DEVELOPING AND EVALUATING A COMPREHENSIVE MULTIPLEX PEPTIDE ARRAY FOR SEROLOGICAL DIAGNOSTIC AND SURVEILLANCE OF INFECTIOUS DISEASES IN ZIMBABWE

By

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PREFACE

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

The research described in this dissertation was carried out in Zimbabwe, in the Department of Biotechnology and Biochemistry at the University of Zimbabwe Bulunder the supervision of Professor T. Mduluza (based at the University of Zimbabwe) and Professor T. Naicker (based at UKZN). The field work and sample collection were conducted in Murewa, Shamva and Makoni rural districts and Bulawayo in Zimbabwe. Peptide microarray immunoassays were conducted by PEPperPRINT GmbH (Heidelberg, Germany)

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DECLARATION 1: PLAGIARISM

I, Arthur Vengesai, declare that:

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vi. This thesis is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster or oral presentations at conferences. In some cases, additional material has been included;
vii. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed: Arthur Vengesai

Date: 11-November-2021

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PUBLICATIONS EMANATING FROM THIS THESIS

My role in each paper and presentation is indicated. The * indicates corresponding author.

Manuscripts, Preprints and manuscripts under peer review

- **Arthur Vengesai***, Herald Midzi, Maritha Kasambala, Hamlet Mutandadzi, Tariro L. Mduluza-Jokonya, Simbarashe Rusakaniko, Francisca Mutapi, Thajasvarie Naicker and Takafira Mduluza. A systematic and meta-analysis review on the diagnostic accuracy of antibodies in the serological diagnosis of COVID-19. Submitted June 3 BMC Systematic Reviews. https://doi.org/10.1186/s13643-021-01689-3.

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- **Arthur Vengesai***, Maritha Kasambala, Hamlet Mutandadzi, Tariro L. Mduluza-Jokonya, Takafira Mduluza and Thajasvarie Naicker. Scoping review of the applications of peptide microarrays on the fight against human infections. Submitted to PLOS ONE February 2021. Preprint available at doi.org/10.1101/2021.03.04.433859

- **Arthur Vengesai*,** Thajasvarie Naicker, Herald Midzi, Maritha Kasambala, Tariro L. Mduluza-Jokonya, Simbarashe Rusakaniko Francisca Mutapi and Takafira Mduluza. Multiplex peptide microarray profiling of antibody reactivity against neglected tropical diseases derived B-cell epitopes for serodiagnosis in Zimbabwe. Submitted to PLOS ONE manuscript number PONE-D-21-23533

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LIST OF ABBREVIATIONS

Artificial neural network based B-cell epitope prediction server	ABC PRED
African Research Network for Neglected Tropical Diseases	ARNTD
Centers for Diseases Control	CDC
Immunochromatographic card	ICT
Lymphatic filariasis	LF
Mass drug administration	MDA
National Center for Biotechnology Information	NCBI
Neglected Tropical Diseases	NTDs
Soil transmitted helminths	STHs
Polymerase Chain Reaction	PCR
Coronavirus disease 2019	COVID-19
Severe acute respiratory syndrome corona virus 2	SARS-CoV-2
Real time reverse transcriptase polymerase chain reaction	RT-PCR
Ribonucleic acid	RNA
Immunoglobulin G	IgG
Immunoglobulin M	IgM
Enzyme-linked immunosorbent assay	ELISA
Tackling Infection to Benefit Africa	TIBA
Hemagglutinin glyprotein of influenza	HA
DyLight Fluors with absorption from 350nm to 777nm,	Dy Light
orange-fluorescent dye excited at 532 nm	Cy3
Interquartile range	IQR
Receiver operating characteristic curve	ROC curve
Area under the curve	AUC

Amino acids	aa
Relative fluorescence intensity units	RFU

ABSTRACT

Introduction: Peptides that mimic B-cell linear epitopes may be used as biomarkers for the diagnosis and surveillance of diseases. Peptide microarray technology provide rapid and high-throughput immunoassay platforms, for the simultaneous of identification of B-cell linear epitopes. In this framework, a peptide microarray was designed for the integrated surveillance of infectious diseases endemic in Zimbabwe. The peptide microarray was evaluated against peptides derived from *Ascaris lumbricoides, Necator americanus, Shistosoma haematobium, Schistosoma mansoni*, *Trichuris trichiura*, *Bacillus anthracis, Mycobacterium leprae, Wuchereria bancrofti, Rabies lyssavirus, Chlamydia trachomatis, Trypanosoma brucei* and severe acute respiratory syndrome coronavirus (SARS-CoV-2).

Methods: Published peptides were retrieved from the Tackling Infection to Benefit Africa infectious diseases epitope microarray. Novel peptides were predicted using ABCpred. The peptide microarrays were printed in a laser based approach. IgG and IgM reactivity against the derived peptides were evaluated using peptide microarray immunoassays. Positive response was defined as fluorescence intensity ≥ 500 relative fluorescence units. Immunodominant peptides were identified using heat maps and bar graphs reflecting the obtained fluorescence signal intensities. Receiver Operating Characteristic (ROC) analysis and Mann-Whitney-U test were performed to determine the diagnostic validity of the peptides.

