



STEM CELL LABORATORY (STCL)



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CAR T-Cell Testing Protocols JA2

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FLOW-GEN-007 JA2: CAR T-CELL TESTING PROTOCOLS

1. EUREKA Pre Screening only- Peripheral blood (**Dilute sample if white blood cell concentration is $\geq 10.0/\mu\text{l}$**)
 - a. 3-TUBES (NO TRUCOUNT)
 - i. Setup first 3 tubes of NK selection panel (refer to FLOW-GEN-007):
 1. (BD Simultest Isotype)/7AAD/IgG1 APC
 2. (BD Simultest CD45/CD14)/7AAD
 3. CD3Fitc/CD56PE/CD19APC/7AAD
 - ii. Use the Adult Allo ACQ template for tube acquisition. Analyze using the Eureka analysis template (Example A) in the Eureka/Novartis folder of the Routine Test template folder on FACSCalibur workstation desktop.

Results:

Calculate by converting the cell concentration provided to cells/ μl . Record the calculations at bottom or on back of printed result page. Multiply by the lymph% and CD3% to obtain total CD3/ μl . For example if the cell concentration is $32.5 \times 10^6/\text{ml}$, convert to $32.5 \times 10^3/\mu\text{l}$. If there are 32% lymphocytes and 73% CD3 the calculation is: $32.5 \times 10^3/\mu\text{l} \times 0.32 \times 0.73 = 7.592 \times 10^3/\mu\text{l}$ or 7592/ μl .

The CD14/ μl is calculated by multiplying the cell concentration/ μl by the CD14%(ex:15%) obtained from the analysis: $32.5 \times 10^3 \times 0.15 = 4.875 \times 10^3$ or 4875/ μl .

Obtain a second review of all analysis and calculations prior to reporting results.

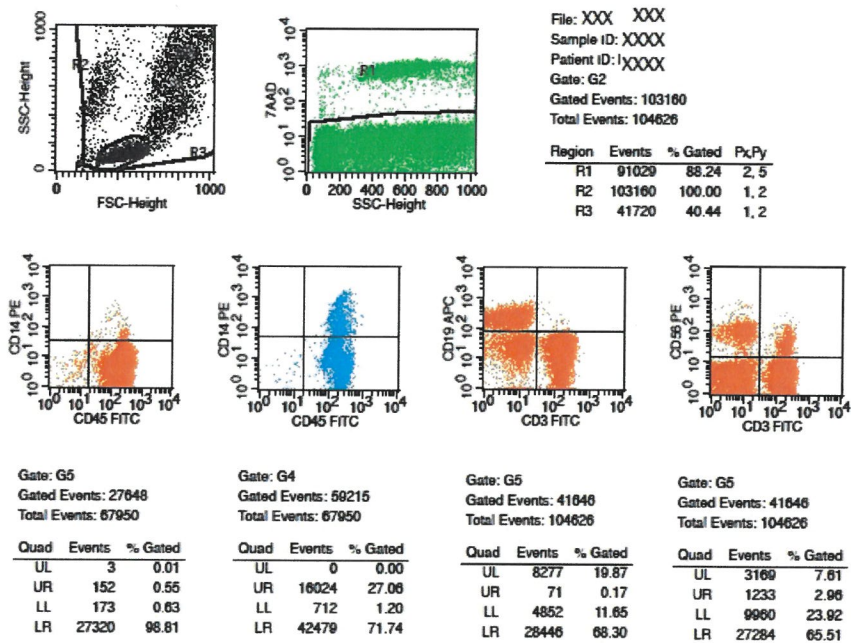
2. Novartis (CTL-019, KYMRIAH) Pre-screen PB (ordered as HPC Pheno) and Product(may be called DLI) (**Dilute sample if white blood cell concentration is $\geq 10.0/\mu\text{l}$**)
 - a. Set up 2-tubes BD Multitest lymphocyte markers as first 2 tubes of IR panel without using a Trucount tube for tube 1. (refer to FLOW-GEN-007)
 - i. Plain 12x75 test tube for MT CD3/CD16+56+/CD45/CD19 (lymphosum qc)
 - ii. Trucount tube for MT CD3/CD8/CD45/CD4
 - iii. Use the 4-color acq. template for tube acquisition. Analyze using the Novartis analysis template (Example B) found in the Eureka/Novartis folder. Always input the current bead count and dilution factor using the expression editor tool (fx) in Cellquest Pro.

