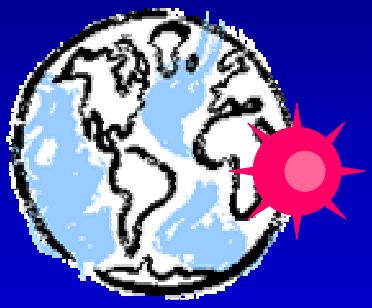
Herpes Not So Simplex

The Detection of HSV-2



In the Developing World

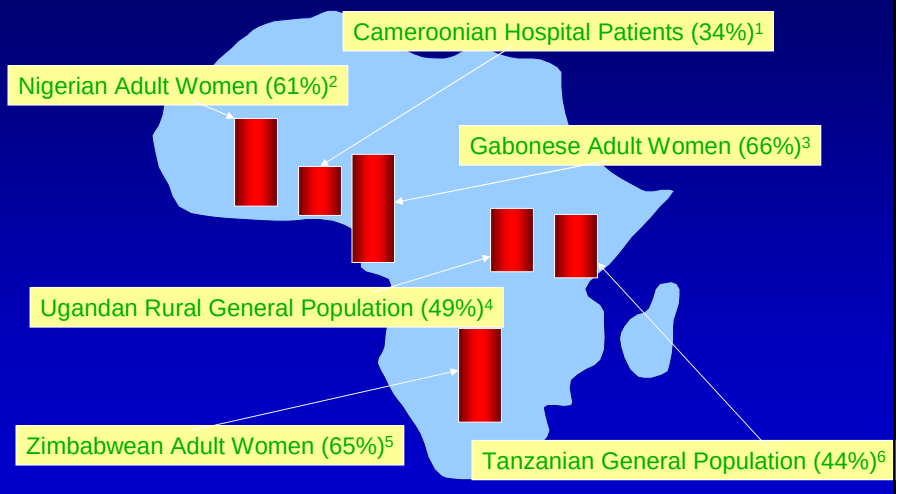
Objectives

- Why is HSV-2 detection in the developing world important
- What are the performance characteristics of HSV-2 serologic tests in the developing world
- Utility of the HSV-2 rapid test in the developing world
- What needs to be done to improve testing performance in the developing world

Why is HSV-2 Detection Important in the Developing World

- High prevalence
- It plays a major role to HIV transmission
- HSV-2 co-infection exacerbates HIV infection

Africa
Low-risk Populations



1) Volpi 2004; 2) Thomas 2004; 3) Ozouaki 2006; 4) Kapiga, 2006; 5) Kjetland 2005 6) Ao 2006

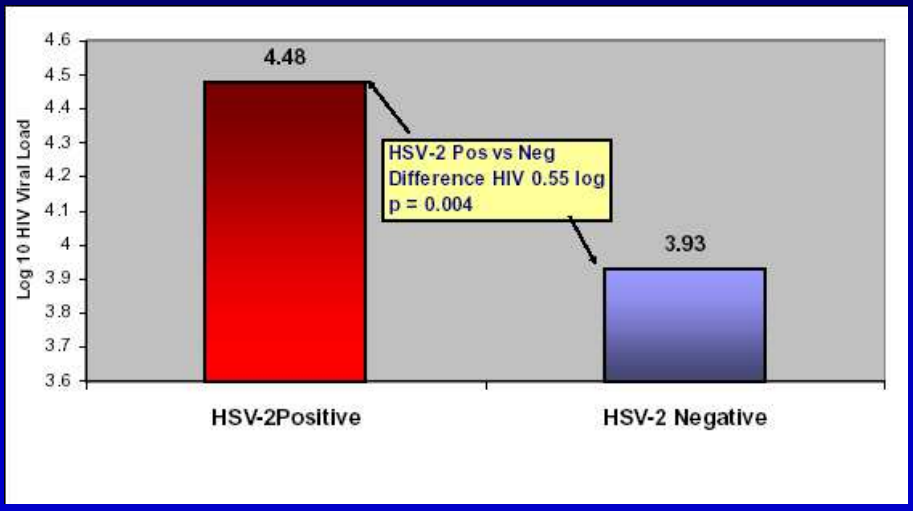
Per-Contact Probability of HIV-1 Acquisition in Relationship to HSV-2