Results: Species-specific responses with at least one peptide derived from each NTD pathogen were observed. The reactive peptides included; for *S. haematobium,* XP_035588858.1-206-220 and XP_035588858.1-206-220 immunodominant for IgG and IgM respectively, for *S.mansoni,* P20287.1-58-72 immunodominant for both antibodies and for *T. trichuria,* CDW52482.1-326-340 immunodominant for IgG and CDW57769.1-2017-2031 and CDW57769.1-1518-1532 immunodominant for IgM. For SARS-CoV-2 derived peptides, 4 (QTH34388.1-1-14, QRU89900.1-41-54, QTN64908.1-136-149 and QLL35955.1-22-35) showed reactivity against IgG. Four peptides (QRU89900.1-41-54, QSM17284.1-76-89, QTN64908.1-136-149 and QPK73947.1-8-21) also showed reactivity against IgM. The SARS-CoV-2 reactive peptide were derived from the membrane glycoprotein and nucleocapsid protein.

Conclusion: Species-specific sero-reactivity was indicative of exposure to the different NTDs parasites antigens. Multiplex peptide microarrays are a valuable tool for integrated NTDs surveillance and for screening parasites exposure in endemic areas. *In silico* peptide prediction and peptide microarray technology may provide a powerful platform for the discovery of SARS-CoV-2 B-cell epitopes.

ABSTRACT-YECHISHONA

Nhanganyaya: Peptides akafanana nema B-cell linear epitopes akakosha nekuti anogona kushandiswa kuongorora kuti munhu anechirwere here nekurakidza kutenderera kwezvirwere munharaunda . Peptide microarray inotibatsira kutsvaka ma B-cell linear epitopes nekukasika panguva imwe. Nekuda kweizvozvo, peptide microarray yakagadzirirwa kuti tiongorore zvirwere zvinotapukira munyika yeZimbabwe. Iyo peptide microarray yakaongororwa mashoja emuviri anorwisa zvirwere zvinosanganisira chirwere cheelephantiasis, bhiraziya, chirwere chemakonye emudumbu (intestinal worms), chirwere chemaziso chetrachoma, chirwere chemapere mbudzi, chirwere cherabis chirwere che COVID-19 nechirwere cheanthrax.

Maitiro: Mamwe mapeptides akawanikwa kubva ku Tackling Infection to Benefit Africa infectious diseases epitope microarray. Mapeptides matsva akawandikwa pachishandiswa chirongwa cheABCpred. Ma peptide microarray akagadzirwa ku Germany nemhando ye laser printer tekinoroji. Masoja emumiviri anoti IgG ne IgM ezvirwere zvambotaurwa akarongororwa tichishandisa peptide microarray tekinoroji.

Zvakabuda muwongororo: Takaona kuti vanhu vemuZimbabwe vane masoja emuviri anokwanisa kurwisa mapeptides anowanikwa pahosha dzinokonzeresa zvirwere zvinosanganisira chirwere cheelephantiasis, bhiraziya, chirwere chemakonye emudumbu (intestinal worms), chirwere chemaziso chetrachoma, chirwere chemapere mbudzi, chirwere cherabis chirwere che COVID-19 nechirwere cheanthrax. Takaona zvakare kuti peptide microarray tekinoroji inokwanisa kushandiswa kuongorora zvirwere zvakawanda panguva imwe.

OUTLINE OF THESIS

Chapter 1: **Introduction and Literature Review**

Chapter 2: **Scoping review of the applications of peptide microarrays on the fight against human infections.**

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.**Chapter 3:** **Aims study design and general methodology**

Chapter 4: **Multiplex peptide microarray profiling of antibody reactivity against neglected tropical diseases derived B-cell epitopes for serodiagnosis in Zimbabwe.**

Citation: Arthur Vengesai, Thajasvarie Naicker, Herald Midzi, Maritha Kasambala, Tariro L. Mduluza-Jokonya, Simbarashe Rusakaniko, Francisca Mutapi and Takafira Mduluza. Multiplex peptide microarray profiling of antibody reactivity against neglected tropical diseases derived B-cell epitopes for serodiagnosis in Zimbabwe. Submitted to PLOS ONE manuscript number PONE-D-21-23533

Chapter 5: **Peptide microarray analysis of *in silico* predicted b-cell epitopes in sars-cov-2 seropositive humans in Zimbabwe.**

Citation: Arthur Vengesai, Thajasvarie Naicker, Maritha Kasambala, Herald Midzi, Simbarashe Rusakaniko, Francisca Mutapi, Takafira Mduluza. Peptide microarray analysis of *in silico* predicted B-cell epitopes in SARS-CoV-2 seropositive humans in Zimbabwe. Tropical Medicine & International Health

