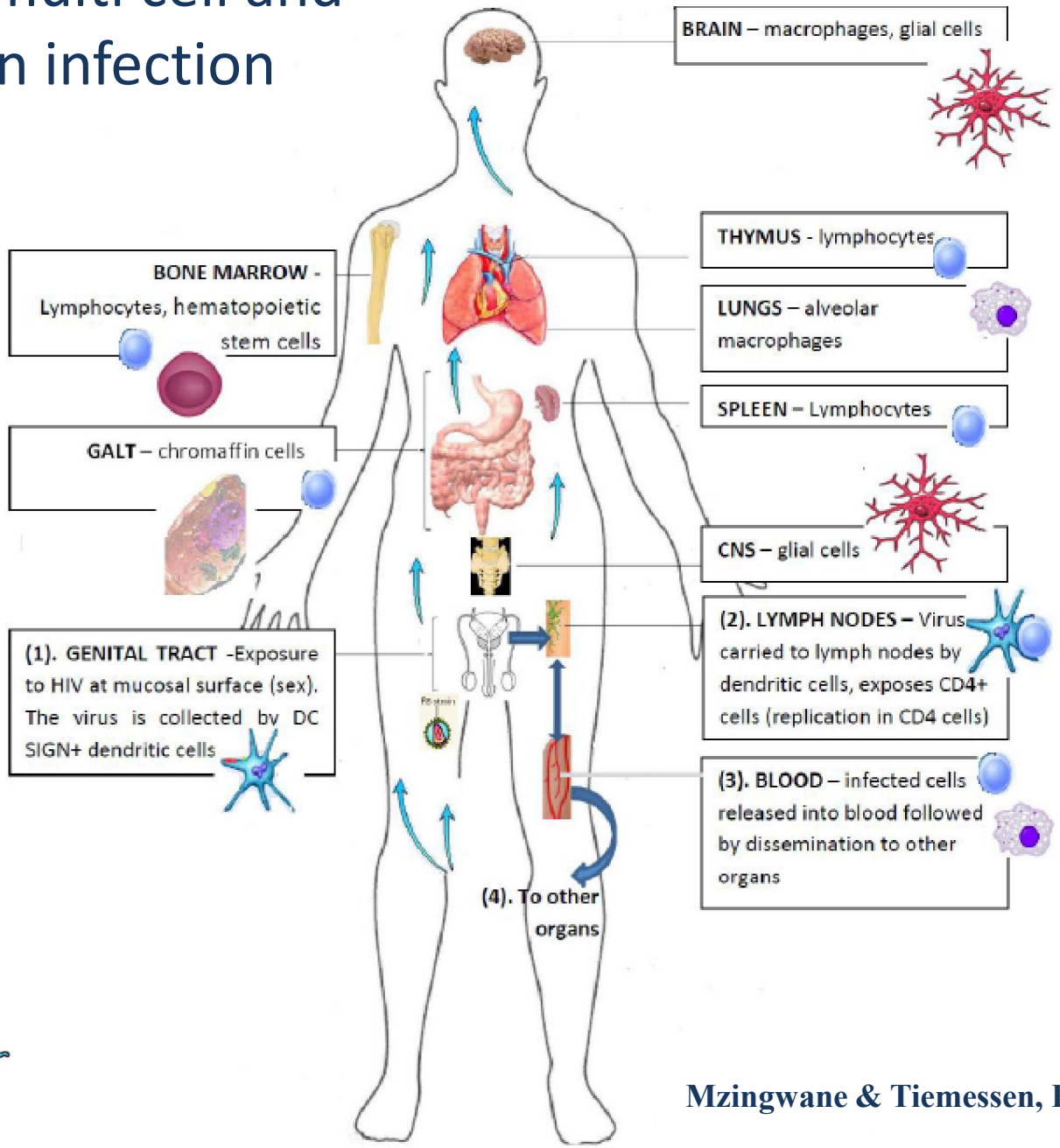
HIV-1 replication, compartmentalization and persistence in the urinary tract.

