MOLECULAR DIAGNOSIS OF HIV: CHALLENGES IN AFRICAN COUNTRIES

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INTRODUCTION

Although there are numerous advantages in using rapid HIV antibody tests and non-rapid serological tests, there may be certain limitations.

• They can yield false-negative results for acute HIV infection (before or early after seroconversion) and

• false-positive results for uninfected HIV vaccine recipients and for infants with passive maternal antibodies derived from HIV-seropositive mothers.

• In addition, current rapid antibody testing strategies may yield false-positive interpretations unless relatively costly confirmation and/or follow-up tests are conducted.
The development of molecular techniques that access viral load and the development of genotypic resistance have revolutionized the diagnosis and treatment of HIV disease. Molecular methods are critical in detecting early infection and for managing patients on anti retroviral therapy whose viral infection may become resistant to therapy. Techniques involving PCR are, however more complex, time consuming and expensive than serological testing.
The HIV genome

vpr: G2 cell cycle arrest and nuclear import of the preintegration complex

net: down-regulation of cell surface CD4 and MHC class I

vif: virion infectivity factor

vpu: enhancement of virion release and CD4 degradation

tat: trans-activator of HIV promoter

rev: nuclear export of late, unspliced RNA
HIV has several major genes coding for structural proteins that are found in all retroviruses as well as several nonstructural ("accessory") genes unique to HIV. The HIV genome contains three major genes, 5'gag-pol-env-3', encoding major structural proteins as well as essential enzymes.

HIV-1 has two important regulatory elements: Tat and Rev and few important accessory proteins such as Nef, Vpr, Vif and Vpu which are not essential for replication in certain tissues.
Genomic Organization of HIV-1

LTR

gag

pol

vif

vpr

env

vpu

tat

nef

MA

CA

NC

p17

p24

p7

p6

PR

RT

IN

SU

gp120

TM

gp41

Structural Proteins

Enzymes

Coat Proteins

100 nm
HIV Replication cycle

1. Binding and Fusion: HIV uses CD4 molecules on the surface of the lymphocytes as a primary receptor.
   - Viral gp 120 binds to the CD4 molecule on the surface of CD4 T lymphocyte leading to the conformational changes exposing binding site for coreceptors (chemokine receptors) present on the surface of a CD4 T lymphocyte.

2. Reverse Transcription: Viral reverse transcriptase transcribes the single-stranded HIV RNA to double-stranded HIV DNA that subsequently moves to the nucleus.
During the process of reverse transcription the two single stranded (\(+\))sense RNA molecules that comprise the virus genome are converted into a double-stranded DNA molecule somewhat longer than the RNA templates due to the duplication of direct repeat sequences at each end—the long terminal repeats (LTRs).
3. **Integration**: An HIV enzyme called “integrase” integrates the HIV DNA to the host cell's own DNA. The integrated HIV DNA is called **provirus**. The provirus may remain inactive for several years, producing few or no new copies of HIV.

4. **Transcription**: When the host cell is activated, the provirus integrated in the host genome is also transcribed by host’s RNA polymerase to create copies of the HIV genomic material, as well as shorter strands of RNA called messenger RNA (mRNA).
   • The mRNA is used as a blueprint to make long chains of HIV proteins.
Integrate holds ends of linear DNA in close proximity and introduces staggered cuts at ends of LTRs and in cell DNA.

Integrated provirus:
- 2 bp lost at end of each LTR
- 6 bp direct repeats of cell DNA flanking the insertion
Integration of viral DNA

Viral genome (DNA from Reverse Transcription) → DNA splits → Host genome (DNA) → Spliced viral genome → Provirus
5. Synthesis/Assembly: An HIV enzyme “protease” cuts the long chains of HIV proteins into smaller individual proteins.