Chapter 6: **Synthesis.**

CHAPTER 1

BACKGROUND AND LITERATURE REVIEW

1.1 Neglected tropical diseases

1.1.1 Introduction

Neglected tropical diseases (NTDs), such as schistosomiasis, dengue, lymphatic filariasis, trachoma, and leishmaniasis, are a group of debilitating communicable diseases that affect over 1.6 billion people globally, 40% of whom live on the African continent (1). Notably, NTDs affect the world's poorest, most marginalized and remote communities, where access to clean water, sanitation and health care is limited. The impact of NTDs on communities are devastating; they cause severe pain, disabilities, deformities, malnutrition, stunted growth, cognitive impairment, social isolation, and humiliation. These diseases may be fatal, in fact anaemia caused by some NTDs have a direct impact on maternal mortality. Importantly, NTDs have a disruptive impact on the productivity of already unstable economies. They keep children out of school and adults out of work hence trapping poor communities in endless cycles of poverty (1–3).

1.1.2 Epidemiology

Sub-Saharan Africa is estimated to account for the following worldwide NTDs proportions; approximately 25%-33% soil-transmitted helminth (STH) infections, more than 33% of the lymphatic filariasis (LF), 50% of trachoma infections, most of the world's cases of schistosomiasis, human African trypanosomiasis, loiasis and onchocerciasis, and all of the world's cases of dracunculiasis and human African trypanosomiasis (4,5). Different NTDs can occur in the same poor populations (6) (illustrated in **Figure 1**). In most regions of sub-Saharan Africa it is not uncommon to find five or more NTDs in one area—the three major soil-transmitted helminth infections, schistosomiasis, LF, onchocerciasis, and trachoma (7). Zimbabwe a country in Southern Africa is endemic to four of the most common NTDs, lymphatic filariasis, schistosomiasis, STH and trachoma (8–10). **Figure 2** contains and illustrates the distribution of endemicity and co-endemicity of Schistosomiasis, lymphatic filariasis and STH in Zimbabwe (11).

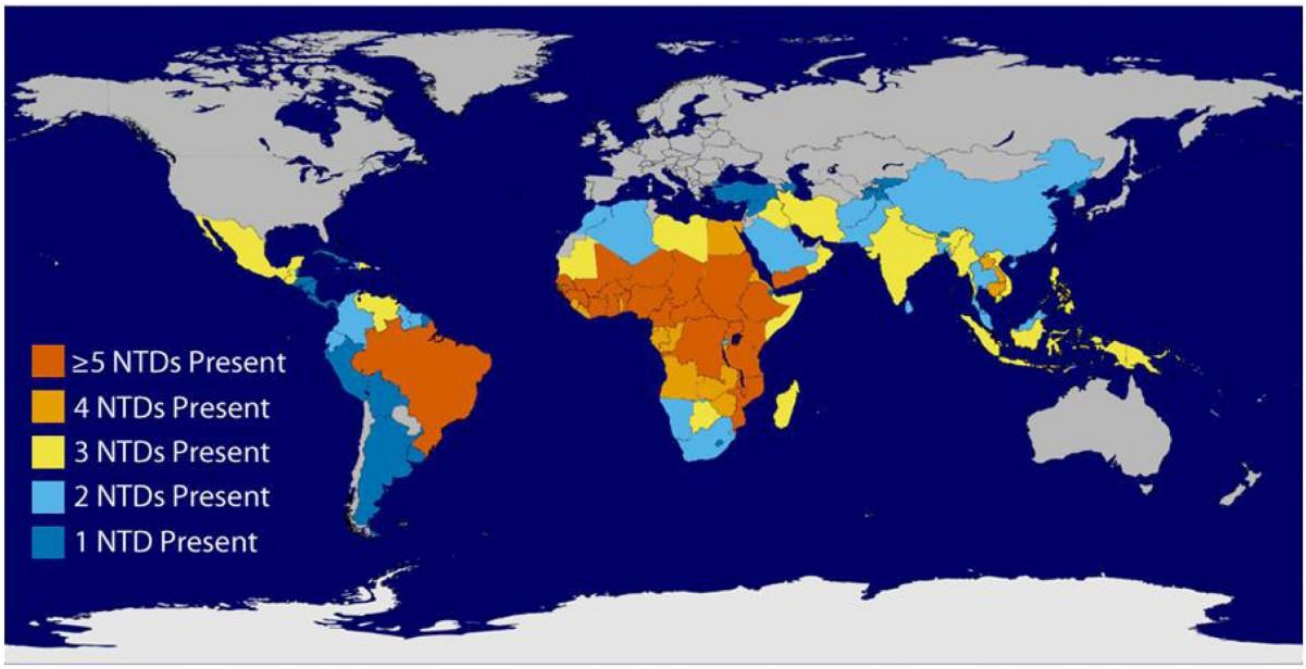

Figure 1. 1 : Global overlap of the six most common NTDs (12).

