UNIVERSITY OF
KWAZULU-NATAL

INYUVESI
YAKWAZULU-NATALI

Impact of *Chlamydia trachomatis* on inhibitory capabilities of broadly neutralizing antibodies *in vitro*

By

Nzuzo Magini

Student number: 207526179

Submitted in fulfilment of the requirements for the degree of
Master of Medical Sciences

CAPRISA Mucosal Immunology Laboratory
Department of Medical Microbiology
School of Laboratory Medicine and Medical Sciences (LMMS)
College of Health Sciences
Nelson Mandela School of Medicine, University of Kwa-Zulu Natal

Supervisor: Dr Sinaye Ngcapu

Co-Supervisor: Dr Gugulethu Mzobe

Co-Supervisor: Dr Bongiwe Ndlovu

PLAGIARISM DECLARATION

I, Nzuzo Magini, hereby declare that research represents original work by the author and this dissertation has not been published or submitted before, to this or any other tertiary institution. Work that has been previously published and was referred to in this dissertation has been adequately referenced, and that the data presented here is original. The original protocol for research in this dissertation was written by the author. The Laboratory tests were performed in CAPRISA Laboratory. The Data was collected and analyzed by the author. The statistician at CAPRISA was consulted for advice on complex statistical tests. This dissertation was compiled under the supervision of Dr Sinaye Ngcapu, co-supervised by Dr Gugulethu Mzobe and Dr Bongiwe Ndlovu, at the Centre for the Aids Programme of Research in South Africa.

[redacted]

Nzuzo Magini Date: 17 December 2021

[redacted]

Dr Sinaye Ngcapu Date: 24 March 2022

[redacted]

Dr Gugulethu Mzobe Date: 24 March 2022

[redacted]

Dr Bongiwe Ndlovu Date: 24 March 2022

DEDICATION

I dedicate this dissertation to my late father.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to my co-supervisor, Dr Gugulethu Mzobe for her guidance and critique of my thesis. Most importantly, I would like to express my sincere thanks and gratitude to my supervisor, Dr Sinaye Ngcapu for his guidance, mentorship and support throughout my master's research. I have been well trained under their supervision, and this has broadened my scientific knowledge. I would like to acknowledge Dr Rubina Bunjun, Dr Bongiwe Ndlovu and Dr. Mellisa Rose-Abrahams for the cell lines, viruses, bNAbs and chlamydia they provided me with for my project, as well as their scientific input. A special thanks goes to Dr Andile Mtshali, Boitumelo Setlhare, Nelisiwe Nxele, Portia Motheo and Qiniso Mkhize, for their assistance and continued support throughout my research. I would like to thank CAPRISA organization management for the opportunity given to study further. My sincere thanks to Natasha Samsunder for allowing me access, to carry out my laboratory work and to complete my master's research within the Laboratory. I am also thankful to the Laboratory team for supporting me, in the duration of my master's degree.

To my mother, wife and siblings, thank you for your support, encouragement, and motivation throughout my dissertation. You were my pillar of strength throughout the challenging times.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Clinic
ART	Antiretroviral therapy
ARV	Antiretroviral drug
bNAbs	Broadly neutralizing antibodies
CAPRISA	Centre for the AIDS Program of Research in South Africa
CCR5	C-C chemokine receptor type 5
CI	Confidence interval
CD4	Cluster of differentiation 4
CD40L	Cluster of differentiation 40 Ligand
CDR	Complementarity-determining regions
C. trachomatis	*Chlamydia trachomatis*
CDRH3	Heavy chain complementarity determining region 3
CXCL13	C-X-C Motif Chemokine Ligand 13
HIV	Human immunodeficiency virus
Fc-receptor	Fragment crystallizable region
IC50	Half-maximal inhibitory concentration
IFN-γ	Interferon gamma
PrEP	Pre-exposure prophylaxis
MOMP	Major outer membrane protein
mAbs	Monoclonal antibodies
MPER	Membrane-proximal external region
Th1	T helper type 1

ENV	Envelope
STI	sexually transmitted infections
NIH	National Institute of Health
IP10	Interferon-γ Inducible Protein 10
TNF-α	Tumour necrosis factor-alpha
RLU	Relative Light Units
FTC	Emtricitabine
TDF	Tenofovir disoproxil fumarate
SHIV	Simian–human immunodeficiency virus
NHS	Normal human serum
MOI	Multiplicity of infection
IQR	Interquartile range
GM	Growth Medium
RANTES	Regulated on Activation, Normal T Expressed and Secreted
UNAIDS	Joint United Nations Programme on HIV/AIDS
EB	Elementary bodies

ABSTRACT

Broadly neutralizing antibodies (bNAbs) have been demonstrated to have significant potential as an alternative to ARV-based HIV prevention strategies due to their potency and breadth against various clades of viruses. However, the influence of the most common sexually transmitted infections (particularly *Chlamydia trachomatis*) on inhibitory capabilities of bNAbs against HIV subtypes remains unknown. Here, we determined whether TZM-bl cells can support *C. trachomatis* growth and characterized in vitro HIV replication in the presence of *C. trachomatis* serovars L2 and E. Furthermore, we investigated the impact of *C. trachomatis* serovars on inhibitory capabilities of CAP256-VRC26.25, VRC07-523LS and PGT121 against a panel of 6 subtype C viruses. In this study, *C. trachomatis* inclusion bodies were observed in TZM-bl cell line after 48 hours post-infection by immunofluorescence staining. *In vitro*, significantly increased concentrations of HIV were observed in TZM-bl cells coinfected with *C. trachomatis* serovars. We also found that *C. trachomatis* serovars did not significantly alter the potency of bNAbs against subtype C viruses. bNAbs were able to neutralize majority of viruses tested at lowest inhibitory concentration (IC_{50} <0.9 μg/ml) in the absence of serovars L2 and E, but required higher concentrations (IC_{50} >0.9 μg/ml) in the presence of serovars L2 and E. The profile of viruses that were profoundly resistant to neutralization by bNAbs remained unchanged even in the presence of serovars L2 and E. In summary, we showed for the first time that TZM-bl cells are appropriate for chlamydial growth, paving way for *C. trachomatis* to be included in TZM-bl cell-based neutralizing antibody assays for HIV. In addition, we showed that *C. trachomatis* induced HIV replication, but the mechanism remains unclear. Furthermore, the potency of bNAbs against subtype C viruses was not significantly affected by the presence of chlamydia, confirming bNAbs use as a viable alternative to antiretroviral-based HIV prevention. More *in vitro* and *in vivo* studies are needed to further investigate the key biological factors mediating effective antibody blocking of HIV-1 infection within the female genital tract.

Chapter 1: Introduction

HIV continues to be a major global health challenge, with approximately 37.7 million people living with HIV worldwide (1, 2). Approximately 70% of global infections are found in Sub-Saharan Africa, with women disproportionately affected (1-4). In particular, young women (15-24 years) are at high risk of acquiring HIV than older women (3, 5, 6). The ongoing high rates of HIV infection in young African women continue to be the major challenge to obtain the United Nations goal of "ending AIDS" by 2030. Therefore, there is an urgent need to develop new strategies to prevent HIV infection.

Although the implementation of pre-exposure prophylaxis (PrEP) has drastically reduced the incidence of HIV infection (7), the efficacy of PrEP may be undermined if the transmitted virus is resistant to antiretrovirals (ARVs) (7, 8). New approaches that overcome some of the challenges for reduced efficacy of PrEP remain a priority. The recent discovery of broadly neutralizing antibodies (bNAbs) has created an opportunity to explore passive immunization as a viable alternative to ARV-based HIV prevention. Due to the potency and breadth of bNAbs against various clades of viruses, the combination of bNAbs with PrEP has been proposed as a promising intervention to prevent HIV infection and transmission (9-17). Broadly neutralizing antibodies are specific and efficacious against HIV and are expected to retain efficacy against viruses that are ARV resistant. *In vitro* (18, 19) and *in vivo* (20, 21) studies have shown that bNAbs can neutralize up to 99% of heterologous viruses across different clades of HIV at low doses and are safe to use in HIV-infected and HIV-uninfected adults and non-human primate models (22). There have been sophisticated efforts to design scaffolded immunogens and immunization strategies to elicit neutralizing antibodies through vaccination (19, 23-25).

Novel technologies in single-cell sorting of antigen-specific memory B cells and soluble Envelope (Env) selection tools have led to the isolation of monoclonal antibodies which exhibit increased potency and neutralization breath against a broad spectrum of HIV strains (17, 19, 26-29). Broadly neutralizing antibodies target major epitopes, including the membrane-proximal external region (4E10, 10E8, 2F5), the CD4 binding site (3BNC117, VRC01, VRC07-523LS, VRC13, b12, NIH 45-46), the V1/V2-glycan site (PG9, PG16, PGT145, PGDM1400, CAP256-VRC26.25), the V3-glycan site (10-1074, PGT121, PGT128) (12, 17, 23, 30-33) and the gp120-gp41 interface (CAP248-2B, PGT151 8ANC195, 35O22) (Figure 1.1) (29, 34-38). A better understanding of these epitopes, including assessing the immunological development of bNAbs can provide important insights for vaccine design and strategies.

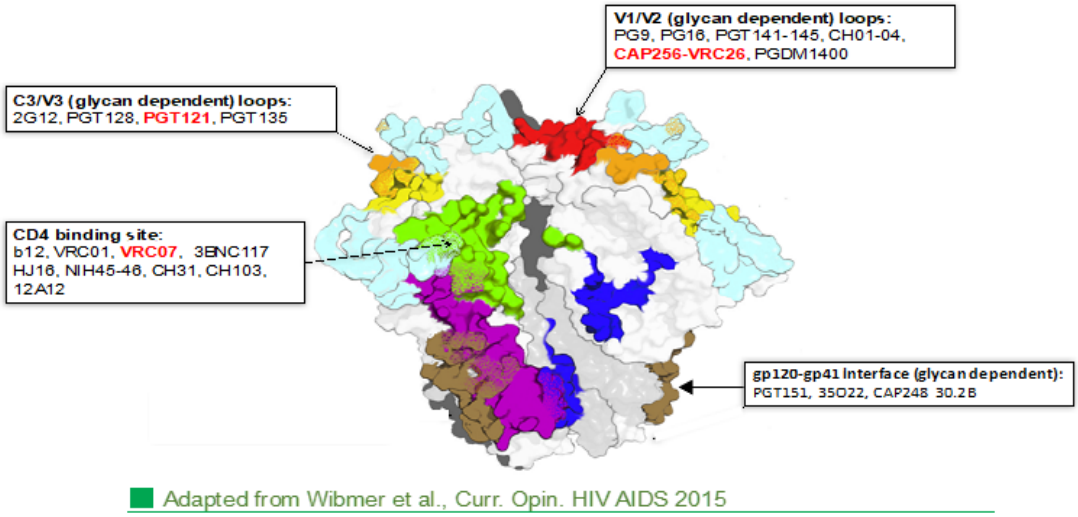


Figure 1.1: Location of broadly neutralizing antibody epitopes on HIV envelope trimer. Epitopes in red represent V2 region, V3 (orange), V4 (yellow), the CD4 binding site (green), the gp120-gp41interface [brown (8ANC195), purple (35O22) and blue (PGT151)], hypervariable regions in cyan (V1 and V2). VRC07 is located in the CD4 binding site, while CAP256 is found in the V1/V2-glycan site and PGT121 in the V3-glycan site.

Recently, a single-cell sorting of antigen-specific memory B cells and soluble Env selection tools were used to isolate the CAPRISA 256 from an HIV-1 subtype C-infected woman infected for 5 years before initiating antiretroviral therapy (ART) (39). The CAP256-VRC26.25 lineage is a series of V2-glycan-dependent bNAbs which exhibited increased potency and neutralization breath against a broad spectrum of HIV strains (17, 19, 24, 26-28). These antibodies have long heavy chain Complementarity determining region 3 (CDRH3) which have the ability to penetrate the glycan shield of the V1/V2 regions of HIV (19, 24, 39, 40). Recently, the CAP256-VRC26.25LS antibody was shown to be the most potent antibody within the lineage, with 10-fold greater neutralization potency.

Although preclinical and clinical studies have demonstrated remarkable protective efficacy of bNAbs against HIV (41) or chimeric simian-human immunodeficiency virus (SHIV) (42-44), breakthrough infections or viral rebound and escape were observed once the antibody titers were below the therapeutic range (41, 45-48). The viral rebound and escape were more pronounced in studies using a single antibody instead of a combination of bNAbs to treat HIV-infected mice (49-51). In light of this, studies have now suggested a combination of bNAbs with multiple epitope specificities to prevent breakthrough infections in HIV uninfected individuals or the emergence of viable escape variants in HIV-infected individuals (45, 46, 48-50). Animal and *in vitro* studies demonstrated that CAP256-

VRC26.25LS, VRC07-523LS and PGT121 showed great potency and breath against diverse HIV clades when selected to complement each other or other antibodies (42, 52).

bNAbs have been associated with a unique inflammatory cytokine profile and enhanced immune activation (53, 54). Studies have shown that high viremia, low CD4+ T cell count, high levels of chemokine (C-X-C Motif Chemokine Ligand 13, Cluster of differentiation 40 Ligand, Interferon-γ Inducible Protein 10, Regulated on Activation, Normal T Expressed and Secreted, and Tumour necrosis factor-alpha) are parameters linked to the development of bNAbs (53-55). Pro-inflammatory cytokines and chemokines play an integral role in immune activation and HIV pathogenesis (56). It has been found that inflammation is caused by sexually transmitted infections (STIs), including *Chlamydia trachomatis* (57) and other biological and behavioral factors. However, less is known about the ability of bNAbs to reduce HIV replication capacity in the presence of *C. trachomatis*. This study explored the HIV prevention capabilities of CAP256-VRC26.25 when used alone and in combination with VRC07-523LS or PGT121 in the presence of *C. trachomatis*. Understanding key biological factors mediating effective antibody blocking of HIV-1 infection within the female genital tract may have important implications for bNAbs as a viable alternative to ARV-based HIV prevention.

Chapter 2: Literature Review

2.1 Human Immunodeficiency Syndrome Virus (HIV) Overview

2.1.1 The HIV Epidemic in South Africa

South Africa, is home to about 1% of the global population but contributes a disproportionate 20% of the world's burden of HIV infection with about 200 000 (4500 weekly) new infections annually (58). In 2020, approximately 7.8 million South Africans living with HIV, 72 000 AIDS-related deaths occurred and over 4 million people received antiretroviral therapy (ART) (58, 59). The province of KwaZulu-Nata, South Africa, remains the epicenter of the HIV pandemic with 4 of its 11 districts having an HIV prevalence among pregnant women of greater than 40% and the remaining seven districts having an HIV prevalence between 30%-40% (60). In Vulindlela, one of the heaviest HIV burden districts in rural KwaZulu-Natal, by age 16, one in ten women utilizing antenatal services are already infected with HIV and this increases to one in three by age 20 and one in two by age 24 (Table 2.1) (61). The incidence rates in this community are extremely high; in the placebo arm of the CAPRISA 004 trial that evaluated the safety and effectiveness of 1% tenofovir gel in urban and rural women in KwaZulu-Natal, HIV incidence was 9.1% per 100 women-years of follow-up despite monthly HIV risk reduction counseling and HIV testing (62). High HIV incidence rates have also been observed in post-partum women in peri-urban communities in KwaZulu-Natal.

Table 2.1: HIV prevalence in Antenatal Clinic (ANC) clients utilizing ANC services in rural KwaZulu-Natal

Overall HIV prevalence in rural Vulindlela (2001-2013)	
Age Group (Years)	**HIV Prevalence (N=4818)**
≤16	11.5%
17-18	21.3%
19-20	30.4%
21-22	39.4%
23-24	49.5%
>25	51.9%

Table modified from (61)

2.1.2 HIV incidence in young women

Women of child-bearing age are more vulnerable to HIV infection, even more so during late pregnancy and postnatal periods. South Africa, particularly KwaZulu Natal, has been described as a region with the highest HIV infection rates in the world (40% antenatal HIV prevalence) (63). In 2015, the national HIV seroconversion rate of women who attended antenatal clinics was 10.7% (95% Confidence interval (CI): 6.2%–16.8%) with 50% of the seroconversions occurring after 32.8 weeks of gestation (64). Several behavioral and biological factors are thought to contribute to this unique vulnerability of young women. Inconsistent or lack of condom use, engagement in age-disparate and/or transactional relationships, men who have other sexual partners during their partner's pregnancy are some of the well-documented risk factors for HIV infection during pregnancy and postpartum (65-68). Several studies suggest that young non-pregnant women become infected with HIV after fewer acts of unprotected sex compared to their male counterparts (69, 70) due to several biological factors including the presence of co-factors such as increased levels of progesterone, mucosal inflammation, and BV, which is often asymptomatic and therefore untreated in women (71-73). Given their disproportionately high risk for HIV infection (74), young women have been identified by UNAIDS as a key population to target in order to end the AIDS epidemic (75), emphasizing the need to find urgent and novel prevention technologies to prevent and reduce incident infections in this key population.

2.1.3 HIV prevention

Since 2010, the outcomes of several trials have demonstrated the prevention benefit of the prophylactic use of antiretroviral (ARV) drugs that have generated new hope in preventing sexually acquired HIV infection. These PrEP clinical trials using tenofovir alone, or in combination with emtricitabine (Truvada®), have demonstrated safety and efficacy in preventing HIV acquisition among men who have sex with men, transgender women, injecting drug users, serodiscordant couples, and heterosexual men and women (76-81). Importantly, no major safety concerns have been identified. A key and consistent message from these trials is that PrEP needs to be used in order for it to work and that efficacy is dependent on adherence. While the PrEP data from men is consistent across all trials with a protective benefit ranging from 44%-96% using both daily and intermittent dosing strategies (76-81), the data in women, in trials that have evaluated oral and topical PrEP in southern Africa have yielded mixed results (62, 82-84). Clinical trials in African women have produced inconsistent levels of protection ranging from 49% to 76% (14, 85). Varying levels of adherence among women mainly accounted for the inconsistent efficacies (14, 85, 86).

