Cervix cancer screening and prevalence of HPV in Mont Ngafula health district/ Kinshasa/ DR Congo.

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on behalf of the women profile for Africa

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- CESVI Foundation, Bergamo, Italy
- APOF Associazione Patologi Oltre Frontiera
- Fondazione Umberto Veronesi, Milan, Italy

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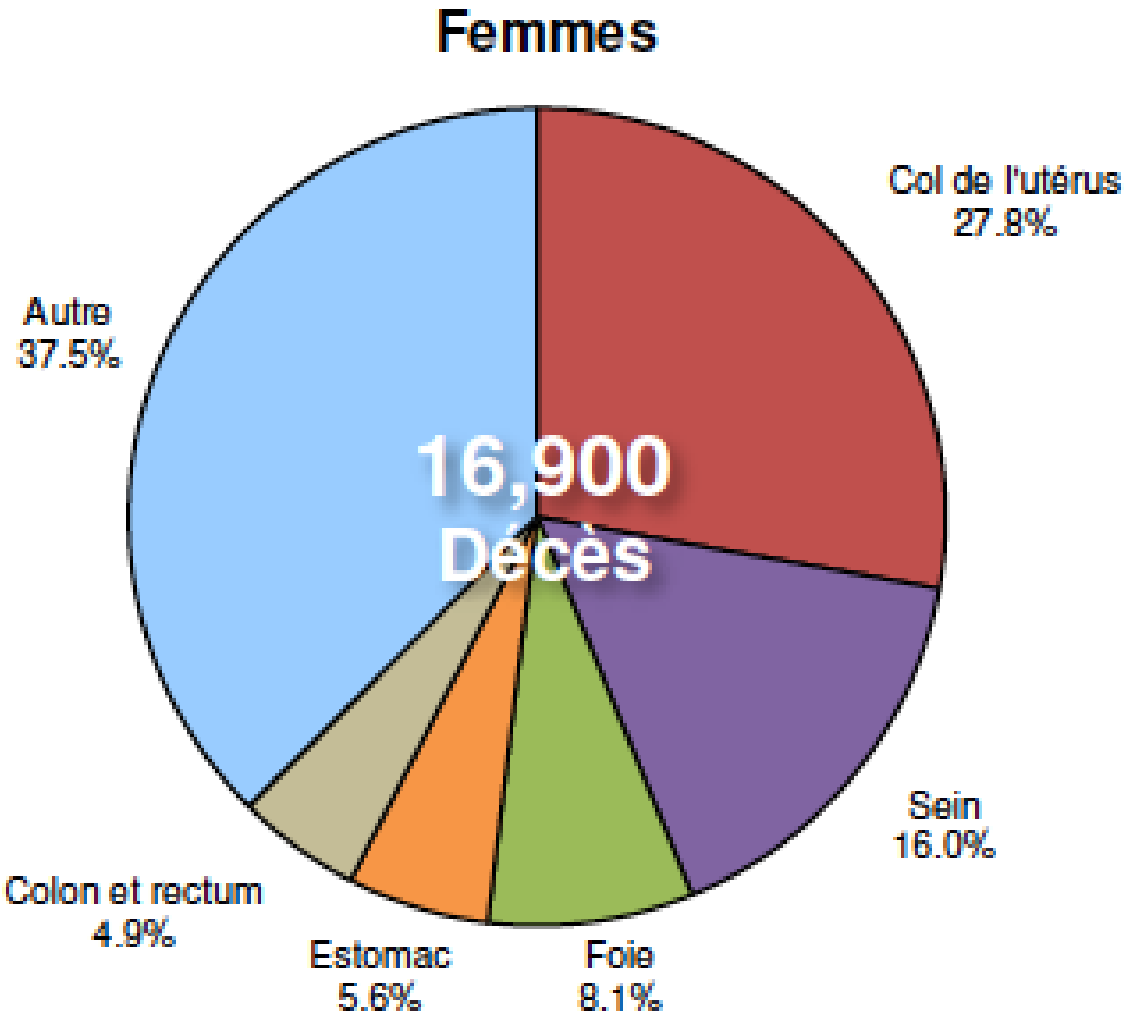
Introduction

