# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer

**Purpose**

- The purpose of this procedure is to describe preparation and staining sample for 10-color Lymphoma phenotyping using Navios Flow Cytometer.

**Work Place Safety:**

All laboratory employees are expected to maintain a safe working environment and an injury-free workplace. Laboratory employees are responsible for their own safety, the safety of others and adhering to all departmental and medical center safety policies and procedures.

- For standard precautions and safety practices in the laboratory; see LAMC-PPP-0123, specifically, but not limited to, equipment safety, proper body mechanics, sharps exposure and proper use of personal protective equipment (PPE).
- For proper handwashing, see LAMC-PPP-0132, specifically, but not limited to, proper handwashing.
- For proper handling of regular and infectious waste, see LAMC-PPP-0130, specifically, but not limited to proper disposal of regular and biohazardous waste.
- For proper cleaning of work area, see LAMC-PPP-0130- Cleaning Work Areas.
- For proper handling of chemicals and reagents, see the Chemical Hygiene Plan.
- For proper storage and disposal of chemical hazardous waste, see LAMC-PPP-0134.

**Policy**

- Always wear personal protective equipment when working with Ammonium Chloride Lyse. This may include, but not limited to, protective eyewear (eye goggles), gloves, and suitable laboratory attire (long sleeved laboratory coat) when working with Ammonium Chloride.
- Store Ammonium Chloride crystals in a tightly closed container in locked cabinet. Refer to Uncontrolled Document – Safety Data Sheets: Millipore Sigma and ThermoFisher Scientific.
- The turnaround time (interval between specimen receipt by laboratory personnel and result reporting) for 10-color Lymphoma Panel order is 2 days for peripheral blood samples and 3 days for bone marrows and other flow cytometry specimens.
- Assigned CLS informs Hematopathologist and requester when testing is delayed.
- In case of instrument downtime, follow instructions as stated in LAMC-PPP-0560 for prioritizing acquisition and analysis of Flow Cytometry.

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

**Principle**

- This procedure describes the steps in performing cell surface marker study for Hairy Cell Leukemia (immunophenotyping) performed from whole blood, bone marrow aspirate, effusion, solid tissue cell suspension, or other body fluids using panel of monoclonal antibodies conjugated with fluorochromes such Fluorescein Isothiocyanate (FITC), Phycoerythrin, (PE), Phycoerythrin-Texas Red® X (ECD), Phycoerythrin-Cy5 (PC5), PC-7, APC, APC Alexa-700, APC-Alexa-750, Pacific Blue, and Krome Orange.
- Immunophenotyping of leukocytes by flow cytometry requires removal of red blood cells, and this can be achieved by lysing erythrocytes. Ammonium chloride solution has been used for many years for lysis of red blood cells.

- Viable cell suspension is incubated with specific monoclonal antibodies, and antigens on the surface of the cell will react with the presence of their respective antibodies.
- After lysis of red cells, samples are stained with monoclonal antibodies and analyzed using flow cytometer by light scatter patterns, fluorescence intensity, and results are expressed as percentage of positive events in relation to all events acquired by electronic gating.
- The Navios is an instrument for phenotyping surface markers of white blood cells. The scatter plots of cells are measured and analyzed as cells pass through a LASER beam in a single-fine using the method of hydrodynamic focusing.

**Specimen Requirement**

- Use cell suspension or body fluid less than 48 hours old.
    - $K_3$EDTA blood – 2ml minimum. ACD and heparin is acceptable if EDTA anti-coagulated specimen is not available.
    - Sodium heparinized bone marrow aspirate – minimum of 2ml. Wash once with Modified PBS before processing.
    - Effusion (pericardial, pleural, or peritoneal) may be treated with anticoagulant with minimum volume of 10 ml.
    - Body fluids cell suspension of washed or mashed solid tissue in cell media, specimen volume 5 to 10ml.
    - CSF must be collected on CSF vial submit all available specimen, minimum volume 2ml.

