Validation Protocol for the BD® Stem Cell Enumeration Kit on the BD FACSLyric™ Flow Cytometer

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1. Introduction

Before reporting patient test results, the flow cytometry laboratory must go through a process of validation to demonstrate that it can obtain performance specifications comparable to those established by BD Biosciences. The following performance characteristics are included in this protocol:

- Accuracy (Method Comparison)
- Repeatability (Within-Run) and Within-Laboratory precision
- Reproducibility (Inter-assay precision)
- Interfering Substances
- Carryover
- Linearity
- Limit of Blank
- Analytical Sensitivity
- Reference Range
- Specimen Stability

The process of validation includes the following steps:

- Review the instruction manual of the test to be implemented
- Review technical data sheets (TDSs) of tests to be implemented
- Review the standard operating procedure (SOPs)
- Review the validation protocol
- Collect appropriate specimens for the studies
- Perform testing
- Analyze data

The laboratories are responsible for determining their own requirements and appropriate acceptance criteria for each of the performance characteristics in accordance with any relevant accreditations and regulations.

2. Specimens, Instruments, and Reagents

2.1. Specimens

The following specimens were validated by BD Biosciences with this kit:

- Normal and mobilized peripheral blood
- Fresh and thawed leukapheresis products
- Fresh and thawed bone marrow
- Fresh and thawed cord blood

The following anticoagulants have been validated for use with this kit:

- EDTA, ACD-A, and heparin for peripheral blood (normal and mobilized), cord blood (fresh and thawed), bone marrow (fresh and thawed), and leukapheresis products (fresh and thawed).
- CPD for peripheral blood (normal and mobilized) and cord blood (fresh and thawed).
- For leukapheresis products, a mixture of ACD-A, heparin, and EDTA can also be used with this assay.
- EDTA, ACD-A, heparin, and CPD anticoagulants were validated with this assay. For leukapheresis, a mixture of ACD-A and heparin, and EDTA, can also be used with this assay.

Stability of fresh specimens (peripheral blood, bone marrow, leukapheresis products) was validated within 24 hours of collection. Stability of fresh cord blood was validated within 48 hours of collection. Stained samples were stored on wet ice (ice in a small amount of water, which allows better contact with the tube so that the contents chill quickly) and acquired within 1 hour of lysing.

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Frozen specimens were stained immediately after thawing. Stained samples were acquired immediately post-lysis.
If other specimens or anticoagulants are to be used for your testing, then validation testing needs to be done for each specimen/anticoagulant.

2.2. BD instruments and reagents

BD FACSLyric™, BD FACSCanto™ II, or BD FACSCalibur™ Flow Cytometer
BD® Stem Cell Control, BD® Stem Cell Enumeration Kit with BD Trucount™ Tubes
BD® CS&T Beads, BD® FC Beads 7-Color Kit
BD® 7-Color Setup Beads
BD FACSFlow™ Sheath Fluid
Falcon™ Test Tubes (from Corning) or equivalent

2.3. Other reagents required

Phosphate buffered saline (PBS) with 0.5% bovine serum albumin (BSA)

2.4. Statistical software

Data Innovations EP Evaluator™ Software or equivalent

2.5. Before beginning

- On a BD FACSLyric™ Flow Cytometer, ensure that the Setup & QC has valid CQC, PQC, and Reference Settings. Refer to *BD FACSLyric Reference System*.
- On a BD FACSLyric™ Flow Cytometer, install the BD® Stem Cell Enumeration Assay Module and refer to *BD Stem Cell Enumeration Application Guide for FACSLyric flow cytometer* for the assay workflow.

3. Accuracy Verification Protocol

Reference: CLSI documents EP-09; H42 for specimen storage and handling

Accuracy is the closeness of the agreement between the measured value of an analyte and the true value of an analyte. Parallel studies are performed to determine the relative bias (accuracy) between the method under evaluation and the reference method. Bias is defined as the difference in mean values between each method or the average of the paired differences.