- As the smaller HIV proteins come together with copies of HIV's RNA genetic material, a new virus particle is assembled.
- Free viral nucleic acid exerts control over the host’s synthetic and metabolic machinery
  - DNA enters the nucleus and is transcribed into RNA
  - The RNA becomes a message for synthesizing viral proteins (translation)
- HIV is replicated and assembled in the cytoplasm
6. Release/ Budding

• The newly assembled virus pushes out ("buds") from the host cell.
• During budding, the HIV envelope also acquires host membrane proteins and lipid bilayer.
• HIV are liberated by **budding** or **exocytosis**.
• Anywhere from 3,000 to 100,000 virions may be released, depending on the virus.
• Entire length of cycle- anywhere from 8 to 36 hours.
MOLECULAR DIAGNOSIS OF HIV
Reaction Components

- DNA template
- Primers
- Enzyme
- dNTPs
- Mg$^{2+}$
- buffers
1- DNA template

- DNA containing region to be sequenced
- Size of target DNA to be amplified: up to 3 Kb
2- Primers

- 2 sets of primers
- Generally 20-30 nucleotides long
- Synthetically produced
- Complimentary to the 3’ ends of target DNA
- Not complimentary to each other
- Not containing inverted repeat sequences to avoid formation of internal structures
- 40-60% GC content preferred for better annealing
3-Enzyme

• Usually Taq Polymerase or anyone of the natural or Recombinant thermostable polymerases
• Stable at T\(^0\) up to 95\(^0\) C
• High processivity
• Taq Pol has 5’-3’ exo only, no proofreading
The PCR Cycle

- Comprised of 3 steps:
  1. Denaturation of DNA at 95°C
  2. Primer hybridization (annealing) at 40-50°C
  3. DNA synthesis (Primer extension) at 72°C
PCR Cycle - Step 1 - Denaturation Template DNA by Heat (95°C)
PCR Cycle - Step 2 –

Temperature is lowered ($T_m$) and primers anneal to target sequences.
At 72 °C *Taq* DNA polymerase catalyses primer extension as complementary nucleotides are incorporated.
End of the 1st PCR Cycle –

Results in two copies of target sequence
Target Amplification

1 cycle = 2 Amplicon
2 cycle = 4 Amplicon
3 cycle = 8 Amplicon
4 cycle = 16 Amplicon
5 cycle = 32 Amplicon
6 cycle = 64 Amplicon
7 cycle = 128 Amplicon

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Standard thermocycle

- Step 1: Denature Template
- Step 2: Anneal Primer
- Step 3: Primer Extension

- One "Cycle"
RT-PCR

- Reverse Transcriptase PCR
- Uses RNA as the initial template
- RNA-directed DNA polymerase (rTh)
- Yields ds cDNA
Reverse Transcription - Step 1 –
Primer Anneals to Target RNA Sequence
Reverse Transcription - Step 2 –

*rTth* DNA Polymerase also has RT activity Catalyses Primer Extension by Incorporating Complementary Nucleotides
PCR Step 1 - Denaturation by Heat
PCR Step 2 - Annealing of Primer to cDNA
PCR Step 3 - \textit{rTth} DNA Polymerase Catalyses Primer Extension
End of 1st PCR Cycle - Yields a Double-Stranded DNA Copy (Amplicon) of the Target Sequence
Detection of amplification products

• Gel electrophoresis
• Sequencing of amplified fragment
• Southern blot
• etc...
APPLICATION OF MOLECULAR DIAGNOSIS IN HIV RESEARCH

• HIV Drug Resistance Studies
• HIV Treatment Failure and Success (Virologic)
• HIV Genotyping and Sub-types
• Coinfections
HIV Drug Resistance: An Overview

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Keywords: HIV drug resistance mechanism, Antiretroviral Drug, Antiretroviral Drug Resistance

ABSTRACT

HIV-1 belongs to the retrovirus family. Highly active antiretroviral therapy (HAART) is the current standard of care for HIV infection. The ability of HIV to mutate and reproduce itself in the presence of drugs is called “HIV drugs resistance” (WHO). Resistance of HIV to Antiretroviral Drugs (ARV) is one of the most common causes for therapeutic failure in people infected with HIV. The first report of HIV-1 drug resistance was to zidovudine (ZDV) in 1989. If the viral load rises above 200 copies/ml, it might be sign of emergence of drug resistance mutations. Viral load monitoring is still not available for everyone in limited resource settings. There are several resistance testing tests: genotype, phenotype, virtual phenotype and integrase inhibitor resistance sequencing tests. Genotype, phenotype and virtual phenotyping testing’s used for NRTI, NNRTI and PI resistance mutations. ARV drug resistance in HIV is growing global concern. We should apply latest and current findings related to ARV drug resistance mechanisms. We should use resources wisely and in an effective manner.
Role of HIV Subtype Diversity in the Development of Resistance to Antiviral Drugs

