Innovative HORIBA Medical compact 6-Diff hematology analyzer (Yumizen H500-ESR) performing FBC and ESR compared with ESR devices (RR Mechatronics Inversa and ALIFAX Test 1)

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Abstract

The studied parameter is the erythrocyte sedimentation rate (ESR). ESR is a common screening test worldwide. This test is usually used to detect and monitor diseases accompanied by an inflammatory syndrome. Nevertheless, the manual standard method to measure ESR is poorly reproducible, and its automated gold-standard method remains time consuming. On the other hand, measurement of the precursor stage of sedimentation, red blood cell aggregation, allows to assess ESR with a very quick measurement. Erythrocytes associate in long chains : rouleaux formation. Rouleaux sediment faster than separated red blood cells (RBCs). Measurement of aggregation is well documented in the literature and is usually performed by following the extinction of light by RBCs rouleaux over time. The International Council for Standardization in Haematology made a point about the issue in 2017 : ESR parameters related to aggregation measurement should be expressed in mm/hour in correlation with the Westergren method.
The purpose of this study is the evaluation of the quality of analysis performed by Yumizen H500-ESR prototype device and ALIFAX-Test1, two devices deriving ESR from RBCs aggregation measurement compared to a reference machine using Westergren automated method (Inversa). ALIFAX-Test1 is a stand alone ESR device and the Yumizen H500-ESR, a device performing Full Blood Count (FBC) and ESR. Blood samples from 232 patients were taken into EDTA tubes for alternate methods and Inversa. Precision and stability of the methods were performed.
For linear regression, the Yumizen H500-ESR (n=232) has a correlation coefficient r of 0,9 versus Automated Westergren method and Alifax (n=226) has a correlation coefficient of 0,92 versus Automated Westergren method. However, the HORIBA device shows significantly less dispersion on low values than the ALIFAX Test 1 and also has a better sensitivity and specificity. With a threshold of 15 mm/h, Test 1 and Yumizen H500-ESR had 2 false negatives but Test 1 has 62 false positives (FP) against 12 FP for Yumizen H500-ESR. Stability at 4°C during 24 hours gave better results with Yumizen H500-ESR (r=0,96) than ALIFAX (r=0,94). In Passing Bablok regression, the ALIFAX method (n=226) yielded a slope of 1,65 (95% CI, 1,5-1,79) with an intercept of 1,88 (95% CI, 0,68-3,31). The Yumizen H500-ESR (n=232) yielded a slope of 1,14 (95% CI, 1,03-1,21) with an intercept of -0,68 (95% CI, -1,08- -0,12). For intra-run precision, the mean CV was 7% for 6 runs on Yumizen H500-ESR.

Objectives

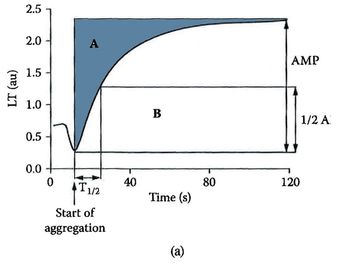
This evaluation compared two test machines, ALIFAX-Test 1, a stand alone ESR device commonly used in ALPHABIO and the Yumizen H500-ESR, a prototype device performing FBC and ESR, against a reference machine using Westergren method (Inversa). This study took place at the European Hospital of Marseille, on ALPHABIO site in Marseille.
Our target was to obtain enough samples with a significant variety of pathologies to understand the function of the prototype with high ESR samples and evaluate the prototype with patient bloods spanning the entire analytical range (2-120 mm). The study should bring data to try to understand biological interferences with our results.
The study was carried out in compliance with the rules of good practice. Specific care was taken to respect pre-analytical conditions (delay before analysis, transport, time of passage between test and reference machine …)

Background – Photometric Method

The optical behavior of blood is determined by the quantity of RBCs (Hematocrit) and also by the RBCs status in the suspension (Zijlstra, 1958). Light beams are either absorbed or reflected if they hit RBCs in suspension (O. Baskurt, 2019). The kinetics of aggregation can be followed by recording the changes in light transmission as a function of time after the flow has stopped abruptly. This recording is called a syllectogram (Fig 1). To clarify the syllectogram, we can divide the phenomenon into stages during the measurement according to the physical properties of RBCs. Firstly, we need a disaggregation of the blood sample by shearing (Fig 3). Under shear stress, RBCs deform, they are oriented along flow stream an in elongated form and blood samples become more transparent: light transmittance through the blood increases.

The abrupt cessation of shearing is followed by a decrease in transmitted light due to disorientation of the scattered cells, followed by an increase in transmission corresponding to erythrocyte aggregation (Fig 2).

Figure 1: Syllectogram



(a)

Figure 2: Mechanisms of aggregation (O. Baskurt, 2019)

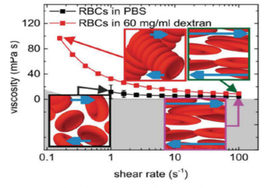


Figure 3: Disaggregation of the blood sample by shear
ref: Daniel Amadeus Dominic Flormann (2017)

Materials & Method

Inversa (RR Mechatronics)

Semi-automatic ESR analyzer, Inversa can be configured to give a first result after 30 minutes or 1hour.
The Mechatronics method uses whole blood anticoagulated with EDTA diluted in the device with sodium citrate (4 :1) with an accuracy of ± 2%.The carousel contains twenty-four Westergren pipettes. Each of these pipettes is made of precision glass.
The measurement is corrected according to the temperature, to give a value equivalent to a temperature of 18°C (Manley 1957). The test results are expressed in millimeters/hour . Quantity of sample (whole blood) aspirated: around 1 ml.

