

WAKA HPV Africa Symposium

DRC, Kinshasa

02 December 2015



HPV and STIs Training Centre for Africa

Dr. Ramokone Lisbeth Lebelo
Sefako Makgatho Health Sciences
University



Centre location



14th of April 2015.



To be one of the top 100 universities
in the world in teaching, research,
knowledge transfer

Sefako Makgatho
Health Science University



HPV and STIs Training Centre for Africa



Official opening on the 27th May 2015

Collaborators and Partners

- VLIR-IUC
 - Vlaamse Interuniversitaire Raad (Flemish Interuniversity Council-Institutional University Cooperation).
- NSS-VLIR (WAKA HPV AFRICA Network).
 - North South South-Vlaamse Interuniversitaire Raad (Flemish Interuniversity Council).
- Sefako Makgatho Health Sciences University (SMU).
 - Department of Microbiology
 - Department of Virology
 - Department of Anatomical Pathology.



Aims WAKA/VLIR

- Set up international reference centre.
 - To establish internationally recognized centre for HPV and cervical cancer research.
 - To capacitate individuals from African countries on LBC testing and HPV testing.
 - To strengthen research in the HPV field and other STIs in African countries/institutions.
 - To encourage collaborations between African countries/institutions.
 - To generate HPV genotype epidemiology in African countries .
 - Investigate cervical cancer disease burden in low resource African countries.
- To train and produce PhD candidates.

Tests and the type of samples

- Cytology (ThinPrep T5000)
 - Cervical LBC samples
 - Self collected cervical LBC samples
 - Oral LBC samples
 - Anal LBC samples
 - Penile LBC samples
- HPV detection & genotyping (Panther system, Abbott m2000 & BioPlex, MagPix)
 - Cervical LBC samples
 - Self collected cervical LBC samples
 - Oral LBC samples
 - Anal LBC samples
 - Penile LBC samples
 - Formalin fixed paraffin embedded tissue samples

Sample transportation

- Samples import permit
 - Information on sample import permit form
 - Address and details of the importer.
 - Address and details of the centre.
 - The type of material transported.
 - The number of samples.
 - Details of the samples
 - Age
 - HIV status if avai