- Cervical cancer is a leading cause of death in women in Africa,
- with high incidence and mortality rates in the Democratic Republic of Congo (DRC).
- The women profile for Africa project aimed to:
  - estimate the validity of screening tools,
  - estimate the prevalence of HPV infection and HPV-genotypes
  - assess the correlation between HPV and cervical cytology.
  - pilot a web-based cancer registry.
  - Understand community perception and perspectives.
  - Comparing performance on screening tools between gynaecologists versus MD versus nurses, Pathologists versus lab technicians

Deaths related to cancer in women from DRC: 2014
Source: DRC MoH



Studies done in DRC

1: Laga M, Icenogle JP, Marsella R, Manoka AT, Nzila N, Ryder RW, Vermund SH, Heyward WL, Nelson A, Reeves WC. Genital papillomavirus infection and cervical dysplasia--opportunistic complications of HIV infection. Int J Cancer. 1992 Jan 2;50(1):45-8. PubMed PMID: 1309459.

2: Icenogle JP, Laga M, Miller D, Manoka AT, Tucker RA, Reeves WC. Genotypes and sequence variants of human papillomavirus DNAs from human immunodeficiency virus type 1-infected women with cervical intraepithelial neoplasia. J Infect Dis. 1992 Dec;166(6):1210-6. PubMed PMID: 1331247.

3: Sangwa-Lugoma G, Mahmud S, Nasr SH, Liaras J, Kayembe PK, Tozin RR, Drouin P, Lorincz A, Ferenczy A, Franco EL. Visual inspection as a cervical cancer screening method in a primary health care setting in Africa. Int J Cancer. 2006 Sep 15;119(6):1389-95. PubMed PMID: 16619217.

4: Ali-Risasi C, Praet M, Van Renterghem L, Zinga-Ilunga B, Sengeyi D, Lokomba V, Mukamina L, Ndarabu A, Kayembe NN, Tshilolo L, Mukumbi MH. [Human papillomavirus genotype profile in Kinshasa, Democratic Republic of the Congo: implications for vaccination]. Med Trop (Mars). 2008 Dec;68(6):617-20. French. PubMed PMID:19639831.

5: Mahmud SM, Sangwa-Lugoma G, Nasr SH, Kayembe PK, Tozin RR, Drouin P, Lorincz A, Ferenczy A, Franco EL. Comparison of human papillomavirus testing and cytology for cervical cancer screening in a primary health care setting in the Democratic Republic of the Congo. Gynecol Oncol. 2012 Feb;124(2):286-91. doi:10.1016/j.ygyno.2011.10.031. Epub 2011 Nov 4. PubMed PMID: 22062546.

6: Sankaranarayanan R, Anorlu R, Sangwa-Lugoma G, Denny LA. Infrastructure requirements for human papillomavirus vaccination and cervical cancer screening in sub-Saharan Africa. Vaccine. 2013 Dec 29;31 Suppl 5:F47-52. doi: 10.1016/j.vaccine.2012.06.066. Review. PubMed PMID: 24331747.

7: Ali-Risasi C, Mulumba P, Verdonck K, Vanden Broeck D, Praet M. Knowledge, attitude and practice about cancer of the uterine cervix among women living in Kinshasa, the Democratic Republic of Congo. BMC Womens Health. 2014 Feb 18;14(1):30. doi: 10.1186/1472-6874-14-30. PubMed PMID: 24548698; PubMed Central PMCID: PMC3937079.

- Several attemps have been done to start screening and/or sensibilisation activities on cervical cancer since 1992.
- Studies achieved mainly in specific group as HIV population
- Recommendations formulated:
  - VIA et VILI but results on sensitivity are largely differents from country to country but also from differents studies.
  - Pap-tests. WHO does not recommend Pap-test in developping countries.
  - HPV strains are different from the strains in Europe and North America although HPV 18 was identified in 33% of 54 HIV infected patients stating that vaccination could be problematic if targetted strains are disconcordant.
- Until 2014 still no screening or vaccination program that has been iniated in DRC.
- Cervival Cancer is mostly identified at a late stage with a high mortality.

