CCTC Clinical Chemistry Trainee Council | AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

www.traineecouncil.org

TITLE: The Molecular Testing of HIV

PRESENTER: Neil Anderson MD

Slide 1:

Hello, my name is Neil Anderson and I'm the assistant director of clinical microbiology at Barnes Jewish Hospital in Saint Louis, Missouri. Welcome to this Pearl of Laboratory Medicine on "**The Molecular Testing of HIV**."

Slide 2:

Human Immunodeficiency Virus was discovered in 1983 and is the causative agent of acquired immunodeficiency syndrome, commonly referred to as AIDS. It is responsible for approximately 6,800 infections per day. There are actually two very different HIV viruses, HIV-1 and HIV-2. HIV-1 is derived from a simian immunodeficiency virus that affects chimpanzees and is the virus responsible for the worldwide AIDS pandemic. It is further divided into Groups M, O, N and P. HIV-2, on the other hand is derived from a different simian immunodeficiency virus that affects a different animal, the sooty mangabey. In contrast to HIV-1, this virus has a distribution predominantly limited to Africa and parts of Europe. It is further divided into subgroups A-H.

Slide 3:

On this slide we have a schematic representing the viral structure of HIV. The virus belongs to the Retroviridae family, and like other members of this family, it is an enveloped virus with a single stranded RNA genome. This genome consists of three main genes, the core, polymerase, and envelope genes. The core gene encodes the matrix, capsid and nucleocapsid proteins, represented in this schematic as blue. The envelope gene encodes for the transmembrane glycoproteins and outer glycoproteins which allow the virus to bind to target cells. These are represented as green in this schematic. The polymerase gene encodes for the protease, reverse transcriptase, RNase, and integrase. The actions of these proteins are

important for virus function; therefore they are often targets for antiviral treatment. They are represented as red in this schematic.

Slide 4:

HIV can be acquired a number of different ways. It is commonly transmitted sexually through direct contact with body fluids including blood, semen, and vaginal secretions. It can also be transmitted through inoculation of infected blood through IV drug use, transfusion, transplantation and occupational exposure as well as from mother to child transplacentally, during birth, and during breastfeeding. Upon entering the body, the virus has a tropism for CD4 positive T cells and macrophages. There are three clinical stages in an HIV infection. The first is acute HIV occurring 2-4 weeks following exposure during which time patients experience non-specific symptoms, including fever, rash, malaise, pharyngitis and lymphadenopathy. These symptoms can mimic a number of other viral diseases, including influenza and epstein barr virus. Following acute symptoms, the virus typically enters a period of latency, which can last for years or even decades. This stage is known as clinical latency. Eventual progression to AIDS is marked by increased viral load and a decrease in CD4 positive T lymphocytes, resulting in opportunistic infections.

Slide 5:

The diagnosis of HIV is typically accomplished through serologic methods. Until recently a third generation enzyme immunoassay was used for screening and a second-generation Western blot analysis was used for diagnosis confirmation. The CDC now recommends a different algorithm which involves screening with an antigen/antibody assay followed by supplemental testing with an HIV1/2 antibody differentiation assay. A detailed discussion of these assays is provided in a different Pearl of Laboratory Medicine. Today we will emphasize the important role molecular tests play in the diagnosis of HIV.

Slide 6:

The schematic in this slide represents the “lab result timeline” in a patient infected with HIV. In other words, this schematic shows approximately when each test becomes positive in a typical infection with HIV. Notice that our fourth generation antigen/antibody screening assay becomes positive approximately 15 days following infection whereas the third generation assay becomes positive approximately five days later. There can be discrepant results with a positive 4th generation test and a negative 3rd generation test if a patient is tested during this period. This is an important time interval because it often correlates to the clinical stage of “acute HIV” during which time the patient may be symptomatic. The only other test that will be positive in a patient during this window of time is the nucleic acid detection test. In fact, the nucleic acid detection test is the first test to become positive, approximately 10 days following infection.