While the HIV prevention landscape is changing rapidly, principally with the roll-out of pre-exposure prophylaxis (PrEP) and early antiretroviral therapy (ART) (Treatment as Prevention), current HIV prevention programmes have had limited impact on reducing HIV incidence in young women (79, 81,

84, 87-90). Clinical trials using daily oral tenofovir disoproxil fumarate (TDF) and emtricitabine (TDF//FTC) in African women demonstrated inconsistent results, most likely due to varying levels of medication adherence (81, 84, 87-92). In contrast, effectiveness was consistently shown in men who have sex with men and transgender women, globally (93). Regulatory authorities in South Africa, the European Union, Canada, Kenya and other countries, have recently approved TDF/FTC (marketed as Truvada®) following the United States Food and Drug Administration (FDA) approval. In addition, the 2015 WHO guidelines recommend the use of TDF-containing PrEP (94). However, this still remains a challenge as studies have shown that women may need to take 5 or 6 out of 7 doses in a week, in order to be protected against HIV, increasing the challenges of adherence once again (95). New approaches that overcome some of these adherence challenges, are a high priority. While the development of an efficacious vaccine remains a major challenge, the recent discovery of potent monoclonal antibodies (mAbs) has created the opportunity to explore passive immunization as a long-acting injectable HIV prevention strategy.

Another important public health concern about PrEP use is the potential of breakthrough infections and the development of antiretroviral resistance. Although it remains unclear how breakthrough infections occur; initiation of tenofovir- and emtricitabine (TDF/FTC) as PrEP among individuals with undiagnosed acute HIV; continued PrEP use by individuals who acquire HIV while using PrEP due to subtherapeutic drug levels from incomplete PrEP adherence; and adherent PrEP user acquire HIV infection with viral strains that are resistant to TDF/FTC (96). There is precedent and necessity for implementing novel HIV prevention strategies that overcome the barriers to PrEP adherence especially for at risk, young African women.

2.2 Broadly Neutralizing Antibodies Overview

2.2.1 Broadly Neutralizing Antibodies for passive immunization

First-generation bNAbs were isolated in the early 1990s, mainly by using phage libraries to identify, isolate and amplify antibodies from asymptomatic individuals with HIV-1 infection. These studies reported on antibodies with different specificities, including b12, 447-52D, 2G12, 17b, 2F5, 4E10 and Z13, (97-100). Although these bNAbs neutralized diverse primary strains of HIV-1 in vitro, their breadth and neutralization potency was moderate (101). Clinical studies have demonstrated that the combination of three neutralizing antibodies (2G12, 2F5 and 4E10) had moderate efficacy against the virus in acutely and chronically HIV-1-infected individuals on interrupted HIV-antiviral treatment. Furthermore, viruses that were resistance to 2G12 were found in most (12/14 and 7/8) of the participants, and these escape mutants developed very rapidly and showed high titres (102, 103). Antibodies such 2F5 and 4E10 were self-reactive (104, 105). Although early studies with the first-generation bNAbs were less

efficacious, the development of high-throughput neutralization assays and single cell sorting of antigen-specific memory B cells and soluble Env selection techniques have facilitated the isolation and characterization of a new generation of bNAbs with increased potency and neutralization breadth for immune prophylaxis and therapy of HIV-1. These potent bNAbs were isolated by single-B-cell culture and direct functional screening or antigen-specific single-B-cell sorting. Both methods identified multiple bNAbs and new HIV-1 spike sites of vulnerability to these neutralizing antibodies(106). This new generation of bNAbs displayed a 10- to 100-fold increase in potency and a more than 2-fold improvement in coverage than the earlier bNAbs. In addition to their strong activity in vitro, these new agents showed encouraging effects for both therapy and prevention in vivo. Animal studies have demonstrated that passive immunisation using mAbs can protect monkeys from infection, thus providing a proof-of-concept that HIV antibodies may be able to prevent HIV-1 infection in humans. Furthermore, pharmacokinetic (PK) data has shown that mAb levels can remain high for several months post-injection in monkeys (43, 107), suggesting that this strategy could be developed into a long-acting HIV prevention strategy. In immunotherapy experiments, administration of bNAb to chronically infected animals resulted in a rapid decline of plasma viral RNA to undetectable levels and a reduction in proviral DNA in the peripheral blood, gastrointestinal mucosa and lymph nodes. Moreover, host Gag-specific T cell responses were enhanced with monoclonal antibody treatment (108).

bNAbs are generally recovered from the memory B cells of chronically HIV infected individuals and effectively neutralize diverse strains of HIV (109). Previous data demonstrate that bNAbs development is associated with duration of HIV infection, lower CD4+ T cell count, higher frequency of T follicular helper cells and higher viral load (109). Advances in single-cell cloning techniques have led to identification of bNAbs with high potency and breadth. The bNAbs exceptional potency is attributed unusual structural characteristics of long CDRH3, high levels of somatic hypermutation and insertion or deletion in both CDRH3 and antibody frame work (110). These features enable the HIV bNAbs to access to epitopes that are often recessed or contain conserved glycan or membrane components (110). Previous studies show that bNAbs have minimum epitope glycan dependence which help to increase their potency (110).

HIV vaccine designs are mainly focused on Env trimer since it is the known target of the bNAbs. Env is expressed as a single gp160 protomer that is extensively glycosylated and trimerized in the endoplasmic reticulum, it is highly sequence variable, particularly within the V1-V5 variable loop

regions, and heterogeneously glycosylated because the densely packed glycans afford each other varied protection from glycan processing enzymes (52, 109). This combination of factors favors the formation of strain-specific neutralizing antibodies and it is vital for understanding of epitopes that are susceptible to bNAbs (29). The major epitope targets for bNAbs include MPER (4E10, 10E8, 2F5), the CD4 binding site (3BNC117, VRC01, VRC07-523LS, VRC13, b12, NIH 45-46), the V1/V2-glycan site (PG9, PG16, PGT145, PGDM1400, CAP256-VRC26.25), the V3-glycan site (10-1074, PGT121, PGT128) (12, 17, 23, 30-33) and the gp120-gp41 interface (CAP248-2B, PGT151 8ANC195, 35O22) (52, 109).

Single-cell sorting of antigen-specific memory B cells and soluble Env selection tools have been developed to isolate monoclonal antibodies which exhibit increased potency and neutralization breath against a broad spectrum of HIV strains in humans and immunized animals (17, 19, 26-28). Unlike other antibody types that may be redirected away from the conserved regions of the Env glycoprotein in the presence of highly immunogenic variable loops, bNAb reactivity appears to target the conserved regions such as the membrane-proximal external region (4E10, 10E8, 2F5), the CD4 binding site (3BNC117, VRC01, VRC07-523LS, VRC13, b12, NIH 45-46) and the gp120-gp41 interface (34-37). The bNAbs also target variable regions of the envelope glycoprotein, such as the V1/V2-glycan site (PG9, PG16, PGT145, PGDM1400, CAP256-VRC26.25), the V3-glycan site (10-1074, PGT121, PGT128) (12, 17, 23, 30-33).

Novel strategies to develop soluble immunogens that promote reactive and stable bNAbs against the CD4 binding site, the MPER region and structurally conserved elements of the V3 loop are being pursued for vaccine design efforts (111). Other researchers have made positive strides in the development of effective vaccine goal. The isolation of bNAbs from HIV infected individuals and ability to define their targets developmental pathway has helped in obtaining cross-neutralizing antibodies that may mature into potent bNAbs targeting more conserved regions of Env, and thus neutralize diverse viral strains (55, 112, 113). Taken together, these developments have created the opportunity to investigate whether passive immunization using bNAbs can be used as a potential long-acting injectable (2-3 doses per year) PrEP strategy. For example, bNAbs such as CAP256-VRC26.25, VRC07-523LS and PGT121 have been selected for the CAPRISA 012 phase I/II trial as passive immunization strategies for HIV prevention. The ongoing trial was proposed after the combination of these antibodies demonstrated proof-of-concept in protecting against simian-human immunodeficiency virus (SHIV) in monkey challenge studies, with each antibody targeting a different viral envelope site (V2 loop, CD4 binding site, and V3 loop respectively) (42).

2.2.2 CAP256-VRC26.25 antibody

CAP256 is the study identification number of a 27-year South African school teacher who is a participant in the CAPRISA 002 study since 2005 (114). In 2011, several monoclonal antibodies were isolated from PID CAP256-VRC26.25 and were named CAPRISA 256 (19). CAPRISA, jointly with the VRC isolated a family of 34 related monoclonal antibodies (mAbs) from patient CAP256 (40). One particular mAb, referred to as CAP256-VRC26.25 exhibited increased potency and neutralization breath against a broad spectrum of HIV subtype C strains (39). CAP256-VRC26.25, which targets the V1V2 C-strand at the apex of the viral envelope glycoprotein, does not depend on the N160 glycan site for neutralization (glycan dependence can result in incomplete neutralization) (18, 115). These antibodies have long CDRH3 which have the ability to penetrate the glycan shield of the V1/V2 regions of HIV (Figure 2.1) (19, 24, 39, 40). CAP256-VRC26.25 was modified to produce an improved LS version (CAP256-VRC26.25) that increases the binding affinity for the neonatal fragment crystallizable receptor (Fc-receptor), resulting in increased bNAb half-life and prolonged duration of protection (107). Recently, the CAP256-VRC26.25 LS antibody was shown to be the most potent antibody within the lineage, with 10-fold greater neutralization potency, even at the lowest dose, but it did not neutralize all commonly used simian-human immunodeficiency virus (SHIV) (42). In light of this, studies have now suggested a combination of bNAbs with multiple epitope specificities to prevent breakthrough infections in HIV uninfected individuals or the emergence of viable escape variants in HIV-infected individuals. Animal and *in vitro* studies demonstrated that CAP256-VRC26.25, VRC07-523LS and PGT121 showed great potency and breadth against diverse HIV clades when these V2-glycan and V3-glycan antibodies selected to complement each other (42).

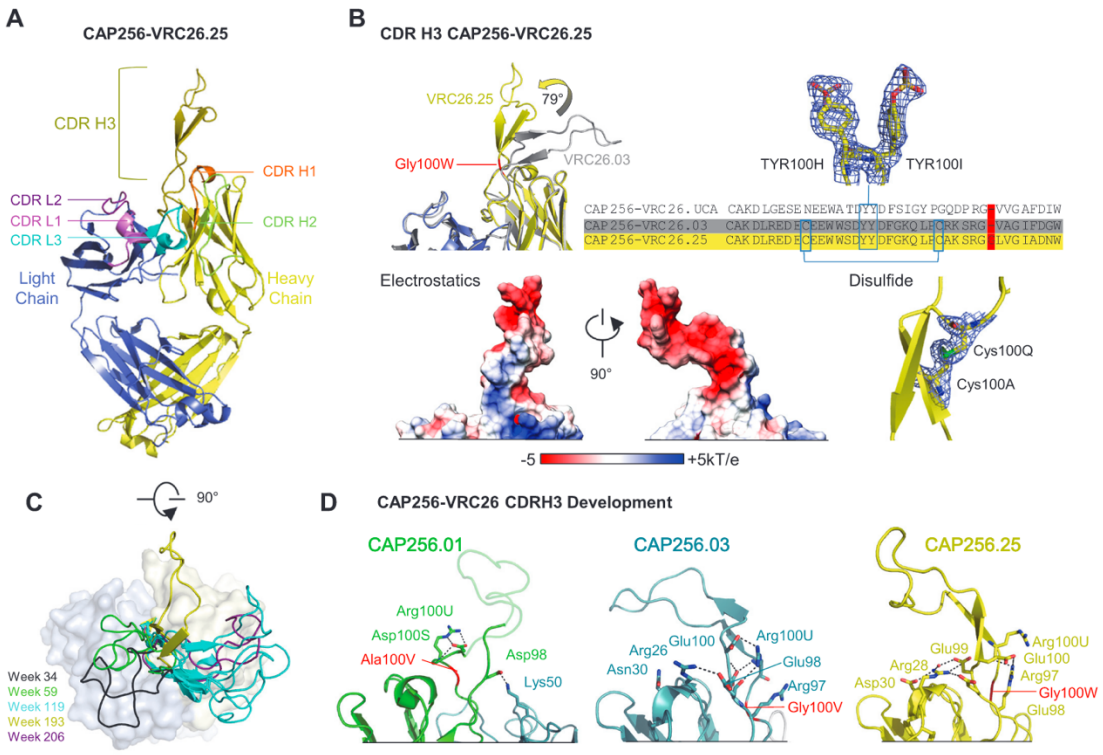


Figure 2.1: Structural characteristics of CAP256. A) Ribbon structure of the antigen-binding fragment (Fab) of CAP256-VRC26.2 with complementarity-determining regions (CDR). B) The 79 degrees rotation of CDRH3 of CAP256-VRC26.25 containing inserted glycine. C) The CDRH3 bend in different directions. D) CDRH3 loop showing interactions for CAP256-VRC26.01, CAP256-VRC26.03, and CAP256-VRC26.25. (Adapted from Doria-Rose et al., Journal of Virology, 2015)

2.2.3 PGT121 antibody

The PGT121 is a recombinant human IgG1 mAb (45). PGT121 and related antibodies target a region of the HIV spike that is heavily glycosylated and shows high levels of somatic mutations. Somatic mutations differentiate the PGT121 lineage that recognizes glycans at N301, N137, and N156 from the 10-1074 or PGT124 lineage that is highly dependent on N332 but less so on the other glycans surrounding the base of the V3 loop (Figure 2.2) (116). Their target epitopes comprise the GlyAspIleArg (GDIR) sequence at the base of the V3 loop and surrounding glycans at positions Asn332, Asn156, Asn301, and Asn137 (N332, N156, N301, N137 (116). Preclinical data in the non-human primate model using the V3 glycan-dependent bNAb PGT121 demonstrated the reduction of proviral DNA in both blood and tissues (117). As a result, several laboratories are exploring the possibility that PGT 121 may contribute to HIV eradication strategies (117). PGT121 has demonstrated potent antiviral activity in both therapeutic and protection studies in rhesus macaques and shows striking complementary coverage with CAP256-VRC26.25 (42, 43). This complementarity increases viral coverage of these antibody

combinations to >90% of clade C viruses, suggesting that PGT121 may be a useful antibody to co-administer with CAP256-VRC26.25 to prevent clade C HIV infection in South Africa (42).

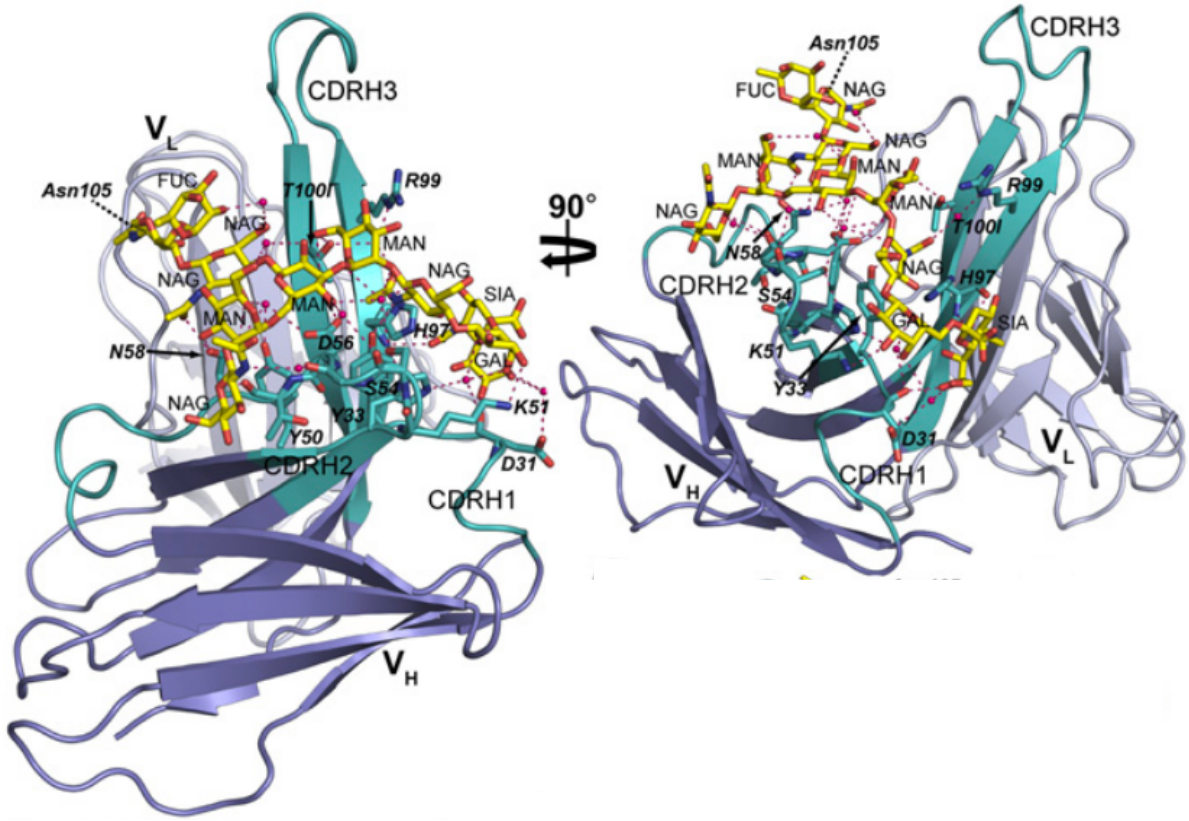


Figure 2.2: Structural characteristics of the PGT121 antibody are shown in the ribbon diagram representation. Yellow sticks depict the variable regions bound to a complex-type N-glycan attached to Asn105$_{HC}$ adjacent to the Fab in the crystal. Blue ribbon shows V_H with CDR (teal), V_L (light blue), side-chain, and backbone atoms of contact residues are shown as sticks, hydrogen bonds as magenta dashed lines, and water molecules in red spheres. (Adapted from Mouquet et al., PNAS, 2012).