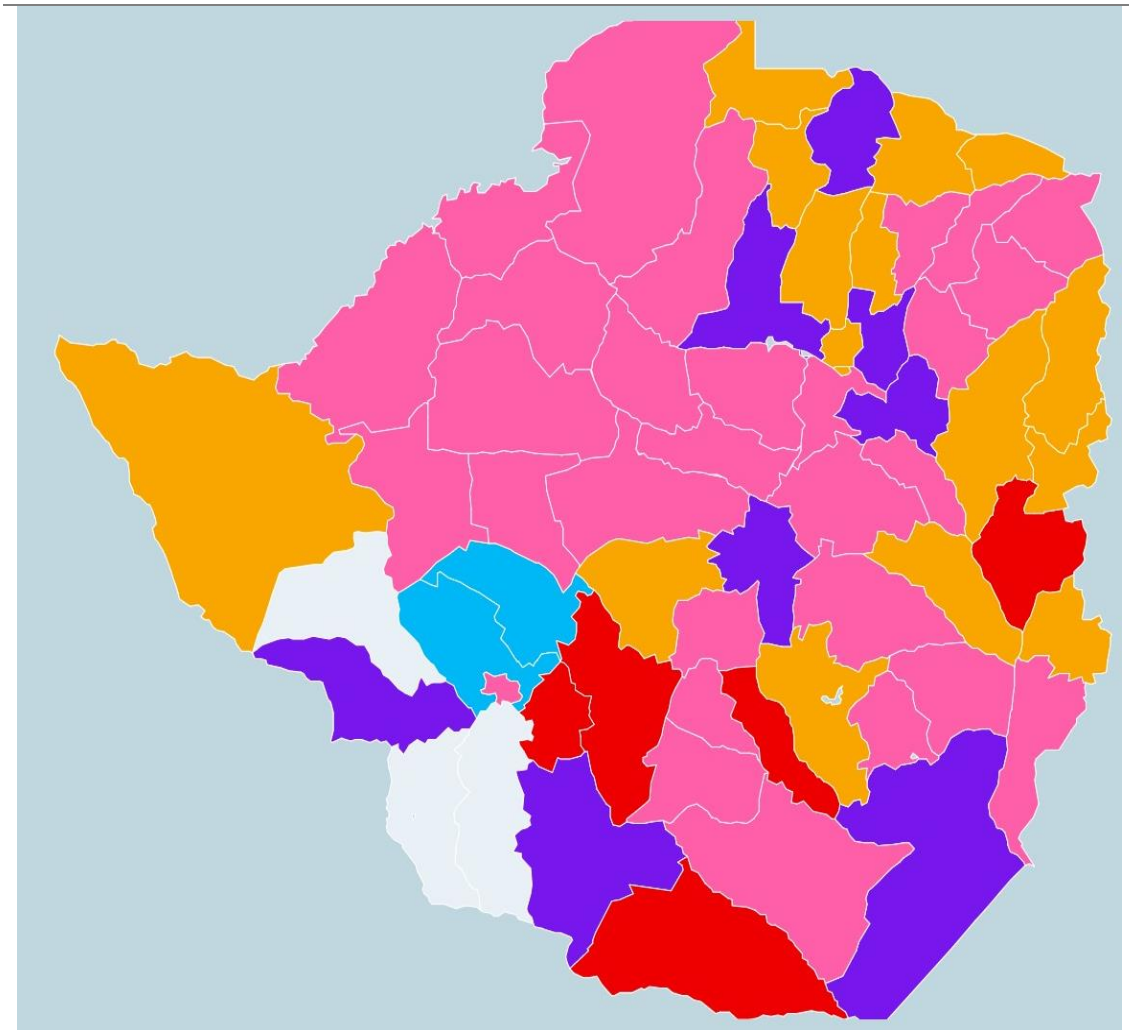

Figure 1.2: Distribution of schistosomiasis, STH, and lymphatic filariasis in Zimbabwe in 2018 Source: Health Ministries and ESPN partnership.

1.1.3 Detection of NTDs

The lack of rapid, accurate, simple-to-use, point-of-care tests for many of the neglected tropical diseases is an important feature for their general neglect and the under-appreciation of their disease burden. Diagnosis plays a crucial role in the surveillance and detection of infectious diseases including NTDs (13). However NTDs remain mostly undiagnosed, the reliable identification of parasitic infections requires in-depth training for specimen preparation, and expertise for subsequent microscopic examination, that are unavailable in most rural clinics and remote areas (14,15). The diagnosis of NTDs, is primarily based on well-established and widely used laboratory techniques, such as the examination of blood, stool and urine samples under a microscope (14). Schistosomiasis is diagnosed through the microscopic detection of parasite eggs in stool or urine specimens using the Kato-Katz and urine filtration techniques respectively (16). The standard method for diagnosing the presence of STHs is to microscopically identify STHs eggs in a stool sample using the Kato-Katz technique or the formal ether concentration technique (17). The standard method for diagnosing LF active infection is the identification of microfilariae in a blood smear by light microscopy (18). Trachoma diagnosis relies on the use of conjunctival examination for the clinical sign of trachomatous inflammation by a trained eye specialist. Polymerase chain reaction (PCR)-based assays for trachroma diagnosis are technically complex and too expensive (19).

