

Verification Protocol for BD Multitest™ Reagents on the BD FACSLyric™ Flow Cytometer

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1. Introduction

BD Multitest™ reagents are intended for use in the immunological assessment of normal individuals and patients having or suspected of having immune deficiency.

Before reporting patient test results, the flow cytometry laboratory typically follows a process to demonstrate it can verify performance specifications comparable to those established by BD Biosciences. Verifications require that the laboratory follow all documented procedures from specimen collection to analysis, as described by the manufacturer. To achieve this, individual laboratories should develop their own requirements in accordance with any relevant accreditations and local regulations for new assays. Within this document are protocols that can be used or guide the laboratory in this process.

- Intra-assay (Repeatability) Precision Protocol
- Inter-assay (Reproducibility) Precision Protocol
- Accuracy Verification Protocol
- Stability Studies Protocol
 - Specimen Stability
 - Process Sample Stability
- Reference Range Verification Protocol
- Additional Optional Protocols
 - Carryover Protocol
 - Linearity Protocol
 - Sensitivity Protocol
 - Interference Protocol

The process of verification must include the following steps:

- Review the manufacturer's Instructions for Use (IFUs) for the test to be implemented
- Review technical data sheets (TDSs) of tests to be implemented
- Review the Standard Operating Procedure (SOPs) for the BD FACSLyric™ Flow Cytometer
- Determine the verification protocols to be utilized
- Establish a verification plan
- Execute the verification plan
- Generate a final assay verification report

2. Specimens, Instruments and Reagents

2.1. Specimens

Collect blood into a sterile BD Vacutainer® EDTA (ethylenediamine-tetraacetic acid) blood collection tube, or equivalent. BD Multitest™ reagents and BD Trucount™ tubes have been validated with both liquid and dry formulations of EDTA.

Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected to ensure proper specimen dilution.

Anticoagulated blood is to be stored at room temperature (20°C - 25°C).

2.2. BD instruments and reagents

- BD FACSLyric™ Flow Cytometer

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- BD FACSuite™ Clinical Application (BD Multitest™ 4-Color Assay Module and/or BD Multitest™ 6-Color Assay Module)
- BD™ FC Beads 7-Color Kit
- BD™ CS&T Beads IVD
- BD™ Multi-Check Whole Blood Control and BD™ Multi-Check CD4 Low Control or equivalent
- BD Multitest™ reagents with BD Trucount™ tubes:
 - BD Multitest™ IMK kit with BD Trucount™ tubes
 - BD Multitest™ CD3/CD8/CD45/CD4 with BD Trucount™ tubes
 - BD Multitest™ CD3/CD16 + CD56/CD45/CD19 with BD Trucount™ tubes
 - BD Multitest™ 6-color TBNK reagent with BD Trucount™ tubes
- BD Multitest™ reagents without BD Trucount™ tubes:
 - BD Multitest™ IMK kit
 - BD Multitest™ CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC reagent
 - BD Multitest™ CD3 FITC/CD16 + CD56 PE/CD45 PerCP/CD19 APC reagent
 - BD Multitest™ 6-color TBNK reagent
 - BD Trucount™ Absolute Counting Tubes
- BD FACS™ Lysing Solution 10X Concentrate
- BD FACSTymer™ Sheath Fluid
- BD Pharmingen™ Stain Buffer (BSA)
- BD Vacutainer® EDTA (ethylenediamine-tetraacetic acid) blood collection tubes
- 12 x 75 tubes Falcon® test tubes (from Corning) or equivalent

2.3. Statistical software

Data Innovations EP Evaluator™ Software or equivalent

2.4. 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 FACSuite™ Application modules: BD Multitest™ 4-Color Assay Module and/or BD Multitest™ 6-Color Assay Module

3. Intra-assay (Repeatability) Precision Protocol

Reference: CLSI EP15-A3 and CLSI H62

Intra-assay precision is defined as replicate measurements of a sample under experimental conditions held as constant as possible. Precision is expressed quantitatively using the mean, standard deviation (SD) and coefficient of variance (CV).

