

# Molecular Virology in the Clinical Laboratory

DEBORAH JOSKO

## LEARNING OBJECTIVES

1. Compare and contrast traditional PCR and real-time PCR methodologies.
2. Describe common real-time PCR fluorescent detection chemistries.
3. Discuss various real-time PCR HIV-1 assays.
4. Explain the principle of transcription mediated assays and hybridization protection assays.
5. Discuss the methodology of the Hybrid Capture 2 High-Risk HPV test.
6. List FDA cleared molecular diagnostic viral assays.

**ABBREVIATIONS:** bDNA = Branched DNA; CD = Cluster of Differentiation; CI = Confidence Interval; CIN = Cervical Intraepithelial Neoplasia; DNA = Deoxyribonucleic Acid; FDA = Food and Drug Administration; FRET = Fluorescence Resonance Energy Transfer; HBV = Hepatitis B Virus; HCV = Hepatitis C Virus; HIV = Human Immunodeficiency Virus; HPA = Hybridization Protection Assay; NASBA = Nucleic Acid Sequence Based Amplification; PCR = Polymerase Chain Reaction; QS = Quantitation Standard; RLU/CO = Relative Light Units/Cutoff Value; RNA = Ribonucleic Acid; RT = Reverse Transcription; RT-PCR = Reverse Transcription Polymerase Chain Reaction; *Taq* = *Thermus aquaticus*; TMA = Transcription-Mediated Amplification.

**INDEX TERMS:** Molecular Methods, Polymerase Chain Reaction, Real-Time PCR, TaqMan Probes, Molecular Beacons, Dual Hybridization Probes, Fluorescence Resonance Energy Transfer; Transcription-Mediated Amplification, Hybridization Protection Assay, Hybrid Capture, FDA Cleared Molecular Viral Assays.

Clin Lab Sci 2010;23(4)231

*Deborah Josko, PhD, M(ASCP)<sup>CM</sup>, MLT, SM, University of Medicine and Dentistry of New Jersey, Scotch Plains, NJ.*

*Address for correspondence: Deborah Josko, PhD, M(ASCP)<sup>CM</sup>, MLT, SM, associate professor, Department of Clinical Laboratory Sciences, Medical Laboratory Science Program, University of Medicine and Dentistry of New Jersey, 1776 Raritan Road, Scotch Plains, NJ 07076. (908) 889-2422. joskotda@umdnj.edu.*

Please note: the author is not endorsing any particular company or product and has no financial gain or otherwise interest in the products presented.

The “gold standard” for viral identification is the conventional cell culture. Although cell culture has proven effective in the identification process of viral pathogens, there are shortcomings to this technology. Turn around times can range anywhere from days to weeks depending on the virus isolated; contamination rates are high; and some viruses are non-culturable making it virtually impossible to identify the microorganism. The need for rapid, sensitive, and accurate identification is imperative to ensure appropriate treatment and improve patient care. Although there are many rapid methods and serological assays available to identify viral agents, nucleic acid based detection and amplification-based assays are proving to be the most accurate and quickest identification processes. Results are reported in two to three hours compared to days or weeks for conventional cell culture.

The invention of the polymerase chain reaction (PCR) by Dr. Kary Mullis in 1983 revolutionized the field of molecular biology and modern medicine. Cetus Corporation, the company Dr. Mullis was working for at the time of his discovery, was awarded the patent for PCR in 1987.<sup>1</sup> Cetus in turn sold the rights to Hoffman La-Roche in 1992 and in 1993, the FDA approved the first diagnostic PCR assay; the AMPLICOR *Chlamydia trachomatis* test, in the U.S.<sup>1</sup> Since then, the use of PCR technology in the clinical laboratories has escalated,

especially in the last two decades. Traditional PCR, however, proved to have several shortcomings. Some of the limitations include detection at the end point of the reaction, which results in low sensitivity and poor precision; the use of agarose gels for detection, which is time consuming; poor resolution of bands on gels making it difficult to detect five fold changes on the gel and, is non-automated.<sup>2</sup> Fortunately, PCR has evolved into real-time PCR technology and can detect products while in progress allowing for quantitation of DNA and RNA as the reaction proceeds.<sup>2,3</sup> In addition, real-time PCR can detect as little as a two-fold change in copies generated, is automated, and eliminates the need for gels and post-PCR processing.<sup>2,3</sup> Since the use of real-time PCR assays is on the rise in the clinical laboratories compared to end-point PCR assays, the majority of this article will focus on some of the more common real-time PCR assays and various instruments (platforms) available for use.