Methodology

Validation of screening tools

- Training on VIA, VILI, Pap test collection and reading, urines collection, Colposcopy for MD, nurses, pathologists, gynecologists was done by experts in specific field from Italy
- We recruited women for 30 years old to 50 years old.
- We performed VIA, VILI (two times by a MD and a nurse), Pap tests to all recruited women
-
- Colposcopy and biopsy were performed on positive on either VIA, VILI or Pap test and also on a sample of negative in all screening tools.
- Bethesda system was used to classify cervical samples
- Biopsy is used as a gold standard
- Control quality was performed on all pap tests and biopsy by cytologist in Italy.

Determination of HPV strains

- We use dried urine spots (DUS) samples
- HPV-DNA was detected by nested-PCR (ORF L1) and infecting genotypes through RFLP technique.

✓ Collection of **cervical samples** is not always easy

- in resource-limited settings
- in populations where these procedures may be less well accepted (ex. for young age or socio-cultural/religious implications)

ALTERNATIVE APPROACH

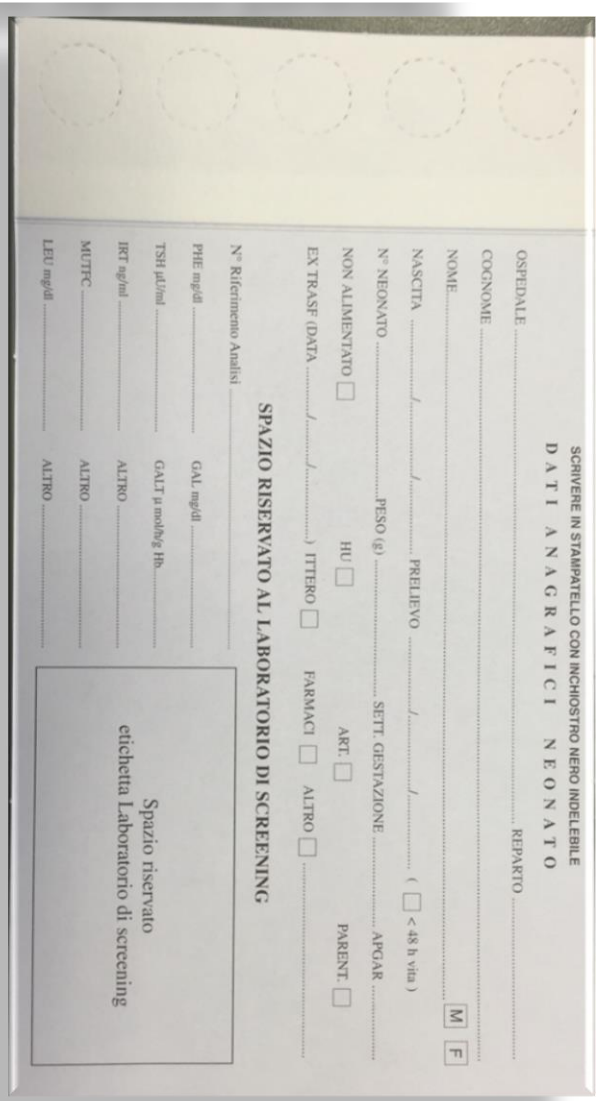
- **Urine sample** for the detection of HPV infection
  - → non-invasive
  - → more accessible and acceptable to women
  - → less expensive
  - → bypasses medical examination
  - → even easier to perform than self-collected vaginal swabs

consequently, the screening coverage could be increased primarily by reaching populations in less developed regions

HPV Testing from Dried Urine Spots (DUS)

DRC HEALTHCARE FACILITIES

- **50μL** of urine samples were spotted on **5** preprinted circles on a filter paper
- DUS was **dried for 3h**
- DUS was **stored** in a paper bag in a dry place at **RT (25–30°C)**