Sample devices and transport solution

Collection devices →



Rovers® Cervex-Brush®

+



Rovers® EndoCervex-Brush®

=



Rovers® Cervex-Brush® Combi



Rovers® Evalyn® Brush



Ilex SA self-sampling device



Transport solutions



Sample collection

10ml or 20ml ThinPrep Preservcyt solution



Rinse the broom/brush

Rinse the broom/brush

1ml for HPV mRNA detection, Panther system

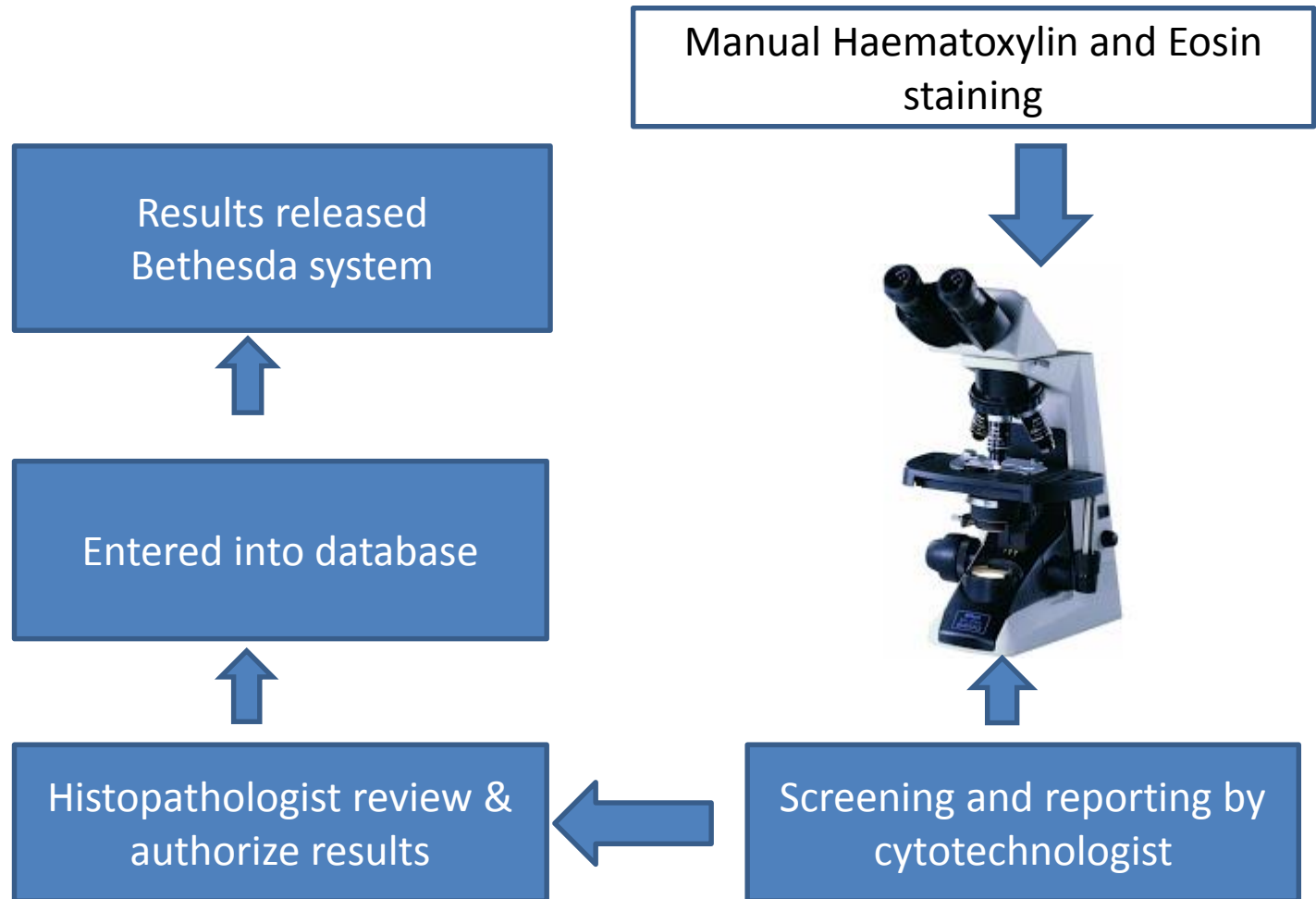
400µl for Abbott HPV DNA extraction & detection,
m2000sp

Refill the ThinPrep Preservcyt vial to 20ml mark for Cytology

ThinPrep T5000 sample processor



Haematoxylin and Eosin staining



Abbott Molecular m2000 RealTime System



Abbott Cervi-Collect
Specimen Collection Kit



Abbott Molecular m2000 RealTime System

- Qualitative PCR that detects HPV DNA.
- Detects 14 HR HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.
- Partially genotype 16, 18.
- Only uses 400µl.

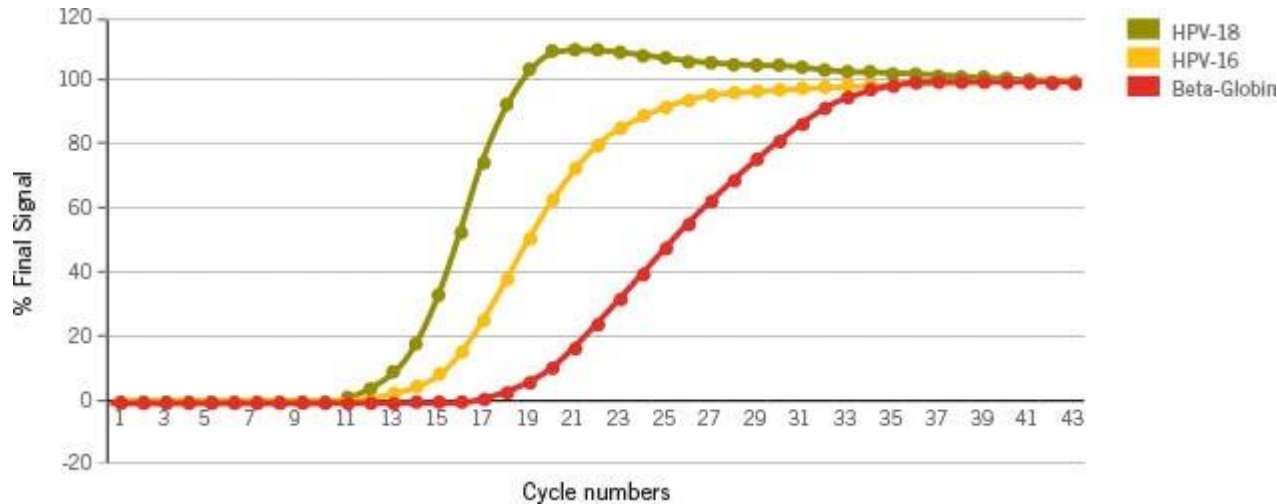


Abbott Molecular m2000 RealTime System

The assay uses four channels for the detection of fluorescent signals:

- First for the detection of an internal process control for
 - Sample adequacy
 - DNA extraction
 - Amplification
- Second for the detection of HPV-16.
- Third for the detection of HPV-18.
- Fourth for the aggregate detection of the 12 HPV types.

