SMARThivDRmos: A complexity-free and Cost Effective Technology for Monitoring HIV Drug Resistance in Resource-Limited Situations

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Abstract

We design an HIV drug resistance monitoring system that is amenable to resource-poor and low expertise countries: SMARThivDRmos. Our system overcomes cost and complexity barriers, the two main factors that have so far limited access of HIV drug resistance monitoring to resource-poor and low expertise countries. We reduce complexity by transferring complex steps to computer execution, and we reduce cost by obviating sequencing requirement using DNA hybridization methods.
We reduce both cost and complexity by importing template sequences for probe design, from freely accessible public HIV drug resistance databases using our SMARTsearch engine. We reduce complexity related to probe design in the DNA hybridization step by incorporating SMARTProbeMarker, a user-friendly version of a probe design algorithm, into the system. We reduce complexity related to HIV drug resistance interpretation, using web-based freely accessible public HIV drug resistance interpretation systems. We reduce cost by obviating sequencing requirement, using SMARThivDRmos DNA hybridization-based protocol for identifying HIV drug resistance mutations.
Compared to previously developed HIV drug resistance monitoring systems that comports a sequencing step, SMARThivDRmos is a sequencing-free analysis system. SMARThivDRmos is a complexity-free and Cost effective alternative technology that is suitable for monitoring HIV drug resistance in resource-poor and low expertise countries.

CONTEXT AND MEDICAL RELEVANCE

Since 2003, through the World Health Organisation (WHO) 3by5 project, AntiRetrovirals (ARVs) are being massively introduced into developing countries. [1] Furthermore through successful WHO and concerned institutions negotiations, cost of ARVs have substantially dropped, thereby creating grounds for increase access to ARVs, to the developing world. [2] Except few countries such as South Africa, no HIV drug resistance monitoring system exists in most recipient countries.

At least three expert panels have recommended HIV drug resistance testing in the clinical management of HIV patients. [3,4,5] Numerous studies have established that patients, whose physicians have access to HIV drug resistance data, showed decreased HIV related morbidity and mortality compared to patients whose physicians lack these data. [6]

Furthermore, increase access to ARVs without a proportional increase in HIV drug resistance monitoring capability could lead to an epidemic of HIV drug resistant strains, that can become an important public health problem in choosing effective therapy. [7,8] According to The Task Force on Innovation, Science and, Technology for the Millennium Developmental Goals, “this is the beginning of a process that recognizes the emergence of a globalized world that requires collective action to deal with issues once considered strictly national “. [9]

HIV drug resistance testing is now part of routine tests in the clinical management of HIV patients in developed countries. [6] Yet cost and complexity have limited access of drug resistance testing to HIV patients in developing countries. [10] The standard Genotypic assays using dideoxynucleoside sequencing cost $ 250-500 USD per test. [11] A Cost, that is far over the reach of the health budget of $1-$2 USD per inhabitant, in most developing countries. [12]

Furthermore, interpretation of HIV genomic sequence for resistance to AntiRetrovirals drugs (ARV) is a complex process that required expertise that is scarce in most developing countries. [12]

Point 6 of the Millennium Developmental Goals (MDG) aims to "have halted by 2015, and begun to reverse, the spread of HIV/AIDS". The Working Group on HIV/AIDS underscores the importance of scaling up both essential HIV prevention services and antiretroviral treatment. "Key to scaling up HIV services, particularly antiretroviral treatment, will be sustained investment in health systems, especially the healthcare workforce". [13] The Task Force on Science, Technology, and Innovation underscores the critical importance of knowledge and innovation for development in every country. [9]

Expert panels have recommended the so-called "Alternative technologies" for the clinical management of HIV patients in the developing world. [2] Under recommendation of the World Health Organisation Network on Diagnostic Support for HAART, researches have been undertaken to develop cheaper and simpler technologies for the clinical management of HIV patients in developing countries. [9,10,12] The importance of "alternative technologies" in the clinical management of HIV patients in the developing world, was previously demonstrated in Senegal (West Africa), through the introduction of the less complex and cheaper alternative Dynabeads technology for monitoring CD4 number. [14] Complexity and cost of the Dynabeads technology were further reduced at the International Medical Center of Japan, thereby creating grounds for its widespread use in "resource-limited situations". [15] Also, a number of simpler and cheaper, yet sensitive HIV viral load monitoring systems, accessible to developing countries, have been developed. [2] $SMART_{hivDRmos}$ is a complexity-free and cost effective, yet sensitive HIV drug resistance monitoring system that is amenable to resource-limited countries.