3.1. Specimens

- Include a minimum of 40 fresh samples collected in the appropriate anticoagulants to be tested.
- Make sure that each sample is within the sample stability limits as listed in *BD Stem Cell Enumeration Kit Technical Data Sheet*.
- Include normal, mobilized peripheral blood specimens and thawed specimens if applicable.
- Set up and run each sample on both the reference method and the BD method within the sample stability as listed in *BD Stem Cell Enumeration Kit Technical Data Sheet*.

3.2. Procedure

- Startup the BD FACSLyric™ Flow Cytometer according to *BD FACSLyric Reference System*.
- Perform Instrument QC with BD® CS&T Beads according to *BD FACSLyric Reference System*.

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- Perform assay setup for the BD® Stem Cell Enumeration Assay according to *BD Stem Cell Enumeration Application Guide for FACSLyric flow cytometer.*
- Prepare samples according to *BD Stem Cell Enumeration Kit Technical Data Sheet*. Include BD® Stem Cell Controls in every run.
- Acquire the samples on the cytometer.
- Review all results and adjust gates as needed. Details are provided in *BD Stem Cell Enumeration Application Guide for BD FACSLyric Flow Cytometers*.
- Enter the results in EP Evaluator or equivalent software for the BD platform and the reference method.

3.3. Analysis of results

- When all the results have been entered into EP Evaluator or equivalent software, calculate the regression value (R^2). Intercept analysis may be performed if comparing dual-platform to single-platform results.
- Check that the regression value agrees with the performance specifications shown in the following charts and in *BD Stem Cell Enumeration Kit Technical Data Sheet*. If there are any highly discrepant values, check that there are no problems with specimen identification, data entry errors, or any method errors in the preparation or running of the samples.
- Print the data chart, regression graph, and bias analysis graph if performed, and put the materials in the validation notebook.

3.4. Performance specifications

Accuracy on the BD FACSLyric™ Flow Cytometer, *BD Stem Cell Enumeration Kit Technical Data Sheet*

Regression statistics by subset percentages and absolute counts by specimen type

Specimen Type	Variable	N	R^2	Slope (95% CI)	Intercept (95% CI)
Peripheral blood	Viable CD34+ (cells/µL)	111 (Normal: 48, Mobilized: 63)	0.99	1.06 (1.02, 1.10)	0.00 (–0.15, 0.16)
	% Viable CD34+ in viable CD45+		0.99	1.04 (0.90, 1.18)	0.00 (–0.03, 0.03)
Leukapheresis product	Viable CD34+ (cells/µL)	137 (Fresh: 68, Frozen: 69)	0.98	1.03 (0.97, 1.10)	0.56 (–2.56, 3.67)
	% Viable CD34+ in viable CD45+		0.99	1.09 (1.03, 1.14)	–0.02 (–0.08, 0.04)
Cord blood	Viable CD34+ (cells/µL)	132 (Fresh: 61, Frozen: 71)	0.98	1.08 (1.04, 1.11)	–0.20 (–0.56, 0.15)
	% Viable CD34+ in viable CD45+		0.98	1.07 (0.98, 1.15)	0.00 (–0.04, 0.05)
Bone marrow	Viable CD34+ (cells/µL)	121 (Fresh 60, Frozen: 61)	0.96	1.07 (1.03, 1.11)	–0.08 (–0.44, 0.28)
	% Viable CD34+ in viable CD45+		0.98	1.15 (1.01, 1.29)	–0.17 (–0.42, 0.08)

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4. Repeatability (Within-Run) and Within-Laboratory (Total) Precision Verification Protocol

Reference: CLSI document EP-15; H42 for specimen storage and handling

Precision is defined as the dispersion of replicate measurements. Precision is expressed quantitatively using standard deviation (SD) and coefficient of variation (CV). Per *CLSI EP15*, repeatability refers to variability due solely to within-run factors whereas within-laboratory or total precision refers to the variability due to run-to-run and day-to-day factors in addition to repeatability.

