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| CISP Comprehensive Immune Status Panel | | | | | | |
| **Purpose** | A 4 color direct immunofluorescence staining of human peripheral blood lymphocytes to assess the number and functional capacity of lymphocyte subsets, reflecting the overall status of the immune competence of an individual. | | | | | |
| **Policy Statements** | This procedure applies to all laboratory technologists performing Flow Cytometry testing, the section  supervisor, and section pathologist. | | | | | |
| **Principle and Clinical Significance** | Lymphocyte subset analysis is used for the diagnosis, prognosis and clinical management of patients with lymphocyte functional disorders. The diagnostic applications are predominantly the detection and monitoring of acquired and congenital immunodeficiency diseases. The Comprehensive Immune Status Panel includes a MultiTEST base panel with additional antigens run in FACSDiva (dual platform analysis). | | | | | |
| **Test Code** | CISP  Test code ITOC may be added to an IMMP accession number to convert to CISP | | | | | |
| Materials | |  |  |  | | --- | --- | --- | | **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 mixer  ● BD FACS CANTO II Flow Cytometer  8. BD FACSCalibur Flow Cytometer 8. BD FACSCalibur Flow Cytometer | | | | | | |
| **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  CD7 / CD5 / CD45 / CD19  TCRαβ / CD3 / CD45 / HLA-DR  TCRγδ / CD3 / 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\_CISP\_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 CISP worksheet. When the total WBC count and relative percentages are entered into Sunquest, absolute counts are automatically calculated. Copy the absolutes to the CISP worksheet. | | | | [Attachment E - CISP Worksheet](https://starnet.childrenshc.org/References/labsop/flow/res/attachment-e-cisp-worksheet.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 | | | |  |
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| **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. | | | | | |
| **Alternate Methods** | An alternative method to using the Lyse-Wash Assistant is lysing and washing manually. Follow the above procedure through step 3, then follow these steps:   1. Incubate at room temperature, protected from light, for 15 minutes. 2. Add 2 mL of 1X FACSLyse solution. Vortex again and incubate for 10 minutes at room temperature. 3. Centrifuge for 3 minutes at 400 X G. Remove supernatant. Vortex gently. 4. Add 2 mL of PBS and centrifuge for 3 minutes at 400 X G. Remove supernatant. Vortex gently. 5. Store at 4° C until ready for analysis.     Occasionally, a patient sample may appear to have non-specific staining which will be especially evident in the isotypic control tube. It will appear as a long "tail" trailing up and to the right of the cluster of unstained cells in the bottom left corner of the dot plot. Repeat the sample preparation, pre-washing the sample with PBS to remove plasma before the monoclonal antibodies are added. | | | | | |
| **Calculations/**  **Result Reporting** | ISPs and CISPs analyzed using the CD45-gating in FACSDiva must have the absolute values manually calculated. The EDTA sample must have a WBC and Differential counted by Hematology in order to calculate the absolute values for each marker. The following equations are used:  Absolute Lymphocytes = WBC x %Lymphocyte  e.g. WBC - 5.0 K/ μL, %L = 35  5000 X 0.35 = 1750 (Absolute Lymph count)  Absolute CD subset = Absolute Lymphs x % CD subset  e.g. CD3 = 70%  1750 x 0.70 = 1225 (Absolute CD3 count)    Record the results of all monoclonal and lymphocyte subpopulations on CISP Flow Cytometry Immunophenotyping Report. [Attachment E - CISP Worksheet](http://khan.childrensmn.org/Manuals/Lab/SOP/Flow/Res/204741.pdf). Sunquest automatically calculates absolute counts in most cases. Any manual calculations must be checked by a second tech, designated by a √ and initialed. Results should also be reviewed for correct entry into the computer.  