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| Auto-Immune Lymphoproliferative Panel on FACSCanto II Flow Cytometer |
| **Purpose** | A 4 color direct immunofluorescence staining of human peripheral blood lymphocytes to assess double negative T-cells (CD3+/TCRαβ+/CD4-/CD8-) seen in ALPS.  |
| **Policy Statements** | This procedure applies to all laboratory technologists performing Flow Cytometry testing, the sectionsupervisor, and section pathologist. |
| **Principle and Clinical Significance** | Autoimmune lymphoproliferative syndrome (ALPS) is an inherited disorder characterized by peripheral lymphocytosis, lymphadenopathy, hepatomegaly, splenomegaly, and autoimmune cytopenias. Patients are also at increased risk of lymphoma. Clinical manifestation usually occurs in pediatric patients with an average age of 22 months at the time of diagnosis. Dysregulated apoptosis is associated with accumulations of T-cell receptor (TCR) alpha-beta positive CD4 and CD8 negative T-cells, CD5 positive B-cells and HLA-DR positive T-cells. Quantifying these lymphocyte subsets provide a rapid and inexpensive screening test to rule out ALPS. A base MultiTEST immune status panel using TruCount tubes is run with additional markers analyzed in FACSDiva mode (dual platform analysis). |
| **Test Code** | ALPTest code CAALP may be added to a CISP accession number to convert to ALP |
| Materials |

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| **Reagents** | **Supplies** | **Equipment** |
| ● PBS Buffer Solution (PBS) with 0.3% fetal calf serum (FCS): Make PBS according to package directions. Add 3 ml of FCS to 1 liter of PBS in a clean container. Label with lot number and expiration date (one month from preparation date).● 1X BD FACSLyse Solution: Prepare a 1X working solution from the 10X FACSLyse stock solution by making a 1:10 dilution with sterile water. Label with lot number and expiration date (one month from preparation date).● Monoclonal Antibodies (MoAbs): Follow manufacturer's insert instructions in handling antibodies. In general, protect from light and store at 2 to 10°. Maintain sterile technique to prevent bacterial or cross contamination of reagents. | ● Various pipettes, tips and glassware● Polystyrene 12 X 75 mm Falcon tubes | Vortex MixerBD FACSCanto II Flow Cytometer  |

