Tan Tock Seng Hospital

IIDE
Institute of Infectious Diseases and Epidemiology

Challenges with HIVDR Testing in a Real-World LMIC Setting

Dr. Arlene Chua

Consultation on Global Trends of HIV Drug Resistance

Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital

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Challenges to HIV VL testing
(= same challenges to HIV GRT)

- Limited access to testing
- Majority of testing – centralized laboratories
- In other areas – rural or peri urban settings – challenges remain
  - Need for transportation (either samples or patients)
  - Results reporting: interpretation, timely reporting
  - Subsequent follow up of patients and access to 2nd and 3rd line ARV
- Cost of testing

HIV VL

- Simplification of sample transport – using DBS (with whole blood)
- Validation of DBS with lab platforms from Abbott, bioMérieux, Roche and Siemens
- Burden and cost of sample transport to a centralized lab

Note: Trade off of DBS for VL monitoring: slightly higher threshold for the lower limit of quantitative detection, due to presence of cell associated RNA and DNA that would have otherwise be extracted during centrifugation.

A2.9 Hold the Microsafe pipette horizontally and allow it to fill by capillary action to the defined volume.

A2.10 Dispense the blood in the center of the first circle of the card.

A2.11 Allow another large drop of blood to form at the puncture site and collect it to fill the next circle.

A2.16 Place completed DBS cards on the rack to dry. Make sure that the cards do not touch each other.

A2.17 Let the DBS cards dry for at least 3-4 hours. Keep out of direct sunlight.

1. Access to GRT (DBS) very limited:

Not many labs have validated DBS procedures for HIV GRT for clinical use (outside of research or surveillance work)

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Field Evaluation of a Broadly Sensitive HIV-1 In-House Genotyping Assay for Use with both Plasma and Dried Blood Spot Specimens in a Resource-Limited Country

Seth Inzaule,[a] Chunfu Yang,[c] Alex Kasembeli,[a] Lillian Nafisa,[a] Jully Okonji,[a] Boaz Oyaro,[a] Richard Lando,[a] Lisa A. Mills,[b] Kayla Laserson,[b] Timothy Thomas,[b] John Nkengasong,[c] Clement Zeh[b]

Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya[a]; Division of HIV/AIDS Prevention, U.S. Centers for Disease Control and Prevention, Kisumu, Kenya[b]; Division of Global HIV/AIDS, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA[c]

HIV-1 drug resistance (HIVDR) assays are important tools in clinical management of HIV-infected patie therapy (ART) and surveillance of drug-resistant variants at population levels. The high cost associated v hinders their use in resource-limited settings. We adopted and validated a low-cost in-house assay using dried blood spot (DBS) samples with a median viral load (VL) of 58,187 copies/ml, ranging from 253 to 3 commercial assay ViroSeq. Results indicated that the in-house assay not only had a higher plasma genoty ViroSeq (94% versus 78%) but also was able to genotype 89.5% (51/57) of the matched DBS samples with V The sensitivity in detecting DR mutations by the in-house assay was 98.29% (95% confidence interval [C plasma and 96.54 (95% CI, 95.93 to 97.15) on DBS, and the specificity was 99.97% (95% CI, 99.91 to 100.(types compared to ViroSeq. The minor DR mutation differences detected by the in-house assay against V clinical significance. In addition, cost analysis showed that the in-house assay could reduce the genotypi both plasma and DBS compared to ViroSeq. This field condition evaluation highlights the potential utili subtype-independent, in-house genotyping assay using both plasma and DBS specimens for HIVDR clin population-based surveillance in resource-limited settings.

JCM

Field Study of Dried Blood Spot Specimens for HIV-1 Drug Resistance Genotyping

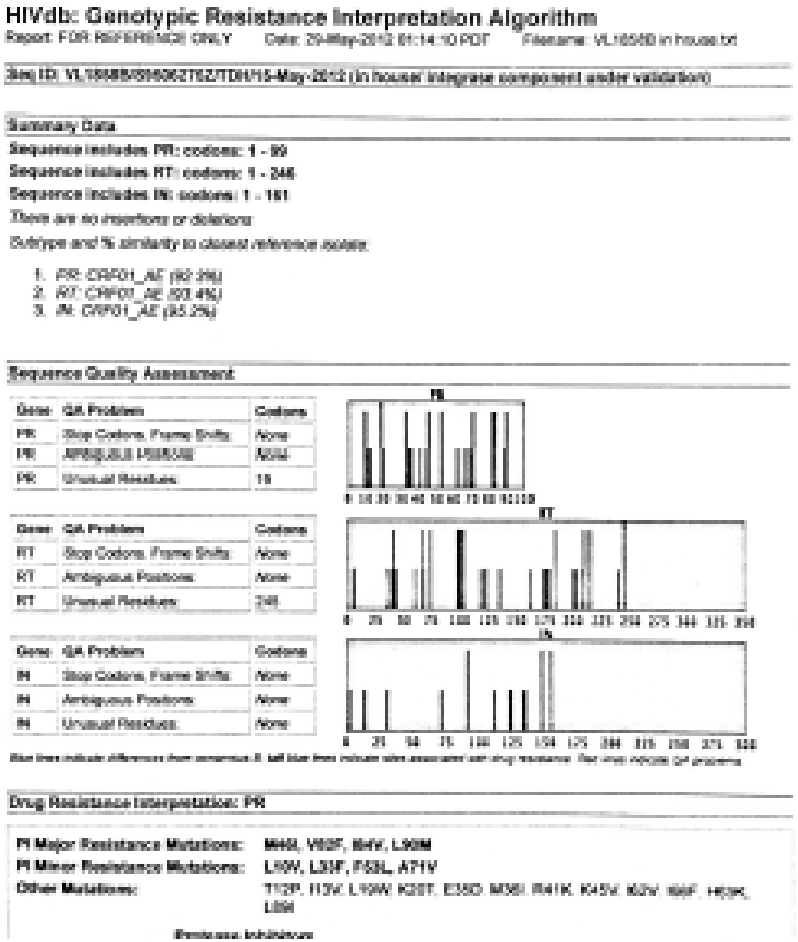
C. M. Parry,[a,b] N. Parkin,[c] K. Diallo,[d] S. Mwebaza,[e] R. Batamwita,[e] J. DeVos,[d] N. Bbosa,[f] F. Lyagoba,[e] B. Magambo,[e] M. R. Jordan,[g] R. Downing,[f] G. Zhang,[d] P. Kaleebu,[e] C. Yang,[d] S. Bertagnolio[h]