$SMART_{hivDRmos}$ alleviates cost related to HIV drug resistance mutation identification, using DNA hybridization-based protocols. A number of cheaper, yet sensitive DNA hybridization-based technologies for identifying HIV drug resistance mutations have been developed. [16, 17, 18] Key to success of these DNA hybridization-based technologies lied in DNA probe design. A complex step that requires expertise in DNA hybridization thermodynamics. Few clinical laboratories in developing countries have the necessary expertise to successfully predict a DNA probe from "raw genomic sequence", for HIV drug resistance mutation identification. A number of DNA probe design algorithms have been developed. [19, 20, 21] The Uppsala University "Probe Marker" can predict DNA probe sequence for hybridization-based genetic analysis. However, the use of "Probe Marker" will require some computer literacy, an expertise barrier that may limit the usefulness of "Probe Marker" in clinical settings, in developing countries. A user-friendly version of "Probe Marker" can overcome the expertise barrier.

Previously designed DNA hybridization-based HIV drug resistance mutation identification technologies comport a sequencing step for template analysis for probe design purpose. [18] SMARThivDRmos obviates sequencing requirement. In lieu of sequencing, our system imports sequences from freely accessible web-based HIV drug resistance databases. The Biotech tropicana, inc SMARTsearch engine is an algorithm designed to retrieve HIV drug resistance genotypes from available HIV drug resistances databases [22], using ARVs as input. SMARTsearch submits the retrieved HIV resistance genotypes to a modified version of the Uppsala University ProbeMarker algorithm (SMARTprobemarker). SMARTprobemarker can design HIV drug resistance mutation identification probes using the SMARTsearch submitted drug resistance genotypes as template. The designed-probes are used for mutation identification in the DNA hybridization-based mutation identification step.

Visualisation technologies, as applied to hybridization-based HIV drug resistance mutation identification, were previously developed at the AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan. [8,18] The Japanese visualisation technologies showed higher sensitivity and specificity compared to previously developed systems. [23, 24, 25] The Japanese visualisation technology was optimized for the recombinant A_E HIV subtypes, the most prevalent HIV subtype in South East Asia. The Biotech tropicana, inc SMARTvis visualisation system aims to expand and optimize visualisation existing systems to HIV subtypes that are prevalent in developing countries world-wide.

Notwithstanding the hurdles of "raw genomic sequence" analysis and interpretation for HIV drug resistance, most developing countries lack DNA sequencing facilities, and cost of extra-facility sequencing is another burden to the weak health budget characteristic of most developing countries. $SMART_{hivDRmos}$ alleviates complexity related to HIV drug resistance mutation interpretation, using web-based HIV drug resistance interpretation algorithms. A number of HIV drug resistance interpretation algorithms have been developed. [26] The web-based freely accessible

HIV drug resistance interpretation algorithms are capable of interpreting HIV drug resistance from HIV "clinical" drug resistance genotypes stored in their databases, thereby obviating interpretation requirement from raw genomic sequence, and alleviating the expertise barrier in HIV drug resistance interpretation that is characteristic of most developing countries. Few, clinical settings in developing countries, have the necessary expertise to interpret HIV drug resistance mutation for medical use, from "raw genomic sequence". However, the analytical capabilities of most computer-based interpretation algorithms are limited to "population" of sequences, thereby limiting their application to individuals in clinical settings. As of a general statistical rule, inference of biomedical parameters for application to "individual", from identical parameters obtained from a biostatistical analysis of a "population-based data", is at best error-prone. [27] Where population-based data can provide guidance in choosing appropriate ARV combination, a confirmatory test at individual level must be done. In developed countries, target fragments of HIV genome from 'individual' patients, are sequenced and submitted to expert interpretation unit for analysis for HIV drug resistance. The Biotech tropicana, Inc HIV Drug Resistance Interpretation Unit (BTI-DRIU) provides human confirmation of computer-based HIV drug resistance interpretation decisions using DNA hybridisation-based identified HIV drug resistance mutations, in lieu of sequence.