Overall	0.0011
HSV2 pos.	0.0020 *
HSV2 neg.	0.0004
HSV2+/GUD+	0.0081 *
HSV2+/GUD-	0.0020
HSV2-/GUD-	0.0004

*** P = 0.01**

Serwadda, JID 2004

HSV-2 Upregulates HIV-1 Viral Load in Acute Infection and at 15 Months



Serwadda, JID 2004

Ongoing Clinical Trials where HSV-2 Detection is Critical

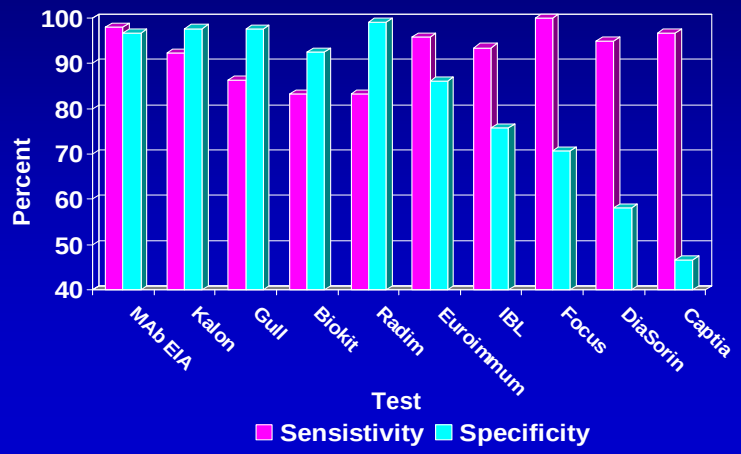
- Randomized, controlled trial of acyclovir prophylaxis of HIV-/HSV2+ partners to decrease HIV acquisition (HPTN 039)
- Randomized, controlled trial of acyclovir prophylaxis of HIV+/HSV2+ partners to decrease HIV transmission (Gates)
- Randomized, controlled trial of acyclovir prophylaxis of HIV+/HSV-2 co-Infected individuals (NIAID 07-I-N032)

Problems

- High rate of false positive ELISA results when confirmed by Western Blot
- No inexpensive way to confirm initial ELISA positive results

Sensitivity & Specificity of HSV-2 Assays vs. Western Blot

Population: 330 Sera from African Subjects



Van Dyck J. Clin. Micro. 2004; 42:2961

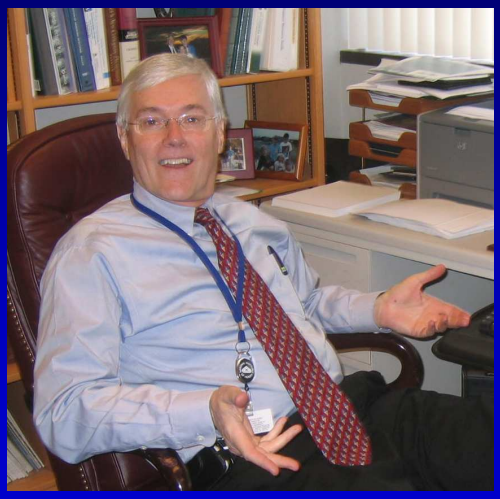
Sequential HSV-2 Testing in Indian STD Prevention trial

Population: Chennai, India two time points from
2186 STD prevention participants
All subjects tested twice
Prevalence 23.4%

	cut off 1.1
Ab- to Ab+	20
Ab+ to Ab-	75

Rizzo-Price 2006 STD meeting poster #126

So Who is Infected??