Abbott Molecular m2000 RealTime System



Example of the result of the Abbott RealTime High Risk HPV test in a cervical sample in which both HPV-16 and HPV-18 were detected.

Positive result for HPV-16 is visible as orange curve.

Positive result for HPV-18 as green curve.

Internal process control based on amplification of housekeeping beta-globin gene gave positive signal in cycle number.

PANTHER System

Fully Automated and Integrated
Positive Sample Identification
Inventory Management

The APTIMA® HPV Assay.

- Target amplification nucleic acid probe test.
- Qualitative test.
- Detects E6/E7 mRNA of 14 HR HPV types (16/18/31/33/35/39/45/51/52/56/58/59/66/68).



PANTHER System

- All-in-one design
- Primary tube sampling
 - No sample prep
- Sample in – Results out
- Optimized workflow & throughput
 - Quick daily start up
 - Minimal hands on time
 - Fast turn around time
- Onboard automated deactivation
- Load-and-go with unattended results processing

PANTHER System

- First penetrable cap approved for molecular testing
- Used on all specimen collection devices:
 - Urine
 - Endocervical Swab
 - Urethral Swab
 - Vaginal Swab
 - Liquid Pap Transport
 - Cervical Brush
- Prevents crossover contamination
- Reduce ergonomic health issues associated with manual steps:
 - Uncapping/recapping of tubes
 - Manual pipetting



MagPix, LUMINEX® Technology

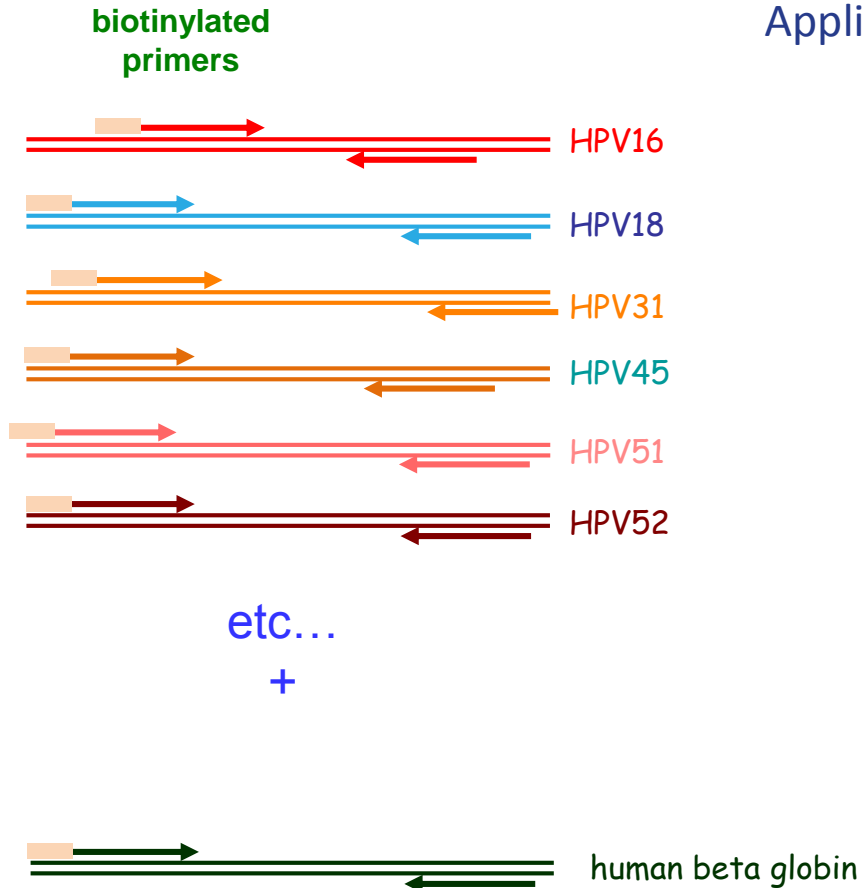


- Quantitative type specific PCR, targeting HPV E7 gene.
- HR HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68a,b, 70, 73, 82).
- LR-HPV types (HPV 6 and 11).
- Detection limits ranges between 10 and 1000 viral genome copies.

