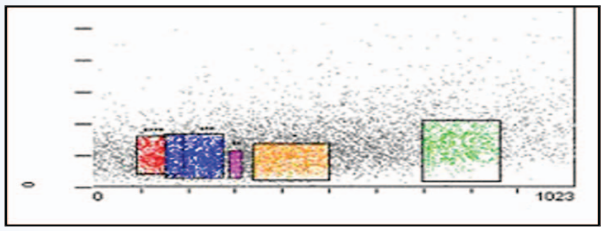
NCTR: Rapid Bacterial Isolation System - A Resource For Counter Bioterrorism Research

The NCTR Biosafety Level 3 (BSL-3) Laboratory is a national resource for rapid isolation and automated manipulation of large sample samples of bacteria.[1] It houses a suite of instruments that perform strain-level identification of nontarget agents. Typically, this identification requires isolation and propagation) prior to confirmation of the agent's identity. Using the instruments in the BSL-3 Laboratory, NCTR anticipates reducing, by almost a week, the time required to isolate and propagate foodborne bacterial agents.

Isolating Bacteria

The NCTR rapid isolation system instruments include an EPICS® Altra™ flow cytometer, an Allegra® centrifuge, and a BioMek® liquid handling robot, all from Beckman Coulter. The EPICS® Altra™ cytometer has a low power laser and a forward scattering detector, which responds to light scattered by typical small diameter (1 to 2 µM) bacterial cells. It also has several side-scatter detectors. Liquid samples are passed through the cytometer in a narrow stream at a high speed. Whenever the laser light reflects off of a particle in the stream, the forward and side scatter reflections indicate the particle's size, number, granularity, and shape. These reflections form a flow cytometer plot.



Flow Cytometer

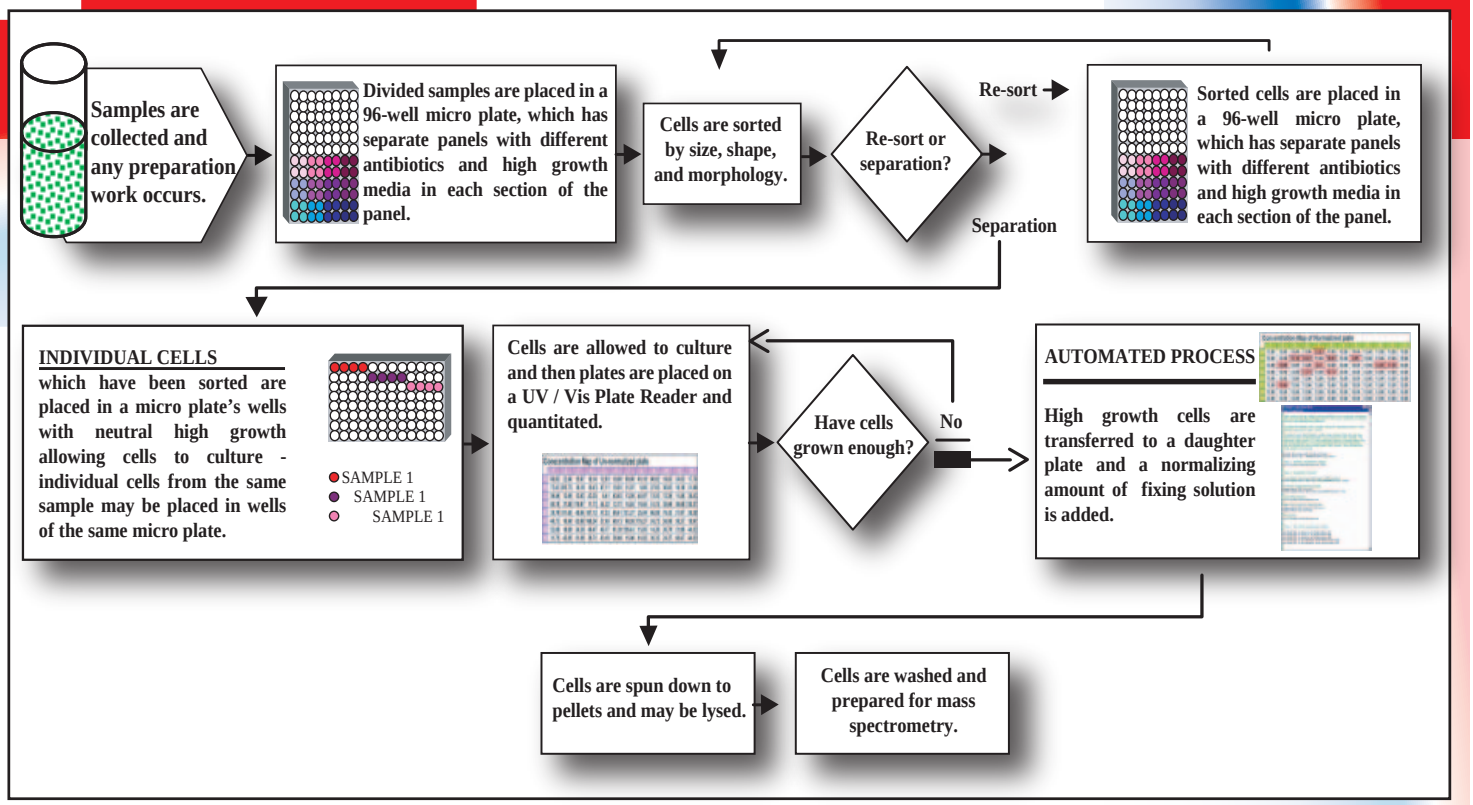
Forward Scatter vs. Side Scatter plot for a mixture of five different rod-shaped bacteria. Each dot represents a light reflection seen simultaneously by the forward and side scatter detectors.

