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ASLM Conference 2014

Symposium "Innovative Solutions for HIV Diagnosis and Monitoring"

Cape Town, Wednesday 3 December 2014

Pooling of Dried Blood Spots for More Cost-Effective Viral Load Monitoring and Early Infant Diagnosis

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University of Stellenbosch / NHLS Tygerberg

NATIONAL HEALTH LABORATORY SERVICE

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What is pooled testing (aka pooling) ?

Idea: Mix several individual specimens together and test the resulting "pool"

Goal: to improve affordability – use only one test for testing several samples

If test result of pool is …

- **… negative: all individuals diagnosed as negative**
- **… positive: retest all samples in pool individually to identify the positive one(s): deconvolution**

Commonly used to reduce cost of screening large numbers of individuals (e.g. blood transfusion)

R. Dorfman (1943): The Detection of Defective Members of Large Populations, The Annals of Mathematical Statistics, 14(4)

Considerations (I)

1. Sensitivity:

How does pooling affect test sensitivity?
We do not want to miss any (relevant) positives!

2. Pool size:

The more samples per pool, the higher the savings; however: The more pools test positive, the more pools will have to be deconvoluted

Considerations (II)

3. Prevalence:

The lower the better:
fewer individuals positive ➔ more pools test negative ➔ no deconvolution required

Lower prevalence ➔ bigger pool sizes possible

4. Throughput

Considerable test volumes required for pooling to be viable; waiting to fill up runs prolongs turnaround times

Possible use: HIV viral load testing

Method of choice to monitor patients on ART

UNDETECTABLE

HOW VIRAL LOAD MONITORING
CAN IMPROVE HIV TREATMENT
IN DEVELOPING COUNTRIES

www.msfaccess.org

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PUTTING HIV TREATMENT TO THE TEST

A PRODUCT GUIDE FOR VIRAL LOAD AND
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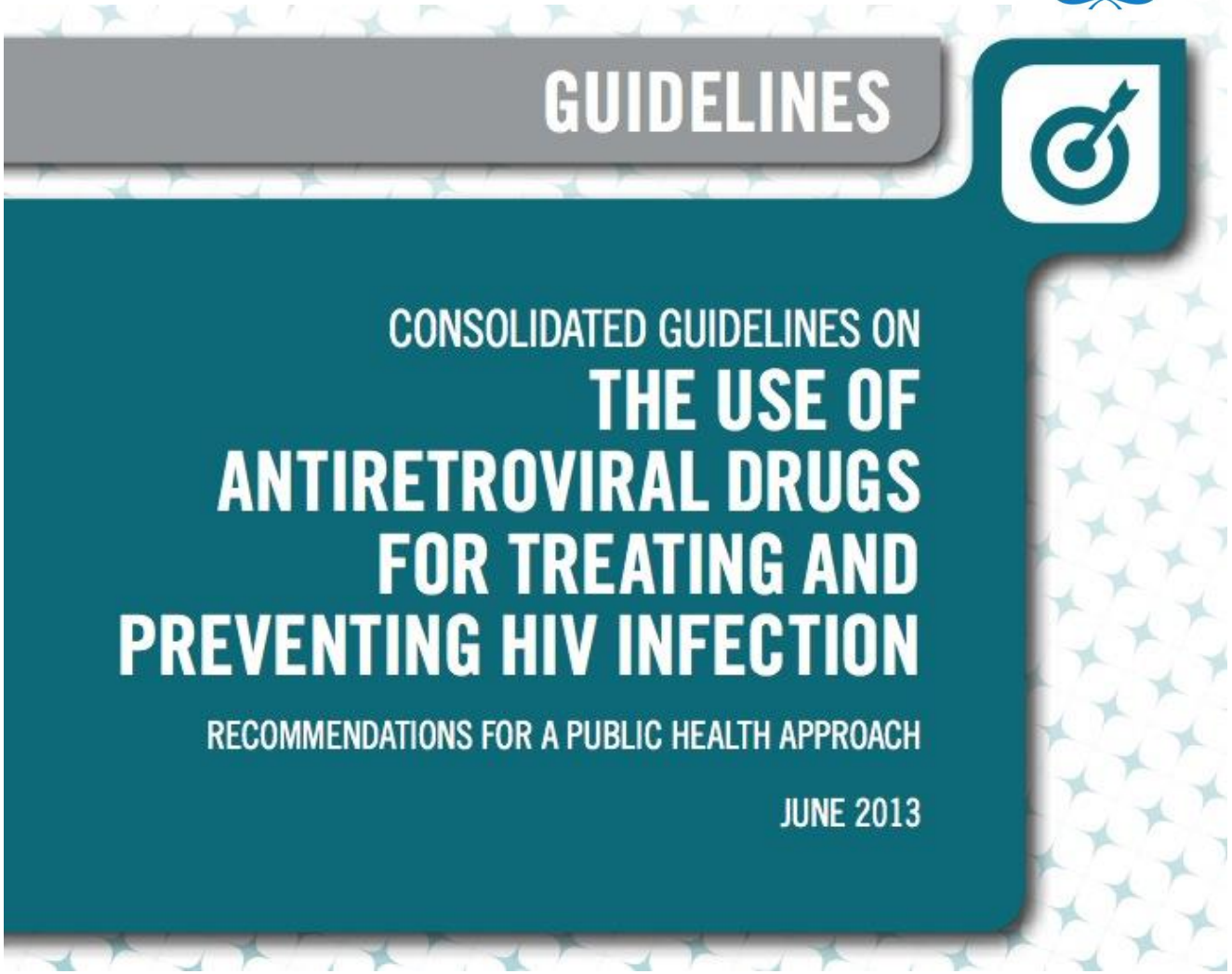
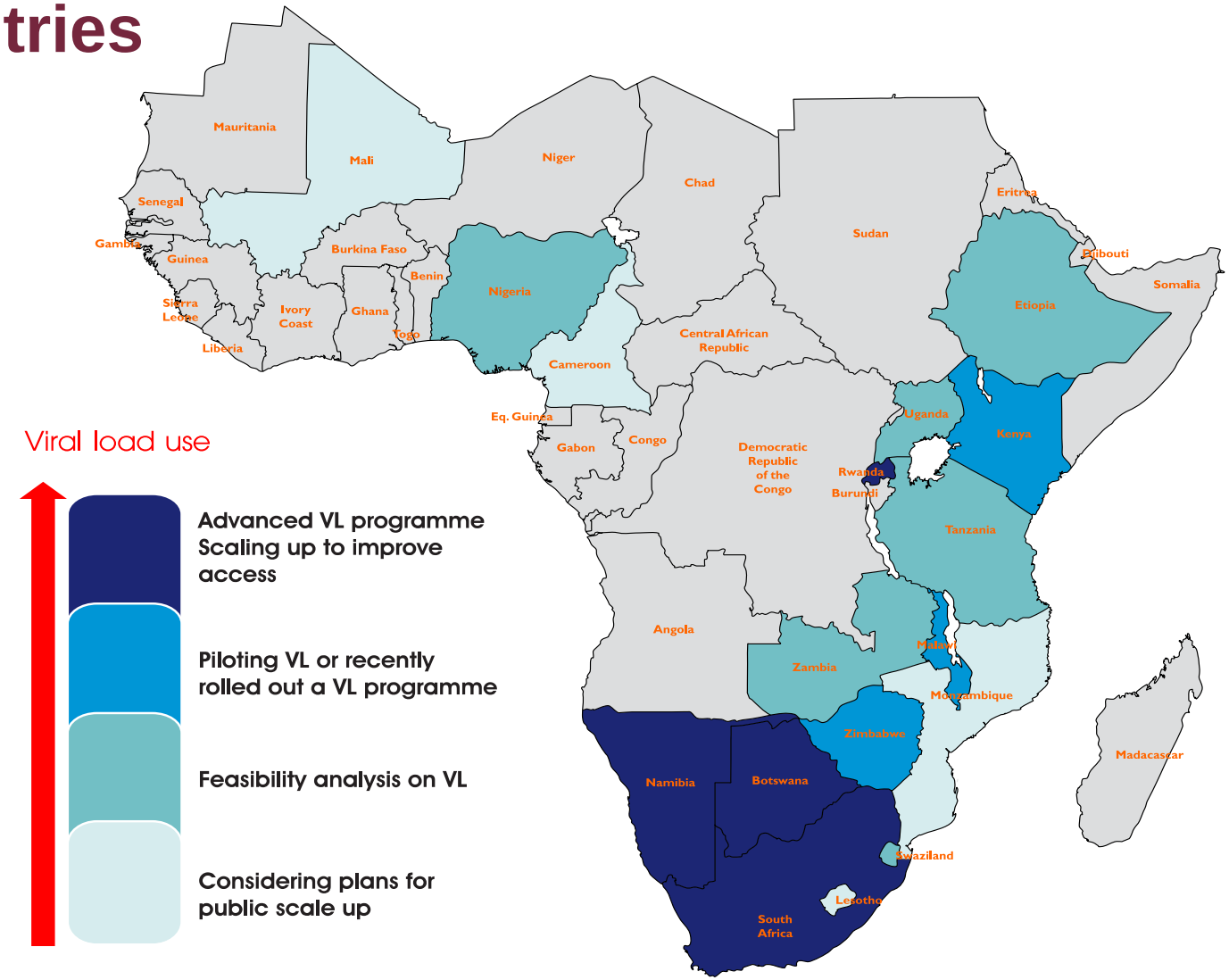
World Health Organization