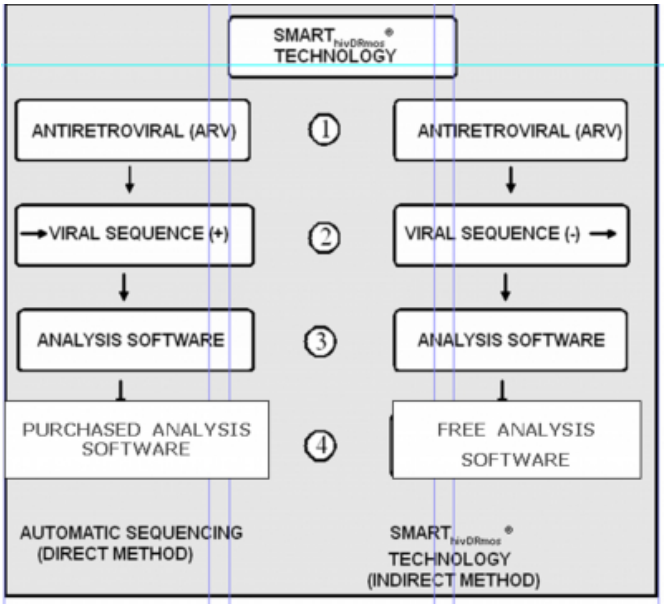
Most cities in developing countries are now internet capable. A Cost of $ 0.5-1 USD per hour internet connection is the norm. Biotech tropicana, Inc HIV drug resistance interpretation unit provides technical support for "individual" confirmatory HIV drug resistance test, to internet capable peripheral health centres, in developing countries.

SYSTEM DESIGN

The main features of SMARThivDRmos are its simplicity and low cost, achieved through a "re-design" of existing principles in standard HIV drug resistances diagnosis systems. [28,29] Specific features are added to optimize the system. [FIG. 1&2]

Figure 1

Figure 1: Comparison of SMARThivDRmos technology with the standard genotypic assays using dideoxynucleoside sequencing.

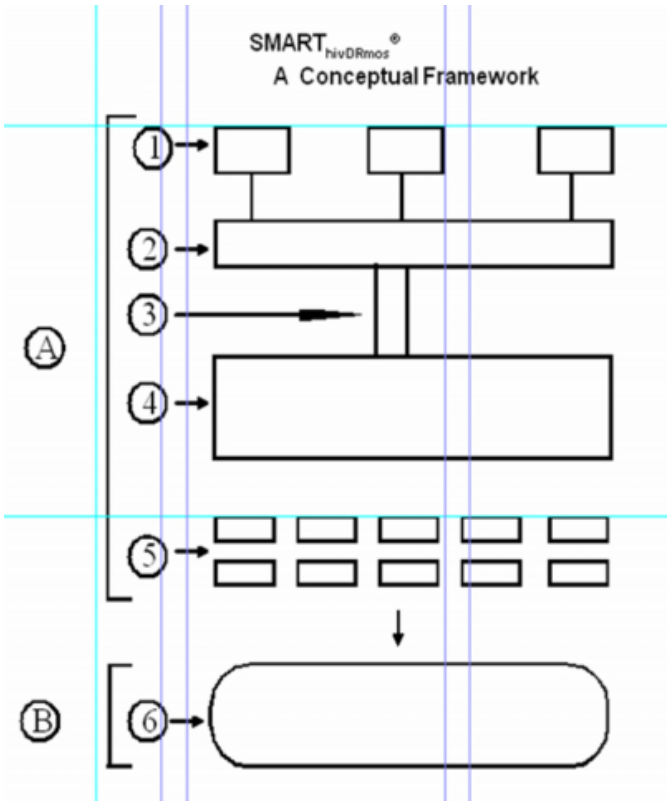


For a given drug regimen (1), the standard assays relied on sequencing (2, left) to determine HIV drug resistance mutations, SMARThivDRmos is a sequencing-free (2, right) mutation identification system.