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| Sample | 1. Peripheral blood, 2mL, collected in EDTA. Minimum acceptable volume is 500 µL in an EDTA microtainer. 2. Specimens should be stored at room temperature until processing. Specimens exposed to extreme temperatures may yield inadequate results.3. Clotted or grossly hemolyzed specimens are not acceptable for analysis.4. Any specimens not properly labeled should be rejected.5. Specimen should be processed within 72 hours of draw per CDC recommendations and our validation studies. (5/13/03). |
| **Special Safety Precautions** | **SAFETY PRECAUTIONS**: BD FACSLyse contains formaldehyde. Formaldehyde is extremely toxic and destructive to tissue of mucous membranes, upper respiratory tract, eyes and skin. It is harmful if swallowed, inhaled or absorbed through the skin. This material is an irritant, a sensitizer, a highly toxic lachrymator and a possible mutagen. Gloves and protective clothing must be worn to prevent contact with skin. See MSDS for further information regarding its irritant, corrosive and possible carcinogenic properties.Formaldehyde Disposal: Tubes containing 1% or less of formaldehyde may be disposed of in red biohazard buckets. Stock and working dilutions of formaldehyde may be disposed of down the drain with copious amounts of water. |
| **Quality Control** | Follow guidelines outlined in *Flow Cytometry Quality Assurance* procedure.  [FLO 1.3 Quality Assurance in Flow Cytometry](http://khan.childrensmn.org/Manuals/Lab/SOP/Flow/Flow/204759.pdf) |
|  | **Step** | Action | **Related Document** |
|  **Procedure** | 1 | Prepare and run FACSCanto MultiTEST base panel according to Immune Status Panel procedure. | [flo-1.7-immp-c48p-immune-status-immunodeficiency-panels](https://starnet.childrenshc.org/References/labsop/flow/flow/flo-1.7-immp-c48p-immune-status-immunodeficiency-panels.pdf) |
|  | 2 | Label tubes for desired antibody panel and add appropriate monoclonal antibodies to each tube: ( FITC / PE/ PerCP / APC ) IgG1 / IgG1 / CD45 / IgG1  TCRαβ / CD3 / CD45 / CD4+CD8 TCRγδ / CD3 / CD45 / HLA-DR CD20 / CD5 / CD45 / CD14 |  |
|  | 3 | Add 50 μL of well mixed whole blood to each tube. Vortex gently to mix. |  |
|  | 4 | Place the uncapped tubes into a carousel rack and load on the Lyse Wash Assistant. Select the "Inc Duo Lyse Wash" program and follow prompts to run. When the program is finished, samples are ready to run on the FACS Canto II flow cytometer.If samples are not analyzed immediately afterpreparation, store them in the dark at 2-8°C and analyze within 4 hours. | [flo-1.6-using-bd-facs-lyse-wash-assistant](https://starnet.childrenshc.org/References/labsop/flow/flow/flo-1.6-using-bd-facs-lyse-wash-assistant.pdf) |
|  | 5 | Log in to FACSDiva software. If daily CS&T beads have not been run, do so at this time.  | [flo-2.9-analyzing-the-performance-setup-on-bd-facscanto-ii](https://starnet.childrenshc.org/References/labsop/flow/flow/flo-2.9-analyzing-the-performance-setup-on-bd-facscanto-ii.pdf) |
|  | 6 | In the Browser, highlight your user name. From the menu bar, select Experiment → New Experiment→ Comprehensive ISP.  |  |
|  | 7 | Right click on the experiment name and select Rename. Use the following format: LAST\_FIRST\_ALP\_ACCN\_MRN\_DOB. The experiment will be archived under this name.  |  |
|  | 8 | Right click “Cytometer Settings” and select “Link Setup.” Choose the current 4-color compensation settings to apply to the experiment. A lock icon will appear.  |  |
|  | 9 | To run tubes manually, click on the pointer next to the first tube, and install the tube on the SIT. Click “Acquire” on the Acquisition Dashboard and make any parameter adjustments if needed. Click “Record” when ready to record patient data. Follow on screen prompt to remove the tube and click “Next Tube” on the Acquisition Dashboard to move on.Alternatively, tubes may be run using the loader. Multiple patients may be run on one carousel if programmed as different specimens under one experiment.  | Using BD FACS Loader |
|  | 10 | When the sample run is complete, gates can be adjusted. Double-click the CD45 vs SSC plot to increase the plot size. Adjust the gate around the lymphocytes as necessary. Quadrants may also be adjusted if needed.  |  |
|  | 11 | Print scatters and fill out the relative percentages of each type of lymphocyte on the ALPS worksheet. When the total WBC count and relative percentages are entered into Sunquest, absolute counts are automatically calculated. Copy the absolutes to the ALPS worksheet. | [Attachment D - ALPS Panel Worksheet](http://khan.childrensmn.org/Manuals/Lab/SOP/Flow/Res/204740.pdf) |
|  | 12 | Highlight the experiment and click File > Export > Experiments on the menu bar. Browse to the appropriate G:\Flow Lab folder (organized by experiment type and year). Check the box to delete experiments after export. Once experiments have been exported to the network drive there is no need to keep them in the browser; excessive data in the browser places strain on the program.  |  |
|  | 13 | After results are entered in Sunquest, reports and CBC printouts are scanned to G: LAB/Hematology/Heme Section Procedures/Scanned Flow Results. Save files as LAST NAME\_FIRST NAME\_MRN\_ACCESSION |  |
| **Sample Analysis** | Throughout the analysis of a particular specimen, the logical relationships between subsets must be maintained. The lymphosum, which is the sum of CD3+, CD19+ and NK (CD16+56+) cells, acts as an internal control and should equal 95 - 105%. Also, the CD4 and CD8 values should add up to the CD3 value ± 10%. The CD3+ cells also serve as a tube to tube variability control. If the CD3 value varies by >5% of any of the others, that tube should be repeated. |
| **Procedure Notes** | If the CD45 vs SSC plot appears to be off-scale or display appears unusual, check that the compensation link is in place (locked icon is displayed). Re-linking compensation may correct this problem.Specimens that have >30.0K/μL WBC count may need to be diluted with PBS if there is a large percentage of lymphocytes and therefore a high absolute lymph count.An appropriate isotypic control should be run to measure auto-fluorescence or non-specific staining, and for guidance in distinguishing fluorescence-negative and fluorescence-positive populations. The cursors should be set on the isotypic control so the <2% of the cells are positive. |
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| **Interpretation/ Reference Ranges** | [Document G - Pediatric Peripheral Blood Reference Ranges](http://khan.childrensmn.org/Manuals/Lab/SOP/Flow/Res/204753.pdf) **Reference Ranges**: TCRab+/CD4-/CD8-% = 0-5%TCRab+/CD4-/CD8-# = 0-335CD5+/CD20+% = 0-2%CD5+/CD20+# = 0-146CD3+/HLA-DR+% = 0-8%CD3+/HLA-DR+# = 0-597 |
| **Result Reporting****(Sunquest)** | Function: MEMWorksheet: CISPEnter accessionSOUR (source): PEBLIWBC: total WBC (x 103/µL)ILYM: Relative percentage of lymphocytes, counted by hematologyALC: Absolute lymphocyte count, calculated by Sunquest

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| CD3: % total CD3+  | 3A: absolute calculated by Sunquest |
| CD4: % CD3+/CD4+ | 4A: absolute calculated by Sunquest |
| CD8: % CD3+/CD8+ | 8A: absolute calculated by Sunquest |
| CD19: % CD19+ | 19A: absolute calculated by Sunquest |
| CD16: % CD16+56+ | 16A: absolute calculated by Sunquest |
| DRP: % HLA-DR+/CD3+ | DRPA: absolute calculated by Sunquest |
| DRM: % HLA-DR+/CD3- | DRMA: absolute calculated by Sunquest |
| TCRAB: % TCRαβ+/CD3+ | ABA: absolute calculated by Sunquest |
| TAB48: % TCRαβ+/CD4-/CD8- | AB48A: absolute calculated by Sunquest |
| TCRGD: % TCRγδ+/CD3+ | GDA: absolute calculated by Sunquest |
| CD205: % CD20+/CD5+ | 205A: absolute calculated by Sunquest |
| HSR: CD4:CD8 ratio, calculated by Sunquest |

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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 |  |  | Initial Version |
| 2 | Janet Schlieman | 01/22/2005 | Updated initial version |
| 3 | Jim Berger | 10/26/2009 |  |
|  | 4 | Jim Berger | 03/31/2011 | Canto II Application |  |  |
| 5 | Al Quigley | 03/31/13 | Reformatted for CMS Web |
|  | 6 | Amanda McCaustland | 08/28/20 | Updated instructions and formatting, removed startup/CS&T instructions (separate procedure), added result entry |