Monitoring schedule

Threshold (5000 cop./ml vs. 1000 cop./ml)

HIV VL monitoring in sub-Saharan African countries



Trevor Peter, IAS-ILF Symposium, Expanding access to viral load monitoring in resource-limited settings, Lusaka, Zambia, 2014

EID point-of-care system: Liat™ analyser (Iquum / Roche)

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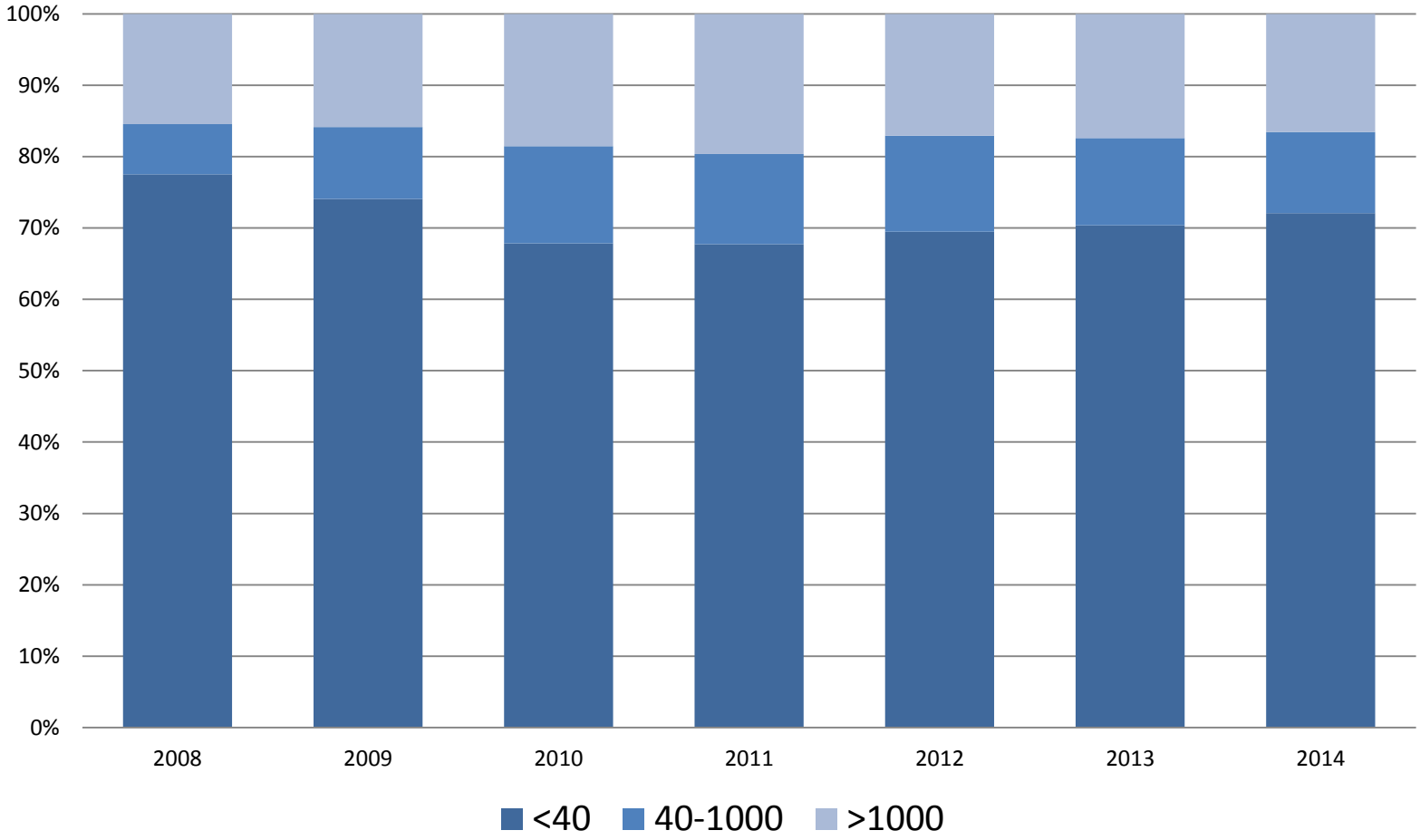
NHLS HIV viral load platform: Abbott m2000 RealTime HIV-1 System