Results:

Results for the pre-screen PB sample come directly from the analysis template and should be given directly to person responsible for result reporting. The final product requires that the total CD3 and CD3/kg be calculated. Do this by taking the CD3/ μl value found in the analysis template and plugging that value into the same formula used to calculate total CD34 and CD34/kg found on the backside of the flow worksheet. Use GMP guidelines to make corrections to formula, converting to CD3 calculation.

Obtain a second review of all analysis and calculations prior to reporting results.

Example A



% CD3-CD56+: 7.61

% CD3: 68.30

%CD19: 20.04

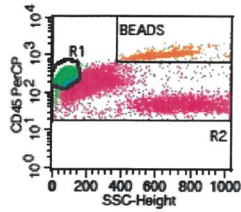
% CD14 of viable cells: 27.06

% Lymphocytes: 46.69

%CD45 of all viable cells= 98.80

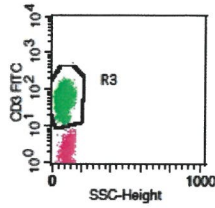
Example B

T-cell analysis



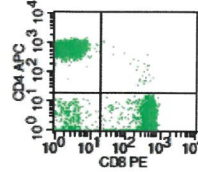
File: XXXXXXXXXXXXXXX
 Patient
 Custom Keyword #1:
 Gate: G2
 Gated Events: 22427

Region	Events	% Gated
R1	9277	41.37
R2	22427	100.00
R3	8218	36.64
BEADS	3115	13.89



File: XXXXXXXXXXXXXXX 02
 Gate: G1
 Gated Events: 9277

Region	Events	% Gated
R1	9277	100.00
R2	9277	100.00
R3	8071	87.00



File: XXXXXXXXXXXXXXX 2
 Gate: T- CELLS
 Gated Events: 8071

Quad	Events	% Gated
UL	3755	46.52
UR	27	0.33
LL	320	3.96
LR	3969	49.18

% Lymph= 48.04

Absolute values
 expressed /ul

CD3: 2559.92

CD4: 1199.56

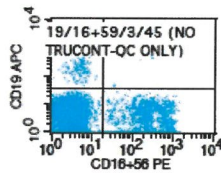
CD8: 1267.43

ALC: 2942.43

CD4+ CD8+: 8.56

CD4/8 RATIO: 0.95

reported CD4 and CD8
 values include dual staining



File: XXXXXXXXXXXXXXX 1
 Gate: G1

Quad	Events	% Gated
UL	126	1.34
UR	7	0.07
LL	7830	83.56
LR	1407	15.02

Trucont Read count: 49400 00

PERCENTAGES

Dilution factor: 1.00

% CD3+ LYMPHS OF CD45+ EVENTS= 41.79

% CD4 OF CD3+ LYMPHS= 46.85

% CD8 OF CD3+ LYMPHS= 49.51

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3. Novartis follow-up testing: Consists of a peripheral blood sample HPC Pheno request with a comment in EPIC stipulating the CD19 request. This testing is set up following the same steps as a 1 tube immune reconstitution sample would be, using the first Multitest reagent (CD3/CD16+56/CD45/CD19) with Trucount tube. It is to be run using Multiset software using the "IR 1 tube" panel.
4. Once acquired, analyzed, and verified by a second tech, give the result to the person responsible for result reporting.

Signature Manifest**Document Number:** FLOW-GEN-007 JA2**Revision:** 01**Title:** CAR T-Cell Testing Protocols JA2

All dates and times are in Eastern Time.

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