Salivary mucin MUC5B inhibits HIV-1 subtype C in an in vitro pseudoviral assay

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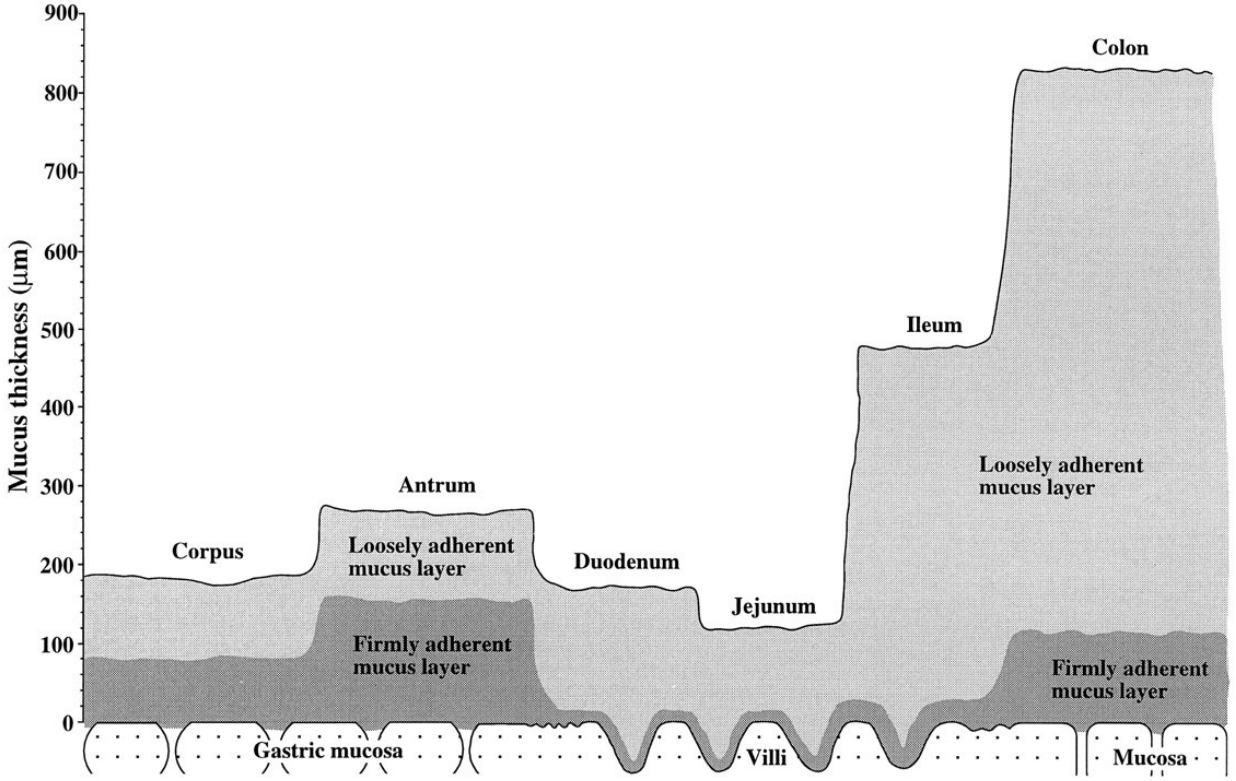
Mucus and Mucins

Slimy, highly viscous secretion coating the surface of epithelial tissues



Mean mucus thickness (μm)

Total	189 ± 11	274 ± 41	170 ± 38	123 ± 4	480 ± 47	830 ± 110
Firmly Adherent	80 ± 5	154 ± 16	16 ± 3	15 ± 2	29 ± 8	116 ± 51
Loosely Adherent	109 ± 12	120 ± 38	154 ± 39	108 ± 5	447 ± 47	714 ± 109
n	6	6	7	6	5 (8 firm)	11

