

Communicable Disease Testing — 11th Annual FDA and the Changing Paradigm for HCT/P Regulation

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Slide 2:

Objectives

- Understand:
 - Required infectious disease tests
 - Testing processes
 - Importance of sample quality
- Evaluate testing lab quality/compliance
- Describe impact of assay changes
- Develop awareness of future changes

The objectives for this presentation today include understanding the required infectious disease screening tests, the testing processes and the importance of sample quality to ensure accurate results in the laboratory. Information will be provided to help with evaluation of testing laboratory quality and compliance, to describe the impact of assay changes on the laboratory as well as the collection and processing centers and help to develop awareness of future changes that will impact the testing processes currently in place.

Slide 3:

Required Tests

- **Purpose of Testing**
 - Detect current or past infection
 - Prevent disease transmission
 - Including viral, parasitic, bacterial, etc.
 - Required by FDA:
 - <http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/TissueSafety/ucm095440.htm>
- **Most Common Technology**
 - Antigen/antibody detection
 - Nucleic acid testing (NAT) for early detection

Its important to keep in mind the purpose of testing samples from donors which is to detect current or past infection. Detection of infection helps to prevent transmission of a variety of viral, parasitic and bacterial diseases. Testing is required by FDA and the link to the FDA website requirements for Tissue Safety is provided on this slide. The most common technologies used for currently required tests is antigen or antibody detection which indicates present or past infection and nucleic acid testing which allows for earlier detection of disease agents that may be present in a donor's blood sample.

Slide 4:

Antigen/antibody detection

- **Enzyme immunoassay (EIA)**
 - Antigen/Antibody complex formation
 - **Enzyme-conjugated antibody** binds to Ag/Ab complexes
 - Substrate activates enzyme bound to Ag/Ab complexes
 - **Colorimetric detection** if Ab/Ab complexes are present
- **Chemiluminescent immunoassay (ChLIA)**
 - Antigen/Antibody complex formation
 - Acridinium-conjugated antibody binds to Ag/Ab complexes
 - Substrate activates acridinium bound to Ag/Ab complexes
 - **Chemiluminescent detection** if Ab/Ab complexes are present

The classic infectious diseases tests are primarily based on an enzyme immunoassay or EIA format which involves detection of basic antigen/antibody complexes. An enzyme-conjugated antibody reagent is added which binds to ag/ab complexes and then a substrate reagent is added to activate the bound enzyme. Colorimetric detection using a spectrophotometer is performed to determine if the specific ag/ab complexes are present.

Many high volume screening laboratories are now using chemiluminescent immunoassay or ChLIA as an ag/ab detection method. The ChLIA includes the use of an acridinium-conjugated antibody which binds to specific ag/ab complexes. The addition of a substrate reagent activates the acridinium and allows for chemiluminescent detection if specific ag/ab complexes are present. The ChLIA technology is available on a large automated instrument which allows scanning of barcoded sample IDs and high throughput for multiple tests at one time.

Slide 5:

Nucleic acid testing (NAT) - early detection

- **Polymerase chain reaction (PCR)**
 - Isolation/purification of viral nucleic acid from sample
 - Reverse transcription/selective amplification (polymerase/primers)
 - Multiple intermittent cycles of high heat disrupt 'amplicons'
 - Specific fluorescent-labeled probes hybridize to PCR products
 - Real-time detection of PCR products measured by fluorescence
- **Transcription-mediated amplification (TMA)**
 - Isolation of viral RNA/DNA from sample using hybridization and target capture onto magnetic microparticles
 - Transcription-based amplification of target viral nucleic acid using reverse transcriptase and polymerase (multiple copies)
 - Detection using specific chemiluminescent-labeled nucleic probes
 - Chemiluminescence measured in Relative Light Units (RLU)

The nucleic acid testing is intended to identify infection with a variety of viruses earlier than an immune response can be detected