Mayibongwe L. Mzingwane, PhD

Department of Pathology

National University of Science & Technology, Zimbabwe

HIV multi cell and organ infection



Mzingwane & Tiemessen, Rev Med Virol. 2017; e1924

Challenges to HIV eradication

- Lack of therapeutic approaches that recognize, target and eliminate cells harbouring HIV-1 provirus (latent HIV reservoir).
  - Traditionally believed to be resting CD4 T cells
- Evidence of new cellular reservoirs
  - HIV persistence in urethral macrophages

Ganor et al. IAS 2019, Mexico city
Gonor et al. *Nature Microbiol. 2019;* **4**:633–644

• Published: 04 February 2019

HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy

Yonatan Ganor, Fernando Real, Alexis Sennepin, Charles-Antoine Dutertre, Lisa Prevedel, Lin Xu, Daniela Tudor, Bénédicte Charmeteau, Anne Couedel-Courteille, Sabrina Marion, Ali-Redha Zenak, Jean-Pierre Jourdain, Zhicheng Zhou, Alain Schmitt, Claude Capron, Eliseo A Eugenin, Rémi Cheynier, Marc Revol, Sarra Cristofari, Anne Hosmalin & Morgane Bomsel

Nature Microbiology **4**, 633–644 (2019)

- Urethral macrophages contain integrated HIV-1 DNA, RNA, proteins and intact virions in virus-containing compartment-like structures, whereas viral components remain undetectable in urethral T cells.
- Urethral cells specifically release replication-competent infectious HIV-1 following reactivation with the macrophage activator lipopolysaccharide

Urinary tract as a potential HIV reservoirs

- Urinary tract, which includes the urethra, bladder and kidneys, has not been well studied as a potential anatomic reservoir
- Wide spectrum of HIV-associated renal diseases
  - HIV-associated nephropathy
  - HIV immune-complex kidney disease
  - Pathogenesis linked to HIV-1 infection of renal epithelium

Wyatt et al. Annu Rev Med 2012; 63:147–159
Marras et al. Nat Med 2002; 8:522–526

Urinary tract as a potential HIV reservoirs

- HIV infection of renal tubular epithelial cells in patients on suppressive ART
- HIV infection of kidney allografts after transplantation in HIV positive recipients with undetectable HIV virus in plasma at the time of transplantation

Chen et al. J Am Soc Nephrol 2011; 22:496 – 507
Blasi M et al. AIDS 2014; 28:2345 – 2353
Canaud et al. J Am Soc Nephrol 2014; 25(2): 407 – 419