#### Real-Time PCR

Real-time PCR was developed in the early 1990s by Higuchi *et al.*<sup>4,5</sup> This modification of traditional PCR utilizes a “closed tube” system, thereby reducing contamination rates. It also allows PCR products to be detected in “real-time” as opposed to end-point PCR where amplicons are detected at the end of the reaction. This allows for quantitation of nucleic acids which is useful in monitoring the progression of certain infections such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV) and, decreases turn around time since no post PCR processing is required.<sup>6</sup> Real-time PCR also differs from end-point PCR by using fluorogenic probes, primers, and amplicons. Although there are several techniques available for fluorescent detection, the most common detection assays include: hydrolysis probe assays such as TaqMan<sup>®</sup>, hybridization probes such as molecular beacons, dual-hybridization probes, scorpion primers, and DNA binding dyes (SYBR green).<sup>6</sup> For the purpose of this article, only the TaqMan<sup>®</sup> probes, molecular beacons, and dual-hybridization probes will be discussed.

#### Hydrolysis Probes

The TaqMan<sup>®</sup> probe (Roche Molecular Systems, Inc.) (see Figure 1) utilizes the 5' exonuclease activity of *Taq*

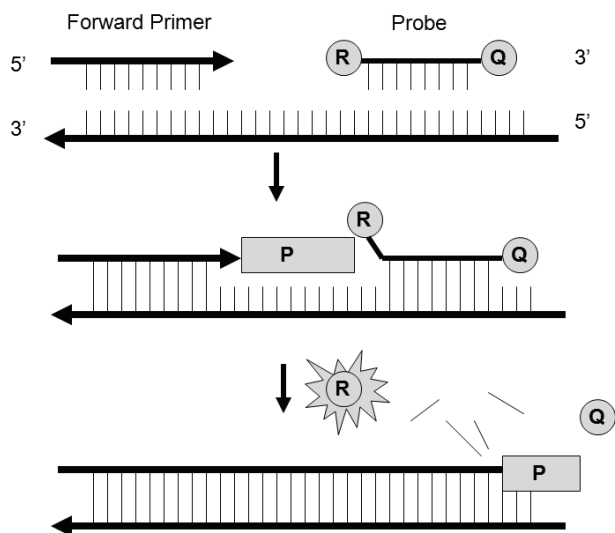
polymerase as originally described in 1991 by Holland *et al.*<sup>7</sup> The assay is based on dual-labeled fluorogenic oligonucleotide probes, which utilize a reporter fluorescent dye attached to the 5' end and a quencher dye near the 3' end.<sup>3</sup> Due to the close proximity of the quencher and the reporter dye, fluorescence is suppressed or “quenched.”<sup>3,8,9,10</sup> During the PCR reaction, the forward primer anneals to the target region, which is extended by *Taq* polymerase. The TaqMan probe along with the reverse primer then anneal to the newly synthesized strand.<sup>9</sup> Once the probe is hybridized to the target it is degraded by the 5' to 3' exonuclease activity of *Taq* polymerase during the extension step in the PCR reaction resulting in release of the reporter dye. The transfer of energy from one molecule to the other is measured indirectly by a process called fluorescence resonance energy transfer (FRET).<sup>6</sup> Since the reporter dye is no longer in close proximity to the quencher, fluorescence is emitted and increases proportionally to the amount of product produced.<sup>3</sup>