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with ag/ab assays. Viral nucleic acid is isolated from the sample of an infected donor and can be amplified using the classic polymerase chain reaction (or PCR) technology which involves reverse transcription of isolated viral nucleic acid followed by selective amplification using polymerase and specific primers. Then multiple intermittent cycles of high heat disrupt the PCR products or 'amplicons' and a specific fluorescent-labeled probe is hybridized to the amplicons. Real-time detection of the PCR products can then be measured by fluorescence. Alternatively, viral RNA and/or DNA from a sample can be hybridized and captured onto magnetic microparticles. Then transcription-based amplification of specific target viral nucleic acid can be performed using reverse transcriptase and polymerase along with specific chemiluminescent nucleic acid probes to make multiple copies of the target nucleic acid. The chemiluminescence can then be measured in relative light units or RLUs.

Slide 6:

Required Tests, continued

- Must test for all relevant communicable diseases
- Licensed, approved or cleared donor screening tests
 - Specifically labeled for cadaveric specimens when applicable and when available
- Blood donor screening
 - HBV, HCV, HIV, HTLV, Syphilis, *T. cruzi*, WNV
 - NAT pooling allowed
- HCT/P screening
 - HTLV-I/II only for viable, leukocyte rich cells/tissues only
 - WNV NAT and *T. cruzi* antibody – draft guidance only

FDA requirements for testing include all relevant communicable diseases which are published on the FDA website. FDA licensed, approved or cleared donor screening tests must be used for routine screening and test kits specifically labeled for use with cadaveric samples must be used for testing post-mortem samples when available. This slide shows the differences between routine blood donor screening and HCT/P screening. All testing requirements are the same except that NAT pooling is allowed for blood donor samples but not for HCT/P samples. Also, HTLV-I/II is required for all blood donors but is only required for viable leukocyte rich cells/tissue and there is only draft FDA guidance available for WNV NAT and *T. cruzi* antibody screening of HCT/P collections.

Slide 7:

Required Tests, continued

- Confirmatory/Supplemental Tests
 - Useful for healthy donor population
 - infection prevalence low so confirmed positive rate low
 - Used by blood banks for donor re-qualification
 - May be helpful for counseling of family members
 - HBsAg = Antigen Neutralization*
 - Anti-HIV-1/2 = HIV-1 Western blot* or HIV-1 IFA*
Anti-HIV-2 EIA, HIV-2 Western blot
 - Anti-HTLV-I/II = Western blot* or Line Immunoassay
 - Anti-*T. cruzi* = Chagas ESA*
 - Syphilis (Anti-*T. pallidum*) = FTA or other
approved diagnostic confirmatory test
 - HIV/HCV HBV NAT = Discriminatory NAT*
 - WNV NAT = WNV IgG/IgM

*FDA-approved confirmatory test(s) available

Most blood donor screening laboratories routinely provide confirmatory and supplemental testing for donors found reactive in one or more of the infectious disease screening tests. These are useful when testing a healthy donor population because the infection prevalence is very low so the confirmed positive rate is also very low. These results can be used for providing counseling to the donor to help them understand whether their test results indicate they are truly infected or possibly just due to a false positive screening test. Some of the confirmatory tests can also be used for donor re-qualification if they are not reactive on a subsequent screening test and they are not confirmed positive for one of the infectious disease agents we currently screen for. In an eye, tissue or organ donation facility these test results might be helpful for counseling family members when abnormal screening results are communicated. The list of tests provided on this slide indicate the most common confirmatory or supplemental test for each of the primary infectious disease agents currently considered relevant communicable diseases. Some of these tests are FDA-approved for confirmation of antigen, antibody or nucleic acid and some of them can be used to determine if a donor is eligible for re-instatement following an unconfirmed reactive screening test.

Slide 8:

Testing process

- Sample Acceptability
- Accessioning
- Sample and reagent preparation
- Testing
- Review of testing documentation
- Result release

Now that we have discussed the testing requirements we will focus on the testing process which includes determination of sample




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acceptability, accessioning of each sample and identification of the testing required for that sample. Determination of sample acceptability is one of the most critical steps in the accessioning process in any laboratory. Once a sample has been determined acceptable for testing, it is routed for preparation and reagents are also prepared. The testing process involves pipetting of controls and samples, followed by a series of incubations and washing steps which can take up to about 4 to 5 hours per batch. Once the testing is complete and all documentation has been verified, it is routed for review by a different qualified individual prior to release of results to the client.

