Using a Rheology-Based ESR Method to Improve Sample Stability

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Abstract

Title: Using a Rheology-Based ESR Method to Improve Sample Stability **Authors:** Thomas Koshy (Beeatus Solutions, Inc., Palatine, IL 60067), Joseph Langella, Megan McCutcheon, Sandra Quaresma, Steve Giarrusso (ALCOR Scientific LLC, Smithfield, RI 02917) **Introduction:** Erythrocyte sedimentation rate (ESR) is a widely used non-specific laboratory test for assessing or monitoring patients with inflammatory conditions. Traditional ESR testing is done via the manual Westergren method, but many laboratories have adopted automated ESR analyzers to minimize hands-on time and decrease turnaround time. Despite these workflow improvements, sample stability for ESR testing remains limited: 4 hours for room temperature samples and 24 hours for refrigerated samples. These stability constraints can be challenging in reference/centralized laboratory setting when specimen collection locations are dispersed and refrigerated sample storage during transport is unavailable or poorly controlled. The iSED® ESR analyzer family (miniiSED®, iSED, iSED ELITE, and iSED PRO) uses novel technology to generate an ESR value by assessing RBC aggregation which significantly improves sample stability by increasing both room temperature and refrigerated stability to 28 hours. **Method:** The iSED analyzer family employs a common analytical unit and utilizes syllectometry-based photometric rheology technology to assess rouleaux formation (which occurs during the lag phase of RBC sedimentation). The degree of RBC aggregation is proportional to the degree of sedimentation. The iSED analyzers have demonstrated equivalence with the Westergren gold standard via analyzer-specific method comparison studies using fresh samples. Stability of iSED analyzer family results was examined by performing a correlation analysis of aged sample results vs. fresh sample (within 4 hours of collection) results from samples spanning the analytical range at defined time intervals. Both refrigerated and room temperature cohorts were evaluated. **Results:** Passing-Bablok regression was used to compare aged sample results to fresh sample results. The storage condition and duration was considered acceptable if 95% CI of slope included 1.0, 95% CI of intercept included 0.0, and the Spearman correlation coefficient was ≥ 0.90. Regression statistics of the 28-hour vs baseline comparison for the room temperature cohort (n=94) were: slope = 0.93 with a 95% confidence interval of 0.8627 to 1.0000, intercept = -1.65 with confidence interval of -4.0000 to 0.1471 and a Spearman correlation coefficient of 0.928. Regression statistics of the 28-hour vs baseline comparison for the refrigerated cohort (n=57) were: slope = 0.94 with a 95% confidence interval of 0.9167 to 1.0000, intercept = -0.67 with confidence interval of -2.0000 to 0.08333 and a Spearman correlation coefficient of 0.994. **Conclusion:** ESR samples tested with the iSED family of analyzers are stable for up to 28 hours when stored room temperature or refrigerated greatly improving transport options and laboratory logistics.

Introduction

Erythrocyte sedimentation rate (ESR), one of the most common laboratory tests in the world, is a widely used non-specific test for assessing or monitoring patients with inflammatory conditions. Traditional ESR testing is done via the manual Westergren method, but many laboratories have adopted automated ESR analyzers to minimize hands-on time and improve turnaround time. Despite these workflow improvements, sample stability for ESR testing remains limited, and it is well documented that results generated by gravity-based ESR methods typically degrade when samples age. Both CLSI and ICSH guidelines recommend testing Westergren ESR samples within 4 hours when stored at room temperature or within 24 hours if stored refrigerated.[1,2] For centralized or reference laboratories, sample collection sites can be a significant distance from the testing laboratory, and courier systems for transporting samples do not allow for effective temperature control. The stringent requirement of needing to test or refrigerate ESR samples within 4 hours is difficult to achieve or even monitor which can lead to off-label use of the assay, due to the sample being older than permitted by the labelling, or a need to re-draw the patient sample. An ESR method or technology that is more robust to changes in sample associated with aging would help ensure result reliability for labs that are challenged by the existing, relatively short, ESR sample stability limitations.