In general, precision assessment will require a minimum of two samples (two levels of process controls or two samples with different concentrations), one run per day, five replicates per run, for five days, to give a total of 25 replicates per sample. Intra-assay precision is defined as replicate measurements that are tested in one run. The mean, SD, and CV are calculated.

4.1. Specimens

- Include at least two samples or BD® Stem Cell Controls High and Low or equivalent.
- Use a freshly opened tube of BD® Stem Cell Controls or equivalent.
- If fresh specimens are used follow the appropriate specimen collection instructions according to *BD Stem Cell Enumeration Kit Technical Data Sheet*.

4.2. Procedure

- Startup the BD FACSLyric™ Flow Cytometer according to *BD FACSLyric Reference System*.
- Perform Instrument QC with BD® CS&T Beads according to *BD FACSLyric Reference System*.
- Perform assay setup for the BD® Stem Cell Enumeration Assay according to *BD Stem Cell Enumeration Application Guide for FACSLyric flow cytometer.*
- Prepare samples according to *BD Stem Cell Enumeration Kit Technical Data Sheet*. Include BD® Stem Cell Controls in every run.
- Acquire the samples on the cytometer.
- Review all results and adjust gates as needed. Details are provided in *BD Stem Cell Enumeration Application Guide for BD FACSLyric Flow Cytometers*.
- Enter the results in EP Evaluator or equivalent software for the BD platform.

4.3. Analysis of results

When all the results have been entered into EP Evaluator or the equivalent software, calculate the mean, SD, and CV.

Check that the CV and SD values agree with the performance specifications in the following charts and in *BD Stem Cell Enumeration Kit Technical Data Sheet*. If there are any highly discrepant values, check that there are no problems with specimen identification, data entry errors, or any method errors in the preparation or running of the samples.

Print the data chart and associated graphs and put the materials in the validation notebook.

4.4. Performance specifications

Precision of CD34+ absolute counts and percentages, *BD Stem Cell Enumeration Kit Technical Data Sheet*

Sample Type	Precision	Total CD34+ (cells/µL)			% Total CD34+ in Total CD45+		
		Mean	SD	%CV	Mean	SD	%CV

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Sample Type	Precision	Total CD34+ (cells/µL)			% Total CD34+ in Total CD45+		
		Mean	SD	%CV	Mean	SD	%CV
CD-Chex Plus™	Within run	3.53	0.56	N/A	0.05	0.01	N/A
	Total		0.62	N/A		0.01	N/A
CD-Chex CD34™ Level 1	Within run	13.23	1.55	N/A	0.21	0.02	N/A
	Total		1.59	N/A		0.02	N/A
CD-Chex CD34™ Level 2	Within run	36.47	N/A	8.50	0.56	N/A	6.63
	Total		N/A	8.93		N/A	6.94
CD-Chex CD34™ Level 3	Within run	120	N/A	5.59	1.8	N/A	3.03
	Total		N/A	6.35		N/A	3.12

5. Reproducibility

Reference: CLSI document EP-15; H42 for specimen storage and handling

Reproducibility includes lab-to-lab variability and/or instrument-to-instrument variability which is needed for multisite laboratory analysis.

Study design, specimens, and analysis will be same as Section 4 except the study would be conducted across multiple instruments and/or laboratories as needed.