#### Hybridization Probes

Molecular beacons (see Figure 2) are similar to TaqMan probes in that they have a fluorescently labeled reporter dye attached to the 5' end and a quencher dye attached to the 3' end.<sup>3,6,9,10,11</sup> The single-stranded oligonucleotide probe however contains complementary sequences at the end, which bind and form a hairpin loop structure. Both the reporter and quencher are held in close proximity resulting in quenching of the fluorescent reporter molecule.<sup>3</sup> During the denaturation process, target DNA is separated which in turn causes the hairpin loop structure to open. Once the temperature decreases to allow annealing of primers and probes, the molecular beacons hybridize to the target sequence, which increases the distance between the reporter and quencher molecules. When they are no longer in close proximity to each other, fluorescence is no longer quenched and is released.<sup>3,6,9,10,11</sup> Fluorescence increases as PCR products accumulate and it is the intensity of the fluorescent signal that is monitored and measured by FRET during each cycle of the PCR.

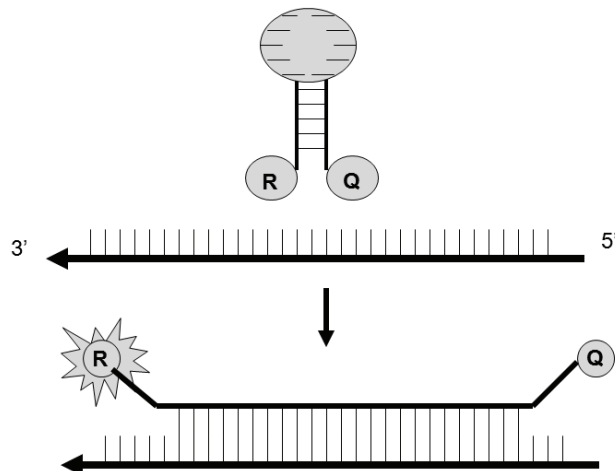
#### Dual-Hybridization Probes

Dual hybridization probes also utilize FRET technology. Two labeled probes are used which incorp-



**Figure 1.** TaqMan® Probe (Roche Molecular Systems, Inc.) Technology. TaqMan® Probe technology utilizes the 5' exonuclease activity of *Taq* polymerase. The forward primer and probe anneal to the template strand. The probe is dual labeled with the reporter fluorescent molecule attached to the 5' end and the quencher molecule attached to the 3' end. Due to the close proximity of both molecules, fluorescence is suppressed. During the reaction, DNA polymerase adds nucleotides to the growing strand and cleaves the reporter molecule from the probe and quencher. As the reporter molecule is released, energy is transferred from one molecule to another in a process called FRET. Since the reporter molecule is no longer in close proximity to the quencher molecule, fluorescence is emitted which increases proportionally to the amount of product produced.

R = Reporter Molecule; Q = Quencher Molecule; P = *Taq* Polymerase. Figure recreated with permission from Elsevier Publishers.<sup>6</sup>



**Figure 2.** Molecular Beacons. Molecular beacons are probes that contain complementary sequences at the ends, which form a hairpin loop structure. There is a fluorescently labeled reporter dye attached at the 5' end and a quencher dye at the 3' end. Due to the close proximity of both molecules, fluorescence is suppressed. During the denaturation step, the molecular beacon probe dissociates causing the hairpin loop structure to open. As the probe anneals to the target sequence, this increases the distance between the reporter and quencher molecule. As the reporter molecule is released from the quencher molecule, energy is transferred from one molecule to another in a process called FRET. Fluorescence is emitted and increases proportionally to the amount of product formed. R = Reporter Molecule; Q = Quencher Molecule. Figure recreated with permission from Elsevier Publishers.<sup>6</sup>

rate a donor fluorescent dye at the 3' end of one probe while the second probe has an acceptor fluorescent dye attached to the 5' end.<sup>6,11</sup> During PCR, both probes anneal to the PCR product in close proximity to each other—usually within a distance of 10 to 100 angstroms.<sup>12,13</sup> The light source in the real time PCR instrument excites the donor dye at the 3' end, which in turn transfers energy to the acceptor dye at the 5' end. The acceptor dye then emits the fluorescent signal, which is proportional to the amount of PCR product generated. The intensity of the fluorescent signal is measured by the PCR instrument.<sup>6,11,12,13</sup>