The colored regions of the flow cytometer plot show how cell types can be preselected to eliminate irrelevant background microflora. Particles in food, which are larger than any of the microflora, reflect in the upper region of the plot. The rods in the green box are longer than the others, so when one passes through at a right angle to the laser, it gives a more intense side scatter signal and is plotted further to the right.

The EPICS® Altra™ cytometer distinguishes individual bacterial cells from groups of two or more cells of the same size and shape. When the cytometer detects a cell of interest, it "captures" that cell for growth and sorts the single cell into a designated well in a 96-well microtiter plate. The next suitable cell is directed into the adjacent well, etc. The entire 96-well plate can be populated, one cell to a well, in less than 60 seconds.

The analyst sets up the flow cytometer for a sorting operation using touch-screen options.

Loading a 96-well microtiter plate for cell sorting with the EPICS® Altra flow cell cytometer.

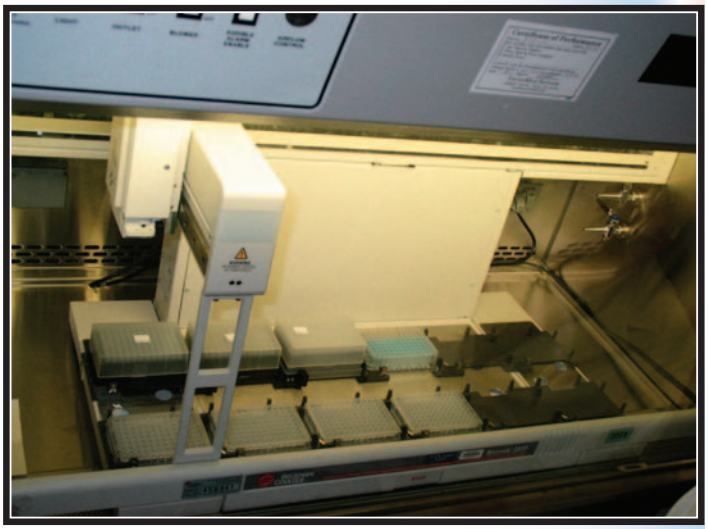


Flow diagram illustrating the rapid isolation process.

Propagating Bacteria

Each well of the 96-well plate contains a nonselective liquid culture medium. After a few hours of incubation, any wells containing bacteria represent isolates (or pure strains) grown under standard conditions. The isolates are initially characterized by placing the entire plate into an Allegra® centrifuge to spin down the cells. Using a BioMek® liquid handling robot, the culture medium is decanted and the cells are diluted with a water disinfecting solution. Cell suspensions are transported to other instruments in a covered 96-well plate for definitive characterization.

The NCTR rapid isolation system allows NCTR to conduct a complicated set of experiments that can properly identify a variety of bioterror, hoax, clinical, or other public health situations.



The Biomek® robot is installed inside a Biosafety Level II enclosure to keep microtiter plate wells free from lab contaminants and to protect analysts from cultured pathogens.

[1]J. G. Wilkes, G. Miertschin, T. Eschler, L. Rushing, D. A. Buzatu, and M. J. Bertrand, "Reproducibility and Spectral Library Assembly for Rapid Bacterial Characterization by Pyrolysis Mass Spectrometry" invited Book Chapter in *Identification of Microorganisms by Mass Spectrometry*, Charles L. Wilkins and Jackson O. Lay, Jr. (Editors), accepted Wiley-Interscience, February 7, 2005.

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