

A Global Perspective on HPV Infection and Cervical Cancer

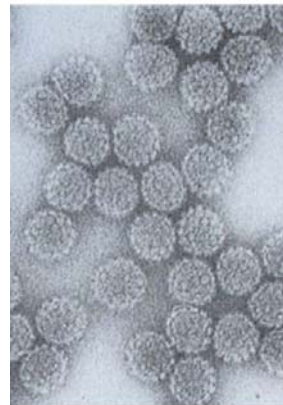
Al Bateman

Assistant Director, Communicable Diseases
Wisconsin State Laboratory of Hygiene

Kaitlin Sundling's February WCLN webinar

"The Biology of HPV Infection and Cervical Cancer"

- Cervical cancer overview
- Pap test
- HPV, HPV disease, HPV vaccine
- Transient and persistent HPV
- Integration of HPV testing and cytologic findings in cervical cancer screening guidelines



Doorbar J, et al. *Rev Med Virol.* 2015;25:2-23.

Learning objectives

- Describe broad epidemiologic patterns of HPV and cervical cancer worldwide
- Explain how HIV infection affects the progression of HPV infection to cervical cancer
- Relate how applied research can have both scientific and policy implications, with the example of HPV genotyping and HPV vaccine implementation in sub-Saharan Africa

My background



HPV

“HPV is so common that nearly all sexually active men and women get the virus at some point in their lives.”

<https://www.cdc.gov/std/hpv/stats.htm>

HPV-related cancer site (ICD-10 code)	Number of incident cases ^{1,2}	Number attributable to HPV	AF (%)
Cervix uteri (C53)	530,000	530,000	100.0
Anus ³ (C21)	40,000	35,000	88.0
Vulva ³ (C51)	34,000	8,500	24.9
Vagina ³ (C52)	15,000	12,000	78.0
Penis ³ (C60)	26,000	13,000	50.0
Oropharynx ³ (C01, C09–10)	96,000	29,000	30.8
Oral cavity ³ (C02–06)	200,000	4,400	2.2
Larynx (C32)	160,000	3,800	2.4
Other pharynx ³ (C12–C14)	78,000	0	0
Total HPV-related sites	1,200,000	630,000	54.0

de Martel, C. Int'l Journal of Cancer. 2017

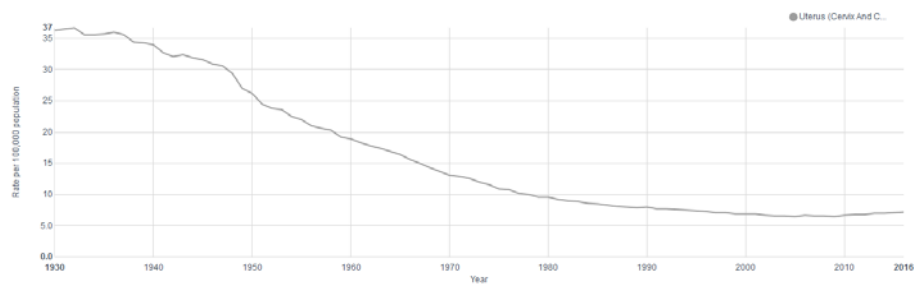
HPV and cervical cancer

- Two-thirds of cervical cancer occur in less developed countries
- The relative contributions of HPV16/18 is 73%, and HPV16/18/31/33/45/52/58 is 90%

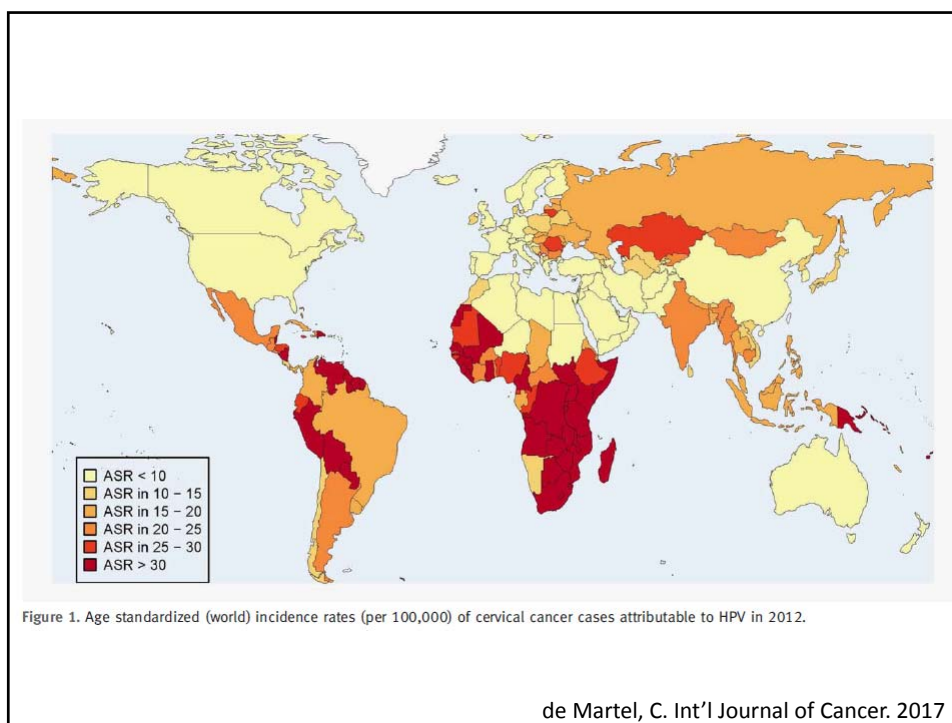
de Martel, C. Int'l Journal of Cancer. 2017