### Viral Detection

The first FDA approved molecular test was the Amplicor *Chlamydia trachomatis* test developed by Roche Molecular Systems, Inc. in 1993.<sup>1</sup> Since then,

many molecular assays utilizing various molecular detection methods have been developed, some for clinical use while others are strictly for research purposes. Since there are numerous assays available from many manufacturers, this article will focus on a few of the most common and widely used FDA cleared tests available in the clinical laboratory. A list of all FDA cleared viral assays to date including the manufacturer, test name, and methodology utilized can be found in Addendum A in the online version of *Clin Lab Sci* 2010;23(4) at [www.ascls.org](http://www.ascls.org).

### HIV-1 Assays

One of the greatest advances in molecular diagnostics has been the development of HIV assays for monitoring and treating HIV-1 infected individuals. Molecular methods were designed to quantify the concentration of

virus in the blood (viral loads) which informs the physician whether anti-retroviral therapy is working, whether the patient is compliant with their medications, and whether a genetic mutation has occurred rendering the antiviral therapy inactive. This information, in conjunction with CD4 counts, is valuable in determining the progression of the disease, the possible complications due to low CD4 counts and high viral loads, as well as predicting the future course of the infection.

In May of 2007, the FDA cleared three new HIV assays for *in vitro* diagnostic use: the Abbott RealTime HIV-1 Assay (ABBOTT Molecular, Inc. Des Plaines, IL), the COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche Molecular Diagnostics, Pleasanton, CA) and Procleix ULTRIO Assay (Gen-Probe, Inc., San Diego, CA (distributed by Chiron)).<sup>14,15</sup>

#### Abbott RealTime HIV-1 Assay

The Abbott RealTime HIV-1 Assay is automated from sample preparation to quantitation and measurement and utilizes reverse-transcription polymerase chain reaction (RT-PCR) technology to quantify and measure the amount of HIV virus present in plasma.<sup>15,16,17</sup> The *m2000* system is the platform where specimen processing, amplification, and detection is performed. Specimen and reagent preparation along with the assembly of the reaction plate is performed on the Abbott *m2000sp* instrument.<sup>16</sup> Once the plate is ready it is transferred to the Abbott *m2000rt* instrument where the amplification and detection steps are completed. Real time detection is based on the use of dual-labeled probes. The HIV-1 probe contains a covalently bound fluorescent molecule attached to the 5' end while a short oligonucleotide probe, which is complementary to the 5' end of the HIV-1 probe, has a quencher molecule attached at its 3' end. If the HIV target sequence is absent, fluorescence is "quenched" and no signal is detected.<sup>16</sup> However, if the HIV target is present, the quencher molecule dissociates from the HIV-1 probe allowing for hybridization of the HIV-1 probe to the target sequence permitting fluorescent emission, which in turn is monitored and measured. The amount of fluorescence generated is proportional to the log of the amount of virus in the initial sample.<sup>16</sup>

#### COBAS AmpliPrep/COBAS TaqMan HIV-1 Assay

The COBAS AmpliPrep/COBAS TaqMan HIV-1 assay is also a fully automated real time PCR assay using RT-PCR and FRET to quantify HIV RNA virus in plasma.<sup>18,19</sup> The specimen preparation and extraction is fully automated using the COBAS AmpliPrep instrument whereas amplification and detection is performed in the COBAS TaqMan analyzer or the COBAS TaqMan 48 analyzer. Quantitation is achieved by placing a quantitation standard (QS) in the master mix with primers and probes specific for both the HIV-1 target and QS. The QS is processed, amplified, and detected along with the target HIV-1 sequence. Once the reaction is complete, the amplicons are detected using dual-labeled probes with different fluorescent reporter dyes (one for the target and one for the QS) and are measured independently at different wavelengths.<sup>19</sup> The intensity of the signal is proportional to the amount of product produced.