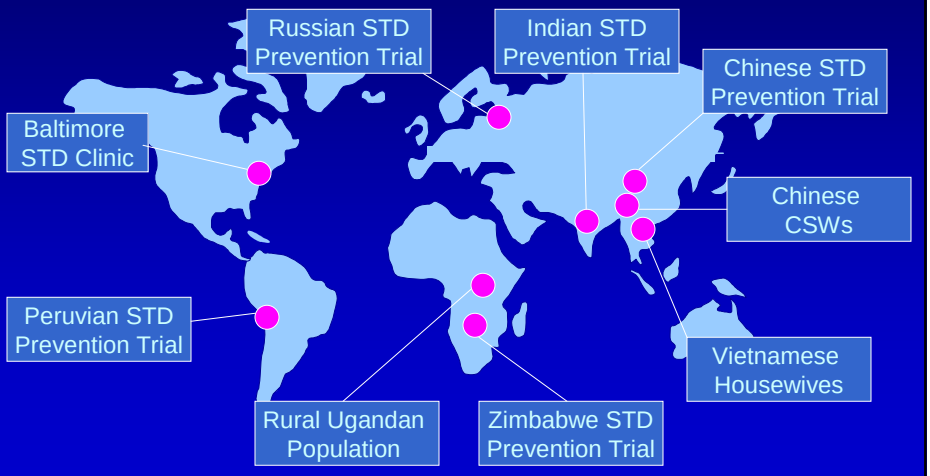


Comparisons of HSV-2 Serology Tests

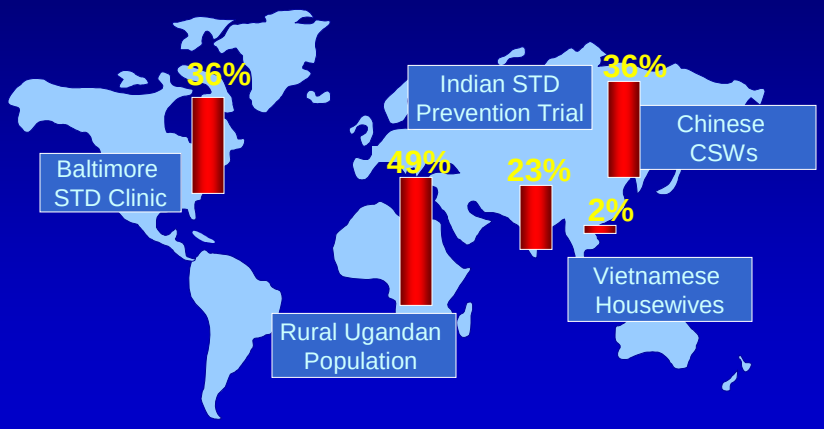
Same technicians
Same lab equipment
Same tests – Focus & Kalon ELISAs, BioKit Rapid test
Western Blot testing was performed at The University of Washington

Location of samples vary

Populations Studied



HSV-2 Prevalence in Populations Studied



HSV-2 Serology Testing Comparison in Low Prevalence Vietnamese Population

Population: 1238 Suburban Housewives outside of Hanoi

HSV-2 prevalence 2% by Western Blot

	Sensitivity	Specificity	PPV	NPV
Focus ELISA (1.1)	100%	93%	23%	100%
Kalon ELISA (1.1)	88%	98%	47%	100%
BioKit Rapid	80%	98%	49%	100%

As compared to HSV-2 Western Blot

Ngo CVI Press

HSV-2 Serology Testing Comparison in High Prevalence Chinese Population

Population: 500 CSWs from Kunming China surveyed in 2004

HSV-2 prevalence 36.8% by Western Blot

HIV-1 Prevalence 4%, HCV Prevalence 32.8%

	Sensitivity	Specificity	PPV	NPV
Focus ELISA (1.1)	86%	98%	96%	92%
Kalon ELISA (1.1)	90%	95%	92%	95%
BioKit Rapid	84%	72%	59%	90%

As compared to HSV-2 Western Blot

Ngo P-251

HSV-2 Serology Testing Comparison in Rural Ugandan Population

Population: Rural Population

HSV-2 prevalence 49% by Western Blot

820 Focus ELISA: 547 HIV-, 273HIV+

538 Kalon ELISA: 361 HIV-, 177 HIV+

535 Rapid test: 360 HIV-, 175 HIV+

	Sensitivity	Specificity	PPV	NPV
Focus ELISA (1.1)	99%	51%	75%	97%
Kalon ELISA (1.1)	95%	88%	90%	92%
BioKit Rapid	96%	56%	72%	92%