2.2.4 VCR07-523LS antibody

VRC07, a potent mAb with excellent breadth across all HIV subtypes, has been shown to be effective in protecting macaques against SHIV infection (118). VRC07-523LS contains a four-amino-acid insertion in the heavy chain CDR3 and 16 additional amino-acid mutations in the heavy-chain variable domain compared with VRC01. VRC07-523LS has increased binding affinity for gp120, which allows this bNAb to bypass conformational and glycan masking that impairs previously identified CD4bs bNAbs (Figure 2.3) (119, 120). It binds to the CD4 binding site of gp120 and neutralizes the viruses since HIV entry into target cells is dependent on viral attachment to the CD4 receptor and is mediated through binding to a conformational epitope on the trimeric Env glycoprotein termed the CD4 binding site (CD4bs) (117). The bNAbs combinations demonstrate superior performance in overall potency and

breadth (52). Major epitope targets include the CD4bs, a glycan-dependent site in variable region 3 (V3) of gp120, a V1/V2 glycan-dependent quaternary site on the apex of the Env trimer, the MPER, and epitopes bridging both gp120 and gp41. VRC07-523 was extensively engineered to improve potency, breadth, expression, and biophysical properties, and modified to include the LS mutation. The resulting VRC07-523LS bNAb is approximately five times more potent than VRC01 against subtype B and C HIV and displays greater breadth, neutralizing 95% of viruses tested (20). A SHIV-SF162P3 challenge study in 6 male and female rhesus macaques showed that all of the monkeys who received a single dose of VRC07-523LS at 20 mg/kg IV were protected (118). The analysis revealed that the neutralization profile of CAP256-VRC26.25LS (which targets the V2 loop) was also well suited as a complementary bNAb with VRC07-523LS (targeting the CD4 binding site). These bNAbs were found to be the best combinations in terms of neutralization breadth and potency (52).

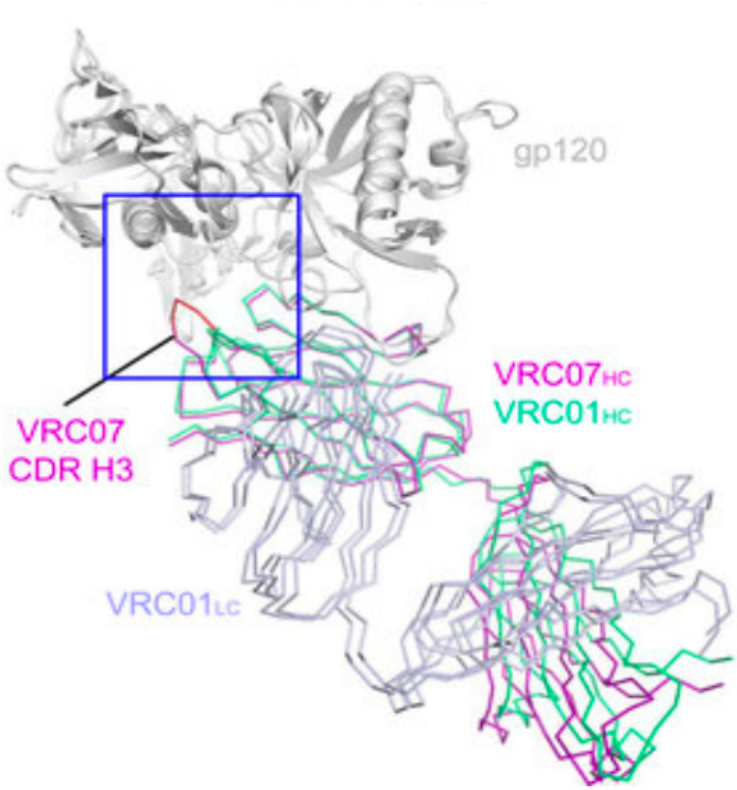


Figure2.3: Ribbon diagram representation of the superposition of the VRC01 and VRC07 antibody-bound gp120 structures. Purple depicts the VRC07 heavy chain, the VRC01 heavy chain (green), light chains (light purple), and four-amino-acid insertion in the CDR H3 of VRC07 VRC01 and VRC07 (red) (Adapted from Rudicell et al., JVI, 2015).

2.3 *Chlamydia trachomatis*

2.3.1 *Chlamydia trachomatis* overview

Chlamydia trachomatis is the most common sexually transmitted bacterial infection globally, which causes different diseases. The most common strains cause disease in the genital tract, while other strains cause disease in the eye or lymph nodes (121-123). This infection translates to a significant public health burden particularly in women healthcare because of disease sequelae such as tubal factor infertility, chronic pelvic pain and ectopic pregnancy (122-124). *C. trachomatis* strains are generally divided into three biovars based on the type of disease they cause. They are further subdivided into several serovars based on the surface antigens recognized by the immune system. Serovars A through C cause trachoma, which is the world's leading cause of preventable infectious blindness. Serovars D through K infect the genital tract, causing pelvic inflammatory disease, ectopic pregnancies, and infertility. Serovars L1 through L3 cause an invasive infection of the lymph nodes near the genitals, called lymphogranuloma venereum(122, 124-126).

C. trachomatis is a spherical obligate intracellular bacteria whose natural hosts are humans (121, 124). *C. trachomatis* has a biphasic life cycle which alternates between two distinct developmental forms, the elementary body (EB), a metabolically inert infectious extracellular form, and the reticulate body (RB), a metabolically active but non-infectious replicative intracellular form (121, 124). Adhesion of *C. trachomatis* is a two-step process that is mediated by a low-affinity interaction with heparan sulfate proteoglycans (HSPGs) followed by high-affinity binding to host cell receptors (121, 125). The chlamydial outer membrane complex protein B (OmcB) interacts with heparan sulfate proteoglycans (HSPGs) on host cell-surface to mediate binding to host cell (121, 125). After attachment and invasion of host epithelial cells through binding of *C. trachomatis* outer membrane protein (MOMP) to both mannose receptor and mannose 6-phosphate receptor and other ligands, interactions modulate infection (121, 124). Studies show that both MOMP and OmcB bind to glycosaminoglycans, favoring synergistic electrostatic interactions required to initiate invasion (121, 124). After invasion of host cell the pre-synthesized T3SS effectors are injected and the elementary body is internalized into the inclusion (121, 125). In 6–8 hours post-infection the transition to reticulate body occurs and early genes are transcribed (125). Early effectors remodel the inclusion membrane, redirect exocytic vesicles to the inclusion and facilitate host–pathogen interactions (125). In 8–16 hours post-infection mid-cycle genes are expressed, which include effectors that mediate nutrient acquisition and maintain the viability of the host cell (125). The bacteria divide by binary fission and the inclusion substantially expands (121, 124, 125). At late stages 24–72 hours post-infection, reticulate bodies transition to elementary bodies (121, 124, 125). An increase of RBs triggers differentiation back into EBs which are rapidly released to disseminate infection into neighboring cells (121).

2.3.2 *Chlamydia trachomatis* Immunopathogenesis

A successful intracellular infection relies on the ability of bacteria to manipulate the host cell molecular machinery to facilitate its entry and invasion, as well as its capacity to avoid lysosomal degradation and evade immune attack (121).Chlamydia infection elicits an aggressive local cellular immune response, both humoral and cell mediated with inflammation and subsequently secrete pro-inflammatory cytokines and chemokines such as interferon gamma (IFN-γ) (122, 124).The outcome of chlamydial infection depends on interaction and balance of cytokines secreted by the activated lymphocytes (124). IFN-γ has been described as a single most important factor in host defence against chlamydia because it activates macrophages and induce class II major histocompatibility complex (MHC) molecule expression (124, 126). While infection susceptibility has been linked with enhanced expression of Interleukin (IL) - 10. Immune system changes or disturbances induced by *C. trachomatis* may favour its own survival in the infected host, and induce persistent infections (124). The damaged and infected epithelial cells secrete numerous pro-inflammatory chemokines and cytokines, including IL-1, IL-6, IL-8, granulocyte - macrophage colony stimulating factor (GM-CSF), growth regulated oncogene, and tumour necrosis factor alpha (TNF-α) (124). The released cytokines cause vasodilatation, increased endothelial permeability, activation and influx of neutrophils, monocytes and T-lymphocytes, and elevated expression of adhesion molecules. In addition, it stimulates other cells to secrete cytokines (124).

Inflammatory response induces a large infiltration of leukocytes, including HIV target cells such as CD4+ bearing lymphocytes and macrophages to which HIV can bind and gain access. Such action may enlarge the portal of entry for HIV by increasing the number of target cells (124, 126). Inflammation can increase the level of body fluids virus-laden and the number of HIV-infected lymphocytes and macrophages present in the genital contact area, increasing the probability of infection and enhancing the efficiency of HIV transmission (126).

2.3.3 *Chlamydia trachomatis* and the efficacy of bNAbs

Although natural immunity can develop during the *Ct* infection, it is generally accepted that both humoral and cell-mediated immune responses are significant in the immune response against *C. trachomatis* however, the exact immune correlates of protection are unknown. (127, 128). The attempts to identify a target for neutralizing antibodies have mainly focused on major outer membrane protein (MOMP) because it has highly conserved species-specific epitope LNPTIAG embedded in the central part of the variable region, but its application as a vaccine antigen has been complicated by the complex structure of the protein (122, 129). Previous data in mice studies has consistently identified a link in cytokine IFN-γ and CD4+ Th1 cells to a key protective immunity role. The protective role of antibodies

to *C. trachomatis* infection is more multifarious and ranges from direct bacterial neutralization to accelerating cell-mediated immune through Fc-mediated uptake in antigen-presenting cells (127). Studies in clinical trials and non-human primates demonstrate that protection can be achieved by the use of vaccine-based strategies of intact Chlamydia bacteria. The presence of antibodies in cervical secretions of women correlates with lower Bacterial shedding (127). However, protection seems to be serovar-specific and a whole-cell vaccine may potentially generate unwanted responses and lead to pathology, emphasizing the need for a broadly protective subunit vaccine (127, 128). Identification of a clinically relevant delivery system that would induce a strong Th1 response, antibodies and long-lived memory has proved to be an obstacle, in addition, to identifying the right antigens for vaccine-induced immunity (127). Vaccine research efforts have led to the identification of multiple antigens with significant protective efficacy (127). The study of nonhuman primates has demonstrated induction of significant potency in cell-mediated immune and humoral immune response with several chlamydial antigens in mouse models, with up to 90% of all infections and showing that vaccine-induced antibodies can modulate Chlamydia infections (127). Research on nonhuman primates showed broadly cross-reactive antibodies toward multiple serotypes (122, 129). Importantly the adjuvant has also been tested in phase I trials with an excellent safety profile and is a promising adjuvant for a recombinant vaccine against Chlamydia (127).

AIMS AND OBJECTIVES

Study Rationale

Several studies have shown bNAbs to inhibit HIV replication, but less is known about the ability of bNAbs alone and in combination with other antibodies to reduce HIV replication capacity in the presence of Chlamydia trachomatis. The findings of this study may confirm the use of broadly neutralizing antibodies as a viable option and inform policy on HIV prevention strategies.

AIM:

To evaluate the ability of CAP256-VRC26.25, VRC07-523LS and PGT121 to reduce HIV replication capacity in the presence of *Chlamydia trachomatis*

OBJECTIVES:

- To screen subtype c viruses for downstream bNAbs neutralization assay against HIV and *C. trachomatis* coinfection
- To determine whether *C. trachomatis* can infect TZM-bl cells commonly used in HIV neutralization assays
- To determine the effect of CAP256-VRC26.25, VRC07-523LS and PGT121 in TZM-bl cells coinfected with *C. trachomatis* and HIV

HYPOTHESIS: bNAbs will be potent and effective at reducing HIV replication regardless of the presence of STIs.

CHAPTER 3: MANUSCRIPT

3.1 Introduction

Over the past decade, major trials have demonstrated the benefits of the prophylactic use of antiretroviral (ARV) in preventing HIV infection among men who have sex with men, transgender women, injecting drug users, serodiscordant couples, and heterosexual men and women (76-81). Although product adherence was a crucial factor for effective HIV pre-exposure prophylaxis (PrEP), but even with high adherence, protection is modest in some studies (9, 76, 81). In addition, the upscale of ARV programs in African countries will most likely coincide with an increased emergence of HIV drug resistance. New approaches that overcome some of the adherence and drug resistance issues are a high priority.

In recent years, the immune milieu of individuals living with HIV has demonstrated an ability to mount effective broadly neutralizing antibody (bNAbs) responses against HIV and their effectiveness was retained even against viruses that are ARV resistant (52, 130). Few percentages of chronically infected HIV patients showed potent bNAbs (115, 131). These bNAbs can effectively bind with a wide range of distinct epitopes and have an increased range of binding affinity, which allows a single clade of similar bNAbs to be able to neutralize many distinct strains of HIV with various mutations and unique envelope spike-proteins (130). While the development of an efficacious vaccine remains a major challenge, the recent discovery of potent bNAbs has created the opportunity to explore passive immunization as a long-acting injectable HIV prevention strategy.

CAPRISA researchers and collaborators used novel technologies in single-cell sorting of antigen-specific memory B cells and soluble Env selection tools to isolate the CAPRISA 256 monoclonal antibodies which exhibited increased potency and neutralization breath against a broad spectrum of HIV strains (17, 19, 24, 26-28). However, CAPRISA CAP256-VRC26.25 monoclonal antibodies alone could not neutralize all commonly used simian-human immunodeficiency virus, suggesting a need for a combination of bNAbs with multiple epitope specificities to prevent breakthrough infections in HIV uninfected individuals or the emergence of viable escape variants in HIV infected individuals. Non-human primate studies showed that a combination of CAP256-VRC26.25 and VRC07-523LS or PGT121 exhibited excellent potency and breath against diverse HIV clades.

While bNAbs have demonstrated remarkable protective efficacy against HIV (41) or chimeric simian-human immunodeficiency virus (42-44), breakthrough infections or the emergence of viable escape variants have been reported, underscoring a role for other biological risk factors. The bNAbs have been

associated with a unique inflammatory cytokine profile and enhanced immune activation (53, 54). Increased viral load and chemokines concentrations, and decreased CD4+ T cell count, are some of the biological factors associated with the development of bNAbs (53-55). In addition, pro-inflammatory cytokines and chemokines are associated with T cell activation and increased risk for HIV infection and pathogenesis (56). Although the causes of inflammation are not fully elucidated, behavioral and biological factors such as sexually transmitted infections (STIs, *Chlamydia trachomatis*), have been reported as the likey drivers (57). However, less is known about the inhibitory effects of bNAbs on HIV replication in the presence of *C. trachomatis*. Here, we investigated the ability of CAP256-VRC26.25 alone and in combination with VRC07-523LS and PGT121 to reduce HIV replication capacity in the presence of *C. trachomatis.* Understanding key biological factors mediating effective antibody blocking of HIV-1 infection within the female genital tract may have important implications for bNAbs as a viable alternative to ARV-based HIV prevention

3.2 Materials and methods

3.2.1 Cell culture

The McCoy cell line (ATCCR number CRL-1696), an adherent mouse fibroblast used for the propagation of *C. trachomatis* was provided by Dr. Rubina Bunjun from the University of Cape Town, South Africa. In addition, the TZM-bl cell line (manufactured by NIH AIDS Reagent Program) was provided by Dr. Mellisa Rose-Abrahams from the University of Cape Town, South Africa. The TZM-bl cell line is a derivative of the HeLa cell line that was engineered to express CD4 and CCR5 coreceptors and is widely used for neutralization assays (132, 133). Briefly, to obtain confluent cell lines, McCoy and TZM-bl cells were respectively cultivated and maintained in vented T-75 cell culture flasks (AEC-Amersham, South Africa) containing minimum essential medium (MEM) and dulbecco's modified eagle medium (DMEM), each supplemented with 10% heat-inactivated foetal bovine serum (FBS), HEPES (25mM) (Thermo Fisher Scientific, USA) and antimycotic solution (10,000 units penicillin, 10 mg streptomycin and 25 µg Amphotericin B per mL) (Merck, USA). The vented T-75 cell culture flasks with attached cell monolayers were incubated at 37°C, 5%CO_2 and maintained until 80% confluency was reached. Cells were then passaged by treatment with 0.25% Trypsin-EDTA solution (Thermo Fisher Scientific, USA), and were either stored or used in an experiment.

3.2.2 Bacterial culture, Immunofluorescent staining and quantification of *C. trachomatis*

Chlamydia trachomatis serovars L2 and E suspended in sucrose-phosphate-glutamate (SPG) was donated by Dr. Rubina Bunjun from the University of Cape Town, South Africa. To propagate serovars

in the vented T-75 cell culture flasks, 500 µl of Chlamydia EB suspension was inoculated into confluent McCoy cell monolayers containing chlamydia growth medium (CGM) supplemented with MEM, 10% FBS, glucose (5.4 mg/ml), 10 mM Hepes, 2 mM L-glutamine, and gentamycin (10 µg/ml). The flasks were centrifuged at 1200 xg for 1 hour and further incubated at 37°C, 5%CO_2 for another additional hour. After incubation, the media was decanted and replaced with fresh CGM, and the cultures were incubated at 37°C, 5%CO_2 for 48 hours. *C. trachomatis* was harvested by vortexing the culture flasks containing glass beads and SPG supplemented with 10% FBS to release Chlamydia EBs from the cells. The obtained SPG-EB suspension was centrifuged at 200 xg at 4°C for 10 minutes to pellet the cell debris. Five hundred microliters of SPG-EB suspension were aliquoted in cryovials and stored at -80°C until required.

We quantified *C. trachomatis* by infecting McCoy cells ($1x10^5$ cells/ml) with seven-fold serial dilutions of the *C. trachomatis* inoculum on Nunc™ Thermanox coverslips (Thermo Fisher Scientific, USA). In addition, we also used the same method to propagate and determine whether *C. trachomatis* can infect TZM-bl cells. Infection was confirmed using immunofluorescence PathoDx chlamydia trachomatis test kit (Thermo Fisher Scientific, USA) and the slides were viewed using the ZEISS Axio Observer Fluorescent inverted microscope (ZEISS, Germany) under Cy5 fluorophore spectrum. Chlamydia inclusions were manually counted, and the inclusion forming units per ml were calculated using formula:$Concentration\ (IFU/ml) = \frac{inclusions}{n(number\ of\ fields)} \times \frac{1000\ \mu l}{V\ (volume\ of\ inoculum)} \times C\ (objective\ lens) \times D$(dilution). The infectious titer was then used to determine chlamydia multiplicity of infections (MOIs) using the formula: $\text{MOI} = \frac{IFU/ml}{number\ of\ cells}$. It is well documented that different MOIs have a different rate of bacterial infectivity, three different MOIs (1, 5, and 10) were tested to determine which MOI for each chlamydia strain supports virus infectivity in the neutralization assays (134, 135).

