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PEARLS OF LABORATORY MEDICINE

The Molecular Testing of HIV

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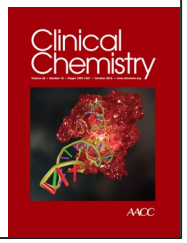


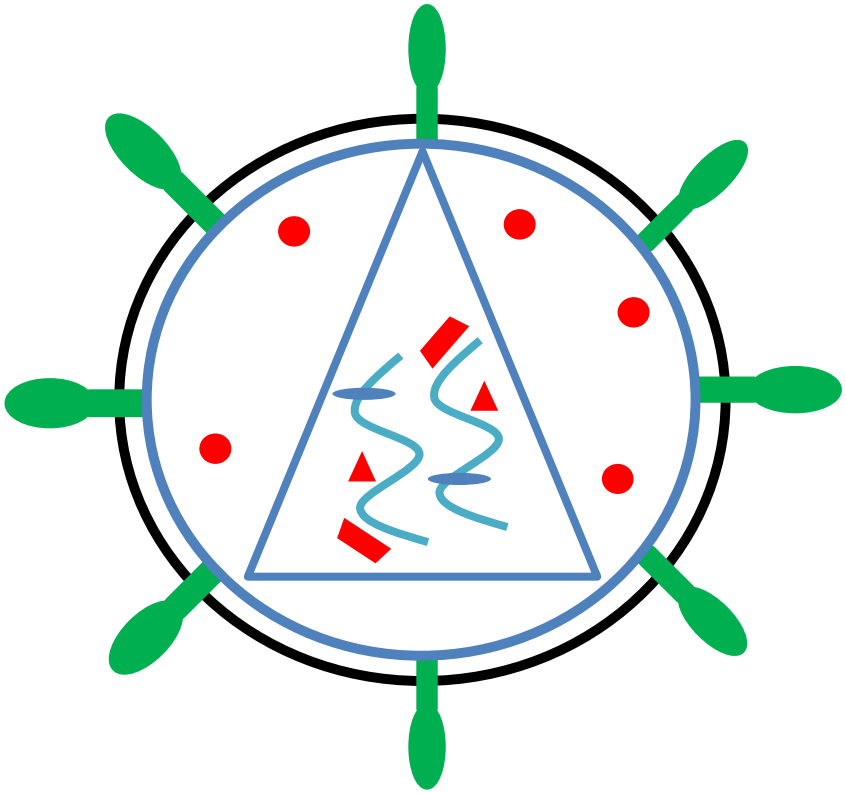
CLINICAL CHEMISTRY TRAINEE COUNCIL

Human Immunodeficiency Virus (HIV)

- Discovered in 1983 as the causative agent of acquired immune deficiency syndrome (AIDS)
- Causes 6,800 infections per day
- HIV-1
  - Derived from chimpanzees
  - Responsible for AIDS worldwide pandemic
  - Groups M (major), O (outlier), N (non-M, non-O), and the newly described group P
- HIV-2
  - Derived from sooty mangabeys
  - Limited geographic distribution (Africa and parts of Europe)
  - Subgroups A-H

Viral structure

- Enveloped single stranded RNA virus
- Belongs to the family Retroviridae
- Genome encodes for three main polyproteins
  - Core (gag): encodes matrix, capsid, and nucleocapsid
  - Envelope (env): encodes outer glycoprotein and transmembrane glycoprotein
  - Polymerase (pol): encodes protease, reverse transcriptase, Rnase, and integrase



