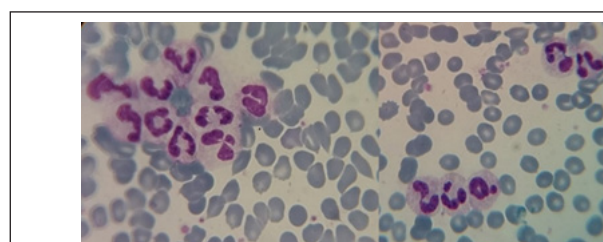


Figure 1) Neutrophil aggregation in EDTA-anticoagulated blood

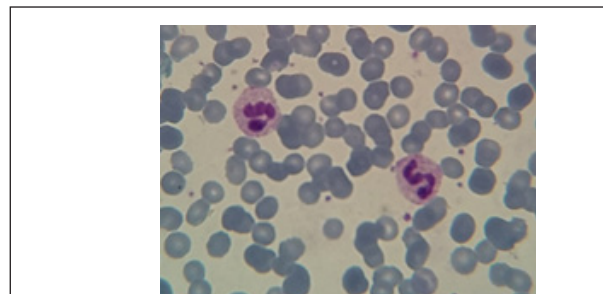


Figure 2) Neutrophils not aggregated in citrate anticoagulated blood

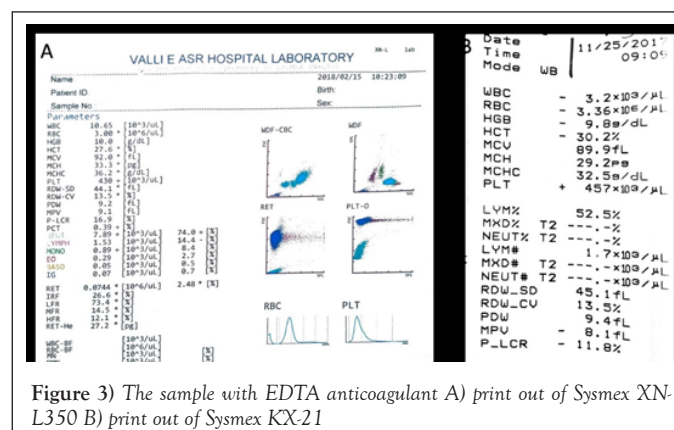


Figure 3) The sample with EDTA anticoagulant A) print out of Sysmex XN-L350 B) print out of Sysmex KX-21

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**RESULTS AND DISCUSSION**

Although the EDTA cause platelet aggregation and satellitism [4], it can neutrophil aggregation [5]. The latter effect has decreased WBC count and change WBC differentiation in automated analyser which use impedance method in counting and shrunken cell size in 3-part differentiation [6]. But this effect is not seen in analyser which uses optic method in counting also in 5 or more-part differentiation on automated analysers use fluorescence staining and optical scatter [7].

**CONCLUSION**

In the impedance method the shrunken cell aggregate and cross over the aperture when the antibody put the cell together. But in the analyser which use the optical method due to use of fluorescent dye and sample stream help to separate cell even in the presence of EDTA mediated antibody neutrophil aggregation.

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