E7 multiplex PCR

96-well gene amp PCR system,
AppliedBiosystems

Reaction in a single tube



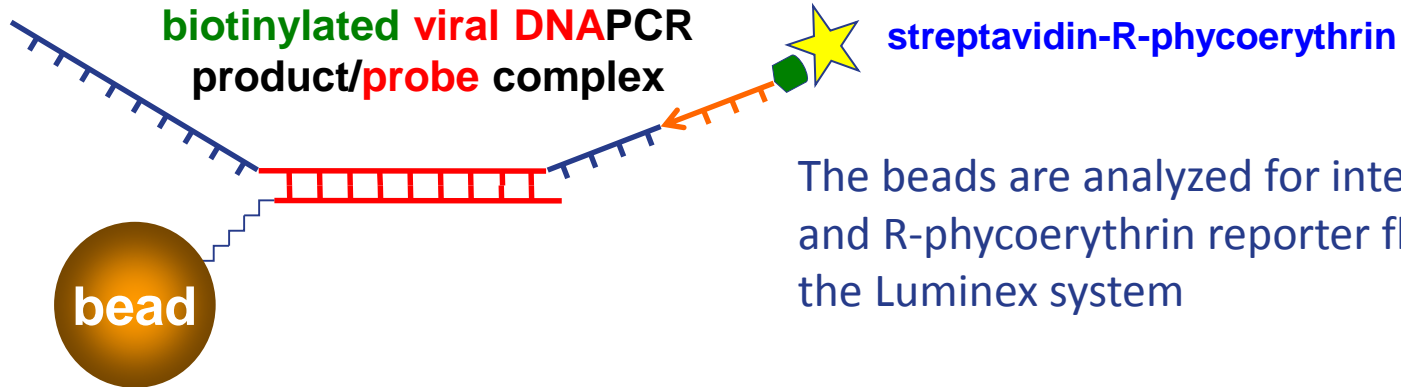
The size of the amplicons generated by PCR is approximately 100 bp.
Bead sets are hybridized with the PCR products

MagPix, LUMINEX® Technology

- Following the PCR amplification
 - PCR products and hybridization solution containing the probes -coupled beads of each set will be transferred to 96-well plates.
 - Hybridization
 - Washing
 - Beads are resuspended in R-phycoerythrin solution.
 - Fluorescence measurement
 - The beads were analyzed for internal bead color and R-phycoerythrin reporter fluorescence on the Luminex

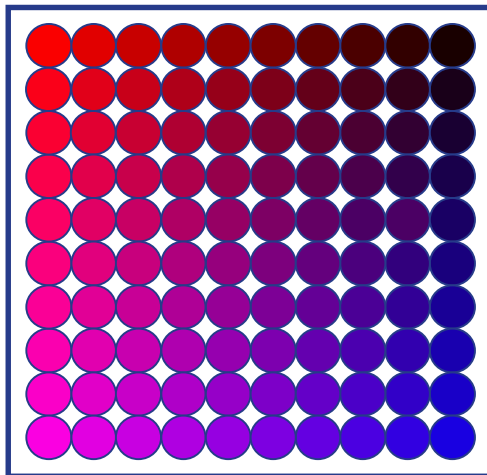
Bio-Plex Luminex-based assay

➤ Multiplex PCR

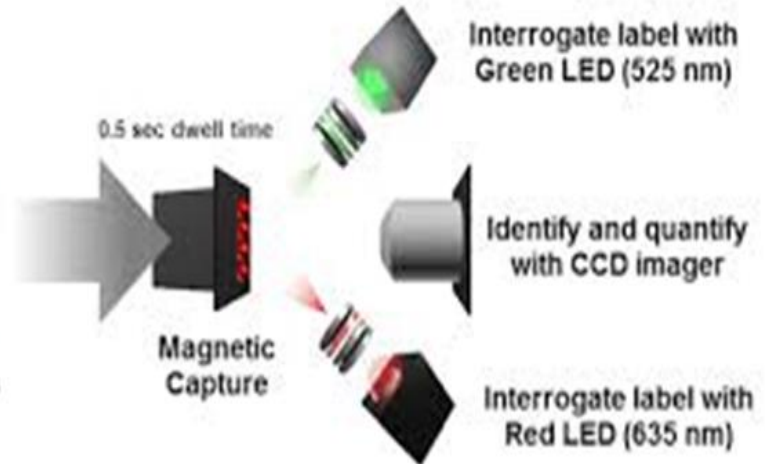
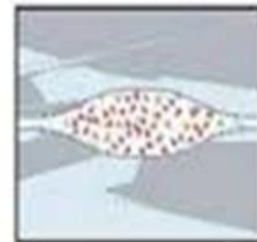