Adult HIV viral load test results, Western Cape, 2008 – 2914

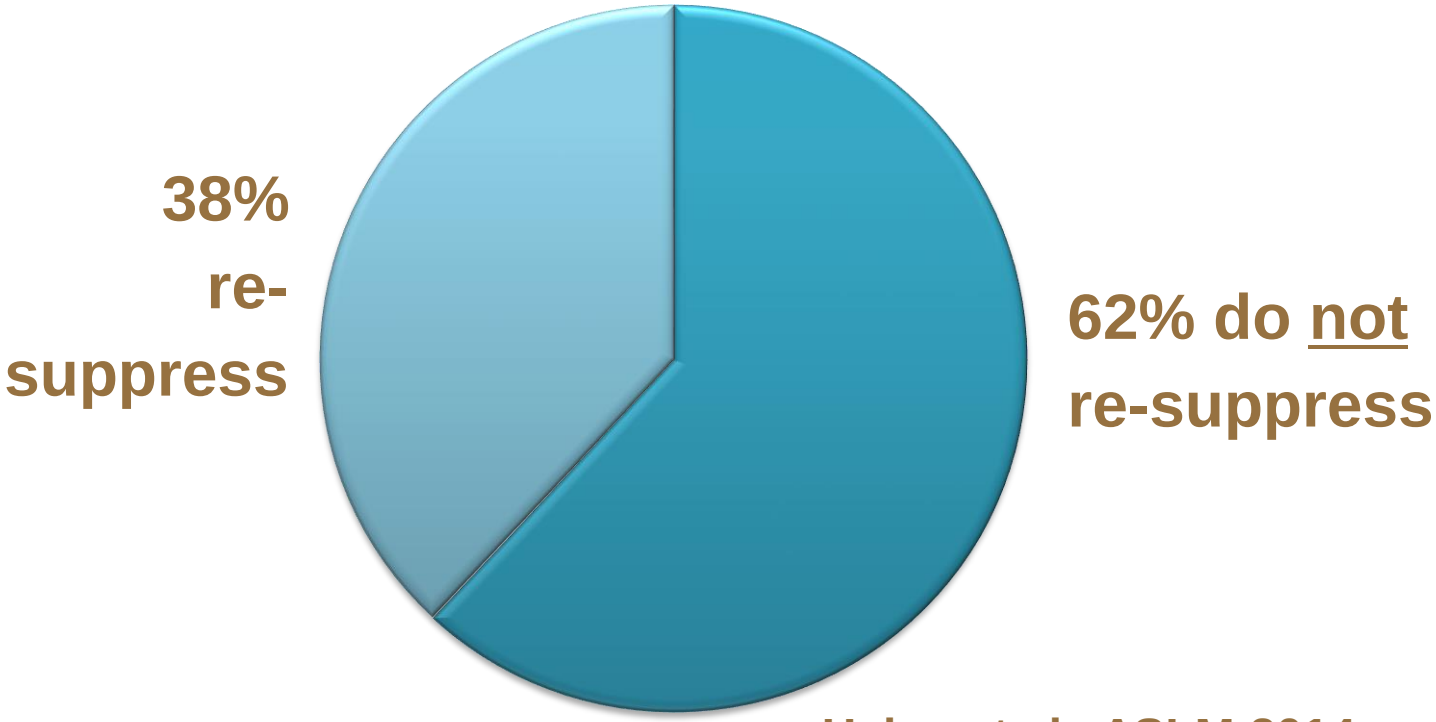


Proportion of VL results >1000 copies/ml remarkably constant at just below 20%.