3.1. Specimen collection, handling and preparation

- Include a minimum of 6 fresh whole blood specimens in BD Vacutainer® EDTA collection tube, or equivalent, that span the analytical measurement range of the instrument as detailed in the Technical Data Sheet (TDS)
- Each specimen should be stained in triplicate and acquired based on the number of instruments and operators in the laboratory
- Testing is conducted over several days.

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- Each sample should be tested in ≥ 1 run(s).
- If > 1 operator and > 1 instrument :
 - 1 run per sample
 - Operator 1 should test half of the samples on instrument 1
 - Operator 2 should test half of the samples on instrument 2
- Samples must be run on the BD FACSLyric™ Flow Cytometer within the sample stability timeframe detailed in the relevant Technical Data Sheet (TDS)

3.2. Procedure

1. Start up the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 1: BD FACSLyric™ Cytometer Startup and QC Procedure*.
2. Prepare samples according to the relevant Lymphocyte Enumeration SOP for 4-color or 6-color Lymphocyte Enumeration. Refer to *Lymphocyte Enumeration SOP 2: Four-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ Reagents Manual Preparation* or *Lymphocyte Enumeration SOP 3: Immunofluorescent Labeling of Whole Blood with BD Multitest™ 6-color TBNK Reagents Manual Preparation* respectively. Controls should be included in every run.
3. Acquire all samples on the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 4: Acquisition of Stained Whole Blood Samples on the FACSLyric™ Cytometer*.
4. Perform analysis and review all results according to *Lymphocyte Enumeration SOP 5: Analysis of Data Acquired on the BD FACSLyric™ Flow Cytometer*.
5. Enter the results in EP Evaluator or equivalent software.

3.3. Analysis of results

- When all the results have been entered into EP Evaluator or the equivalent software, calculate the mean, standard deviation (SD), and coefficient of variation (CV)
- Check that the CV and SD values agree with the performance specifications in the relevant BD Multitest 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 specimen preparation or running of the samples
- Print the data chart and associated graphs and put the materials in the validation notebook

4. Inter-assay (Reproducibility) Precision Protocol

Reference: CLSI EP15-A3 and CLSI H62

Inter-assay precision or between run precision is defined as replicate measurements that are tested under multiple conditions (e.g., days, runs, instruments, and/or operators). Precision is expressed quantitatively using the mean, standard deviation (SD) and/or coefficient of variation (CV).

4.1. Specimen collection, handling and preparation

- Use stabilized whole blood quality control material such as BD™ Multi-Check Whole Blood Control and BD™ Multi-Check CD4 Low Control
- Each specimen should be stained in triplicates and acquired based on the number of instruments and operators in the laboratory

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- Testing is conducted over several days
- Each sample should be tested in 4 runs
- If >1 operator and >1 instrument:
 - Operator 1 should test 2 QC on instrument 1 and perform 2 runs
 - Operator 2 should test 2 QC on instrument 2 and perform 2 runs
- Samples must be run on the BD FACSLyric™ Flow Cytometer within the sample stability timeframe detailed in the relevant Technical Data Sheet (TDS)

4.2. Procedure

1. Set up the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 1: BD FACSLyric™ Cytometer Startup and QC Procedure*.
2. Prepare samples according to the relevant Lymphocyte Enumeration SOP for 4-color or 6-color Lymphocyte Enumeration. Refer to *Lymphocyte Enumeration SOP 2: Four-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ Reagents Manual Preparation* or *Lymphocyte Enumeration SOP 3: Immunofluorescent Labeling of Whole Blood with BD Multitest™ 6-color TBNK Reagents Manual Preparation* respectively.
3. Acquire all samples on the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 4: Acquisition of Stained Whole Blood Samples on the FACSLyric™ Flow Cytometer*.
4. Perform analysis and review all results according to *Lymphocyte Enumeration SOP 5: Analysis of Data Acquired on the BD FACSLyric™ Flow Cytometer*.
5. Enter the results in EP Evaluator or equivalent software.

4.3. Analysis of results

When all the results have been entered into EP Evaluator or the equivalent software, calculate the mean, standard deviation (SD), and coefficient of variation (CV).

Check that the CV and SD values agree with the performance specifications in the relevant BD Multitest 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 specimen preparation or running of the samples.

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

5. Accuracy Protocol

Reference: CLSI EP15-A3 and CLSI H62

Accuracy or trueness is the closeness of the agreement between a measured result and a true value. Parallel studies are performed to determine the correlation 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.