The beads are analyzed for internal bead color and R-phycoerythrin reporter fluorescence on the Luminex system

Fifty of luminex beads with different fluorescent dye are commercially available



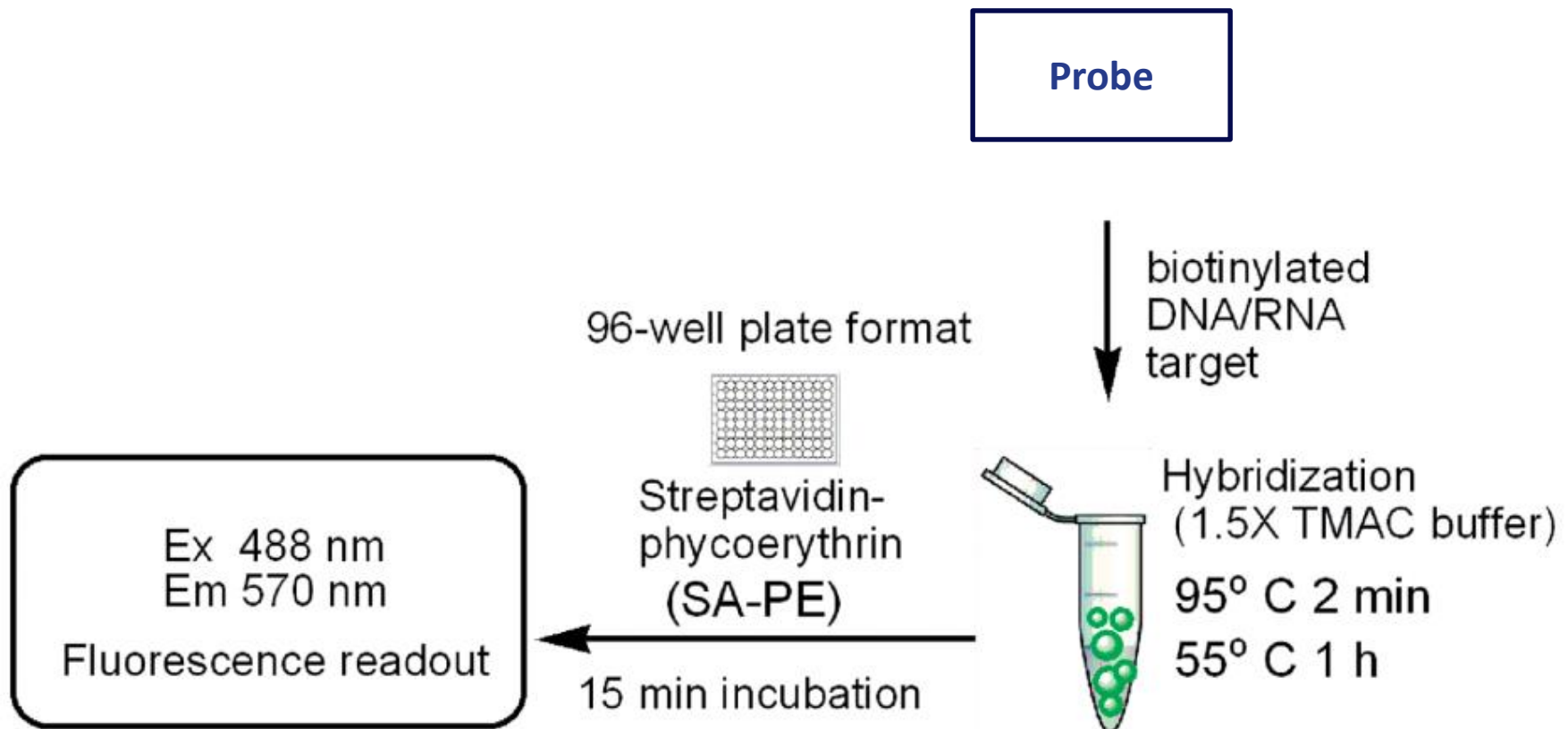
LED-based analysis



High specificity of the Luminex-based assay

| HPV PCR product | HPV type-specific probe | | | | | | | | | | | | | | | | | | | |
|-------------------|-------------------------|------|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----------------|-----|-----|
| | 16_1 | 16_2 | 18 | 26 | 31 | 33 | 35 | 39 | 45 | 51 | 52 | 53 | 56 | 58 | 59 | 66 | 68 | 70 | 73 | 82 |
| 16 | 369 | 289 | 5 | 10 | 4 | 16 | 4 | 13 | 4 | 15 | 4 | 9 | 14 | 9 | 15 | 10 | 8 | 15 | 8 | 6 |
| 18 | 13 | 2 | 701 | 11 | 2 | 9 | 3 | 12 | 4 | 12 | 3 | 9 | 7 | 5 | 16 | 6 | 6 | 15 | 8 | 6 |
| 26 | 14 | 2 | 3 | 152 | 2 | 10 | 3 | 13 | 4 | 12 | 3 | 8 | 8 | 5 | 15 | 6 | 6 | 23 ^b | 7 | 6 |
| 31 | 11 | 4 | 4 | 10 | 791 | 13 | 4 | 12 | 4 | 15 | 4 | 9 | 11 | 8 | 16 | 8 | 8 | 15 | 8 | 7 |
| 33 | 12 | 3 | 5 | 11 | 3 | 152 | 3 | 13 | 4 | 16 | 5 | 10 | 12 | 9 | 16 | 9 | 8 | 17 | 8 | 7 |
| 35 | 12 | 2 | 2 | 11 | 3 | 11 | 218 | 12 | 4 | 14 | 3 | 9 | 10 | 7 | 15 | 7 | 7 | 15 | 7 | 6 |
| 39 | 11 | 2 | 4 | 12 | 2 | 10 | 4 | 189 | 4 | 14 | 3 | 9 | 7 | 4 | 15 | 6 | 6 | 17 | 6 | 6 |
