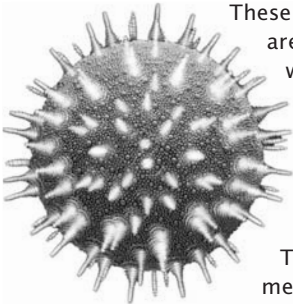


testing for immune responses to hiv: the science in pictures

PATRICIA KAHN and MICHAEL HOELSCHER

Of the roughly two dozen vaccine trials now going on, all but two of them are early-stage studies looking at the safety of candidates and at their ability to induce *immune responses*.



These studies help vaccine developers identify which vaccines are the most promising and which are not worth pursuing, which parts of HIV stimulate the best immune responses, and whether *booster* doses are needed (and at what time intervals). They also yield important information on how to improve vaccine designs. Even trials of candidates that show poor responses contribute to our knowledge of what should—and shouldn't—be included in a vaccine.

These insights are based on the results of laboratory tests that measure immune responses in the blood samples drawn from volunteers at each clinic visit. On the following pages we show what happens to a blood sample after it leaves a volunteer's arm and is used in one important test, which measures *cellular immune responses*. (Not shown here: part of each blood sample is also sent for clinical analysis to monitor the health of the volunteers.)

This sequence was photographed at the site of a vaccine preparedness study by the Mbeya Medical Research Program (MMRP) in Mbeya, Tanzania and at the Uganda Virus Research Institute (UVRI) in Entebbe, Uganda.



©Michael Hoelscher/MMRP

A nurse collects blood from a trial volunteer.

LAB SETUP

In the meantime, technicians in the lab prepare everything they will need to process blood samples from all the volunteers who visit the clinic that day.

The setup shown is for blood samples from 30 people.



©Michael Hoelscher/MMRP



©UVRI/IAVI

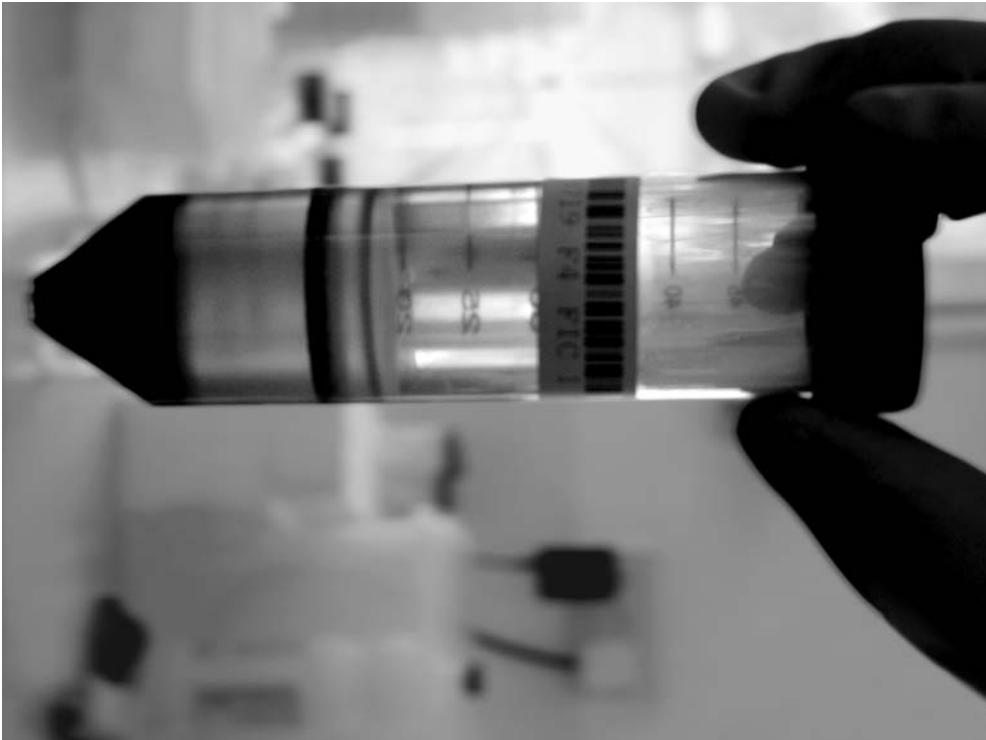
SEPARATING CELLS FROM PLASMA

When test tubes with the samples arrive in the lab, the first step is to separate the blood cells from the liquid part of the blood, called plasma. The cells will be tested for their ability to recognize HIV, while the plasma is used for HIV tests and measuring *antibody* responses, or for *viral load* measurement in the case of an infected person.

The separation is done by spinning the cells at high speed in a machine called a centrifuge. This causes the cells to form a pellet at the bottom of the test tube, with the liquid on top.

ISOLATING LYMPHOCYTES

The next step is to separate *lymphocytes* (the cells relevant for *immunity*) from the rest of the blood cells. This is done by putting the cells from the pellet on top of a very thick liquid (called *ficoll*) in a test tube and spinning them again. The lymphocytes stay on top of the liquid, while other cells move through it and settle at the bottom of the tube.



©Christoph Geldmacher/MMRP



©Michael Hoelscher/MMRP

PREPARING LYMPHOCYTES FOR STUDY

Lymphocytes are “washed” by suspending them in a washing solution and spinning them down again, pouring off the liquid and repeating this cycle a few times. Then they are processed for freezing and placed at -196°C in liquid nitrogen. They can be thawed at a later time for immune analysis, or alternatively, immune tests can be done right away on a portion of the cells.



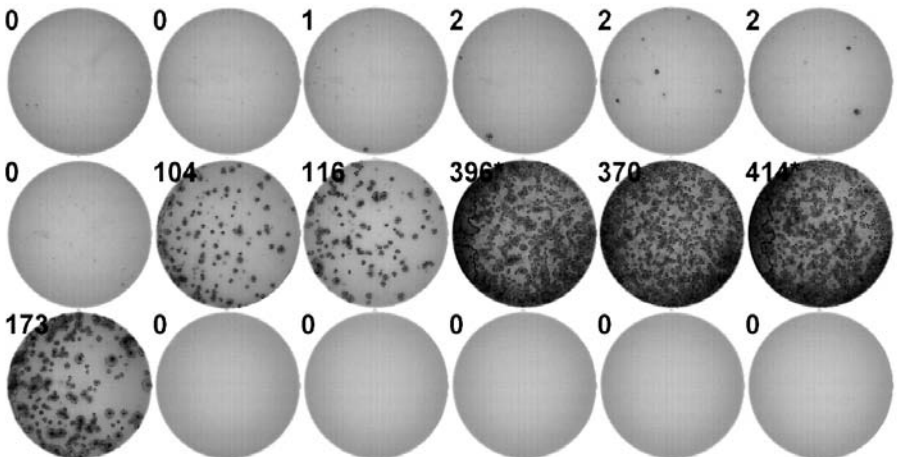
©Michael Hoelscher/MMRP

TESTING FOR T-CELL RESPONSES

To analyze *T-cell* responses using a test called ELISPOT, a technician then counts cells under the microscope and takes a pre-determined number for the immune measurements.

COUNTING "SPOTS"

A fixed number of cells is added to each well in a cell culture plate containing fragments from one or more HIV *proteins*. Cells that recognize the HIV fragment begin producing chemicals called *cytokines*. Then a second ingredient is added, which stains cytokine-producing cells blue. The number of blue spots in each well indicates how many cells recognize the fragment—in other words, how strong the immune response is.



©Christoph Geldmacher/MMRP