MRC/UVRI Uganda Research Unit on AIDS, Uganda Virus Research Institute, Entebbe, Uganda[a]; MRC/UCL Centre for Medical Molecular Virology, UCL, London, United Kingdom[b]; Data First Consulting, Belmont, California, USA[c]; Division of Global HIV/AIDS, CGH, CDC, Atlanta, Georgia, USA[d]; Mildmay Centre, Kampala, Uganda[e]; CDC-Uganda, Entebbe, Uganda[f]; Division of Geographic Medicine and Infectious Disease, Tufts University School of Medicine, Boston, Massachusetts, USA[g]; HIV Department, World Health Organization, Geneva, Switzerland[h]

Dried blood spots (DBS) are an alternative specimen type for HIV drug resistance genotyping in resource-limited settings. Data relating to the impact of DBS storage and shipment conditions on genotyping efficiency under field conditions are limited. We compared the genotyping efficiencies and resistance profiles of DBS stored and shipped at different temperatures to those of plasma specimens collected in parallel from patients receiving antiretroviral therapy in Uganda. Plasma and four DBS cards from anti-coagulated venous blood and a fifth card from finger-prick blood were prepared from 103 HIV patients with a median viral load (VL) of 57,062 copies/ml (range, 1,081 to 2,964,191). DBS were stored at ambient temperature for 2 or 4 weeks or frozen at −80°C and shipped from Uganda to the United States at ambient temperature or frozen on dry ice for genotyping using a broadly sensitive in-house method. Plasma (97.1%) and DBS (98.1%) stored and shipped frozen had similar genotyping efficiencies. DBS stored frozen (97.1%) or at ambient temperature for 2 weeks (93.2%) and shipped at ambient temperature also had similar genotyping efficiencies. Genotyping efficiency was reduced for DBS stored at ambient temperature for 4 weeks (89.3%, $P = 0.03$) or prepared from finger-prick blood and stored at ambient temperature for 2 weeks (77.7%, $P < 0.001$) compared to DBS prepared from venous blood and handled similarly. Resistance profiles were similar between plasma and DBS specimens. This report delineates the optimal DBS collection, storage, and shipping conditions and opens a new avenue for cost-saving ambient-temperature DBS specimen shipments for HIV drug resistance (HIVDR) surveillances in resource-limited settings.

2. Doctors have difficulty in interpreting HIV GRT test results

- Need to simplify test results and focus on “actionable” information
- Linked to availability of 2nd or 3rd line ARV
- Continuing education and training needed



HIVdb: Genotypic Resistance Interpretation Algorithm

Report: FOR REFERENCE ONLY Date: 29-May-2012 01:14:10 PDT Filename: VL16568 in house.txt

Seq ID: VL16568/5560627SZ/TD4/15-May-2012 (in house integrase component under validation)

Summary Data

Sequence includes PR: codons: 1 - 99

Sequence includes RT: codons: 1 - 248

Sequence includes IN: codons: 1 - 181

There are no insertions or deletions

Subtype and % similarity to closest reference isolate:

1. PR: CRF01_AE (92.9%)
2. RT: CRF01_AE (93.4%)
3. IN: CRF01_AE (93.2%)

Sequence Quality Assessment

Gene	QA Problem	Codons
PR	Stop Codons, Frame Shifts	None
PR	Ambiguous Positions	None
PR	Unusual Residues	19

Gene	QA Problem	Codons
RT	Stop Codons, Frame Shifts	None
RT	Ambiguous Positions	None
RT	Unusual Residues	248

Gene	QA Problem	Codons
IN	Stop Codons, Frame Shifts	None
IN	Ambiguous Positions	None
IN	Unusual Residues	None