Hsiao et al., ASLM 2014, poster 96

Adult HIV viral load test results, Western Cape, 2008 – 2914

More than 60% of samples with previous VL>1000 copies/ml failed to re-suppress, highlighting persistent ART failure as an important issue



Hsiao et al., ASLM 2014, poster 96

Pooling Strategies to Reduce the Cost of HIV-1 RNA Load Monitoring in a Resource-Limited Setting

G.U. van Zyl,[1] W. Preiser,[1] S. Potschka,[2] A.T. Lundershausen,[2] R. Haubrich,[3] and D. Smith[3]

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Clinical Infectious Diseases 2011;52(2):264–270

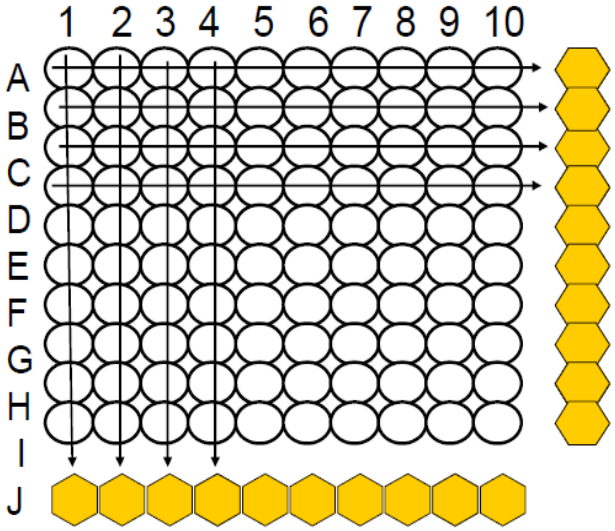
Summary: Materials & Methods

Specimens with low pre-test probability of ART failure

Plasma vs. dried blood spots vs. dried plasma spots

2 different pooling strategies:

matrix pool **vs.** **minipool**



van Zyl et al., 2011

Summary: Methods, Results

Deconvolution algorithm to identify specimens(s) with detectable viral loads

Results:

Method	% failure (>1000c/ml) /100 spec.	NPV	# tests	savings needed
3 matrices (300 specimens)	11 %	98 %	41 %	1,640 $
80 minipools (400 specimens)	9.5 %	100 %	30.5 %	1,220 $

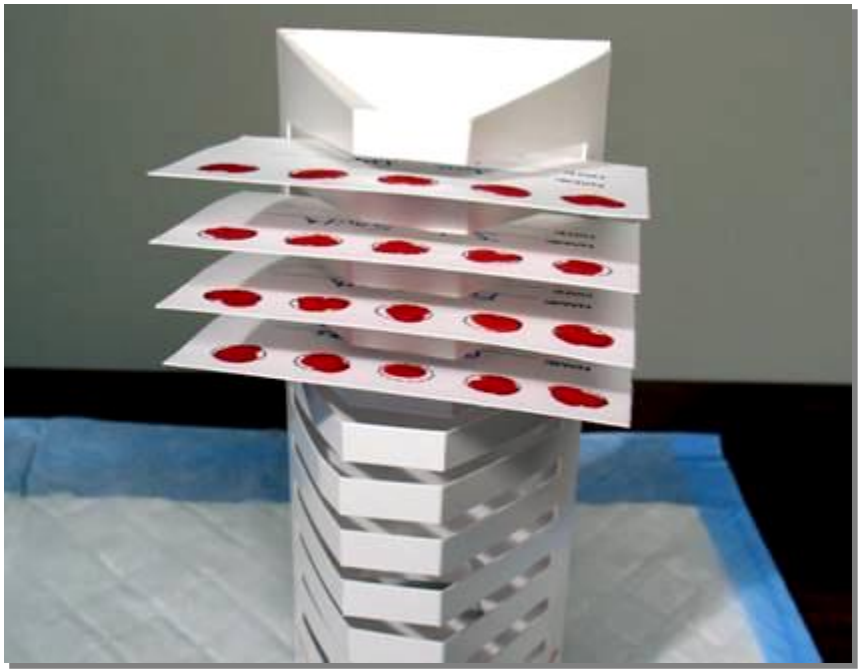
van Zyl et al., 2011

Summary: Conclusions

- Pooling saves 30.5 % – 60 % of HIV RNA tests
- Matrix strategy may be more efficient but is technically demanding
- Minipools of 5 dried blood spots were accurate with NPV >95%
- In resource-constrained settings, combining pre-selection of patients with low pre-test probability of virologic failure and pooled testing can reduce cost of monitoring without compromising accuracy

van Zyl et al., 2011

Suitable for dried blood spots (DBS) and dried plasma spots (DPS)



Pooled HIV-1 Viral Load Testing Using Dried Blood Spots to Reduce the Cost of Monitoring Antiretroviral Treatment in a Resource-Limited Setting

Pieter Pannus, MSc, Emmanuel Fajardo, BSc,* Carol Metcalf, MBChB, MPH,* Rebecca M. Coulborn, MPH,† Laura T. Durán, MBChB,† Helen Bygrave, MA, MBChB,* Tom Ellman, BSc, MBChB, MSc,* Daniela Garone, MBChB, BSc (ID),† Michael Murowa, MBBS,‡ Reuben Mwenda, MSc,§ Tony Reid, MD,‖ and Wolfgang Preiser, MD, PhD¶*

J Acquir Immune Defic Syndr • Volume 64, Number 2, October 1, 2013

Malawi pooling study

- Compared plasma vs. finger prick DBS vs. venous blood DBS
- Compared "minipool" and "minipool + algorithm" strategies on pools of 5 samples
- Accuracy: NPV 97.3% - 100%, PPV 96.2% - 100%
- Efficiency: depends on sample type and threshold (1000 cop./ml vs. 5000 cop./ml)
- Example: with finger prick DBS and 5000 cop./ml threshold, mini pooling reduces number of tests required by 51.4% compared with individual testing

Pannus et al., 2013

And finally:
Do we need the "load" in viral load?

