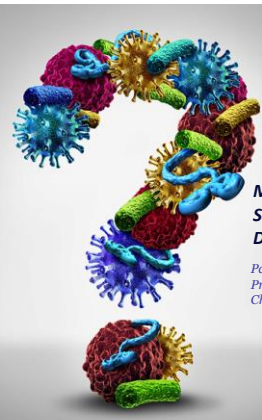

Molecular Diagnostics: The NEW Standard of Care in Virology Detection and Treatment

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Chair, Microbiology Advisory Board IFBLS

Molecular Diagnostics:

The NEW Standard of Care in Virology Detection and Treatment

Objective 1

Identify reasons why molecular diagnostics has become the recommended standard of care for the major viral pathogens.

Objective 2

Describe the clinical efficacy for the use of molecular virology associated with the major viral pathogens.

Objective 3

Identify current FDA status and Proficiency Testing available for molecular platforms and methods for major viral infections

Introduction

Microbiology and the Evolution of Molecular Diagnostics

Contents

Molecular Diagnostics: The NEW Standard of Care in Virology Detection and Treatment

Human Immunodeficiency Virus 1

Viral Syndrome

Retroviridae: Lentivirus

- ***+ RNA➔ DBL Stranded DNA molecule or Provirus***
- ***HIV-1 Enters the cell using CD4 as the receptor and the CXCR4 (T-cells) or CCR5 (macrophages) as the coreceptor.***
- ***Reverse Transcriptase➔ no proofreading capabilities***
  - ***Coinfections and recombination***
- ***Subtypes and Clades***
  - ***Major (M)-worldwide***
  - ***Outlier (O)***
  - ***Nonmajor and Nonoutlier (N)***
  - ***New Group (P)***
  - ***CRF***

HIV

Testing Evolution

Clinical Questions: Whether an individual is infected and how actively is the virus replicating? And the susceptibility to antiretroviral medications

Previous Testing Methods

Serological Assays-high specificity and sensitivity

Screening Tests
- *Phenotype of the virus*
- *Infectious dose*
- *Transmission mode*
- *Sensitivity of the assay*

Combination tests for antigens and antibodies, reduce the diagnostic window by 5 days.

4th Generation testing reduced detection to 3-5 days

Western Blot became the world wide confirmatory method for decades.

LIA-Line assay, more standardized form of WB

Both WB and LIA are prone to carry-over problems.

Component Testing p24 and NAT

Emergence of Molecular Methods

Molecular assays reduce initial window
For diagnosis from 2.5 weeks by an additional week.

HIV1 RNA newer assays, following combination assays, reduced window to 9 days

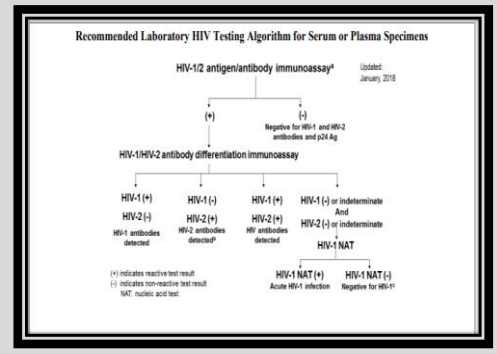
Signal and amplification techniques available from a variety of manufacturers.

Testing was transformed when HIV1 viral load testing became possible and resistance testing for antiviral drugs.

Advantages and Disadvantages of FDA-Approved HIV Assays Used for Screening, *by test category*