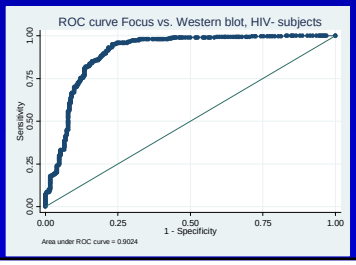
As compared to HSV-2 Western Blot

Gamiel P-172

Performance of Focus ELISA to Western Blot in Rakai Uganda

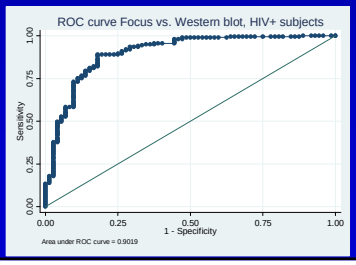
HIV- N= 547

Index value	Sens	Spec
1.1	0.99	0.53
2.2	0.96	0.75
3.5	0.85	0.83



HIV+ N= 273

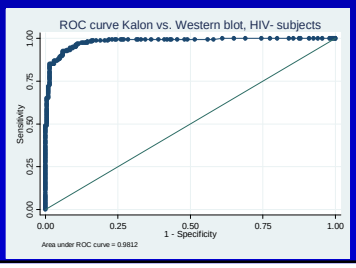
Index value	Sens	Spec
1.1	0.99	0.42
2.2	0.96	0.63
3.5	0.86	0.82



