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# METHOD VALIDATION

## CORRELATION, PRECISION, AND CARRYOVER

# INTRODUCTION

This training is intended to provide an overview of performing method comparison with any of ALCOR® Scientific's iSED® Family of ESR Analyzers.

Method comparisons are performed as part of method validation to assess whether two analytical methods are comparable and is a way for the lab to verify the accuracy claims of the manufacturer.

ALCOR Scientific provides the protocols and worksheets for Correlation, Precision, and Carryover procedures for the iSED Family of Analyzers.

Clinical and Laboratory Standards Institute Procedures for the Erythrocyte Sedimentation Rate Test; Approved Standard – Fifth Ed.; H02-A5, Vol. 31 No. 11., 2011

# ESR truly is unique. Did you know…?

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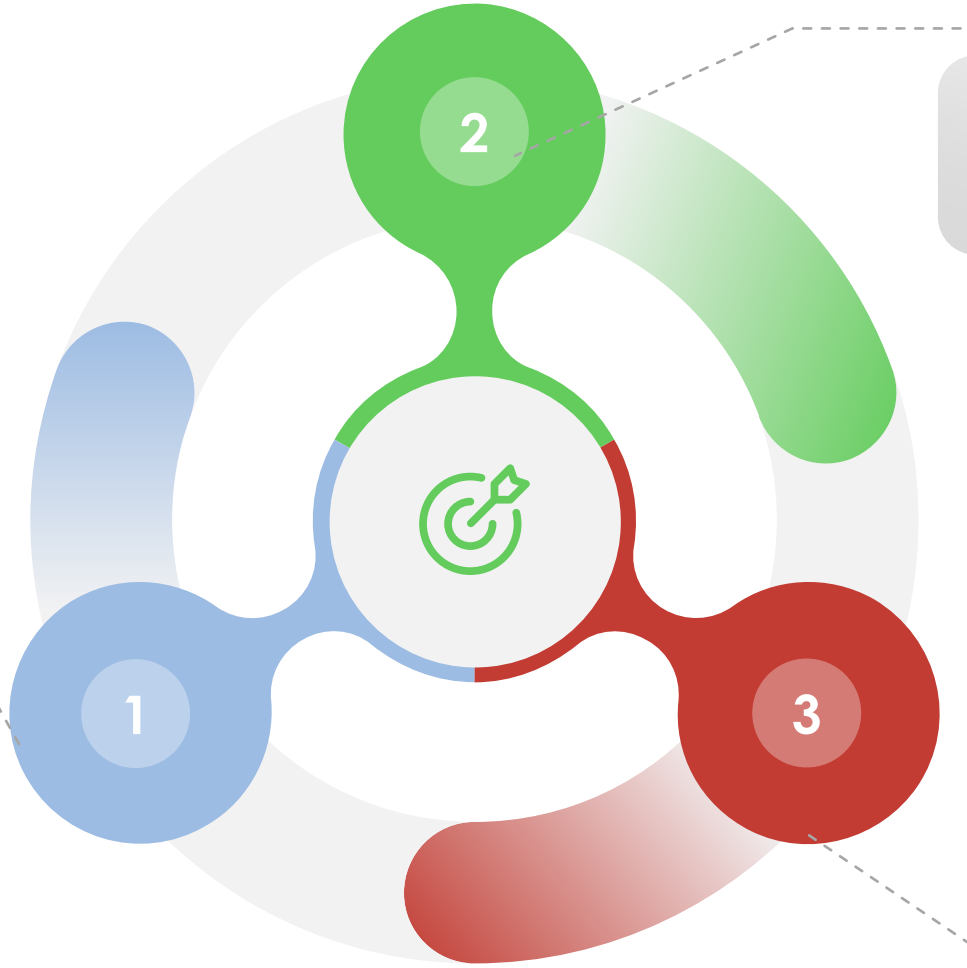


**There is no international standard available for ESR**

**Different ESR methods measure different parts of the erythrocyte sedimentation process[1]**

**Guidelines recognize ESR procedures cannot be calibrated[1] and a "true standardization of ESR assays is by definition impossible"[2]**


1. CLSI. Procedures for the Erythrocyte Sedimentation Rate Test; Approved Standard— Fifth Edition. CLSI document H02-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
2. Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ; on behalf of the International Council for Standardization in Haematology (ICSH). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. Int J Lab Hem. 2017;39:448–457. https://doi.org/10.1111/ijlh.12693






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# Challenges with comparing different ESR methods

ESR is a unique laboratory test and must be evaluated differently than other hematology tests with well-defined analytes.