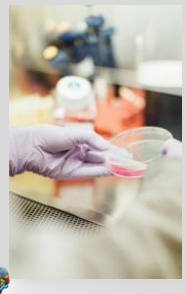
Test Category	HIV Screening Tests	Run Time	Instrument	Report Ag and Ab separately	Detects IgG	Detects IgM	Uses whole blood (WB) specimens	Uses oral fluid (OF) specimens	Uses dried blood spot specimens	Least complex CLIA category	External quality control not required in each run
Nucleic acid laboratory test	Aptima HIV-1 RNA Qualitative Assay	>3 hours	semi-automated							high	
Ag/Ab laboratory test	ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay	<1 hour	automated		✓	✓				moderate	✓
	ARCHITECT HIV Ag/Ab Combo Assay	<30 mins	automated		✓	✓				moderate	✓
	BioPlex 2200 HIV Ag-Ab	45 mins	automated	✓	✓	✓				moderate	✓
	Elecsys HIV combi PT	27 mins	automated		✓	✓				moderate	✓
	GS HIV Combo Ag/Ab EIA	>3 hours	semi-automated		✓	✓				high	
	VITROS HIV Combo Test	48 mins	automated		✓	✓				moderate	✓
Ag/Ab rapid test	Determine HIV-1/2 Ag/Ab Combo	20 mins	single-use	✓	✓	✓	✓			waived	✓
Ab laboratory test	ADVIA Centaur HIV 1/O/2 Enhanced (EHIV) Assay	< 1 hour	automated		✓	✓				moderate	
	Aviosq HIV-1 Microelisa System	>3 hours	semi-automated		✓	✓		✓	✓	high	
	GS HIV-1/2 Plus O	>3 hours	semi-automated		✓	✓				high	
	VITROS Anti-HIV 1+2	< 1 hour	automated		✓	✓				high	✓
Ab rapid test	DPP HIV-1/2 Assay	10 mins WB/ 25 mins OF	single-use		✓		✓	✓		waived	✓
	HIV 1/2 STAT-PAK	15 mins	single-use		✓		✓			waived	✓
	INSTI HIV-1/HIV-2 Antibody Test	<2 mins	single-use		✓	✓	✓			waived	✓
	OraQuick ADVANCE Rapid HIV-1/2 Antibody Test	20 mins	single-use		✓	✓	✓	✓		waived	✓
	Reveal G4 Rapid HIV-1 Antibody Test	<2 mins	single-use		✓		✓			moderate	✓
	SURE CHECK HIV 1/2 Assay	15 mins	single-use		✓		✓			waived	✓
	Uni-Gold Recombigen HIV-1/2	10 mins	single-use		✓	✓	✓			waived	✓

Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens



APTIMA HIV-1 RNA Qualitative Assay

FDA APPROVED



Approved for Diagnostic Use

HIV-1 Diagnostic Use in both acute or primary infection
- Plasma

Three Main Steps in an enclosed system
- Sample Preparation
- HIV-1 target amplification (TMA)
- Detection by Hybrid Capture

99-100% confidence interval
(>100 copies /mL)

Why is there no improvement or more of these tests available?

Transformation of HIV

Complete Transition

Patient Management Improvements

HIV
First viral load testing began in 1996

Stigma about CD4 cell levels has changed over time.

Viral load testing is completed at time of entry into care, when therapy is initiated and about every 3-4 months to monitor therapy.

Treatment regimens vary, some do not work on patient's with high viral loads.

Goal of treatment is to reach 20-50 copies/mL, below the limit of detection of the most sensitive assays.

Viral load assays are NOT FDA approved for diagnostic purposes.

HIV Viral Load Testing

FDA Approved

01 ***Roche Diagnostics***

Ampli Prep/cobas Taqman HIV1
Real-Time PCR
gag, LTR
20-10,000,000 copies/mL

COBAS® 6800 System / COBAS® 8800 System11

02 ***Abbott Diagnostics***

M2000 Real Time
Real-Time PCR
int
40-10,000,000 copies/mL

03 ***Siemens Diagnostics***

Versant HIV RNA
bDNA-signal amplification
pol
75-500,000 copies/mL
Available???