In Sunquest:  Function: MEM  Worksheet: CISP  Enter accession number  SOUR (source): PEBL  IWBC: total WBC (x 103/µL)  ILYM: Relative percentage of lymphocytes, counted by hematology  ALC: Absolute lymphocyte count, calculated by Sunquest   |  |  | | --- | --- | | CD3: % total CD3+ | 3A: absolute calculated by Sunquest | | DRP: % HLA-DR+/CD3+ | DRPA: absolute calculated by Sunquest | | DRM: % HLA-DR+/CD3- | DRMA: absolute calculated by Sunquest | | CD4: % CD3+/CD4+ | 4A: absolute calculated by Sunquest | | 519M: % CD5+/CD19- | 519MA: absolute calculated by Sunquest | | 519P: % CD5+/CD19+ | 519PA: absolute calculated by Sunquest | | CD7: % CD7+ | 7A: absolute calculated by Sunquest | | CD8: % CD3+/CD8+ | 8A: absolute calculated by Sunquest | | CD19: % CD19+ | 19A: absolute calculated by Sunquest | | CD16: % CD16+56+/CD3- | 16A: absolute calculated by Sunquest | | TCRAB: TCRαβ+/CD3+ | ABA: absolute calculated by Sunquest | | TCRGD: % TCRγδ+/CD3+ | GDA: absolute calculated by Sunquest | | HSR: CD4:CD8 ratio, calculated by Sunquest | | | | | | | |
| **Interpretation/ Reference Range** | [Document G - Pediatric Peripheral Blood Normal Ranges T & B Cells](http://khan.childrensmn.org/Manuals/Lab/SOP/Flow/Res/204753.pdf) | | | | | |
| **References** | 1. BD Bioscience FACSCanto Training Manual. 23-9575-00 Rev. A. 2007, Becton, Dickinson and Company, San José, CA  2. BD FACSLyse Wash Assistant User’s Guide, 23-11113-00 Rev. A, Becton Dickenson AND Company, San José, San José, CA  3. MultiTEST IMK Kit product Insert 23-3602-01. Becton Dickenson, San José, CA  4. BD Multitest IMK Kit product Insert 23-3602-04. Becton Dickenson, San José, CA  5. BD TruCOUNT Tubes produce Insert 23-3483-05, Becton Dickenson, San José, CA  6. CD-Chex PLUS package insert. Streck Laboratories, Inc. July 1996.  7. Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes. NCCLS Document H42-T. May 1992.  8. Clinical Applications of Flow Cytometry of Leukemic Cell, Proposed Guideline. NCCLS Document H43-P. December 1993.  9. 1997 Revised Guidelines for performing CD4+ T-cell Determinations in Persons Infected with Human Immunodeficiency Virus (HIV). CDC MMWR Recommendations and Reports, Vol. 46 No. RR-2. Jan 10, 1997.  10. Flow Cytometry Inspection Checklist. College of American Pathologists Commission on Laboratory Accreditation. 1996.  11. Flow Cytometry Principles for Clinical Laboratory Practice. Owens MA, Loken MR 1995. | | | | | |
| **Historical Record** | **Version** | | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** | |
| 1 | | Immune Cell Phenotyping by Flow Cytometry | 03/04/91 | Updated 05/93 | |
| 2 | | Colleen Berglund | 08/05/97 | Supercedes procedure Immune Status Panel | |
| 3 | | Colleen Berglund | 09/11/99 | Updated Procedure | |
| 4 | | Colleen Berglund | 01/13/2000 | Updated Procedure | |
|  | 5 | | Jim Berger | 10/29/2009 | Updated Procedure | |  |  |
| 6 | | Jim Berger | 03/24/2011 | FAACSCanto II Flow Cytometer Application | |
| 7 | | Jim Berger | 07/06/2011 | Addition of CS&T L-J Data Printouts and review | |
|  | 8 | | Al Quigley | 03/31/13 | Updated for CMS Web | |
|  | 9 | | Amanda McCaustland | 08/28/20 | Removed instructions for CS&T and startup/shutdown (moved to separate procedure document), revised instructions, added result entry, formatting | |