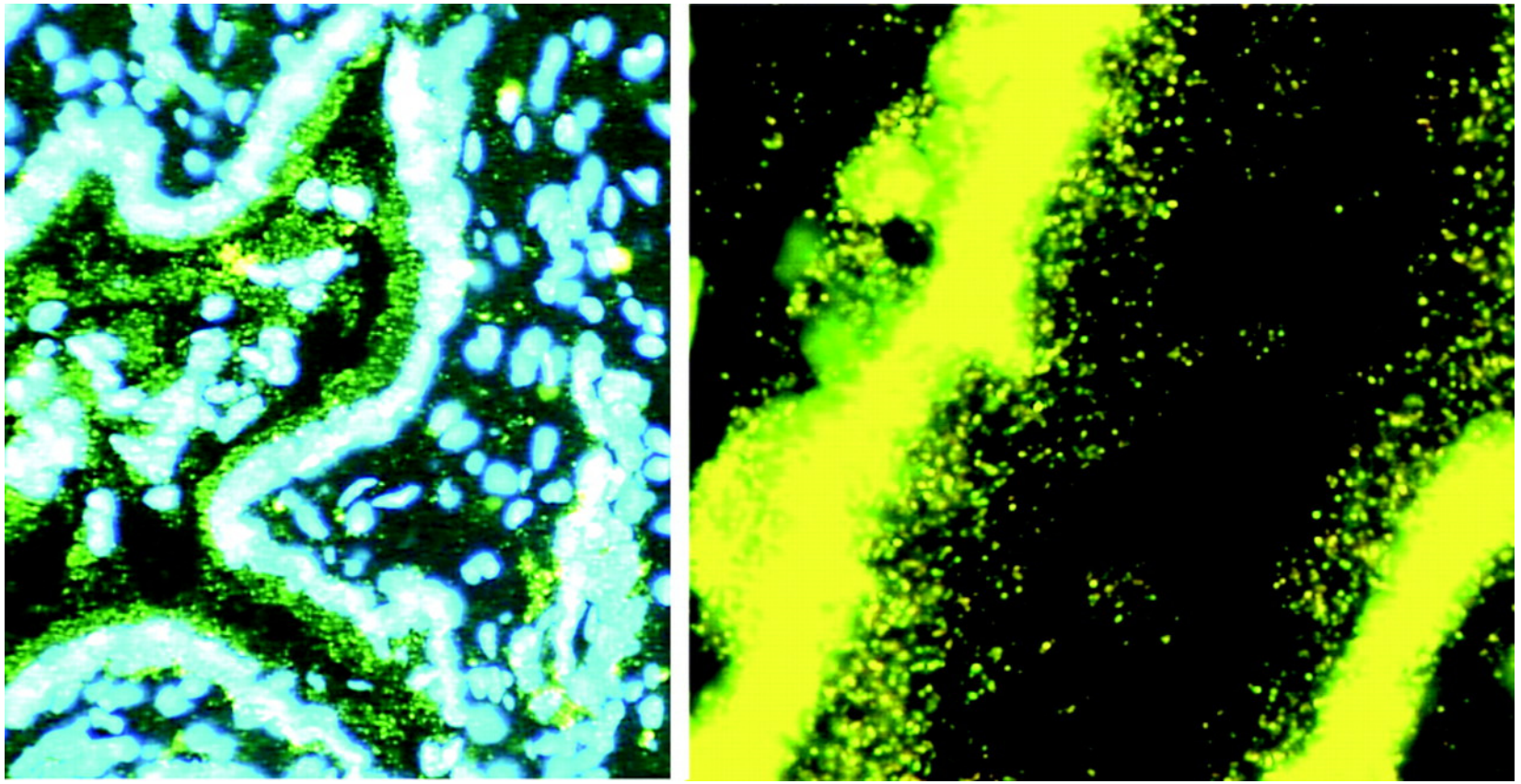
Atuma C et al. Am J Physiol Gastrointest Liver Physiol 2001;280:G922-G929

AMERICAN JOURNAL OF PHYSIOLOGY
Gastrointestinal and Liver Physiology

- ***Protective mucus barrier***
  - Highly elastic and adhesive properties – role in aggregating and removing pathogenic micro-organisms from the oral cavity

  - Insolubility and unstirred nature of the mucus gel layer on gastric epithelium (Rees and Turnberg, 1982)
  - The mucus layer provides spatial separation of the colonic epithelium with intraluminal bacteria
  - **Protection greatly limits the viral infection (SIV) of cervicovaginal epithelial tissues (Miller *et al,* 2005)**

Residual virions from the inoculum trapped in cervical mucus.



Miller C J et al. J. Virol. 2005; doi:10.1128/JVI.79.14.9217-9227.2005

Journal of Virology

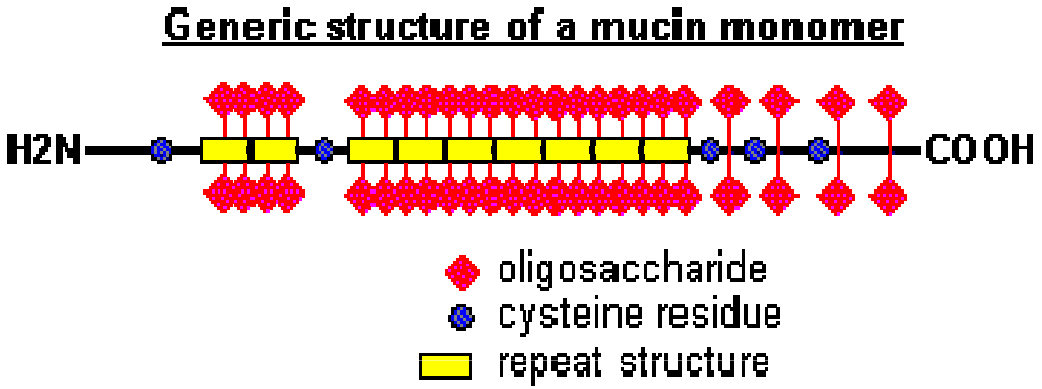
AIM

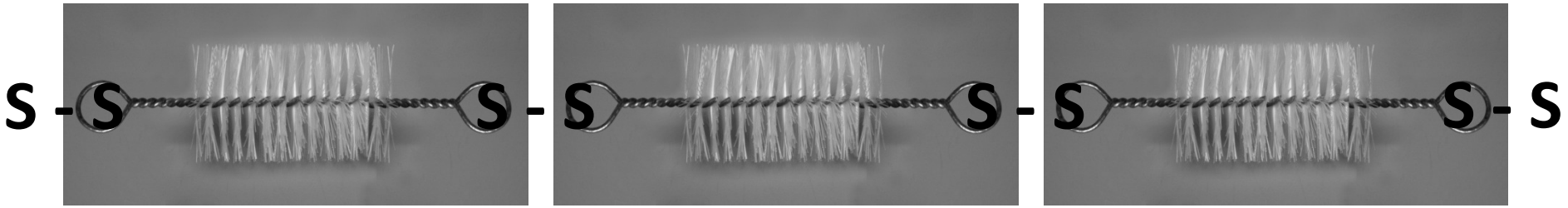
- Investigating this mucus/viral interaction – strategy for preventing transmission of HIV-1?

- Mucus-based microbicide?