A qualitative PCR minipool strategy to screen for virologic failure and antiretroviral drug resistance in South African patients on first-line antiretroviral therapy

Howard Newman[a,*], Lukas Breunig[a,b], Gert van Zyl[a], August Stich[b], Wolfgang Preiser[a]

[a] *Division of Medical Virology, Department of Pathology, National Health Laboratory Service (NHLS), Tygerberg and Stellenbosch University, Cape Town, Western Cape, South Africa*
[b] *Medical Mission Institute and University of Würzburg, Würzburg, Germany*

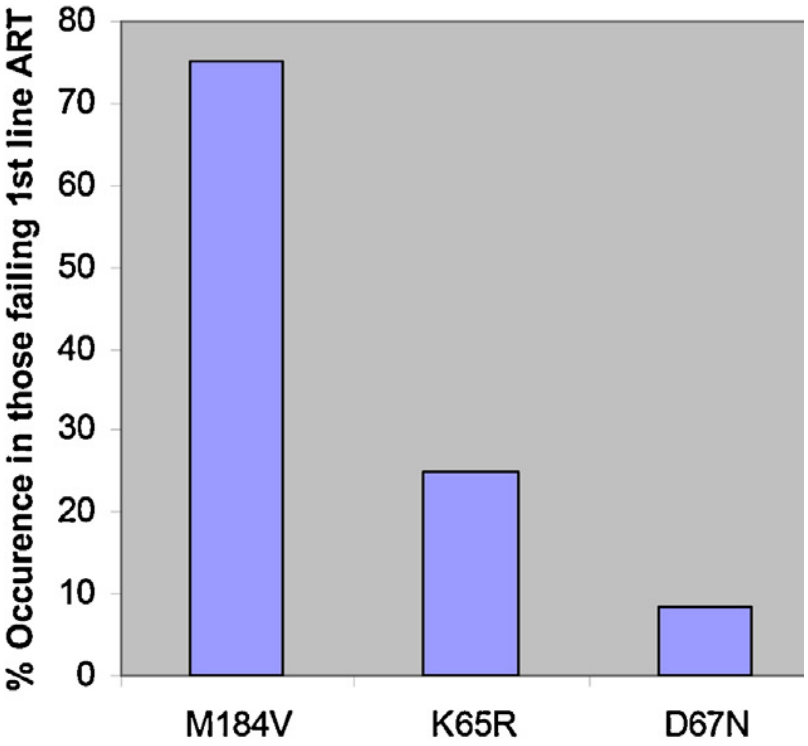
Journal of Clinical Virology 60 (2014) 387–391

Pooled qualitative PCR to detect virological failure ...

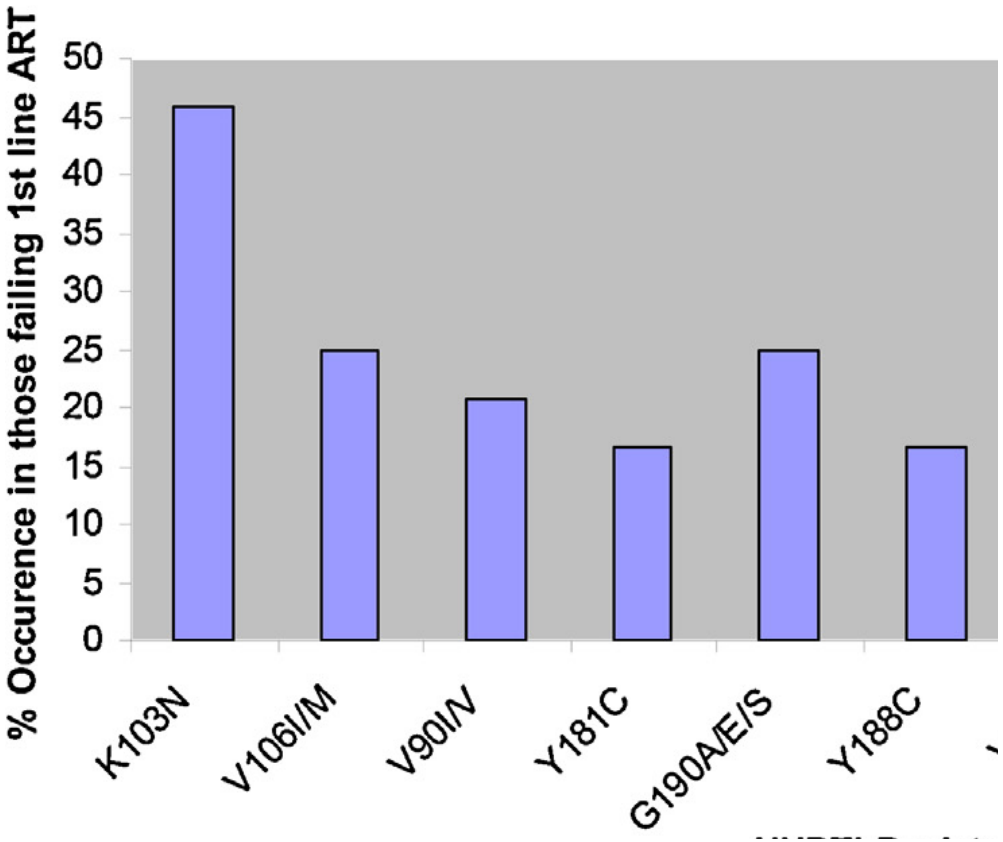
- Qualitative in-house PCR targeting partial RT gene
- 300 routine patient samples (incl. 29 positives, of which 26 with VL >1000 cop./ml)
- 60 minipools of 5 EDTA blood samples each
- 22 / 60 pools tested positive
- Pooling detected 24 / 26 failing patients
- Sensitivity for detecting failure 92%, specificity 98.9%
- NPV 99.3%, PPV 89.7%
- Pooled testing required 43% fewer assays than conventional viral load testing

Newman et al., 2014

... and sequencing of positives



NRTI resistance mutations



NNRTI resistance mutations

Newman et al., 2014

Possible use: HIV early infant diagnosis (EID)

Requires detection of viral nucleic acid (proviral DNA and / or viral RNA) or viral antigen (p24) due to presence of maternal antibodies