Trends in death rates, 1930-2016

Per 100,000, age adjusted to the 2000 US standard population.



<https://cancerstatisticscenter.cancer.org/>

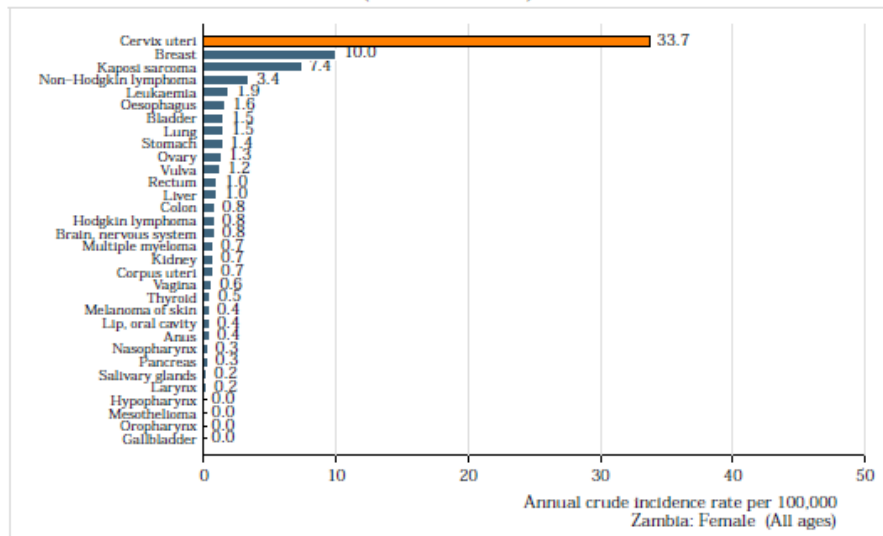


Cervical cancer

- Cancer of the cervix uteri is the 3rd most common cancer among women worldwide, with an estimated 569,847 new cases and 311,365 deaths in 2018 (GLOBOCAN)
- Cervical cancer ranks as the 1st leading cause of female cancer in Zambia

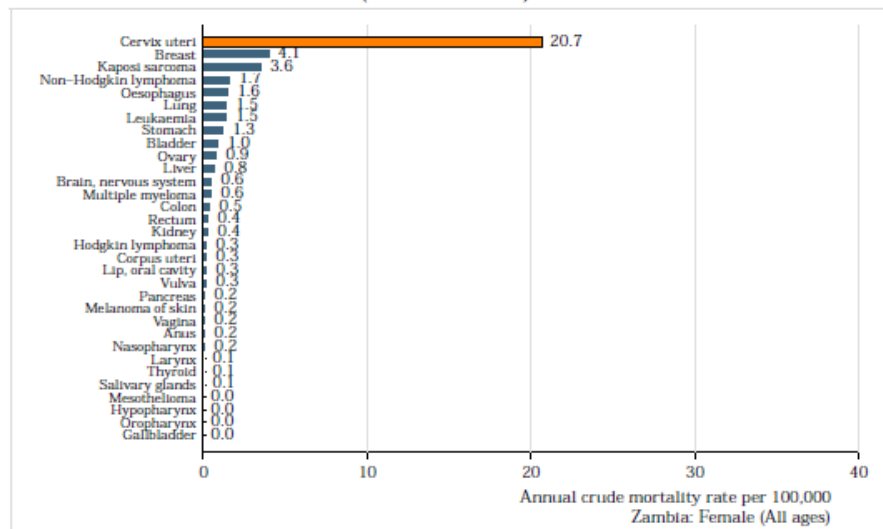
Zambia HPV IARC report, December 2018

Figure 5: Comparison of cervical cancer incidence to other cancers in women of all ages in Zambia (estimates for 2018)