RESISTANT TESTING

FDA Approved: Genotypic and Phenotypic Testing

01 ***Siemens Diagnostics***

Trugene HIV-1 Genotyping
OpenGene DNA Sequencing System

02 ***Abbott Diagnostics***

ViroSeq HIV-1 Genotyping System

The ViroSeq HIV-1 Genotyping System is intended for use in detecting HIV-1 genomic mutations that confer resistance to specific types of antiretroviral drugs, as an aid in monitoring and treating HIV infection

Resistance Testing Recommendations:
- Before initiation of ART in treatment of naïve patients
- Guide the selection when changing regimens
- Management of suboptimal viral load reduction
- All Pregnant women before initiation of ART

Hepatitis Viruses

Blood Borne (Except D)

Hepadnaviridae (B): Flaviviridae (C)

- ***3.2 million people are infected with Hepatitis C***
- ***2 billion people have evidence of Hepatitis B have serological evidence with 350 million displaying chronic infection.***

- ***Wide diverse genomic properties and replication strategies***
- ***Pathological effects on the liver are similar***
  - ***Provide no reliable clinical clues about the specific virus***

HBV-Enveloped virus, partially ds DNA circular genome that uses a reverse Transcriptase and RNA intermediate for reproduction

HCV-Enveloped virus,, + RNA ss genome

Hepatitis Viruses

Testing Evolution

Clinical Questions: Whether an individual is infected and how actively is the virus replicating? And the susceptibility to antiretroviral medications

Previous Testing Methods

HBV

Immunoassays of viral specific proteins and corresponding antibodies (HBsAg, HBcAg, anti-HBc, anti-HBe, and anti-HBs)

This provides an accurate diagnosis of most acute and chronic infections

HCV

Serology is generally the first approach

Viremia precedes biochemical indicators of liver damage by 3-10 weeks, and production of antibodies by several more.

Laboratory rarely diagnoses HCV early, during the window prior to serological evidence.

10% or more of immunosuppressed patients, test antibody negative

Emergence of Molecular Methods

HBV

Because serology is effective in diagnosis, DNA detection was not perceived as urgent.

However, presence and quantity have demonstrated utility in patient management, and validated interpretation of immunological data.

HBeAG serves as a monitor for infection, but there are HBeAG minus variants.

Seroconversion does not exclude viral replication; DNA became the most accurate indicator of HBV infection.

HCV

Due to limitations and complexity of HCV serology, molecular identification of HCV RNA in plasma and quantification has dominated the detection and clinical management of patients.

HCV RNA is present in the blood 1-3 weeks post exposure

Genomes have high variability, therefore target selection is critical

Transformation of HBV

Almost Complete Transition

Patient Management Improvements

HBV
Serological Assays have high specificity, sensitivity and reproducibility

HBV DNA in the serum.
Evaluation of initial infection
Monitoring chronic infections
Assessing efficacy of treatment
Immune escape mutants

Blood Donors should now be screened using HBV DNA qualitative tests to detect EARLY infection

Antiretrovial resistance mutations are detected for drug resistance

HBV DNA Detection and Quantification

FDA Approved

01 *Roche Diagnostics*

Real-Time PCR
Ampli-Prep and Taqman HBV
Cobas
Precore/Core
20-170,000,000 IU/mL

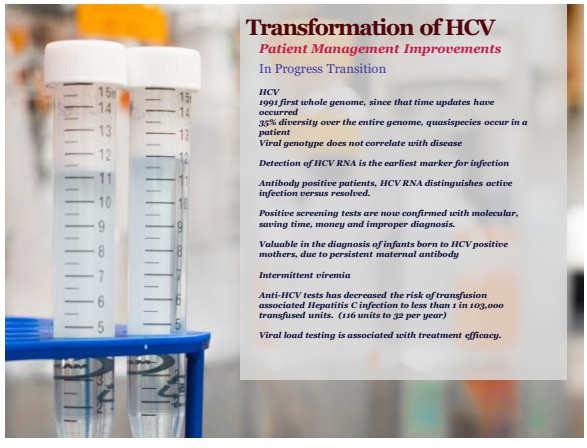
COBAS® 6800 System / COBAS® 8800 System11

WHO HBV Standard;

What about Resistance typing?