While microscopy of parasites is considered as highly specific, its sensitivity depends on the intensity of infection and timing of sample collection. Schistosomiasis eggs tend to be passed at irregular intervals and in small amounts and may not be detected (20). Soil-transmitted helminth eggs may be difficult to find in light infections (17), hence concentration techniques are often recommended for diagnosis. The microfilariae that cause LF circulate in the blood at night and blood should be collected at night to coincide with the appearance of the parasite (18). For trachoma, conjunctival inflammation may persist in the absence of detectable bacteria, an important limitation to decision making based on clinical examination (19).

Serologic tests provide an alternative to microscopic detection of parasites for the diagnosis of schistosomiaisis, STHs, trachoma and LF. Antibodies and/or antigens detected in blood samples are also indications of *Schistosoma* infections (21). Patients with active filarial infection typically have elevated levels of antifilarial IgG4 in the blood and these can be detected using serological assays (18). A dipstick immunoassay based on detection of chlamydial lipopolysaccharide was developed for the diagnosis of trachoma (19). Serologic tests may also be useful in showing exposure to infection and the need for thorough examination, treatment and follow-up of people living in low-transmission areas (21).

Engels and Savioli, (2006) recommended diagnosis of NTDs collectively as a group, as they tend to cluster in the same poor populations and, in order to make progress with their control they should be dealt with in an integrated manner (6). Peptide microarrays may be a solution to these problems, where diagnosis for co-infection can be detected simultaneously using the one tool.

1.1.4 Peptide Microarrays

A microarray is a multiplexed lab-on-a-chip. Peptide microarrays (collections of short peptides of pathogens immobilised on solid planar supports) are large scale screening systems for simultaneous identification of multiple pathogens from small quantities of serum or plasma and other body fluids (22). These microarrays may be used for the diagnosis and surveillance of NTDs. The principle behind microarray chips involves the use of antibody tests that can determine whether an individual is infected or uninfected. Antibody-protein interactions play a critical role in the humoral immune response. B-cells secrete antibodies, which bind antigens. The specific part of antigens that are recognized by antibodies are called B-cell epitopes. These epitopes may be linear, corresponding to a contiguous amino acid sequence fragment of an antigen, or conformational, in which case residues critical for recognition are not contiguous in the primary sequence, but are in close proximity within the folded protein 3D structure (23).

Against this background, one of the objective of this study (**Chapter 4**) was to design and produce a peptide microarray for the integrated surveillance of NTDs endemic in Zimbabwe. The peptide microarray was evaluated against peptides derived from *Ascaris lumbricoides*, *Necator americanus*, *Schistosoma haematobium*, *Schistosoma mansoni*, *Trichuris trichiura*, *Bacillus anthracis*, *Mycobacterium leprae*, *Wuchereria bancrofti*, *Rabies lyssavirus*, *Chlamydia trachomatis* and *Trypanosoma brucei*. Box 1 gives a brief description of the NTDs included in this study.

Box 1: Summary of NTDs

Schistosomiasis also known as bilharzia or the snail fever is an acute and chronic water-born parasitic disease, caused by infection with trematodes (blood borne flukes) of the genus *Schistosoma* (24). There are 2 major forms of schistosomiasis – intestinal and urogenital – caused by 5 main species of blood fluke.

Schistosomiasis affects almost 240 million people worldwide, and more than 700 million people live in endemic areas. Globally there are about 436 million people at risk of infection and 112 million people infected with *Shistosoma haematobium* (Urogenital schistosomiasis). *Shistosoma mansoni* the main cause of intestinal schistosomiasis in Sub Saharan Africa and places 393 million people at risk of infection and infects 54 million people globally. Urogenital schistosomiasis is also considered to be a risk factor for HIV infection, especially in women. Control measures include mass drug administration, improved sanitation, snail control and avoidance of contact with infested water. The disease is found in tropical and subtropical areas of Asia, Africa and Latin America (16,21).

Soil-transmitted helminth infections (intestinal worms) are among the most common parasitic infections worldwide that affect the poorest and most deprived communities. The main species that infect people are *Ascaris lumbricoides* (round worm), *Trichuris trichiura* (whipworm) and the hookworms (*Necator americanus* and *Ancylostoma duodenale*) (25). A large part of the world's population is infected with one or more of these soil-transmitted helminths: approximately 807-1,121 million, 604-795 million and 576-740 million are infected with *Ascaris lumbrioides*, *Trichuris trichiura* and *Nector americanus* respectively (26). Infected children are nutritionally and physically impaired (25). STHs also cause anaemia due to worm induced blood loss and compromised nutrition, intestinal obstruction as well as reduced absorption of vitamin A (9). STHs can be controlled by periodical deworming campaigns to eliminate infecting worms, health education to prevent re-infection, and improved sanitation practices to reduce soil contamination with infective eggs (25).

Trachoma is the leading infectious cause of blindness worldwide. *Chylamydia trachomatis* the causative agent spreads through personal contact (particularly from child to child), commonly through contact with contaminated hands, clothing or bedding and by flies that have been in contact with discharge from the eyes or nose of an infected person. Though it can be clinically diagnosed Trachoma is a public health problem in 44 countries, and is responsible for the blindness or visual impairment of about 1.9 million people. Based on March 2019 data, 142 million people live in trachoma endemic areas and are at risk of trachoma blindness. In 2018, 146 112 people received surgical treatment for advanced stage of the disease, and 89.1 million people were treated with antibiotics (27).