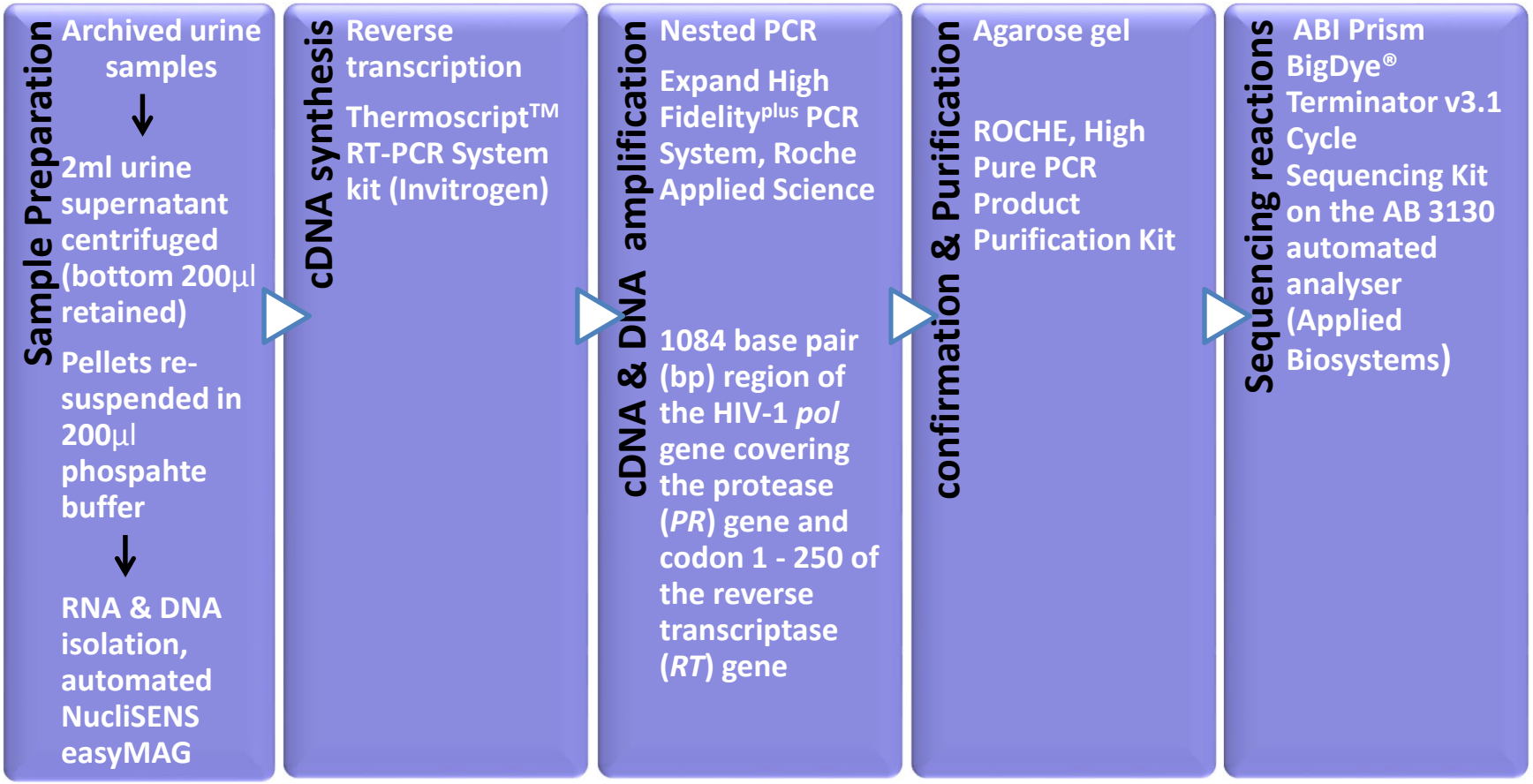
Study aims

- To investigate HIV-1 replication, compartmentalization and persistence in the urinary tract during suppressive ART using urine samples.

Study methods

- Archived urine samples from the Soweto Lung Cohort (n = 756) recruited from patients in Soweto, South Africa between November 2008 and October 2012
- 6 monthly visits
  - Samples & clinical data
- Following collection, urine samples were clarified by centrifugation and stored at -70 °C as supernatants and cell pellets.

Study methods



* Extracted DNA quantitated using the NanoDrop 2000 Spectrophotometer

Zhou et al. PLoS ONE 2011; 6(11): e28184.

RESULTS

	Participants (N = 20)
Age (years)	
Mean (std)	37.8 (6.4)
Gender	
% males	3 (15%)
% females	17 (85%)
Log_{10} viral load at enrolment	
Median (IQR)	4.17 (4.05-4.44)
CD4 count at enrolment (cells/µl)	
Median (IQR)	265 (232-325)
N participants followed up for:	
12 months	1 (5%)
18 months	7 (35%)
24 months	1 (5%)
30 months	3 (15%)
36 months	8 (40%)

Clinical and demographical characteristics of 20 study participants

HIV-1 nucleic acid detection in urine samples

Group	Number of samples	Positive samples			# of Subjects with positive samples		
		Supernat.	Pellet	Total	Supernat.	Pellet	Any
Overall	Samples n = 97 (20 individuals)	13 (13.4%)	14 (14.4%)	27 **(13.9%)**	8 (40%)	7 (35%)	**8 (40%)**
Treatment naïve	Samples n = 84 (20 individuals)	12 (14.3%)	13 (15.5%)	25 (15.2%)	8 (40%)	7 (35%)	8 (40%)
Virological failure (VL>1000 RNA copies/ml)	Samples n = 13 (7 individuals)	0 (0%)	1 (7.7%)	1 (3.8%)	0 (0%)	1 (14.3%)	1 (14.3%)
Virally suppressed/ low level viremia	Samples n = 21 (13 individuals)	1 (4.8%)	0 (0%)	1 (2.4%)	1 (7.7%)	0 (0%)	**1 (7.7%)**

