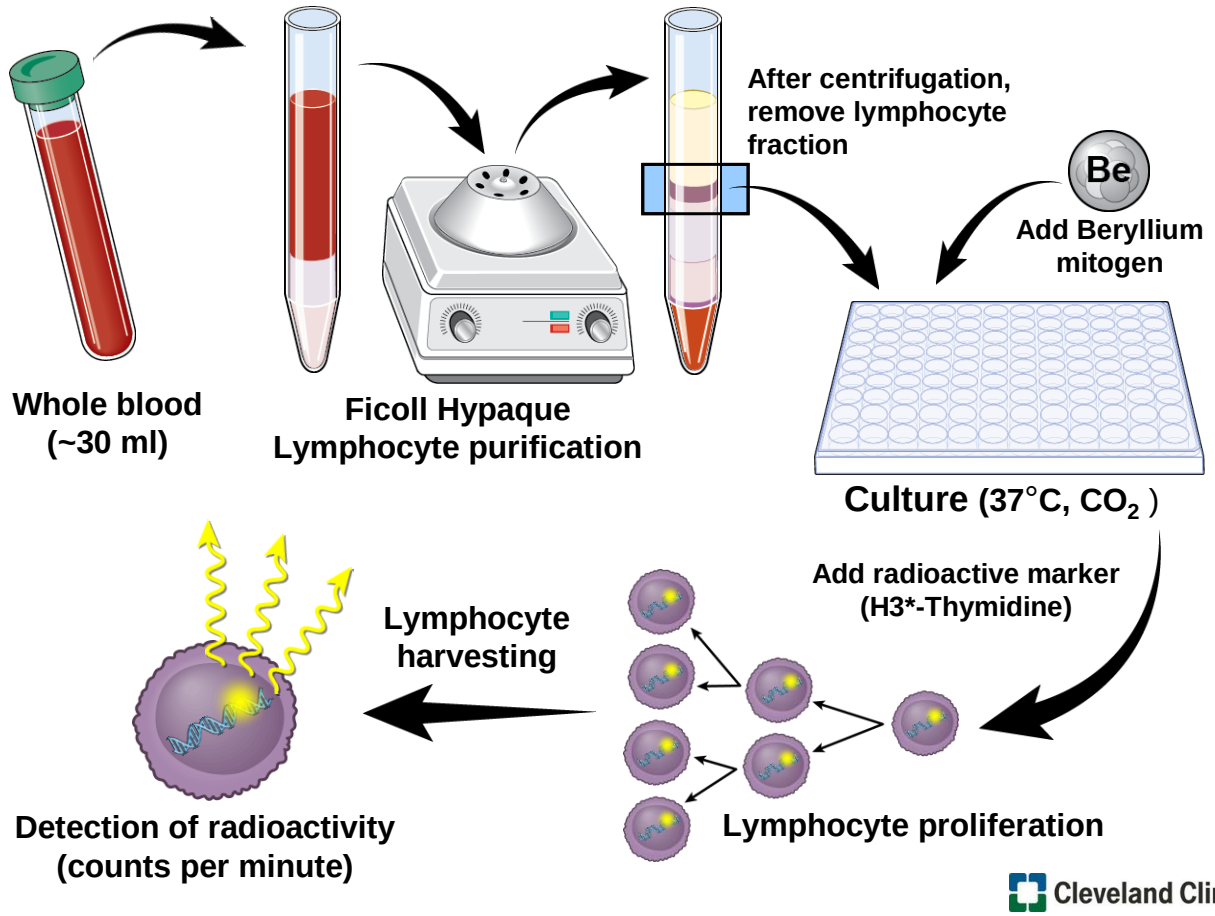
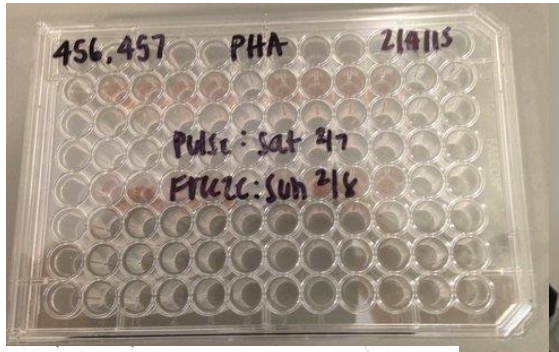
Commonly used
Laboratory Tests used to Evaluate
and Monitor the Immune Status of
Individuals.