Lymphatic filariasis (LF), commonly known as elephantiasis is a mosquito-transmitted diseases caused by parasitic worms (*Wuchereria bancroftii* responsible for 90 % of the cases and *Brugia* spp.) that damage part of the immune system. LF is a painful and profoundly disfiguring disease and can lead to stigma. It is one of the world's leading causes of disability. In communities where LF is transmitted, all ages are affected. The painful and profoundly disfiguring visible manifestations of the disease, lymphoedema, elephantiasis and scrotal swelling occur later in life and can lead to permanent disability. In 2000 over 120 million people were infected, with about 40 million disfigured and incapacitated by the disease. Infection is usually acquired in childhood causing hidden damage to the lymphatic system. 893 million people in 49 countries worldwide remain threatened by LF and require preventive chemotherapy to stop the spread of this parasitic infection (18).

1.2 Coronavirus disease 2019

Coronavirus disease 2019 (COVID-19) is a major contagious pandemic of respiratory disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is also known as the novel (new) coronavirus 2019-nCoV (28–30). COVID-19 cases were first identified in December 2019 in Wuhan, Hubei Province, China and have now spread through out the world (31). As of July 13 2021, according to the European Centre for Disease Prevention and Control, week 2021-26, there were 184 424 524 confirmed cases of COVID-19 and 3 986 982 deaths reported worldwide (32). The virus spreads from person to person through infected air droplets that are projected during coughing or sneezing. COVID-19 can also be transmitted when humans have contact with hands or surfaces that contain the virus and touch their eyes, nose, or mouth with the contaminated hands. Clinical manifestations range from asymptomatic cases to patients with mild and severe respiratory illness, with or without pneumonia, fever, cough and shortness of breath. Older people and people of all ages with severe chronic medical conditions such as lung disease, heart disease and diabetes seem to have a higher risk of succumbing to severe COVID-19 illness (33).

Early and accurate diagnostic testing for COVID-19 is critical for tracking the SARS-CoV-2, understanding the virus epidemiology, informing case management, suppressing transmission and for quarantine purposes (34,35). The standard diagnostic confirmatory test for COVID-19 is based on the detection of nucleic acids of SARS-CoV-2 by nucleic acid amplification tests, such as real-time reverse-transcriptase polymerase chain reaction (rRT-PCR). The test identifies viral nucleic acids when present in sufficient quantity in sputum, throat swabs, and secretions of the lower respiratory tract. In some patients, SARS-CoV-2 RNA detection in blood and oral fluid specimens has been reported, however limited data is available on adequacy of SARS-CoV-2 detection in these specimens (36). The rRT-PCR test is time consuming as it takes between four to six hours for completion. It requires expensive specialist equipment, skilled laboratory personal for sample preparation and testing and PCR reagents, creating diagnostic delays and limiting use in real-life situations when rapid diagnosis is required for fast intervention decisions. Therefore, less expensive and easy implementable tests are required for SARS-CoV-2 detection. Another limitation of using rRT-PCR involves the use of swabs from the upper respiratory tract which can be falsely diagnosed as negative due to the poor quality of the sample or acquiring the sample at an incorrect timeframe; notably viral load in upper respiratory tract secretions peak in the first week of symptoms but may decline below the limit of detection in patients presenting late with symptoms (37–40). Missing the time-window of viral replication may also provide false negative results. Moreover, after a variable period of time, one expects the rRT-PCR result to become negative due to cessation of viral shedding (35).

False-negative rRT-PCR results are common during diagnosis of SARS-CoV-2. The Fever Clinic of the Beijing Haidian Hospital collected data from January 2020 which indicated that only two out of ten negative cases diagnosed by rRT-PCR test were confirmed to be true positive for COVID-19. This yielded an approximately 20% false-negative rate of rRT-PCR (40). Zhang *et al* (2020) also showed that the current strategy for the detection of viral RNA in oral swabs used for SARS-CoV-2 diagnosis is not 100% accurate. The presence of the virus has been detected in anal swabs or blood samples of patients while their oral swabs diagnosis reports a negative result. This observation implies that a patient cannot be discharged based purely on oral swabs samples being negative (41).

A false negative diagnosis may have grave consequences, especially at this stage of the COVID-19 pandemic by allowing SARS-CoV-2 infected patients to spread the infection thereby hampering the efforts to contain the spread of the virus (37). Additional screening methods that can detect the presence of infection despite lower viral titres are highly beneficial to ensure timely diagnosis of COVID-19. Detection of serum specific anti-SARS-CoV-2 antibodies, both immunoglobulin G (IgG) and M (IgM) which are produced rapidly after the infection provide an alternative highly sensitive and accurate solution and compensates for the limitations of rRT-PCR. The serological methods could also be a more practical alternative to chest CT (35,42,43). Immunoglobulin G antibodies permit the use of serological tools to better understand the overall rate of COVID-19 infections including the rate of asymptomatic infections (37).