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

- Obtain WBC count, if available.
- Maintain and transport specimen at room temperature (18-22 $^0$C) in a leak-proof container. Avoid temperature <10 $^0$C and >37 $^0$C.
- Take note on the worksheet and report on the form if the following occur:
    - If the specimen is hot or cold but not obviously hemolyzed or frozen.
    - If the specimen is hemolyzed or frozen.
    - If clots are visible.
    - If the specimen is >48 hours old at the time it arrives at the laboratory.
- Do viability testing. Refer to SOP LAMC-PPP-057001.

**Reagent, Materials and Equipment**

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**Reagent:**

- Ammonium Chloride 500g Sigma-Aldrich Catalog number: 213330-500g.
- Potassium bicarbonate 327205 Sigma-Aldrich Catalog Number: 237205-500g
- Ethylenediaminetetraacetic acid tetrasodium salt.
- Orion Buffer (USA)
    - pH buffer, Orion 910104, Thermofisher
    - pH 7.00 buffer, Orion 910107, Thermofisher
    - pH10.01 buffer, Orion 910110, Thermofisher
    - pH Electrode Storage Solution, Orion 910001, Thermofisher
    - Distilled water
- Deionized Water
- Bleach
- IsoFlow Sheath Fluid, Beckman Coulter, PN 8547008
- Coulter Clenz cleaning agent, PN 8546930
- Bovine Serum Albumin 22% (BSA) Sigma-Adrich A7034-100ML
- Flow Set Pro Fluorospheres – Beckman Coulter PN A63492
- Flow Check Fluorospheres – Beckman Coulter PN A63493
- CD-Chex Plus Streck 213340
- Phosphate buffered saline, pH =7.4. Sigma Catalog # P-3813

Preparation:

| Step | Action |
|---|---|
| 1 | Take 1 packet (foil pouch) and dissolve in 1 liter of deionized water. Yield: 0.01 M PBS pH= 7.4 |
| 2 | Store in a 1 liter amber dispenser at room temperature. |

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

Monoclonal Antibody Reagents: Table 1 (Titered volume) for 10-color Hairy Cell Leukemia Panel:

| Reagent | Catalog # | Manufacturer | ul/test |
|---|---|---|---|
| FMC7-FITC | 340918 | Becton Dickinson | 10 |
| CD25-PE | IM0479U | Beckman Coulter | 7.5 |
| CD22-ECD | B10245 | Beckman Coulter | 7.5 |
| CD11c-PC5.5 | B19719 | Beckman Coulter | 2.5 |
| CD5-PC7 | A51075 | Beckman Coulter | 2.5 |
| CD103-APC | B06204 | Beckman Coulter | 2.5 |
| CD19-APC-AF700 | A78837 | Beckman Coulter | 2.5 |
| CD14-APC-AF750 | A86052 | Beckman Coulter | 2.5 |
| CD20-PB | A74777 | Beckman Coulter | 2.5 |
| CD45-Krome Orange (KrO) | A96416 | Beckman Coulter | 2.5 |
| PBS | NA | NA | 7.5 |

Notes:

a) Cocktail Preparation: Pipette the corresponding volumes of monoclonal reagents above, add 7.5 microliters (ul) of PBS into dark reagent vial for a total volume of 50ul.
b) Cocktailed reagents are stable for one month after preparation.
c) Label cocktailed reagents with the following information: prepared by, date prepared, expiration date, contents of the cocktail, ul/test, and storage temperature.

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

**Materials**

- Spatula
- Weight boat
- Curwood Parafilm 4in W x125L, Catalog #13-374-10 Fisher-Scientific
- Parafilm Dispense/Cutter, Catalog# 06-666-52 Fisher Scientific
- Erlenmeyer Flask -1000ml and 4000ml
- 30 ml graduated beaker (3)
- 200 ml beaker

- Polypropylene round bottom tubes 12X75mm., Fisher Scientific, Catalog # 14-959-5
- Disposable plastic centrifuge, 15ml., Fisher Scientific, Catalog # 35-2099
- Disposable Petri dishes, size 100 x 15mm., Fisher Brand Catalog # 08-757-12
- Pipette Eppendorf
- Pipet tips (Finntip ® 250 Universal) Catalog # 9400260
- Transfer pipette, 1ml Graduated, Fisher Scientific, Catalog # 13-711-9A
- Test tube racks.