The standard genotypic assays using dideoxynucleoside sequencing comports an analysis software that is purchased with the kit (3, left), SMARThivDRmos uses freely accessible web-based public HIV drug resistance analysis software (3, right). The standard genotypic assays using dideoxynucleoside sequencing is a biostatistical analysis-free system that "automatically" displays identified HIV drug resistance mutations (4, left), SMARThivDRmos comports a biostatistical analysis module for identifying the "best candidate probes" for mutation identification (4, right).

Figure 2

Figure 2: SMARThivDRmos, A Conceptual Framework.



Using ARVs as input (1), the SMARTsearch engine (2) imports "clinical" HIV drug resistance sequences from public HIV drug resistance databases. SMARThivDRmos comports a Communication Platform (3) through which imported sequences are transmitted to SMARTProbeMarker (4). SMARTProbeMarker designs the Best Candidate Probes (5) which are used for mutation identification using SMARThybprot DNA hybridization protocol (6).

In SMARThivDRmos the complex steps (1-5) are computer-performed (A). Human input is reduced to execution of the SMARThybprot DNA hybridization protocol (B).

Compared with the standard genotypic assays using dideoxynucleoside sequencing, SMARThivDRmmos is a sequencing-free analysis system. In SMARThivDRmos, sequence is obtained from public HIV drug resistance databases, using the SMARTsearch engine. [FIG.1, point 2.]

HIV genomic drug target sequences from individual patients are analysed for drug resistance mutations, using analysis software contained in the standard assays kits. [28,29] In SMARThivDRmos, a list of mutations known to confer resistance to ARVs, is analysed using freely accessible web-based HIV drug resistance interpretation systems. [30,31,32,33] [FIG1, point 3] The list of HIV drug resistance mutations is obtained from individual patients using SMARThivDRmos DNA hybridization protocol, in lieu of sequencing.

A biostatistical analysis module is used to select the "best candidate probe" for DNA hybridization-based mutation identification. [FIG1, point 4]

THE SMARTSEARCH ENGINE

SMARTsearch is a search engine designed to retrieve HIV genomic sequence from public HIV drug resistance databases, using ARV as input. [FIG2, point 1&2] More than 10 academic systems for interpreting HIV drug resistance are available, 6 of which are freely accessible through the internet. [26] These systems relied on HIV drug resistance genomic sequences stored in databases. The sequences in the databases are linked to other data such as treatment history, including ARV treatment history, of patients from whom the sequences were collected. SMARTsearch is an algorithm designed to retrieve HIV drug resistant sequences from these public databases, using ARVs as input.

THE COMMUNICATION PLATFORM

A communication platform is added between the SMARTsearch engine and the SMARTProbeMarker engine, to allow sequence transmission from SMARTsearch to SMARTProbeMarker. [FIG2, point 3]

THE SMARTPROBEMARKER ENGINE

SMARTProbeMarker is designed to accept HIV drug resistance sequences from the SMARTsearch engine and to design hybridization probes for downstream processing. [FIG2, point 4] ProbeMarker is a probe design program developed at Uppsalla University, Sweden. [23] We modified ProbeMarker (with author permission) by incorporating a specific platform that allows ProbeMarker to accept sequences from the SMARTsearch engine.

THE BIOSTATISTICAL ANALYSIS MODULE

SMARThivDRmos comports a biostatistical analysis module for selecting the "best candidate probe" in response to HIV high genetic variability. For a user given template sequence, ProbeMarker designs a set of probe, and choose candidates probes based on user specified parameters. However, ProbeMarker is designed as a general purpose genetic analysis tool. [19] Considering HIV high polymorphism, we add an analysis module for choosing the "best candidate probe" for point mutation identification in HIV target

sequences. This feature makes SMARThivDRmos specificity and sensitivity "adjustable" to country-specific context, in response to variability in HIV subtypes geographic distribution.