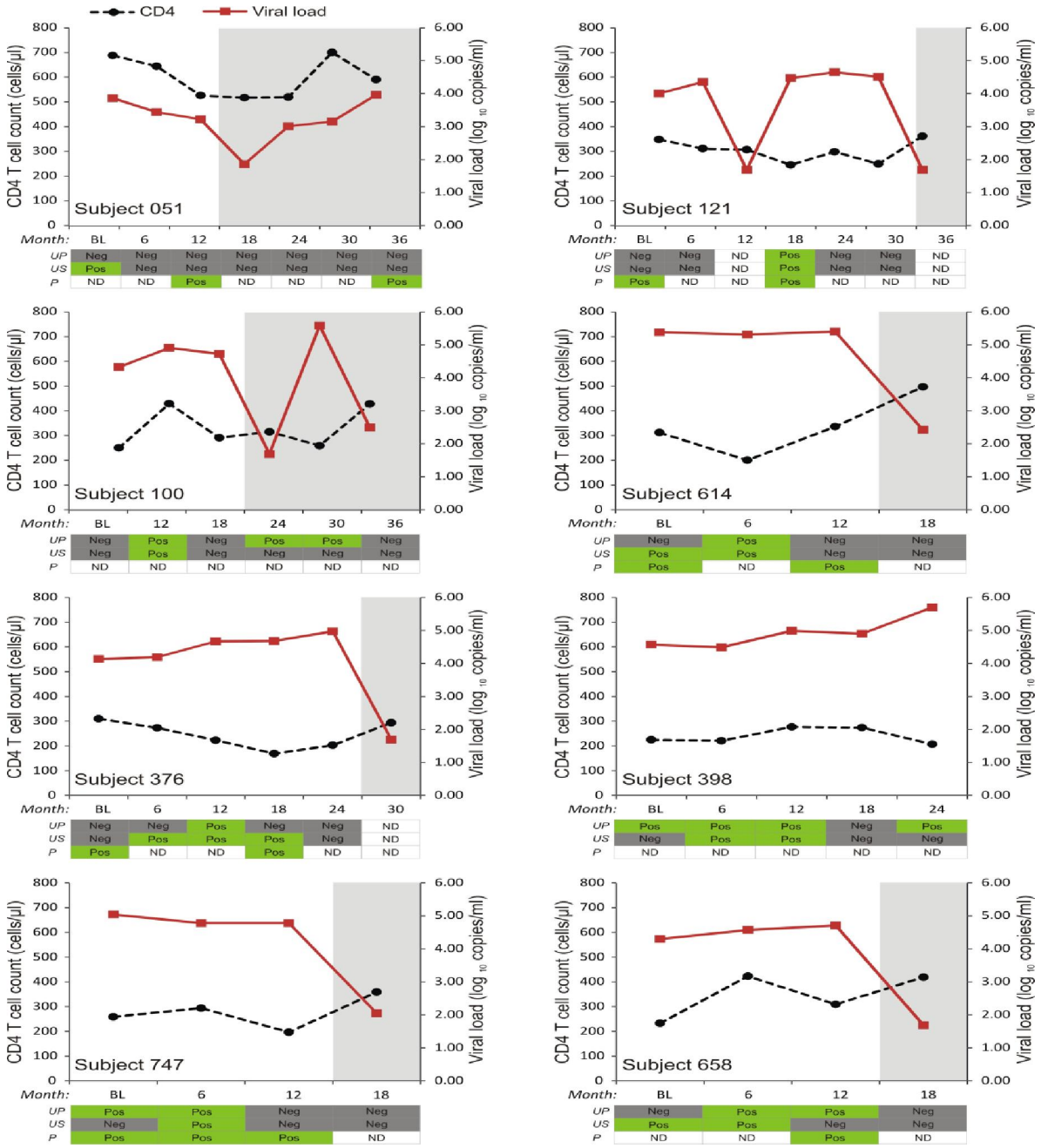
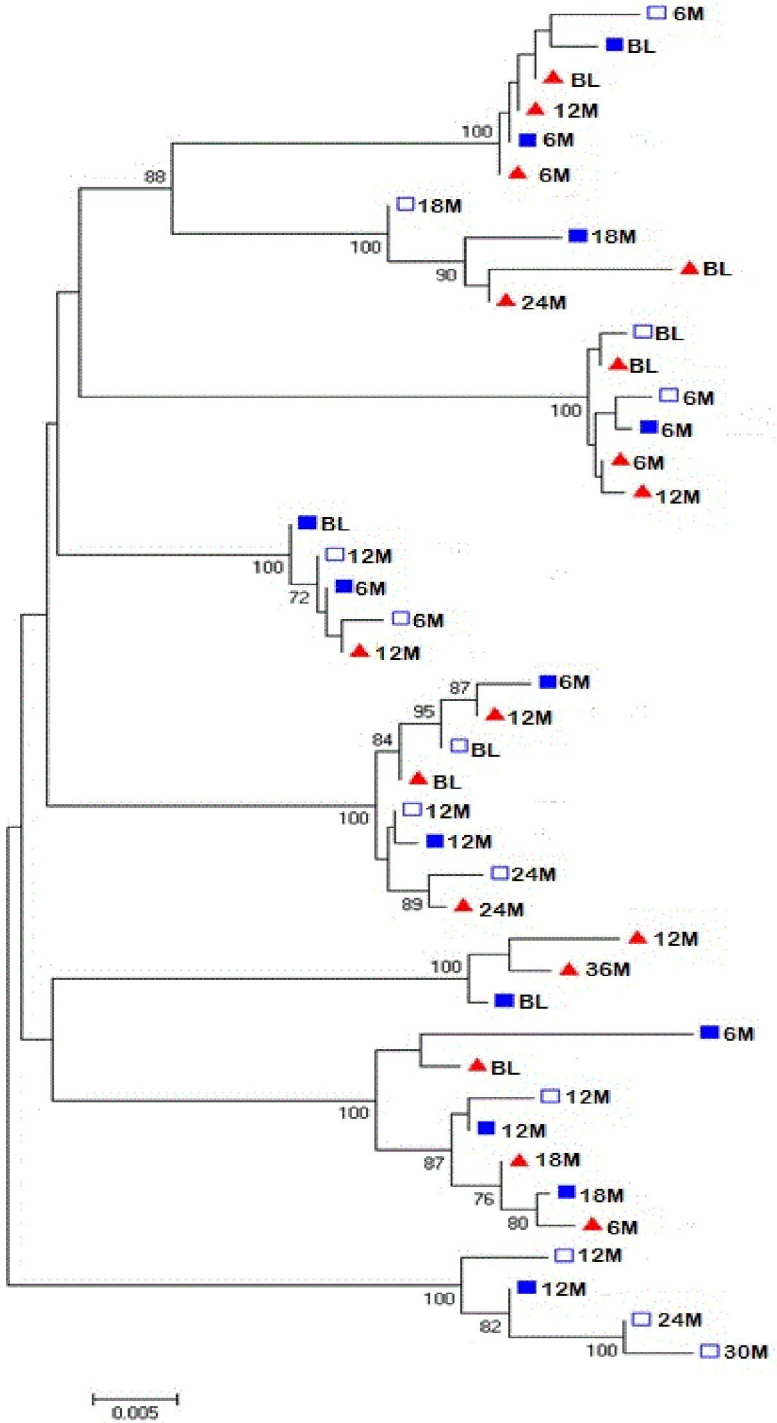