**Equipment**

- pH Meter Fisher Sci Model 510
- Mettler XS Analytical Balance
- Navios Flow Cytometer
- Beckman Centrifuge
- Vortex Mixer
- Biosafety Cabinet
- Non-$CO_2$ Incubator at $37^0$C
- Stirrer and stirrer bar

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

**Quality Control**

See associated Standard Operating Procedures:

- Daily Start-up Procedure for Navios Flow Cytometer, LAMC-PPP-0535
- Quality Control Procedure for Navios Flow Cytometer, LAMC-PPP-0537
- Shutdown Procedure for Navios Flow Cytometer, LAMC-PPP-0536
- Navios Flow Cytometer, Preventive Maintenance, LAMC-PPP-0541

Daily QC:

Preparation of Flow Check Pro for Optical Alignment:

| Step | Action |
|---|---|
| 1 | Label a polypropylene round bottom tubes 12X75mm as Flow Check Pro indicate Lot number and expiration date on the tube. |
| 2 | Mix Flow Check Pro beads thoroughly and add about 1ml of Flow Check Pro beads in the labeled Flow Check Pro tubes. |
| 3 | Load the labeled tube in the first position of the carousel. |

Preparation of Flow Check Pro for Standardization of voltages of PMT.

| Step | Action |
|---|---|
| 1 | Label a polypropylene round bottom tubes 12X75mm as Flow Set Pro and indicate Lot number and expiration date on the tube. |
| 2 | Mix Flow Set Pro beads thoroughly and add about 1ml of Flow Check Pro beads in the labeled Flow Check Pro tubes. |
| 3 | Load the labeled tube in the 2$^{nd}$ position of the carousel. |

Preparation of Verify Tube using CD Chex Plus:

| Step | Action |
|---|---|
| 1 | Take CD Chex Plus from the storage refrigerator, bring to room temperature. Note: Open vial stability is 30 day. Record expiration on form LAMC-PPP-0149. |
| 2 | Stain CD Chex Plus with using T- cell monoclonal antibody reagents. VerifyTube:CD57-FITC/CD16-PE/CD5-PC7/CD7-APC/CD4-A700/CD3-A750/CD8-PB/CD45KO |
| 3 | Load the labeled tube in the 3$^{rd}$ position of the carousel. |

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

How Acquire Data Optical Alignment, Standardization (Voltage and Mode), and compensation check.

| Step | Action |
|---|---|
| 1 | Load tube with 1ml of Flow Check Pro, Flow Set Pro, and Verify Tube into a carousel. |
| 2 | Drag the 10-color daily QC Panel into the Acquisition Manager |
| 3 | Label Daily 10-color QC the file name column of the Acquisition Manager. |
| 4 | Press F9 to run the 10-color Daily QC Panel. |

How to prepare 10-Color Auto-setup tubes: **(As needed only)**

| Step | Action |
|---|---|
| 1 | Reconstitute 1 vials of Cytocomp cells (Part# 6607023) and into a 1ml Cytocomp Buffer. Let the solution sit for 5 to 10 minutes |
| 2 | Label 12x72mm tubes in the following manner: |

| Reagent | Catalog # | Manufacturer | ul/test |
|---|---|---|---|
| Flow Set Pro Fluorospheres | A63492 | Beckman Coulter | NA |
| CD45-FITC | IM0782U | Beckman Coulter | 5 |
| CD45-PE | IM2078U | Beckman Coulter | 5 |
| CD45-ECD | IM2710U | Beckman Coulter | 5 |
| CD45-PC5.5 | A54139 | Beckman Coulter | 5 |
| CD45-PC7 | IM3548U | Beckman Coulter | 5 |
| CD45-APC | IM2473U | Beckman Coulter | 5 |
| CD45-APC-AF700 | A71117 | Beckman Coulter | 5 |
| CD45-APC-AF750 | A71119 | Beckman Coulter | 5 |
| CD45-Pacific Blue | A74765 | Beckman Coulter | 5 |
| CD45-Krome Orange | A96416 | Beckman Coulter | 5 |
| VerifyTube:CD57-FITC/CD16-PE/CD5-PC7/CD7-APC/CD4-A700/CD3-A750/CD8-PB/CD45KO | Stain CD Chex Plus with using T- cell monoclonal antibody reagents. VerifyTube:CD57-FITC/CD16-PE/CD5-PC7/CD7-APC/CD4-A700/CD3-A750/CD8-PB/CD45KO | Beckman Coulter | See Table 1 |