Cellular Immune Function Assays in the Clinical Laboratory

- Leukocyte transformation test-Mitogen screen (LTT-MS)
- The traditional lymphocyte proliferation test (LPT)
- Quantiferon TB gold test
- ImmuKnow Assay
- NBT (Neutrophil functional test)
- Flow cytometry Assays

Lymphocyte Proliferation Test and LTT-MS



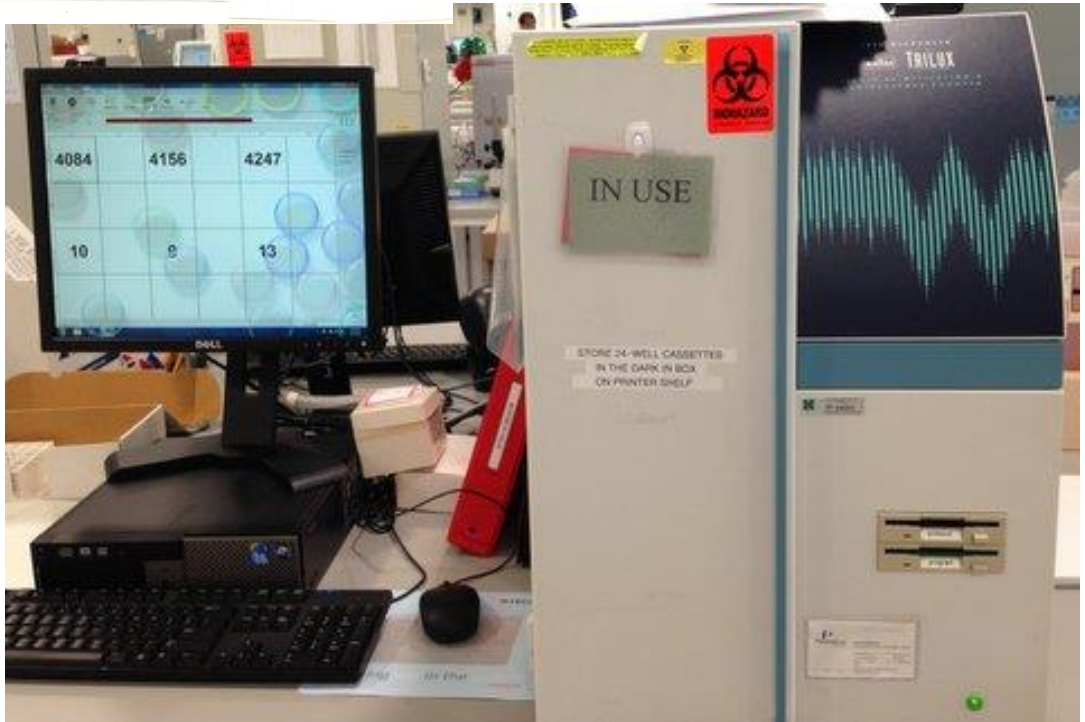


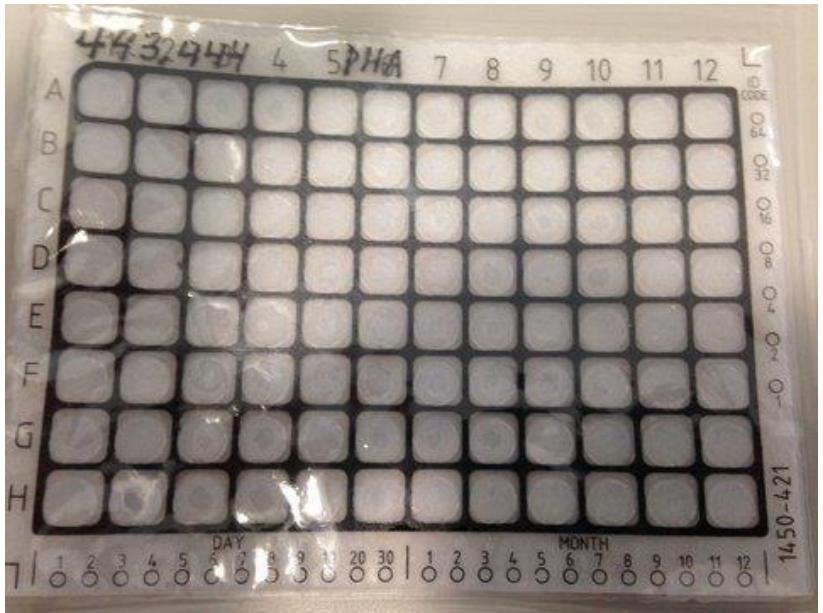
LTT with Mitogens in Peripheral Blood

RESULTS

The expected values for this test are:

Mitogen	Mean CPM	Lower Limit of Normal
PHA	320,627	≥ 188,799
PWM	131,826	≥ 68,549
CON A	169,825	≥ 81,283
SAC	11,940	≥ 3,412





LTT-MS

MITOGENS

- PHA (Phytohemagglutinin) T cells
- PWM (Pokeweed Mitogen) T and B cells
- Con-A (Concanavalin A) T cells
- SAC (S. aureus, Cowen Strain) B cells

QuantiFeron-TB Gold Plus

- All HCP had IGRA test as part of the pre-employment screening (since July, 2007)

- Cell Mediated Immunity

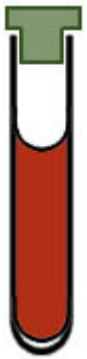
- 1 ml blood collected into each of the four tubes in the following sequence:
  - Nil/control (*grey*)
  - TB 1 antigen (*green*)
  - TB 2 antigen (*yellow*)
  - Mitogen ***(purple)***

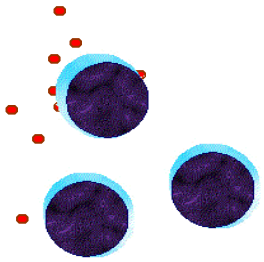
Interferon Gamma Release Assay (IGRA)

- **QuantiFERON TB Gold in-tube Test :**
  - **Nil control** - <8.0 IU/ml.
  - **TB antigens -ESAT-6 & CFP-10** (>0.35 IU/mL)
  - **PHA-Phytohemagglutinin (Mitogen)** (> 0.50 IU/mL)

A test is considered positive for an IFN-y response to the TB Antigen tube that is significantly above the Nil IFN-y IU/mL value (>25%).

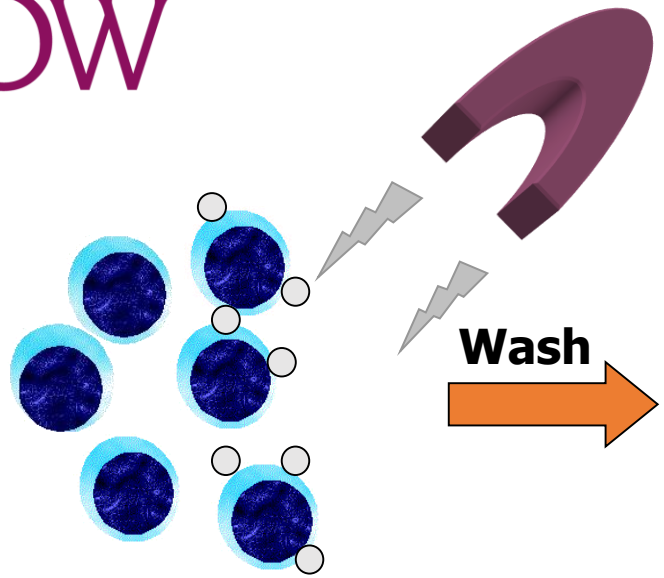
ImmuKnow™





Lymphocyte Stimulation w/PHA

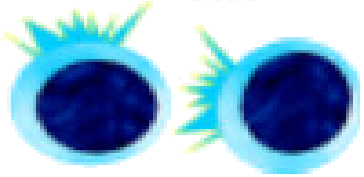
Incubate 15-18 hrs.



Magnetic separation of CD4 cells.