Urine samples collection and DUS preparation

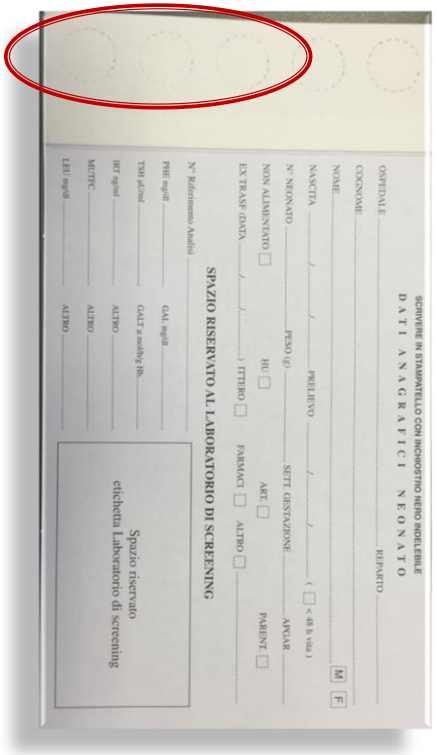
November 2014 - January 2015

N = 456

Asymptomatic women, 30-49 years of age

UNIVERSITY OF MILAN, STIS LABORATORY

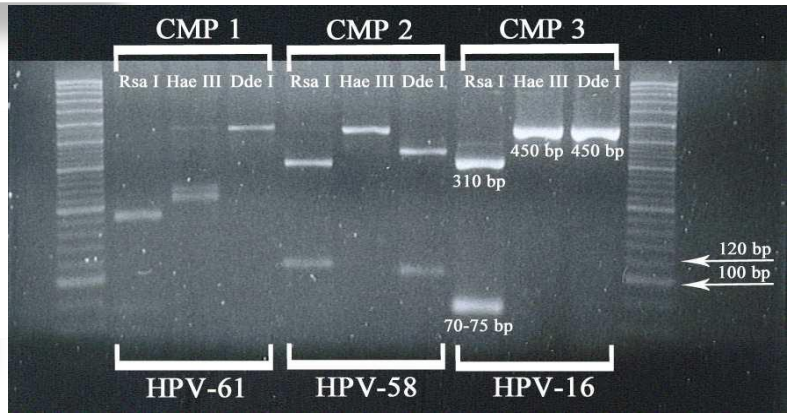
- **3** circles were cut out using a sterile scalpel blade, transferred into 1mL of Lysis Buffer, incubated on a roller mixer for 30’ at RT and then centrifuged for 15’’ at 1500 ×g
- **Nucleic acids extraction**: lysate (750μL) was extracted using the NucliSENS EasyMAG method
- **HPV detection**: nested PCR amplifying a fragment of 150 bp of **ORF L1** region
- **HPV genotyping**: first step (450 bp) of HPV positive samples was genotyped using RFLP technique



HPV detection and genotyping (31/03/2015)

N = 242/456 (53%)

(all DUS prepared in November-December 2014)



HPV genotyping (RFLP technique)

Community percpetion and perceptives

- We used:
  - focus group discussions
  - Interviews of health workers

Preliminary results

Validation of screening tools

n=864 with age range from 30 to 50 years old.