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

| | |
|---|---|
| 3 | Add corresponding volume CD45 antibody. |
| 4 | Incubate for 15minutes. |
| 5 | Add 2 ml pf PBS, spin for 1 minute using Clay Adams Centrifuge. |
| 6 | Decant supernatant and a 1 ml of PBS. |
| 7 | Run Auto-setup in Navios. |

**How to run 10-color Auto-Setup: (as needed)**

| Step | Action |
|---|---|
| 1 | From the Main menu, click on **Tools** and choose Auto-Setup Scheduler. |
| 2 | From Auto-Setup Scheduler window, choose 10-color Auto-Setup. |
| 3 | Load Carousel. Enter carousel number and click on **Schedule**, click **Close.** |
| 4 | At Acquisition Manager (BT File name column), enter Flow Set Pro 10-C and at the Name column, Enter date. |
| 5 | Press F9 to run Auto-setup. The instrument will acquire Auto-Setup tubes. |

**How to use LAMC-FORM-0147, LAMC-FORM-0148, and LAMC-FORM-0149.**

| Step | Action |
|---|---|
| 1 | After acquisition of Flow Check Pro, review LJ chart to ensure all parameters (Half Peak CV) are within range, and indicate check mark on FSC-HPCV, SSC-HPCV, FL1-HPCV up to FL10-HPCV on LAMC-FORM-0147, otherwise indicate action taken on outliers using LAMC-FORM-0109. |
| 2 | After acquisition of Flow Set Pro, review LJ chart to ensure all parameters (Detector Modes) are within range, and indicate check mark on FSC-Mode, SSC-Mode, FL1-Mode up to FL10-Mode on LAMC-FORM-0148, otherwise indicate action taken on outliers using LAMC-FORM-0109. |
| 3 | After acquisition of verify tube (CD Chex Plus), review LJ chart to ensure all parameters are within range, and indicate check mark on CD57, CD16, CD19, CD56, CD5, CD7. CD3, CD4, CD8 on LAMC-FORM-0149, otherwise indicate action taken on outliers using LAMC-FORM-0109. |

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

Quality Control:

Ammonium Chloride performance as lysing agent must be check before lysing

| Step | Action |
|---|---|
| 1 | Pipet 2-3 ml of sample (normal peripheral blood) in to a 15 ml conical tube. |
| 2 | Add 10 ml of freshly prepared $NH_4Cl$ (1X) |
| 3 | Mix the specimen after adding the lysing agent and start timing for 7 minutes. |
| 4 | Check for completeness of erythrocyte lysing, by holding a printed page, and when the print can be read through the tube, then the lysis is complete. |
| 5 | Document the completeness of erythrocyte lysing by using a "$NH_4Cl$ pH Reading and Completeness of Erythrocyte Lysing" form. |

Patient samples. The amount of time it takes to lyse red cells is measured. The lysis time of erythrocyte must be complete within 7 minutes after addition of Ammonium Chloride.

How to make 1X Ammonium Chloride solution:

| Step | Action |
|---|---|
| 1 | Make 1:10 Dilution of Lysing solution (NH4Cl)<br>Add 9 volumes of de-ionized water to a 10X volume of stock solution. Do not refrigerate. |
| 2 | The 1X NH4Cl working solution surplus must be discarded after expiration date. Use adjust pH to 7.4.± 0.1. |

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

**Note:** For bone marrow specimen and peripheral blood: To ensure ideal flow rate, the WBC count should be from 5,000 to 10,000 cells/cu mm. WBC above 15,000 requires adjustment of the cell concentration to an appropriate level.