- There is **no perfect substitute for human blood**; reference material for ESR (even if human-blood based) does not aggregate at the same speed as fresh human blood.
- **Reference material is method-specific** based on the part of the ESR process that is being assessed.
- Since a **true standardization of ESR methods is not possible,** the analytical performance of all new ESR methods should be correlated to the Westergren method[1] if possible.

***"Because the phenomenon of erythrocyte sedimentation is confined to fresh blood and is transient, presently, the only feasible way of providing a control material is for the manufacturer of test developer to specify a method of production of such material in the laboratory where it will be used. Because of the nature of the human erythrocyte sedimentation reaction, reference or control material of the usual type are not available for the ESR test."***[2]

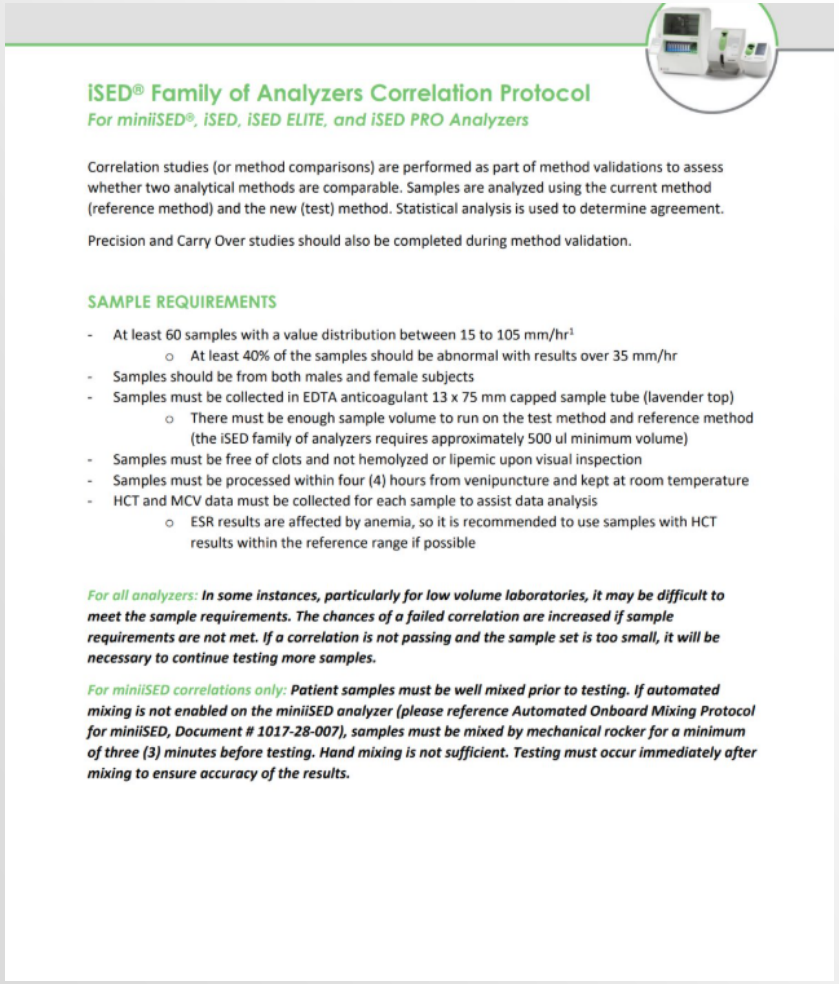

1. Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ; on behalf of the International Council for Standardization in Haematology (ICSH). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. Int J Lab Hem. 2017;39:448–457. https://doi.org/10.1111/ijlh.12693
2. CLSI. Procedures for the Erythrocyte Sedimentation Rate Test; Approved Standard— Fifth Edition. CLSI document H02-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