However the dynamics of blood or serum antibodies in the cases of COVID-19 are not well evaluated. Currently the serological dynamics of COVID-19 patients remain limited. Also, before diagnostic assays are widely deployed, their performance characteristics need to be evaluated. Therefore, the third objective of this study (**Chapter 5**) to discover diagnostic linear B-cell epitopes for SARS-CoV-2 using *in silico* predicted linear B-cell epitopes and multiplex peptide microarray immunoassay. The validated peptide sequences may then be adapted to conventional ELISA and chemiluminescent immunoassays and point-of-care lateral flow tests the main types of SARS-CoV-2 serological test currently available (44). The lateral flow assay may be used in field settings in low resources countries.

1.3 Rationale

The climate in Southern African countries including Zimbabwe is generally tropical hence creates favourable conditions for the prevalence of several infectious diseases. The tropical climate makes Zimbabwe endemic to several infectious diseases such as malaria, meningitis, enteric pathogen diseases, and NTDs. In light of fierce competition for global health funding, surveillance of a single infection is difficult to defend as a priority. The possibility of testing the same serum sample simultaneously for the presence of antibodies against multiple antigens is an added value particularly in seroprevalence.

Accurate surveillance of multiple-infections enable public health officials to identify opportunities for implementation of integrated control programs. Infectious diseases multiplex peptide microarray tools, could represent an integrated surveillance platform that could be used for monitoring exposure to pathogens and for detection of infections.

1.4 Research questions

a) What are the clinical applications of peptide microarrays on the fight against human infections?
b) Are peptide microarrays effective in diagnosing and screening schistosomiasis, lymphatic filariasis, trachoma and the STHs (roundworm, whipworm and hookworm) in Zimbabwe?
c) Are peptide microarrays effective in screening *Bacillus anthracis, Mycobacterium leprae, Wuchereria bancrofti, Rabies lyssavirus, Chlamydia trachomatis* and *Trypanosoma brucei*?
d) Do in silico prediction of linear B-cell epitopes coupled with peptide microarray technology identify linear B-cell epitopes for SARS-CoV-2?

1.5 Aims and objectives of the thesis

1.5.1 Aim

The aim of the study was the prediction and selection of immunogenic B-cell epitopes (peptides) using the bioinformatics software and the validation of the epitopes using multiplex peptide microarray immunoassays.

1.5.2 Objectives

The objectives of the study were:

a) To determine the applications of peptide microarrays on the fight against human infections.

b) To develop and evaluate a multiplex peptide microarray for the simultaneous serological detection of *S. mansoni, S. haematobium, Trichuris trichiura, Ascaris lumbricoides, Necator americanus, Bacillus anthracis, Mycobacterium leprae, Wuchereria bancrofti, Rabies lyssavirus, Chlamydia trachomatis* and *Trypanosoma brucei* in people living in NTDs endemic areas in Zimbabwe.
c) To establish linear B-cell epitopes for SARS-CoV-2 using *in silico* predicted linear B-cell epitopes and multiplex peptide microarray immunoassay.

CHAPTER 2

SCOPING REVIEW OF THE APPLICATIONS OF PEPTIDE MICROARRAYS ON THE FIGHT AGAINST HUMAN INFECTIONS.

This chapter is scoping review that explores the clinical applications of peptide microarrays in the fight against infectious diseases. The article has been submitted to PLOS ONE. The article is also available as a preprint in medRxiv at doi.org/10.1101/2021.03.04.433859.

Scoping review of the applications of peptide microarrays on the fight against human infections.

Short title: Peptide microarrays application

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Abstract

Introduction

This scoping review explores the use of peptide microarrays in the fight against infectious diseases. The research domains explored included the use of peptide microarrays in the mapping of linear B-cell and T cell epitopes, antimicrobial peptide discovery, immunosignature characterisation and disease immunodiagnostics. This review also provides a short overview of peptide microarray synthesis.

Methods

Electronic databases were systematically searched to identify relevant studies. The review was conducted using the Joanna Briggs Institute methodology for scoping reviews and data charting was performed using a predefined form. The results were reported by narrative synthesis in line with the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews guidelines.

Results

Eighty-six articles from 100 studies were included in the final data charting process. The majority (93%) of the articles were published during 2010–2020 and were mostly from Europe (44%) and North America (34 %). The findings were from the investigation of viral (44%), bacterial (30%), parasitic (25%) and fungal (2%) infections. Out of the serological studies, IgG was the most reported antibody type followed by IgM. The largest portion of the studies (78%) were related to mapping B-cell linear epitopes, 10% were on diagnostics, 9% reported on immunosignature characterisation and 6% reported on viral and bacterial cell binding assays. Two studies reported on T-cell epitope profiling.