3.2.3 Neutralization assay on TZM-*bl cells*

Neutralization assays were performed to determine the inhibitory capabilities of bNAbs (CAP256-VRC26.25LS, PGT121 and VRC07-523LS donated by Professor Penny Moore and Dr Bongiwe Ndlovu) against subtype C, tier 2 viruses isolated in South Africa KwaZulu Natal. The inhibitory capabilities was achieved by measuring viral reduction in luciferase gene expression after a single round of infection in TZM-bl cells (1×10^5cells/ml) supplemented with DEAE-Dextran (37.5µg/ml) (Merck, USA) in the presence and absence of *C. trachomatis*. The neutralization assay for each virus was performed in three different flat bottom 96 well plates (Greiner Bio-One, Austria), with plate 1 served as a control, while plates 2 and 3 were used for experimental purposes (Figure 3.1). Serial dilutions of individual bNAbs with a starting concentration of 200 µg/ml were tested against each virus. Each plate

was designed to assay 3 bNAbs (CAP256-VRC26.25, PGT121, VRC07-523LS) and normal human serum (negative control) in duplicates. Initially, growth medium, serial diluted bNAbs and viruses were added to each plate sequentially, followed by TZM-bl cells. All 3 plates were simultaneously incubated for 48 hours at 37°C. After 48 hours of incubation, half of the media was removed, and Bright-Glo Luciferase (Anatech, South Africa) was added. The suspension was transferred into a black 96 well plates (Merck, USA) and read in a luminometer (Perkin Elmer multimode plate reader, USA). Data was calculated as a reduction in the number of luminescence units (RLU) compared with the number for the control wells and were reported as the 50% inhibitory concentration (IC_{50}).

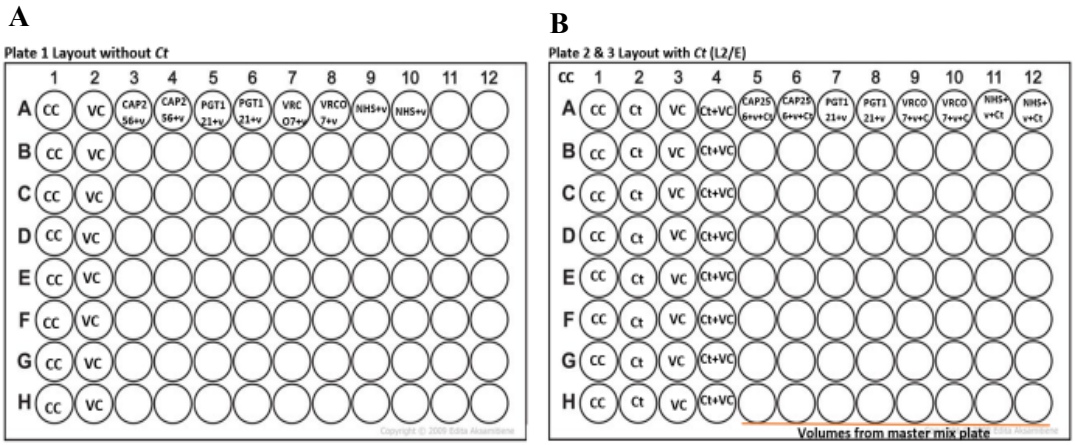


Figure 3.1: Plate layout of neutralization assay in the absence and presence of *C. trachomatis* serovars E and L2. Plate 1 (A) is the control plate with cells, virus and bNAbs only, and Plates 2 and 3 (B) are modified to include *C. trachomatis* (serovars E (MOI 10) and L2 (MOI 1)) and TZM-bl cells. CC=cell control, VC=virus control, Ct=*Chlamydia trachomatis* control, CAP 256=bNAb (CAP256-VRC26.25 LS), PGT12=bNAb (PGT121), VRC07=bNAb (VRC07-523LS), NHS=non-human serum.

3.2.4 Statistical considerations

The statistical analyses were performed using GraphPad Prism 9 (GraphPad, San Diego, CA). Non-parametric t-test analysis was performed and p-values <0.05 were considered significant. Adjustment of multiple comparisons was performed using false discovery rates.

3.3 Results

3.3.1 Detection of *C. trachomatis* EBs in TZM-bl cells

To assess if TZM-bl cells support the growth of *C. trachomatis,* TZM-bl cells (including McCoy as controls) were infected with dilutions of Chlamydial serovars and EB inclusions were confirmed using immunofluorescence microscopy. The presence of Chlamydia inclusions

was observed in TZM-bl cell lines and appeared as circular bright red and smooth-edged spheres (Figure 3.2). Compared to cell control (McCoy), TZM-bl cells had fewer EB inclusions and infectivity titer. Serovar L2 in McCoy cells had more (588) compared to EBs inclusions (336) in TZM-bl cells. Similar observations were found for serovar E, with EB inclusions (394) compared to L2 EBs inclusions (232) in TZM-bl cells. The infectivity of serovar L2 in McCoy cells was 1.8-fold higher (9,73 x 10^7 IFU/ml) compared to TZM-bl cells (5,50 x 10^7 IFU/ml). Similarly, the calculated infectivity of serovar E in McCoy cells was 1.7-fold higher (6,52 x 10^7 IFU/ml) compared to TZM-bl cells (3,84 x 10^7 IFU/ml) (Table 3.1).

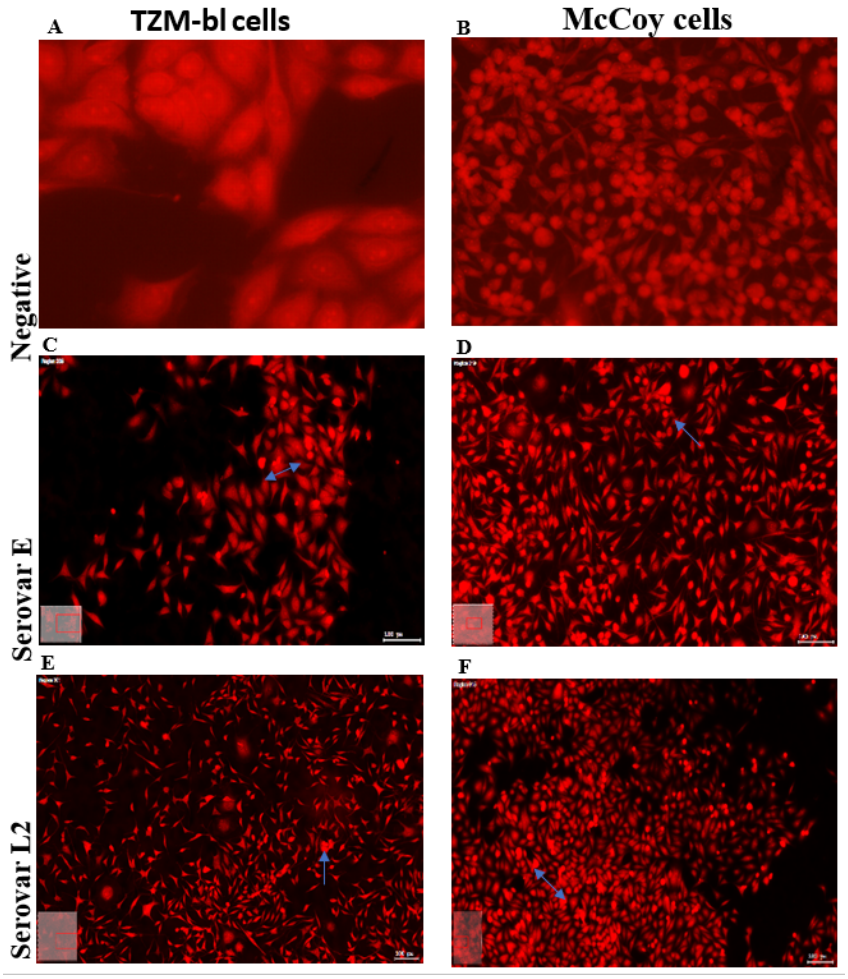


Figure 3.2: Images of *C. trachomatis* inclusions viewed under a fluorescent microscope (100X). The inclusions are shown in bright red spheres as indicated by blue arrows. **A and B** represent uninfected cell controls TZM-bl (A) and McCoy cells (B). **C:** represent TZM-bl cells infected with *C. trachomatis* serovar E strain, **D:** McCoy cells infected with *C. trachomatis* serovar E strain, **E:** TZM-bl cells infected with *C. trachomatis* serovar L2 strain, and **F:** McCoy cells infected with *C. trachomatis* serovar L2 strain.

Table 3.1: *C. trachomatis* EB inclusions and infectivity titer

		TZM-bl cells	McCoy cells	Fold difference
Serovar L2	EB inclusions	336	588	1.8 Log
	Infectivity titer	5.5 x 10^7 IFU/ml	9.73 x 10^7 IFU/ml	
	Serovar L2 Infectivity in McCoy cells was 1.7-fold higher compared to TZM-bl cells			
		TZM-bl cells	**McCoy cells**	**Fold difference**
Serovar E	EB inclusions	232	394	1.7 Log
	Infectivity titer	3.84 x 10^7 IFU/ml	6.52 x 10^7 IFU/ml	
	Serovar E Infectivity in McCoy cells was 1.8-fold higher compared to TZM-bl cells			

3.3.2 *C. trichomatis* multiplicity of infection in TZM-bl cells

We then determined *C. trichomatis* MOI by coinfecting TZM-bl cells with *C. trichomatis* serovar E/L2 (MOIs 1, 5 and 10) virus (308) which is HIV subtype C, tier2 isolated from CAPRISA cohort at KwaZulu Natal, South Africa. Virus 308 had increased infectious activity in serovar E, MOI 10 (51420 RLU) compared to MOI 1(43360 RLU) and 5 (38707 RLU, Figure 3.3). In addition, virus 308 coinfected with serovar L2 had higher virus activity using MOI 1(50265 RLU) compared to MOI 5 (43915 RLU) and MOI 10 (33547 RLU). Therefore, MOI 10 for serovar E and MOI 1 for L2 were selected for downstream neutralization assays.

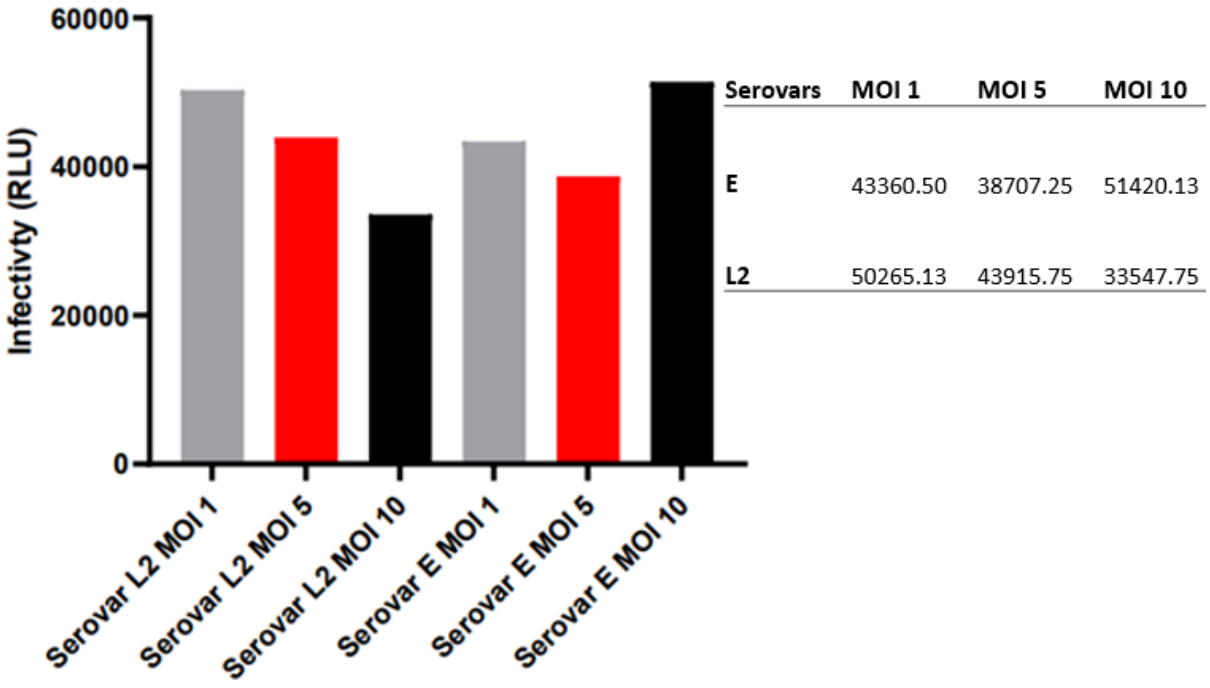


Figure 3.3: TZM-bl cells coinfected with virus 308 and serovar E and L2 at 3 different MOIs 1, 5 and 10. Grey bar graph indicates virus infectivity in presence of serovars E and L2 at MOI 1, red bar graph indicates infectivity using MOI 5 and black bar graph indicates infectivity using MOI 10. The actual MOI values are depicted on the right.

3.3.3 Impact of *C. trachomatis* on virus replication activity in TZM-bl cells

Analysis was performed by t-test to demonstrate the impact of *C. trachomatis* on replication of subtype C, tier 2 viruses isolated from CAPRISA cohort at South Africa KwaZulu Natal (Figure 3.4). A significant increase in viral infection was observed in cells coinfected with *C. trachomatis* serovars E and L2. Cells infected with *C. trachomatis* serovar E and virus 308 (median 5.0, interquartile range (IQR) 5.0-5.1 vs, median 4.7, IQR 4.6-4.8 RLU, p=0.0002), 331 (median 4.8, IQR 4.7-4.8 vs median 4.6, IQR 4.5-4.7 RLU, p=0.0148), 355 (median 5.0, IQR 4.9-5.0 vs median 4.6, IQR 4.6-4.6 RLU, p=0.0002), 357 (median 4.8, IQR 4.8-4.9 vs median 4.5, IQR 4.4-4.5 RLU, p=0.0002), 367 (median 4.6, IQR 4.5-4.6 vs median 4.3, IQR 4.2-4.3 RLU, p=0.0002), 370 (median 4.3, IQR 4.3-4.3 vs median 4.2, IQR 4.1-4.2 RLU, p=0.0006) had increased viral infections compared to cells infected with a virus in absence of *C. trachomatis*. Cells infected with viruses in absence of chlamydia serovar L2 had low viral infections compared to cells infected with viruses in the presence of serovar L2. These include virus 308 (median 4.7, IQR 4.7-4.8 vs, median 5.2, IQR 5.1-5.2 RLU, p=0.0002), 331 (median 4.8, IQR 4.7-4.8 vs median 5.1, IQR 4.9-5.2 RLU, p=0.0070), 355 (median 4.6, IQR 4.6-4.7 vs median 4.9, IQR 4.9-5.0 RLU, p=0.0002), 357 (median 4.5, IQR 4.5-4.5 vs, median 4.8, IQR 4.8-4.9 RLU, p=0.0002), 367 (median 4.2, IQR 4.2-4.3 vs, median 4.6, IQR 4.6-4.7 RLU, p=0.0002) (Table 3.2).

Table 3.2: Impact of *C. trachomatis* on virus replication activity in TZM-bl cells

	Median	IQR (RLU)	P- value		Median	IQR (RLU)	P- value
Virus 308	4.7	4.6-4.8	0.0002	**Virus 308**	4.7	4.7-4.8	0.0002
Virus 308 + Serovar E	5	5.0-5.1		**Virus 308 + Serovar L2**	5.2	5.1-5.2	
Virus 331	4.6	4.5-4.7		**Virus 331**	4.8	4.7-4.8	
Virus 331 + Serovar E	4.8	4.7-4.8	0.0148	**Virus 331 + Serovar L2**	5.1	4.9-5.2	0.007
Virus 355	4.6	4.6-4.6	0.0002	**Virus 355**	4.6	4.6-4.7	0.0002
Virus 355 + Serovar E	5	4.9-5.0		**Virus 355 + Serovar L2**	4.9	4.9-5.0	
Virus 357	4.5	4.4-4.5	0.0002	**Virus 357**	4.5	4.5-4.5	0.0002
Virus 357 + Serovar E	4.8	4.8-4.9		**Virus 357 + Serovar L2**	4.8	4.8-4.9	
Virus 367	4.3	4.2-4.3	0.0002	**Virus 367**	4.2	4.2-4.3	0.0002
Virus 367 + Serovar E	4.6	4.5-4.6		**Virus 367 + Serovar L2**	4.6	4.5-4.6	
Virus 370	4.2	4.1-4.2	0.0002				
Virus 370 + Serovar E	4.3	4.3-4.3					

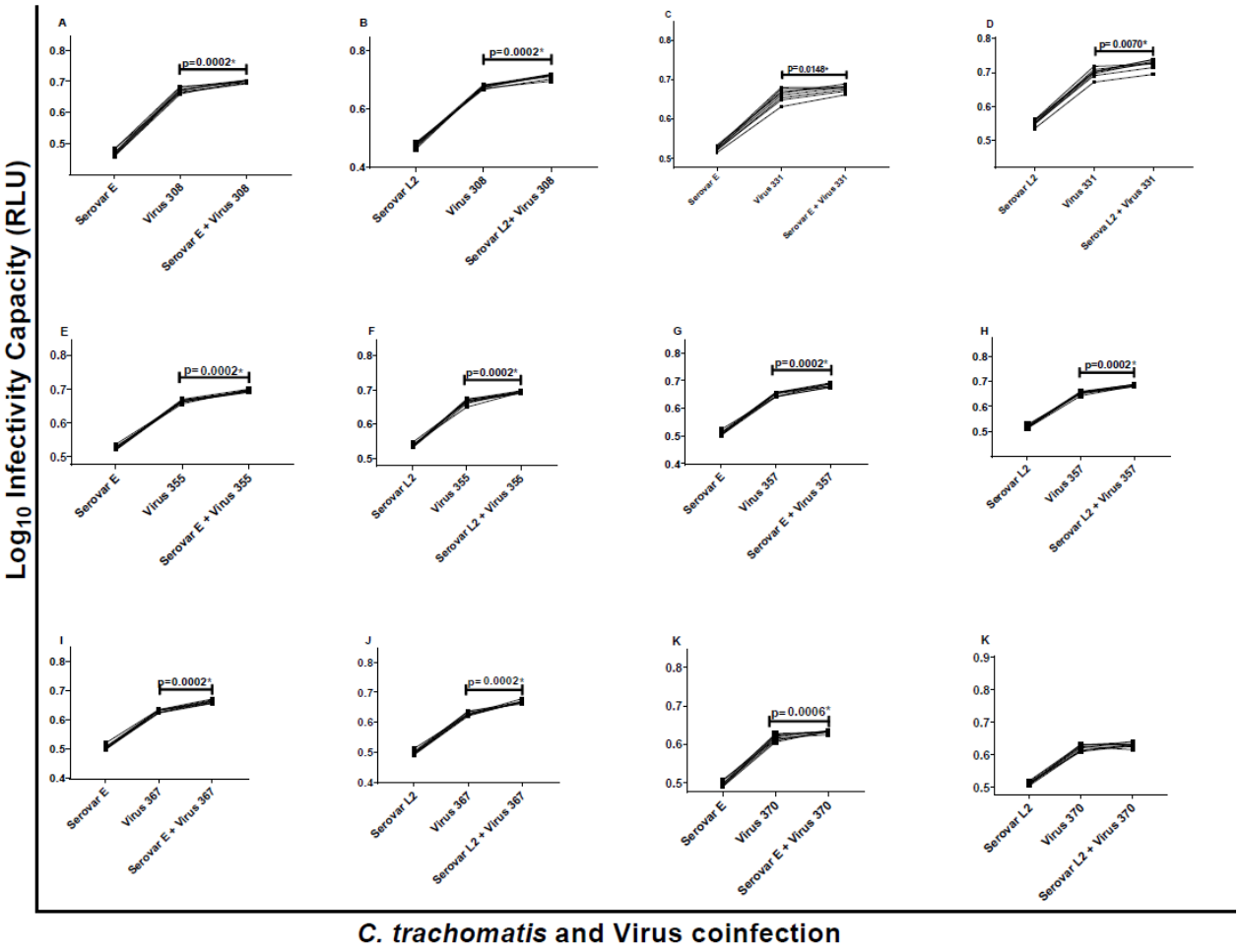


Figure 3.4: Log_{10}-transformed impact of *C. trachomatis* on virus replication in TZM-bl cells. Non-parametric t-test analysis was used to assess the impact of *C. trachomatis* in virus infectivity capacity by measuring viral reduction in luciferase gene expression, after a single round of infection in TZM-bl cells. Virus infectivity capacity was measured in (RLU) represented on the y-axis and TZM-bl cells infected with *C. trachomatis*, virus and coinfected with *C. trachomatis* and virus are on the x-axis. Statistical significance represented as ($p \leq 0.05$), * = Data remained statistically significant, after adjustment of multiple comparisons was performed using the Bonferroni adjustment.

