

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
INSTRUMENT ONLY TEMPLATE**

A. 510(k) Number:

k131301

B. Purpose for Submission:

Modify Intended Use to include FACSCalibur

C. Manufacturer and Instrument Name:

BD Biosciences

BD FACS™ Sample Prep Assistant III

D. Type of Test or Tests Performed:

Specimen Processor

E. System Descriptions:

1. Device Description:

The BD FACS™ Sample Prep Assistant III (SPA III) is a microprocessor-controlled pipetting and diluting system which automatically prepares whole blood samples using the lyse / no-wash sample preparation method for flow cytometry. Used as an accessory to the BD FACSCalibur™ and BD FACSCanto™ II flow cytometers, the SPA III combines fluidic, optic, robotic, and electronic components to automatically prepare samples for acquisition and analysis.

The SPA III pierces the sample tube cap to withdraw sample, aliquots blood and reagent into daughter tubes, and mixes the sample according to preprogrammed protocols. The device also adds lysing solution and automates cleaning procedures.

The unit consists of an enclosure, one robotic pipetting module moving in the X/Y/Z axes, a power supply, a central controller unit, fluid pumps, and a barcode reader.

2. Principles of Operation:

Two syringe pumps volumetrically measure fluids and move them through the system for aspiration and delivery.

3. Modes of Operation:

Automated

4. Specimen Identification:

Manual entry or bar code reader.

5. Specimen Sampling and Handling:

Specimens are whole blood specimens collected into approved tubes

6. Calibration:

Not applicable.

7. Quality Control:

The laboratory follows the manufacturer's instructions for gravimetric verification of accuracy of pipetting and quality control instructions for BD cleared flow cytometric IVD reagents in the context of the BD flow cytometer systems.

8. Software:

FDA has reviewed applicant's Hazard Analysis and Software Development processes for this line of product types:

Yes X or No _____

F. Regulatory Information:

1. Regulation section:

21 CFR § 862.2750, Pipetting and Diluting System for Clinical Use

2. Classification:

Class I

3. Product code:

PER, Automated pipetting, diluting and specimen processing workstations for flow cytometric analysis

4. Panel:

Clinical Chemistry (75)

G. Intended Use:

1. Intended Use:

The BD FACSTM Sample Prep Assistant III is intended to prepare human whole blood for flow cytometric analysis on BD FACSCanto™ II and BD FACSCalibur™ flow cytometry systems.

2. Indications for Use:

Pipetting blood, reagents, and lysing solution using the following previously cleared assays for flow cytometric analysis on BD FACSCanto™ II flow cytometry systems:

- BD Multitest 6-Color TBNK Reagent with or without BD Trucount Tubes
- BD Multitest IMK Kit with or without BD Trucount Tubes
- BD Multitest CD3 FITC/CD16+CD56 PE/CD45 PerCP/CD19 APC with or without BD Trucount Tubes
- BD Multitest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC with or without BD Trucount Tubes

Pipetting blood, reagents, and lysing solution using the following previously cleared assays for flow cytometric analysis on BD FACSCalibur™ flow cytometry systems:

- BD Multitest IMK Kit with or without BD Trucount Tubes
- BD Multitest CD3 FITC/CD16+CD56 PE/CD45 PerCP/CD19 APC with or without

BD Trucount Tubes
 BD Multitest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC with or without BD Trucount Tubes
 BD Tritest CD3/CD16+56/CD45 with or without BD Trucount Tubes
 BD Tritest CD3/CD19/CD45 with or without BD Trucount Tubes
 BD Tritest CD3/CD4/CD45 with or without BD Trucount Tubes
 BD Tritest CD3/CD8/CD45 with or without Trucount Tubes
 BD Tritest CD4/CD8/CD3 with BD Trucount Tubes

For in vitro diagnostic use.

3. Special Conditions for Use Statement(s):

For prescription use only.