PR

RT

IN

Drug Resistance Interpretation: PR

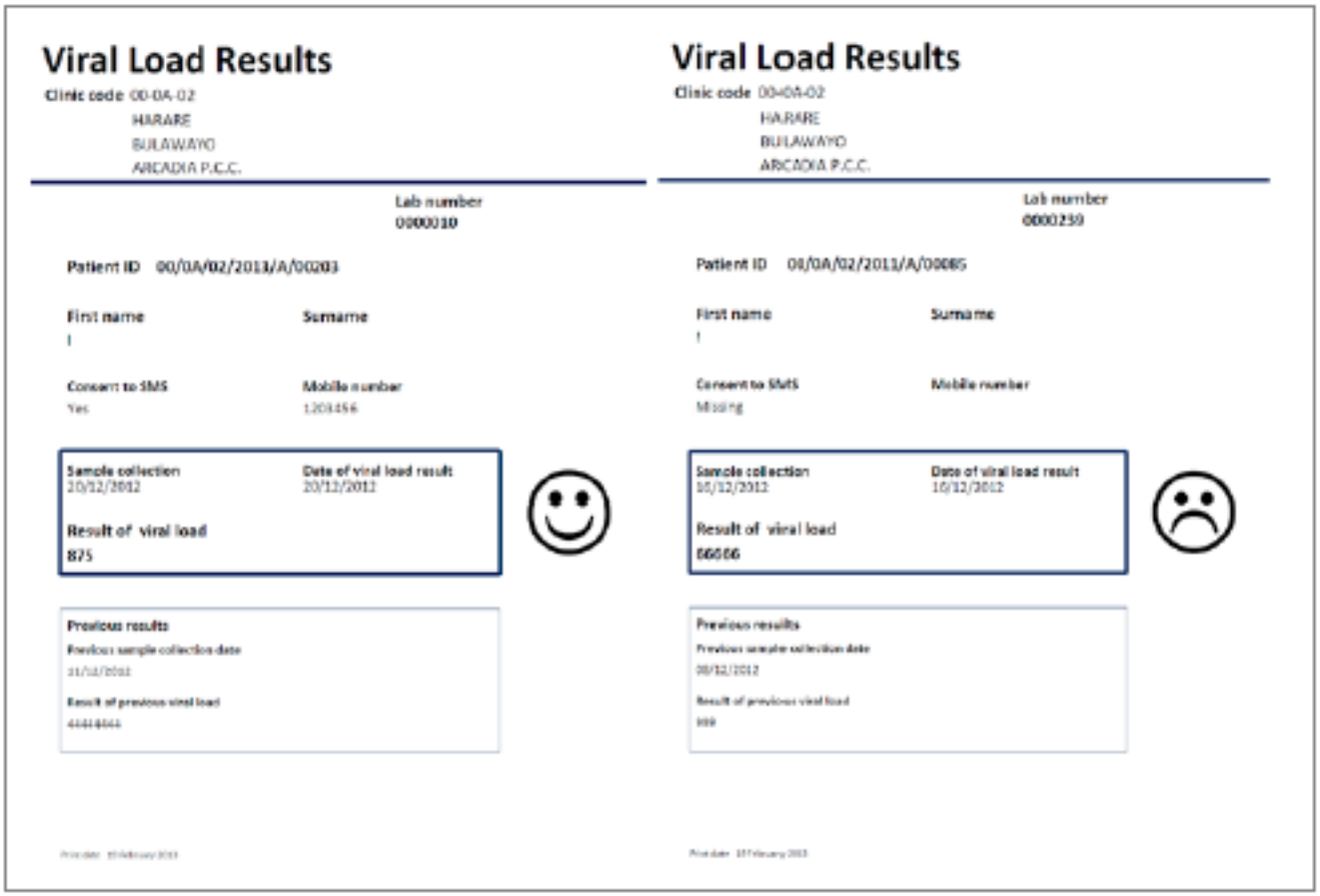
PI Major Resistance Mutations:	M46I, V82F, I84V, L90M
PI Minor Resistance Mutations:	L10W, L33F, F53L, A71V
Other Mutations:	T12P, I13V, L19W, K20T, E35D, M36I, R41K, K45V, I62V, I66F, H69K, L89I