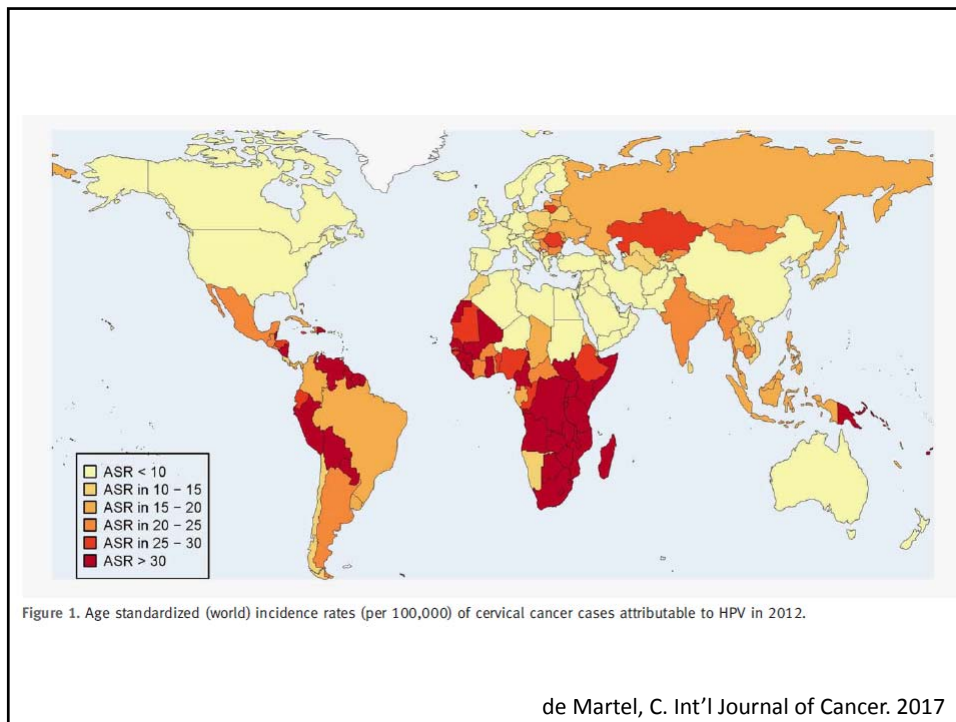


Zambia HPV IARC report, December 2018

Figure 11: Comparison of cervical cancer mortality to other cancers in women of all ages in Zambia (estimates for 2018)



Zambia HPV IARC report, December 2018



HIV exacerbates HPV

- HIV-positive women have a greater prevalence of
 - persistent HPV infection
 - infection with multiple hrHPV types
- Leads to
 - increased incidence of precancerous lesions
 - faster progression to invasive cervical cancer
- Recommendation that HIV-positive women be screened for cervical cancer annually, compared with screening every 3–5 years for HIV-negative women

HPV vaccines available globally

- Three currently available vaccines
 - Cervarix (GlaxoSmithKline)
 - two-valent vaccine targeting HPV16/18
 - Gardasil (Merck)
 - four-valent vaccine targeting HPV16/18 and HPV6/11
 - Gardasil 9 (Merck)
 - nine-valent vaccine targeting HPV6/11/16/18 and the next five most carcinogenic types (HPV31/33/45/52/58)

de Martel, C. Int'l Journal of Cancer. 2017

Zambia



- British colony
- Independence in 1964
- Peaceful
 - 4 democratic changes of power



- Zambian NGO, affiliated with UNC-Chapel Hill and UAB
- Implementing organization for PEPFAR
 - Through CIDRZ-supported programs, more than 1,000,000 have been tested for HIV and over 120,000 are receiving care
- 3 main aspects: Research, health services, and training
- Focus areas: HIV/AIDS, TB, Women's health, Newborn and Child Health, Community, and Health Systems Strengthening
- Women's health: breast cancer and cervical cancer
- Me:
 - HPV genotyping
 - Cervical cancer screening

CIDRZ Central Laboratory

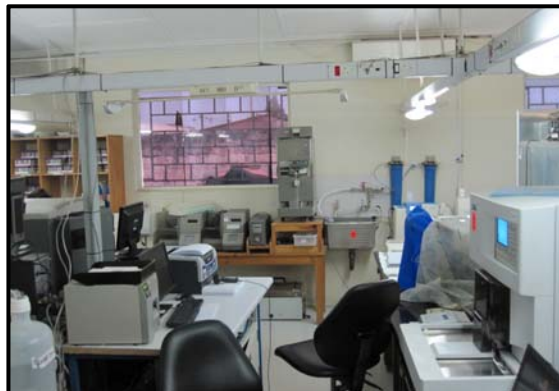
Major reference lab for the country

- TB testing
- HIV viral load
- HIV genotyping by Sanger sequencing
- Dried blood spot testing for early infant diagnosis of HIV
- Hematology, chemistry, immunochemistry, serology



CIDRZ Central Laboratory

- Major reference lab for the country
 - ~70 employees
 - Tens of thousands of tests per month
 - Testing for clinical care (reference lab for PEPFAR, plus business/outreach), international HIV research studies, and individual research projects