Disease

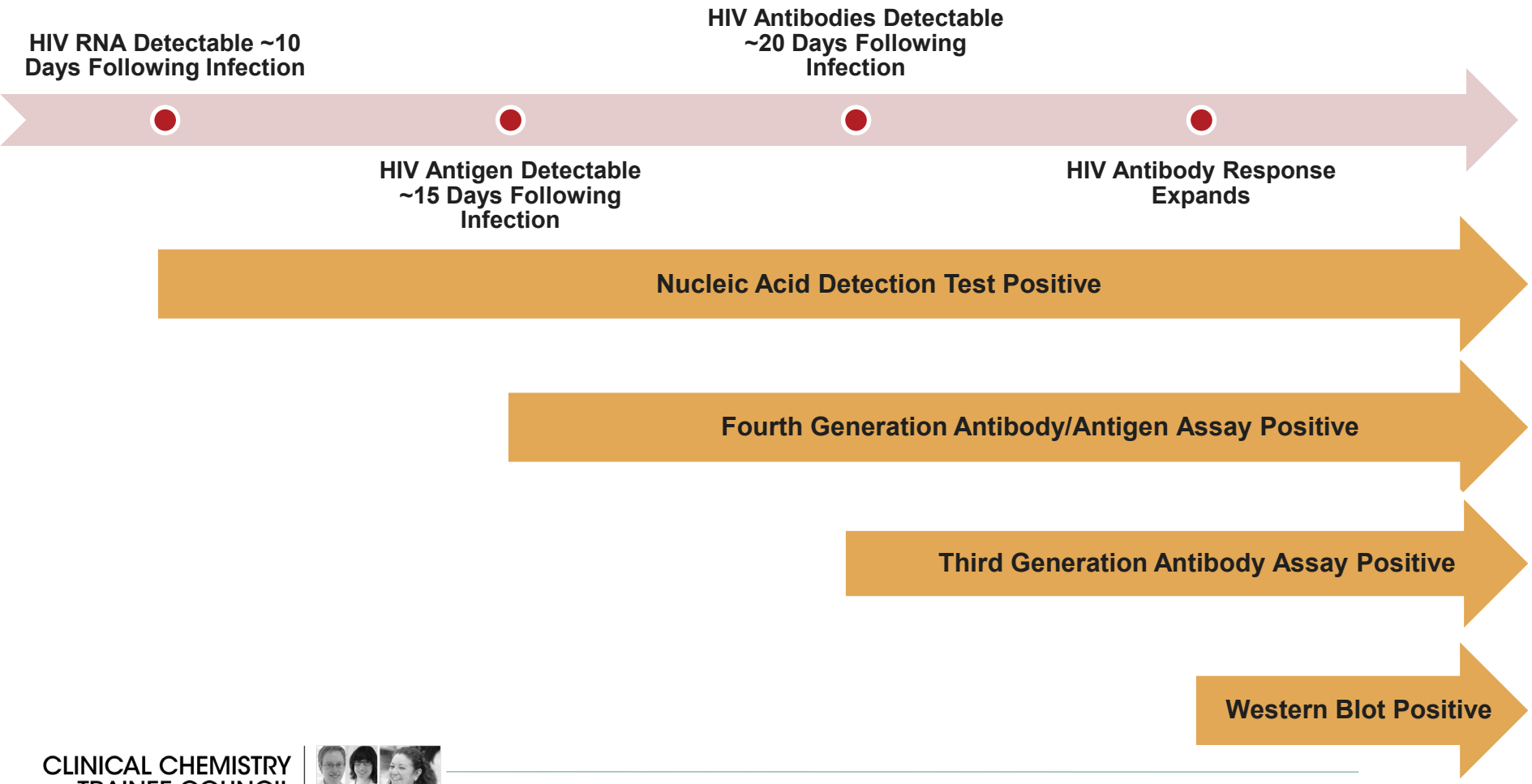
- Commonly transmitted sexually through direct contact with body fluids including blood, semen, and vaginal secretions
  - Can also be transmitted through inoculation of infected blood as a result of IV drug use, transfusion/transplantation, or occupational exposure
  - Can be transmitted from mother to child transplacentally, during birth, and during breastfeeding
- Virus has a tropism for CD4+ T cells and macrophages
- Three clinical stages of HIV infection
  - Acute HIV infection- associated with non-specific symptoms (fever, rash, malaise, lymphadenopathy, and pharyngitis)
  - Clinical latency- symptom free period with low level viral replication
  - AIDS- marked by increased viral load, decrease in CD4 positive T lymphocyte, and resultant opportunistic infections

Diagnosis of HIV

- Typically through serology
  - Until recently the diagnostic algorithm involved a third generation enzyme immunoassay (EIA) and a second-generation Western blot confirmatory test
  - Fourth generation diagnostic algorithm involves an antigen antibody screen with supplemental testing by HIV1/2 antibody differentiation assay