Protease Inhibitors

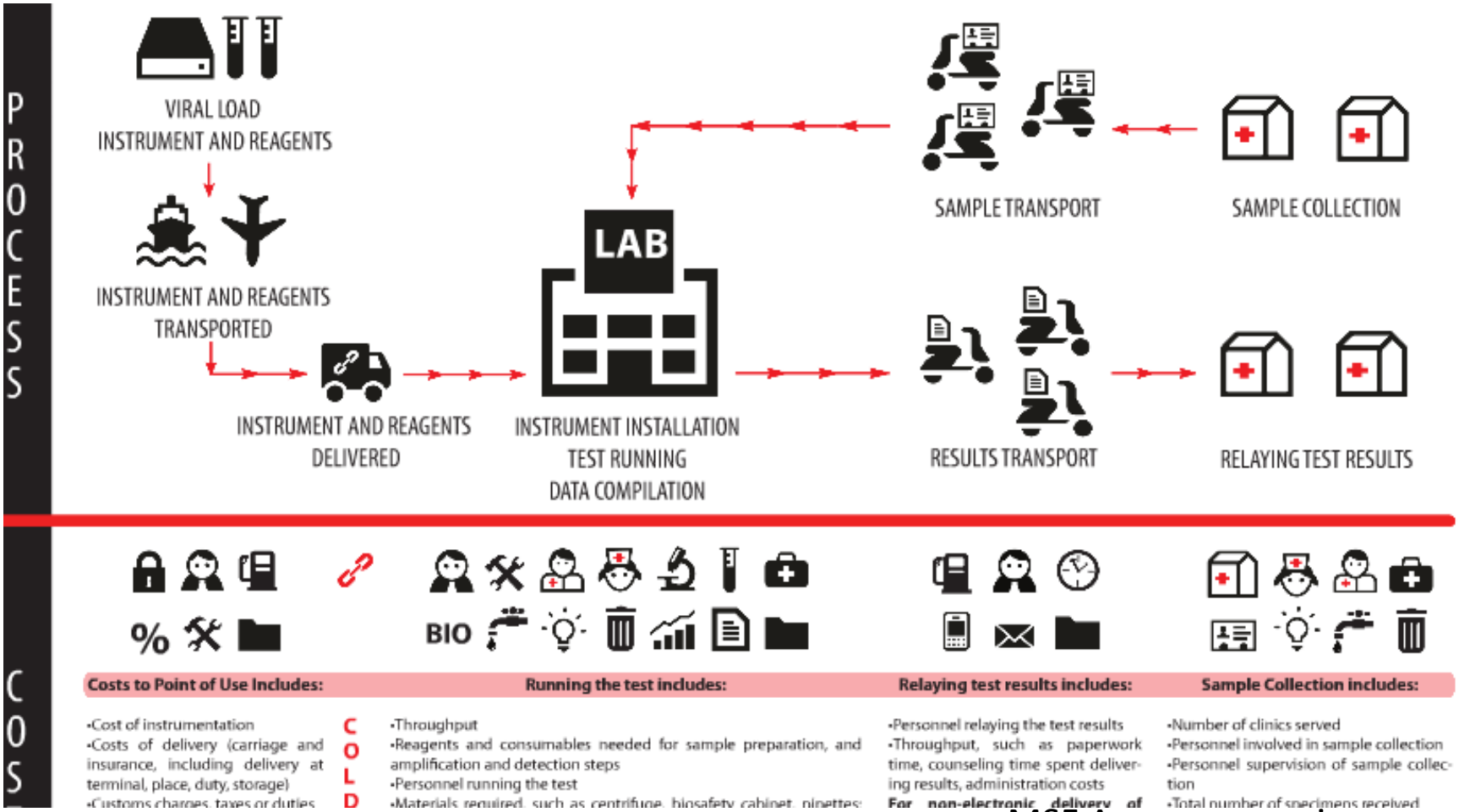
“flagging system” for abnormal results – to assist interpretation of VL results, particularly where HIV care has been task shifted to lower levels of health care workers

(MSF Viral Load Toolkit – Implementer’s guide)