Slide 7:

For this reason, we are able to use molecular testing to investigate discrepant serology tests. The CDC fourth generation algorithm recommends that any patient with a positive antigen/antibody screen and a negative HIV1/2 differentiation assay be tested using a molecular method. For purposes of diagnosis, HIV qualitative tests should be used. Currently only one FDA approved molecular test is available for the diagnosis of HIV-1. This test detects viral RNA. Qualitative HIV testing is very useful in the diagnosis of acute HIV. It can be used in patients who have discrepant screening and confirmatory tests, as previously described, or it can be used in patients believed to be too early in the infection course to detect disease by serology. Qualitative testing is also very helpful for the diagnosis of HIV in neonates. In neonates serologic testing is unreliable given the transfer of maternal antibodies, therefore the only viable option in these patients is molecular testing. A drawback of FDA approved qualitative HIV testing is that it is not as readily available as quantitative HIV molecular tests. Quantitative HIV molecular tests are FDA approved for viral load monitoring only in patients with established diagnosis. However, recently developed quantitative tests demonstrate excellent sensitivity and specificity and as such can be used as qualitative diagnostic tests following appropriate validation. One important caveat to consider when using molecular methods to diagnosis HIV is that HIV-2 is not detected by commercially available molecular tests in the US used to monitor and diagnose HIV-1. Therefore, if HIV-2 is suspected based on patient history and a diagnosis cannot be achieved through serology, standard molecular tests used for HIV-1 cannot be used to rule out infection. While several lab developed HIV-2 assays have been described, the reliability of HIV-2 molecular testing during acute HIV-2 infection is unknown.

Slide 8:

Let's shift discussion to the management of patients with HIV. While molecular testing plays an important role in diagnosis, its primary role is in patient management. Very early on in the HIV epidemic it was recognized that the virus could develop resistance to antiviral therapy. Given the dangers of inadequately treated infection, patients should therefore be monitored for drug resistance. We know there are several patient parameters that change throughout an infection, leaving us with different options for monitoring. CD4 count is a useful measurement of disease status at the time of diagnosis. It also conveys the urgency of starting or changing therapy as well as starting treatment for opportunistic infections. Viral load, however, has emerged as the most useful marker of disease progression in both untreated and treated patients.

Slide 9:

Similar to qualitative HIV assays, quantitative HIV assays only detect HIV-1. Early iterations of these assays used a variety of technologies, including conventional reverse transcriptase PCR, branched DNA signal amplification, and Nucleic Acid sequence based amplification. Common to all these methods is the ability to quantify target virus. Although updated versions of these

assays are available, recent viral load assays use an entirely different technology, real-time PCR. These assays generate signal throughout the amplification process, in “real time”.

There are two main formats of real-time PCR utilized in HIV diagnostics. The top schematic represents real-time PCR using hydrolysis probes. A probe conjugated to a fluorophore (in yellow) and a quencher (in red) binds to target DNA. During the elongation phase of PCR the probe is hydrolyzed and the quencher is released from the fluorophore, allowing for signal generation. The bottom schematic depicts the other methodology commonly used. A partially double stranded probe reacts with the target DNA and disassociates, releasing the quencher from the fluorophore and creating signal. A major advantage to real time PCR is less specimen manipulation, since the whole process can occur in a closed tube during PCR amplification. This decreases the risk of contamination. This technology is also very sensitive, resulting in limits of detection ranging from 20-40 copies/ml.

Slide 10:

How should viral loads be used to monitor patients? The department of health and human services has specific guidelines for monitoring HIV positive patients which they continuously update. Current recommendations are for the testing of patients immediately prior to treatment and 2-8 weeks later in order to assess initial treatment response. Patients should have a 2log decrease in viral load after the first 2-3 months in order to signify an adequate drug response. Viral load should be completely undetected by week 24 and patients should then be monitored every 3-4 months to ensure continuation of treatment response. These guidelines also specify that in order for a change in viral load to be considered clinically significant it must be greater than 0.5 log copies/ml. In other words, viral loads need to roughly triple in magnitude before being considered clinically significant. This is mathematically explained by the fact that biological variation (that is variation within a patient) is approximately 0.3 log copies/ml whereas assay variation is approximately 0.2 log copies/ml.