| 45 | 12 | 2 | 4 | 11 | 2 | 13 | 3 | 14 | 1055 | 14 | 5 | 9 | 11 | 8 | 17 | 9 | 7 | 16 | 7 | 7 |
| 51 | 10 | 3 | 3 | 10 | 2 | 12 | 4 | 12 | 4 | 216 | 3 | 9 | 9 | 6 | 15 | 8 | 7 | 15 | 6 | 6 |
| 52 | 11 | 2 | 4 | 11 | 2 | 14 | 4 | 13 | 5 | 13 | 114 | 8 | 10 | 7 | 16 | 8 | 7 | 16 | 7 | 6 |
| 53 | 11 | 2 | 4 | 9 | 3 | 12 | 3 | 12 | 4 | 14 | 4 | 116 | 9 | 7 | 16 | 7 | 8 | 15 | 7 | 6 |
| 56 | 12 | 3 | 4 | 10 | 2 | 12 | 4 | 12 | 4 | 15 | 5 | 9 | 705 | 7 | 16 | 9 | 7 | 16 | 7 | 6 |
| 58 | 11 | 2 | 3 | 9 | 3 | 12 | 3 | 13 | 3 | 14 | 4 | 8 | 9 | 218 | 16 | 7 | 6 | 15 | 6 | 6 |
| 59 | 8 | 2 | 3 | 7 | 2 | 10 | 3 | 10 | 4 | 12 | 3 | 7 | 9 | 7 | 292 | 6 | 6 | 12 | 7 | 5 |
| 66 | 10 | 2 | 3 | 10 | 3 | 10 | 3 | 12 | 3 | 13 | 4 | 8 | 10 | 7 | 15 | 577 | 6 | 14 | 6 | 5 |
| 68 | 12 | 2 | 3 | 11 | 2 | 12 | 4 | 12 | 4 | 12 | 3 | 9 | 9 | 7 | 16 | 8 | 332 | 16 | 7 | 6 |
| 70 | 11 | 2 | 3 | 11 | 2 | 12 | 3 | 13 | 3 | 14 | 4 | 9 | 9 | 7 | 18 | 8 | 8 | 216 | 7 | 6 |
| 73 | 7 | 2 | 3 | 6 | 2 | 11 | 3 | 8 | 4 | 13 | 3 | 5 | 8 | 7 | 12 | 7 | 6 | 11 | 215 | 5 |
| 82 | 11 | 2 | 3 | 8 | 2 | 12 | 3 | 11 | 3 | 13 | 3 | 8 | 8 | 7 | 14 | 7 | 6 | 15 | 6 | 227 |
| None ^a | 10 | 2 | 4 | 10 | 2 | 10 | 4 | 13 | 4 | 13 | 4 | 8 | 11 | 9 | 13 | 10 | 8 | 13 | 8 | 7 |
| Cutoff | 17 | 7 | 8 | 17 | 7 | 18 | 8 | 18 | 9 | 20 | 9 | 15 | 15 | 13 | 23 | 13 | 13 | 22 | 13 | 12 |