Wash

ATP ATP ATP



Cell lysis to release ATP

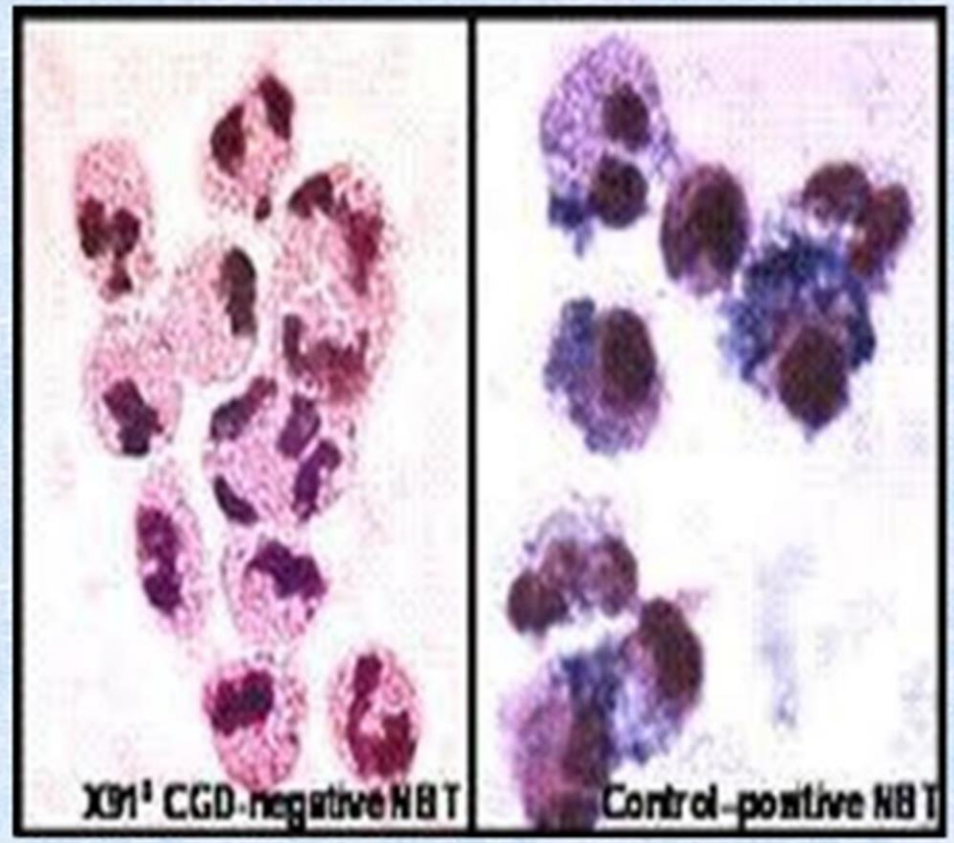
ATP detection reagents

Measure light intensity

J. Britz et. al.,"In Vitro CMI: Rapid Assay for Measuring Cell-Mediated Immunity", CRC Press, 2002

Chronic Granulomatous Disease (CGD)

- **NITROBLUE TETRAZOLIUM (NBT) DYE TEST**
- Leukocytes in a test tube are incubated with the NBT dye, which turns blue if superoxide FRs are present, indicating that the respiratory (oxidative) burst is intact
- The NBT dye test is **negative in the X-linked type of CGD** (NBT dye is not converted to a blue dye), because the NADPH oxidase enzyme complex is dysfunctional
- NBT test is being replaced by more accurate flow cytometry using Dihydrorhodamine 123 fluorescence (DHR test)