Performance of Kalon ELISA to Western Blot in Rakai Uganda

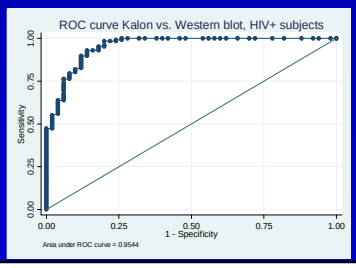
HIV- N= 361

Index value	Sens	Spec
1.1	0.95	0.90
1.5	0.93	0.94
2.4	0.81	0.99

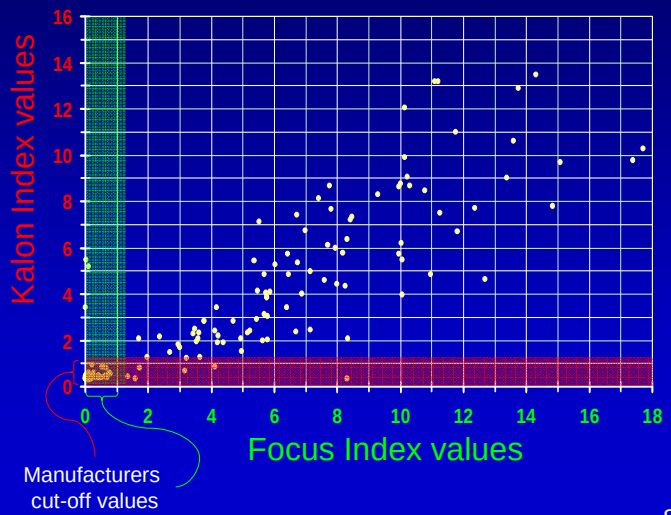


HIV+ N= 177

Index value	Sens	Spec
1.1	0.95	0.80
1.5	0.91	0.86
2.4	0.74	0.94



Comparison of Focus vs. Kalon ELISAs on Samples from Baltimore, Maryland

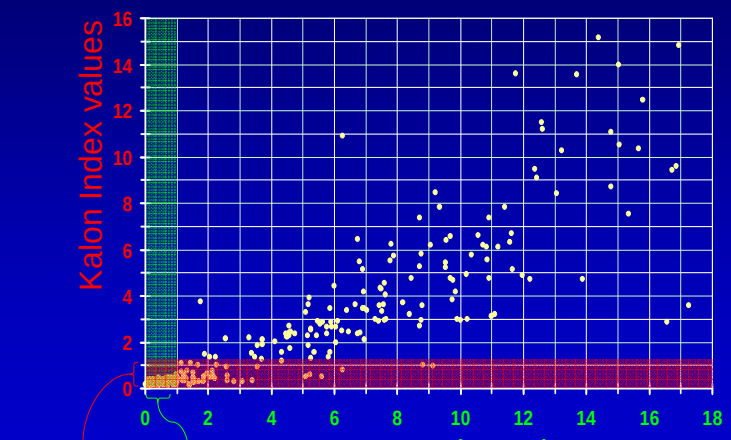


STD clinic
2% HIV+

N = 280

Summerton CVI in press

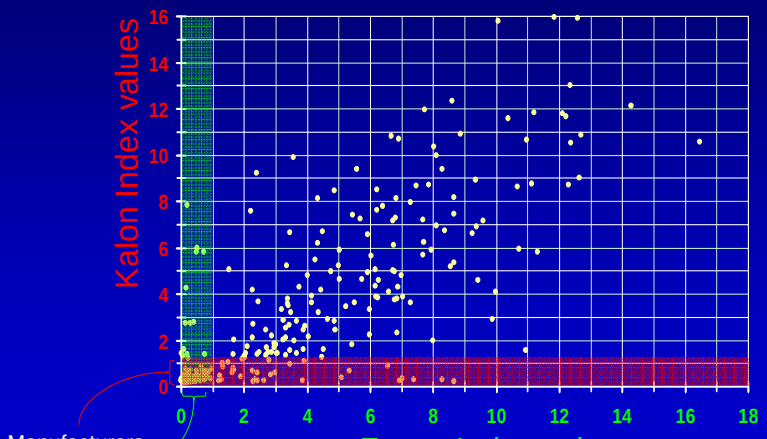
Comparison of Focus vs. Kalon ELISAs on Samples from Chennai, India



N = 279

Rizzo-Price 2006 STD Meeting Poster #126

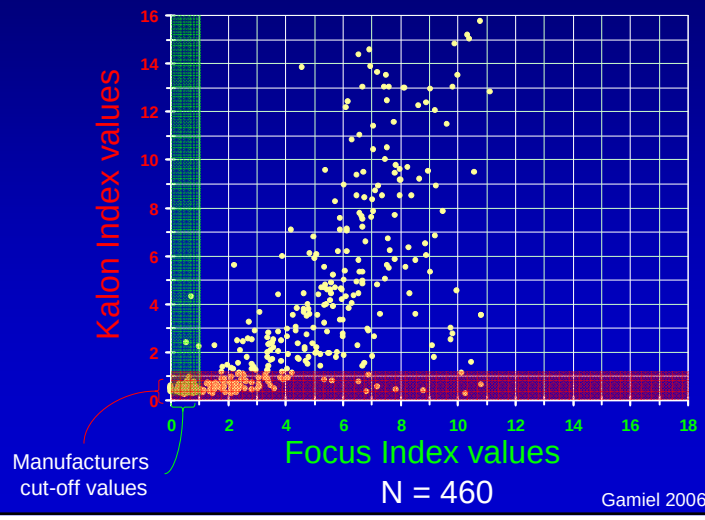
Comparison of Focus vs. Kalon ELISAs on Samples from Kunming, China



