BECKMAN COULTER
Life Sciences

Comparison of anti-CD19 conjugates and performance of newly developed SuperNova polymer dyes

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Introduction

Performance of newly developed SuperNova polymer dye conjugated antibodies from Beckman Coulter were compared with other dyes from various providers to obtain further intelligence on the strengths and weaknesses of each. All dyes compared have similar excitation/emission. Three main parameters were assessed using CD19-conjugated antibodies: brightness, non-specific staining and spillover.

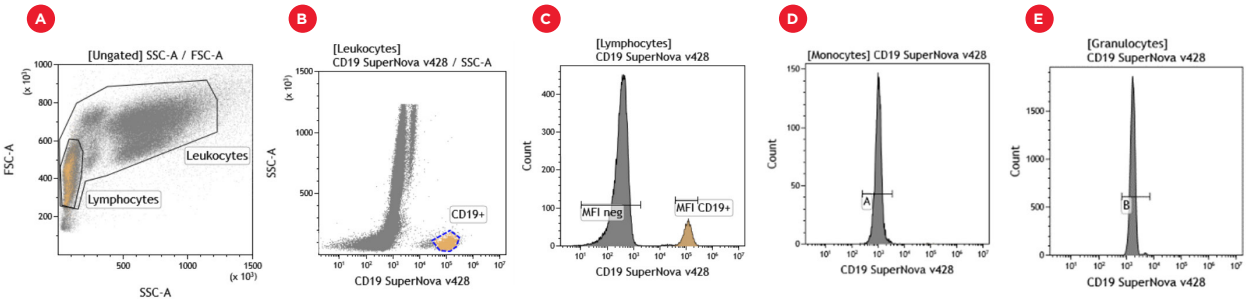
Methods

The SuperNova v428 anti-CD19 conjugate (Beckman Coulter) was compared to BV421 (BD Biosciences) and Super Bright 436 (ThermoFisher) on fresh whole blood samples from healthy donors (n = 50).

The samples were prepared and analyzed on a CytoFLEX flow cytometer (Beckman Coulter) according to standardized procedures under hygrometry and temperature-controlled environment, and analyzed using Kaluza Analysis Software (Beckman Coulter).

Comparison between conjugated antibodies was based primarily on the assessment of the Staining Index.