Flow Cytometry – T cells Subsets

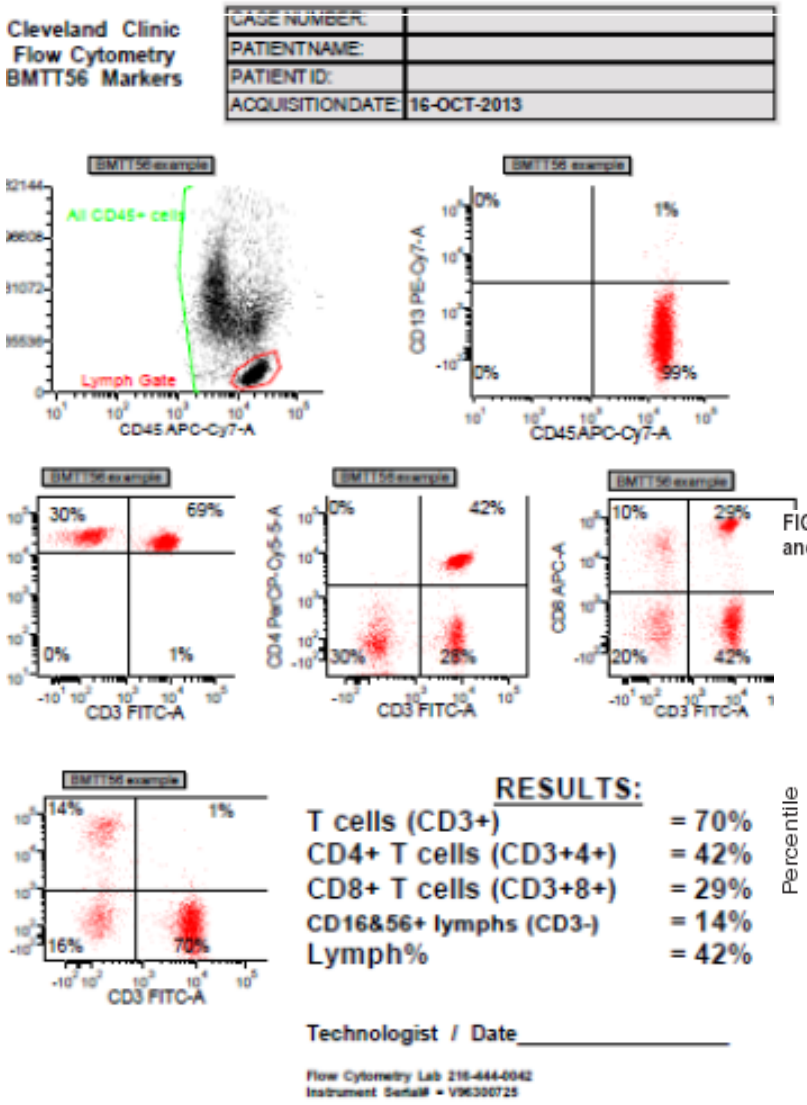
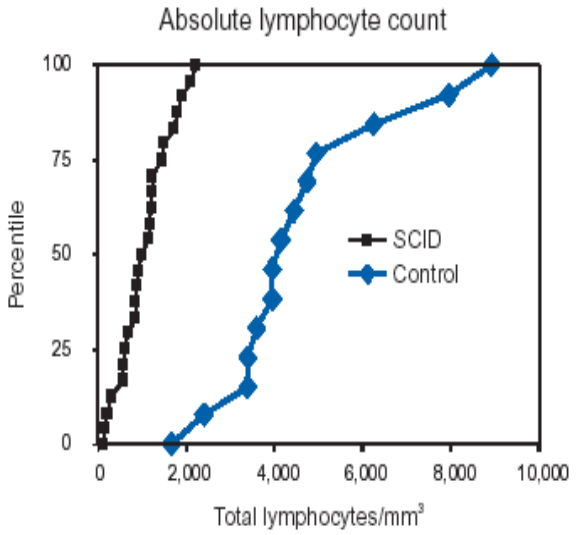
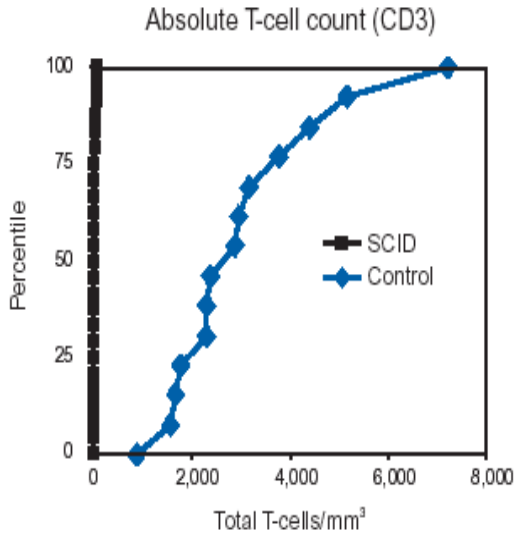


FIGURE 3. Absolute lymphocyte count distributions in severe combined immunodeficiency (SCID) — 25 newborns with SCID and 14 healthy newborns at birth evaluated at Duke University*





Immune Cell Function Tests

- Measure the Net State of Immune Function
- Identify Patients at Greater Risk
- Manage Treatment Strategies