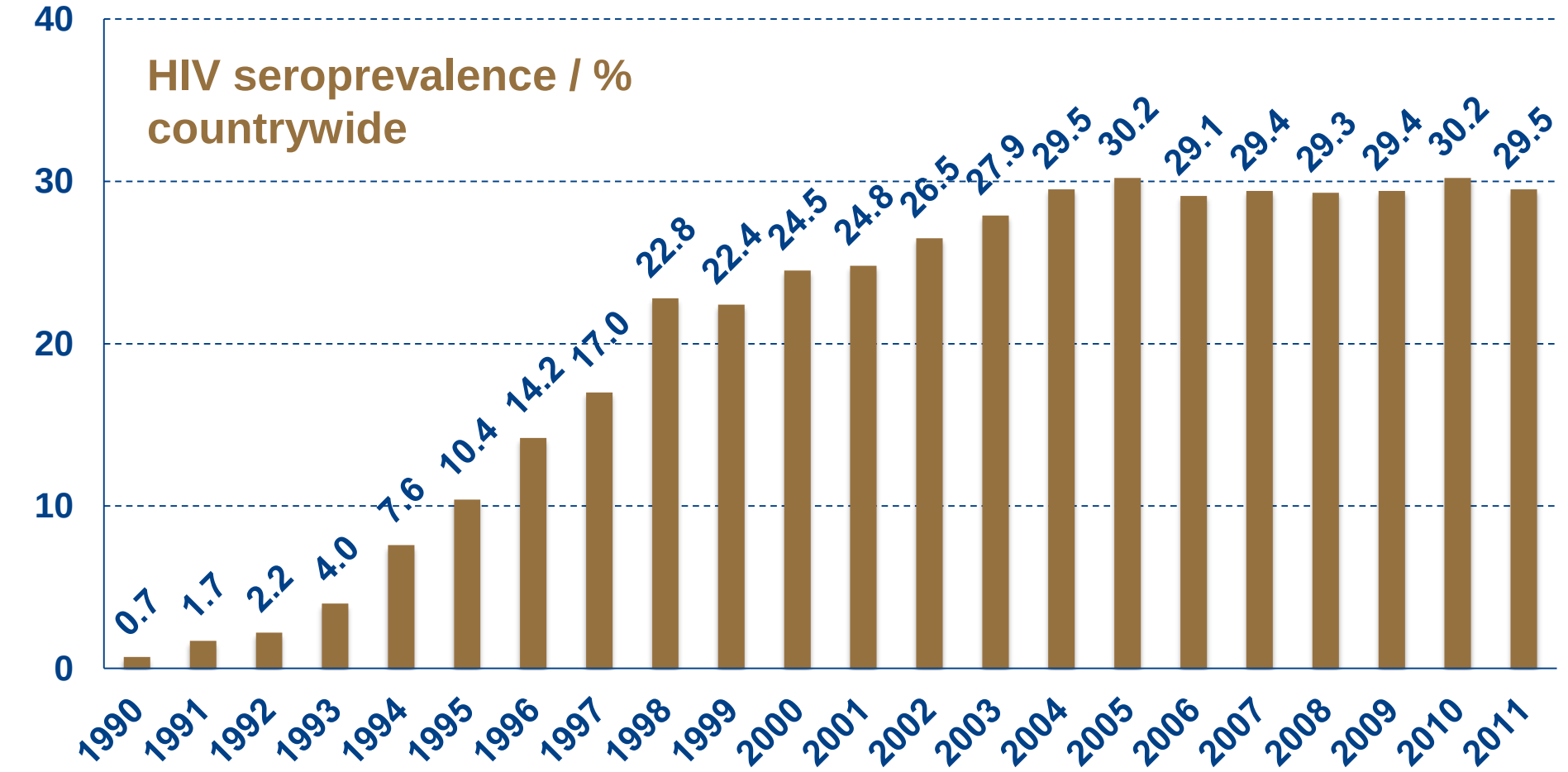
Complex, expensive; centralised ➔ delays

Infant testing at 6 weeks is not enough:
3/4 of HIV-infected babies can be diagnosed at birth (intra-uterine infection)

CHER study: Infant mortality peaks at 3 months of age ➔ early ART improves outcomes!

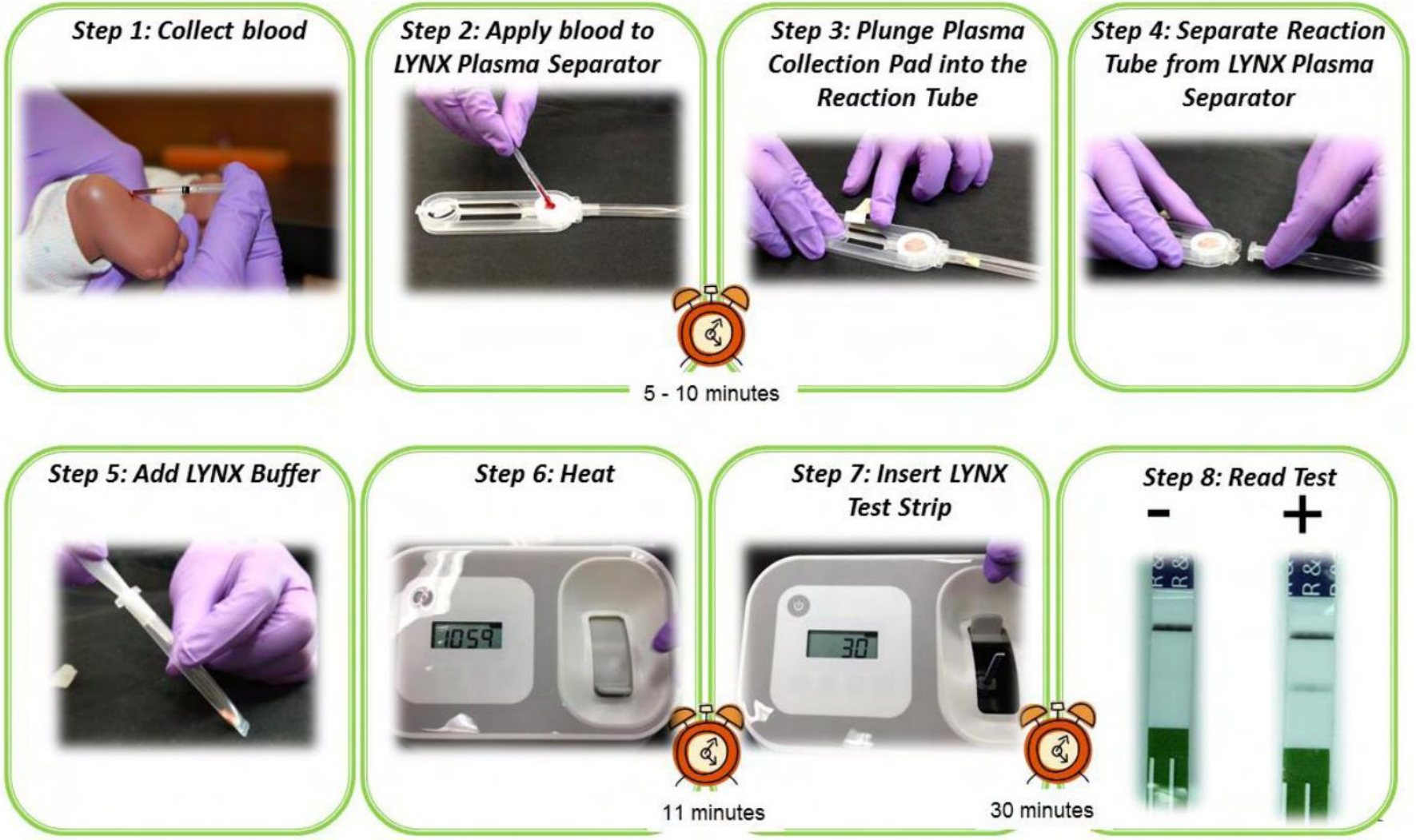
Maritz et al. 2012; Maritz and Preiser 2011; Maritz et al. 2014; Lilian et al. 2012; WHO 2012; Violari et al. 2008; Bourne et al. 2009; van Schalkwyk et al. 2013; Nachega et al. 2012