FIGURE 4: SCHEMATICS FOR FLAGGING VIRAL LOAD RESULTS



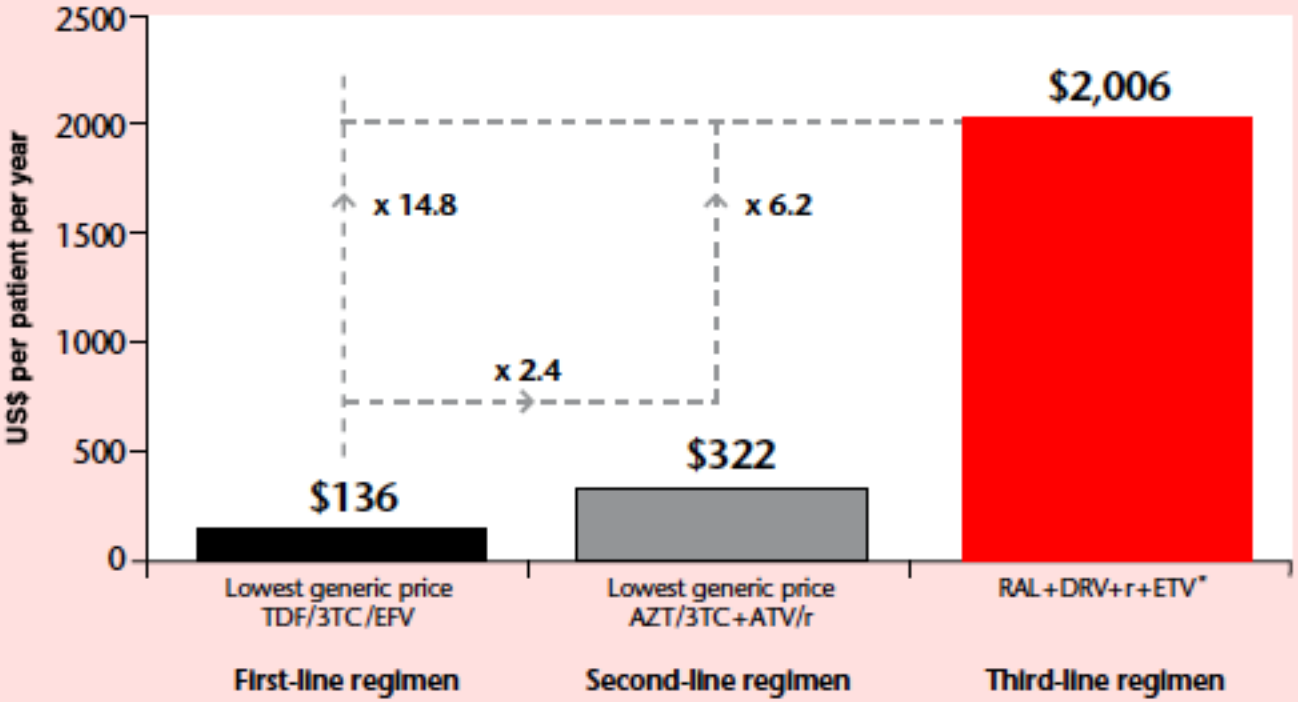
3. Long turnaround time – test results



MSF Access campaign,
HIV VL briefer

4. Access to 3rd line ARV regimen

GRAPH 6: PRICE COMPARISON OF TREATMENT REGIMENS



*Note: The price of the third-line ARV regimen of US$2,006 was calculated by adding the three individual prices of the originator product.

MSF Access Campaign, Untangling the web, 2014

5. Cost of testing

Cost of lab based VL tests (per test); not including platform

(MSF Access Campaign, Putting HIV and HCV to the test – A product guide, 2015)

LABORATORY-BASED HIV AND HCV VIROLOGICAL TESTS	
Abbott ARCHITECT HCV Ag *The only fully automated, highly sensitive, commercially available, quality approved, HCV core antigen test; chemiluminiscent microparticle immunoassay*	**$25 - 50**
Abbott RealTime HIV-1 Qualitative (EID), RealTime HIV-1 (viral load) and RealTime HCV (viral load) and RealTime HCV Genotype II *Fully polyvalent single m2000 platform for HIV EID and viral load, as well as HCV viral load and genotyping; different throughput options (m24sp and m2000sp); RNA specific for HIV viral load*	**HIV: $13 – 30** **HCV: $13 – 35**
Biocentric Generic HIV DNA Cell (EID), Generic HIV Charge Virale and Generic HCV Charge Virale *Open platform for HIV and HCV; platform has a small footprint; allows for low instrument and test prices without the need for high volumes to bring costs down*	**EID: $13** **HIV: $15** **HCV: $23**
bioMérieux NucliSENS EasyQ HIV-1 *Only platform that has received regulatory approval to use DBS as a sample type for HIV viral load*	**$23**
Cavidi ExaVir Load *Non-molecular platform and therefore not affected by amplicon contamination; not as dependent on precision pipetting; not automated and very hands-on; medium throughput; can only be used with plasma*	**$12 - 25**
Hologic Aptima HIV-1 Quant Dx Assay and Aptima HCV Quant Dx Assay *New automated platform for HIV and HCV; awaiting market launch of HCV test*	**HIV: $10 - 25**
Qiagen artus HI Virus-1 RG RT-PCR, artus HI Virus-1 QS-RGQ, artus HCV RG RT-PCR and artus HCV QS-RGQ (viral load) *Different options available for HIV and HCV viral load testing; platform not widely used in low-resource settings*	**$16 - 45**
Roche CAP/CTM HIV-1 Qualitative (EID), CAP/CTM HIV-1 (viral load), CAP/CTM HCV Qualitative and CAP/CTM HCV (viral load) *Different throughput options (Taqman 48 and Taqman 96); current extraction method extracts DNA and RNA but HIV viral load is currently being optimised on DBS using the "Free Virus Elution" protocol, which is RNA-specific*	**EID: $12.50** **HIV: $9.40** **HCV: dependent on country income level and volume commitments**
Sacace HIV Real-TM Quant Dx, HCV Real-TM Quant Dx and HCV Genotype Plus Real-TM *Open platform for HIV and HCV; platform has a small footprint; allows for low instrument and test prices without the need for high volumes to bring costs down*	**>$20**
Siemens VERSANT HIV-1 RNA Assay, VERSANT HCV RNA Assay (viral load) and VERSANT HCV Genotype 2.0 Assay *Widely used for HCV viral load and genotyping, but not widey found in low-resource settings; expensive*	**HIV: $54 – 72** **HCV: $72 – 100** **GT: $132 – 350**

‘Volume-based’ pricing model for HIV GRT may be difficult (with current use)

- VL platforms can cost more than US $150,000 plus cost of reagents (previous slide)
- Lowering the costs further by volume-based pricing
  - Global fund procurement strategy
  - Program plans to establish benchmarks for cost of test and support
  - Procurement framework will establish baseline prices for all inclusive VL testing – up to 1/3 reduction in current costs

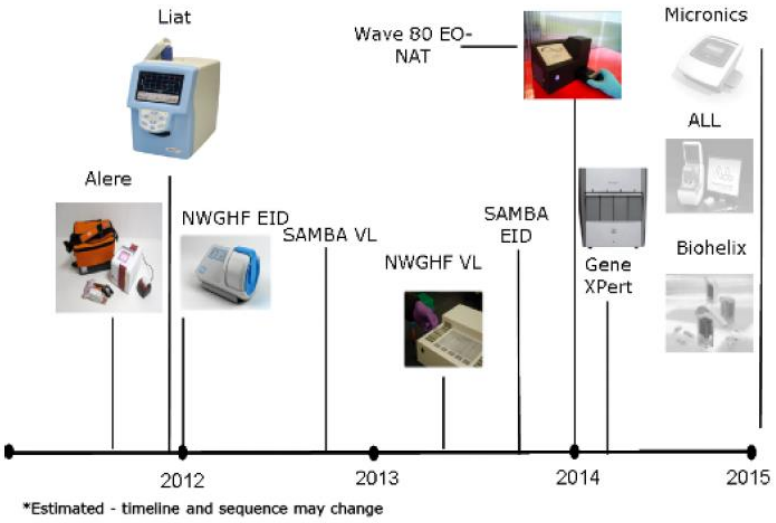
Unitaid HIV/AIDS Diagnostics Technology Landscape, 5th edition