02 *Abbott Diagnostics*

Real-Time System
Surface Antigen
10-1,000,000,000 IU/mL



Transformation of HCV

Patient Management Improvements

In Progress Transition

HCV
1991 first whole genome, since that time updates have occurred
35% diversity over the entire genome, quasispecies occur in a patient
Viral genotype does not correlate with disease

Detection of HCV RNA is the earliest marker for infection

Antibody positive patients, HCV RNA distinguishes active infection versus resolved.

Positive screening tests are now confirmed with molecular, saving time, money and improper diagnosis.

Valuable in the diagnosis of infants born to HCV positive mothers, due to persistent maternal antibody

Intermittent viremia

Anti-HCV tests has decreased the risk of transfusion associated Hepatitis C infection to less than 1 in 103,000 transfused units. (116 units to 32 per year)

Viral load testing is associated with treatment efficacy.

HCV Viral Load Testing

FDA Approved

01 *Roche Diagnostics*

Ampli Prep/cobas Taqman HIV1
Real-Time PCR
5' UTR
20-10,000,000 IU/mL

COBAS® 6800 System / COBAS® 8800 System11

02 *Abbott Diagnostics*

M2000 Real Time
Real-Time PCR
5' UTR
12-100,000,000 IU/mL

03 *Siemens Diagnostics*

Versant 3.0
bDNA-signal amplification
5' UTR
615-7,700,000 IU/mL
Available?

Calibrated against; WHO CMV standard

CAP has an extensive proficiency testing program

CMV and EBV

Transplant Patients

Herpesviridae

- ***CMV-dsDNA, enveloped virus with 95% homology among strains***
- ***EBV-ds DNA, enveloped virus, two primary strains with significant divergence in sequence***

- **CMV**
  - *Asymptomatic to minor infections- resembles mononucleosis*
  - *Pathogen in Immunocompromised*
  - *Lifelong latent infection*
  - *Immunocompromised-reactivation and clinical syndromes*
  - *Seroprevalence varies from 30-100% (40-50% in the United States)*
- EBV
  - Mononucleosis to a variety of malignancies including lymphomas (oncogenic virus)
  - Immunocompetent- mononucleosis is rarely fatal, symptoms more associated with increased lymphocyte proliferation
  - Immunocompromised- lymphoproliferative disease (PT-LPD)
  - 87% B-cell origin, 12.5% T-cell, 0.5% null cell

Herpesviridae: CMV and EBV

Testing Evolution

Clinical Questions: Whether an individual is infected and how actively is the virus replicating? And the susceptibility to antiretroviral medications

Previous Testing Methods

Histopathology, Viral Culture, Serology, Antigen Detection and NAT for detection

CMV
Histopathology of infected cells on autopsy-CPE
Viral Culture requiring 1-2 weeks incubation
Shell Vial, 24-48 hours
Serology 2-4 weeks post-infection, IgG appearing after 4 weeks (low versus high avidity assays)
Antigenemia- PMN's expressing pp65

EBV
Serology standard method, EBNA, EA and VCA

Nonspecific rise in heterophile antibodies, predominantly IgM

Histology-in LPD in tissue biopsy
RNA probes will identify viral infected tissue

Emergence of Molecular Methods

Nucleic Acid Testing: Diagnosis

CMV
Variety of amplification and hybridization techniques
Blood, BAL, CSF and tissue samples
Whole blood has a higher viral load than plasma

Lacks standardization for specimen types, target gene, calibrators, detection and extraction, assay comparison is difficult.

Development of mRNA testing versus DNA

EBV
Variety of amplification techniques
Normal latent load and PT-LPD significantly different
Early detection, therapeutic intervention.

Transformation of Herpesviridae Testing

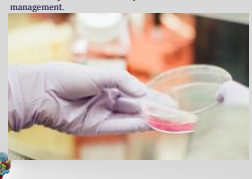
Viral Load-CMV

Patient Management Improvements

Why Molecular Assays for CMV?
1. Initiation of preemptive therapy
2. Diagnosis of active disease
3. Monitoring response to therapy

Despite improvements, until recently, laboratories used LDT's to detect and quantify CMV DNA. The threshold and viral load cutoff is varied among laboratories and transplant populations.