Test-1 (ALIFAX)

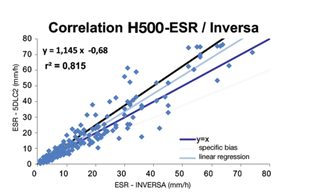
The Test-1 is provided by ALIFAX. Able to deliver results in 20 seconds, ALIFAX ESR analyzers measures red blood cell aggregation. Using 175 µl of EDTA blood sample, the test result is expressed in mm/h. The device has a capacity of 60 samples at a time.

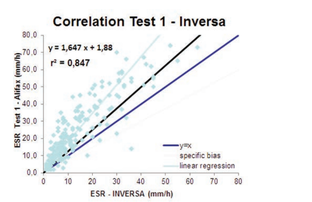
Yumizen H500-ESR (HORIBA)

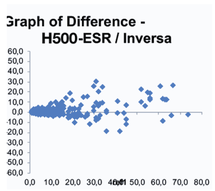
H500-ESR is a closed-tube instrument, configured for anticoagulated samples (EDTA mode). There is no sodium citrate dilution inside the device.
The prototype is able to deliver ESR results in around 60 seconds. The test results are expressed in millimeters/ hour. The quantity of sample (whole blood) aspirated is 180 µl. After presentation of a mixed sample tube, the blood sample is aspirated, and the optical transmittance measurement is started simultaneously.
Disaggregation is performed by shearing the whole blood sample. After sample collection, the shearing is abruptly interrupted. RBC relaxation is followed by RBC aggregation: a syllectogram is obtained. The acquisition is stopped and the ESR is computed from syllectogram.

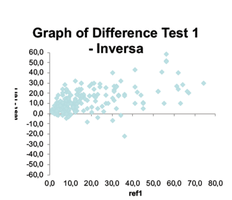
Results

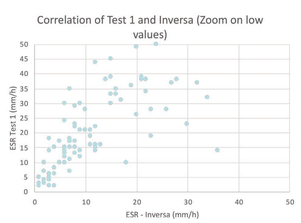
Passing-Bablok regression, the ALIFAX method (n=226) yielded a slope of 1,65 (95% CI, 1,5-1,79) with an intercept of 1,88 (95% CI, 0,68-3,31):

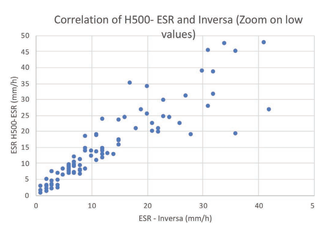


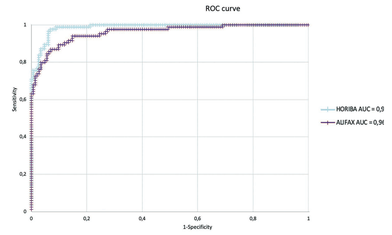




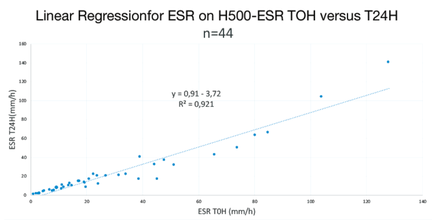


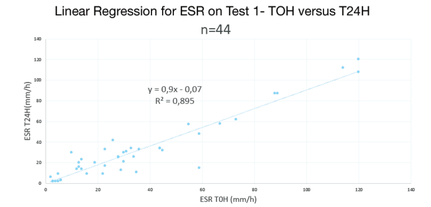






H500-ESR has less dispersion on the low values than Test 1 from ALIFAX ; H500-ESR has a better area under the curve than Test 1 (AUC of 0,99 for H500-ESR and 0,96 for Test1), Yumizen H500-ESR is better in both sensitivity and specificity.
With a threshold of 15 mm/h, Yumizen H500-ESR reaches 91% of specificity against 54% for ALIFAX. The same 44 samples were run at T0 and T+24 Hours on both machines at (4°C during 24 hours). This stability testing gave better results with Yumizen H500-ESR (r=0,96) than ALIFAX (r=0,94).





Discussion & Conclusions

"Despite the fact that ESR testing could, in some cases, be replaced by CRP, ESR testing remains widely prescribed in worldwide clinical laboratories. ESR still has an important clinical interest in rheumatology, particularly for assessing disease severity, especially for rheumatoid arthritis.

For emerging markets, affordable testing of ESR inside a hematology analyzer is of high value (significant technical gap). Despite its limitations and the introduction of other more specific markers of inflammation, ESR remains a widely used test.

This study is a proof of concept demonstration of integration of ESR into a haematology analyser. The blood sample volume required by the standard Westergren method is excessive in comparison to alternate methods. In this study, we demonstrated a blood consumption equivalent to the Test 1 and lower than the reference method by a 6 fold.

We also demonstrated the integration within a hematology analyzer with an equivalence between both devices of correlations versus the reference method. Thus, we provide a less labor-intensive workflow, costs reduction and elimination of a dedicated ESR tube.

We also observed an equivalent stability for aged samples up to 24H. Moreover, the specificity and sensitivity shown better results with the HORIBA Yumizen H500-ESR.

The performance for values above 60 mm/h remains to be fully understood by future study, although this specific threshold is significantly above the decision threshold. »

This evaluation study demonstrates the reliable performances of combined CBF-ESR testing on the Yumizen H500-ESR, as a suitable diagnostic tool for clinical laboratories seeking cost effective solution for cellular blood disorders and inflammatory syndrome.

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