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Abstract: Despite the fact that over 90% of HIV-1 infected people worldwide harbor non-subtype B variants of HIV-1, knowledge of resistance mutations in non-B HIV-1 and their clinical relevance is limited. Due to historical delays in access to antiretroviral therapy (ART) on a worldwide basis, the vast majority of reports on drug resistance deal with subtype B infections in developed countries. However, both enzymatic and virological data support the concept that naturally occurring polymorphisms among different nonB subtypes can affect HIV-1 susceptibility to antiretroviral drugs (ARVs), the magnitude of resistance conferred by major mutations, and the propensity to acquire some resistance mutations. Tools need to be optimized to assure accurate measurements of drug susceptibility of non-B subtypes. Furthermore, there is a need to recognize that each subtype may have a distinct resistance profile and that differences in resistance pathways may also impact on cross-resistance and the selection of second-line regimens. It will be essential to pay attention to newer drug combinations in well designed long-term longitudinal studies involving patients infected by viruses of different subtypes.

Keywords: HIV; drug resistance; subtypes; reverse transcriptase; protease; integrase
HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance

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**Objectives:** Monitoring regional levels of transmitted HIV-1 resistance informs treatment guidelines and provides feedback on the success of HIV-1 prevention efforts. Surveillance programs for estimating the frequency of transmitted resistance are being developed in both industrialized and resource-poor countries. However, such programs will not produce comparable estimates unless a standardized list of drug-resistance mutations is used to define transmitted resistance.

**Methods:** In this paper, we outline considerations for developing a list of drug-resistance mutations for epidemiologic estimates of transmitted resistance. First, the mutations should cause or contribute to drug resistance and should develop in persons receiving antiretroviral therapy. Second, the mutations should not occur as polymorphisms in the absence of therapy. Third, the mutation list should be applicable to all group M subtypes. Fourth, the mutation list should be simple, unambiguous, and parsimonious.

**Results:** Applying these considerations, we developed a list of 31 protease inhibitor-resistance mutations at 14 protease positions, 31 nucleoside reverse transcriptase inhibitor-resistance mutations at 15 reverse transcriptase positions, and 18 non-nucleoside reverse transcriptase inhibitor-resistance mutations at 10 reverse transcriptase positions.

**Conclusions:** This list, which should be updated regularly using the same or similar criteria, can be used for genotypic surveillance of transmitted HIV-1 drug resistance.

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**Keywords:** HIV-1, Antiretroviral drug resistance, protease, reverse transcriptase, mutations, epidemiology, surveillance
Molecular Epidemiology of HIV-1 Subtypes and Drug Resistant Strains in Taiwan

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The Taiwanese government has provided free highly active antiretroviral therapy since April 1997. Previously, we have reported on the molecular epidemiology of HIV-1 in Taiwan from 1988 to 1998. In addition, an outbreak of circulating recombinant form (CRF) 07_BC among intravenous drug users was noted in 2004. Therefore, the purposes of this study were to elucidate the distribution of HIV-1 subtypes among different high-risk groups in Taiwan from 1999 to 2000 and to conduct surveillance on drug resistance among treatment-naïve patients from 1997 to 2000. Blood samples from 239 HIV-1/AIDS patients and their subtypes were examined using DNA sequencing and phylogenetic analysis. The results showed that among 226 male patients, 213 (94.2%) had subtype B, 11 (4.9%) had CRF01_AE, 1 had unique recombinant strain related to both CRF07 BC and CRF08 BC (strain T12-99TW) and 1 had BC (strain L9312-00TW). The patients infected with T12-99TW and L9312-00TW were intravenous drug users and had needle-sharing experiences in Yunnan Province, China. Of the 13 HIV-1-infected females, 7 (53.8%) had subtype B, 5 (38.5%) had CRF01_AE, and 1 (7.7%) had subtype C. Phylogenetic analysis demonstrated that the neither strain T12-99TW nor L9312-00TW clustered with any CRF subtypes isolated from Taiwanese intravenous drug users in 2004. In addition, 126 treatment naïve patients were selected for genotypic DR analysis and the results showed that 4.3% (2/47) homosexual males had M184V mutations. This is the first report on the identification of CRF08 BC and a unique recombinant strain related to both CRF07 BC and CRF08 BC in Taiwan. J. Med. Virol. 80:183–191, 2008. © 2007 Wiley-Liss, Inc.