| WBC COUNT | DILUTION (with PBS) |
|---|---|
| 10,000 up to 20,000 | 1:2 |
| 20,000 up to 30,000 | 1:3 |
| 30,000 up to 40,000 | 1:4 |
| 40,000 up to 50,000 | 1:5 |
| 100,000 up to 200,000 | 1:10 |
| Above 200,000 | 1:20 |

For specimens with low WBC use the Table below to determine number of panels to stained:
**Note:** Spin to concentrate cells and q.s. to 0.5ml.

| Number of Panels | Events/60 seconds | Approximate WBC |
|---|---|---|
| No flow | <200 | 0.002-.02 |
| 1 Panel/tube | 201-400 | 0.02-0.04 |
| 2 Panels/tubes | 401-600 | 0.04-0.06 |
| 3 Panels/tubes | 601-800 | 0.06-0.08 |
| 4 Panels/tubes | 801-1000 | 0.08-0.1 |

**Procedure:**

**Kaiser SCPMG Flow Cytometry Lab 10-color Hairy Cell Leukemia Panel:**

| 10-color Hairy Cell Panel | FMC7 FITC | CD25 PE | CD22 ECD | CD11c PC5.5 | CD5 PC7 | CD103 APC | CD19 APC-AF700 | CD14 APC-AF750 | CD20 PB | CD45 KrO | # of test |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Test/ul | 10 | 7.5 | 7.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5ul | 60 |
| 50ul | 600 | 450 | 450 | 150 | 150 | 150 | 150 | 150 | 150 | 3000 | |

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

How stain 10-color Hairy Cell Leukemia Panel:

| Step | Action |
|---|---|
| 1 | Lyse 2-3 ml of specimen using 10ml of 1X NH4Cl (working solution) for 7 minutes. |
| 2 | After 7 minutes, centrifuge lysed specimen for 5 minutes at 1500rpm. |
| 3 | Decant and add 10 ml of 1X NH4Cl, centrifuge for 5 minutes at 1500rpm.<br><br>**Note:** For specimen with weak or suspicious monoclonal population (to further block extrinsic or cytophilic immunoglobulin staining) after step #3, add 3 drops of 22% Albumin, and incubate suspension of cells in water bath (37 degrees Celsius) for 20 minutes. |
| 4 | Add 10 ml of PBS and vortex. |
| 5 | Wash cells with PBS 5 minutes at 1500rpm, decant and add 1 ml of RPMI. |
| 6 | Filter lysed samples using 210um filter to remove large tissue aggregates. |
| 7 | Obtain initial count of WBC and viability:<br>a) Add 100ul and 0.5ml of 50ugm/ml of Propidium Iodide solution.<br>b) Incubate for 5 minutes.<br>c) Run viability using Navios Flow Cytometer<br>d) adjust cell concentration to 5 to 10 cells x $10^3$ cells/cumm. |
| 8 | **Label secondary round bottom tubes (12x75mm): c with internal number (BT number) based from primary sample.** |
| 9 | Add titered amount of (10-color Mab Panel) into each tube. (see table above) |
| 10 | Add 100ul of sample from Step 8 into each tube. |
| 11 | Incubate for 20 minutes |
| 12 | Add 2ml of PBS or 1X NH4Cl. |
| 13 | Centrifuge tubes using Serofuge Clay Adams 2002 for 2 minutes. |
| 14 | Decant and add 0.5ml of PBS. |
| 15 | Run samples using Navios Flow Cytometer. |

How to acquire sample for 10-color Hairy Cell Leukemia Panel:

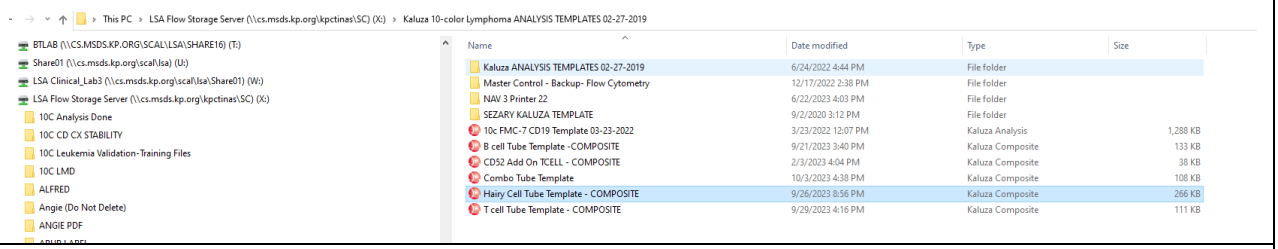
| Step | Action |
|---|---|
| 1 | Clear Worklist by clicking **New Worklist** icon. |
| 2 | At the Resource Explorer, Click Folder containing 10 color lymphoma screen panel (BT Folder). |
| 3 | Drag 10-color lymphoma panel into Acquisition Manager. |
| 4 | At Acquisition Manager, type file names of samples at (BT# column): See file name format below:<br>Year-Sample type-case number- last name<br>XX- BT-XXX-XXXX. |
| 5 | Enter Carousel number, load Carousel, and Press F9. |
| 6 | Check verify tube: Inspect verify tube to ensure proper color compensation |

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

**Data Acquisition and Gating Strategy: Combo, T, B Lymphoma Panels:**

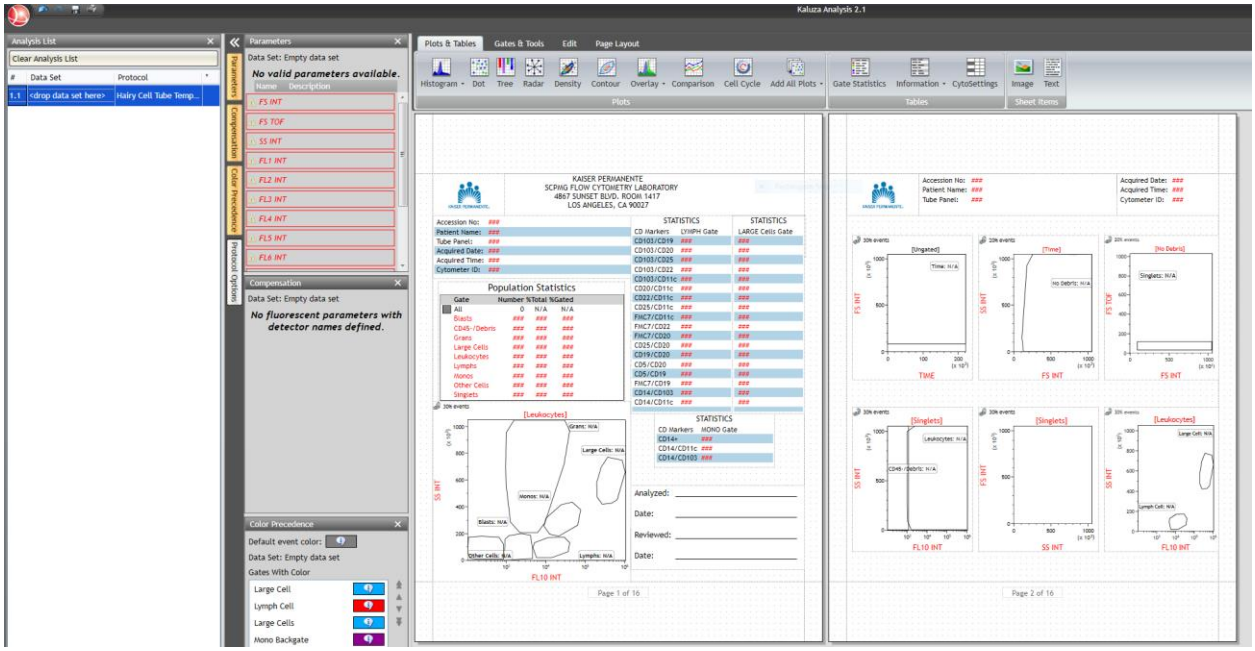
| Step | Action |
|---|---|
| 1 | For 10-color gating: Select Set-up mode:<br>To capture abnormal population (monocytoid or lymphoid), CD45 vs Side Scatter (SSC) gating is used, drag the gate to the population of interest. |
| 2 | De-select Set-up Mode. Press **Play** button or **F9**. |
| 3 | Continue to acquire subsequent tubes, and during acquisition, check for gating consistency.<br>Note: for underlyzed samples, re-stain and re-acquire samples. |