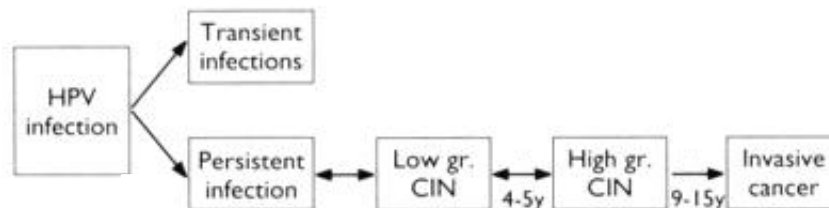




HPV genotyping cervical cancer specimens in Zambia

- Over 120 genotypes of HPV
 - 14 or 15 are oncogenic (aka, high-risk HPV)
- HPV vaccines at the time (Cervarix and Gardasil)
 - include HPV-16 and HPV-18, the two high-risk genotypes that cause 60-70% of cervical cancer worldwide
 - Gardasil 9, is now available
- Immunity is relatively genotype-specific, and it wasn't known which genotypes were most prevalent in cervical cancer in Zambia

Cervical cancer progression



Previous studies

- Studies using pre-cancerous samples from women in Zambia and South Africa: a wide diversity of HPV genotypes in HIV-positive patients
 - HPV-16 and HPV-18 not the most common
- Pre-cancerous lesions often regress
- To determine which HPV genotypes cause invasive cervical cancer (ICC) in Zambia, the correct type of specimen to test is cancer samples (not pre-cancer samples)
- To determine how common HPV-16 and HPV-18 are in cervical cancer in Zambia, extracted and genotyped HPV DNA from formalin-fixed paraffin-embedded (FFPE) specimens of pathology-confirmed cervical cancer

Methods

- Use FFPE specimens from pathology-confirmed ICC from UTH Pathology Department (220 specimens from 2007-2012)
- Section the FFPE specimens at UTH
- Put sections into 2.0ml screwtop tubes, take to CIDRZ Central Lab for DNA extraction and HPV genotyping



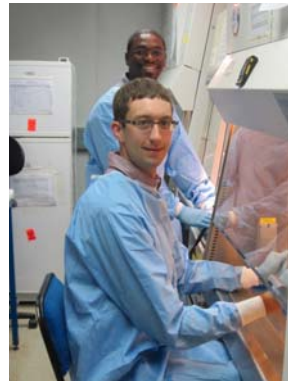
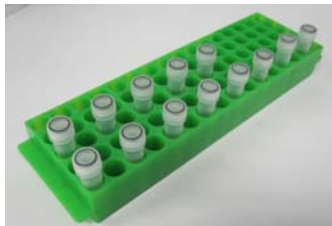
Methods

- Use FFPE specimens from pathology-confirmed ICC from UTH Pathology Department (about 220 specimens from 2007-2012)
- Section the FFPE specimens at UTH (being very careful not to have DNA carry-over from one specimen to the next)
- Put sections into 2.0ml screwtop tubes, take to CIDRZ Central Lab for DNA extraction and HPV genotyping



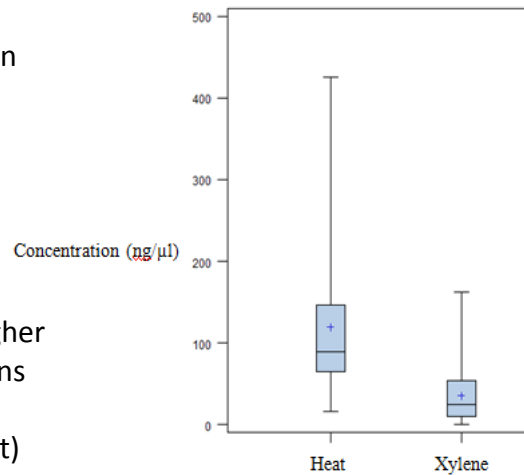
Methods

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Objective 1: To determine which DNA extraction technique performs the best

- Nucleic acid concentration after heat extraction and xylene extraction
- Boxplot, 27 specimens extracted in parallel
- Heat extraction led to higher nucleic acid concentrations than xylene extraction ($p < 0.0001$ by paired t-test)