Mucin composition, structure and conformation

- High molecular weight glycoproteins



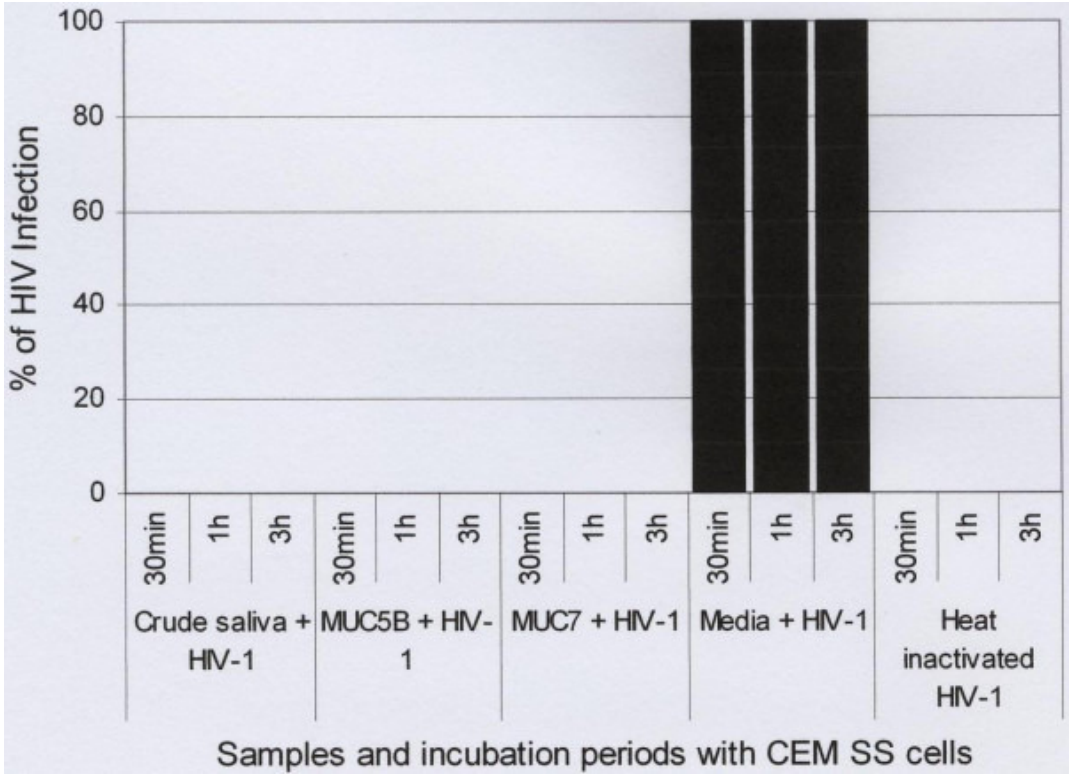


- Secreted ***salivary mucins*** - **MUC5B** (gel forming) and **MUC7** (not gel forming)

Transmission of HIV-AIDS occurs rarely in oral fluid exchange

Cohen MS, Shugars DC, Fiscus SA. **Limits on oral transmission of HIV-1.** Lancet. 2000 Jul 22;356(9226):272.

H. Habte *et al* (2006) in our laboratory:



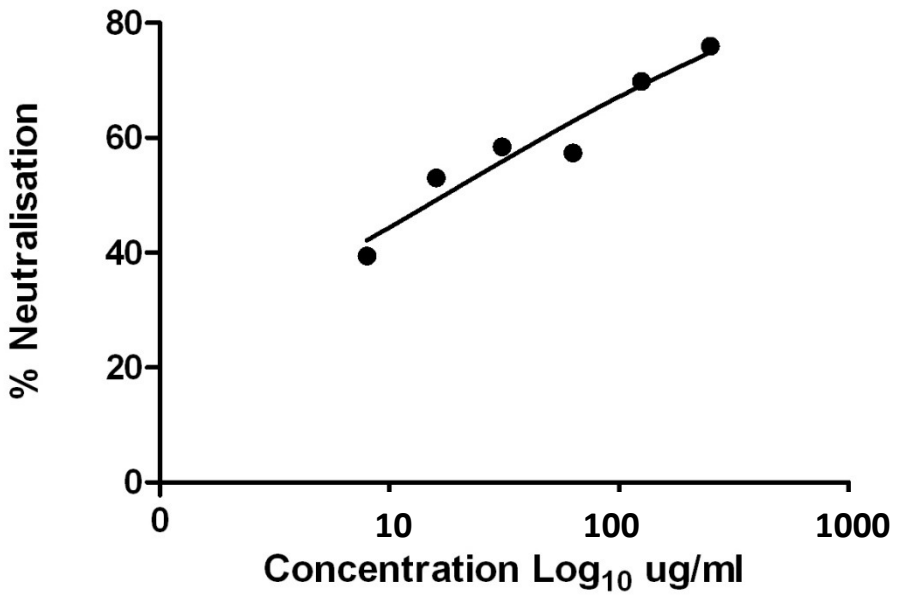
Habte *et al. Virology Journal* 2006 **3**:99