3.3.4 Efficacy of CAP 256-VRC26.25, PGT121 and VRC07-523LS against HIV in presence of *C. trachomatis* in TZM-bl cells

Here, we determined the inhibitory capabilities of bNAbs against a panel HIV subtype C viruses in the presence of *Chlamydia trachomatis* (Figure 3.5). A panel of three antibodies (CAP256-VRC26.25, VRC07-523LS and PGT121) was tested against six viruses with high (331, 355, 357 and 367), medium (308) and low (370) infectivity rate. Virus 308 exhibited sensitivity to CAP 256- VRC26.25 (IC_{50} of <

0.1 µg/ml) but was resistant to VRC07-523LS and PGT121 was less potent in the absence and presence of *C. trachomatis* serovars L2 and E. In the absence of serovars L2 and E, CAP 256- VRC26.25 was able to neutralize viruses 331 (IC_{50} of 1.20 µg/ml), 367 (IC_{50} of 0.21 µg/ml) and 370 (IC_{50} of 0.77 µg/ml) at lower concentrations compared to when serovars L2 (331 IC_{50} of 4.14 µg/ml, 367 IC_{50} of 6.1 µg/ml; and 370, IC_{50} of 5.35 µg/ml) and E (331 IC_{50} of 2.19 µg/ml and 370, IC_{50} of 8.69 µg/ml) were present. Viruses 331 (IC_{50} of 0.15 µg/ml), 357 (IC_{50} of 0.6 µg/ml), 367 (IC_{50} of 0.39 µg/ml) and 370 (IC_{50} of 0.35 µg/ml) demonstrated neutralization sensitivity to PGT121 in the absence of *C. trachomatis* serovars. In addition, similar sensitivity to PGT121 was observed in viruses 331, 357, 367 and 370 in the presence of serovars E and L2. Lower concentrations of VRC07-523LS neutralized virus 331, 357 367, and 370 in the absence of serovars L2 and E, but higher concentrations were required to neutralize viruses 331, 357 and 370 in the presence of serovars L2 and E. In the presence and absence of serovars L2 and E, viruses 357 were resistant to CAP256-VRC26.25 and VRC07-523LS, while virus 355 (IC_{50} >10 µg/ml) was resistant to all bNAbs tested, with an exception of VRC07-523LS in the absence of serovars. As expected, all viruses (IC_{50} >10 µg/ml) were resistant to NHS in the absence and presence of *C. trachomatis* serovars E and L2.

bNAbs		Viruses					
		308 (Mid)	355 (High)	357 (High)	367 (High)	370 (Low)	331 (High)
CAP256-VRC26.25	Ab + V	<0.1	>10	>10	0.21	0.77	1.2
	Ab + V+ E	<0.1	>10	>10	>10	8.69	2.19
	Ab + V+ L2	<0.1	>10	>10	6.1	5.35	4.14
PGT121	Ab + V	10	>10	0.6	0.39	0.35	0.15
	Ab + V+ E	10	>10	1.15	1.42	0.8	0.24
	Ab + V+ L2	10	>10	1.71	1.82	0.69	0.24
VRC07-523LS	Ab + V	>10	7.71	5.81	0.27	1.52	1.27
	Ab + V+ E	>10	>10	8.62	0.66	4.03	2.64
	Ab + V+ L2	>10	>10	9.27	0.7	4.23	2.76
NHS	Ab + V	>10	>10	>10	>10	>10	>10
	Ab + V+ E	>10	>10	>10	>10	>10	>10
	Ab + V+ L2	>10	>10	>10	>10	>10	>10

IC_{50} titers µg/ml)
<0.1
0.1-0.9
1.0-3.0
3.1-10
>10

(V = Virus) (E = *C. trachomatis* serovar E) (L2 = *C. trachomatis* serovar L2)
(Ab = Broadly Neutralizing Antibody)

Figure 3.5: Neutralization activity of bNAbs against subtype C viruses. Heatmap showing the impact of *C. trachomatis* on neutralization sensitivity by potency-breadth represented by IC_{50}. The bNAbs are assigned by target epitopes and neutralization score is colour coded.

3.4 Discussion

Although the efficacy in reducing viremia and maintaining viral suppression has been observed in pre-clinical and clinical studies, the protective efficacy of broadly neutralizing antibodies has not been fully characterized in the presence of *C. trachomatis* (42, 109, 136). Research on growth kinetics of C. trachomatis has been mainly on McCoy or HeLa cells and not on the TZM-bl cell line (135, 137).

Therefore, we determined whether *C. trachomatis* can infect TZM-bl cells and influence the inhibitory capabilities of CAP256-VRC26.25, VRC07-523LS and PGT121 against subtype C viruses. In this study, several TZM-bl cells containing *C. trachomatis* inclusion bodies were observed 48 hours post-infection by immunofluorescence staining. We also established that *C. trachomatis* infected TZM-bl cells significantly enhanced HIV replication compared to TZM-bl cells infected with the virus alone. Furthermore, lower concentrations of CAP256-VRC26.25, VRC07-523LS and PGT121 were able to neutralize majority of viruses tested in the absence of serovars L2 and E, but higher concentrations were required in the presence of serovars L2 and E.

While *C. trachomatis* is usually grown *in vitro* in HeLa (135, 138), McCoy (137-139) and keratinocytes (140) cell lines; it was unknown whether TZM-bl cells support chlamydial growth. This study showed that the TZM-bl cell line was susceptible to infection with *C. trachomatis* serovars L2 and E. Although a low infectivity rate of TZM-bl cells was observed compared to McCoy cells, our findings confirm that TZM-bl cells support chlamydial growth. In addition, these findings suggest that the chlamydial mode of entry into the host cell was not affected during the modification of HeLa to TZM-bl cells. Furthermore, these findings also pave way for future studies to investigate the impact of *C. trachomatis* in TZM-bl cell-based neutralization assays involving monoclonal antibodies and viruses. However, this study did not determine whether TZM-bl cells can support all stages of the developmental cycle, including the transcriptional expression of multiple chlamydial genes.

Individuals with clinical manifestation of lymphogranuloma venereum, caused by *C. trachomatis* serovar L2, are often co-infected with HIV (141, 142), but the mechanisms remain unknown. Here, we established an *in vitro* model of coinfection to determine whether *C. trachomatis* serovar L2 or a clinical isolate serovar E influences HIV replication in TZM-bl cells. We found that both *C. trachomatis* serovars L2 and E were associated with increased replication of HIV. These findings are consistent with previous *in vitro* studies that found increased levels of HIV infection in presence of chlamydia (143, 144). Ho et al., (1997) demonstrated that chlamydia in the presence of polymorphonuclear leukocytes increased HIV p24 production in U1 cell lines, possibly through the production of reactive oxygen products secreted by granulocytes and inflammatory cytokines such as Interleukin 6 and Tumour Necrosis Factor-alpha (143). Another study showed that endocervical epithelial cells of women infected with *C. trachomatis* had an increased expression of HIV-1 C-C chemokine receptor type 5+ and C-X-C chemokine receptor type 4+ HIV target cells, which resulted in increased HIV viral copies (144). Interestingly, an *in vitro* study demonstrated that HIV does not influence the replication and function of *C. trachomatis* (145), a correlation that has not been elucidated in this study. Although our study did not assess soluble factors in coinfected cultures, it is reasonable to suggest that the association between

Chlamydial infection and increased risk of HIV infection is likely due to enhanced secretion of proinflammatory cytokines and an increased frequency of activated HIV target cells. More studies, including transmission electron microscopy, Luminex multiple array and flowcytometry assays are needed to better understand the mechanism by which *C. trachomatis* impact cellular and cytokine markers of genital inflammation and thereby increase the risk of acquiring HIV.

Next, we assessed whether *C. trachomatis* mediates effective antibody blocking of HIV infection within TZM-bl cells *in vitro*. Our data demonstrate that *C. trachomatis* serovars did not significantly alter the effectiveness of bNAbs against subtype C viruses tested in this study. In addition, viruses that were initially resistant to bNAbs remained so even in the presence of *C. trachomatis*. Lower concentrations of CAP256-VRC26.25, VRC07-523LS and PGT121 had a greater neutralization potency against our panel of subtype C viruses but slightly higher concentrations of bNAbs were required in the presence of *C. trachomatis* serovars. When tested *in vitro* against a genetically diverse panel of 6 HIV subtype C viruses in the absence of *C. trachomatis* serovars, PGT121 neutralized 67% (4/6) while 50% (3/6) were neutralized by CAP256-VRC26.25 at inhibitory concentrations (IC_{50}) of <0.9 µg/ml. However, these viruses required an IC_{50} >0.9 µg/ml to achieve complete neutralization by CAP256-VRC26.25 (67%, 2/3) and PGT121 (50%, 2/4) in the presence of *C. trachomatis* serovars. Our findings are consistent with previous studies demonstrating the capacity of CAP256-VRC26.25 and PGT121 to confer complete inhibition at extremely low titers (42, 146). PGT121 is a recombinant of human IgG1 monoclonal antibodies targeting the V3 glycan dependent region on the gp120 of the HIV envelope protein by weakening the Env-receptor engagement by an allosteric mechanism that interferes with CD4 binding and virus entry (45, 116, 117, 147). CAP256-VRC26.25 is a V2 targeting antibody with long CDRH3's which can penetrate the glycan shield of the V1/V2 regions of HIV (19, 24, 39, 40). In contrast to previous studies, our study showed that VRC07-523LS was less potent against the panel of viruses tested in this study, with only (17%, 1/6) virus demonstrating neutralization sensitivity at inhibitory concentrations (IC_{50}) of <0.9 µg/ml in the absence and presence of *C. trachomatis* serovars. The majority (83%, 5/6) of viruses required IC_{50} >0.9 µg/ml to achieve complete neutralization by VRC07-523LS. Previous studies have demonstrated a remarkable protective efficacy of VRC07-523LS at inhibitory concentrations (IC_{50}) of <0.1 µg/ml against 92% of diverse clade pseudoviruses (118). Animal and *in vitro* studies demonstrated that CAP256-VRC26.25, VRC07-523 and PGT121 showed great potency and breath against diverse HIV clades when selected to complement each other or other antibodies(42, 52). These findings convinced researchers to establish efficacy trial aiming to select the ideal dose and monoclonal antibody combination (VRC07-523 and PGT121) for coformulation and testing with CAP256-VRC26.25 for HIV prevention strategy. However, this study did not assess the potency of CAP256-VRC26.25 in combination with VRC07-523 and PGT121. Taken together, our findings suggest a need to further determine the role of *C. trachomatis* on the potency of CAP256-

VRC26.25 alone and in combination with VRC07-523 and PGT121 using a large number of viruses with diverse clades.

This is the first study to demonstrate the growth of *C. trachomatis* in TZM-bl cells and to successfully evaluate TZM-bl cell-based neutralization assays in the presence of *C. trachomatis*. However, we could not determine whether TZM-bl cells can support all stages of the chlamydial developmental cycle, including inclusion size and number, morphological changes and gene expression in *C. trachomatis* organisms. Another limitation included the use of a small number of viruses that were mainly subtype C, which prohibited us from determining the variability of neutralization potency and breadth of the tested bNAbs against a broader range of viruses with diverse subtypes. In addition, our study only used monoclonal and not combination antibodies which are currently recommended by recent studies as it increases potency, breadth, and multiple epitope specificities. Furthermore, our experiments were performed in duplicates, whereas most studies perform experiments in triplicates to get a good representation of the results.

3.5 Conclusion

In conclusion, our study demonstrated that TZM-bl cells support chlamydia growth and are appropriate to interrogate cell differentiation, growth kinetics, and gene expression of *C. trachomatis* infection. Our findings also suggest that *C. trachomatis* can be included in TZM-bl cell-based neutralization assays. Furthermore, we showed that *C. trachomatis* serovars E and L2 induces viral replication in TZM-bl cells infected with HIV, suggesting a causal relationship between STIs and enhanced HIV transmission. However, more studies are required to evaluate the potential causal relationship between coinfection with other STIs and enhanced HIV transmission. Lastly, our study also shows that *C. trachomatis* does not completely alter the efficacy of bNAbs *in vitro*. Although our study supports the use of bNAbs as a viable alternative to ARV-based HIV prevention, a better understanding of the key biological factors mediating effective antibody blocking of HIV-1 infection within the female genital tract remains important.

4. Budget

This study was conducted as part of the DST-NRF Centre of Excellence (CoE) in HIV Prevention, which is supported by the Department of Science and Innovation and the National Research Foundation (grant 96354). NM and GFM were funded by DST-NRF CoE in HIV Prevention (grant 96354). GFM was funded by Poliomyelitis Research Foundation Research Grant (grant# 18/16) and National Research Fund Thuthuka Research Grant (grant# SFP180507326699). SN was supported by Columbia

University-Southern African Fogarty AITRP Programme (grant# D43TW00231), National Research Fund Thuthuka Research Grant (grant# TTK160510164586), and Poliomyelitis Research Foundation Research Grant (grant# 16/17).

REFERENCES

1. Moosa A, Gengiah TN, Lewis L, Naidoo K. Long-term adherence to antiretroviral therapy in a South African adult patient cohort: a retrospective study. BMC infectious diseases. 2019;19(1):775.
2. Sather DN, Carbonetti S, Malherbe DC, Pissani F, Stuart AB, Hessell AJ, et al. Emergence of broadly neutralizing antibodies and viral coevolution in two subjects during the early stages of infection with human immunodeficiency virus type 1. J Virol. 2014;88(22):12968-81.
3. Gómez-Olivé FX, Angotti N, Houle B, Klipstein-Grobusch K, Kabudula C, Menken J, et al. Prevalence of HIV among those 15 and older in rural South Africa. AIDS care. 2013;25(9):1122-8.
4. Welz T, Hosegood V, Jaffar S, Bätzing-Feigenbaum J, Herbst K, Newell M-L. Continued very high prevalence of HIV infection in rural KwaZulu-Natal, South Africa: a population-based longitudinal study. Aids. 2007;21(11):1467-72.
5. Hansoti B, Stead D, Eisenberg A, Mvandaba N, Mwinnyaa G, Patel EU, et al. A window into the HIV epidemic from a South African emergency department. AIDS research and human retroviruses. 2019;35(2):139-44.
6. Mabaso M, Makola L, Naidoo I, Mlangeni L, Jooste S, Simbayi L. HIV prevalence in South Africa through gender and racial lenses: results from the 2012 population-based national household survey. International journal for equity in health. 2019;18(1):167.
7. Edessa D, Sisay M, Asefa F. Second-line HIV treatment failure in sub-Saharan Africa: A systematic review and meta-analysis. PloS one. 2019;14(7):e0220159.
8. Cuevas JM, Geller R, Garijo R, López-Aldeguer J, Sanjuán R. Extremely high mutation rate of HIV-1 in vivo. PLoS Biol. 2015;13(9):e1002251.
9. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science. 2010;329(5996):1168-74.
10. Abdool Karim SS, Richardson BA, Ramjee G, Hoffman IF, Chirenje ZM, Taha T, et al. Safety and effectiveness of BufferGel and 0.5% PRO2000 gel for the prevention of HIV infection in women. AIDS. 2011;25(7):957-66.
11. Dezzutti CS, Brown ER, Moncla B, Russo J, Cost M, Wang L, et al. Is wetter better? An evaluation of over-the-counter personal lubricants for safety and anti-HIV-1 activity. PLoS One. 2012;7(11):e48328.
12. Eroshkin AM, LeBlanc A, Weekes D, Post K, Li Z, Rajput A, et al. bNAber: database of broadly neutralizing HIV antibodies. Nucleic Acids Res. 2014;42(Database issue):D1133-9.
13. Euler Z, Bunnik EM, Burger JA, Boeser-Nunnink BD, Grijsen ML, Prins JM, et al. Activity of broadly neutralizing antibodies, including PG9, PG16, and VRC01, against recently transmitted subtype B HIV-1 variants from early and late in the epidemic. J Virol. 2011;85(14):7236-45.
14. Marrazzo JM, Ramjee G, Richardson BA, Gomez K, Mgodi N, Nair G, et al. Tenofovir-based preexposure prophylaxis for HIV infection among African women. N Engl J Med. 2015;372(6):509-18.
15. Rees H, Delany-Moretlwe SA, Lombard C, Baron D, Panchia R, Myer L, et al., editors. FACTS 001 Phase III Trial of Pericoital Tenofovir 1% Gel for HIV Prevention in Women. Conference on Retroviruses and Opportunistic Infections (CROI); 2015; Seattle, Washington.
16. Van Damme L, Ramjee G, Alary M, Vuylsteke B, Chandeying V, Rees H, et al. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. Lancet. 2002;360(9338):971-7.
17. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL, et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science. 2009;326(5950):285-9.
18. Bhiman JN, Anthony C, Doria-Rose NA, Karimanzira O, Schramm CA, Khoza T, et al. Viral variants that initiate and drive maturation of V1V2-directed HIV-1 broadly neutralizing antibodies. Nature medicine. 2015;21(11):1332-6.