# CORRELATION PROTOCOL

**Sample Requirements**

- ✓ Minimum of 60 samples, from both males and females
- ✓ Adequate volume for both test and reference methods
- ✓ Visually acceptable (no clots, hemolysis, or lipemia)
- ✓ Samples within 4 hours of venipuncture and kept at room temperature
- ✓ HCT and MCV results available for analysis
- ✓ Samples within HCT values within the reference range (if possible; ESR results are affected by anaemia)

Refer to **iSED Family of Analyzers Correlation Protocol** (Document #100-07-011) when performing the correlation. **Following the established Correlation Protocol will provide the best outcome.**



iSED® Family of Analyzers Correlation Protocol

*For miniiSED®, iSED, iSED ELITE, and iSED PRO Analyzers*

Correlation studies (or method comparisons) are performed as part of method validations to assess whether two analytical methods are comparable. Samples are analyzed using the current method (reference method) and the new (test) method. Statistical analysis is used to determine agreement.

Precision and Carry Over studies should also be completed during method validation.

SAMPLE REQUIREMENTS

- At least 60 samples with a value distribution between 15 to 105 mm/hr[1]
  - At least 40% of the samples should be abnormal with results over 35 mm/hr
- Samples should be from both males and female subjects
- Samples must be collected in EDTA anticoagulant 13 x 75 mm capped sample tube (lavender top)
  - There must be enough sample volume to run on the test method and reference method (the iSED family of analyzers requires approximately 500 ul minimum volume)
- Samples must be free of clots and not hemolyzed or lipemic upon visual inspection
- Samples must be processed within four (4) hours from venipuncture and kept at room temperature
- HCT and MCV data must be collected for each sample to assist data analysis
  - ESR results are affected by anemia, so it is recommended to use samples with HCT results within the reference range if possible

*For all analyzers:* ***In some instances, particularly for low volume laboratories, it may be difficult to meet the sample requirements. The chances of a failed correlation are increased if sample requirements are not met. If a correlation is not passing and the sample set is too small, it will be necessary to continue testing more samples.***

*For miniiSED correlations only:* ***Patient samples must be well mixed prior to testing. If automated mixing is not enabled on the miniiSED analyzer (please reference Automated Onboard Mixing Protocol for miniiSED, Document # 1017-28-007), samples must be mixed by mechanical rocker for a minimum of three (3) minutes before testing. Hand mixing is not sufficient. Testing must occur immediately after mixing to ensure accuracy of the results.***

# CORRELATION PROTOCOL



**Running Quality Controls**

SEDiTROL® Quality Controls Level 1 & 2 should be run on the miniiSED®, iSED iSED ELITE, or iSED PRO analyzer each day of correlation testing. Quality Control protocols for the reference method should be adhered to during correlation testing. This helps ensure both methods are performing as expected.

Refer to Document #315-09-011 SEDiTROL Erythrocyte Sedimentation Rate Control, Instructions For Use for additional details along with LOT specific Instructions For Use found in your SEDiTROL pack.

# CORRELATION PROTOCOL



**Running Samples**

It is recommended that samples be tested first on the test method (miniiSED , iSED , iSED ELITE, or iSED PRO) followed by the reference method. Samples should be run on both methods within **60 minutes** of each other.

Please refer to iSED Family of Analyzers Correlation Protocol (Document #100-07-011) for step-by-step instructions.

**Data Analysis**

The Correlation Worksheet (Document #100-23-011) should be completed electronically and returned to ALCOR Scientific: **techservice@alcorscientific.com**

# DATA ANALYSIS AND ACCEPTANCE CRITERIA

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The correlation report is generated using Passing-Bablok regression analysis which is recommended for ESR.