Testing near site of patient care: POC VL

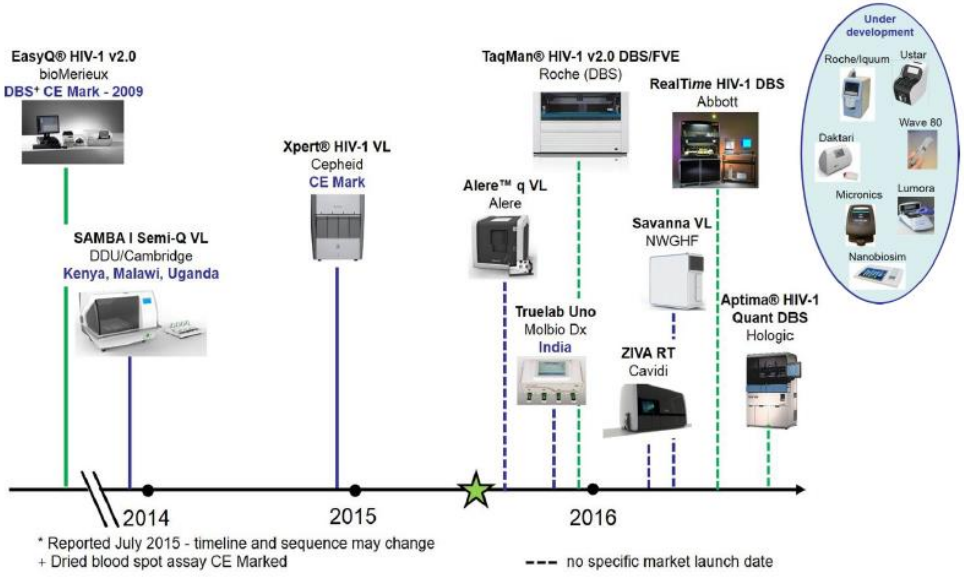
Delay in availability and rollout

(Unitaid HIV diagnostics technology landscape report, 2011 and 2015)

Viral Load and EID Platforms



Appendix 4: Point-of-care (POC) viral load (VL) technologies in the pipeline



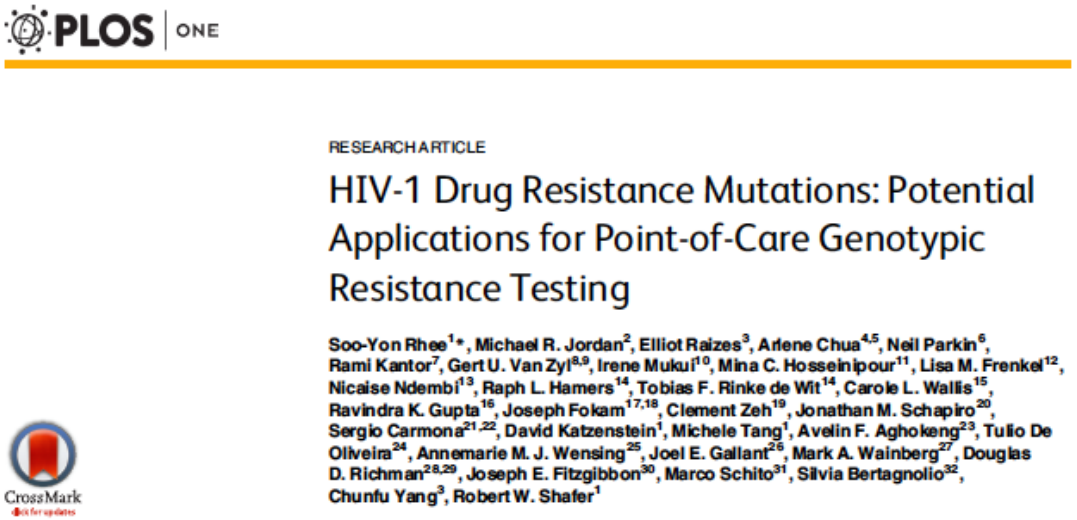
Cost

(MSF Access Campaign, Putting HIV and HCV to the test – A product guide, 2015)