Objective 1: To determine which DNA extraction technique performs the best

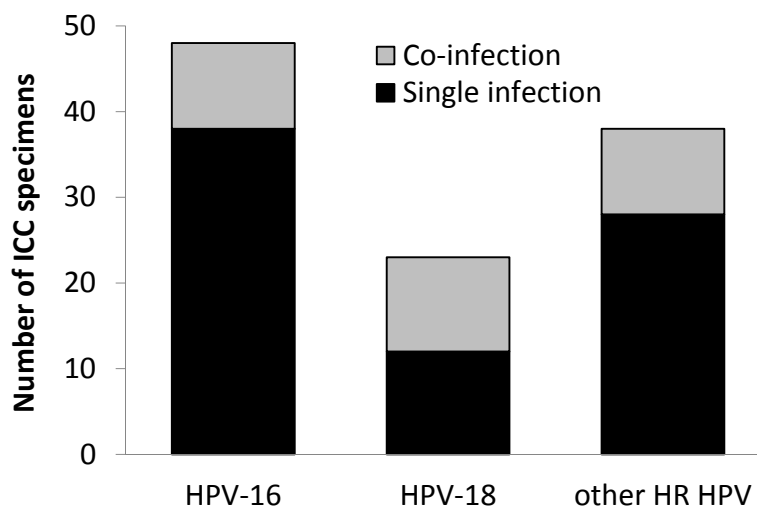
	Heat	Xylene
Internal control error	2	2
No HPV detected	2	4
Single HPV infection	18	19
Dual HPV infections	4	2
Triple HPV infections	1	0
Total	27	27

- Tested extracts with Abbott RealTime HR HPV assay (real-time PCR)
- Number of specimens with HPV identified was higher after heat than xylene
- Number of specimens with dual or triple HPV infections identified was higher after heat than xylene
- Ct values were lower after heat than xylene
- Conclusion: heat extraction works better than xylene extraction

Objective 2: To determine the combined prevalence of HPV-16 and HPV-18 in ICC specimens

	ICC		CIN 2/3	
	n	Prevalence (95% CI)	n	Prevalence (95% CI)
Total tested	114	--	75	--
Total with valid results	100	--	69	--
Positive for any HPV	93	--	65	--
HPV-16 positive	48	51.6% (41.6–61.5)	36	55.4% (43.3–66.8)
HPV-18 positive	23	24.7% (17.1–34.4)	5	7.7% (3.3–16.8)
Other HR HPV positive	38	40.9% (31.4–51.0)	43	66.2% (54.0–76.5)
HPV-16 and/or HPV-18 positive	65	69.9% (59.9–78.3)	38	58.5% (46.3–69.6)

Objective 2: To determine the combined prevalence of HPV-16 and HPV-18 in ICC specimens



Conclusion and future directions

Conclusion

- ICC specimens: 69.9% prevalence of HPV-16/18
- CIN 2/3 specimens: 58.5% prevalence of HPV-16/18
- **Conclusion: HPV vaccines can prevent approximately 2/3 of cervical precancer and cancer in vaccinated girls in Zambia**

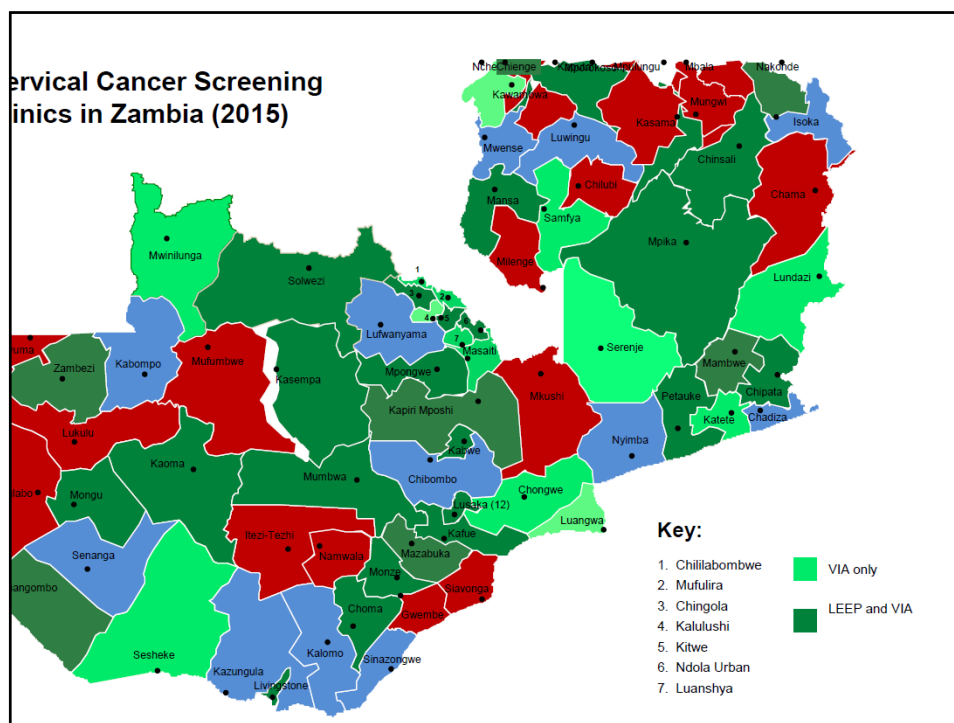
F Cancer Medicine

Open Access

- ORIGINAL RESEARCH
- **HIV infection and domestic smoke exposure, but not human papillomavirus, are risk factors for esophageal squamous cell carcinoma in Zambia: a case-control study**