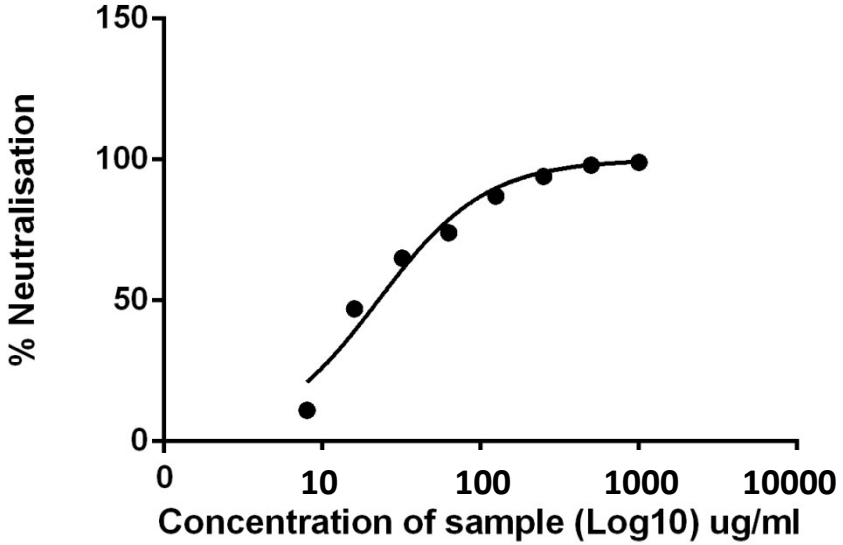
- ***Mechanism*** – postulate broad interaction between the extensive glycosylation of the mucin with that of the viral capsid – aggregating and removing virus from the oral cavity – preventing viral interaction with susceptible cells

- **Further studies by J.Peacocke *et al* (2012):**

  - **HIV-negative** crude saliva inhibited HIV-1
    - Subtype C strain
    - 70% HIV-negative group
    - 75% HIV-positive group
  - **HIV-positive crude saliva** mucus ***also inhibited*** the virus
  - Purified HIV-negative ***and HIV-positive mucins*** inhibited the virus
    - Unlike the previous study
  - ***No significant difference in inhibition with HIV-status***

Neutralisation of HIV-1 by Crude Saliva





- **Crude saliva IC_{50} – 17.34ug/ml protein conc.**

IC_{50} - 22.54ug/ml protein conc.

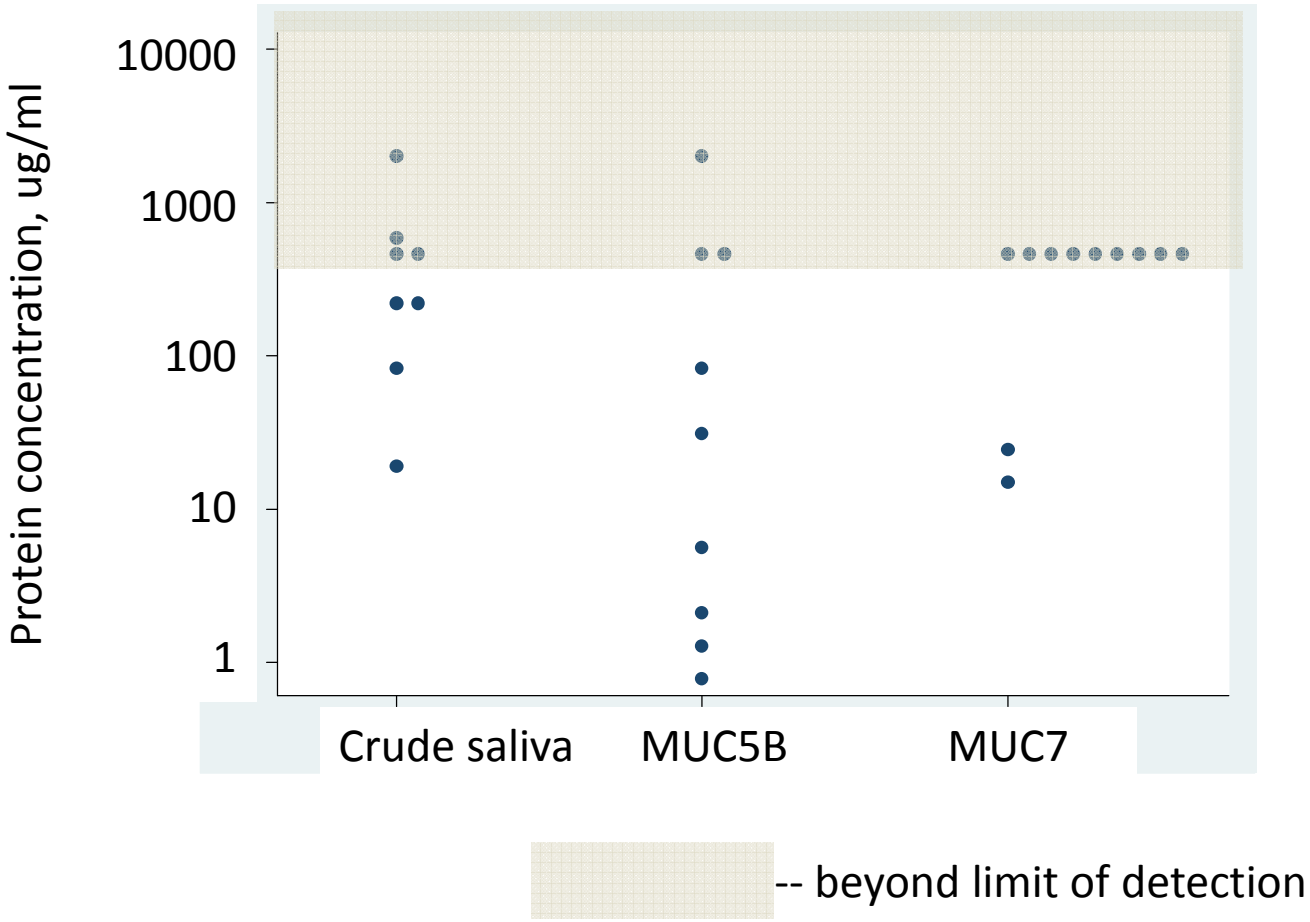
- **Pseudovirus - CAP45, subtype C, KZN**
- **Representative samples**