Luminex-based assay



Bio-Plex Data Pro software with manager software

| | |
|---------------------------|--|
| Configurations | Workstation version: Performs system control, data collection, data analysis Desktop version: Performs data analysis on a single computer Netshare version: Performs data analysis for multiple users from a file server |
| Computer requirements | Minimum (Recommended) |
| Operating system | Data acquisition: Windows 7 32-bit Data analysis: Windows 7 32-bit (Windows 7 64-bit) |
| Processor | Pentium III or equivalent, 733 MHz (Pentium III or higher, 1 GHz or higher) Hyperthreading must be turned off |
| Disk space | 4 GB (40 GB) |
| Memory | 128 MB (256 MB) |
| Minimum screen resolution | 1024 x 768 |
| Bit depth | 8-bit, 256 colors (24-bit True Color) |
| Ports | 1 RS232 serial port, 1 USB port, and 1 USB port with USB-to-serial converter(2 RS232 serial ports and 1 USB port) |
| Other software | Internet Explorer 6.0 or higher Microsoft Excel 2000 or higher |

HPV genotypes are generated automatically using Bio-Plex Data Pro Software (Luminex) that recognizes fluorescent peaks according to specific color and size.

Data storage and retrieval

Software - eLab system

- Full-scale clinical Laboratory Information Management System (LIMS).
- Single lab deployment or local or regional chain of labs connected together.
- Access control and audit trails keep data secure.
 - In the process to procure a laptop for the centre to be able install the LIMS software.
- It assist conforming to legislative guidelines concerning data integrity and patient security.

Quality control and quality assurance

- External quality control programs.
- Inter-laboratory quality control programs.
- Exchange of samples with other laboratories
 - Create a Bio-bank of all the samples received
 - Store all the extracted and tested material.
 - Panther
 - Abbott



Important logistics

- Ethical Clearance of the studies.
- Collection of samples.
- Packaging of the samples.
- Transportation of samples.
- Reporting/access of the results.



Funding

- WAKA-VLIR NSS funding
 - Cytology testing
 - ThinPrep Vials
 - HPV detection
 - HPV genotyping
 - Approved by Profs JP Bogers & JP Van geertruyden
- VLIR-IUC funding in other African countries.
- External funding application for all the projects supported by WAKA HPV Africa Network.



HPV & STIs Training Centre for Africa

- Head: Dr Ramokone Lisbeth Lebelo
 - Medical Scientist
- Histopathologists
- Cytotechnologists/Cytotechnicians
- Virology Pathologists
- Medical Scientists



Progress

- Prepared and screened 300 LBC samples from Burundi for cytology.
- Organized permit for DRC sample transportation.
- Piloted oral collection brushes for LBC cytology.

Acknowledgements

- Prof JP Bogers (University of Antwerp)
- Prof JP Van geetruyden (University of Antwerp)
- Prof M Nchabeleng (SMU)
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- Prof MJ Mphahlele (SAMRC)
- Prof N Bida (SMU)
- Dr Tarik Gheit & Dr Massimo Tommasino (IARC-France)
- VLIR-IUC
- VLIR-NSS
- WAKA HPV Africa Network
- Hologic
- Ilex SA
- Separation Scientific

Address of the centre

- HPV and STIs Training Centre for Africa
- Sefako Makgatho Health Sciences University
- Department of Virology
- Clinical Pathology Building
- 3rd floor, Room S301
- Molotlegi street
- Next to Dr George Mukhari Academic Hospital
- Pretoria
- South Africa
- 0204
- <https://www.uantwerpen.be/en/projects/waka-hpv-af>