The sensitivities (linear range) for both the Abbott RealTime HIV-1 and the COBAS AmpliPrep/COBAS TaqMan HIV-1 assays according to the manufacturer package inserts are 40 copies/mL to 10 million copies/mL and 48 copies/mL to 10 million copies/mL, respectively.<sup>16,19</sup> In 2009, Sloma *et. al* performed a comparison study comparing the Abbott RealTime HIV-1 and the COBAS AmpliPrep/COBAS TaqMan HIV-1 assays. According to their study, "the analytical sensitivities of the Abbott RealTime HIV-1 and COBAS TaqMan assays were determined to be 32.2 copies/mL (95% CI, 24.7 to 51.8) and 45.3 copies/mL (95% CI, 36.3 to 64.5), respectively, closely approximating the lower limits of quantification (40 copies/mL) set by the manufacturers of both assays."<sup>20</sup>

#### Procleix ULTRIO Assay

The Procleix ULTRIO assay which utilizes the Procleix TIGRIS system is a fully automated system that produces qualitative results.<sup>14</sup> This *in vitro* assay has been approved to screen donor blood and blood components as well as organ donations for the presence of HIV-1, hepatitis B virus (HBV) and hepatitis C virus (HCV).<sup>14,21</sup> The assay, which takes place in one tube, involves three steps: specimen preparation of HIV-1, HBV, and HCV; amplification by transcription-mediated amplification (TMA); and detection of



amplicons by the hybridization protection assay (HPA).<sup>21,22</sup>

TMA is an RNA transcription amplification methodology where the entire reaction takes place in one tube at one temperature.<sup>21,22</sup> This isothermal reaction differs from PCR in that PCR requires the use of a thermal cycler to raise and lower the reaction temperature in order for denaturation, annealing, and extension of the target to take place. TMA technology utilizes two enzymes: RNA polymerase and reverse transcriptase (RT). RT is used to generate a copy of the DNA target whereas RNA polymerase generates many copies of RNA from the DNA template strand.<sup>21</sup> Since RNA polymerase and RT are used in this reaction, TMA technology can amplify both DNA and RNA and generate RNA amplicons unlike some of the other amplification systems.<sup>22</sup> Once the reaction is complete, detection is achieved by using the hybridization protection assay (HPA).<sup>23</sup> If the target is present and has been amplified, single-stranded complementary chemiluminescent labeled probes will hybridize to the specific amplicon.<sup>21</sup> The signal generated can be detected and measured in a luminometer.<sup>21,22,23</sup> Both TMA and HPA can be done in the same tube, which aids in the reduction of possible contamination. The sensitivity for all three assays: HIV-1, HBV, and HCV were over 98% according to the manufacturer's package inserts.<sup>21</sup>

### Human Papillomavirus (HPV) Testing

The last FDA-cleared molecular assay discussed in this article, will be the Hybrid Capture 2 High-Risk HPV DNA test by Qiagen, Inc. (Germantown, Maryland), marketed by Digene.<sup>15</sup> This *in vitro* nucleic acid hybridization assay can detect 13 high-risk HPV serotypes in cervical specimens using Hybrid Capture 2 technology with signal amplification and chemiluminescent detection.<sup>24,25</sup> Once the nucleic acids are released and denatured, specific HPV RNA probes (specific for the 13 high-risk types) are added to the mixture. These hybridize to the DNA target sequence if present. The resultant RNA:DNA hybrids are then "captured" on the wells of a microplate coated with surface antibody specific to the hybrids.<sup>24</sup> A second alkaline phosphatase labeled antibody is added to the wells, which binds to the RNA:DNA hybrids. The light

emitted as a result of cleaved substrate by bound alkaline phosphatase is measured in a luminometer.<sup>24</sup> The intensity of the light emitted is proportional to the amount of target present.