POINT-OF-CARE HIV AND HCV VIROLOGICAL TESTS	
Alere q HIV 1/2 Detect (EID) *Market launched and quality assured, fully decentralisable; cartridge-based*	**$15 - 25**
Cepheid Xpert HIV-1 qual (EID), Xpert HIV-1 Viral Load and Xpert HCV Viral Load *Market launched and quality assured; GeneXpert is modular and near POC; but not fully decentralisable, cartridge-based*	**<$20**
Diagnostics for the Real World SAMBA HIV-1 Qual Test, SAMBA II HIV-1 Qual Whole Blood Test, SAMBA HIV-1 Semi Q Test and SAMBA II HIV-1 Semi Q Plasma Test *Semi-quantitative test for viral load at the 1,000 copies/mL virological failure threshold, SAMBA II is more decentralisable than SAMBA, is fully automated and has random access but has a lower throughput, SAMBA operates by batch testing and requires additional pipetting steps compared to SAMBA II; cartridge-based*	**$17 - 28**
Molbio Diagnostics Truenat HIV and Truenat HCV (viral load) *Not yet market launched; may be launched in India first; cartridge-based*	**$15**
NWGHF LYNX HIV p24 Antigen Test *Not yet market launched; non-molecular test, simple, affordable and fully decentralisable; cartridge-based*	**$6.50 - 15**
NWGHF/Quidel Savanna Quantitative RealTime HIV-1 Assay *Not yet market launched; 50µL plasma (capillary whole blood separated by plasma separator) and 200µL plasma options; cartridge-based*	**$11**

HIV GRT POC?

- Experience on POC HIV VL will inform the role of a POC HIV GRT in decentralized setting
- Need to simplify test results



PLOS ONE

RESEARCH ARTICLE

HIV-1 Drug Resistance Mutations: Potential Applications for Point-of-Care Genotypic Resistance Testing

Soo-Yon Rhee[1]*, Michael R. Jordan[2], Elliot Raizes[3], Arlene Chua[4,5], Neil Parkin[6], Rami Kantor[7], Gert U. Van Zyl[8,9], Irene Mukui[10], Mina C. Hosseinipour[11], Lisa M. Frenkel[12], Nicaise Ndembi[13], Raph L. Hamers[14], Tobias F. Rinke de Wit[14], Carole L. Wallis[15], Ravindra K. Gupta[16], Joseph Fokam[17,18], Clement Zeh[19], Jonathan M. Schapiro[20], Sergio Carmona[21,22], David Katzenstein[1], Michele Tang[1], Avelin F. Aghokeng[23], Tulio De Oliveira[24], Annemarie M. J. Wensing[25], Joel E. Gallant[26], Mark A. Wainberg[27], Douglas D. Richman[28,29], Joseph E. Fitzgibbon[30], Marco Schito[31], Silvia Bertagnolio[32], Chunfu Yang[3], Robert W. Shafer[1]

Current HIV GRT testing needs/priorities for individual patients

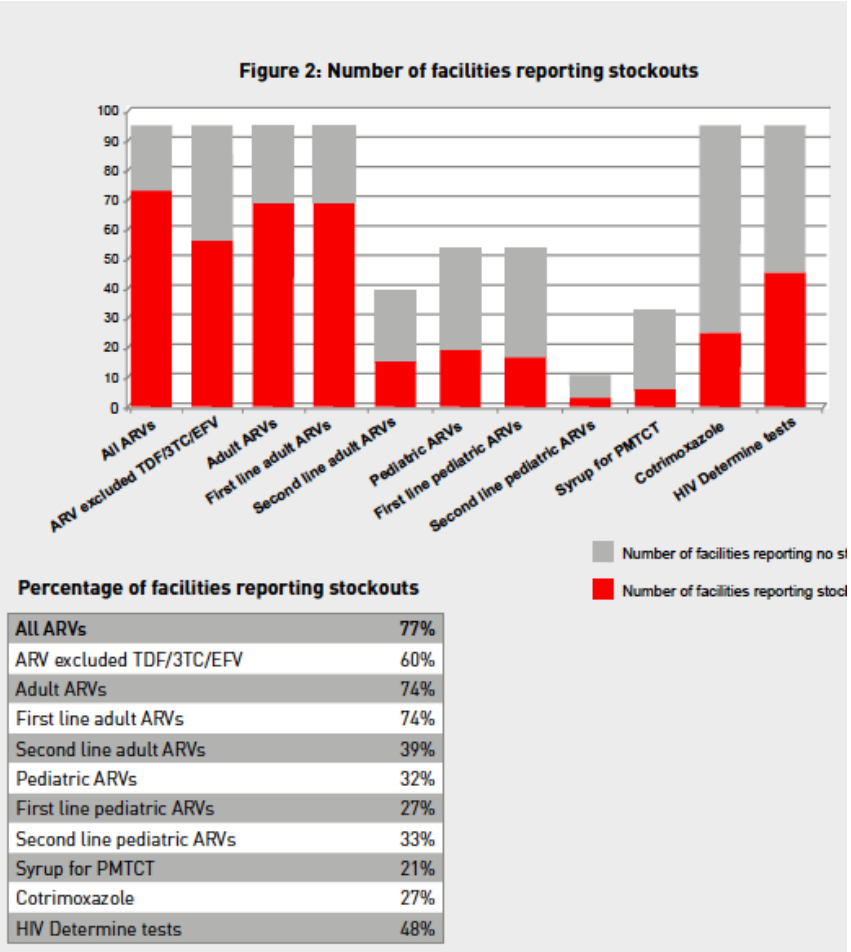
- Failure of 2nd line ARV treatment
- Failure in children, adolescents, pregnant women
- Failure of 1st line ARV treatment
- Prior to starting ARV treatment

Improving access to HIV GRT testing

- Strengthen DBS network for HIV VL
- Allow affordable access to HIV GRT testing for individual patients (outside of research and surveillance work) on DBS validated laboratories
- POC HIV GRT placement may follow HIV VL POC use, but could also potentially be placed in more ‘centralized settings’ when HIV GRT capacity don’t exist
- Testing (beyond 2^{nd}/3^{rd} line failure) will increase specially if tests are made available and affordable regardless of whether they are lab based or near patient care.