- Molecular tests also play an important role…

Lab Result Timeline



Role of Molecular Tests in Diagnosis

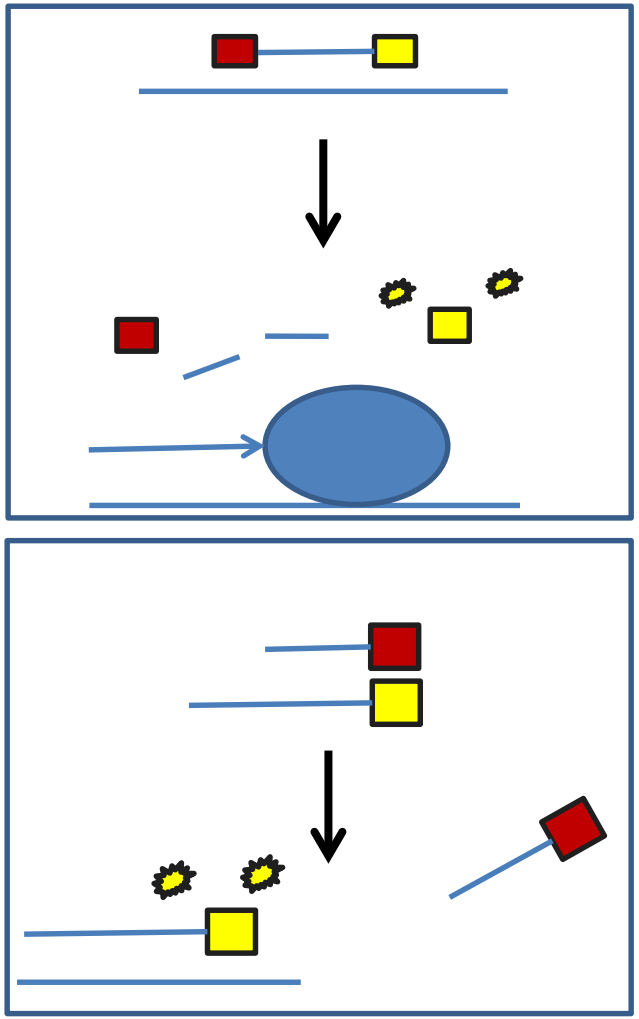
- Molecular testing can be used to investigate discrepant results
  - Recommended for patients with positive antigen/antibody screens, with negative HIV1/2 differentiation assays
- Currently only one FDA approved molecular test is available for the diagnosis of HIV-1
  - Detects viral RNA
  - Useful for the diagnosis of acute HIV
  - Useful for diagnosis in neonates
- Quantitative tests are FDA approved for monitoring only
  - More readily available than qualitative tests
  - Can be used as qualitative diagnostic tests following appropriate validation
- Important caveat: HIV-2 is not detected by these assays!

Managing HIV Positive Patients

- Patients receiving HIV treatment should be monitored for drug resistance
- CD4
  - Useful measurement of immune status at diagnosis
  - Conveys urgency of starting treatment for HIV and opportunistic infections
- Viral load
  - Most useful marker of disease progression both in untreated patients and patients on therapy

HIV-1 Quantitative Assays

- Only detect HIV-1
- Earlier developed assays used a variety of technologies
  - Conventional reverse transcription PCR
  - Branched DNA signal amplification
  - Nucleic Acid Sequence Based Amplification
- More recent viral load assays use “real time PCR”
  - Signal generated during amplification process by fluorescent probes
  - Less specimen manipulation, higher sensitivity and low limits of detection (20-40 copies/ml)



