

## Implementation of NAT for HCV and HIV: Testing, Donor and Product Management

FDA is developing draft algorithms for the implementation of NAT screening of blood and plasma for HCV and HIV, in anticipation of the eventual licensure of these methods... Today we are going to focus on the portions of the algorithms dealing with “test resolution”, which is the process determining which individual donations in a “Reactive” Master Pool are responsible for the reactivity. At a later date we will bring the topic of reentry issues to BPAC.

At the present time, all NAT screening done under INDs is being done on pooled donor samples, because the current NAT methods are so labor intensive. To resolve a reactive pool into reactive and non-reactive individual donations necessarily leads to at least two “layers” of testing. When the tests in all layers of the testing are in agreement (all positive or all negative), product management strategies are clear. However, when the layers disagree with one another, product management is problematic.

We are going to discuss what should be done when the master pool is NAT reactive, EIA-non-reactive, but one or more of the subsequent “layers” (sub-pools or individual donations) are all NAT non-reactive.

Generally we can consider several approaches to resolving discrepancies between the layers of testing.

1. One possibility is to retest the negative layer using a different NAT method for the same virus. However, if the initial test is picking up a true positive sample in the master pool, then it clearly is using the primers and probes capable of detecting the culprit virus. Switching away from any of these primers or probes would bias the results away from the truth.
2. Another way to resolve discrepancies is to test dilute individual donations or sub-pools and retest them using the same NAT method. The rationale behind this is that the individual samples in the more concentrated state (individual donations or subpools) are non-reactive and therefore, must have some contaminant which inhibits NAT at high concentration but not at low concentration (similar to a so-called “prozone” effect). However, all NAT tests have an internal control and if this works, there is no reason to believe any sample has contributed a general NAT inhibitor.
3. Further possibilities include, under certain circumstances, repooling and retesting or simply releasing on the basis of the negative subpool testing.

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One of the first issues to be considered is whether or not replicate testing should be done on reactive pools or subpools to confirm initially reactive results. If so, how many replicates are enough and whether or not the pool can be considered non-reactive if any of these are reactive. We will come to this question at several points during the discussion of the individual algorithms.

The first algorithm goes directly from testing the master pool to testing individual donations. Thus, it may be more applicable to whole blood screening than to screening source plasma, although it could be used for either at the discretion of the blood establishment.

The difficult issue is what to do when all the Individual Donations test non-reactive. (Question 1, for the committee.) Should all the donations be released, or should establishments use additional testing to track down the positive sample(s) that might be in the pool? If additional testing is chosen, should establishments do replicate testing of the Master Pool (if not already done) or should they do alternate NAT on Individual Donations or should they dilute individual donations and retest using the same NAT method?

The second algorithm (which contains a separate, sub-algorithm) uses tests of sub-pools to resolve discrepant results.

The first difficult issue here arises when all sub-pools test non-reactive after the master pool has tested positive. (See “sub-algorithm” and Question 2 for the committee.) Should donations be released or should the discrepancy be resolved by additional testing? If additional testing is chosen, should there be replicate testing of the Master Pool? Should the subpools be diluted and retested? Should the subpools be tested with an alternate NAT. Should Individual Donations be tested with the same NAT?

The objections to alternate NAT and dilution with retesting have already been noted. The real choice thus seems to be Release vs. test individual donations. Establishments that use pool sizes such as 512 and 1200 donations are going to be very reluctant to re-test an entire Master Pool using individual donations.

Another problem arises when one or more of the sub-pools has tested reactive, but all Individual Donations were non-reactive (Question 3 for the committee). We feel that it is essential that re-testing in this situation should be performed on sub-pools obtained from a pooling which is independent from the pooling of the Master Pool. If the sub-pools were archived during the formation of a Master Pool and are not independent of that pooling event, FDA is recommending test results be obtained on re-pooled subpools. If after re-pooling, a pool still tests reactive there arises the question of whether or not more testing should be done on the individual donations. Should Individual Donations be tested with an alternate NAT? Should Individual donations be diluted and retested with the same NAT.