KEY WORDS: HIV drug resistance; molecular epidemiology; HIV subtype

INTRODUCTION

Phylogenetic analyses of human immunodeficiency virus type-1 (HIV-1) strains have identified three distinct groups—major (M), outlier (O), and novel (N) groups. More than 99% of the HIV-1 strains in this pandemic belong to group M and can be classified into nine subtypes (A, B, C, D, F, G, H, J, and K), 16 circulating recombinant forms (CRFs) and at least 30 unique recombinant forms (URFs) [Los Alamos National Laboratory, 2004; Takebe et al., 2004]. If an intersubtype recombinant virus is transmitted from one person to another, and becomes one of the circulating strains in the HIV epidemic, it can be classified as a CRFs [Robertson et al., 2001]; if there is no evidence of

Dr. Yu-Ching Lan and Dr. Tarek Elbeik contributed equally to this study.

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Studies on antiretroviral treatment failure among HIV positive clients attending Aminu Kano Teaching Hospital, Kano, Nigeria

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Antiretroviral (ART) treatment failure is a major obstacle in the management of HIV positive clients. This study was carried out to determine the prevalence of ART treatment failure among HIV positive patients attending Aminu Kano Teaching Hospital. The study was conducted by assessing immunological (CD4 cells count) and virological (viral load test) parameters. Of the 120 HIV positive patients tested, 27 (22.5%) had immunological failure, while seven (5.8%) had virological failure. Overall, 5 (4.1%) met the criteria for treatment failure. There was no significant difference between CD4 cells count at first and second visits (P = 0.118). There was also no significant difference between viral load at first and second visits (P = 0.671). When subjected to correlation analysis, the study showed negative correlation between CD4 cells count and viral load test (r = -0.68201). The number of patients with treatment failure deduced from the research is ascribed to non-adherence of patients to medication, which is a major obstacle in the treatment and management of HIV. Plans to counter this obstacle are highly recommended. Government and concerned organizations can also greatly make a positive impact with regards to the cost of the tests carried out so as to ensure adequate monitoring of HIV patients’ health status.

Key words: Treatment failure, HIV, CD4 lymphocyte count, viral load.
HIV/AIDS Epidemiology in Kano, Nigeria

Various governmental and non-governmental efforts are being exerted to combat the HIV/AIDS pandemic by sentinel surveys. Unfortunately, there has not been any organized survey in Nigeria to extensively determine the opportunistic infections associated with HIV/AIDS. This book therefore, highlights specifically on some pulmonary bacterial and fungal opportunistic infections in Kano metropolis, the isolation and identification of opportunistic bacterial and fungal agents causing pulmonary infections, the relationships between pulmonary opportunistic pathogens and CD4 count and immune status and viral load of HIV positive clients. This will contribute towards management and care of AIDS clients and will provide the need for monitoring by health care facilities and related agencies.

Usman Aliyu Dutsinma

Human resources and infrastructure that are required for successful Molecular assays, such as trained personnel, clean water, and electricity, may not be found in most African cities. Even the very few Reference laboratories that have the personnel, equipment, and infrastructure to perform CD4 cell counts and viral load testing may lack the resources to purchase the kits. Thus, what works for one country in Africa, or even for one city within a country, may not apply in all resource-limited settings.
The challenge of sustainability is expedient for the long-term viability of quality diagnostic testing. This includes:

• Tapping into local economies for manufacturing,

• Developing a supply chain, and working to ensure that distribution channels meet the needs.

Lack of capacity for Molecular studies on HIV in Tertiary Health centres and Higher Institutions.
CONCLUSION

Although PCR may offer some advantages over serologic techniques, efforts to build the capacity of more laboratories to perform PCR techniques should not out phase efforts to maintain laboratories that are able to perform reliable serologic diagnosis of HIV.

Even though numerous laboratories in major African cities are employing highly sophisticated laboratory protocols, including PCR-based assays, they are however, too few to keep pace with the scale of the HIV epidemic.

The combination of information collected using PCR technology with virologic and immunologic assays will help to broaden our understanding of HIV genetic variation and its role in transmission, pathogenesis, disease progression, drug resistance, and vaccine development.
During Training workshop on HIV diagnosis with Prof. Robert Gallo, Director, Institute of Human Virology, University of Maryland Baltimore, USA 2016
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Thank you for Listening