4. Special instrument requirements:

For use on the BD FACSCanto II (previously cleared under k041074) and BD FACSCalibur (previously cleared under k923790) flow cytometry systems.

H. Substantial Equivalence Information:

1. Predicate Device Name(s) and 510(k) numbers:

BD FACSTTM Sample Prep Assistant III accessory to the BD FACSCantoTM system with BD FACSCanto clinical software (k102064)

2. Comparison with Predicate Device:

Similarities/Differences		
Item	New Device	Predicate
	The BD FACST TM Sample Prep Assistant III as an accessory to BD FACSCanto II and BD FACSCalibur flow cytometry systems.	BD FACST TM Sample Prep Assistant III accessory to the BD FACSCanto TM system with BD FACSCanto clinical software (K102064)
Intended Use	The BD FACS Sample Prep Assistant III is intended to prepare human whole blood for flow cytometric analysis on BD FACSCanto II and BD FACSCalibur flow cytometry systems.	The BD FACS Sample Prep Assistant III is intended to prepare human whole blood for flow cytometric analysis on BD FACSCanto II flow cytometry systems.
Controlling software	BD FACS SPA software version 4.0.2.1 (did not include any software changes. The software revision only added the cleared BD FACS Sample Prep Assistant III Instructions for Use with the software and also updated the Online Help Files)	BD FACS SPA software version 4.0.2

Similarities/Differences		
Item	New Device	Predicate
	The BD FACST TM Sample Prep Assistant III as an accessory to BD FACSCanto II and BD FACSCalibur flow cytometry systems.	BD FACST TM Sample Prep Assistant III accessory to the BD FACSCanto TM system with BD FACSCanto clinical software (K102064)
Sample Type	Whole blood	Same
Preparation Method	Automated	Same
Pipetting Syringe	1 mL sample/reagent syringe 10 mL lyse syringe	Same
Supported primary blood sample tubes	Vacutainer Sarstedt	Same
Probe Rinse	3 pulses of approximately 1 second	Same
Quality Control Techniques	Gravimetric calibration	Same

I. Special Control/Guidance Document Referenced (if applicable):

CLSI EP5-A2: Evaluation of Precision Performance of Clinical Chemistry.
 CLSI EP9-A2: Method Comparison and Bias Estimation Using Patient Samples. Class II Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells; Final Guidance for Industry and FDA, December 4, 2001.

J. Performance Characteristics:

1. Analytical Performance:

a. *Accuracy:*

The performance of the SPA III was compared to manual pipetting. Normal peripheral blood specimens (defined as specimens with normal CD4+ counts) and HIV-infected patient peripheral blood specimens were evaluated. Samples were stained with BD Multitest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC and BD Multitest CD3 FITC/CD16+CD56 PE/CD45 PerCP/CD19 APC with Trucount Tubes reagents using manual pipetting and using the SPAIII. The samples were acquired on BD FACSCalibur systems containing the BD FACSTTM Loader (k953302). Both Sarstedt and BD Vacutainer EDTA tubes were used for blood collection. Three testing sites were used for the study.

Acceptance Criteria:

Parameter	95% CI on Mean Bias
CD3+ absolute counts	≤ 10%
CD3+CD4+ absolute counts	≤ 10%
CD3+CD8+ absolute counts	≤ 10%
B cell absolute counts (CD19+, CD3-)	≤ 20%

Parameter	95% CI on Mean Bias
NK cell absolute counts (CD16++ CD56+, CD3-)	$\leq 20\%$
% CD3, CD3+CD4+, CD3+CD8+, CD19+CD3-, CD16+CD56+CD3-	If the mean of % positive values of all specimens is $\leq 30\%$ as determined by reference method, 95% CI of mean bias must be within ± 3 .
NK cell (CD16+ + CD56+, CD3-) %Positive	If the mean of % positive values of all specimens is $> 30\%$ as determined by reference method, 95% CI of relative mean bias must be within $\pm 10\%$.