**Data Analysis using Kaluza Software:**

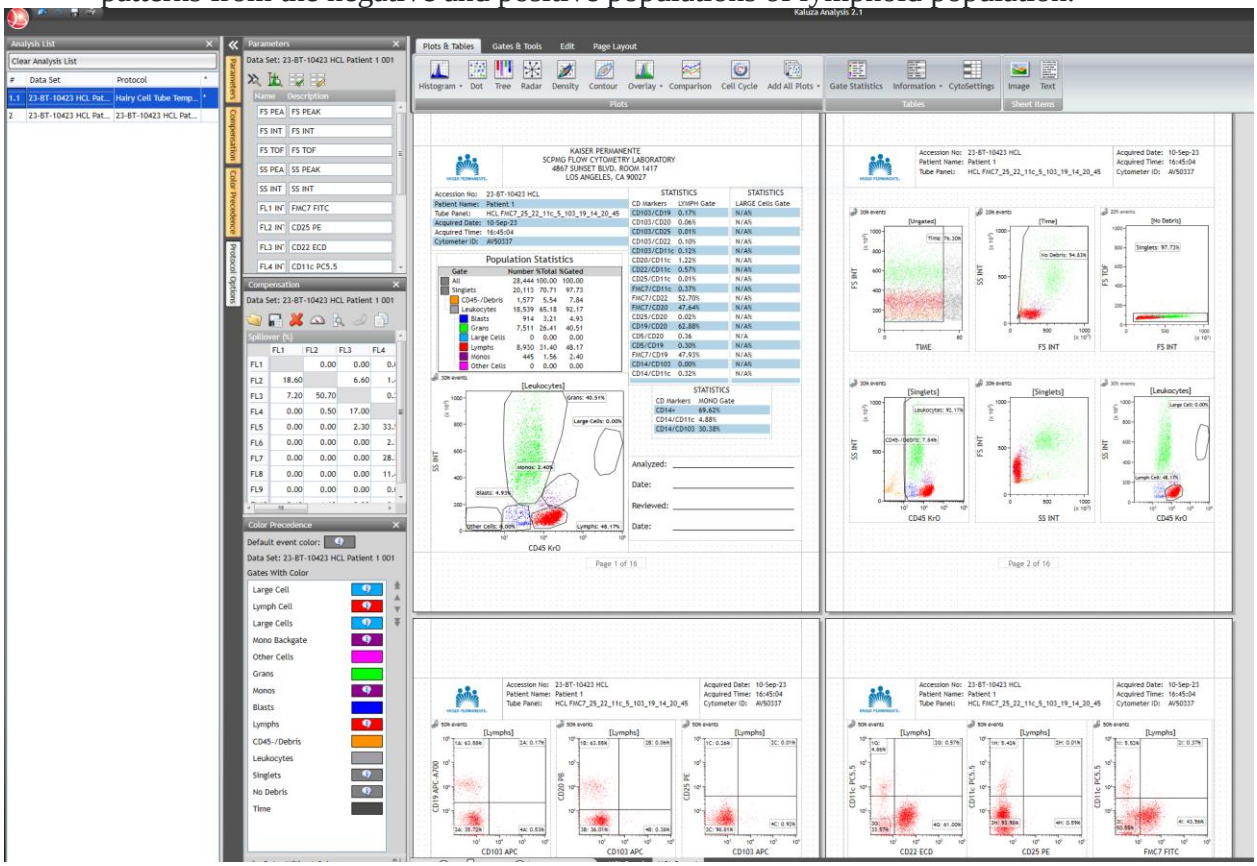
| Step | Action |
|---|---|
| 1 | Open Kaluza Software – double click Kaluza software icon, click on share drive – LSA Flow Storage Server, select analysis template folder: 10-color Kaluza 10-color Hairy Cell Analysis template.<br>  |
| 2 | Double click to open 10-color Kaluza Hairy Cell Analysis template. |