Slide 11:

This panel has also defined what is meant by a “viral load blip”. Viral load blips are transiently detectable low level viral loads seen in patients on adequate antiviral treatment. They’re typically reactive to some stimulus to patient’s immune system, such as a transient infection. Viral load blips have even been seen following vaccination for influenza, tetanus, or pneumococcus! It is very important that these blips are not interpreted as treatment failures. Viral loads often return to undetectable levels following blips and these patients do not appear to experience any adverse outcomes. Unnecessarily switching a patient from effective treatment, however, would be considered an adverse event!

Slide 12:

We will finish our discussion of HIV molecular tests by talking about resistance testing. Resistance testing should be performed in patients prior to starting treatment and whenever treatment failure is suspected. Resistance testing can be summarized nicely by this Venn diagram. Available approaches include genotypic methods, phenotypic methods, and methods that are a combination of the two.

Slide 13:

Genotyping is the most frequently performed method for resistance testing. These tests involve extraction, amplification and sequencing of target regions within the viral genome, typically the reverse transcriptase and protease genes. Obtained sequence data is then queried for resistance mutations by using curated databases of known mutations. Lab developed tests have been used for the sequencing of other genes of concern regarding resistance, such as the gene encoding integrase.

Slide 14:

Phenotypic methods are far more involved than genotypic methods. During phenotyping, viral RNA is isolated and amplified. It is then cloned into an HIV vector with a reporter gene (the exact type of reporter gene varies by method). This cloned vector is then allowed to grow in various cell cultures in the presence of varying concentrations of antivirals. The amount of drug necessary to inhibit 50% and 90% of viral prorogation is reported. An advantage to this method is the ability to test more antivirals than commercially available genotypic methods.

Slide 15:

Virtual phenotype is a third method of resistance testing and is actually a clever combination of both genotyping and phenotyping. Through virtual phenotyping, a database of isolates characterized by both phenotype and genotype is used. Sequence data obtained from a patient's genotyped isolate is then queried against this database, obtaining a predicted phenotype based on database matches. This method allows one to obtain the same information gained during phenotyping without the lengthy procedure. The final resistance tests I'll mention today are tropism assays. These assays are designed specifically to predicted response to maraviroc, a relatively new antiviral. HIV binds CCR5 or CXCR-4 as a co-receptor for cellular entry. Maraviroc blocks binding with CCR5, therefore it is only effective when the virus is CCR5 tropic. During tropism testing viral genes are cloned into HIV vectors with reporter genes. The ability of the cloned vector to infect CCR5 positive cell lines and CXCR-4 positive cell lines is then measured. If the virus infects CCR5 cell lines, though not CXCR4 cell lines it is considered CCR5 tropic and maraviroc may be used for treatment.

Slide 16:

This slide is a summary of the different mechanisms for resistance detection. Importantly, while sequencing and virtual phenotyping have relatively similar and straightforward procedures, both phenotyping and tropism assays are quite complicated and require lengthy cloning steps. These are important factors to consider when deciding which test is most appropriate for a given patient and when designing appropriate algorithms for resistance testing.

Slide 17:

In assessing the appropriateness of resistance testing one must keep in mind the limitations. Currently the only FDA approved genotypic tests are for reverse transcriptase and protease inhibitors. If physicians are concerned about resistance associated with other genes, it may become necessary to find a laboratory that performs this specific testing. Also, the interpretation of genotypic results may be challenging since new mutations are constantly being identified, though they may not be present in databases that are not up to date. Finally, it is important to consider that both genotyping and phenotyping are not appropriate for all specimens. Most of these methods require viral loads of at least 500 copies/ml in order to be successful. Even when sequencing or cloning can be performed, a mutation must compromise approximately 25-30% of the viral population in order to be detected. Given the high cost and lengthy turnaround times of these tests, physicians should be aware of these limitations prior to testing.

Slide 18:

In summary, here are the key points from this Pearl of Laboratory Medicine:

-HIV Molecular testing plays an important role in the diagnosis of acute HIV.

-Resistance testing should be performed in patients prior to starting therapy.

-HIV viral load should be monitored during treatment to assess for drug resistance.

-Sustained or large increases in HIV viral load are suggestive of treatment failure and should be followed up with resistance testing.

-While genotyping is most common, several different resistance detection assays are available.

-All forms of HIV resistance testing have limitations which should be considered prior to testing.

Slide 19: References

Slide 20: Disclosures

Slide 21: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**The Molecular Testing of HIV**."