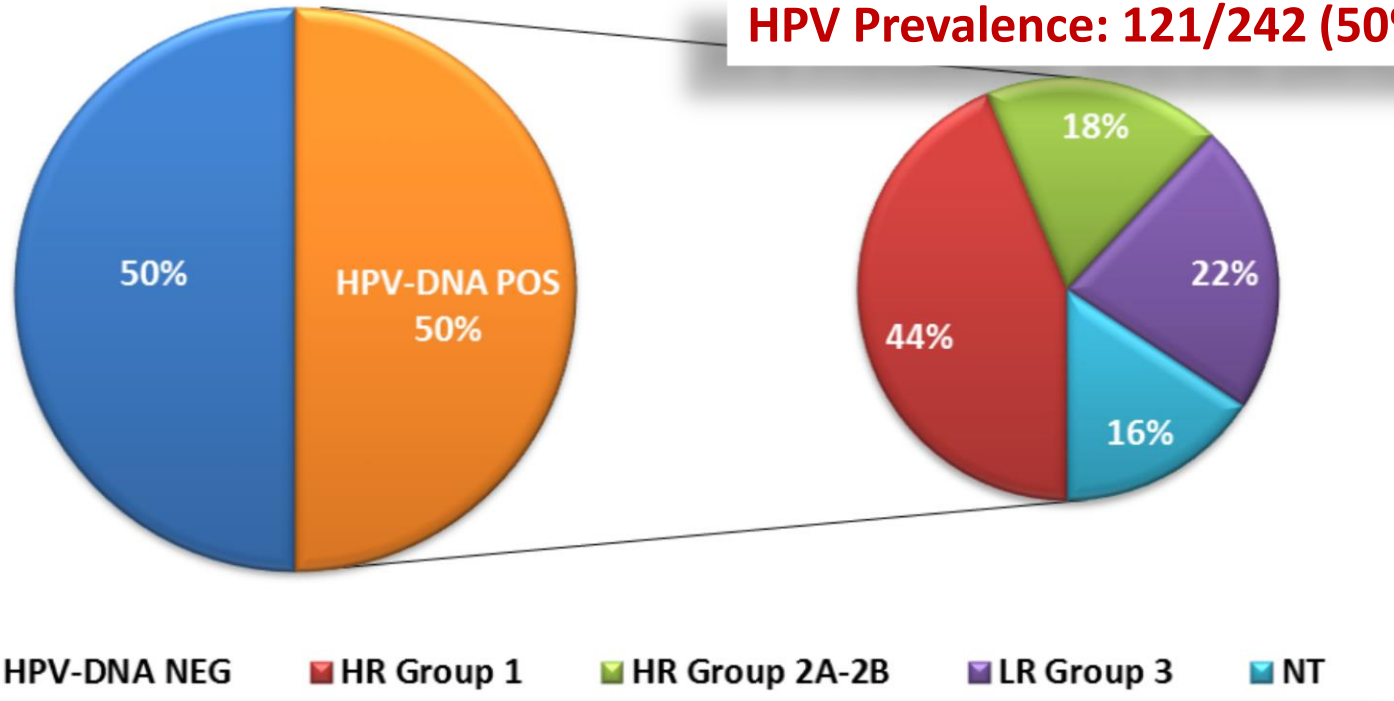
	positive	negative
VIA	65(8%)	747(92%)
VILI	104 (12.1%)	759 (87.9%)
VIA+VILI*	143 (**16.6%**)	721 (83.4%)

*= positive women on either test (either VIA or VILI positive)

PAP	VIA_VILI	
	POSITIF	NEGATIF
Atypical cellular reactive (ACR)	2	15
NEGATIVE	111	617
Atypical Glandular Cells (AGC)	1	2
atypical squamous cells of undetermined significance ASCUS	11	44
atypical squamous cells cannot exclude HSIL ASC-H	5	4
LSIL	2	25
HSIL	4	9
Squamous cells carcinoma	7	5
TOTAL	143 (16.6)	721 (83.4)

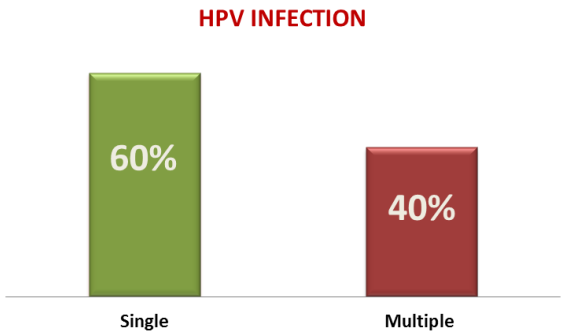
Determination of HPV strains testing from urine and Dried Urine Spots (DUS)

HPV Prevalence: 121/242 (50%)