Summary of the Bland Altman (bias) Absolute Count Results

Vacutainer Tubes

Absolute Count Subsets	Rep	N	Mean %Bias	SD	95% CI	Criteria	Pass/Fail
CD4	1	236	-3.3	9.6	(-4.3, -2.3)	10%	Pass
CD8	1	236	-3.2	8.8	(-4.1, -2.2)	10%	Pass
CD3	1	236	-3.5	7	(-4.2, -2.7)	10%	Pass
CD19	1	236	-3.7	17.5	(-5.6, -1.8)	20%	Pass
CD16+56	1	236	-1.5	13.2	(-2.9, -0.1)	20%	Pass

Sarstedt

Absolute Count Subsets	Rep	N	Mean %Bias	SD	95% CI	Criteria	Pass/Fail
CD4	1	100	0.9	9.1	(-0.6, 2.4)	10%	Pass
CD8	1	100	0.0	8.4	(-1.4, 1.4)	10%	Pass
CD3	1	100	-1.0	5	(-1.9, -0.2)	10%	Pass
CD19	1	100	-1.5	10.8	(-3.3, 0.3)	10%	Pass
CD16+56	1	100	1.8	14.3	(-0.6, 4.1)	10%	Pass

Summary of the Bland Altman (bias) Percent Positive Results

Vacutainer

Percent Positive Subsets	Rep	N	Mean %Bias	SD	95% CI	Criteria	Pass/Fail
CD4	1	236	-0.03	1.2	(-0.2, 0.1)	3	Pass
CD8	1	236	0.3	3.6	(0, 0.7)	10%	Pass
CD3	1	236	0.2	1.8	(0, 0.4)	10%	Pass
CD19	1	236	-0.1	0.9	(-0.2, 0)	3	Pass
CD16+56	1	236	0.1	1	(0, 0.2)	3	Pass

Sarstedt

Percent Positive Subsets	Rep	N	Mean %Bias	SD	95% CI	Criteria	Pass/Fail
CD4	1	100	0.1	1.2	(-0.1, 0.3)	3	Pass
CD8	1	100	-0.5	3.6	(-1.1, 0.1)	10%	Pass
CD3	1	100	-0.3	1.4	(-0.5, -0.1)	10%	Pass
CD19	1	100	0	0.6	(-0.1, 0.1)	3	Pass
CD16+56	1	100	0.2	0.9	(0, 0.4)	3	Pass

Summary of the Deming Regression Absolute Count results

Vacutainer Tubes

Abs Counts	N	R ²	Intercept	Confidence Limit (L/U)	Slope	Confidence Limit (L/U)
CD4	236	0.96	4.64	(-20.45, 29.78)	0.96	(0.93, 0.99)
CD8	236	0.98	1.67	(-9.88, 13.23)	0.96	(0.92, 0.99)
CD3	236	0.93	11.27	(-12.16, 34.71)	0.95	(0.90, 0.99)
CD19	236	0.96	-0.65	(-3.94, 2.64)	0.96	(0.93, 0.98)
CD16+56	236	0.97	0.23	(-1.92, 2.4)	0.97	(0.95, 0.99)

Sarstedt

Abs Counts	N	R ²	Intercept	Confidence Limit (L/U)	Slope	Confidence Limit (L/U)
CD4	100	0.98	-45.53	(-77.4, -13.7)	1.02	(0.99, 1.04)
CD8	100	0.98	6.6	(-17.6, 30.8)	0.99	(0.93, 1.04)
CD3	100	0.97	-24.5	(-53.3, 4.3)	1.02	(0.99, 1.05)
CD19	100	0.97	-1.08	(-4.91, 2.76)	0.99	(0.96, 1.01)
CD16+56	100	0.96	4.22	(-0.006, 8.44)	0.98	(0.94, 1.01)