5.1. Specimen collection, handling and preparation

- Include a minimum of 10 fresh whole blood specimens in BD Vacutainer® EDTA collection tube, or equivalent

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- Stabilized whole blood quality control material such as BD Multi-Check Whole Blood Control and BD™ Multi-Check CD4 Low Control should be included in the method validation run to enhance the accuracy claim. Ensure the recovery of expected values from certified reference material
- Both normal and abnormal specimens should be tested that represent the analytical measurement range of the instrument as detailed in the Technical Data Sheet (TDS)
- Samples must be run on both the BD FACSLyric™ Flow Cytometer and the reference method within the sample stability timeframe detailed in the relevant Technical Data Sheet (TDS)

5.2. Procedure

1. Set up the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 1: BD FACSLyric™ Cytometer Startup and QC Procedure*.
2. Prepare samples according to the relevant Lymphocyte Enumeration SOP for 4-color or 6-color Lymphocyte Enumeration. Refer to *Lymphocyte Enumeration SOP 2: Four-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ Reagents Manual Preparation* or *Lymphocyte Enumeration SOP 3: Immunofluorescent Labeling of Whole Blood with BD Multitest™ 6-color TBNK Reagents Manual Preparation* respectively.
3. Acquire all samples on the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 4: Acquisition of Stained Whole Blood Samples on the FACSLyric™ Flow Cytometer* and on the reference method to be used.
4. Perform analysis and review all results according to *Lymphocyte Enumeration SOP 5: Analysis of Data Acquired on the BD FACSLyric™ Flow Cytometer*.
5. Enter the results in EP Evaluator or equivalent software.

5.3. Analysis of results

When all the results have been entered into EP Evaluator or equivalent software, calculate the regression value (R²).

Check that the regression value agrees with the performance specifications shown in the relevant Multitest Technical Data Sheet (TDS). 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 specimens or running of the samples.

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

6. Stability Study Protocol

Reference: CLSI H52 and CLSI H62

Stability is the capability of a specimen/sample material to retain the initial property of a measurand constituent for a period of time within specified limits when the specimen/sample is stored under defined conditions. The confirmation of the manufacturer's claims is required. Specimen collection, preparation and stability information can be found in the relevant Multitest™ Technical Data Sheet (TDS). Any claims for stability are based on the use of EDTA as an anticoagulant.

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6.1.A. Specimen stability

Specimen collection, handling and preparation

- Collect 3-6 peripheral blood specimens in BD Vacutainer® EDTA collection tubes, or equivalent
- Specimens should be tested within the time frames listed on the TDS under Specimen Collection and Preparation to be considered baseline testing
- Store specimens at room temperature (20°C - 25°C) during the testing period
- Stain the same specimens at the limit of the stability time point relevant to the MultiTest™ Reagent being confirmed
- All Samples must be run on the BD FACSLyric™ Flow Cytometer

6.1.B. Processed sample stability

Specimen collection, handling and preparation

- Collect 3-6 peripheral blood specimens in BD Vacutainer® EDTA collection tubes, or equivalent.
- Stain and Acquire samples to establish baseline testing
- Acquire the same samples at the limit of the processed sample stability time for the relevant MultiTest™ Reagent being confirmed
- All Samples must be run on the BD FACSLyric™ Flow Cytometer

6.2. Procedure

1. Set up the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 1: BD FACSLyric™ Cytometer Startup and QC Procedure*.
2. Prepare samples according to the relevant Lymphocyte Enumeration SOP for 4-color or 6-color Lymphocyte Enumeration. Refer to *Lymphocyte Enumeration SOP 2: Four-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ Reagents Manual Preparation* or *Lymphocyte Enumeration SOP 3: Immunofluorescent Labeling of Whole Blood with BD Multitest™ 6-color TBNK Reagents Manual Preparation* respectively. Controls should be included in every run.
3. Acquire all samples on the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 4: Acquisition of Stained Whole Blood Samples on the FACSLyric™ Cytometer*.
4. Perform analysis and review all results according to *Lymphocyte Enumeration SOP 5: Analysis of Data Acquired on the BD FACSLyric™ Cytometer*.
5. Enter the results in EP Evaluator or equivalent software.