N = 500

Unpublished Data

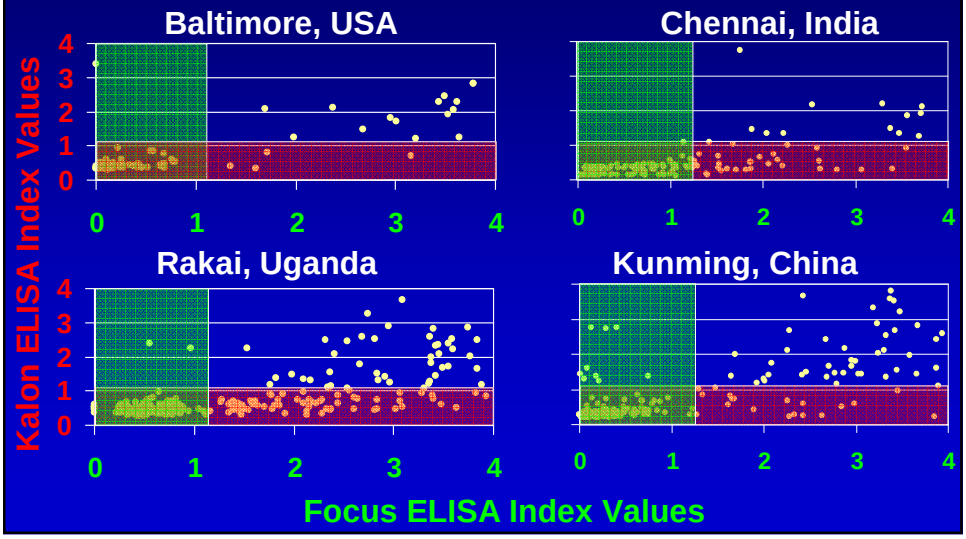
Comparison of Focus vs. Kalon ELISAs on Samples from Rakai, Uganda



N = 460

Gamiel 2006 STD Meeting poster # 320

Low Positive Analysis of Kalon vs. Focus ELISAs on Four Different Populations



Summary

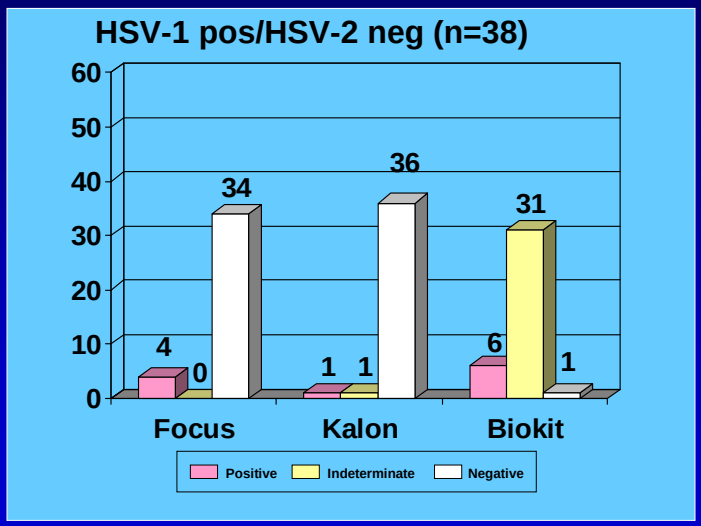
- The Focus and Kalon ELISAs have different performance characteristics depending on where the samples come from
- HIV co-infection does not affect ELISA performance

Utility of HSV-2 Rapid Test in the Developing World

Point of Care (POC) tests for HSV-2

- Great for a setting without reliable water and electricity
- Do not need expensive laboratory infrastructure
- The Biokit HSV-2 Rapid test (the old POCkit*) showed sensitivity of 96% and specificity of 98% in pre-market evaluation in a US population
- Not much known about its performance in the developing world

Effect of HSV-1 Co-Infection on HSV-2 Serology in Samples from Baltimore



Summerton CVI, in press

Biokit test for HSV-2 on Baltimore STD patients

Biokit using Western blot results as the gold standard.

	If Indeterminate results considered negative	considered positive
Sensitivity	**64.9%**	**48.1%**
Specificity	**71.4%**	**94.6%**
PPV	**69.8%**	**90.9%**