Viral Load Monitoring Guidelines

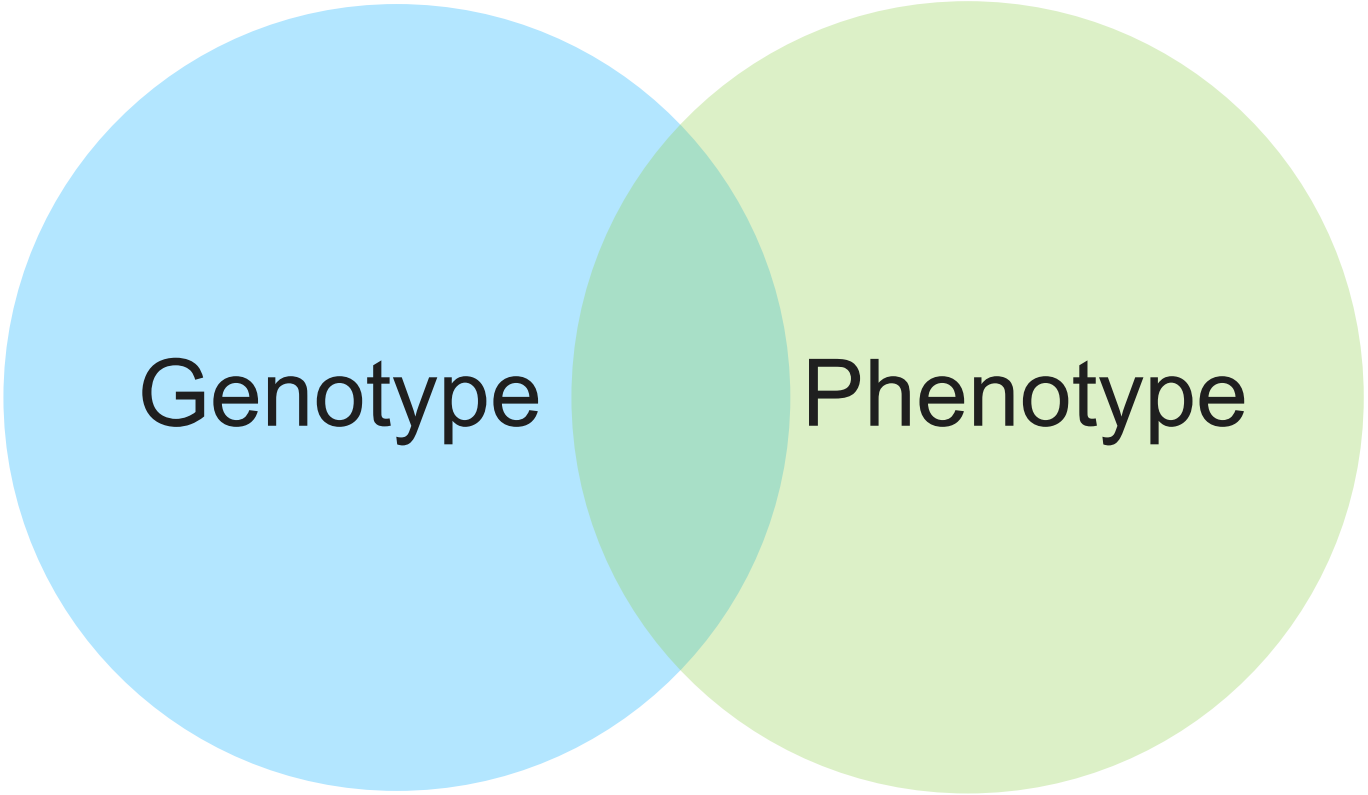
- Measure immediately before treatment and 2-8 weeks later
- Usually a 2 log decrease in viral load after first 2-3 months signifies drug response
- Should be undetectable by week 24
- Monitor every 3-4 months to ensure response
- Clinically significant change is >0.5 log copies/ml
  - Biologic variation 0.3 log copies/ml
  - Assay variation 0.2 log copies/ml

Viral Load Monitoring Guidelines

- Viral load blips
  - Defined by the Panel on Antiretroviral Guidelines (Department of Health and Human Services)
  - Viral loads transiently detectable at low levels (typically <400 copies/ml)
  - May be seen in patients with transient infections
  - May be after vaccination for influenza, tetanus, or pneumococcus
- Viral load blips should not be interpreted as treatment failure

Resistance Detection

- Performed before treatment is started and whenever treatment failure is suspected



Resistance Detection

- Genotyping
  - Commercially available tests involve extraction, amplification and sequencing of viral genomic material
  - Involve sequencing of reverse transcriptase and protease genes
  - Identify known resistance mutations through querying mutation databases
  - Other lab developed assays for the sequencing of other genes of concern (ie. integrase)