6.3. Analysis of results

When all the results have been entered into EP Evaluator or equivalent software, calculate the regression value (R²).

Check that the regression value agrees with the performance specifications shown in the relevant Multitest Technical Data Sheet (TDS). 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 specimens or running of the samples.

Print the data chart, regression graph and put the materials in the validation notebook

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7. Reference Range Verification Protocol

Reference: CLSI EP-28 and CLSI H62

Reference intervals describe the limits of the distribution for a measurand within a selected population. The reference range as published by the manufacturer is acceptable if the verification studies determine that the patient population undergoing tests at the laboratory falls within the published reference range. If patient demographics are outside the ages of 19 to 80, further reference intervals validation studies should be performed to determine the appropriate range.

7.1. Specimen collection, handling and preparation

- Include a minimum of fresh whole blood specimens from 20 healthy individuals collected in BD Vacutainer® EDTA collection tube, or equivalent
- Samples must be run on the BD FACSLyric™ Flow Cytometer within the sample stability timeframe detailed in the relevant Technical Data Sheet (TDS)

7.2. Procedure

1. Set up the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 1: BD FACSLyric™ Cytometer Startup and QC Procedure*.
2. Prepare samples according to the relevant Lymphocyte Enumeration SOP for 4-color or 6-color Lymphocyte Enumeration. Refer to *Lymphocyte Enumeration SOP 2: Four-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ Reagents Manual Preparation* or *Lymphocyte Enumeration SOP 3: Immunofluorescent Labeling of Whole Blood with BD Multitest™ 6-color TBNK Reagents Manual Preparation* respectively. Controls should be included in every run.
3. Acquire all samples on the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 4: Acquisition of Stained Whole Blood Samples on the FACSLyric™ Cytometer*.
4. Perform analysis and review all results according to *Lymphocyte Enumeration SOP 5: Analysis of Data Acquired on the BD FACSLyric™ Cytometer*.
5. Enter the results in EP Evaluator or equivalent software.

7.3. Analysis of results

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

Calculate the mean and standard deviation (SD). Verify that the +/- 2SD range agrees with the 95% range found in the relevant Technical Data Sheet (TDS). 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 in the validation notebook.

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8. Additional Optional Protocols

The following protocols are considered optional in accordance with the CLSI H62 document.

8.1. Carryover Protocol

Reference: CLSI H44-A2 and CLSI H62

The effect of one sample should have no clinical significance on subsequent samples. This can be evaluated by determining the amount of carryover from a high leukocyte count sample into a low leukocyte count sample. Carryover testing should be conducted during instrument qualification and as needed.

8.1.A. Specimen collection, handling and preparation

- Include a minimum of 2 fresh whole blood specimens in BD Vacutainer® EDTA collection tube, or equivalent, one with a high-frequency cell of interest and one having a low frequency cell of interest
- Prepare 3 replicates each of the high-frequency and low frequency specimen
- Samples must be run on the BD FACSLyric™ Flow Cytometer within the sample stability timeframe detailed in the relevant Technical Data Sheet (TDS)

8.1.B. Procedure

1. Set up the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 1: BD FACSLyric™ Cytometer Startup and QC Procedure*.
2. Prepare samples according to the relevant Lymphocyte Enumeration SOP for 4-color or 6-color Lymphocyte Enumeration. Refer to *Lymphocyte Enumeration SOP 2: Four-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ Reagents Manual Preparation* or *Lymphocyte Enumeration SOP 3: Immunofluorescent Labeling of Whole Blood with BD Multitest™ 6-color TBNK Reagents Manual Preparation* respectively. Controls should be included in every run.
3. Acquire all samples in the following order according to *Lymphocyte Enumeration SOP 4: Acquisition of Stained Whole Blood Samples on the FACSLyric™ Cytometer*. Tube 1-3: High frequency count (H1), (H2), and (H3) respectively, followed by Tube 4–5: Low frequency count (L1), (L2), and (L3)
4. Perform analysis and review all results according to *Lymphocyte Enumeration SOP 5: Analysis of Data Acquired on the BD FACSLyric™ Cytometer*.