Summerton CVI, in press

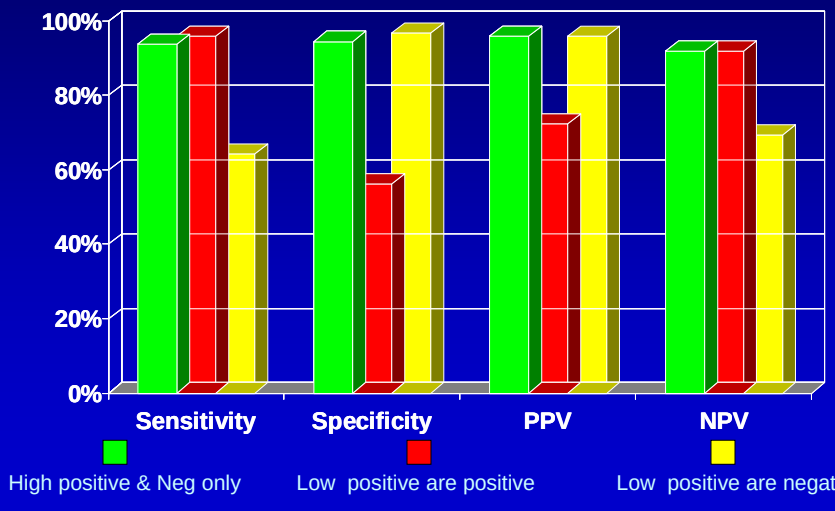
Focus HSV-2 ELISA followed by Biokit Rapid Test – Does not work in Uganda

	HIV+	HIV-
Positive	81	110
Light Positive	64	123
Negative	28	118

36% light positive

11 indeterminate

Consequences of Low Positive Rapid Test Results



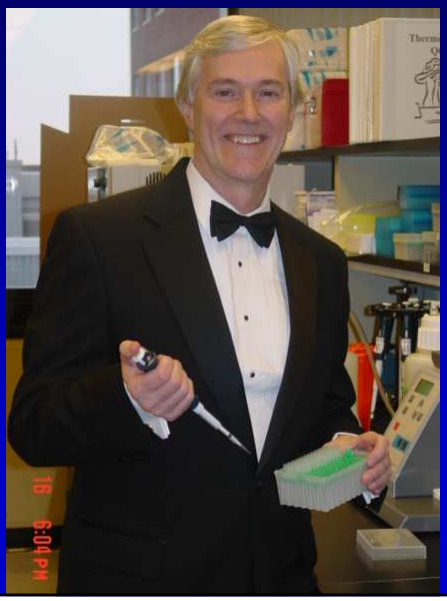
Future Directions

What needs to happen to improve performance detection of HSV-2

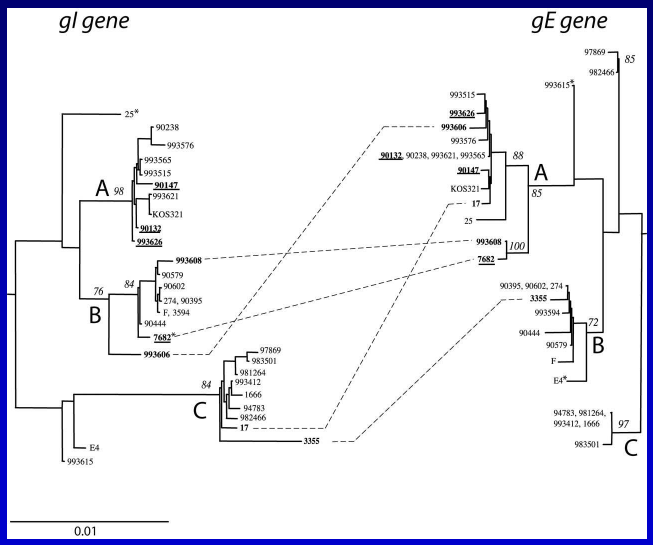
Need to Characterize HSV-2 Virus and the Hosts response to infection in the Developing World

- Sequence data from the virus – especially the gG-2 region
- Map out antibody responses to gG-2 protein from individuals in developing countries
- The effect of co-infection on assay performance