5.1. Performance specifications

Precision of CD34+ absolute counts and percentages, *BD Stem Cell Enumeration Kit Technical Data Sheet*

Sample Type	Precision	Total CD34+ (cells/µL)			% Total CD34+ in Total CD45+		
		Mean	SD	%CV	Mean	SD	%CV
CD-Chex Plus™	Repeatability	2.30	0.44	N/A	0.04	0.01	N/A
	Reproducibility		0.47	N/A		0.01	N/A
CD-Chex CD34™ Level 1	Repeatability	12.19	1.35	N/A	0.18	0.02	N/A
	Reproducibility		1.38	N/A		0.02	N/A
CD-Chex CD34™ Level 2	Repeatability	34.70	N/A	6.40	0.51	N/A	5.63
	Reproducibility		N/A	6.69		N/A	5.80
CD-Chex CD34™ Level 3	Repeatability	118.33	N/A	4.88	1.72	N/A	3.77
	Reproducibility		N/A	5.37		N/A	3.87

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6. Interfering Substances

Reference: CLSI document EP-07, EP-37; H42 for specimen storage and handling

Substances and their concentrations tested for interference with the BD® Stem Cell Enumeration Kit are listed in *BD Stem Cell Enumeration Kit Technical Data Sheet.* Substances that are not in this list and are applicable to the sample cohort will need to be tested for interference.

6.1. Specimens

- Do not use previously fixed and stored specimens.
- Any samples that are clotted or hemolyzed should be rejected for testing.

6.2. Procedure

- Check *BD Stem Cell Enumeration Kit Technical Data Sheet* for a list of interfering substances.
- If additional substances are applicable, list them in the SOP with appropriate plan of action when present.

7. Carryover Protocol

Reference: CLSI document H26; H42 for specimen storage and handling

The carryover results for the BD FACSLyric™ Cytometer is <0.1% for viable CD34+ stem cells when assessed with the BD® Stem Cell Enumeration Kit. These values are published in *BD FACSLyric Technical Specifications and BD FACSLyric Reference System*. The carryover was established at default settings.

7.1. Specimens

A sample containing high viable CD34 concentration (1,000–1,500 cells/µL), e.g., leukapheresis.
A sample containing low viable CD34 concentration (0 cells/µL), e.g., normal peripheral blood.

7.2. Procedure

- Startup the BD FACSLyric™ Flow Cytometer according to *BD FACSLyric Reference System*.
- Perform Instrument QC with BD® CS&T Beads according to *BD FACSLyric Reference System*.
- Perform assay setup for the BD® Stem Cell Enumeration Assay according to *BD Stem Cell Enumeration Application Guide for FACSLyric flow cytometer.*
- Prepare 3 replicates of samples with high CD34 concentration and 3 replicates of samples with low CD34 concentration according to *BD Stem Cell Enumeration Kit Technical Data Sheet*. Include BD® Stem Cell Controls in every run.
- Acquire 3 replicates of High samples immediately followed by 3 replicates of Low samples on the cytometer.
- Review all results and adjust gates as needed. Details are provided in *BD Stem Cell Enumeration Application Guide for BD FACSLyric Flow Cytometers*.
- Enter the results in EP Evaluator or equivalent software for the BD platform.

7.3. Analysis of results

When all the results have been entered into EP Evaluator or equivalent software, calculate the %Carryover using the formula below. The %carryover should be within the stated carryover

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specifications of the instrument. Verify that there are no entry errors or mistakes in the analysis to account for the differences shown.

$$\%\text{Carryover} = \frac{(\text{LTV1} - \text{LTV3})}{(\text{HTV3} - \text{LTV3})} \times 100$$

LTV1 – Low target value replicate 1
LTV3 – Low target value replicate 3
HTV3 – High Target value replicate 3

Print the data chart and carryover graph and put the materials in the validation notebook.

8. Linearity Verification Protocol

Reference: CLSI document EP-06; H42 for specimen storage and handling

The purpose of this study is to verify the linear range established by the manufacturer for the BD® Stem Cell Enumeration Kit and determine any deviation from the expected value.

8.1. Specimens

A sample containing a high viable CD34 concentration (at least 1,500 CD34 cells/µL, e.g., leukapheresis) and a sample containing a low viable CD34 concentration (0 cells/µL, e.g., normal peripheral blood) are mixed to create a series of evenly distributed dilutions with known concentrations spanning the linear range of 0–1,000 viable CD34 cells/µL as listed in the table below.