Figure shows frequency of nucleic acid detection in plasma and urine over time compared to CD4 T cell counts and viral loads at time of testing



Phylogenetic tree of the sequenced *pol* gene from the urine and blood viruses of 8 individuals with detected NA in urine.

- plasma RNA
- urine supernatant variants
- urine pellet variants.

- Sequences are named using time points (e.g 6M = 6 months, BL = baseline

Neighbour-Joining method. The bootstrap values (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method. Evolutionary analyses were conducted in MEGA6.

Discussion and conclusions

- We isolated and molecularly characterized HIV-1 viruses in urine samples collected prospectively from twenty HIV-1 infected individuals, before and after antiretroviral treatment initiation and compared them to blood-derived viruses
- Urine samples may be used to study HIV activity in the urinary tract.
  - Non invasive
  - Difficulty in getting kidney biopsies

Discussion and conclusions

- Suppressive ART reduces HIV-1 replication in the urinary tract but HIV-1 DNA may persist and be shed from some cells in the urinary tract despite treatment.
  - HIV-1 DNA detected in urine samples in 23% of virally suppressed subjects on ART in another study
  - Need to characterize & quantify cell types involved
  - Replication competence and potential for rebound?

Winston et al. N Engl J Med 2001; 344:1979 - 1984

Discussion and conclusions

- The frequency of detection for each individual over time varied substantially.
  - Detection of the HIV-1 did not occur at all the time points tested regardless of the associated higher plasma viral loads.
  - Specific factors may modulate the presence of HIV in urine.
    - HIV-1 shedding may be intermittent leading to absence of virus in some samples.
    - Volume used
    - Presence or degree of renal injury, which may affect the rate of shedding of renal epithelial cells, was unknown in our subjects.

Discussion and conclusions

- Possibility of independent HIV-1 replication in the urinary tract but also direct virus importation from blood resulting in equilibrium between blood viruses and viruses shed from the urinary
  - larger number of sequences would be required to confirm compartmentalization.

Poliomyelitis Research Foundation

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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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SA NICD Centre for HIV and Sexually Transmitted Infections

- Caroline Tiemessen
- Gillian Hunt
- Ria lassauniere
- Monalisa Kalimashi
- Aisha Bongwe
- Johanna Ledwaba

University of Pretoria Medical Virol. dept

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- Karin Richter

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- Richard Chaiss

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