Dose-response nature of inhibition of HIV-1 pseudovirus

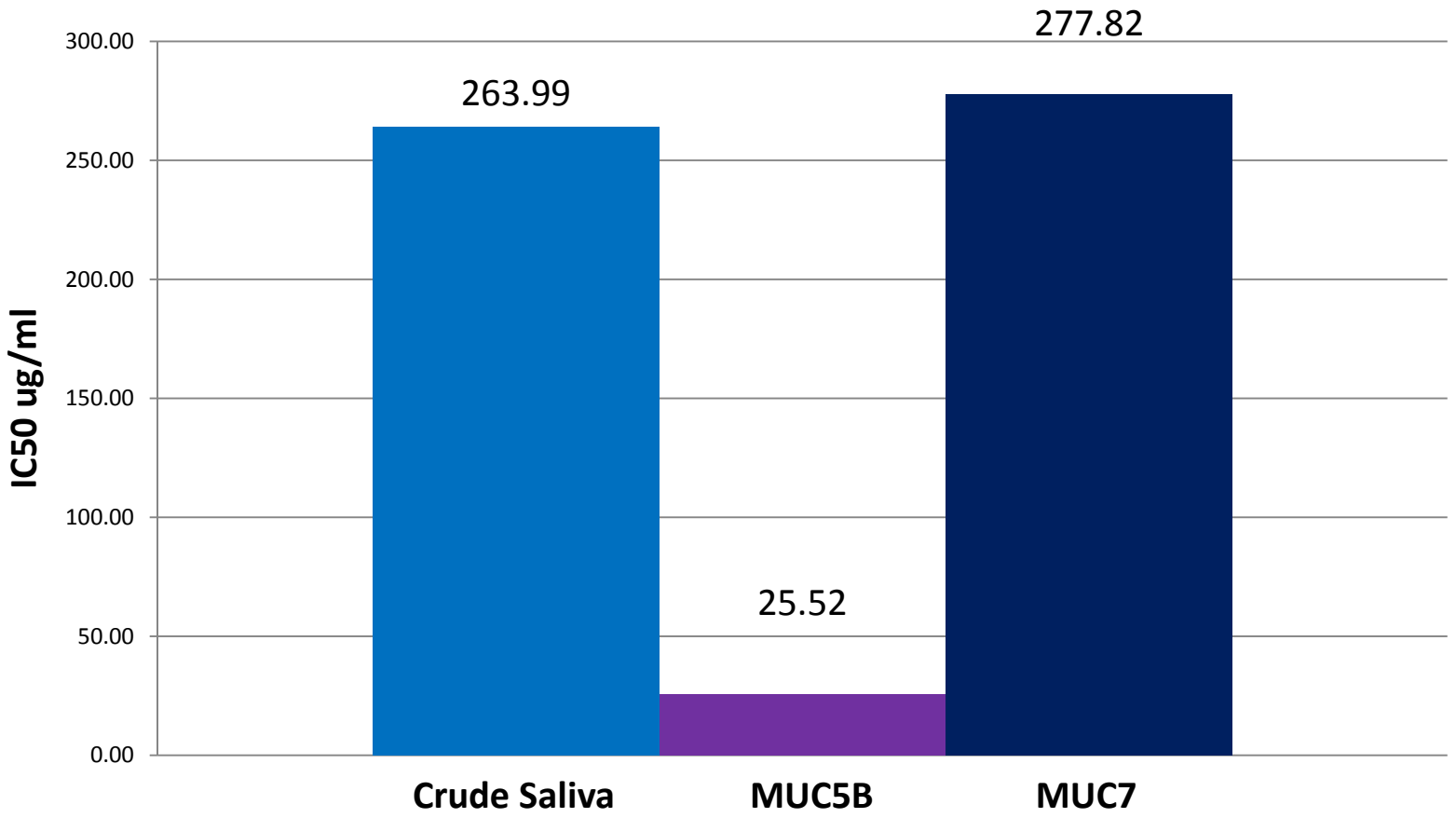
IC_{50} is the concentration of the inhibitor at which 50% of the response is observed – calculated using a curve fit (GraphPad Prism)

Neutralisation of Subtype C HIV-1 Pseudovirus by Crude Saliva and its Purified Mucins

Subtype C viruses DU422.1 and CAP45, KZN



Salivary mucus and mucin samples tested against subtype C pseudovirus



MUC5B appears to have greater neutralising activity of HIV-1 than MUC7 (and crude saliva)

CONCLUSION

- ***Salivary MUC5B neutralises*** HIV-1 pseudoviruses CAP45 (KZN) and DU422 (Durban) of **subtype C** when purified from HIV-negative and HIV-positive individuals.
- **The neutralisation activity of MUC5B IC_{50} 25.52ug/ml appears greater than MUC7 IC_{50} 277.82ug/ml**
- Neutralising activity irrespective of subtype (A, Q168a.2 from Kenya)

THANKS

- Head of research, supervisor Prof Mall, HOD Prof Kahn, and our laboratory staff

- Dr Paul Roux from the HIV-clinic, Groote Schuur Hospital, for recruitment of donors

- Collaboration with Dr Jeff Dorfman and PhD student Rajesh Jacob at the ICGEB – International Centre for Genetic Engineering and Biotechnology

- Surgery Department, UCT, NRF for funding

GROOTE SCHUUR HOSPITAL

ICGEB

NRF National Research Foundation

UNIVERSITY OF CAPE TOWN

'If all these players do their part, we will move forward, as fast as science can take us, to discoveries that can help block the transmission of HIV. This goal is worth our greatest efforts; it could very well be the turning point that leads to the end of this disease'

