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| Using BD FACS Loader with FACSCanto II Flow Cytometer |
| **Purpose** | This procedure provides instructions for using the BD FACS Loader with the FACSCanto II cytometer. The loader uses a carousel to introduce prepared samples to the cytometer, enabling automated sample runs. The loader is controlled by FACSCanto and FACSDiva software programs.  |
| **Policy Statements** | This procedure applies to all laboratory technologists performing Flow Cytometry testing. |
| **Materials** |

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| **Supplies** | **Equipment** |
| 12 x 75 polystyrene Falcon tubesBD MultiTEST TruCount tubes | BD FACSCanto II flow cytometerBD FACS LoaderBD Sample Prep Ready Carousel Racks |

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| **Sample** | Any flow cytometry specimens that have been fully prepped may be run using the loader. Use only polystyrene Falcon tubes, BD Trucount tubes or BD 7-Color Setup bead tubes.  |
| **Special 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. |
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|  | **Step** | **Action** | Related Document |
| **Procedure (FACSCanto)** | 1 | Enter information for all samples to be run into the worklist. Ensure the correct panel is selected (4 Color TBNK + TruC). | [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 | Load uncapped sample tubes on to the carousel in the same order as they are programmed; the worklist will show the sample's assigned positions. The 3/4/45/8 MultiTEST tube is run first, 3/16+56/45/19 is run second.  |  |
|  | 3 | Check that the tube guide and aspirator arm are positioned for automatic loading; the black tube guide should be in the forward position around the SIT and the aspirator arm bar should be horizontal. | TUBE GUIDE.jpg |
|  | 4 | Place the carousel on the loader drawer spindle. Gently insert the drawer until it clicks into place. Close both loader doors and allow the loader to scan the carousel. The loader recognizes the ID number of the carousel and whether a tube is present in each position, but it is not equipped with a specimen bar code reader. It is critical that tube placement matches the worklist.  |  |
|  | 5 | Click PLAY.jpg to begin the run. If the loaded carousel's ID number does not correspond with what is displayed in the "Carousel" window, a dialog box will pop up. Either swap the carousels or click "Ignore" to update and use the currently loaded carousel. |  |
|  | 6 | The software will automatically collect the needed number of events and move on to the next tube until the worklist run is complete. |  |
|  | 7 | Additional samples may be added to the same worklist and carousel later in the day. Open the worklist (Worklist > Open or ) and enter new sample information. Pick up loading where the previous run left off (rather than starting over in position 1.) Follow the tube positions indicated on the worklist. |  |
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|  | **Step** | Action | **Related Document** |
| **Procedure****(FACSDiva)** | 1 | Set up experiment (LLP, CISP, etc) according to procedure. Delete any pre-programmed tubes in the experiment that will not be run by right-clicking the tube and selecting "Delete." If any other changes to the panel need to be made, such as experiment layout, do this now. |  |
|  | 2 | On the menu bar, select Carousel > Carousel Setup. The carousel setup window will appear. Select the carousel ID number you wish to use and adjust preferences as needed (mixing, recording delay, etc.) Once an experiment has been assigned to a carousel, changes cannot be made. Carousel assignment can be removed by changing the carousel ID to “None,” but only if the run has not been started.  |  |
|  | 3 | Starting in position 1, load tubes in the order they are listed in the experiment.  |  |
|  | 4 | Check that the tube guide and aspirator arm are positioned for automatic loading; the black tube guide should be in the forward position around the SIT and the aspirator arm bar should be horizontal. |  |
|  | 5 | Place the carousel on the loader drawer spindle. Gently insert the drawer until it clicks into place. Close both loader doors and allow the loader to scan the carousel. The loader recognizes the ID number of the carousel and whether a tube is present in each position, but it is not equipped with a specimen bar code reader. It is critical that tube placement matches the experiment. |  |
|  | 6 | On the Acquisition Dashboard, click “Run Carousel.”  |  |
|  | 7 | The software will automatically collect the default number of events and move on to the next tube. A dialog box and Carousel Report summary will pop up at the end of the run. Carousel reports do not need to be printed.  |  |
|  | **To Run Multiple Specimens Under One Experiment:** |
|  | 1 | Experiments can include multiple specimens. This is useful for batching CISPs, or adding additional tubes to a leukemia/lymphoma panel that had been run using the carousel and therefore cannot be modified.  | experiment hierarchy.jpg |
|  | 2 | If batching CISPs, create the experiment per procedure. Patient information is entered at the specimen name level rather than experiment name. Use the test code and date as the experiment name.  |
|  | 3 | To add additional specimens to an experiment, click Experiment > New Specimen on the menu bar. A dialog box will appear asking if you would like to create another global worksheet. This is not necessary if adding additional tubes to a single patient experiment, but each patient specimen in a batch should have its own worksheet. (Copying a specimen by using the “Duplicate Without Data” function does not create a new worksheet.)  |  |
|  | 4 | Edit tubes to be run if necessary.  |  |
|  | 5 | Go to Carousel Setup. All specimens of your experiment will be listed with their assigned places on the carousel. If adding tubes to a previously run experiment, a bold black line will be present between specimens indicating that the new specimen will be run on a different carousel. Carousel breaks can be manually added if necessary.  |  |
|  | 6 | Assign the carousel and run as usual. If adding tubes to a previously run experiment, a box will pop up asking which specimens to run. Select the checkboxes to run only the additional tubes. |  |
| **Procedure Notes** | To prevent sticking, tubes should have no more than two labels. Flatten labels completely before loading on the carousel. |
| **References** | BD FACSCanto II Instructions for Use [BDFACSCantoII\_Users\_Guide.pdf](BD_FACS_Canto_II_Users_Guide.pdf) |
| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | Amanda McCaustland | 08/28/20 | Initial Version |
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