Violet Kayamba¹, Allen C. Bateman², Akwi W. Asombang³, Aaron Shibemba⁴, Kanekwa Zyambo¹, Themba Banda¹, Rose Soko¹ & Paul Kelly^{1,5}





Cervical cancer screening: visual inspection with acetic acid

- Is WHO-recommended as a safe and relatively accurate test that allows for same-day screen-and-treat (cryotherapy)
- Many studies have estimated test characteristics (sensitivity, specificity, etc) of VIA in HIV-uninfected women
- Few studies have looked at test characteristics of VIA in HIV-infected women
- A project enrolled 303 HIV-infected women in Lusaka
 - All women received VIA screening, liquid cytology (Pap), and biopsy for histologic results
 - Data were scattered and incomplete
- Consolidated/merged/cleaned the data, and used data to estimate test characteristics of VIA and Pap (histology as gold standard)

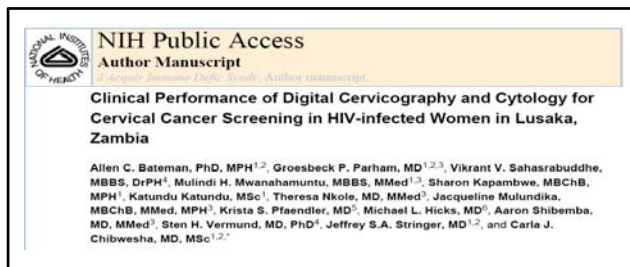
Results - 1

	CIN2+ threshold on histopathology					
	True +	False +	True -	False -	Sensitivity (95%CI)	Specificity (95%CI)
DC positive	51	102	140	10	84% (72-91)	58% (52-64)
HSIL+	37	101	141	24	61% (48-72)	58% (52-64)
LSIL+	55	157	85	6	90% (80-95)	35% (29-41)

- CIN2+: gold standard histopathology, high-grade pre-cancer and cancer
- DC = VIA
- HSIL+, high-grade pre-cancer and cancer by Pap smear
- LSIL+, low-grade pre-cancer, high-grade pre-cancer, and cancer by Pap smear

Results - 2

- Take-away : VIA is at least as good as, and possibly more accurate than, Pap smears in HIV-infected women
- Good, the CCPZ uses a relatively accurate test that identifies most disease
- However, probably quite a bit of over-treatment (because of low specificity)
- Can we use an even more accurate test?



Newer cervical cancer screening tests

- Identify HPV directly
 - HPV DNA
 - HPV RNA
 - HPV protein
- Likely more accurate than visual inspection screening (more sensitive and more specific)
- However, few studies have investigated these new screening tests in HIV-infected women

Cervical cancer screening study #2

- “Comparison of point-of-care tests and VIA for cervical cancer screening in HIV-infected women in Lusaka, Zambia”
- Cross-sectional study, n=200
 - All women receive VIA screening, biopsy for histology, and three molecular tests
 - GeneXpert HPV
 - OncoE6
 - Abbott HR HPV
- Objective: to determine the sensitivity, specificity, positive predictive value, and negative predictive value of VIA, GeneXpert, OncoE6, and Abbott HPV for the detection of pre-cancer in HIV-positive women.

Cepheid GeneXpert tests offered

- MTB/RIF
- MRSA
- *C. difficile*
- Group A Strep
- Group B Strep
- CT/GC, TV
- Flu
- Flu/RSV
- Enterovirus
- Norovirus
- Carba-R
- vanA



Cepheid GeneXpert MTB/RIF

- Qualitative, nested real-time PCR
- Specimen types: raw sputum or concentrated sediments prepared from induced or expectorated sputum
- Targets *rpoB* gene for identification of TB and identification of rifampin resistance
- Rifampin resistance often associated with resistance to other anti-TB drugs
- Simple to run, 2 hour TAT



Roll-out of GeneXpert MTB/RIF in Sub-Saharan Africa

Xpert MTB/RIF strategy: from endorsement to scale-up



WHO endorsement 2010

- Global Consultation
- WHO Policy Guidance
- Roadmap for implementation

Phased implementation 2011

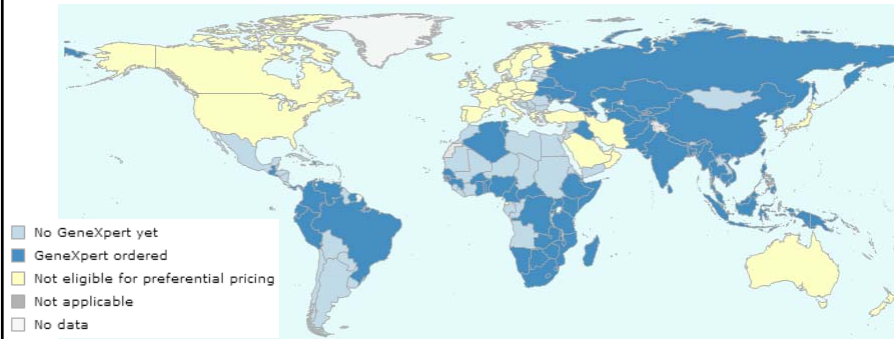
- Through UNITAID, TBREACH, TBCARE, PEPFAR
- Selected countries, different health service levels