19. Doria-Rose NA, Schramm CA, Gorman J, Moore PL, Bhiman JN, DeKosky BJ, et al. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. Nature. 2014;509(7498):55-62.
20. Gaudinski MR, Houser KV, Doria-Rose NA, Chen GL, Rothwell RSS, Berkowitz N, et al. Safety and pharmacokinetics of broadly neutralising human monoclonal antibody VRC07-523LS in healthy adults: a phase 1 dose-escalation clinical trial. The Lancet HIV. 2019;6(10):e667-e79.
21. Mendoza P, Gruell H, Nogueira L, Pai JA, Butler AL, Millard K, et al. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. Nature. 2018;561(7724):479-84.
22. Klein F, Mouquet H, Dosenovic P, Scheid JF, Scharf L, Nussenzweig MC. Antibodies in HIV-1 vaccine development and therapy. Science. 2013;341(6151):1199-204.
23. Burton DR, Ahmed R, Barouch DH, Butera ST, Crotty S, Godzik A, et al. A Blueprint for HIV Vaccine Discovery. Cell Host Microbe. 2012;12(4):396-407.
24. Doria-Rose NA, Joyce MG. Strategies to guide the antibody affinity maturation process. Curr Opin Virol. 2015;11:137-47.
25. Liao HX, Lynch R, Zhou T, Gao F, Alam SM, Boyd SD, et al. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature. 2013;496(7446):469-76.
26. Corti D, Langedijk JP, Hinz A, Seaman MS, Vanzetta F, Fernandez-Rodriguez BM, et al. Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals. PLoS One. 2010;5(1):e8805.
27. Scheid JF, Mouquet H, Feldhahn N, Walker BD, Pereyra F, Cutrell E, et al. A method for identification of HIV gp140 binding memory B cells in human blood. J Immunol Methods. 2009;343(2):65-7.
28. Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science. 2010;329(5993):856-61.
29. Wibmer CK, Gorman J, Ozorowski G, Bhiman JN, Sheward DJ, Elliott DH, et al. Structure and recognition of a novel HIV-1 gp120-gp41 interface antibody that caused MPER exposure through viral escape. PLoS Pathogens. 2017;13(1):e1006074.
30. Haynes BF, Moody MA, Alam M, Bonsignori M, Verkoczy L, Ferrari G, et al. Progress in HIV-1 vaccine development. J Allergy Clin Immunol. 2014;134(1):3-10; quiz 1.
31. Julien JP, Lee JH, Cupo A, Murin CD, Derking R, Hoffenberg S, et al. Asymmetric recognition of the HIV-1 trimer by broadly neutralizing antibody PG9. Proc Natl Acad Sci U S A. 2013;110(11):4351-6.
32. Mascola JR, Haynes BF. HIV-1 neutralizing antibodies: understanding nature's pathways. Immunol Rev. 2013;254(1):225-44.
33. Walker LM, Huber M, Doores KJ, Falkowska E, Pejchal R, Julien JP, et al. Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature. 2011;477(7365):466-70.
34. Blattner C, Lee JH, Sliepen K, Derking R, Falkowska E, de la Pena AT, et al. Structural delineation of a quaternary, cleavage-dependent epitope at the gp41-gp120 interface on intact HIV-1 Env trimers. Immunity. 2014;40(5):669-80.
35. Falkowska E, Le KM, Ramos A, Doores KJ, Lee JH, Blattner C, et al. Broadly neutralizing HIV antibodies define a glycan-dependent epitope on the prefusion conformation of gp41 on cleaved envelope trimers. Immunity. 2014;40(5):657-68.
36. Huang J, Kang BH, Pancera M, Lee JH, Tong T, Feng Y, et al. Broad and potent HIV-1 neutralization by a human antibody that binds the gp41-gp120 interface. Nature. 2014;515(7525):138-42.
37. Scharf L, Scheid JF, Lee JH, West AP, Jr., Chen C, Gao H, et al. Antibody 8ANC195 reveals a site of broad vulnerability on the HIV-1 envelope spike. Cell Rep. 2014;7(3):785-95.
38. Sievers SA, Scharf L, West Jr AP, Bjorkman PJ. Antibody engineering for increased potency, breadth and half-life. Current opinion in HIV and AIDS. 2015;10(3):151.

39. Moore PL, Gray ES, Sheward D, Madiga M, Ranchobe N, Lai Z, et al. Potent and broad neutralization of HIV-1 subtype C by plasma antibodies targeting a quaternary epitope including residues in the V2 loop. J Virol. 2011;85(7):3128-41.
40. Doria-Rose NA, Bhiman JN, Roark RS, Schramm CA, Gorman J, Chuang GY, et al. New Member of the V1V2-Directed CAP256-VRC26 Lineage That Shows Increased Breadth and Exceptional Potency. J Virol. 2015;90(1):76-91.
41. Caskey M, Klein F, Lorenzi JC, Seaman MS, West AP, Jr., Buckley N, et al. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature. 2015;522(7557):487-91.
42. Julg B, Tartaglia LJ, Keele BF, Wagh K, Pegu A, Sok D, et al. Broadly neutralizing antibodies targeting the HIV-1 envelope V2 apex confer protection against a clade C SHIV challenge. Sci Transl Med. 2017;9(406).
43. Moldt B, Rakasz EG, Schultz N, Chan-Hui PY, Swiderek K, Weisgrau KL, et al. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. Proc Natl Acad Sci U S A. 2012;109(46):18921-5.
44. Pegu A, Yang ZY, Boyington JC, Wu L, Ko SY, Schmidt SD, et al. Neutralizing antibodies to HIV-1 envelope protect more effectively in vivo than those to the CD4 receptor. Sci Transl Med. 2014;6(243):243ra88.
45. Barouch DH, Whitney JB, Moldt B, Klein F, Oliveira TY, Liu J, et al. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. Nature. 2013;503(7475):224-8.
46. Klein F, Halper-Stromberg A, Horwitz JA, Gruell H, Scheid JF, Bournazos S, et al. HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. Nature. 2012;492(7427):118-22.
47. Klein F, Nogueira L, Nishimura Y, Phad G, West AP, Jr., Halper-Stromberg A, et al. Enhanced HIV-1 immunotherapy by commonly arising antibodies that target virus escape variants. J Exp Med. 2014;211(12):2361-72.
48. Shingai M, Nishimura Y, Klein F, Mouquet H, Donau OK, Plishka R, et al. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. Nature. 2013;503(7475):277-80.
49. Diskin R, Klein F, Horwitz JA, Halper-Stromberg A, Sather DN, Marcovecchio PM, et al. Restricting HIV-1 pathways for escape using rationally designed anti-HIV-1 antibodies. J Exp Med. 2013;210(6):1235-49.
50. Horwitz JA, Halper-Stromberg A, Mouquet H, Gitlin AD, Tretiakova A, Eisenreich TR, et al. HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. Proc Natl Acad Sci U S A. 2013;110(41):16538-43.
51. Poignard P, Sabbe R, Picchio GR, Wang M, Gulizia RJ, Katinger H, et al. Neutralizing antibodies have limited effects on the control of established HIV-1 infection in vivo. Immunity. 1999;10(4):431-8.
52. Wagh K, Bhattacharya T, Williamson C, Robles A, Bayne M, Garrity J, et al. Optimal Combinations of Broadly Neutralizing Antibodies for Prevention and Treatment of HIV-1 Clade C Infection. PLoS Pathog. 2016;12(3):e1005520.
53. Dugast AS, Arnold K, Lofano G, Moore S, Hoffner M, Simek M, et al. Virus-driven Inflammation Is Associated With the Development of bNAbs in Spontaneous Controllers of HIV. Clin Infect Dis. 2017;64(8):1098-104.
54. Sather DN, Armann J, Ching LK, Mavrantoni A, Sellhorn G, Caldwell Z, et al. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J Virol. 2009;83(2):757-69.
55. Gray ES, Madiga MC, Hermanus T, Moore PL, Wibmer CK, Tumba NL, et al. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. J Virol. 2011;85(10):4828-40.

56. Masson L, Passmore JA, Liebenberg LJ, Werner L, Baxter C, Arnold KB, et al. Genital Inflammation and the Risk of HIV Acquisition in Women. Clin Infect Dis. 2015.
57. Mlisana K, Naicker N, Werner L, Roberts L, van Loggerenberg F, Baxter C, et al. Symptomatic vaginal discharge is a poor predictor of sexually transmitted infections and genital tract inflammation in high-risk women in South Africa. The Journal of infectious diseases. 2012;206(1):6-14.
58. UNAIDS D, Update AE. Geneva: Joint United Nations Programme on HIV. AIDS. 2019.
59. Shroufi A, Van Cutsem G, Cambiano V, Bansi-Matharu L, Duncan K, Murphy RA, et al. Simplifying switch to second-line antiretroviral therapy in sub Saharan Africa: predicted effect of using a single viral load to define efavirenz-based first-line failure. AIDS (London, England). 2019;33(10):1635.
60. Department of Health. The 2013 National Antenatal Sentinel HIV Prevalence Survey South Africa. Pretoria: Department of Health; 2015.
61. Kharsany AB, Frohlich JA, Yende-Zuma N, Mahlase G, Samsunder N, Dellar RC, et al. Trends in HIV prevalence in pregnant women in rural South Africa. J Acquir Immune Defic Syndr. 2015.
62. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, et al. Effectiveness and Safety of Tenofovir Gel, an Antiretroviral Microbicide, for the Prevention of HIV Infection in Women. Science. 2010;329:1168-74.
63. Welz T, Hosegood, V, Jaffar, S, Batzing-Feigenbaum, J, Herbst, K, & Newell, M. L. Continued very high prevalence of HIV infection in rural KwaZulu- Natal, South Africa. AIDS Res Hum Retroviruses. 2007;11 1467-72.
64. Dinh TH, Delaney KP, Goga A, Jackson D, Lombard C, Woldesenbet S, et al. Impact of Maternal HIV Seroconversion during Pregnancy on Early Mother to Child Transmission of HIV (MTCT) Measured at 4-8 Weeks Postpartum in South Africa 2011-2012: A National Population-Based Evaluation. PLoS One. 2015;10(5):e0125525.
65. Chetty V, Moodley D, Chuturgoon A. Evaluation of a 4th generation rapid HIV test for earlier and reliable detection of HIV infection in pregnancy. J Clin Virol. 2012;54(2):180-4.
66. Keating MA, Hamela G, Miller WC, Moses A, Hoffman IF, Hosseinipour MC. High HIV incidence and sexual behavior change among pregnant women in Lilongwe, Malawi: implications for the risk of HIV acquisition. PLoS One. 2012;7(6):e39109.
67. Lawoyin TO, Larsen U. Male sexual behaviour during wife's pregnancy and postpartum abstinence period in Oyo State, Nigeria. J Biosoc Sci. 2002;34(1):51-63.
68. Maman S, Moodley D, McNaughton-Reyes HL, Groves AK, Kagee A, Moodley P. Efficacy of enhanced HIV counseling for risk reduction during pregnancy and in the postpartum period: a randomized controlled trial. PLoS One. 2014;9(5):e97092.
69. Glynn JR, Carael M, Auvert B, Kahindo M, Chege J, Musonda R, et al. Why do young women have a much higher prevalence of HIV than young men? A study in Kisumu, Kenya and Ndola, Zambia. AIDS. 2001;15 Suppl 4:S51-60.
70. Pettifor AE, Rees HV, Kleinschmidt I, Steffenson AE, MacPhail C, Hlongwa-Madikizela L, et al. Young people's sexual health in South Africa: HIV prevalence and sexual behaviors from a nationally representative household survey. AIDS. 2005;19(14):1525-34.
71. Cohen MS. HIV and sexually transmitted diseases: lethal synergy. Topics in HIV medicine : a publication of the International AIDS Society, USA. 2004;12(4):104-7.
72. McKinnon LR, Nyanga B, Chege D, Izulla P, Kimani M, Huibner S, et al. Characterization of a human cervical CD4+ T cell subset coexpressing multiple markers of HIV susceptibility. Journal of immunology. 2011;187(11):6032-42.
73. Sheffield JS, Wendel GD, Jr., McIntire DD, Norgard MV. The effect of progesterone levels and pregnancy on HIV-1 coreceptor expression. Reproductive sciences. 2009;16(1):20-31.
74. Dunbar MS, Kang Dufour MS, Lambdin B, Mudekunye-Mahaka I, Nhamo D, Padian NS. The SHAZ! project: results from a pilot randomized trial of a structural intervention to prevent HIV among adolescent women in Zimbabwe. PLoS One. 2014;9(11):e113621.

75. UNAIDS. EMPOWER YOUNG WOMEN AND ADOLESCENT GIRLS: FAST-TRACKING THE END OF THE AIDS EPIDEMIC IN AFRICA 2015 [Available from: http://www.unaids.org/sites/default/files/media_asset/JC2746_en.pdf.
76. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med. 2012;367(5):399-410.
77. Choopanya K, Martin M, Suntharasamai P, Sangkum U, Mock PA, Leethochawalit M, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. The Lancet. 2013;381(9883):2083-90.
78. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N Engl J Med. 2010;363(27):2587-99.
79. McCormack S, Dunn D, On behalf of the PROUD Study Group. Pragmatic Open-Label Randomised Trial of Preexposure Prophylaxis: The PROUD Study. [Abstract 22LB]. Conference on Retroviruses and Opportunistic Infections (CROI), 23-26 February; Seattle, Washington2015.
80. Molina J-M, Capitant C, Spire B, Pialoux G, Chidiac C, Charreau I, et al. On Demand PrEP With Oral TDF-FTC in MSM: Results of the ANRS Ipergay Trial. [Abstract 23LB]. Conference on Retroviruses and Opportunistic Infections (CROI); Seattle, Washington23-26 February 2015.
81. Thigpen MC, Kebaabetswe PM, Paxton LA, Smith DK, Rose CE, Segolodi TM, et al. Antiretroviral preexposure prophylaxis for heterosexual HIV transmission in Botswana. N Engl J Med. 2012;367(5):423-34.
82. Rees H., Delany-Moretlwe S.A., Lombard C, Baron D, Panchia R, Myer L, et al. FACTS 001 Phase III Trial of Pericoital Tenofovir 1% Gel for HIV Prevention in Women. [Abstract 26LB]. Conference on Retroviruses and Opportunistic Infections (CROI); Seattle, Washington23-26 February 2015.
83. Van Damme L, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, et al. Preexposure Prophylaxis for HIV Infection among African Women. New England Journal of Medicine. 2012;367:411-22.
84. Marrazzo JM, Ramjee G, Richardson BA, Gomez K, Mgodi N, Nair G, et al. Tenofovir-Based Preexposure Prophylaxis for HIV Infection among African Women. New England Journal of Medicine. 2015;372(6):509-18.
85. Baeten JM, Palanee-Phillips T, Brown ER, Schwartz K, Soto-Torres LE, Govender V, et al. Use of a vaginal ring containing dapivirine for HIV-1 prevention in women. New England Journal of Medicine. 2016;375(22):2121-32.
86. Nel A, van Niekerk N, Kapiga S, Bekker L-G, Gama C, Gill K, et al. Safety and efficacy of a dapivirine vaginal ring for HIV prevention in women. New England Journal of Medicine. 2016;375(22):2133-43.
87. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science. 2010;329(5996):1168-74.
88. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. New England Journal of Medicine. 2012;367(5):399-410.
89. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. New England journal of medicine. 2011;365(6):493-505.
90. Van Damme L, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, et al. Preexposure prophylaxis for HIV infection among African women. New England Journal of Medicine. 2012;367(5):411-22.

91. Fonner VA, Dalglish SL, Kennedy CE, Baggaley R, O'reilly KR, Koechlin FM, et al. Effectiveness and safety of oral HIV preexposure prophylaxis for all populations. AIDS (London, England). 2016;30(12):1973.
92. Jewkes RK, Levin JB, Penn-Kekana LA. Gender inequalities, intimate partner violence and HIV preventive practices: findings of a South African cross-sectional study. Social science & medicine. 2003;56(1):125-34.
93. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. New England Journal of Medicine. 2010;363(27):2587-99.
94. Organization WH. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV: World Health Organization; 2015.
95. Donnell D, Baeten JM, Bumpus NN, Brantley J, Bangsberg DR, Haberer JE, et al. HIV protective efficacy and correlates of tenofovir blood concentrations in a clinical trial of PrEP for HIV prevention. Journal of acquired immune deficiency syndromes (1999). 2014;66(3):340.
96. Dimitrov DT, Boily M-C, Hallett TB, Albert J, Boucher C, Mellors JW, et al. How much do we know about drug resistance due to PrEP use? Analysis of experts' opinion and its influence on the projected public health impact. PLoS One. 2016;11(7):e0158620.
97. Gama L, Koup RA. New-generation high-potency and designer antibodies: role in HIV-1 treatment. Annual review of medicine. 2018;69:409-19.
98. Gorny M, Xu J-Y, Karwowska S, Buchbinder A, Zolla-Pazner S. Repertoire of neutralizing human monoclonal antibodies specific for the V3 domain of HIV-1 gp120. The Journal of Immunology. 1993;150(2):635-43.
99. Thali M, Moore J, Furman C, Charles M, Ho D, Robinson J, et al. Characterization of conserved human immunodeficiency virus type 1 gp120 neutralization epitopes exposed upon gp120-CD4 binding. Journal of virology. 1993;67(7):3978-88.
100. Zwick MB, Labrijn AF, Wang M, Spenlehauer C, Saphire EO, Binley JM, et al. Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. Journal of virology. 2001;75(22):10892-905.
101. West AP, Scharf L, Horwitz J, Klein F, Nussenzweig MC, Bjorkman PJ. Computational analysis of anti–HIV-1 antibody neutralization panel data to identify potential functional epitope residues. Proceedings of the National Academy of Sciences. 2013;110(26):10598-603.
102. Mehandru S, Vcelar B, Wrin T, Stiegler G, Joos B, Mohri H, et al. Adjunctive passive immunotherapy in human immunodeficiency virus type 1-infected individuals treated with antiviral therapy during acute and early infection. Journal of virology. 2007;81(20):11016-31.
103. Trkola A, Kuster H, Rusert P, Joos B, Fischer M, Leemann C, et al. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nature medicine. 2005;11(6):615-22.
104. Haynes BF, Fleming J, St. Clair EW, Katinger H, Stiegler G, Kunert R, et al. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science. 2005;308(5730):1906-8.
105. Yang G, Holl TM, Liu Y, Li Y, Lu X, Nicely NI, et al. Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. Journal of Experimental Medicine. 2013;210(2):241-56.
106. Sok D, Burton DR. Recent progress in broadly neutralizing antibodies to HIV. Nature immunology. 2018;19(11):1179-88.
107. Gautam R, Nishimura Y, Gaughan N, Gazumyan A, Schoofs T, Buckler-White A, et al. A single injection of crystallizable fragment domain–modified antibodies elicits durable protection from SHIV infection. Nature medicine. 2018;24(5):610-6.
108. Barouch DH, Whitney JB, Moldt B, Klein F, Oliveira TY, Liu J, et al. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. Nature. 2013;503(7475):224-8.