8.1.C. 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 specifications of the instrument. The carryover specification for the BD FACSLyric™ Flow Cytometer is $\leq 0.1\%$ with the default SIT flush between samples. Verify that there are no entry errors or mistakes in the analysis to account for the differences shown.

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$$\% \text{Carryover} = \frac{(L1 - L3)}{(H3 - L3)} \times 100$$

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

8.2. Linearity verification protocol

Reference: CLSI EP06 and CLSI H62

Linearity is defined as the ability to provide results that are directly proportional to the concentration of the analyte in the sample to be tested. The purpose of this protocol is to verify that results are linear throughout the Analytical Measurement Range (AMR) provided in the relevant Multitest™ Technical Data Sheet (TDS).

Eleven levels with two replicates at each level are recommended to verify that the linear range is valid.

The lowest detectable result as determined can be further used in the sensitivity (Lower Detection Limit) protocol. It is suggested that you use this serial diluted specimen for staining to determine the sensitivity (Lower Detection Limit) within the sample stability timeframe.

8.2.A. Specimen collection, handling and preparation

- Obtain a peripheral blood specimen in BD Vacutainer® EDTA collection tube, or equivalent with a high lymphocyte count of 9,000 cells/μL
- Stain 11 levels; each in duplicate
- Make two-fold serial dilutions with a stain buffer such as BD Pharmingen Stain Buffer (BSA) or equivalent
- Samples must be run the BD FACSLyric™ Flow Cytometer within the sample stability timeframe detailed in the relevant Technical Data Sheet (TDS)

Perform the dilution of the specimen in a similar manner as below:

Tube	Dilution Factor	Expected Lymphocyte counts	Volume of Stain Buffer	Volume of previous dilution
1	Neat	9,000	0 μL	1000 μL of whole blood
2	1:2	4,500	500 μL	500 μL of Neat
3	1:4	2,250	500 μL	500 μL of 1:2
4	1:8	1,125	500 μL	500 μL of 1:4
5	1:16	563	500 μL	500 μL of 1:8
6	1:32	281	500 μL	500 μL of 1:16
7	1:64	141	500 μL	500 μL of 1:32
8	1:128	71	500 μL	500 μL of 1:64
9	1:256	36	500 μL	500 μL of 1:128
10	1:512	18	500 μL	500 μL of 1:256
11	1:1,024	9	500 μL	500 μL of 1:512

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8.2.B. Procedure

1. Set up the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 1: BD FACSLyric™ Cytometer Startup and QC Procedure*.
2. Prepare samples according to the relevant Lymphocyte Enumeration SOP for 4-color or 6-color Lymphocyte Enumeration. Refer to *Lymphocyte Enumeration SOP 2: Four-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ Reagents Manual Preparation* or *Lymphocyte Enumeration SOP 3: Immunofluorescent Labeling of Whole Blood with BD Multitest™ 6-color TBNK Reagents Manual Preparation* respectively. Controls should be included in every run.
3. Acquire all samples in the following order according to *Lymphocyte Enumeration SOP 4: Acquisition of Stained Whole Blood Samples on the FACSLyric™ Cytometer*. Tube 1-3: High frequency count (H1), (H2), and (H3) respectively, followed by Tube 4–5: Low frequency count (L1), (L2), and (L3)
4. Perform analysis and review all results according to *Lymphocyte Enumeration SOP 5: Analysis of Data Acquired on the BD FACSLyric™ Cytometer*.
5. Enter the results in EP Evaluator or equivalent software.

8.2.C. Analysis of results

The data can be plotted to determine linearity with a regression value. For each level that falls within the AMR, ensure the regression values meets the laboratory's acceptance level of agreement for each reportable result.

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

8.3. Sensitivity (Lower Detection Limit) Protocol

Reference: CLSI EP-17 and CLSI H62

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The minimum detection (lowest concentration that can be distinguished from zero) is determined by testing replicates of the lowest detectable result as determined previously in the Linearity protocol.