Method

The iSED analyzer family employs a common analytical unit and utilizes syllectometry-based photometric rheology technology to assess rouleaux formation (which occurs during the initial lag phase of the erythrocyte sedimentation process). Red blood cells (RBCs) are disaggregated and pumped into a flow cell. When the pump stops, RBCs immediately begin to aggregate in the rouleaux formation. Light transmission through the flow cell, which increases with RBC aggregation (Figure 1), is monitored and and converted to an ESR result in mm/hr. The degree of RBC aggregation is proportional to the degree of sedimentation, and the iSED analyzers have demonstrated equivalence with the Westergren method via analyzer-specific method comparison studies using fresh samples.

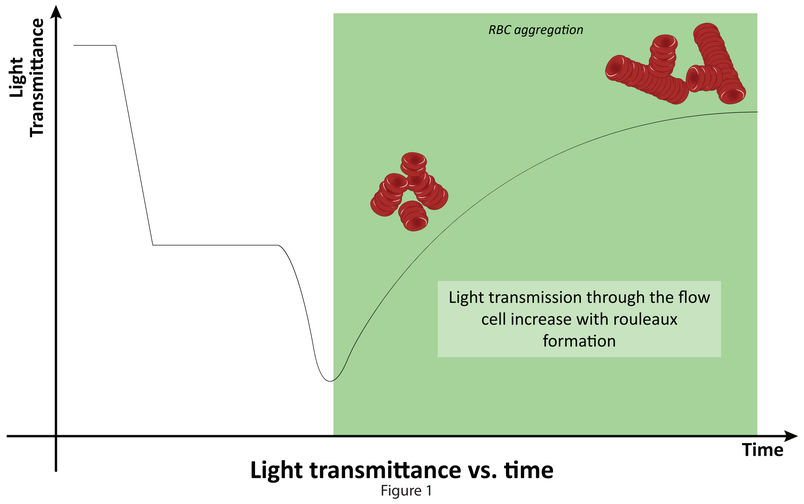


Figure 1

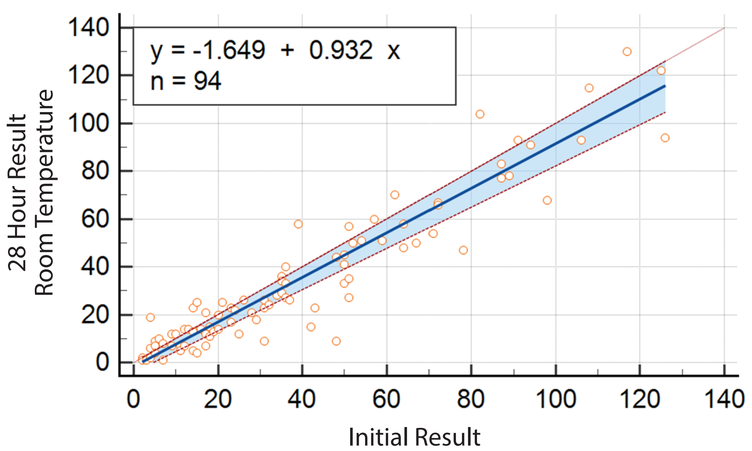
Sample stability on the iSED analyzer was examined by testing remnant clinical samples spanning the analytical range at defined time intervals at both room temperature (18-25°C) and refrigerated (4-8°C) storage conditions. Testing was performed on the iSED ELITE and miniiSED ESR analyzers (sample stability is consistent between iSED analyzer models due to common technology/analytical unit). Baseline testing was performed within 4 hours of sample collection on samples kept at room temperature. The samples were then stored either at room temperature or refrigerated and tested again at 24 hours and 28 hours on miniiSED (for refrigerated samples) or iSED ELITE (for room temperature samples). Sample tubes remained capped and refrigerated samples were brought to room temperature before testing. Stability was assessed by performing a correlation analysis of aged sample results vs. fresh sample results.

