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| Six Color Compensation Set Up (COG) Application |
| **Purpose** | This procedure was developed in order to perform MRD testing for B-ALL patients that are enrolled in COG ALL trials. |
| **Policy Statements** | • Applies to Becton Dickinson FACSCanto II Flow Cytometer and technologist analyzing flow cytometryspecimens. |
| **Principle and Clinical Significance** | Minimal Residual Disease (MRD) is the name given to small numbers of leukemic cells that remain inthe patient during treatment, or after treatment when the patient is in remission (no symptoms or signsof disease). It is the major cause of relapse in cancer and leukemia.MRD is strongly associated with outcome. Day 29 MRD >0.01% is the most strongly correlated parameter that we currently have with outcome.Day 8 MRD in peripheral blood is also associated with outcome (the rate of leukemic cells disappearing from blood).Day 8 MRD and day 29 MRD are not duplicative. There is some additional information that can be gained when looking at day 8 in the blood over that of just looking at day 29 in the bone marrow. |

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| **Materials** |

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| **Reagents** | **Supplies** | **Equipment** |
| **Prepare NH4Cl Lyse – Stock;** Weigh out the following and add to a 1000ml volumetric flask; NH4Cl ( chc# 29138) – 80g KHCO3 (chc# 29141)– 10g NaEDTA ( chc# 29139) –  3.7g  Add RO water to a final volume of 1000ml. Check pH on i-STAT (7.2-7.4)  Adjust pH by using 1N HCl or 1N NaOH (usually 6-8ml NaOH). Check pH on i-STATPoint of care analyzer.Validate by staining 100ul of normal whole blood with CD45 APC-Cy7 using the Surface staining, all types procedure, check for acceptable staining.Store at 2-8°C.Stability – 2 years.**Prepare NH4Cl Lyse**  **working solution;** Add 90ml RO water to glass  bottle. Add 10ml NH4Cl stock  solution. Invert to mix. Remove 2.5ml and discard. Add 2.5ml 10% Ultrapure  Formaldehyde (chc# 22479). Invert to mix. Prepare fresh daily. Store at room temp.**Monoclonal Antibodies** **(MoAbs):**  Follow manufacturer's insert instructions in handling antibodies. In general, protect from light and store at 2 to 10° C. Maintain sterile technique to [prevent bacterial or cross contamination of reagents.**Bovine Serum;**Chc# 21302 Sigma Aldrich F4135 **Dulbecco’s PBS 1L;**Chc#24276Invitrogen Life Tech 21600-010 **BD Comp Beads;**Chc#26350MS IGK CompbeadBD Biosciences 552843**Syto 16;**Chc# 2898316 green fluorescent nucleic acid stain\*1mM solution in DMSO\***Prepare Syto 16 working solution;**Add 10ul stock Syto 16 to 490ul DMSO, aliquot 50ul to 10 tubes. Freeze at -20°C for future use. (No more than 4 freeze/thaw cycles).**Dimethyl Sulfoxide (DMSO)**Chc# 29140Sigma Aldrich 472301  | Various pipettes, tips andglasswarePlastic 12 X 75 mm snapcap tubes | CentrifugeVortex mixerBD FACS CANTO II FlowCytometerLyse Wash Assistant, BDBiosciences |

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| **Procedure** |

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| **Step** | **Action** | **Related Document** |
| 1 | **Preparation of Flourochrome tubes;**Label tubes 1-7, add the following

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| Unstained negative control beads | CD20FITC | CD20PE | CD38 PerCpCy5.5 | CD19PC7 | CD58APC | CD45 APC-H7 |
|  | 5ul | 5ul | 5ul | 5ul | 5ul | 5ul |
| 1-2 drops | 1-2 drops | 1-2 drops | 1-2 drops | 1-2 drops | 1-2 drops | 1-2 drops |

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| 2 | Vortex 5 seconds, incubate at room temperature 15 minutes in the dark. |  |
| 3 | Add 350ul 0.3%Bovine Serum/PBS\* to each tube, vortex. | \*Prepared by adding 3ml of Bovine Serum to 1L of PBS.Stability 1 month. |
| 4 | **Preparation of Whole Blood Controls;**Label two tubes ( 1 and 2 ), add 100ul normal whole blood to each tube. |  |
| 5 | Add 2ml of working NH4Cl Lyse solution to each tube vortex for 5 seconds.Incubate for 10 minutes at room temperature, vortex once more for 5 seconds before 10 minutes are up. |  |
| 6 | Centrifuge for 5 minutes. |  |
| 7 | Remove approximately 1.8ml of supernatant ( to the line on the tube ). |  |
| 8 | Place in Lyse Wash Assistant, select dual wash program, push run. |  |
| 9 | Remove tubes 1 and 2, add 1ul of working Syto 16\*\* to tube 2, incubate at room temperature for 10 minutes in the dark.\*\*Prepare working Syto16 by adding 10ul of a prepared 50ul aliquot to 190ul of 0.3%Bovine Serum/PBS (1:20 dilution). |  |
| 10 | Add 350ul 0.3%Bovine Serum/PBS to both tubes. |  |
| 11 | **Prepare analyzer to verify Compensation settings;**Go into Diva software on analyzer, select 6 Color Compensation (Right Click). |  |
| 12 | Select Duplicate without data. |  |
| 13 | Select new file, right click, select rename, enter date, click select. |  |
| 14 | Select first tube (Unstained tube 1). Then run tubes 2-7, mean channel should fall within preset range. |  |
| 15 | **Run Control Tubes 1 and 2;**Run tube 1 (whole blood negative control) collect 5000 events.(Select acquire, append, collect 5000 events).Run tube 2 (whole blood Syto 16 positive control) collect 5000 events.(Select acquire, collect 5000 events).Verify separation between negative and positive populations. |  |
| 16 | Right click, select calculate Compensations, rename 6C Compensation, date. |  |

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| **Special Safety Precautions** | [MSDS Search | MSDSonline](https://msdsmanagement.msdsonline.com/a07dc954-23d8-42a9-b591-ef5763cdfd33/ebinder/?nas=True) Childrens Star Net |
| **References** | BD Bioscience FACSCanto Training Manual. 23-9575-00 Rev. A. 2007, Becton, Dickinson and Company, San José, CA BD FACSLyse Wash Assistant User’s Guide,Rev.23-11113-00 Rev. A ,Becton Dickenson, San José, San José, CA  |
| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Al Quigley | 07/16/18 | Initial Version ( COG Application ) |
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