Although the sensitivity has been reported to be >96% for the detection of high-grade cervical intraepithelial neoplasia (CIN) and cancer when performed with cytology,<sup>26</sup> false negative results have been reported when the HPV viral load is below the detectable limit (<1.0 RLU/CO).<sup>25,27</sup>

### FDA Cleared Diagnostic Tests

There are several FDA cleared *in vitro* molecular diagnostic assays on the market, which utilize various molecular methodologies. This article focused on a few assays, which use real-time PCR, TMA, HPA, and Hybrid Capture technology. A list of all FDA cleared viral molecular assays as of February 2010 can be found in Addendum A in the online version of *Clin Lab Sci* 2010;23(4) at [www.ascls.org](http://www.ascls.org).

The Association for Molecular Pathology lists all FDA cleared viral assays at [www.amp.org](http://www.amp.org). There are many other assays that are available outside the U.S. or for research purposes only that are not FDA cleared and are therefore not included in this list.

### Summary

As one can see by the tests listed at [www.amp.org](http://www.amp.org), molecular diagnostic techniques have enabled the laboratory professionals to play an integral role in the identification and quantitation of viral infectious agents. Viral loads can be determined for HIV, HBV, and HCV using a variety of molecular methods such as real-time PCR, TMA, NASBA, and bDNA. Determining the amount of viral particles in a sample can not only monitor the status and progression of the disease, but can also guide recommendations for antiviral therapy.<sup>28</sup> Other assays listed include cytomegalovirus, enterovirus, and human metapneumovirus detection, HPV testing, influenza and respiratory virus panels, and West Nile virus detection in blood donations using a variety of molecular methodologies.<sup>15</sup>

The use of molecular methodologies in the detection of viral pathogens has grown at an astounding rate,

especially in the past two decades. It is now widely accepted that PCR is the “gold standard” for nucleic acid detection in the clinical laboratory as well as in research facilities.<sup>11</sup> This article only touched on some of the common, widely used assays and platforms used in the identification process. With more and more assays being developed, the cost behind molecular testing has decreased since there are more competitors on the market. At one point, laboratorians may have thought of routine molecular testing as the wave of the future. It is obvious the future is upon us. Molecular diagnostics has become part of the daily, routine workload in most clinical laboratories. The advent of fully automated systems with faster turn around times has given laboratory professionals the tools necessary to report out accurate and sensitive results to clinicians who can ultimately improve patient care and outcomes by rendering a correct and rapid diagnosis.