For the data to **pass**, the following criteria must be met:

**INTERCEPT (A)**

**The value of the Intercept (A) is close to 0 and the 95% confidence limits contain the value of 0.0**

*The intercept (A) is an expression of the systemic differences between the methods.*

**SLOPE (B)**

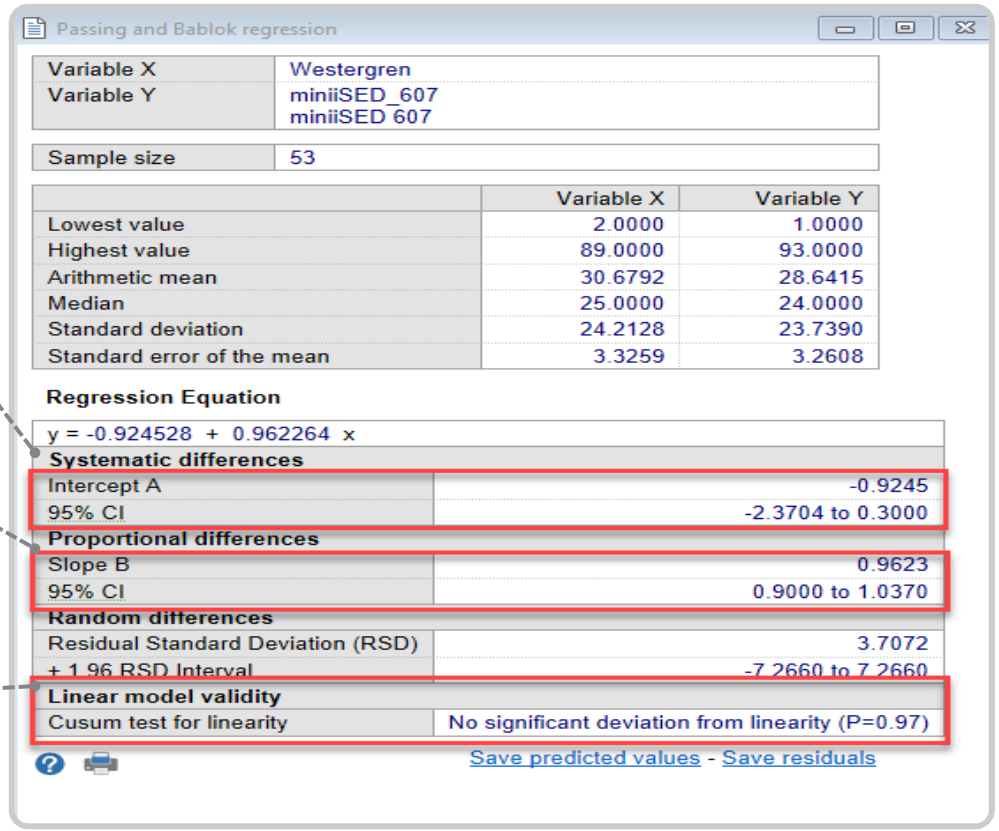
**The value for the Slope (B) is close to 1 and 95% confidence limits contain the value of 1**

*The Slope (B) is an expression of the proportional difference between the methods.*

**CUSUM TEST**

**The Cusum test value of P>0.05 indicates that the Passing-Bablok method is satisfactory as a method for analysis.**

*The Cusum test for linearity is used to evaluate how well a linear model fits the data for the Passing-Bablok method.*



Passing and Bablok regression

| | |
|---|---|
| Variable X | Westergren |
| Variable Y | miniiSED_607<br>miniiSED 607 |
| Sample size | 53 |

| | Variable X | Variable Y |
|---|---|---|
| Lowest value | 2.0000 | 1.0000 |
| Highest value | 89.0000 | 93.0000 |
| Arithmetic mean | 30.6792 | 28.6415 |
| Median | 25.0000 | 24.0000 |
| Standard deviation | 24.2128 | 23.7390 |
| Standard error of the mean | 3.3259 | 3.2608 |

**Regression Equation**

y = -0.924528 + 0.962264 x

| | |
|---|---|
| **Systematic differences** | |
| Intercept A | -0.9245 |
| 95% CI | -2.3704 to 0.3000 |
| **Proportional differences** | |
| Slope B | 0.9623 |
| 95% CI | 0.9000 to 1.0370 |
| **Random differences** | |
| Residual Standard Deviation (RSD) | 3.7072 |
| +-1.96 RSD Interval | -7.2660 to 7.2660 |
| **Linear model validity** | |
| Cusum test for linearity | No significant deviation from linearity (P=0.97) |

Save predicted values - Save residuals





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# The correlation failed. What could be the cause?