HIV seroprevalence in pregnant women in South Africa: 1990 – 2011



National Dept. of Health, RSA:
2011 National Antenatal Sentinel HIV & Syphilis Prevalence Survey

LYNX p24 antigen point-of-care assay (Northwestern Global Health Foundation)



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NHLS EID platform: COBAS AmpliPrep / COBAS TaqMan

Maritz et al., 2012; Maritz et al., 2014

Infant HIV PCR results, Western Cape, South Africa, 2008 – 2014

Year	# tests	Positive	Community positivity rate *
2008	19058	8.79%	3.55%
2009	19518	8.32%	3.54%
2010	17681	5.79%	2.86%
2011	18042	4.14%	1.52%
2012	19716	3.41%	1.36%
2013	21560	3.14%	1.24%
2014	24873*	3.26%	0.99%

* tests requested from primary level facilities for infants 5 – 7 weeks of age

Maritz et al., ASLM 2014, poster 94

Pilot study: Test results of pooled samples

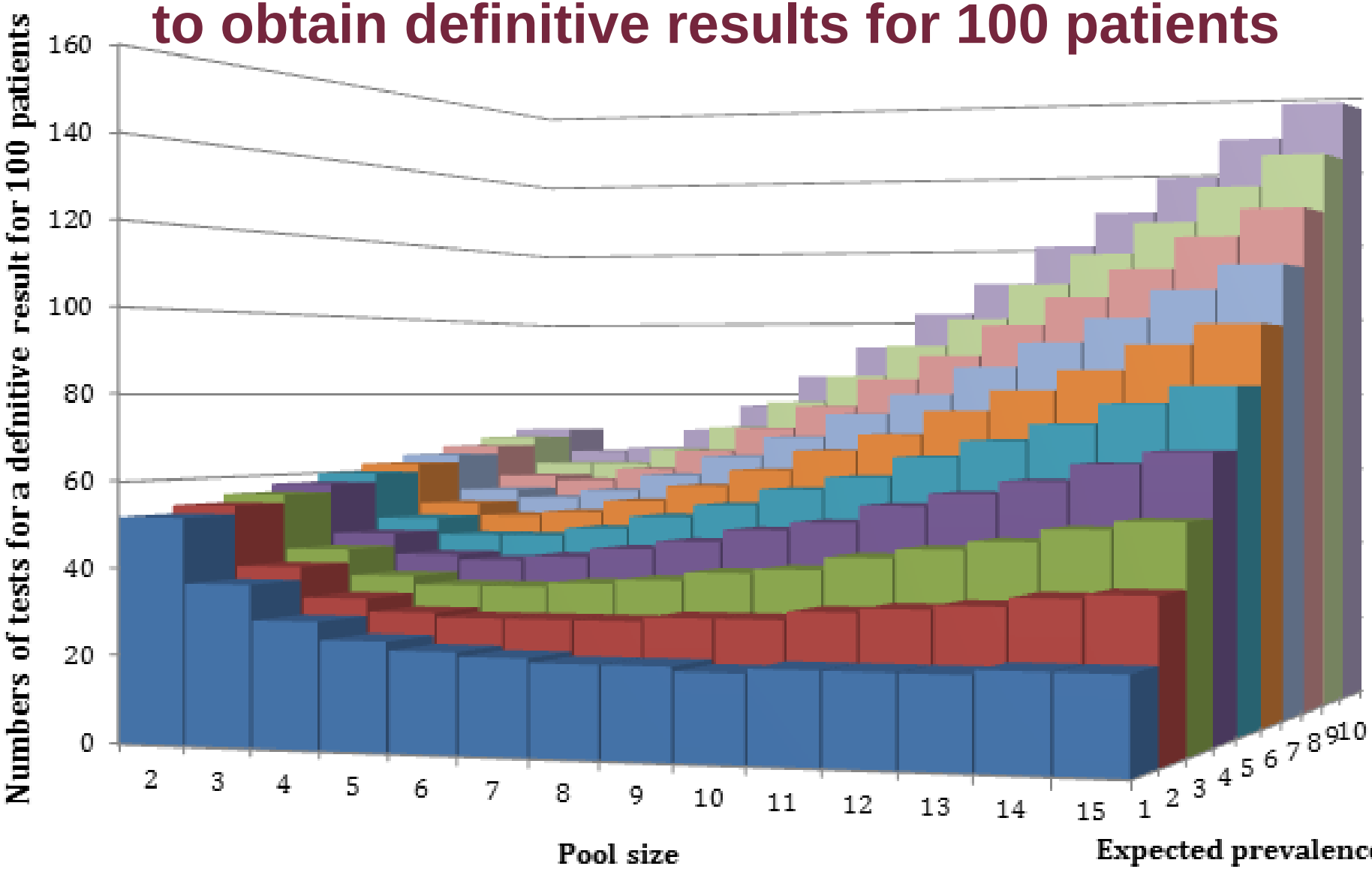
	HIV reactive pools		HIV non-reactive pools
	HIV reactive DBS	Total HIV reactive DBS	
Reactive result	35	38	0
Negative result	0	1	19
Sensitivity		97%[γ]	
Specificity			100%[γ]
Positive predictive value[α]		100 %[γ]	
Negative predictive value[α]			99.9%[γ]