Summary of the Deming Regression Percent Positive results

Vacutainer Tubes

Percent Positive Subsets	N	R ²	Intercept	Confidence Limit (L/U)	Slope	Confidence Limit (L/U)
CD4	236	0.98	-0.57	(-1.94, 0.81)	1.0	(0.99, 1.02)
CD8	236	0.99	0.22	(-0.18, 0.63)	0.99	(0.97, 1.0)
CD3	236	0.98	-0.39	(-1.31, 0.52)	1.01	(0.99, 1.03)
CD19	236	0.98	-0.008	(-0.30, 0.28)	0.99	(0.96, 1.02)
CD16+56	236	0.96	0.19	(-0.035, 0.42)	0.99	(0.97, 1.02)

Sarstedt

Percent Positive Subsets	N	R ²	Intercept	Confidence Limit (L/U)	Slope	Confidence Limit (L/U)
CD4	100	0.99	-0.27	(-1.69, 1.15)	1.00	(0.98, 1.01)
CD8	100	0.99	0.71	(-1.06, 2.49)	0.97	(0.89, 1.04)
CD3	100	0.97	0.39	(-1.22, 1.99)	0.99	(0.95, 1.02)
CD19	100	0.98	0.032	(-0.21, 0.27)	0.99	(0.97, 1.02)
CD16+56	100	0.96	0.33	(-0.06, 0.72)	0.99	(0.94, 1.03)

The results showed that the 95% confidence interval for the two collection tubes and for all parameters tested met the acceptance criteria. When used with the BD FACSCalibur system, the SPA III and manual pipetting yield equivalent performance.

b. Precision/Reproducibility:

Precision for the SPA III was evaluated in three studies; two for Dispense Accuracy and Precision, and one for Assay Precision.

Dispense Accuracy and Precision (1)

The pipetting accuracy and precision were assessed gravimetrically for the BD-defined Multitest protocol using normal donor blood, BD Multi-Check process Control and reagent buffer (to represent reagent). These studies were run on three instruments. The study design is shown in the following table.

Table 1: Study Design

Testing Mode	Single Dispense	Multi Dispense
Blood - “Multitest/Tritest/Absolute Count”	50 µL blood (20 primary Tubes x1 daughter tube=20 daughter tubes)	50 µL Multi-Check Control (5x4=20) 50 µL TruCount Control (3x7=21)
Reagent - “Multitest/Tritest/Absolute Count”	20 µL blood (1x20=20)	5 µL blood (20x1=20) Repeat three times (20x3=60)

Table 2: Acceptance Criteria Table

Sample Type	Volume Dispensed	N	Mean Value	%CV
Blood	50 µl	20	50 µl ± 3%	≤ 3%
Reagent	20 µl	20	20 µl ± 7%	≤ 5%

Conclusion: The SPA III demonstrated pipetting accuracy and precision within specification.

Dispense Accuracy and Precision (2)

Testing was performed gravimetrically using reagent buffer.

The studies were run on three SPAIII instruments. Multitest/Tritest/Absolute Count mode were evaluated for accuracy and precision as well as reagent liquid detection, aspirate and dispense.

Table 3: Acceptance Criteria Table

Sample Type	Volume Dispensed	N	Mean Value	%CV
Reagent	20 µl	20	20 µl ± 7%	≤ 5%

Table 4: Accuracy and Precision

Instrument	Mean Volume	% CV	90% Upper Limit of % CV	Pass/Fail
T0069	19.49	1.18	1.43	Pass
T0412P	21.12	1.90	2.30	Pass
T0413P	19.91	2.74	3.32	Pass

Conclusion: The SPA III demonstrated pipetting accuracy and precision within specification.

Assay Precision:

Precision was assessed based on Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline, CLSI document EP5-A2.

Pipetting precision was assessed gravimetrically for the BD-defined Multitest protocol using normal donor blood, BD Multi-Check process control, reagent buffer (to represent reagent). The performance for pipetting blood was found to be within the manufacturer's specifications of $50 \mu\text{L} \pm 3\%$ and the performance for pipetting reagent was found to be within the manufacturer's specifications of $20 \mu\text{L} \pm 7\%$.