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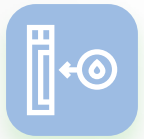
## Sample Age

Samples used in the correlation must be tested within 4 hours from collection. Differences between sedimentation-based and aggregation-based methods can be more significant with older samples.

## Sample Mixing

Samples must be thoroughly mixed prior to testing, particularly for miniiSED.



## ALCOR Scientific Correlation Protocol Deviation

The closer the correlation protocol is followed, the better the correlation outcome.

## Patient Population

While we know it can be challenging to obtain high ESR samples, it is very important to have a wide range of results. At least 40% should be in the abnormal range. To achieve the best data set, it is recommended to perform the correlation over multiple days.





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# The correlation failed. What's next?

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Repeat correlation following the ALCOR Scientific Correlation Protocol.

Consider running a 3-way correlation between the current method, the miniiSED or iSED, and manual Westergren (if they did not use Westergren initially). This will compare both methods to the Westergren reference method.

Provide ALCOR Scientific Technical Support the Config File from the analyzer. This is to ensure that the analyzer settings are correct.

Provide ALCOR Scientific Technical Support a description of how the correlation was performed.

Investigate if current ESR method is performing correctly (if they use their incumbent method instead of Westergren as suggested in the Correlation Protocol). If the current method is an automated or semi-automated analyzer, evaluate the service history.





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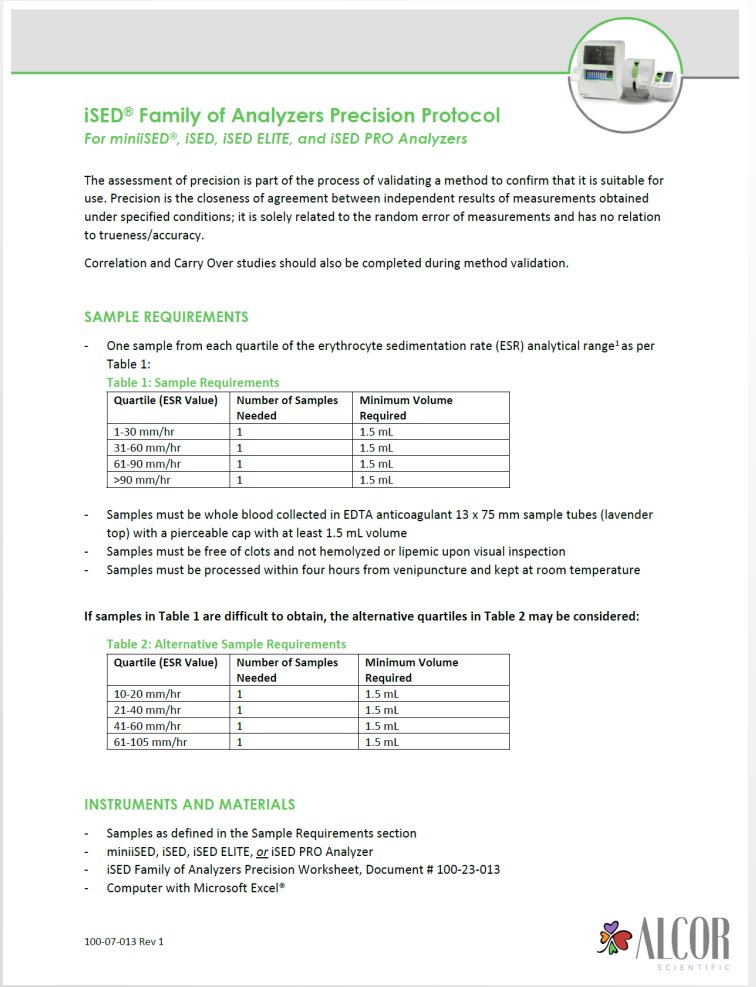
# PRECISION

The assessment of Precision is part of the process for validating a method confirming it is suitable for use.