Results

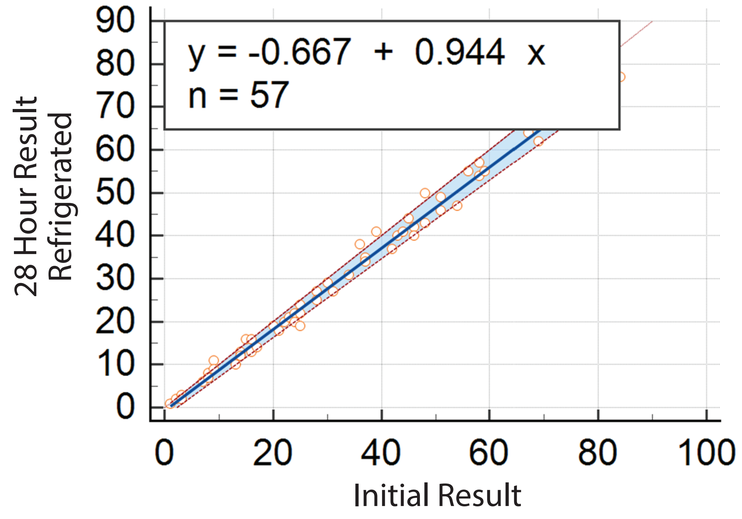
Ninety-four samples were used in the room temperature stability evaluation and 57 samples in the refrigerated temperature stability evaluation. Samples with initial results greater than 30 mm/hr were reasonably represented in this data set. Passing-Bablok regression was used to compare aged sample results to fresh sample results. The storage condition and duration was considered acceptable if:

- ✓ 95% CI of slope included 1.0
- ✓ 95% CI of intercept included 0.0
- ✓ The Spearman correlation coefficient was ≥ 0.90

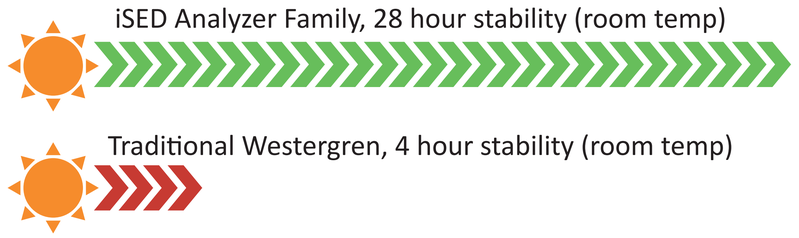
Regression statistics of the 28-hour vs baseline comparison for the room temperature cohort (n=94) were: slope = 0.93 with a 95% confidence interval of 0.8627 to 1.0000, intercept = -1.65 with confidence interval of -4.0000 to 0.1471 and a Spearman correlation coefficient of 0.928.



Regression statistics of the 28-hour vs baseline comparison for the refrigerated cohort (n=57) were: slope = 0.94 with a 95% confidence interval of 0.9167 to 1.0000, intercept = -0.67 with confidence interval of -2.0000 to 0.08333 and a Spearman correlation coefficient of 0.994



Compared to traditional ESR methods, this represents a 600% or 24-hour increase in room temperature stability.



Conclusion

By using novel technology to measure an earlier part of the erythrocyte sedimentation process, ESR sample stability dramatically increases from 4 hours room temperature/24 hours refrigerated to 28 hours when samples are stored room temperature or refrigerated.

The degree of RBC aggregation is proportional to the degree of sedimentation, but it appears that the charges governing RBC aggregation are more stable over time vs. the variables affecting sedimentation. Using photometric rheology to assess ESR increases sample stability, greatly improving transport options and laboratory logistics and helping ensure result reliability.

References

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2. Jou JM, Lewis SM, Briggs C, Lee SH, De La Salle B, McFadden S; International Council for Standardization in Haematology. ICSH review of the measurement of the erythocyte sedimentation rate. *Int J Lab Hematol. 2011* Apr;33(2):125-32. doi: 10.1111/j.1751-553X.2011.01302.x. Epub 2011 Feb 25. PMID: 21352508. https://medlineplus.gov/lab-tests/erythrocytesedimentation-rate-esr/