CENTER FOR AIDS RESEARCH
University of California San Diego

Translational Virology Core Protocols

Rapid HIV Testing

The OraQuick- Rapid HIV-1 Antibody Test is manually performed, visually read, 20-minute immunoassay for the qualitative detection of antibodies to HIV-1 in human whole blood obtained from finger puncture. The OraQuick-Rapid Test is comprised of a single-use test device and vial containing a pre-measured amount of a buffered developer solution (stored at 2-27°C). The device plastic housing holds an assay test strip composed of several materials that provide the matrix for the immunochromatography of the specimen and platform for indication of the test results. The assay test strip, which can be viewed through the test device result window, contains synthetic peptides representing the HIV envelope region and the goat anti-human IgG procedural control immobilized onto a nitrocellulose membrane in the Test (T) zone and Control (C) zone, respectively. A whole blood specimen is collected and transferred into the vial of developer solution, followed by the insertion of the test device. The developer solution facilitates the flow of the specimen into the device and onto the strip. As the diluted specimen flows through the device, it rehydrates the protein. As the specimen continues to migrate up the strip, it encounters the T zone. If the specimen contains antibodies that react with the antigens immobilized on the nitrocellulose membrane, a reddish-purple line will appear, qualitatively indicating the presence of antibodies to HIV –1 in the specimen.

For Rapid Test quality control we will have an internal control: A reddish-purple line in the Control (C) area of the Result Window indicates that specimen was added and that the fluid migrated appropriately through the Test Device and an external control (a known reactive and non-reactive specimens controls): Positive control will produce a Reactive test result. Negative control will produce a Non-Reactive test result.

For training purposes, each new operator, prior to performing testing on patient specimens will complete the State Office of AIDS HIV Single Session 2-day training, which includes completing a set of proficiencies: (1) On each new shipment of test kits to be performed by QA assignee; (2) On each new open test kit lot to be performed by QA assignee; (3) If the temperature of the storage area and testing area falls outside acceptable range; (4) When there is a significant environmental change (e.g. the air conditioning shuts down); (5) At periodic intervals—the first testing day of the week (usually this will fall on a Monday) to be completed by the QA assignee at each HIV testing site where the Rapid Test is implemented; (6) Whenever two consecutive invalid tests results are obtained on a client, repeat the HIV test using EIA and ELISA.

Contact
Demetrius dela Cruz, Core Administrator
Tel: 858-642-1620
Fax: 858-552-7445
E-mail: ddelacruz@ucsd.edu
URL: http://cfar.ucsd.edu/core-facilities/translational-virology-core

More

Specimen Collection, Storage, Tracking and Shipping for Nucleic Acid Testing (NAT)

Blood is collected in NAT specific PPT tubes by standard phlebotomy by individuals who are trained and certified phlebotomists. These NAT tubes are labeled by the [phlebotomist in front of the individual tester with a unique barcoded identifier, which matches the number given to the participant, which is to be used for result retrieval. Participant data is entered into the project database, which is linked to the specimen's barcoded identifier. Collected tubes and paperwork for each test are sent to the AVRC lab everyday. Specimens will be processed, aliquoted and appropriately cryo-preserved (<4°C). Storage location and inventory will be linked to the initial barcode label and a laboratory masterbook code, and both will be unique to the participant, the day and time of collection and testing site. Specimen inventory and storage databases are linked and will be used for tracking processes. Additionally, it will be documented in the database when specimens are shipped to the American Red Cross laboratories. Shipping to American Red Cross Laboratories will occur at least two times a week (Wednesday and Friday), which is the present practice; however, this will be expanded as more testing and counseling sites are added. When results are expected for a particular assay, an estimated date of result will be logged for this assay. This will allow for real-time tracking of results, and Dr. Smith will be notified if a result has not been entered by the estimated date. A schematic of the NAT program is detailed in Figure 1.

American Red Cross NAT Procedures

The American Red Cross laboratories in St. Louis, MO perform the following standard operating procedures to decontaminate work spaces and evaluate levels of contaminations during the amplification of nucleic acids (i.e. false positives). Firstly, after each shift of work, all work surfaces and equipment used is decontaminated either with a validated cleaning solution pre-amplification or with bleach per the manufacturer's recommendations. Additionally, the work flow is linear from the area of least to highest potential for contamination, which includes each room being environmentally contained to prevent cross contamination and entry and exit of personnel and the flow of materials from pre- and post amplification areas is controlled. All wastes are decontaminated as well prior to leaving post-amplification areas. We also have procedures to perform swab testing for any amplicon that may exist in pre- and post-amplification areas followed by decontamination procedures if amplicons are detected. Also, standard operating procedure is if there is an unexpectedly high number of NAT+ per given run (greater than 3 or 5 depending on the nature of the run) further investigation of equipment, personnel and reagents is initiated. Data for rates of contamination are tracked by laboratory and reagent lot to ensure that background contamination levels are kept to a minimum. The current rate of reactive pools from standard blood donation by lot ranges from 0.08 to 0.2% and rate of false positive individual tests is approximately 1:40,000.

We use the standard pool of 16 as recommended by the manufacturer (GeneProbe) and validated by our pooling equipment. If the population being tested were to exceed a rate where the number of reactive pools of 16 exceeds the amount of testing required during resolution, then testing individual donations would be more efficient. This equates to greater than 1 of every 16 pools being reactive, i.e., if the background rate of NAT positivity exceeds 1:256. This is obviously not the case when testing routine blood donors, but may be the case when screening for acute HIV infection among very high risk clients. As added value, samples that are NAT positive (and antibody negative) are sent to the National Genetics Institute (Los Angeles, CA) for viral load determinations using their validated SuperQuant procedure.