Precision of the staining and lysing of samples was also assessed. This was demonstrated by measuring the closeness of agreement between independent test/measurement results obtained under stipulated conditions. These conditions include multiple operators (3), multiple instruments (3 SPA III), and duration of testing (21 days) run twice daily in duplicate. The study samples were CD-Chex normal (CDN) and CD-Chex CD4 low (CDL) stained with BD Multitest 6-Color TBNK Reagent in BD Trucount Tubes. Measurements for this study were the absolute counts and percentages of the following lymphocyte subsets: CD3+CD4+, CD3+CD8+, CD3+, CD3-CD19+ and CD3-CD16+CD56+. The manufacturer's acceptance criteria were as follows:

Absolute counts:

95% upper CI on the CV: $\leq 10\%$ for CD3+, CD4+ and CD8+

95% upper CI on the CV: $\leq 20\%$ for CD19+ and CD16+56+

Percent positives:

95% upper CI on the SD: ≤ 2.5

Study results indicate that the device performed according to the manufacturer's acceptance criteria.

Conclusion: This study confirms that the SPA III system aspirates and dispenses specified volumes of blood and reagent accurately and precisely.

c. *Linearity:*

Not applicable

d. *Carryover:*

Sample Carryover

Carryover was assessed based on recommendations contained in Class II Special Controls Guidance Document: Premarket Notifications for Automated Differential Cell Counters for Immature or Abnormal Blood Cells; Final Guidance for Industry and FDA, December 4, 2001.

Sample carryover was tested using three donors (in duplicate) on three SPA III instruments by running three consecutive high cell count samples followed by three consecutive low cell count samples. The carryover acceptance criterion was carryover $\leq 0.2\%$. High cell count samples ($\geq 50,000 \text{ WBC}/\mu\text{L}$) were prepared by addition of autologous RBC-lysed whole blood. Low cell counts samples ($400 \pm 200 \text{ WBC}/\mu\text{L}$) were prepared by dilution with buffer (PBS). Comparison between the gold standard of manual pipetting and SPA III was done with three instruments using

three normal donor samples run twice per day [as Replicates 1 and 2 (Rep1 and Rep2)]. Samples were stained with the pan-leucocyte marker CD45 in BD Trucount Tubes.

Sample carryover was calculated using the following formula:

$\% \text{ carryover} = [(First\ Low - Third\ Low)/(Third\ High - Third\ Low)] * 100$
from “Guidelines for the Evaluation of Blood Cell Analysers including those use for Differential Leucocyte and Reticulocyte Counting and Cell Marker Applications,” Clinical Laboratory Haematology 16: 157-174 [1994].

$\% \text{ carryover} = [(First\ Low - Third\ Low)/(Third\ High - First\ Low)] * 100$
from CLSI (formerly known as NCCLS) Guideline H52-A “Approved Guideline for Fetal Red Cell Detection.”

These studies, when analyzed using both carryover formulae indicated that the device performed within the manufacturer’s specification of <0.2%.

Reagent Carryover

Reagent carryover was assessed by determining carryover of reagent to buffer for reagent tube to reagent tube carryover and for secondary specimen tube to secondary specimen tube. The acceptance criteria was carryover < 0.01%. High cell count samples ($\geq 50,000$ WBC/ μ L) were used and were prepared by addition of autologous RBC-lysed blood. Comparison between manual pipetting and SPA III was done with three instruments using six donor samples run twice per day [as Replicates 1 and 2 (Rep1 and Rep2)]. The reagent dispensed was BD Multitest 6-Color TBNK Reagent (three donors) for CD45+ cells or BD Leucocount (three donors) for White Blood Cells (WBCs).

e. Interfering Substances:

Not applicable

2. Other Supportive Instrument Performance Data Not Covered Above:

Not applicable

K. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

L. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.