8.3.A. Specimen collection, handling and preparation

- Stain the diluted fresh whole blood specimen with results at the lowest detectable value (within the AMR) as defined by the linearity protocol five times. Acquire each of these samples three times
- Samples must be run on the BD FACSLyric™ Flow Cytometer within the sample stability timeframe detailed in the relevant Technical Data Sheet (TDS)

8.3.B. Procedure

1. Set up the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP BD FACSLyric™ Cytometer Startup and QC Procedure*.
2. Prepare samples according to the relevant Lymphocyte Enumeration SOP for 4-color or 6-color Lymphocyte Enumeration. Refer to *Lymphocyte Enumeration SOP 2: Four-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ Reagents Manual Preparation* or *Lymphocyte Enumeration SOP 3: Immunofluorescent Labeling of Whole Blood with BD*

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Multitest™ 6-color TBNK Reagents Manual Preparation respectively. Controls should be included in every run.

3. Acquire all samples on the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 4: Acquisition of Stained Whole Blood Samples on the FACSLyric™ Cytometer* and on the reference method to be used.
4. Perform analysis and review all results according to *Lymphocyte Enumeration SOP 5: Analysis of Data Acquired on the BD FACSLyric™ Cytometer*.
5. Enter the results in EP Evaluator or equivalent software.

8.3.C. Analysis of results

Perform statistical analysis on results calculating the mean, Standard Deviation (SD), and Coefficient of Variation (CV). Check that the %CV and SD values agree with the performance specifications in the relevant TDS. The laboratory is responsible for determining the acceptance criteria for each reportable result. 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.

8.4. Interference Protocol

Reference: CLSI H62

Interfering substances due to specimen handling or biologics can be the cause of aberrant results. Interfering substances should be identified, and appropriate plans of actions should be documented. Additional validation is only required if testing in the presence of a biologic that interferes with the assay.

Refer to the Interfering Conditions section of the product TDSs for interfering substances or conditions. Perform the following validation only if testing will be performed in the presence of potential interfering biological agents.

8.4.A. Specimen collection, handling and preparation

- Include a minimum of 3 fresh whole blood specimens in BD Vacutainer® EDTA collection tube, or equivalent
- Each specimen should be stained in triplicate and acquired in one or more runs
- Samples must be run on the BD FACSLyric™ Flow Cytometer within the sample stability timeframe detailed in the relevant Technical Data Sheet (TDS)

8.4.B. Procedure

1. Check the relevant MultiTest™ Technical Data Sheet (TDS) for all possible interfering substances and precautions.
2. Set up the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 1: BD FACSLyric™ Cytometer Startup and QC Procedure*.
3. Prepare samples according to the relevant Lymphocyte Enumeration SOP for 4-color or 6-color Lymphocyte Enumeration. Refer to *Lymphocyte Enumeration SOP 2: Four-color Immunofluorescent Labeling of Whole Blood with BD Multitest™ Reagents Manual Preparation* or *Lymphocyte Enumeration SOP 3: Immunofluorescent Labeling of Whole Blood with BD*

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Multitest™ 6-color TBNK Reagents Manual Preparation respectively. Controls should be included in every run.

4. Acquire all samples on the BD FACSLyric™ Flow Cytometer according to *Lymphocyte Enumeration SOP 4: Acquisition of Stained Whole Blood Samples on the FACSLyric™ Cytometer*.
5. Perform analysis and review all results according to *Lymphocyte Enumeration SOP 5: Analysis of Data Acquired on the BD FACSLyric™ Cytometer*.
6. Enter the results in EP Evaluator or equivalent software.

8.4.C. Analysis of results

Perform statistical analysis on results calculating the mean, standard deviation (SD), and coefficient of variation (CV). Check that the %CV and SD values agree with the performance specifications in the relevant TDS. The laboratory is responsible for determining the acceptance level of agreement for each reportable result.

9. References

- Validation of Assays Performed by Flow Cytometry; Approved Guideline-First Edition. Wayne PA: Clinical and Laboratory Standards Institute; 2021. CLSI document H62.
- Methods for Reticulocyte Counting (Automated Blood Cell Counters, Flow Cytometry, and Supravital Dyes); Approved Guideline-Second Edition. Wayne PA: Clinical and Laboratory Standards Institute; 2004. CLSI document H44-A2.
- User Verification of Precision and Estimation of Bias; Approved Guideline – Third Edition. Wayne PA: Clinical and Laboratory Standards Institute; 2014. CLSI document EP15-A3.
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Verification Protocol for BD Multitest™ Reagents on the BD FACSLytic™ Flow Cytometer

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