Slide 9:

Testing process, continued

- Testing
 - Reagent preparation
 - Multiple incubation and washing steps (3.5-4 hours)
 - Automated or semi-automated reading
- Review of testing documentation
 - Operator verification
 - Primary review and transfer to LIS
- Result Release
 - Secondary review
 - Release to client



Many of the tests require preparation of several reagents before or during the testing process. As mentioned during the previous slide, multiple incubation and washing steps are required for the primary screening tests which routinely take at least 4 to 4 ½ hours. Most laboratories use automated or semi-automated reading devices to detect the colorimetric or chemiluminescent signal for all the controls and samples in a test batch. This allows electronic transfer of results to a laboratory information system or LIS following the completion of an independent review of all the testing documentation by a qualified staff member. Following this electronic transfer, a second level of review is performed prior to release of results to a client.

Slide10:

Testing process, continued

What happens when things go wrong?

- Unacceptable samples
 - Contact client, request additional sample
- Run failures
 - Repeat testing (+3.5-4 hours minimum)
- Initial reactive results
 - Duplicate retests required for ag/ab tests
- Reagent shortages
 - Contact vendor AND client

This slide illustrates a variety of things that laboratory staff don't like to think about but have to be able to manage. Frequently

a laboratory will receive unacceptable samples which require them to contact the submitting facility to determine if an alternate acceptable sample is available. In some instances there is no other option and testing cannot be completed. Another reason that test results might be delayed is a run failure. These are usually due to unacceptable kit control results and all samples must be retested. In this case, the testing process must start over which can cause delays of at least 4 to 4 ½ hours. In some cases, the laboratory cannot report results until duplicate retests are completed. Most of the antigen or antibody tests require this duplicate testing for all initial reactive samples and a final interpretation cannot be reported until the duplicate retests are complete. The worst case situation that a laboratory must manage is a reagent shortage. This can sometimes be caused by ineffective inventory management but can also sometimes be due to a manufacturer back-order situation. When this occurs the most critical action is to contact the vendor to determine if reagents can be made available and also to contact the client so they are aware of the issue and the potential impact on their operation.

Slide 11:

Importance of sample quality

“Specimen integrity dictates test result integrity “

- **Sample requirements** - specified by manufacturers
 - Ambient storage/shipping limits
 - Refrigeration requirements based on storage time
 - Centrifugation and separation of serum/plasma
 - Frozen storage at specific temperatures
- Excessively icteric, lipemic and hemolyzed samples are not acceptable
 - Various restrictions in manufacturer instructions
 - Labs frequently use the most restrictive limits for initial sample acceptability

By far the most common issue related to inability to perform testing is the quality of the sample provided to the laboratory. The integrity of the specimen will always dictate the integrity of the test results and the collection facility has the ability to control that quality. Paying strict attention to the requirements specified by the assay manufacturer will help ensure that the laboratory receives acceptable, high quality samples. Most manufacturers provide specific details for ambient storage, shipping limits and refrigeration requirements based on the storage time. Some tests also have requirements for centrifugation and separation of serum or plasma from the red cells within a specified time following collection. There are also specific times and temperatures provided for frozen storage of serum or plasma aliquots. Collection facilities must follow these requirements to ensure their samples are acceptable for testing. In addition to the sample handling requirements, a variety of restrictions are included in the manufacturer instructions related to acceptable levels of icterus, lipemia and hemoglobin. Laboratories frequently use the most restrictive limits for evaluation of initial sample acceptability.