Precision is the closeness of agreement between independent results of measurements obtained under specified conditions; it is solely related to the random error of measurements and has no relation to trueness/accuracy.

Please refer to iSED Family of Analyzers Precision Protocol for sample requirements and process.



## iSED® Family of Analyzers Precision Protocol

*For miniiSED®, iSED, iSED ELITE, and iSED PRO Analyzers*

The assessment of precision is part of the process of validating a method to confirm that it is suitable for use. Precision is the closeness of agreement between independent results of measurements obtained under specified conditions; it is solely related to the random error of measurements and has no relation to trueness/accuracy.

Correlation and Carry Over studies should also be completed during method validation.

### SAMPLE REQUIREMENTS

- One sample from each quartile of the erythrocyte sedimentation rate (ESR) analytical range[1] as per Table 1:

Table 1: Sample Requirements

| Quartile (ESR Value) | Number of Samples Needed | Minimum Volume Required |
|---|---|---|
| 1-30 mm/hr | 1 | 1.5 mL |
| 31-60 mm/hr | 1 | 1.5 mL |
| 61-90 mm/hr | 1 | 1.5 mL |
| >90 mm/hr | 1 | 1.5 mL |

- Samples must be whole blood collected in EDTA anticoagulant 13 x 75 mm sample tubes (lavender top) with a pierceable cap with at least 1.5 mL volume
- Samples must be free of clots and not hemolyzed or lipemic upon visual inspection
- Samples must be processed within four hours from venipuncture and kept at room temperature

**If samples in Table 1 are difficult to obtain, the alternative quartiles in Table 2 may be considered:**

Table 2: Alternative Sample Requirements

| Quartile (ESR Value) | Number of Samples Needed | Minimum Volume Required |
|---|---|---|
| 10-20 mm/hr | 1 | 1.5 mL |
| 21-40 mm/hr | 1 | 1.5 mL |
| 41-60 mm/hr | 1 | 1.5 mL |
| 61-105 mm/hr | 1 | 1.5 mL |

### INSTRUMENTS AND MATERIALS

- Samples as defined in the Sample Requirements section
- miniiSED, iSED, iSED ELITE, *or* iSED PRO Analyzer
- iSED Family of Analyzers Precision Worksheet, Document # 100-23-013
- Computer with Microsoft Excel®

100-07-013 Rev 1

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# PRECISION WORKSHEET



**Data Analysis**

For each sample, evaluate the series mean, standard deviation, and CV% of the 10 replicates. The **Precision Worksheet** contains formulas for these calculations.