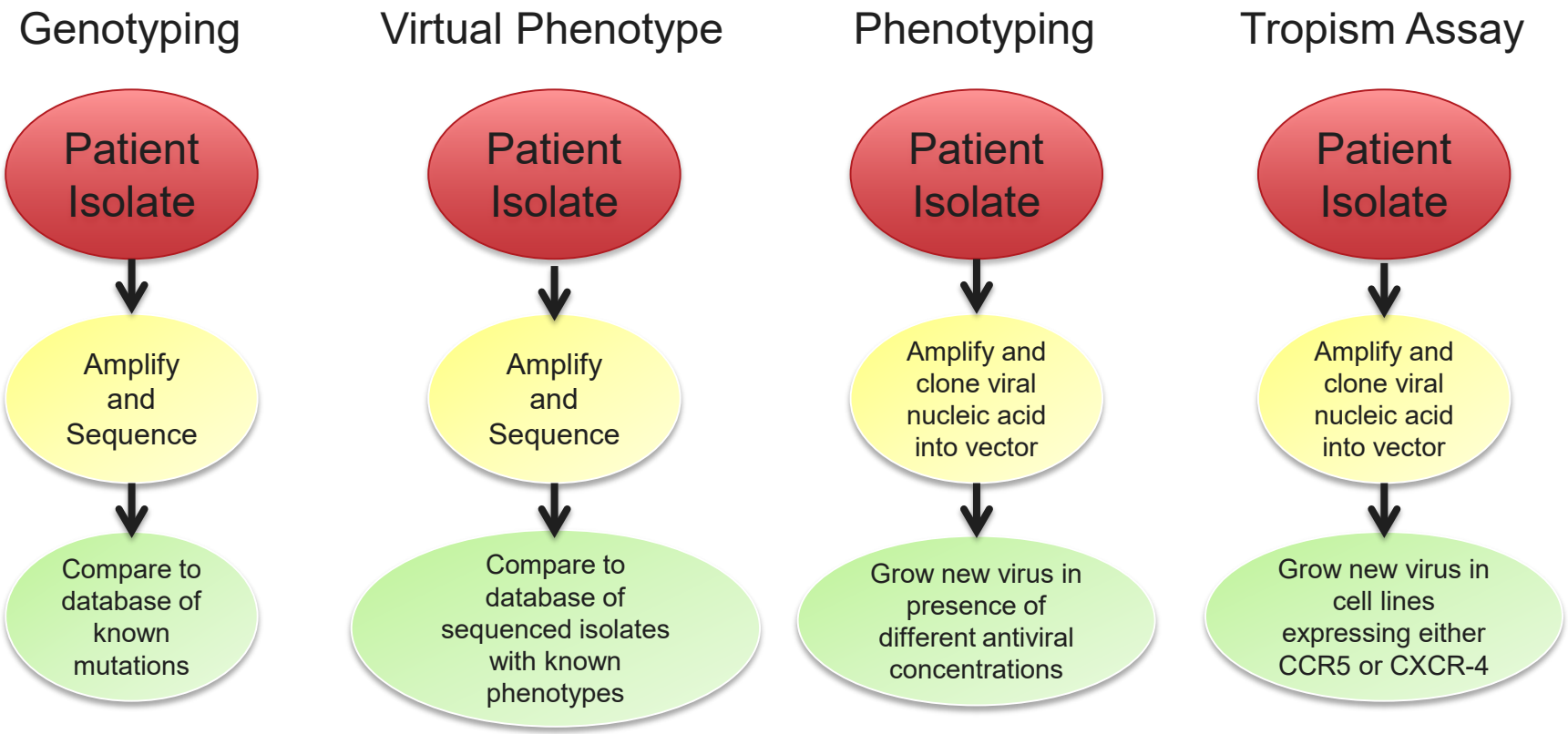
Resistance Detection

- Phenotypic methods
  - Several FDA approved tests available
  - Viral RNA isolated and amplified
  - Cloned into HIV vector with a reporter gene
  - Growth in cell culture quantified by the measurement of signal in the presence of varying concentrations of antivirals
  - The amount of drug necessary to inhibit 50% and 90% is reported
  - Typically able to test more drugs than those tested by commercial genotype assays

Resistance Detection

- Virtual phenotype
  - Combination of genotype and phenotype
  - Utilizes database of isolates characterized by both genotype and phenotype
  - When a patient isolate is genotyped this database is queried to obtain predicted phenotype
- Tropism assay
  - HIV binds either CCR5 or CXCR-4 as a co-receptor for cell entry
  - Miravaroc is a CCR5 entry inhibitor and only effective when virus is CCR5 tropic
  - To test for tropism viral genes are cloned into HIV vector with reporter gene
  - The ability of cloned vector to infect CCR5 positive cell lines and CXCR-4 positive cell lines is measured

Summary of Resistance Detection



Resistance Detection Limitations

- Genotyping Limitations
  - Only FDA approved tests are for reverse transcriptase and protease inhibitors
  - Interpretation is challenging since new mutations are constantly being identified
- Limitations for all methods
  - Require at least 500 viral copies/ml to be performed successfully
  - Mutation must comprise of 25-30% of viral population

Points to Remember

- 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 ordering

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Disclosures/Potential Conflicts of Interest

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