109. Ndlovu B, Gounder K, Muema D, Raju N, Hermanus T, Mthethwa Q, et al. Envelope characteristics in individuals who developed neutralizing antibodies targeting different epitopes in HIV-1 subtype C infection. Virology. 2020;546:1-12.
110. Mahomed S, Garrett N, Karim QA, Zuma NY, Capparelli E, Baxter C, et al. Assessing the safety and pharmacokinetics of the anti-HIV monoclonal antibody CAP256V2LS alone and in combination with VRC07-523LS and PGT121 in South African women: study protocol for the first-in-human CAPRISA 012B phase I clinical trial. BMJ open. 2020;10(11):e042247.
111. Barouch DH. Challenges in the development of an HIV-1 vaccine. Nature. 2008;455(7213):613-9.
112. Doria-Rose NA, Klein RM, Manion MM, O'Dell S, Phogat A, Chakrabarti B, et al. Frequency and phenotype of human immunodeficiency virus envelope-specific B cells from patients with broadly cross-neutralizing antibodies. Journal of virology. 2009;83(1):188-99.
113. Hraber P, Seaman MS, Bailer RT, Mascola JR, Montefiori DC, Korber BT. Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. AIDS (London, England). 2014;28(2):163.
114. Van Loggerenberg F, Mlisana K, Williamson C, Auld SC, Morris L, Gray CM, et al. Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 acute infection study. PloS one. 2008;3(4):e1954.
115. Gorman J, Chuang G-Y, Lai Y-T, Shen C-H, Boyington JC, Druz A, et al. Structure of Super-Potent Antibody CAP256-VRC26. 25 in Complex with HIV-1 Envelope Reveals a Combined Mode of Trimer-Apex Recognition. Cell Reports. 2020;31(1):107488.
116. Escolano A, Steichen JM, Dosenovic P, Kulp DW, Golijanin J, Sok D, et al. Sequential immunization elicits broadly neutralizing anti-HIV-1 antibodies in Ig knockin mice. Cell. 2016;166(6):1445-58. e12.
117. Stephenson KE, Barouch DH. Broadly neutralizing antibodies for HIV eradication. Current Hiv/aids Reports. 2016;13(1):31-7.
118. Rudicell RS, Do Kwon Y, Ko S-Y, Pegu A, Louder MK, Georgiev IS, et al. Enhanced potency of a broadly neutralizing HIV-1 antibody in vitro improves protection against lentiviral infection in vivo. Journal of virology. 2014;88(21):12669-82.
119. Kwon Y, Georgiev I, O'Dell S, Shi W, Chuang G, Yang Y, et al. Structure-guided modification and optimization of antibody VRC07. Retrovirology. 2012;9(S2):O34.
120. Kwong PD, Nabel GJ, Rudicell RS, Mascola J, Connors M, Georgiev I, et al. Broadly neutralizing HIV-1 VRC07 antibodies that bind to the CD4-binding site of the envelope protein. Google Patents; 2017.
121. Lujan AL, Croci DO, Tudela JAG, Losinno AD, Cagnoni AJ, Mariño KV, et al. Glycosylation-dependent galectin–receptor interactions promote Chlamydia trachomatis infection. Proceedings of the National Academy of Sciences. 2018;115(26):E6000-E9.
122. Olsen AW, Lorenzen EK, Rosenkrands I, Follmann F, Andersen P. Protective effect of vaccine promoted neutralizing antibodies against the intracellular pathogen Chlamydia trachomatis. Frontiers in immunology. 2017;8:1652.
123. Pinto VM, Tancredi MV, Silva RJdCd, Khoury Z, Buchalla CM. Prevalence and factors associated with Chlamydia trachomatis infection among women with HIV in São Paulo. Revista da Sociedade Brasileira de Medicina Tropical. 2016;49:312-8.
124. Malhotra M, Sood S, Mukherjee A, Muralidhar S, Bala M. Genital Chlamydia trachomatis: an update. The Indian journal of medical research. 2013;138(3):303.
125. Elwell C, Mirrashidi K, Engel J. Chlamydia cell biology and pathogenesis. Nature Reviews Microbiology. 2016;14(6):385-400.
126. Redgrove KA, McLaughlin EA. The role of the immune response in Chlamydia trachomatis infection of the male genital tract: a double-edged sword. Frontiers in immunology. 2014;5:534.

127. Bøje S, Olsen AW, Erneholm K, Agerholm JS, Jungersen G, Andersen P, et al. A multi-subunit Chlamydia vaccine inducing neutralizing antibodies and strong IFN-γ+ CMI responses protects against a genital infection in minipigs. Immunology and cell biology. 2016;94(2):185-95.
128. Hafner LM, Wilson DP, Timms P. Development status and future prospects for a vaccine against Chlamydia trachomatis infection. Vaccine. 2014;32(14):1563-71.
129. Olsen AW, Follmann F, Erneholm K, Rosenkrands I, Andersen P. Protection against Chlamydia trachomatis infection and upper genital tract pathological changes by vaccine-promoted neutralizing antibodies directed to the VD4 of the major outer membrane protein. The Journal of infectious diseases. 2015;212(6):978-89.
130. Jia M, Lu H, Markowitz M, Cheng-Mayer C, Wu X. Development of broadly neutralizing antibodies and their mapping by monomeric gp120 in human immunodeficiency virus type 1-infected humans and simian-human immunodeficiency virus SHIVSF162P3N-infected macaques. Journal of virology. 2016;90(8):4017-31.
131. Gorman J, Soto C, Yang MM, Davenport TM, Guttman M, Bailer RT, et al. Structures of HIV-1 Env V1V2 with broadly neutralizing antibodies reveal commonalities that enable vaccine design. Nature structural & molecular biology. 2016;23(1):81.
132. Montefiori DC. Measuring HIV neutralization in a luciferase reporter gene assay. HIV protocols: Springer; 2009. p. 395-405.
133. Moore PL. The neutralizing antibody response to the HIV-1 Env protein. Current HIV research. 2018;16(1):21-8.
134. Chiarelli TJ, Grieshaber NA, Omsland A, Remien CH, Grieshaber SS. Single-inclusion kinetics of Chlamydia trachomatis development. Msystems. 2020;5(5):e00689-20.
135. Foschi C, Bortolotti M, Marziali G, Polito L, Marangoni A, Bolognesi A. Survival and death of intestinal cells infected by Chlamydia trachomatis. Plos one. 2019;14(4):e0215956.
136. Kumar R, Qureshi H, Deshpande S, Bhattacharya J. Broadly neutralizing antibodies in HIV-1 treatment and prevention. Therapeutic advances in vaccines and immunotherapy. 2018;6(4):61-8.
137. Lantos I, Virok DP, Mosolygó T, Rázga Z, Burián K, Endrész V. Growth characteristics of Chlamydia trachomatis in human intestinal epithelial Caco-2 cells. Pathogens and disease. 2018;76(3):fty024.
138. Guseva NV, Dessus-Babus S, Moore CG, Whittimore JD, Wyrick PB. Differences in Chlamydia trachomatis serovar E growth rate in polarized endometrial and endocervical epithelial cells grown in three-dimensional culture. Infection and immunity. 2007;75(2):553-64.
139. Shao L, Guo Y, Jiang Y, Liu Y, Wang M, You C, et al. Sensitivity of the standard Chlamydia trachomatis culture method is improved after one additional in vitro passage. Journal of clinical laboratory analysis. 2016;30(5):697-701.
140. Joubert BC, Sturm AW. Differences in Chlamydia trachomatis growth rates in human keratinocytes among lymphogranuloma venereum reference strains and clinical isolates. Journal of medical microbiology. 2011;60(11):1565-9.
141. Jebbari H, Alexander S, Ward H, Evans B, Solomou M, Thornton A, et al. Update on lymphogranuloma venereum in the United Kingdom. Sexually transmitted infections. 2007;83(4):324-6.
142. Ward H, Martin I, Macdonald N, Alexander S, Simms I, Fenton K, et al. Lymphogranuloma venereum in the United Kingdom. Clinical infectious diseases. 2007;44(1):26-32.
143. Ho JL, He S, Hu A, Geng J, Basile F, Almeida M, et al. Neutrophils from human immunodeficiency virus (HIV)-seronegative donors induce HIV replication from HIV-infected patients' mononuclear cells and cell lines: an in vitro model of HIV transmission facilitated by Chlamydia trachomatis. The Journal of experimental medicine. 1995;181(4):1493-505.
144. J Schust D, A Ibana J, R Buckner L, Ficarra M, Sugimoto J, M Amedee A, et al. Potential mechanisms for increased HIV-1 transmission across the endocervical epithelium during C. trachomatis infection. Current HIV research. 2012;10(3):218-27.

145. Broadbent A, Horner P, Wills G, Ling A, Carzaniga R, McClure M. HIV-1 does not significantly influence Chlamydia trachomatis serovar L2 replication in vitro. Microbes and infection. 2011;13(6):575-84.
146. Pegu A, Cully M, Wang K, Schmidt S, Doria-Rose N, Julg B, et al., editors. Broadly Neutralizing Antibodies Provide Potent Dose-dependent Protection to Rhesus Macaques Against Mucosal SHIV Challenge. AIDS RESEARCH AND HUMAN RETROVIRUSES; 2018: MARY ANN LIEBERT, INC 140 HUGUENOT STREET, 3RD FL, NEW ROCHELLE, NY 10801 USA.
147. Julien J-P, Sok D, Khayat R, Lee JH, Doores KJ, Walker LM, et al. Broadly neutralizing antibody PGT121 allosterically modulates CD4 binding via recognition of the HIV-1 gp120 V3 base and multiple surrounding glycans. PLoS pathogens. 2013;9(5):e1003342.

APPENDIX A:

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

16 October 2019

Mr Stanley Nzuzo Magini (207526179)
School of Lab Med & Medical Sc
Medical School

Dear Mr Stanley Nzuzo Magini,

Protocol reference number: BREC/00000527/2019
Project title: Inhibitory capabilities of broadly neutralizing antibodies on HIV replication in the presence of Chlamydia trachomatis in vitro: **Degree Purposes: MMedSc**

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 16 October 2019. **Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.**

This approval is valid for one year from **16 October 2019.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

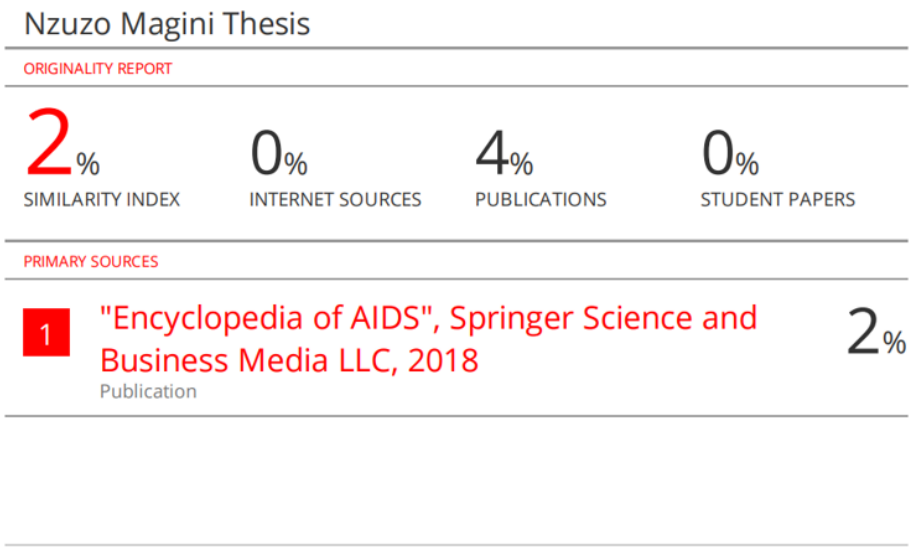
The sub-committee's decision will be noted by a full Committee at its next meeting taking place on **12 November 2019.**

Yours sincerely

Prof V Rambiritch (Chair)

Biomedical Research Ethics Committee
Prof V Rambiritch (Chair)
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Website: http://research.ukzn.ac.za/Research-Ethics/

APPENDIX B: Turnitin Report



Nzuzo Magini Thesis

ORIGINALITY REPORT

2%	0%	4%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Encyclopedia of AIDS", Springer Science and Business Media LLC, 2018 Publication	2%

APPENDIX C

Cell culture

Cell lines and antibodies of choice

McCoy cells were used for the propagation of *C. trachomatis* and TZM-bl cells were used for the experimental purpose.

The McCoy cell line (ATCCR number CRL-1696) an adherent mouse fibroblast cell line, was provided by Dr. Rubina Bunjun from the University of Cape Town. This cell line is widely utilized for the propagation of Chlamydiae.

TZM-bl is an adherent HeLa cell line. The parental cell line (JC.53) stably expresses large amounts of CD4 and CCR5. The TZM-bl cell line is highly sensitive to infection with diverse isolates of HIV. Both cell lines were provided by Dr. Mellisa Rose-Abrahams from the University of Cape Town

Cell culture

Cell culture media

Cells used in this study had different nutritional requirements, 3 different cell culture media was used to ensure optimal growth of each cell line.

The McCoy cell line was cultured in Eagles Minimum Essential Medium (EMEM), supplemented with 10% Foetal Bovine Serum (FBS), HEPES (25mM) to facilitate the maintenance of pH 7.4 and Antimycotic.

TZM-bl cell lines were grown in a complete growth medium consisting of D-MEM supplemented with 10% fetal bovine serum (FBS, heat-inactivated), HEPES (25mM) to facilitate the maintenance of pH 7.4 and Antimycotic.

Cultivation of cells

All cells were stored in 2ml cryovials with original medium and kept at -80°C for short-term storage and in a liquid nitrogen freezer at -196°C for long-term storage. In order to reconstitute frozen cells, the cells were rapidly thawed by placing the cryovial in a water bath at a temperature of 37°C and gently swirled. Once thawed, the cryovial was removed from the water bath, swabbed with 70% ethanol to decontaminate and transferred to a class II biosafety cabinet. The cryoprotective agent was removed by transferring the vial contents to a 15 ml centrifuge tube containing 0.8 ml pre-warmed growth media. The cell suspension was centrifuged at 2000rpm for 7 minutes. The supernatant was discarded, the cell pellet was resuspended in 2 ml fresh growth media. The cell suspension was transferred into an equilibrated 75cm^2 tissue culture flask containing a complete cell culture medium. The flask was incubated at 37°C, 5% CO_2. Cells were viewed using an inverted microscope daily to monitor cell confluency and to check for any possible contamination. Every 2 to 3 days the cell culture medium changed. The old medium was discarded and the monolayer, washed with phosphate-buffered saline (PBS) to remove unattached cells, added fresh complete cell culture medium. The flask was reincubated at 37°C, 5% CO_2.

Passaging of cells

Once the adherent cell monolayer is approximately 90% confluent it was passaged and the cells were either seeded into several new flasks to propagate more cells, used for experiments, or cryopreserved. The passage procedure was similar for all three cell lines; Old medium was discarded from the cell culture flask. The monolayer was washed three times with prewarmed (37°C) cell culture PBS to remove unattached cells and to ensure that the old medium was removed. Two millilitres of 0.25% trypsin 1mM was added to the flask made sure it covered the whole monolayer of cells. The flask was incubated for 4 minutes at 37°C temperature. The side of the flask was gently tapped to detach cells. The proteolytic action of trypsin-EDTA was neutralized by adding 1ml of FBS. The cell suspension was transferred into a 15ml centrifuge tube and centrifuged at 2000 rpm for 7 minutes. The supernatant was discarded cells were resuspended in a growth medium. The cell suspension was aliquoted into a 75cm^2 cell flask with a 20 ml complete growth medium incubated at 37°C, 5% CO_2 until confluent.

Cryopreservation of cells

Cells were cultured and passaged as described above. The cell suspension obtained after passaging the cells was cryopreserved by adding an equal quantity of the appropriate freezing fluid. One millilitre aliquot was prepared and stored at -80ºC overnight, transferred into liquid nitrogen. The McCoy cell freezing fluid consisted of 80% EMEM, 10% dimethyl sulphoxide (DMSO) and 10% FBS. The TZM-bl cell freezing fluid consisted of 80% FBS and 20% dimethyl sulphoxide (DMSO). The freezing fluid was added drop by drop to the cell-FBS suspension. The suspension was gently swirled between drops to slowly mix the cells with the freezing fluid. The cell suspension was aliquoted into cryovials 1.0 ml per cryovial, tightly capped. Each vial was labeled with the date of storage, passage number and concentration of cells. Cells were temporally frozen in Mr-frosty at -80°C. Cells were stored in vials into a liquid nitrogen freezer (-196°C) for long-term storage.