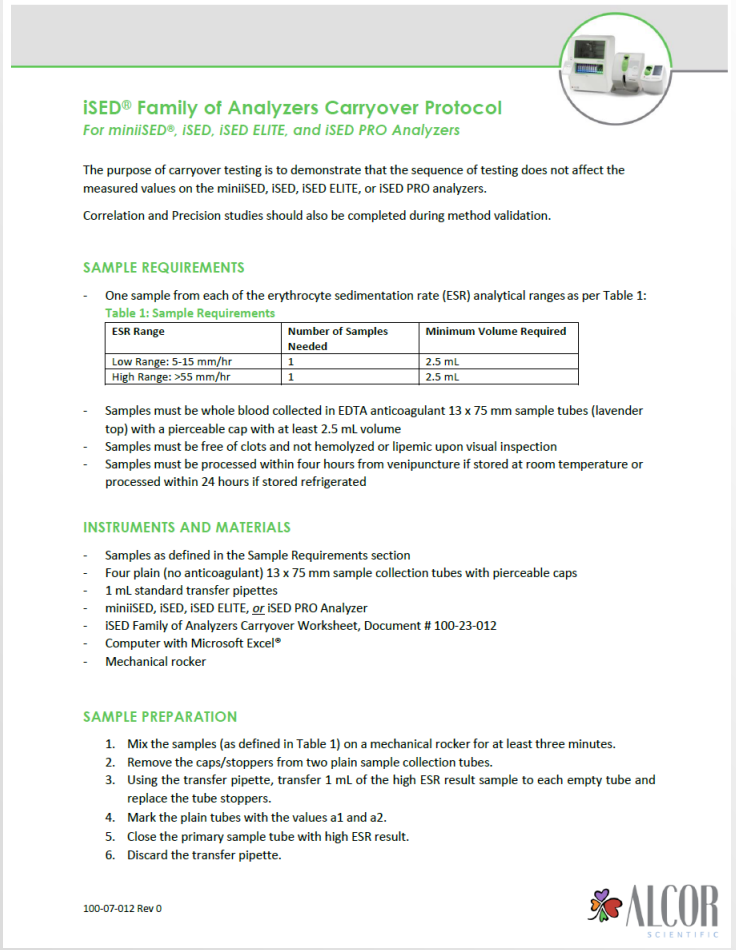
**Acceptable criteria for precision testing is as follows:**

| ESR Result | Acceptable CV% |
|---|---|
| Values 1-30 mm/hr | Less than 15% |
| Values 31-60 mm/hr | Less than 15% |
| Values 61-90 mm/hr | Less than 15% |
| Values >90 mm/hr | Less than 15% |

# CARRYOVER

The purpose of Carryover testing is to demonstrate that the sequence of testing does not affect the measured values on the miniiSED, iSED, iSED ELITE, or iSED PRO analyzers.

Please refer to iSED Family of Analyzers Carryover Protocol for details about sample requirements, preparation, and procedure.



**iSED® Family of Analyzers Carryover Protocol**

*For miniiSED®, iSED, iSED ELITE, and iSED PRO Analyzers*

The purpose of carryover testing is to demonstrate that the sequence of testing does not affect the measured values on the miniiSED, iSED, iSED ELITE, or iSED PRO analyzers.

Correlation and Precision studies should also be completed during method validation.

**SAMPLE REQUIREMENTS**

- One sample from each of the erythrocyte sedimentation rate (ESR) analytical ranges as per Table 1:

Table 1: Sample Requirements

| ESR Range | Number of Samples Needed | Minimum Volume Required |
|---|---|---|
| Low Range: 5-15 mm/hr | 1 | 2.5 mL |
| High Range: >55 mm/hr | 1 | 2.5 mL |

- Samples must be whole blood collected in EDTA anticoagulant 13 x 75 mm sample tubes (lavender top) with a pierceable cap with at least 2.5 mL volume
- Samples must be free of clots and not hemolyzed or lipemic upon visual inspection
- Samples must be processed within four hours from venipuncture if stored at room temperature or processed within 24 hours if stored refrigerated

**INSTRUMENTS AND MATERIALS**

- Samples as defined in the Sample Requirements section
- Four plain (no anticoagulant) 13 x 75 mm sample collection tubes with pierceable caps
- 1 mL standard transfer pipettes
- miniiSED, iSED, iSED ELITE, *or* iSED PRO Analyzer
- iSED Family of Analyzers Carryover Worksheet, Document # 100-23-012
- Computer with Microsoft Excel®
- Mechanical rocker

**SAMPLE PREPARATION**

1. Mix the samples (as defined in Table 1) on a mechanical rocker for at least three minutes.
2. Remove the caps/stoppers from two plain sample collection tubes.
3. Using the transfer pipette, transfer 1 mL of the high ESR result sample to each empty tube and replace the tube stoppers.
4. Mark the plain tubes with the values a1 and a2.
5. Close the primary sample tube with high ESR result.
6. Discard the transfer pipette.

100-07-012 Rev 0

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# CARRYOVER



The **Carryover Worksheet** from ALCOR® Scientific will automatically calculate carryover.

**Acceptable criteria for carryover testing is <10%.**

# THANK YOU!

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