Alternatively, a sample with a CD34 count of lower than 500 CD34 cells/µL can be concentrated by centrifuging the sample at 350*g* for five minutes and aspirating the plasma. The sample can then be tested to determine the concentration of viable CD34 cells.

Dilute of the sample in the following manner:

Dilution	% Volume of high concentration CD34 sample	% Volume of low concentration CD34 sample	Approximate expected concentration (cells/µL)
D1	0	100	0
D2	12.5	87.5	87.5
D3	25.0	75.0	375.0
D4	37.5	62.5	562.5
D5	50.0	50.0	750.0
D6	62.5	37.5	937.5
D7	75.0	25.0	1,125.0
D8	87.5	12.5	1,312.5
D9	100	0	1,500.0

8.2. Procedure

- Startup the BD FACSLyric™ Flow Cytometer according to *BD FACSLyric Reference System*.
- Perform Instrument QC with BD® CS&T Beads according to *BD FACSLyric Reference System*.

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- Perform assay setup for the BD® Stem Cell Enumeration Assay according to *BD Stem Cell Enumeration Application Guide for FACSLyric flow cytometer.*
- Prepare the dilution levels according to the above table. Stain 3 replicates of each dilution level according to *BD Stem Cell Enumeration Kit Technical Data Sheet*.
- Acquire all dilution levels in a non-sequential order on the flow cytometer. (Replicates of one dilution level may be acquired sequentially). Include BD® Stem Cell Controls in every run.
- Review all results and adjust gates as needed. Details are provided in *BD Stem Cell Enumeration Application Guide for BD FACSLyric Flow Cytometers*.
- Enter the results in EP Evaluator or equivalent software for the BD platform.

8.3. Analysis of results

Check that the regression values for CD34 absolute counts meet the statistical requirements of your laboratory.

Print the data chart and put the materials in the validation notebook.

8.4. Performance specifications

Linearity for the BD® Stem Cell Enumeration Kit was assessed for the BD FACSLyric™ System within a WBC concentration up to 40,000 cells/µL. Linearity for the BD® Stem Cell Enumeration Kit was determined to be 1–1,000 viable CD34+ cells/µL as described in *BD Stem Cell Enumeration Kit Technical Data Sheet*.

9. Limit of Blank

Reference: CLSI document EP-17; H42 for specimen storage and handling

The purpose of the study is to verify detection capability using a small number of samples in replicates over a few days using one reagent lot

9.1. Specimens

At least 2 blank specimens (e.g.: plasma extracted from normal peripheral blood) stained in duplicates each, one run per day and run over 5 days to give a total of 20 replicates per sample.

9.2. Procedure

- Startup the BD FACSLyric™ Flow Cytometer according to *BD FACSLyric Reference System*.
- Perform Instrument QC with BD® CS&T Beads according to *BD FACSLyric Reference System*.
- Perform assay setup for the BD® Stem Cell Enumeration Assay according to *BD Stem Cell Enumeration Application Guide for FACSLyric flow cytometer.*
- Prepare the samples according to *BD Stem Cell Enumeration Kit Technical Data Sheet*. Include BD® Stem Cell Controls in every run.
- Acquire all samples on the flow cytometer.
- Review all results and adjust gates as needed. Details are provided in *BD Stem Cell Enumeration Application Guide for BD FACSLyric Flow Cytometers*.
- Enter the results in EP Evaluator or equivalent software for the BD platform and the reference method.

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9.3. Analysis of results

Identify potential outliers. Calculate the percentage of all blank measurement results that are less than or equal to the LoB claim. Analyze the data per recommendations in CLSI EP-17.

Print the data chart and put the materials in the validation notebook.

9.4. Performance specifications

The Limit of Blank is 0 cells/µL as described in *BD Stem Cell Enumeration Kit Technical Data Sheet*.

