Standard Operating Procedure

(Laboratory Practices)

Infectious Agent and Strain: Unfixed Human Primary Cells

Lab/Location(s): Mayer 583, Mayer 522a

Principle Investigator: John Daley

Protocol Name & Number: Analysis Unfixed Human Primary Cells

Current Revision Date 07/23/2015

Unfixed Human Primary Cells usage practice:

- 1. Unfixed Human Primary Cells will be analyzed on the designated instruments:
 - a. BD LSRFortessa X-20 analyzer
 - b. BD FACSCanto II HTS (mayer 583)
 - c. BD FACSCanto II HTS (mayer584)

Researchers must run their **Unfixed Human Primary Cells** samples in 96 or 384-well micro titer plates on the HTS (High Throughput Sampler) attached to the above stated analyzers **ONLY**. You may not run Unfixed Human Primary Cells on the CantoII Carousel. Usage of the HTS system allows for fully automated sample acquisition.

2. Researcher must use universal safety precautions; wear disposable lab coats and gloves.

96 Well Plate Procedure

Analyzer (HemNeo Facility HTS instruments) Disinfectant protocol for 96well micro titer plate is as follows:

a) Last row on EACH 96 well plate must be treated as a disinfectant row
 (no exceptions). This will sanitize the HTS tubing as well as the sample tubing on the analyzer.

Plate Loader Settings for Row: H Wells: 1-12

Sample Flow Rate: 3.0
Sample Volume: 175ul
Mixing Volume: 100
Mixing Speed: 200
Number of Mixes: 0
Wash Volume: 200ul

- •Wells 1-6 will contain 200ul (10x) Bleach
- •Wells 7-9 will contain 200ul BD FACSRinse
- •Wells 10-12 200ul ddH₂0

384 Well Plate Procedure

Analyzer (HemNeo Facility HTS instruments) Disinfectant protocol for 384well micro titer plate is as follows.

a) The last row on EACH 384 well plate must be treated as a disinfectant row

(no exceptions). This will sanitize the HTS tubing as well as the sample tubing on the analyzer.

Plate Loader Settings for Row: P Wells: 1-24

Sample Flow Rate: 3.0
Sample Volume: 90ul
Mixing Volume: 45
Mixing Speed: 200
Number of Mixes: 0
Wash Volume: 200ul

- •Wells 1-12 will contain 100ul (10x) Bleach
- •Wells 13-17 will contain 200ul BD FACSRinse
- •Wells 18-24 200ul ddH₂0

Post-analyses procedure:

After each analysis the researcher is responsible for disinfecting the areas used for **Unfixed Human Primary Cells** analysis work.

Please spray the following with 10% Bleach and use the Super Sani-cloth wipes to clean accordingly

- Analyzer
- Analyzer Sample Station
- •All affected areas
- The researcher is responsible for removal and disposal of their gloves; disposable lab coat and all wipes used to disinfect the analyzer and affected areas.
- Contact the Flow Cytometry Facility (617-632-3179) or Suzan Lazo (617-632-4571) for more information.