Results



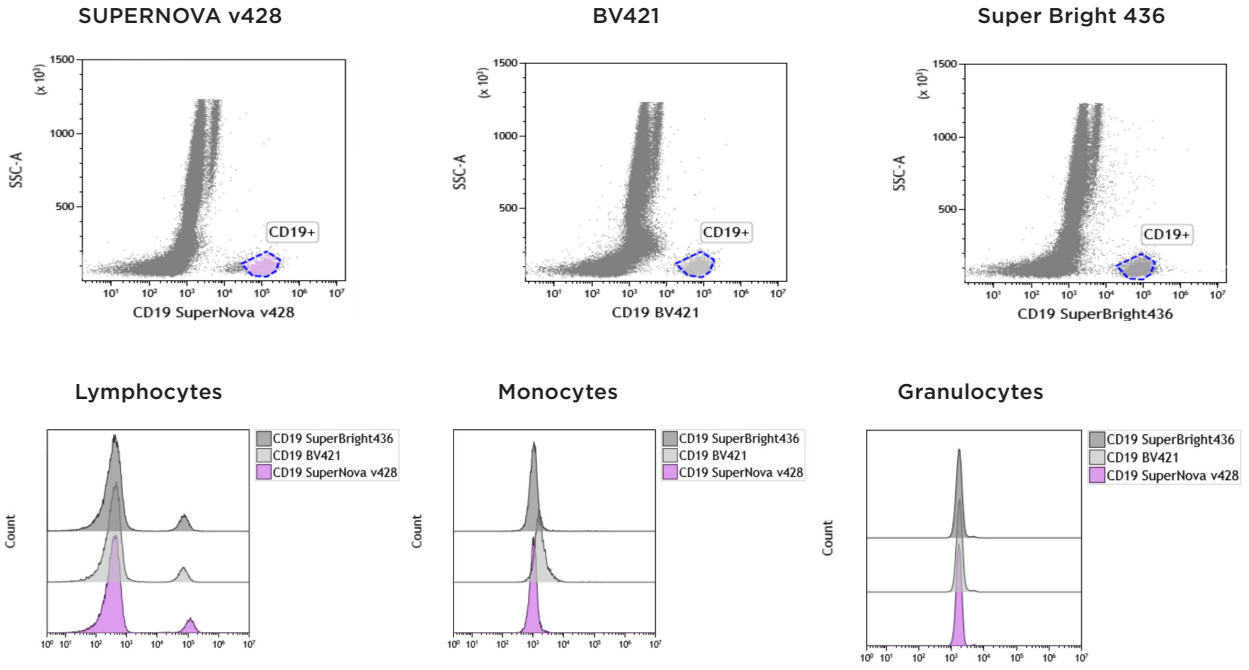
Gating strategy: Cytometric profile of whole leukocytes population **(A)** is shown on dot plot SSC-A vs CD19 Dye **(B)**. Mean Fluorescence Intensity (MFI) and Standard Deviation (SD) from the CD19 negative population lymphocytes (MFIneg and SDneg) and MFI from the CD19 positive population (MFICD19+) are collected from histogram Count vs CD19 Dye **(C)** gated on the whole lymphocyte population **(A)**. Non-specific binding was evaluated on monocytes **(D)** and granulocytes **(E)**.

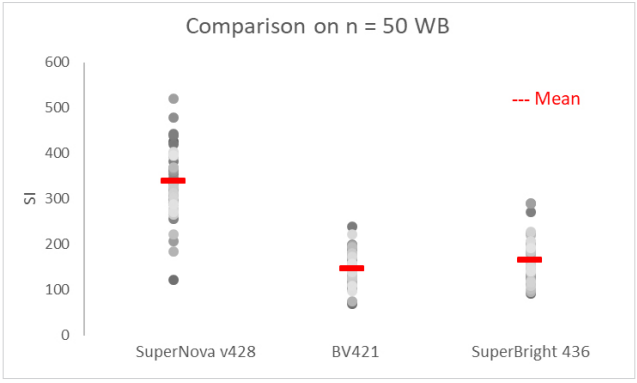
Staining Indexes (SI) were calculated by applying this formula:

$$SI = \frac{[(MFI_{CD19+} - MFI_{neg})]}{2 \times SD_{neg}}$$

Statistical analysis: comparison of SI on B cells and MFI for non-specific staining on monocytes and granulocytes

Antibody	supplier	Staining Index (mean)	Difference	MFI Monocytes (mean)	MFI Granulocytes (mean)	Conclusion
SNv428	Beckman Coulter	338	-	1069	1511	Highest SI
BV421	BD Biosciences	145	-57,2 %	2095	1660	Statistically relevant
Super Bright 436	ThermoFisher	165	-51,2 %	1277	1834	Statistically relevant





Assessment of Staining Indexes: SuperNova v428 consistently demonstrated a higher staining index compared with BV421 and Super Bright 436. Lower non-specific binding on monocytes was observed with SuperNova v428 compared to BV421 and Super Bright 436, while non-specific binding on granulocytes was similar from all suppliers. Spillover in other channels was also similar for all suppliers.

Conclusion

This evaluation showed that the staining index comparison on 50 whole blood samples using SNv428 anti-CD19 conjugate was 134% higher on average than BV421. This suggests that the new SuperNova polymer dye, which shows increased brightness and an improved staining index, enables clear differentiation among positive and negative populations for dimly expressed markers, which can enhance accurate assessment of results in critical laboratory tests.

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