Melinda Gates

EVIDENCE

- MUC7 inhibitory potential against fungal and bacterial infection in the oral cavity? But not HIV-1?
  - Wei GX, Bobek LA.**Human salivary mucin MUC7 12-mer-L and 12-mer-D peptides: antifungal activity in saliva, enhancement of activity with protease inhibitor cocktail or EDTA, and cytotoxicity to human cells.** Antimicrob Agents Chemother. 2005 Jun;49(6):2336-42.
- MUC5B is a larger and more extensively glycosylated and gel-forming molecule than MUC7 – refined approach to defining the mechanism of inhibition
  - Broad/physical interaction due to charge?
  - Specific binding between mucin and virus?
  - Wu Z, Golub E, Abrams WR, Malamud D. **gp340 (SAG) binds to the V3 sequence of gp120 important for chemokine receptor interaction.** AIDS Res Hum Retroviruses. 2004 Jun;20(6):600-7.
  - Earl Stoddard, Houping Ni, Georgetta Cannon, Chunhui Zhou, Neville Kallenbach, Daniel Malamud, and Drew Weissman **gp340 Promotes Transcytosis of Human Immunodeficiency Virus Type 1 in Genital Tract-Derived Cell Lines and Primary Endocervical Tissue** J Virol. 2009 September; 83(17): 8596–8603.

- Postulate altered glycosylation –
  - Further work using LC-MS to analyse glycosylation between mucins
  - Manipulation within neutralisation assay

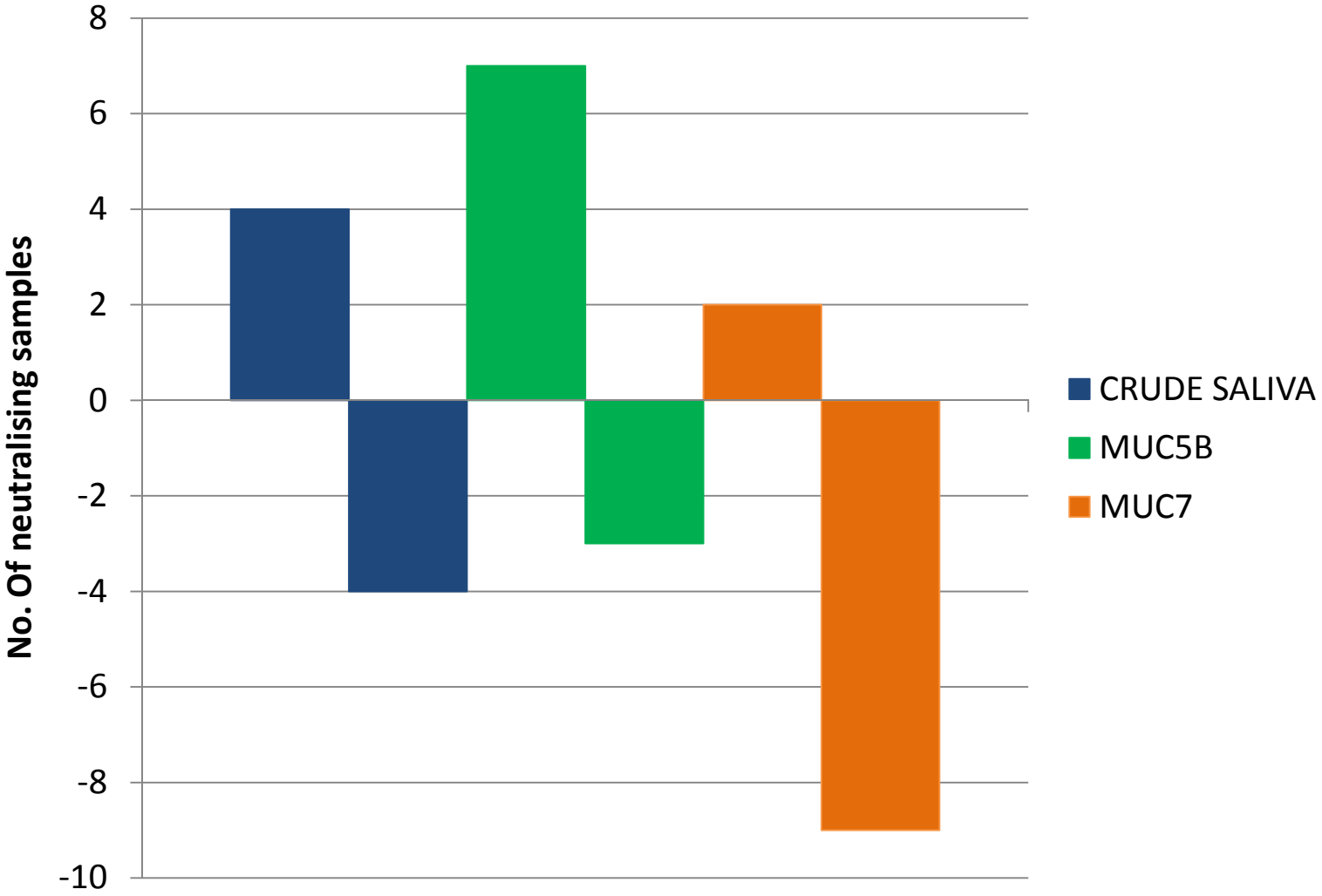
Neutralisation of virus	MUC5B	
CAP45		
Yes	11	100%
No	0	0%
Total	11	
DU422		
Yes	10	83%
No	10	17%
Total	20	
Q168a.2		
Yes	4	100%
No	1	0%
Total	5	