10. Analytical Sensitivity Protocol

Reference: CLSI document EP-15, EP-17; H42 for specimen storage and handling

Analytical sensitivity represents the smallest amount of substance in a sample that can be accurately measured by an assay. Accuracy and repeatability at the low end of the range of viable CD34 (≤ 5 cells/µL) can be determined.

10.1. Specimens

At least 10 normal peripheral blood specimens with known CD34 counts ≤5 cells/µL. Each specimen should be stained in at least five replicates according to *BD Stem Cell Enumeration Kit Technical Data Sheet*.

10.2. Procedure

- Startup the BD FACSLyric™ Flow Cytometer according to *BD FACSLyric Reference System*.
- Perform Instrument QC with BD® CS&T Beads according to *BD FACSLyric Reference System*.
- Perform assay setup for the BD® Stem Cell Enumeration Assay according to *BD Stem Cell Enumeration Application Guide for FACSLyric flow cytometer.*
- Prepare the samples according to *BD Stem Cell Enumeration Kit Technical Data Sheet*. Include BD® Stem Cell Controls in every run.
- Acquire all samples on the flow cytometer.
- Review all results and adjust gates as needed. Details are provided in *BD Stem Cell Enumeration Application Guide for BD FACSLyric Flow Cytometers*.
- Enter the results in EP Evaluator or equivalent software for the BD platform and the reference method.

10.3. Analysis of results

Calculate the bias, mean, SD, and %CV. A %CV of 10% to 20% is acceptable.

When all the results have been entered into EP Evaluator or equivalent software, check that the Bias and SD values agree with the performance specifications in the following charts and in *BD Stem Cell Enumeration Kit Technical Data Sheet*. If there are any highly discrepant values, check that there are no problems with specimen identification or any other method errors.

Print the data chart and put the materials in the validation notebook.

10.4. Performance characteristics

Sensitivity of viable CD34+ absolute count, *BD Stem Cell Enumeration Kit Technical Data Sheet.*

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Viable CD34+ cells (≤5 cells/µL)		
Accuracy	**Repeatability**	
Mean absolute difference (95% CI)	**Source**	**SD (Upper 97.5% CL of SD)**
0.15 (–0.06, 0.37)	Instrument	0.10 (0.60)
	Within run	0.60 (0.66)
	Total precision	0.61 (0.67)

11. Reference Range Verification Protocol

Reference: CLSI document EP-28; H42 for specimen storage and handling

Samples from at least 20 healthy individuals need to be acquired to verify the published reference range. The 95% reference range published by the manufacturer is acceptable if no more than two of the 20 tested samples (or 10% of the test results) fall outside the published range in the verification studies. If more than 10% of the test values exceed manufacturer reference range, samples from at least 120 healthy individuals with patient demographics ages of 18 to 77, need to be acquired to determine the appropriate reference range. Some state licensures require that the reference range be validated in healthy donors according to age, gender, and a mix of races.

11.1.Specimens

- Include a minimum of samples from 20 healthy individuals to be tested.
- Set up and run each sample on both the reference method and the BD method within a close time frame.

11.2.Procedure

- Startup the BD FACSLyric™ Flow Cytometer according to *BD FACSLyric Reference System*.
- Perform Instrument QC with BD® CS&T Beads according to *BD FACSLyric Reference System*.
- Perform assay setup for the BD® Stem Cell Enumeration Assay according to *BD Stem Cell Enumeration Application Guide for FACSLyric flow cytometer.*
- Prepare the samples according to *BD Stem Cell Enumeration Kit Technical Data Sheet*. Include BD® Stem Cell Controls in every run.
- Acquire all samples on the flow cytometer.
- Review all results and adjust gates as needed. Details are provided in *BD Stem Cell Enumeration Application Guide for BD FACSLyric Flow Cytometers*.
- Enter the results in EP Evaluator or equivalent software for the BD platform.