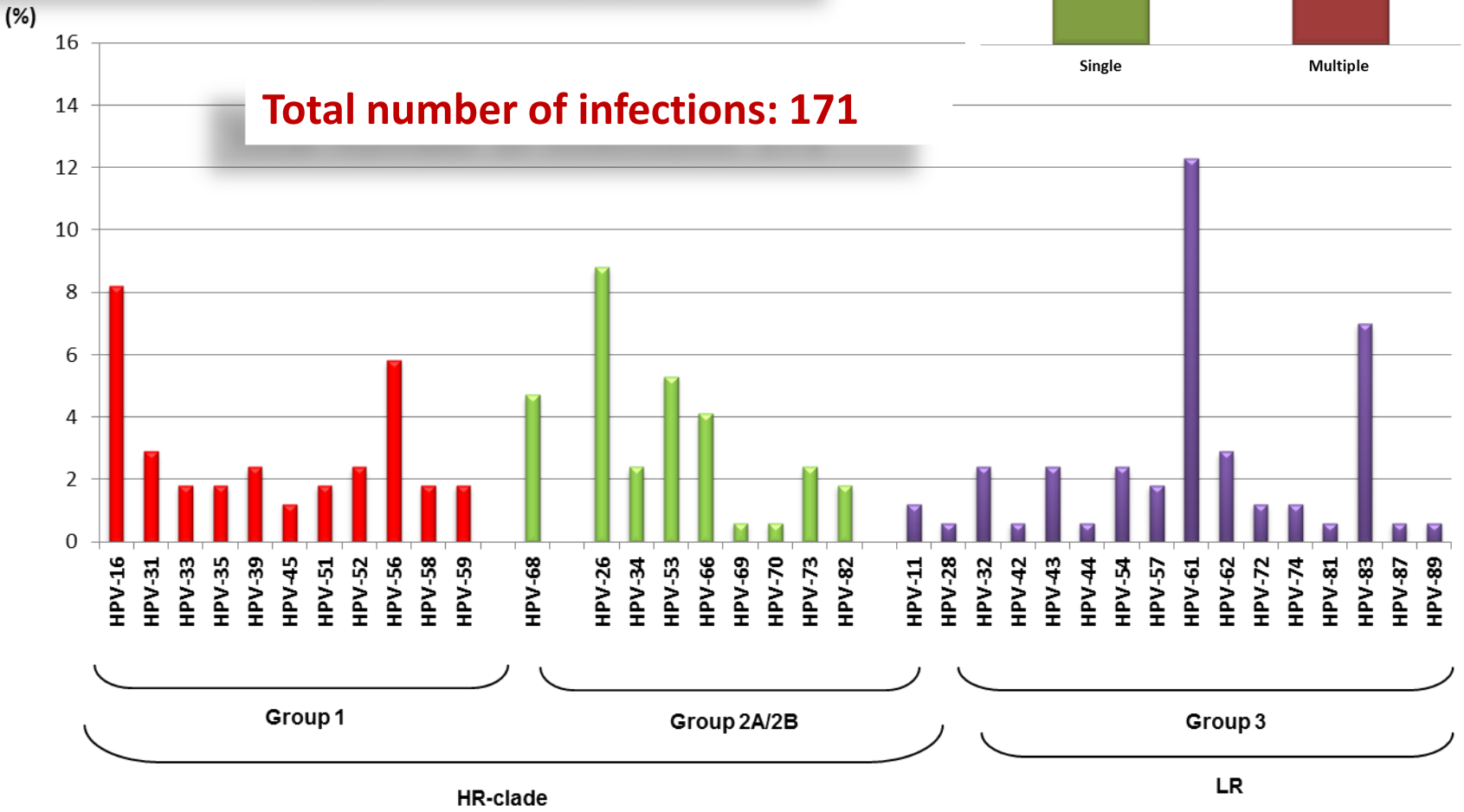


HPV: IARC 2011 classification			
HR Clade	Group 1	Carcinogenic to humans	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
	Group 2A	Probably carcinogenic to humans	68
	Group 2B	Possibly carcinogenic to humans	26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97
LR	Group 3	Not classifiable as carcinogenic to humans	6, 11, 28, 32, 40, 42, 43, 44, 54, 55, 57, 61, 62, 71, 72, 74, 81, 83, 84, 86, 87, 89

HPV Genotype distribution



Total number of infections: 171



Correlation between HPV and cytology

- During November 2014-February 2015, DUS and cervical samples were collected from 209 women (median age, 37.2y)
- HPV-DNA prevalence was 56.5% (IC95% 49.7-63).
- Overall, 167 infections and 39 genotypes were detected.
- 73.6% (IC95% 66.5-79.7) of all infections was sustained by at least one genotype of HR-clade,
- HPV-26 (8.4%) and HPV-16 (6%) were the prevalent.