[α] = Calculated using Bayes' rule and a prevalence of 2.0% as determined by CAP/CTM assay tested on individual whole blood samples of 100 µL during the preceding 12-month period

[γ] = Calculation based on all reactive pools, including weakly reactive DBS pools

J. Maritz, S. Douma, W. Preiser, 2014 (study ongoing)

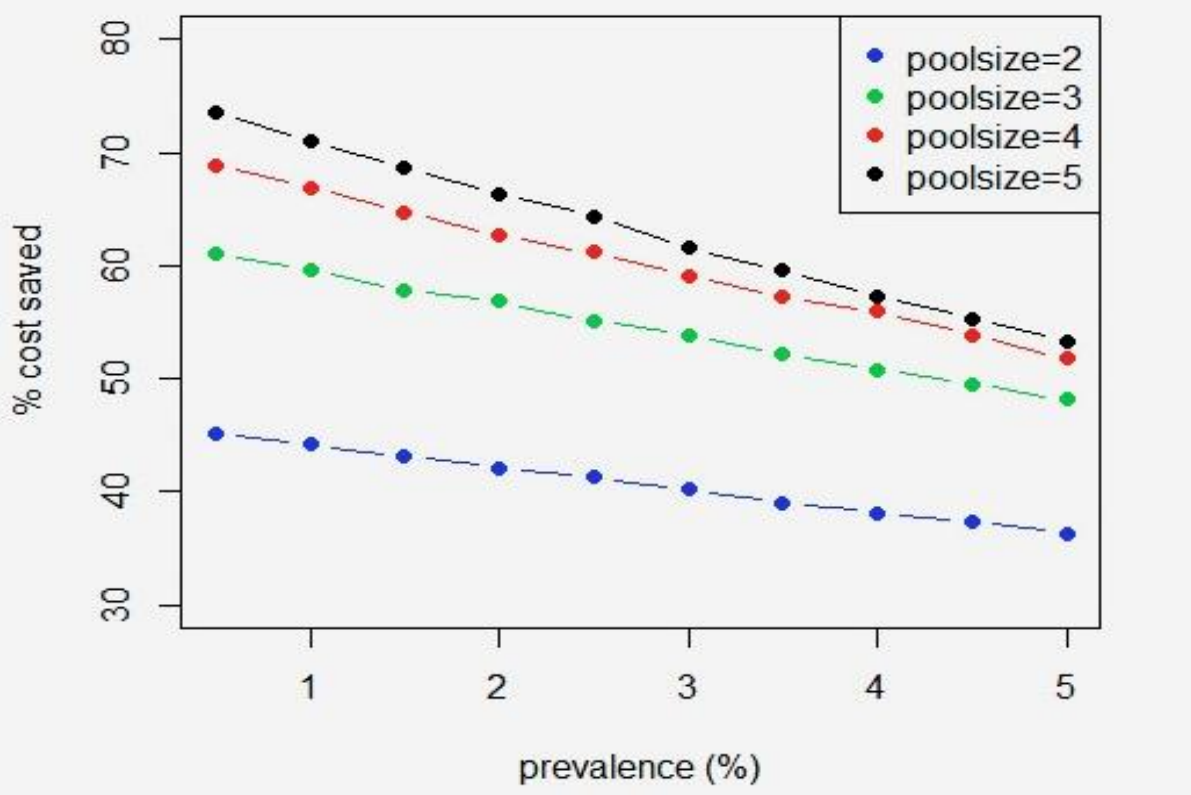
Pilot study: Maximal number of tests needed to obtain definitive results for 100 patients



J. Maritz, S. Douma, W. Preiser, 2014

Modelling

"Cost- effectiveness of pooled PCR testing of dried blood spots for infant HIV diagnosis" accepted for poster presentation at CROI2015



The next step: implementation where EID is not always available and has long TATs

Bugando Medical Centre, Mwanza, Tanzania

Conclusions: Pooled testing ...

... can help meet the enormous and largely unmet need for HIV EID and HIV viral load testing in many African settings

... is feasible if relatively few patients are infected / failing as is increasingly the case in Africa

... can be done without inacceptable decreases in sensitivity and accuracy

... reduces number of tests needed ➔ cost reduction

Outlook

Centralised, high throughput setting: optimises use of scarce qualified staff and sophisticated facilities

Option: highly automate e.g. pipetting robot and computer-guided pooling and deconvolution

Defining appropriate pooling strategies (pool size, pool type (mini, matrix, 3D), ...) needs to take into account both economical and practical perspectives


freedom evo
TECAN

Outlook

Pooled testing should be part of a "package"

Stratify patients:

- **'low risk' (e.g. those in ART adherence clubs) ➔ pooled testing**
- **'high risk' (e.g. those who would receive targeted VL testing) ➔ individual testing**

Pooled testing can improve affordability and thus availability of virological diagnosis and monitoring in resource-constrained settings

Collaborators

Tygerberg: Jean Maritz, Gert van Zyl, Howard Newman, Sanne Douma

MSF: Pieter Pannus, Emmanuel Fajardo, Carol Metcalf, ...

SACEMA: Alex Welte, Cari van Schalkwyk

University of California SD: Davy Smith, Richard Haubrich

Medical Mission Institute Würzburg: Gustl Stich, Lukas Breunig, Susanne Potschka, Anna-Theresa Lundershausen

Thank you, baie dankie,

enkosi kakhulu, vielen Dank!