"Resistance testing should not be seen as a technological solution to the problem of drug resistance."
AIDS reviews, 2013

- Patient support will be crucial as we move towards 'Treat for all' to ensure good adherance
- Address stock outs and ensure reliable ARV supply



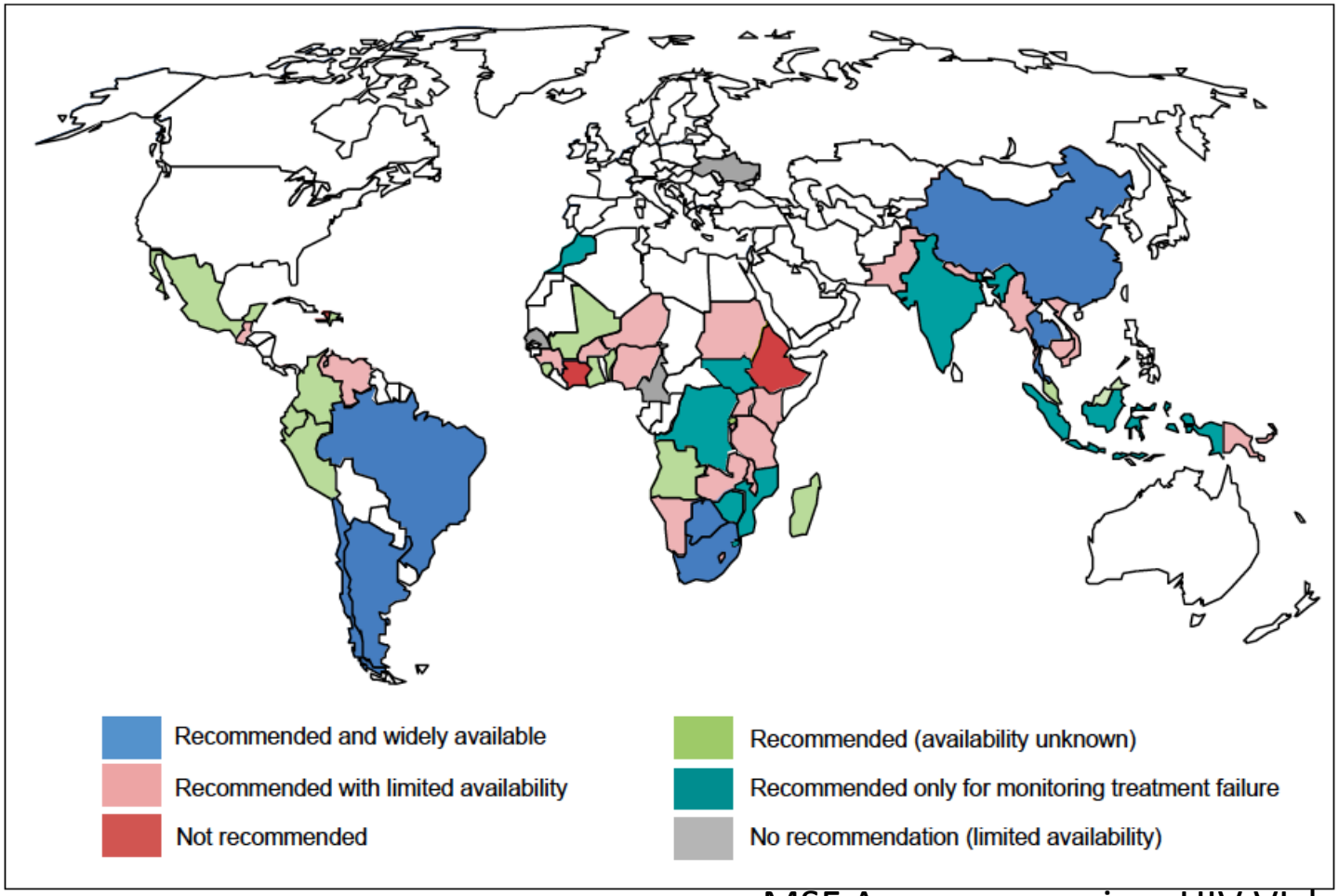
Percentage of facilities reporting stockouts

All ARVs	77%
ARV excluded TDF/3TC/EFV	60%
Adult ARVs	74%
First line adult ARVs	74%
Second line adult ARVs	39%
Pediatric ARVs	32%
First line pediatric ARVs	27%
Second line pediatric ARVs	33%
Syrup for PMTCT	21%
Cotrimoxazole	27%
HIV Determine tests	48%

From MSF report: Empty shelves come back tomorrow, 2015

Routine HIV VL should be made accessible for all patients to detect early failure

Recommendation on Use of Viral Load Testing for ART Monitoring and its Availability (Source: UNAIDS)



Note: Tanzania recommends routine VL for monitoring but it is not essential

MSF Access campaign, HIV VL briefer, Dec 2014 supplement

Thank you