Performing of cell count

The trypan blue dye exclusion assay in manual cell count or automated cell counter was used to enumerate viable and nonviable cells. Twenty microlitres cell suspension was added to 20 µl 0.4% trypan blue solution (1:2 dilution) and mixed well by pipetting up and down. A pipette was used to place a drop of this Trypan blue-cell suspension into the slide. The trypan blue dye stained non-viable cells blue and viable cells colourless. The automated counter determined cell viability percentage, non-viable cell percentage and cell count. The manual cell count was determined using the formula: cells/ml = $\frac{\text{total no. cells counted}}{\text{no. squares counted}}$ x dil factor x vol of suspension x 10^4

Bacterial Culture

Chlamydia trachomatis strains of choice

Experiments were conducted using: Serovar L2 clinical isolate (US151) and serovar E strain isolated by Maleka and coworkers (1996) from a male patient at the Prince Cyril Zulu Communicable Diseases Clinic in Durban, South Africa with urethritis. This strain was typed in the Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington.

Propagation, isolation and storage

Monolayer preparation

McCoy cells were cultured in tissue culture flasks and passaged as described above. Approximately 1 x 10^6 cells suspended in 1ml chlamydia growth media with 10% FBS were seeded into 75cm^2. Cells were incubated for 24hr at 37°C with 5% CO2, checked if cells adhered by inverted microscope. We monitored the integrity of cells, ensured that the monolayer reached 80 to 90% confluent.

Infection

The frozen stock of *Chlamydia* elementary bodies was removed from the −80°C freezer and transferred immediately to a 37°C water bath. The tube was gently agitated until EBs were thawed, after the preparation of chlamydia growth media. Wiped the tube with 70% ethanol and stored it on ice until use. Removed old culture medium from the flask and added 20 ml complete growth media infected with 0.5 ml inoculum of Chlamydia EB suspended in a sucrose-phosphate-glutamate buffer. Flask was centrifuged at 1200xg for 1hr at room temperature. Flask was removed and incubated for 1h at 37°C. The infected medium was removed, replaced with 20 ml of Chlamydia growth media. The flask was incubated for 48 hours at 37°C. After incubation, the flask was viewed in an inverted microscope to verify the presence of chlamydial inclusions in the monolayer. Once verified the chlamydia EBs were harvested. Prepared the required media, 8 ml SPG + 2 ml FBS in 50 ml tube. Old excess Chlamydia growth media remaining in the flask was aspirated and replaced with SPG containing 10% FBS, added 10 glass beads. Vortexed the flask with glass beads to disrupt the McCoy cell monolayer and release the chlamydial EB. The EB-SPG suspension from the flask was pooled into a 50 ml tube, ran through a hollow 23G needle 5 times, and centrifuged at 200g for 10 min. Supernatant with *Chlamydia trachomatis* was aliquoted into sterile safe-lock cryovials, labelled with the isolate number, date and stored at -80 °C. Since more than 1 serovar was harvested on the day, it was done sequentially, not simultaneously, and the biosafety cabinet and all equipment used were swabbed with 70% ethanol between serovars. The safety cabinet's air circulation system was allowed to run for 5-10 minutes to prevent cross-contamination between chlamydial strains.

Determination of the infectious titer

The viability of chlamydial EB decreases during freezing, therefore frozen EB suspensions used for experiments were determined for their infectious titer on 80 to 90% confluent McCoy cell monolayers grown in a 24-well plate with coverslips, at 1×10^5 cells/ml. One hundred microlitres of serial diluted EB-SPG suspension was used to infect 80-90% confluent McCoy

cell monolayers in the 24-well plate using the infection procedure described above. SPG was used for the negative control. After 48 hours of incubation, wells were stained and viewed with an electron microscope. The number of chlamydial inclusions per field of view were counted. The infectious titre was calculated using the following formula:

$$\text{Concentration (IFU/ ml)} = \frac{\text{inclusions}}{N} \times \frac{1000\mu l}{V} \times C \times D$$

Where: n = number of fields counted

V = volume of inoculum (Ll)

C = objective lens conversion factor

D = dilution (ml)

Cultures were stained using the PathoDx *Chlamydia trachomatis* Kit. This staining kit contains anti-*C. trachomatis* monoclonal antibodies raised against the Chlamydiae LPS. Chlamydiae LPS present in serovars of *C. trachomatis* and in both EBs and RBs. These monoclonal antibodies are labeled with fluorescein isothiocyanate (FITC) to facilitate species-specific identification of the organism when present in a monolayer of cultured cells, which causes chlamydial inclusions to fluoresce. Immunofluorescence staining was performed as follows. Media was carefully aspirated from each well of the 24-well plate, added 95% ethanol. The cell monolayers were washed twice with 1 ml PBS. Following fixation, we added 1 drop of the Chlamydiae reagent to the fixed monolayers and rocked the plate to ensure complete coverage of the monolayer by the Reagent. The plate was covered and incubated for 15 minutes at room temperature, constantly agitated to assure even distribution of the reagent during incubation. Excess reagent was aspirated and the stained monolayers were washed twice with 1-ml of de-mineralized water. Removed de-mineralized water by aspiration. Added 1 drop of Mounting fluid, placed coverslip, left to air-dry and examined the stained monolayers using a fluorescence microscope

Optimization of bacterial culture on TZM-bl cells

- Bacterial culture on TZM-bl cells was performed as described above
- DMEM was used for TZM-bl cells instead of EMEM used for McCoy cells.

Neutralization assay on TZM-bl cells

Assay measured neutralization in TZM-bl cells as a function of a reduction in Tat-induced luciferase (Luc) reporter gene expression after a single round of infection. DEAE dextran was

used in the medium during neutralization assays to enhance infectivity. Expression of the reporter genes was induced in trans by viral Tat protein soon after infection. Luciferase activity is quantified by luminescence and is directly proportional to the number of infectious virus particles present in the initial inoculum.

Neutralization experiment was measured in high infectivity, low infectivity and Pseudoviruses in TZM-bl cells. Viruses were provided by Dr. Mellisa Rose-Abrahams from the University of Cape Town.

Used the format of a 96-well flat-bottom culture plate for lay-out of plate1, 2 and 3 neutralization assay.

APPENDIX D

Neutralization assay Protocol

Plate setup (1 master plate)	Master Mix	
	1	**3**
Pippette 25 µl GM into all wells (except H3-H12)	25	75
Add 33.8 µl GM into wells H3-H10	33.8	101.4
Add 3.75 µl of sample (200 µg/ml) to wells H3-H10	3.75	11.25
Transfer 12.5 µl (= 3 fold serial dil) to row G and repeat until row A (only columns 3-10)	12.5	37.5
Aliquot 25 µl to experimental plates(Plate 2 and 3 (Direction: A–H))		

Virus and *C. trachomatis* setup

Plate 1 set up

Thaw virus and dilute in GM to 20000-60000 RLU RLU using formula: $\frac{\text{Number of ml required}}{\text{dilution}} \times 1000$

Dispense 25 µl to all wells in columns 2-10 in plate 1 (Rotate plates Direction: Row A–H)

Add 25 µl of GM into 1st column

Incubate at 37°C for 1 hour

Plate 1 Layout without *Ct*

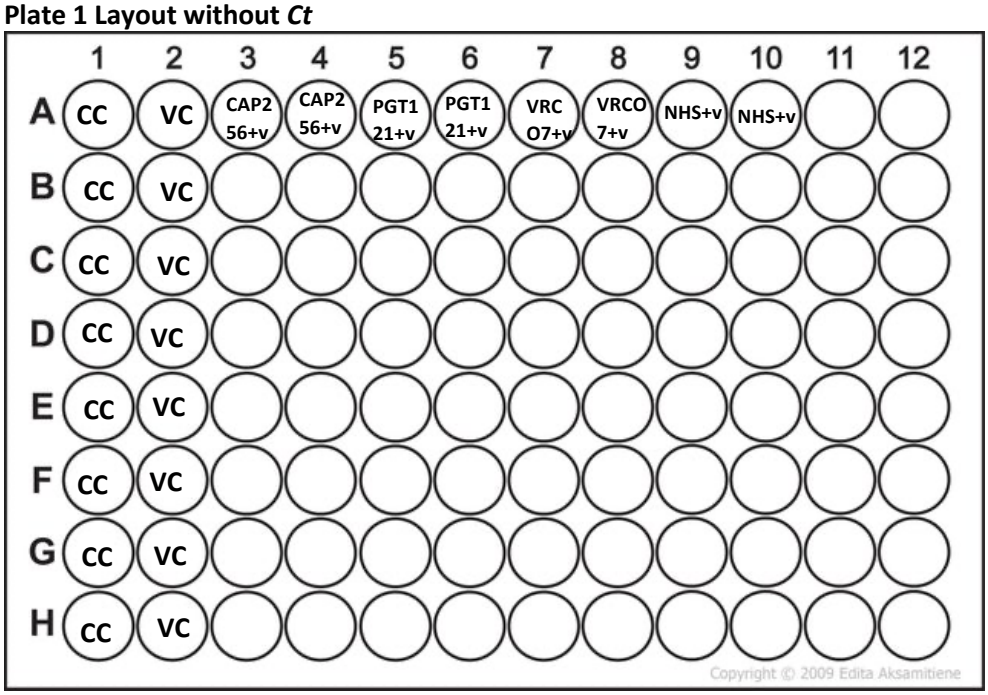


Plate 2 & 3 Ct: E or L2 set up

Thaw virus and dilute in GM to 20000-60000 RLU using formula: $\frac{\text{Number of ml required}}{\text{dilution}} \times 1000$

Dispense 25 µl to all wells in columns 3-12 in plate 2 & 3 (Rotate plates Direction: Row A–H)

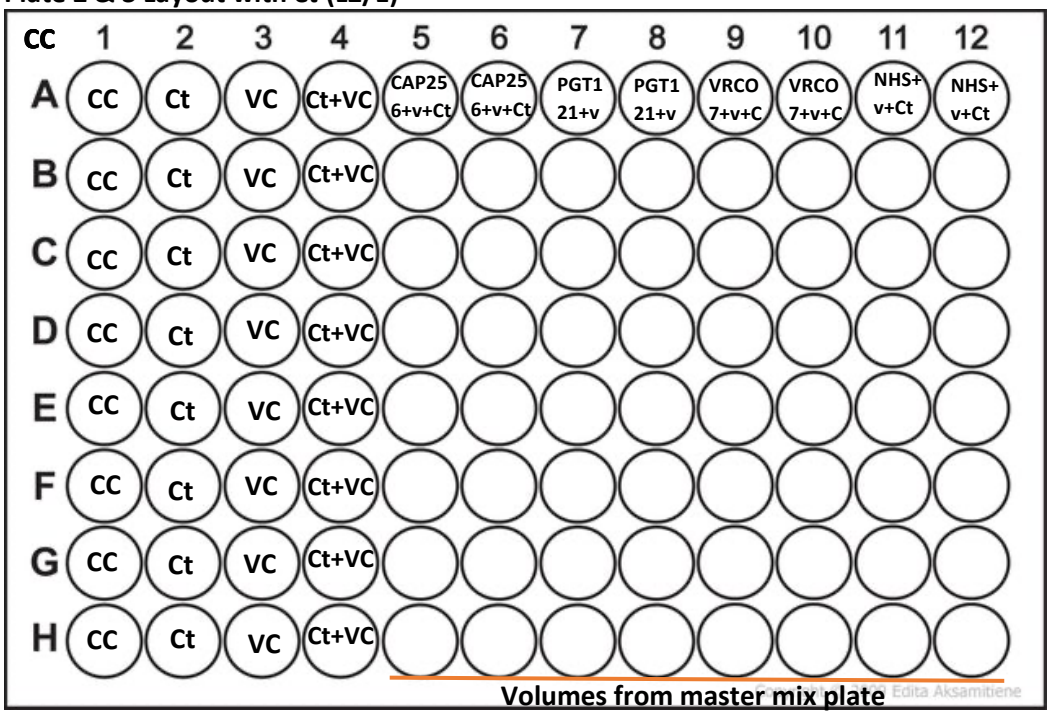
Thaw and dilute serovars E (MOI 10) and (L2 MOI 1)

Dispense 25 µl to all wells in except columns 1-2 in plate 2 & 3 (Rotate plates Direction: Row A–H)

Add 25 µl of GM into 1st column

Incubate at 37°C for 1 hour

Plate 2 & 3 Layout with *Ct* (L2/E)



Cell setup

Require 0.5 x 10^6 cells/ml, 2 ml of cells/plate and 28 µl of DEAE-dextran/plate

Dispense 20 µl of cells to all wells (Directions: Row H – A)

Day 2

Plate 1

After 24 hours Add 130 µl GM to all wells (Direction: Row H-A)

Plate 2 & 3

After 24 hours Add 130 µl GM to column 1,2&3

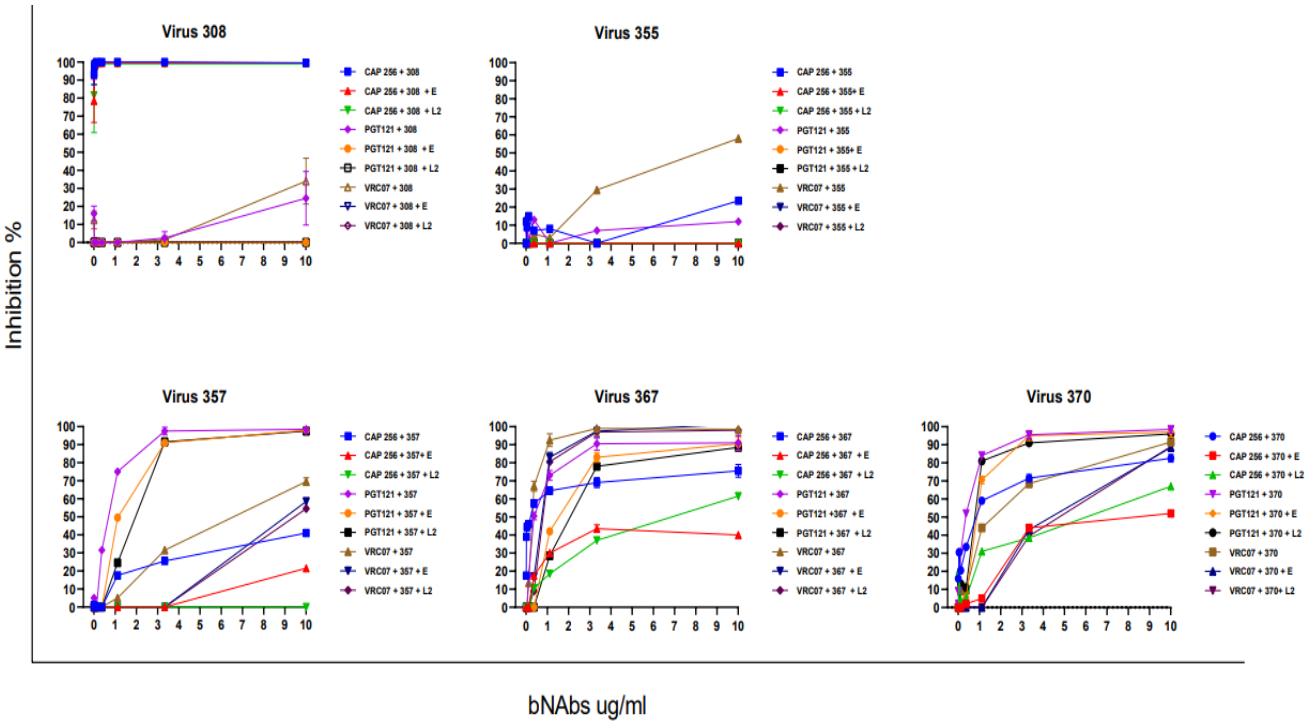
Added 105 µl GM from column 4 to 12

Day 3

After 48 hours remove 100 µl of GM then dispense 100 µl of Bright Glow reagent (Direction: H-A). Incubate for 2 minutes at room temperature. Mix-pipette 5-10 times and transfer 150 µl to 96-well black plate. (Directions: H-A) Read the plate immediately in a luminometer.

APPENDIX E

Comparison of virus inhibition percentage by bNAbs in TZMbl cells Neutalization assay in presence and absence of *C trachomatis*



APPENDIX F

Neutralization activity of bNAbs against subtype C viruses with fold difference

Neutralization score

bNAbs		Viruses					
		308 (Mid)	355 (High)	357 (High)	367 (High)	370 (Low)	331 (High)
CAP256-VRC26.25	Ab + V	<0.1	>10	>10	0.21	0.77	1.2
	Ab + V+ E	<0.1	>10	>10	>10	8.69	2.19
	Ab + V+ L2	<0.1	>10	>10	6.1	5.35	4.14
PGT121	Ab + V	10	>10	0.6	0.39	0.35	0.15
	Ab + V+ E	10	>10	1.15	1.42	0.8	0.24
	Ab + V+ L2	10	>10	1.71	1.82	0.69	0.24
VRC07-523LS	Ab + V	>10	7.71	5.81	0.27	1.52	1.27
	Ab + V+ E	>10	>10	8.62	0.66	4.03	2.64
	Ab + V+ L2	>10	>10	9.27	0.7	4.23	2.76
NHS	Ab + V	>10	>10	>10	>10	>10	>10
	Ab + V+ E	>10	>10	>10	>10	>10	>10
	Ab + V+ L2	>10	>10	>10	>10	>10	>10

IC_{50} titers µg/ml)
<0.1
0.1-0.9
1.0-3.0
3.1-10
>10

(V = Virus) (E = *C. trachomatis* serovar E) (L2 = *C. trachomatis* serovar L2)
(Ab = Broadly Neutralizing Antibody)

Fold difference

bNAbs		Viruses					
		308 (Mid)	355 (High)	357 (High)	367 (High)	370 (Low)	331 (High)
CAP256-VRC26.25	(Ab + V+ E)/(Ab + V)	N/A	N/A	N/A	N/A	11	1.8
	(Ab + V+ L2)/(Ab + V)	N/A	N/A	N/A	29	7	3.5
PGT121	(Ab + V+ E)/(Ab + V)	N/A	N/A	1.9	3.6	2.3	1.6
	(Ab + V+ L2)/(Ab + V)	N/A	N/A	2.9	4.7	2	1.6
VRC07-523LS	(Ab + V+ E)/(Ab + V) +	N/A	N/A	1.5	2.4	2.7	2.1
	(Ab + V) + (Ab + V+ L2	N/A	N/A	1.6	2.6	2.8	2.2
NHS	(Ab + V+ E)/(Ab + V)	N/A	N/A	N/A	N/A	N/A	N/A
	(Ab + V+ L2)/(Ab + V)	N/A	N/A	N/A	N/A	N/A	N/A

N/A = Fold difference statistical insignificant