Preliminary data – MUC5B neutralises Subtype C pseudoviruses CAP45 and DU422.1 as well as subtype A virus Q168a.2

MUC7				
	HIV status of donor			
Neutralisation of virus	Negative		Positive	
CAP45				
Yes	0	0%	4	100%
No	0	0%	0	0%
Total	0		4	
DU422				
Yes	1	20%	4	44%
No	4	80%	5	56%
Total	5		9	
Q168a.2				
Yes	0	0%	0	0%
No	2	100%	2	100%
Total	2		2	

Preliminary data – MUC7 neutralises Subtype C pseudovirus DU422.1, potentially CAP45

Salivary mucus and mucin samples tested against subtype C pseudovirus



Greater number of neutralising samples for MUC5B than both crude saliva and MUC7

HIV-1 pseudovirus neutralisation assay

Genetically modified pseudovirus

- Multiple strain testing
- Any cloned *env* gene may be used
- Panels of env's will be used

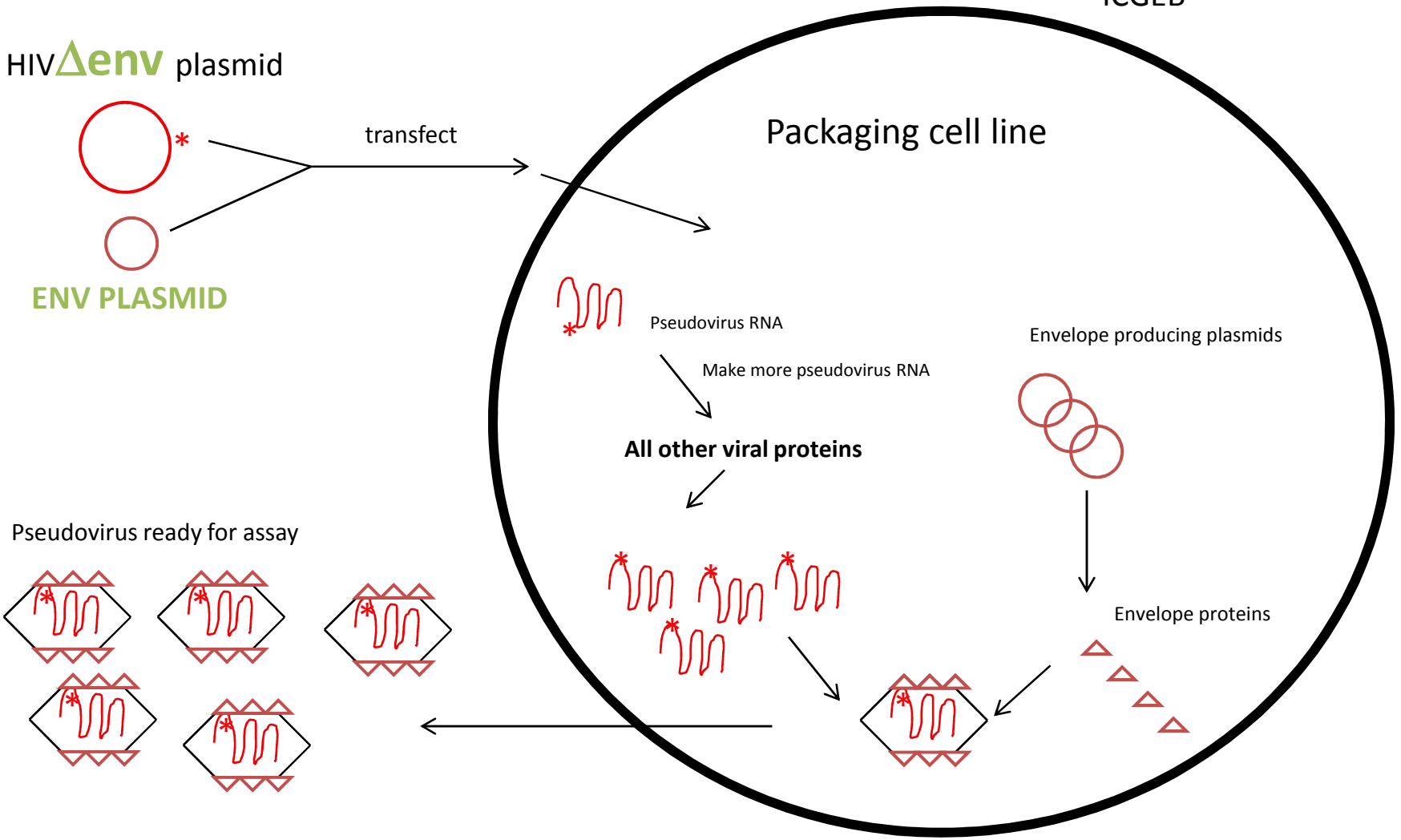
- All virus produced normally invade only once
  - "single cycle pseudo-virus"
    - (low risk of recombination to infectious virus)
  - Much safer
  - BSL2 lab

- Sensitive and technically easy assay readout
  - luminescence.