SMARTHYBPROT HYBRIDIZATION PROTOCOL

The SMARTProbeMarker designed DNA probes are used for point mutation identification using the SMARThybprot DNA hybridization protocols. Standard DNA hybridization protocols are optimized through appropriate modifications to increase specificity and sensitivity. [8,18,23,24,25]

SMARTVIS VISUALISATION SYSTEM

Systems for visualization of DNA hybridization-based identified point mutations were previously developed. These systems relied essentially on enzyme-linked immunosorbent assays [18,24], or modified DNA gel electrophoresis [8], or radiolabelling. [25] Compared to the standard genotypic assays using dideoxynucleoside sequencing, some of the DNA hybridization-based visualisation systems showed higher sensitivity and specificity. [8,18] SMARTvis protocol is designed to allow appropriate modifications to be added to increase visualization specificity and sensitivity, on case by case basis. For SMARTvis adopts and improves previous systems that demonstrated higher sensitivity and specificity than the standard genotypic assays using dideoxynucleoside sequencing, SMARTvis is of higher sensitivity and specificity than the standard genotypic assays using dideoxynucleoside sequencing.

CONCLUSION

We demonstrate the feasibility of HIV drug resistance monitoring in resource-poor and low expertise countries. Our system overcomes cost and complexity barriers, the two main obstacles that have so far limited access of HIV drug resistance testing to HIV patients in developing countries. Compared to previously developed DNA hybridization-based mutation identification protocols, our system is far more simpler, for we transfer complex steps requiring complex analysis to computer execution. We reduce human input to a mere execution of the hybridization protocol. Furthermore, our system is more sensitive than the standard genotypic assays using dideoxynucleoside sequencing that is complex and expensive. Considering the flexibility of SMARTProbeMarker, the sensitivity and specificity of SMARThivDRmos can be adjusted to country specific context in response to HIV high genetic variability, at the level of probe design. SMARThivDRmos is a "simple", "low cost", "high sensitivity and specificity" HIV drug resistance monitoring technology that is amenable to resource-poor and low expertise countries.

FUTURE DIRECTIONS

We are completing the development and optimization of the SMARTsearch program, the SMARTProbeMarker program, and the biostatistical analysis module.

Development and optimization of the SMARThivDRmos DNA hybridization protocol is in progress.

$SMART_{hivDRmos}$, is an intended component of the Biotech tropicana, inc SMARThivpack , a three tests combo kit for the clinical management of HIV patients in developing countries, consisting of:

$SMART_{hivDRmos}$ A Simple (complexity-free) and affordable (cost effective) Technology for Monitoring HIV Drug Resistance in Resource-Limited Countries,

$SMART_{hivCD4mos}$ A Simple (complexity-free) and affordable (cost effective) Technology for Monitoring CD4 in Resource-Limited Countries, and

$SMART_{hivVLmos}$ A Simple (complexity-free) and Affordable (cost effective) Technology for Monitoring viral load in Resource-Limited Countries.

The Biotech tropicana, Inc SMARThivpack is an intended "generic" HIV patient clinical management box, for the developing world. Most equipment required for executing the three-tests are "cross-usable", thereby reducing equipment cost. Considering HIV treatment objectives for the developing world, under point 6 of the Millennium Developmental Goals, [13] a large number of clinical follow up centres will be required for compliance with experts set treatment guidelines. [3,4,5] The modest savings in equipment cost per follow up centre, through the development of a "generic" follow up box, would yield huge savings in follow up cost on global scale. SMARThivpack is an ideal companion to generic AntiRetroViral drugs.

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NOTE

ProbeMarker is a probe design algorithm developed by

Johan Stenberg at Uppsala University. ProbeMarker is accessible for use and modification under the terms of GPL license. We receive confirmation from the author to use and modify the software under the terms of the GPL license. SMARTProbeMarker must be and is freely accessible for use and modification by others under the terms of GPL license.

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