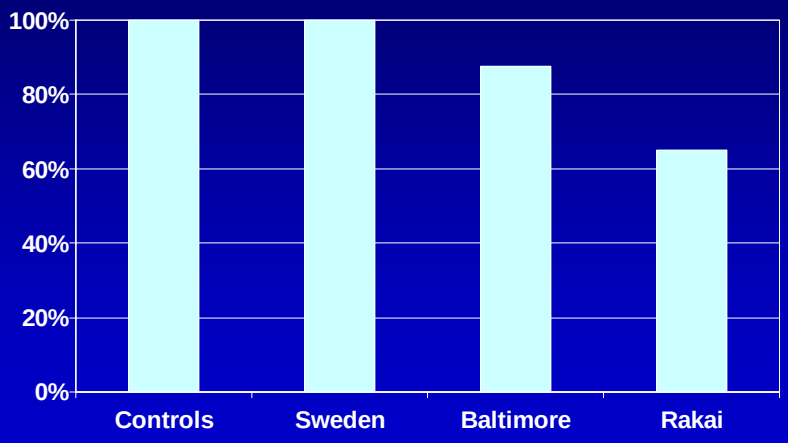
More Lab Work Required



Sequence Variation of HSV-1

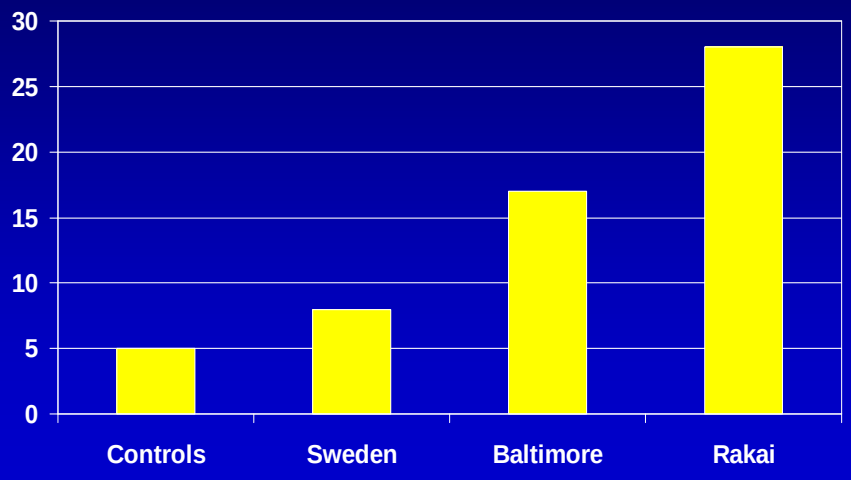


Length variation of mgG-2 Protein Baltimore USA vs. Rakai Uganda



Brankin P-258

Number of Mutated Amino Acid Sites in mgG-2 Protein Relative to Strain HG52 per Isolate by Location

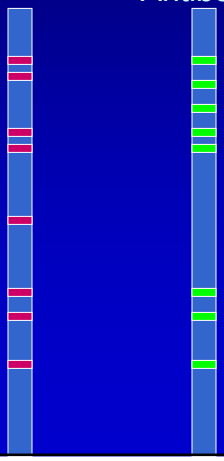


Brankin P-258

Is the Western Blot a "Gold Standard" in the Developing World

US Virus US Antibody

US Virus African Antibody



- Antigen on blot is from Western Europe or US Strain of HSV-2
- Antibody form Africa either binds or does not to Western control viral protein
- Banding pattern is interpreted
- Country specific antigen greatly improves specificity*

*Görander *Clin Vaccine Immunol 2006*

Conclusions

- Focus and Kalon ELISA have different perform characteristics on samples from different parts of the world
- mgG-2 Protein sequences are different across the world
- An Afrocentric Western Blot is needed

Acknowledgements

- Amy Brankin
- Jordyn Gamiel
- Thoai Ngo
- Melissa Riedesel
- Andrew Hardick
- Aaron Tobian
- Tara Suntoke
- Patti Rizzo-Price
- Charlotte Gaydos
- Thomas Quinn
- Ron Gray
- Maria Wawer
- David Serwadda
- Nelson Serwankombo
- Fred Mukibe
- Rhoda Ashley-Morrow
- Wayne Hogrefe (Focus)
- Michael Childerstone (Kalon)