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

| | |
|---|---|
| 3 | Open LMD file from Kaluza (Red ball) icon on the upper left, choose LSA Flow Storage service share drive and open 10C LMD folder, choose instrument folder where the file was acquired, select file to be analyzed. |



# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

| | |
|---|---|
| 4 | a) For 10-color gating: To capture abnormal population (monocytoid or lymphoid), CD45 vs Side Scatter (SSC) gating is used, drag the gate to the population of interest.<br>b) Quadrant Marker or cursor settings must be determined based on the fluorescence patterns from the negative and positive populations of lymphoid population.<br>  |
| 5 | Save file as "Analysis List" on the 10c Done folder. To print file, save as pdf, and print. |
| 6 | Printed pdf files and accompanying slides will be forward to assigned Hematopathologists for interpretation. |

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

**Reporting and Documentation**

- To detect clerical errors, analytical, and unusual laboratory results, a CLS aside from the one who acquired and analyzed the data from the cytometer, will review histogram and immunophenotying results. The CLS who reviewed the results shall initial the worksheet containing the immunophenotyping report before release from the laboratory.
- Assigned Hematopathologist may consult with Medical Director of Flow Cytometry to review unusual histograms and suggest resolution of flow cytometric issues.
- Print-out of immunophenotyping histograms shall be submitted to the assigned pathologist together with accompanying stained slides.
- Results of the percentage positivity of monoclonal antibodies may be reported through the telephone or faxed to the assigned pathologist upon his/her request.
- Viability testing acceptable result: Greater than 90% viable cells. For viabilities <90%, results are reported to hematopathologist for interpretation.
- If histograms are needed for interpretation immediately by Hematopathologist, or critical results are observed by the performing CLS, the requesting Hematopathologist shall be notified and flow cytometry histograms will be scanned directly to the Hematopathologist's e-mail for interpretation.

**Controlled Document:**

- LAMC-PPP-0123 - Safety Practices.
- LAMC-PPP-0132 – Handwashing Policy.
- LAMC-PPP-0127 – Infection Control.
- LAMC-PPP-0130 – Cleaning Work Areas.
- LAMC-FORM-0145 for pH meter readings.
- LAMC-FORM- 0146: for recording complete lysis of erythrocytes.
- LAMC-FORM-0147: for Flow Check Pro Optical Alignment beads.
- LAMC-FORM-0418: Standardization use Flow Set Pro Fluorospheres.
- LAMC-FORM-0149: Verification – CD-Chex Plus, 10-color QC
- LAMC-FORM-0150: Summary Shift/Trend Monitoring 10-color Flow Cytometry
- LAMC-Form-0102: LASER Usage Log Navios Flow Cytometer.
- LAMC-Form-0103: Daily Maintenance and QC
- LAMC-FORM-0109: Navios Quality Control – Outlier Action Log

# 10-Color Hairy Cell Leukemia Immunophenotyping Using Navios Flow Cytometer, continued,

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**Uncontrolled Document**


- RBC Lysing Solution and Cell Lysing Procedure: Retrieved, April 21, 2015: https://depts.washington.edu/flowlab/Cell%20Analysis%20Facility/RBC%20Lysing%20Solutions%20and%20Cell%20Lysing%20Procedure.pdf
- Lysing Solution IO Test 3 10X concentrate Ref A07799, January 01, 2003, Beckman Coulter.
- Ammonium Chloride: Safety Data Sheet (SDS) – Sigma Millipore – Retrieved, April 14, 2020, Version 6.3, Revised 01/15/2020.
- Ammonium Chloride: Safety Data Sheet (SDS) – ThermoFisher Scientific – Retrieved April 13, 2020, Revision 4 Dated: 01/1/2018.


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**SelfAssessment:**

1. How long is the lysing time of specimen with 1X ammonium chloride?
    a) 10 minutes
    b) Maximum of 7 minutes
    c) It does not matter.
2. True or False:
   WBC above 15,000 requires adjustment of the cell concentration to an appropriate level.
3. True or False:
   Hairy cell Analysis Kaluza analysis panel and acquired LMD files are both located on the LSA Flow Server share drive.

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