Slide 12:

Importance of sample quality, continued

- Testing cannot be performed on samples that do not meet manufacturer instructions
 - Ag/Ab tests within 7 - 10 days
 - Number of days depends on manufacturer
 - Syphilis tests within 5 days
 - NAT/PCR tests within 8 - 11 days
 - Samples centrifuged within 72 hours
 - Cadaveric samples often within 2 days
 - Depends on manufacturer and test

Unfortunately, testing cannot be performed on samples that do not meet manufacturer instructions. Most antigen and antibody assays require samples less than 7 to 10 days old, depending on the manufacturer. Samples intended for syphilis antibody screening can only be tested up to five days following collection. NAT and PCR test must be performed within 8 to 11 days from collection and samples must be centrifuged within 3 days of collection. Additional restrictions are frequently in place for cadaveric samples which must be tested within 2 days of collection for some tests. This means that the collection facility must work very closely with the laboratory to ensure samples are transported and received in sufficient time to allow testing to be performed.

Slide 13:

Testing lab quality/compliance

- Licensure and Registration
 - FDA registration for HCT/P testing
 - CLIA/CMS certification for testing
- Quality plan
 - Required by all regulatory agencies
 - Evidence of ongoing monitoring required



Now that we have discussed the testing process and the importance of sample management, we will focus on the importance of assuring overall quality and compliance. Since many collection and processing facilities send samples to an outside laboratory for testing, it is important to exercise due diligence in evaluating the capabilities of the laboratory. FDA registration is required by any laboratory performing infectious disease testing of HCT/P collections and most labs are also required by their state health departments to be CLIA/CMS certified. A formal documented quality plan is required by all regulatory agencies so it is important to ensure

that the laboratory has a written quality plan and that there is evidence of ongoing monitoring of deviations and complaints.

Slide 14:

Testing lab quality/compliance, continued

- Quality control
 - Evidence of appropriate equipment and assay control (validation plus ongoing monitoring)
 - Evidence of successful proficiency survey participation for all analytes
 - Evidence of document control, training, etc.
- Client communications
 - Adequate response time for technical inquiries
 - Sufficient notification of changes

It is also important to verify that the laboratory has documented evidence for appropriate equipment and assay control. Validation prior to implementation of any new equipment, assays or processes as well as ongoing monitoring of equipment and assay performance should be readily available. Laboratories must also show successful participation in proficiency surveys for all tests performed in the lab and there should be evidence of document control (version control, MD approvals, etc.) as well as staff training. Another area that is critical to the client/laboratory relationship is communication. The laboratory should be responding to technical inquiries as quickly as possible and should provide sufficient notification of changes so the collecting/processing facility has adequate time to manage the changes.

Slide 15:

Impact of assay changes

- Assay differences
 - Sensitivity (false negatives) – rare due to FDA oversight/lot release processes
 - Reactive rates (specificity/false positives)
 - FDA focus on increasing **sensitivity** of donor screening assays
 - Corresponding loss of **specificity**
 - Sample limitations
 - Refrigerated and/or frozen storage time
 - Cadaveric sample claims
 - Cost (impact of long-term contracts)

Another issue that can impact a collection and/or processing facility is a change in the test or tests performed by the laboratory. This impact can occur due to potential differences between various manufacturer tests for the same infectious disease. Sensitivity (which is the potential for an assay to have a false negative result) is not usually an issue with different tests because of the stringent FDA oversight of assay manufacturing. The FDA requires manufac-

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urers of licensed donor screening tests to submit an example of each new kit lot so it can be tested with an FDA 'lot release' panel prior to allowing distribution of test kits. This process is intended to ensure that the assay sensitivity is adequate prior to allowing the test kits to be used in the laboratory. The issue of specificity is a little different. Specificity is the potential for an assay to have a false positive result and this can be affected when manufacturers are focused on increasing the sensitivity of a test because there is usually an inverse relationship between specificity and sensitivity. There is also a tendency for differences in specificity between manufacturers because of the variations in the raw materials that are used to prepare the various reagents in the test kit. When a laboratory changes from one manufacturer to another, there is frequently a change in the reactive rate due to these differences in specificity. There are also differences in sample requirements from manufacturer to manufacturer. The sample requirements are based on the clinical trial data that is submitted to the FDA for licensure of a new test so the manufacturers are required to include those limitations in their instructions for use of the test. Additionally, some manufacturers have submitted data on cadaveric sample testing and some have not so that's why there may be differences in cadaveric sample acceptability as well. Another reason for assay changes may be related to cost since most donor testing facilities have been under significant cost reduction pressure due to the overall decline in blood usage in the US. Many of these donor testing laboratories are entering into long-term reagent contracts with their test kit suppliers in order to help reduce their expenses so will sometimes change assays for this reason.