Scale up 2012

- EXPAND-TB, Global Fund R11, TBREACH, TBCARE, PEPFAR, country budgets, etc



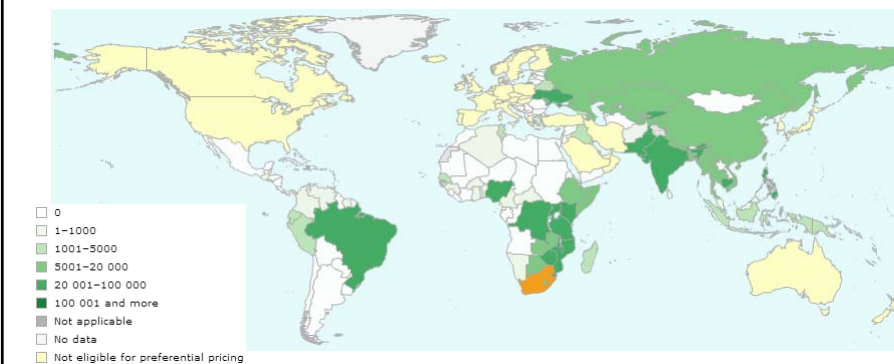
Xpert MTB/RIF global landscape 2012



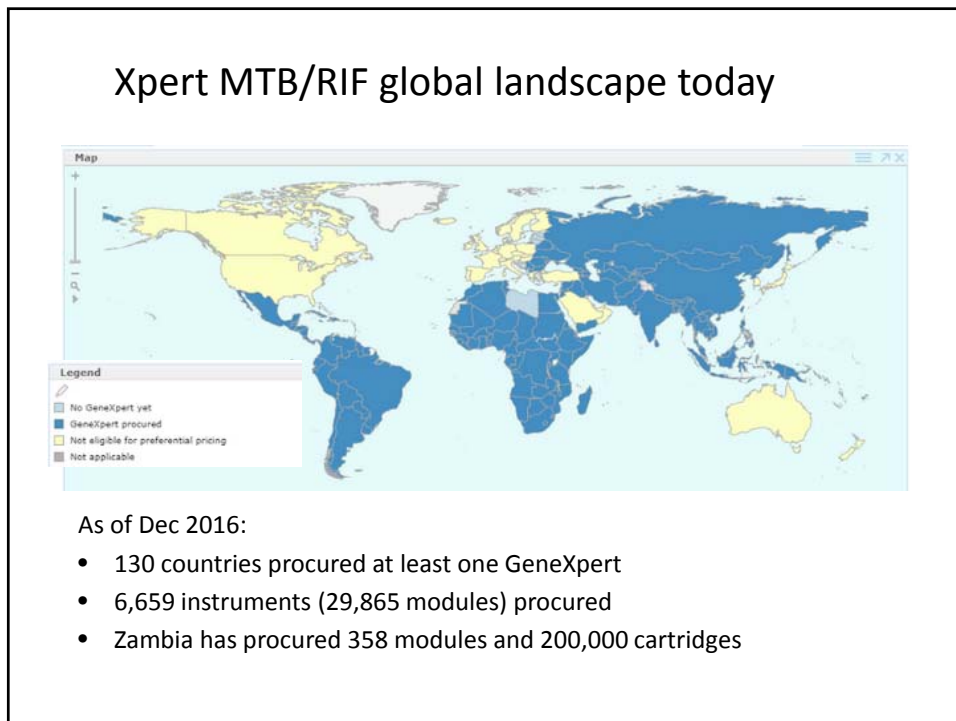
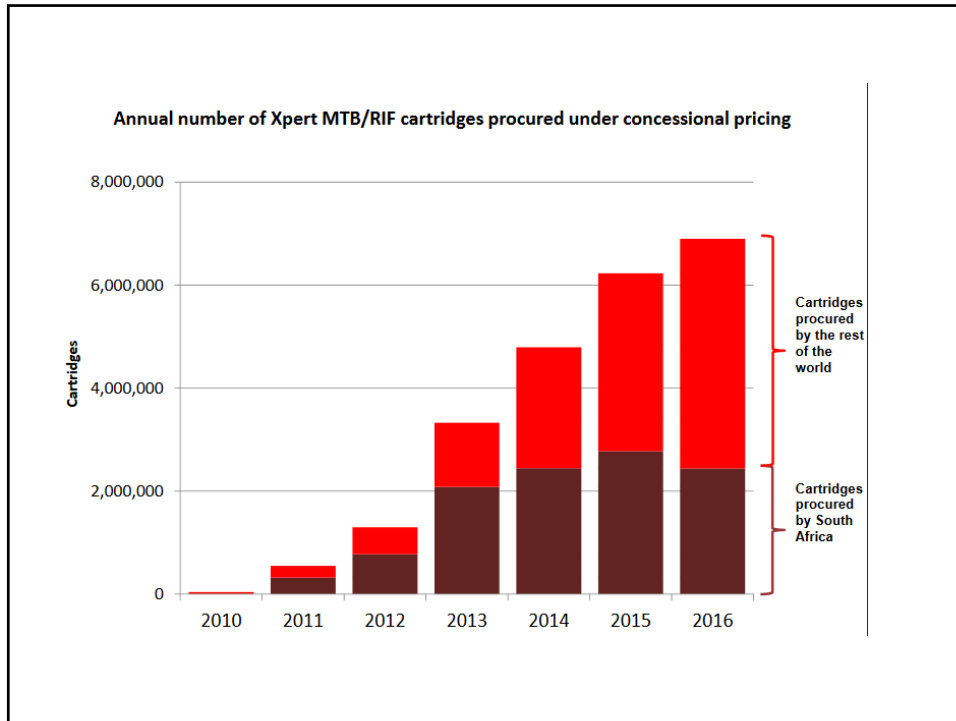
As of Sept 2012:

- 73 countries procured at least one GeneXpert
- 898 instruments (4,660 modules) procured

Xpert MTB/RIF global landscape 2012



- 1,482,550 Xpert MTB/RIF cartridges procured



Good news, but challenges keeping Xpert running...

- Power cuts
- Retaining qualified staff
- Room temp issues in hot season
- Space
 - Airtight room without ventilation
- Dust control
 - fan filters block
 - instruments overheat



"Challenges during implementation of Xpert MTB/RIF at health centers and district hospitals in Zambia."
Winnie Mwanza, Kaunda Kaunda, Monde Muyoyeta, Stewart Reid, Annika Krüüner



HPV piggybacking?



Current cervical cancer screening in Zambia: visual inspection with acetic acid

- Is WHO-recommended as safe and relatively accurate
- Women who have a positive screening test result are immediately treated (“screen-and-treat”)
- Test characteristics (sensitivity, specificity) not ideal
 - Low sensitivity necessitates routine screening

Future cervical cancer screening in Zambia: GeneXpert HPV?

- Likely more accurate than visual inspection screening (more sensitive and more specific)
- Fast turn-around-time of GeneXpert means “screen-and-treat” is still feasible
- GeneXpert HPV identifies high-risk HPV and individually identifies HPV-16, HPV-18, and HPV-45



GeneXpert HPV by Cepheid

Simple to run:

- Add specimen to cartridge (which contains all extraction and PCR reagents), using a transfer pipet
- Put cartridge into machine
- Press “Start Test”
- Result available in 1 hour



ORIGINAL RESEARCH ARTICLES: CERVIX AND HPV

Clinical Performance Validation of 4 Point-of-Care Cervical Cancer Screening Tests in HIV-Infected Women in Zambia

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 Aaron Shibemba, MMed,³ Sharon Kapambwe, MBChB,² Mulindi H. Mwanahamuntu, MMed,^{2,4} Susan Banda,²
 Chalwa Hamusimbi,² Pascal Polepole, MSc,⁵ and Groesbeck P. Parham, MD^{1,4}

Test performance characteristics of VIA and Xpert HPV

	VIA	Xpert HPV
True positive	15	28
False positive	14	66
True negative	153	100
False negative	16	4
Sensitivity (95% CI)	48 (30–67)	88 (71–97)
Specificity (95% CI)	92 (86–95)	60 (52–68)
PPV (95% CI)	52 (33–71)	30 (21–40)
NPV (95% CI)	91 (85–95)	96 (90–99)

- Histology was used as the gold standard
- Xpert HPV, high sensitivity and NPV
- With Xpert HPV, may only need 1 or 2 tests per lifetime
 - VIA is needed every 3 years (low sensitivity)



Also evaluated OncoE6 by Arbor Vita

- Dipstick (lateral flow) format
- No complex equipment or refrigeration required
- 2 1/2 hour turn-around time

E6 oncoprotein assay result:	Yes	No
HPV-16	<input type="radio"/>	<input type="radio"/>
HPV-18	<input type="radio"/>	<input type="radio"/>
HPV-45	<input type="radio"/>	<input type="radio"/>
HPV-16/18/45 not detected	<input type="radio"/>	<input type="radio"/>
No HR HPV detected	<input type="radio"/>	<input type="radio"/>
Other	<input type="radio"/>	<input type="radio"/>