Health care providers must relay on the trend over-time rather than a single reference range for diagnosis and patient management.



CMV

FDA Approved

01 ***Roche Diagnostics***

CAP/CATM CMV Test
Cobas Amplliprep/Taqman Test
UL54 gene-CMV polymerase
137-9,100,000 UU/mL

Both real-time

Calibrated against; WHO CMV standard

Despite standardization, Interlaboratory variation exists in proficiency testing.

02 ***Qiagen***

Artus CMV RGQ MDx
MIE gene-antigen
119-79,400,000
10 fold greater dynamic range

COBAS® 6800 System / COBAS® 8800 System11

Transformation of Herpesviridae Testing

EBV Management in PTLD

Patient Management Improvements

PTLD ranges from benign lymphocytic hyperplasia to fatal malignant lymphoma
- Most occur within first year following transplant

Treatment is challenging, after disease is established antiretrovirals are not effective
- Immunosuppression must be reduced.

Accurate measurement of viral load
- Prior to the development of EBV-associated PTLD
- Monitoring effective therapy

Limitations
- Assays lack standardization and optimal assay technique
- Sample types vary (no consensus)
- Sampling schedule not defined
- No target consensus

No FDA approved Viral load tests

WHO International Standard is Available!

BK Virus

Viral Syndrome

Polyomaviridae: (JC virus and Simian Virus 40)

- ***Enveloped, DNA DBL Stranded***
- ***Seroprevalence reaches nearly 100% in early childhood, declines to 60-80% in adulthood***
- ***Virus remains latent***
  - ***Urinary tract and lymphoid cells***
  - ***Reactivates in immunosuppressed patients***
  - ***Polyovirus associated nephropathy (PVAN)***

BK Virus

Testing Evolution

Clinical Questions: Whether an individual is infected and how actively is the virus replicating? And the susceptibility to antiretroviral medications

Previous Testing Methods

Histopathology of kidney biopsy and CPE
However, not pathognomic

Urine cytology and intranuclear viral inclusions

Molecular tests were proposed in 2002.

Emergence of Molecular Methods

Nucleic Acid Testing: Diagnosis

Patients with HC, have higher viruria than asymptomatic

PVAN detection and quantitation in urine
Essential, viruria appears before PVAN

Recommend screening renal transplant patients every 3 months for up to 2 years.
Positive patients are followed up with a renal biopsy

No effective anti-virals exist
Lower immunosuppression

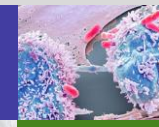
No consensus on the design of the PCR assays or standardization

Complicated due to homology with other viruses and matrix (urine)

Molecular Transition: Standard of Care

Evidence is in the Patient Care

Early Detection



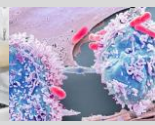
Treatment Efficacy

Independent of Immune Status

Disease Progression and Prevention

The NEW Standard of Care in Virology Detection and Treatment

Molecular Diagnostics



HIV

HIV there is a clear transition and acceptance of the NEW STANDARD of PRACTICE and CARE

Hepatitis B/C

Hepatitis B slow to transition due to serological efficacy.

Hepatitis C is also slow, but nearly there…

Transfusion medicine!

CMV/EBV

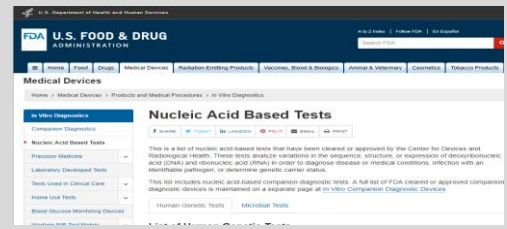
CMV has transitioned

EBV is beginning a transition

BK Virus

Disease association and clinical presentations are still problematic.

Simplicity of molecular testing; transitioning to Standard of Care is not apparent, yet.



https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm

UPDATED LISTING…