Slide 16:

Impact of assay changes, continued

- Reagent shortage
 - Single manufacturer contract to reduce cost
 - Reduced ability to maintain two different technologies
 - Good lab practice requires inventory management
- Manufacturer discontinuation
 - Long history of adequate manufacturing processes
 - Significant manufacturing changes are rare
 - Recently an issue for confirmatory and supplemental tests
 - Manufacturing and licensing expense
 - Low volume of testing does not allow cost recovery

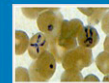
One of the other critical issues related to assay changes is reagent shortage. If a lab has entered into a single manufacturer contract for all their infectious disease tests in order to help control their expenses then they are at risk if that manufacturer is not able to meet their needs. In the past, many labs tried to maintain two different vendors so they had the capability of substituting one manufacturer's test for another. Although this prevented issues related to a reagent shortage from one vendor, the expense of maintaining two different sets of equipment, reagents and procedures has led to reduced ability to maintain two different assay technologies. This means that good inventory management is even more critical than it used to be. Manufacturer discontinuation of an assay has also been an issue in the past but there is now a long history of adequate manufacturing processes by the two primary donor screening

manufacturers. Significant manufacturing shortages or manufacturing changes are very rare now except for some recent issues related to confirmatory and supplemental tests. These are sometimes due to manufacturing problems but are more frequently due to the expense of manufacturing and licensing a test that is only used for a small proportion of the donor samples being screened. Since the confirmatory tests are only used for reactive samples, the low volume of this testing does not allow the manufacturer an opportunity to recover the costs of test development, licensing, or improvements that might be needed. All of these are issues that seem to be primarily laboratory issues but ultimately they impact the collection/processing facilities as well.

Slide 17:

Potential future changes

- Draft FDA Guidance
 - Syphilis testing requirements
 - WNV NAT recommendation
 - Anti-*T. cruzi* recommendation
- Potential new tests – selective or universal?
 - *Babesia* antibody/NAT
 - Dengue
 - Chikungunya
 - What is next?
- Potential upgrades
 - Enhanced sensitivity for NAT
 - Upgrades to automation
 - On-board discrimination for multiplex NAT



Now we will spend a little time discussing potential future changes in infectious disease testing. Currently, there are several draft FDA guidance documents pending finalization. These include syphilis testing requirements for HCT/P collections as well as WNV NAT and Anti-*T. cruzi* recommendations. Once finalized, the FDA usually provides some period of time (often around 6 months) to allow for implementation whenever new or modified testing recommendations are finalized. There are several new tests on the horizon but there is still quite a bit of discussion about whether these should be selective (only for certain regions of the country or only during certain times of the year). These include *Babesia* antibody and/or NAT screening for donors in the Northeast and upper Midwest regions of the US as well as Dengue and Chikungunya NAT screening for diseases which appear to be spreading from the southern hemisphere into the US. Who knows what emerging disease will be next? In addition to these potential new tests, laboratories are watching for some potential upgrades for existing assays. These include enhanced sensitivity for some of the current NAT assays and upgrades to the automation that is used in the laboratory. Another change on the horizon is automated discrimination for the 'multiplex' or 'triplex' nucleic acid tests. This 'on-board' functionality will allow a laboratory to perform discrimination of the NAT reactive samples for HIV, HCV or HBV at the time of the initial individual test which will reduce turn around time for samples with NAT reactivity. Some of the clinical trials for these upgrades are in progress now and will be submitted to the FDA for approval in the future. All of these potential changes will impact the testing laboratories and thus also impact the collection and processing facilities that we serve.