## REFERENCES

1. Roche PCR Timeline. Available from [http://molecular.roche.com/roche\\_pcr/pcr\\_timeline.html](http://molecular.roche.com/roche_pcr/pcr_timeline.html). Accessed 2010 April 16.
2. Real-Time PCR vs. Traditional PCR. Available from [http://www.appliedbiosystems.com/support/tutorials/pdf/rtPCR\\_vs\\_tradPCR.pdf](http://www.appliedbiosystems.com/support/tutorials/pdf/rtPCR_vs_tradPCR.pdf). Accessed 2010 April 16.
3. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. *Genome Res.* 1996;6:986-94.
4. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. *Nat Biotechnol.* 1992;10:413-17.
5. Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Nature Biotechnology.* 1993;11:1026-30.
6. Mahon CR, Lehman DC, Manuseles G, editors. *Textbook of Diagnostic Microbiology.* 4<sup>th</sup> ed. Missouri Saunders Elsevier; 2011.
7. Holland PM, Abramson RD, Watson R, Gelfand DH. Detection of specific polymerase chain reaction product by utilizing the 5'-3' exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc. Natl. Acad. Sci. USA* 1991;88:7276-80.
8. Hydrolysis Probes. Available from <http://www.realtimeprimers.org/Hydrolysis/Hydrolysis%20Probe%20Page.html>. Accessed 2010 April 27.
9. Biosearch Technologies : TaqMan Probes. Available from [http://biosearchtech.com/download/flash\\_guides/formats\\_explained.html](http://biosearchtech.com/download/flash_guides/formats_explained.html). Accessed 2010 April 27.
10. RT-PCR: The Basics. Available from <http://www.ambion.com/techlib/basics/rtPCR/index.html>. Accessed 2010 April 27.
11. Mackay IM, Arder KE, Nitsche A. Survey and Summary Real-Time PCR in Virology. *Nucleic Acids Research.* 2002;30:1292-1305.
12. Clegg RM. Fluorescence resonance energy transfer and nucleic acids. *Methods Enzymology.* 1992;211:353-88.
13. Stryer L, Haugland RP. Energy transfer: a spectroscopic ruler. *Proc. Natl. Acad. Sci. USA.* 1967;58:719-26.
14. Food and Drug Administration. Three New HIV Assays Approved. Available from <http://www.fda.gov/ForConsumers/ByAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm124218.htm>. Accessed 2010 May 5.
15. FDA-Cleared/Approved Molecular Diagnostics Tests. From the Association for Molecular Pathology. Available from [www.amp.org](http://www.amp.org). Accessed 2010 May 5.
16. ABBOTT Molecular Inc. Abbott RealTime HIV-1 Package Insert. 2007. REF 6L18.
17. Abbott Molecular RealTime HIV-1. Available from [http://www.abbottmolecular.com/RealTimeHIV1\\_22838.aspx](http://www.abbottmolecular.com/RealTimeHIV1_22838.aspx). Accessed 2010 May 5.
18. Fully Automated Human Immunodeficiency Virus Type 1 (HIV-1) Quantification Test. Available from [http://molecular.roche.com/diagnostics/virology/products\\_virology\\_12.html](http://molecular.roche.com/diagnostics/virology/products_virology_12.html). Accessed 2010 May 5.
19. Roche Molecular Diagnostics, Inc. COBAS AmpliPrep/COBAS TaqMan HIV-1 Test. Package insert. 2007. Doc Rev. 1.0.
20. Sloma CR, Germer JJ, Gerads TM, Jayawant N, Mandrekar P, Mitchell S, et al. Comparison of the Abbott RealTime Human Immunodeficiency Virus Type 1 (HIV-1) Assay to the Cobas AmpliPrep/Cobas TaqMan HIV-1 Test: Workflow, Reliability, and Direct Costs. *J. Clin Micro.* 2009;47:889-95.
21. Gen-Probe, Inc. Porcleix Ultrio Assay Package Insert. 2009.
22. Gen-Probe, Inc. Transcription-Mediated Amplification. Available from <http://www.gen-probe.com/science/amplification.aspx>. Accessed 2010 May 5.
23. Gen-Probe, Inc. Hybridization Protection Assay. Available from <http://www.gen-probe.com/science/hpa.aspx>. Accessed 2010 May 5.
24. Qiagen, Inc. Hybrid Capture 2 High-Risk HPV DNA Test Package Insert. 2007 Digene Corporation.
25. Digene HC2 High-Risk HPV DNA Test. Available from <http://www.qiagen.com/products/digenehighriskhpvtstc2.aspx#Tabs=t2>. Accessed 2010, May 10.
26. Hybrid Capture 2 High-Risk HPV DNA Test. Available from <http://www.medcompare.com/details/42815/Hybrid-Capture-Andreg-2-High-Risk-HPV-DNA-TestAndreg.html>. Accessed 2010, May 10.
27. Raid J, Geddie WR, Chapman W, Boerner S. Characteristics of Apparently False-Negative Digene Hybrid Capture 2 High-Risk HPV DNA Testing. *Am J Clin Pathol.* 2006;125:223-228.
28. HIV Viral Load. Available from [http://www.labtestsonline.org/understanding/analytes/viral\\_load/test.html#when](http://www.labtestsonline.org/understanding/analytes/viral_load/test.html#when). Accessed 2010, May 10.