Correlation between HPV and cytology

- As for cytology results,
  - 32% resulted inadequate,
  - 48.3% negative,
  - 19.7% positive (6.7% ASC-us, 7.2% L-SIL, 2.9% ASC-H, 2.9% H-SIL).
- The prevalence of HR-HPV infections increased with abnormal cervical cytology result:
  - 30.7% in negative
  - and 35.7%, 40%, 50%, 83.3% in ASC-us, L-SIL, ASC-H, H-SIL respectively.

Perception and barriers for cancer screening program

1. Economical barrier

- **Poverty**
  - Fees for a consultation: 25 USD, Transport fees to reach the centre: 3USD, Fees for screening tests and collection of pap samples:10 USD, Fees for pap test reader: 30-50 USD. (lack of cytotech.), Fees for biopsy if needed: around 250 USD. Treatment: ? Depend on the level of lesion. Chemotherapy and radiotherapy can reach 2000 or 3000 USD per course.

Resolution: providing free access to screening? But How and by who??

- **Opportunity lost:** Women who survive daily with small trade do not adhere because they perceive it as loss of income during the time they are at the screening facility.

«…who will sell for me? I cannot abandon my table…»

. **Treatment cost** for precancerous lesions but also for cancer because at the begining of screening certainly some invasive cancers will be detected. Chemotherapy and radiotherapy are not affordable in DRC and there is no mutual or health insurance.

2. Geographical barriers

- Screening facilities need to be accessible and as close as possible to the population.
    - *Problem*: service not available for women.
    - *Solution*: Set up 4 pilot screening centers in kinshasa with pap test as screening tool.
    - But in rural area, facilities are rare and we need to think how to solve this problem.

3. Cultural barriers

Lack of information on cervical cancer screening: women continue to be ignorant and dont seek correct information

Habits: In general, women don't consult when they are not feeling ill. Their husband cannot let them to see a gynecologist when they are not seek. Problem of privacy.

Ashamed to have an examination when not ill, even the husband does not accept that his wife goes to a consulation when she's not ill.

Fear of knowing that they have cancer *« I want to die if I find out that I have cancer »*

Beliefs: The « bad » illness = bad luck related to bad spirits *« God cannot permit that this illness comes into my family » « Accept screening is opening the door for the devil to enter our family»*

What these preliminary results suggest?

- ✓ Pre-cancerous lesions are frequent in women between 30 and 50 years
- ✓ Validation tools are ongoing but pap-tests seems the best with the possibility of remote quality control. Its feasibility and its costs (30-50 US dollars for readers) in remote area is a limitation. The is a need of cyto-technicians in DRC in support of pathologist.
- ✓ HPV prevalence (50%) is high, but not so far from that observed in studies conducted in Africa using the same molecular methods in women with normal cytology (Kenya: about 40%, Mozambique: 32-41%)
- ✓ The genotype distribution is similar to that found in women of other regions, including those in developed countries (and Italy)

- ✓ More than 60% of HPV-DNA positive women is infected with at least one genotype of HR-clade, about 40% with HR genotypes of Group 1

- ✓ These data, although preliminary, support the need for prevention interventions targeted at women of this age group (> 30 yrs)

- ✓ DUS could be a useful tool for planning cervical cancer screening strategies, especially in less developed regions

- ✓ DUS could also be useful for
  - epidemiological/virological surveillance where pelvic examination is not practical (ex. post-vaccination surveillance in adolescent women) or where other strategies are difficult to apply

  - monitoring of type-specific prevalence (vaccine-preventable HPV types - other non-vaccine types)

- Based on generated data and evidence, we will build a rationale, effective and efficient algorythm for cervix cancer screening in DRC combining HPV test, pap test, VIA, VILI and having in mind the rural remoate are.

- but according to the prevalence, **colposcopy** can become a real bottelneck in case of VIA or HPV testing with this prevalence,
  - too many colposcopy will cost more and will be impossible due to
  - lack of colposcopes
  - and trained colposcopists.

- Finally, we forgot to have a PhD student on this project.
- Thanks for your attention.