- **Easy to reproduce**

Step 1. make pseudovirus:

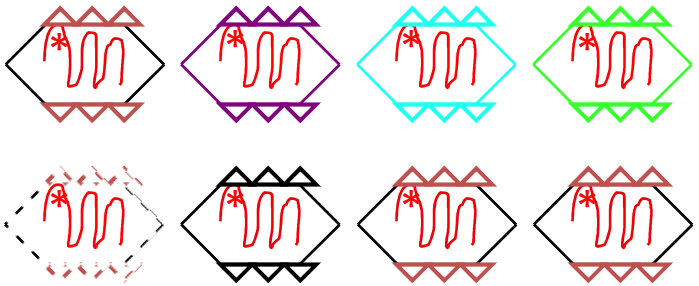
Jeffrey Dorfman
ICGEB



Step 2. assay:

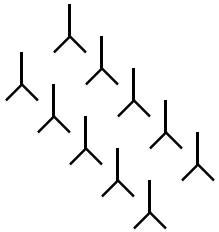
Jeffrey Dorfman
ICGEB

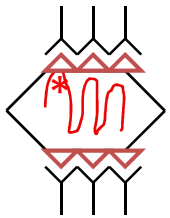
Pseudovirus env mutant panel



+

Add test sample:
-- dilution of serum
-- **MUCIN**





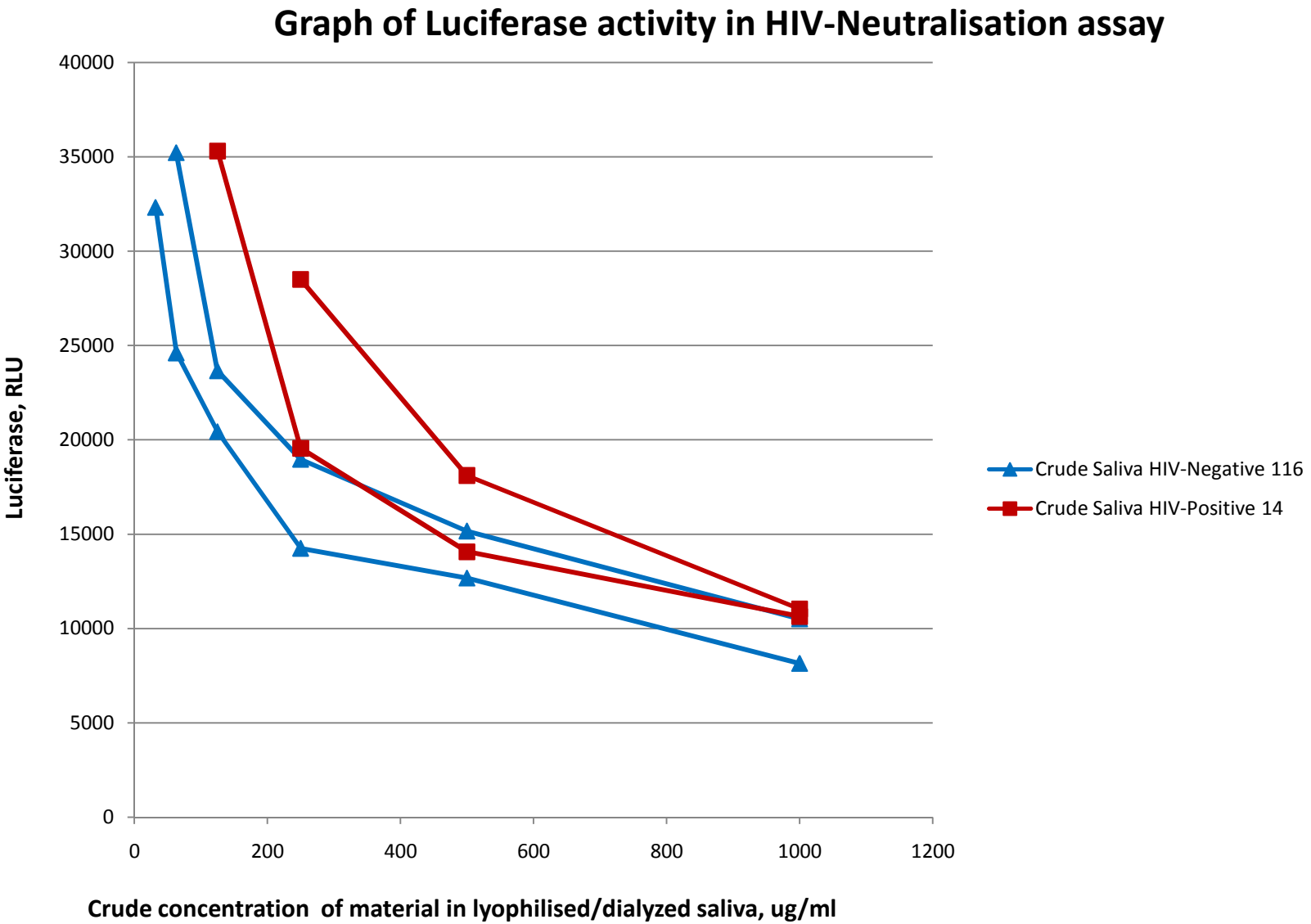
Blocks

No virus entry

Does not block

Virus enters target cell

Luciferase
(expression in target cell line driven by Tat protein) from the virus


Graph of Luciferase activity in HIV-Neutralisation assay
Luciferase, RLU
40000
35000
30000
25000
20000
15000
10000
5000
0
0
200
400
600
800
1000
1200
Crude concentration of material in lyophilised/dialyzed saliva, ug/ml
Crude Saliva HIV-Negative 116
Crude Saliva HIV-Positive 14

Crude saliva				
	HIV status of donor			
Neutralisation of virus	**Negative**		**Positive**	
CAP45				
Yes	1	100%	0	0%
No	0	0%	0	0%
Total	1		0	
DU422				
Yes	7	100%	2	67%
No	0	0%	1	33%
Total	7		3	
Q168a.2				
Yes	2	67%	0	0%
No	1	33%	2	100%
Total	3		2	