11.3.Analysis of results

When all the results have been entered into EP Evaluator, calculate the mean, SD of the data generated.

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Check that no more than two of the 20 tested samples (or 10% of the test results) fall outside the 95% Reference Interval in the performance specifications below. If there are any highly discrepant values, check that there are no problems with specimen identification or any other method errors.

The study may be expanded to include more specimens, if the results of the validation study are inconclusive, or the parallel (accuracy) studies have demonstrated a significant positive or negative bias.

Print the data chart and put in the validation notebook.

11.4.Performance specifications

Reference ranges for the BD® Stem Cell Enumeration Kit from *BD Stem Cell Enumeration Kit Technical Data Sheet*. The following reference intervals were determined using normal peripheral blood from healthy adults.

Measure reported	N	Mean	SD	95% Reference Interval	
				Lower (90% conf. bounds)	Upper (90% conf. bounds)
Viable CD34 absolute counts (cells/µL)	130	2.35	1.98	0 (0, 1)	7 (6, 13)
%CD34 cells	130	0.04	0.03	0.01 (0, 0.01)	0.1 (0.09, 0.18)

We recommend that laboratories and other users establish their own reference intervals for their patient populations using the BD® Stem Cell Enumeration Kit to reflect potential sources of variability such as patient gender, race, and age, and sample preparation techniques.

12. Specimen Stability Protocol

Reference: CLSI document EP-25; H42 for specimen storage and handling

Stability is the capability of a specimen to retain the same components over a period of time under specified conditions. The purpose of this study is to verify the manufacturer claims for specimen and stained sample stability. The specimen type to be tested is based on individual laboratory requirements. Timepoints selected for testing would be based on specimen stability claims for each specimen type. One timepoint beyond the claim should be selected to ensure that stability claim is encompassed in the testing.

12.1.Specimens

- Collect at least 3-6 specimens in the appropriate anticoagulant.
- Samples should be fresh (less than six hours old since draw), to be considered for time 0 testing.
- Store specimens at 2 to 8 °C throughout the duration of testing.
- Prepare and stain the samples according to *BD Stem Cell Enumeration Kit Technical Data Sheet* every day for each of the test timepoints.

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12.2. Procedure

- Set up the flow cytometer according to *Stem Cell Enumeration SOP 1: BD FACSLyric Cytometer Startup Procedure*.
- Prepare samples according to *BD Stem Cell Enumeration Kit Technical Data Sheet*. Include controls in every run.
- Acquire the samples on the cytometer.
- Review all results and adjust gates as needed. Details are provided in *BD Stem Cell Enumeration Application Guide for BD FACSLyric Flow Cytometers*.
- Enter the results in EP Evaluator or equivalent software for the BD method.

12.3. Analysis of results

When all the results have been entered into EP Evaluator or equivalent software, calculate the CV. Calculate %bias between each test timepoint and time zero.

Check that the CV value agrees with the total precision. Check the %bias for each test timepoint compared to time zero. If there are any highly discrepant values, check that there are no problems with specimen identification or any other method errors.

Print the data chart and CV graph and put the materials in the validation notebook.

12.4. Performance specifications

Specimen and stained sample stability for the BD® Stem Cell Enumeration Kit from *BD Stem Cell Enumeration Kit Technical Data Sheet*.

Specimen stability testing was carried out by BD Biosciences at internal or clinical trial sites and sample stability was determined to be 24 hours after collection for fresh specimens (peripheral blood, bone marrow, leukapheresis products) when held at 2 to 8 °C. Stability of fresh cord blood was determined to be 48 hours after collection when held at 2 to 8 °C. Stained samples are to be stored on wet ice (ice in a small amount of water, which allows better contact with the tube so that the contents chill quickly) and acquired within 1 hour of lysing.

Frozen specimens are to be stained immediately after thawing. Stained samples are to be acquired immediately post-lysis.

13. References

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