Conclusion

The most important application of peptide microarrays was found to be B-cell epitope mapping or antibody profiling to identify diagnostic and vaccine targets. Immunosignatures identified by random peptide microarrays were found to be applied in the diagnosis of infections and interrogation of vaccine responses. The analysis of the interactions of random peptide microarrays with bacterial and viral cells using binding assays enabled the identification of antimicrobial peptides. Peptide microarray arrays were also used for T-cell linear epitope mapping which may provide more information for the design of peptide-based vaccines and for the development of diagnostic reagents.

Introduction

Infectious diseases also known as communicable diseases are a major growing concern worldwide (1) and are a significant burden on public health (2). They account for a large proportion of death and disability globally. At least 25% of 60 million deaths that occur worldwide each year are estimated to be due to infectious diseases (2).

There are countless examples that highlight the severity of the impact of infectious diseases on human health (2). Since 31 December 2019 and as of 13 July 2021, 187 086 096 cases and almost 4 million deaths of COVID-19 have been reported world-wide (3). HIV infection continues to be a major pandemic where approximately 33 million people have died of HIV-related illnesses since the start of the pandemic. In 2019, 690 000 people died from HIV-related illnesses and 1.7 million people acquired new infections (4). Currently there are 20 neglected tropical diseases' (NTDs) affecting over 1.7 billion people and killing more than 200 000 people every year (5). Historically, the Black Death (1348–1350) killed 30%–60 % of Europe's population (2). In the 20th century, smallpox was responsible for an estimated 300–500 million deaths (2). The 1918-1919 Spanish Influenza pandemic killed more people than the World War 1 (2).

The threat posed by infectious diseases is further deepened by the continued emergence of new, unrecognized, and old infectious disease epidemics (2). Outbreaks caused by SARS-CoV-2, HIV, Ebola, influenza, and Zika viruses, have increased over the past decade, underlining the need for the rapid development of diagnostic tools and vaccines (6). Epidemics that occurred in the WHO African regions during the period 2016-2018 **(Figure. 1)**, included 41countries and 87% had at least one epidemic, while 21 countries (45%) had at least one epidemic per year (7).

Figure. 1: Infectious disease epidemics in the WHO African region, that occurred during the period 2016 to 2018.

A reasonable public health response towards addressing the infectious disease problem aims to address the fundamental factors that promote their occurrence and persistence, whilst implementing appropriate control measures (2). The field of medical biotechnology offers innovative devices for fighting infections, such as peptide microarrays (1,8).

Peptide microarrays are collections of short peptides immobilized on solid planar supports (9). They provide rapid, reproducible ways to simultaneously screen and detect hundreds to thousands of different pathogen related peptides or epitopes on standard microscope slides from small quantities of serum, plasma and cerebrospinal fluid (10–12).

Peptide microarrays offer a wide range of applications in the fight against infectious diseases, such as, B-cell and T-cell epitope discovery for development of diagnostics and rationally designed vaccines, drug discovery (antimicrobial peptides discovery), immunosignature characterisation and pathogen immunodiagnostics (13–17). Additionally, peptide microarrays are used for autoimmune disease research, cancer research and enzyme profiling (18)**.** In spite of the growing number of studies utilizing peptide microarrays, there is a paucity of systematic and narrative type reviews that reflect their clinical importance. This review focuses on the applications and use of peptide microarrays to fight infections

Review aim and objectives

In order to systematically summarize the literature on the applications of peptide microarrays, we have conducted a scoping review. This scoping review aims to explore the use of peptide microarrays, in the mapping of B-cell linear epitopes, antimicrobial peptide discovery through bacterial cells glyco-profiling, immunosignature characterisation, immunodiagnostics and T-cell epitope mapping. This review also provides a short overview of peptide microarray synthesis. It is hoped that this review will highlight and enable recommendations that may aid future peptide microarray biomedical research, systematic and meta-analysis reviews.

Methods

Study design

The scoping review protocol was developed using the methodological framework proposed by Arksey and O'Malley (2005) and further refined by the Joanna Briggs Institute (19,20). The completed review followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines (**S1 Table**) (21).

The review team consisted of four authors (AV, MK, HM and TLJ) who developed clear research questions, search strategies, identified relevant articles, selected articles, extracted and charted data. The discussion and reporting of the results were done in consultation with TN and TM.

Eligibility criteria

The inclusion criteria was developed using the population-concept-context framework (19). The 'population' of the review were human participants of all ages, ethnicity and gender diagnosed with infectious diseases. Animal models for human infectious diseases studies, viruses and bacteria in antimicrobial activity investigations were also included as the review population. The 'concept' of the review was peptide microarrays. The review 'context' was a primary research study from any healthcare settings or institution from any country. All narrative reviews, studies investigating animal diseases, and duplicate articles were excluded. The search strategy was not restricted by the publication date or language. Hence, all related studies up to November 30, 2020, that met the inclusion criteria were assessed. As a scoping review is an iterative process, the eligibility criteria was amended as the study progressed.

Search strategy information sources and search terms

The following online data bases PubMed, Medline complete, The Cochrane Central Register of Controlled Trials (CENTRAL) and Web of Science were systematically searched from their inception without any restrictions on language or date of publication. The data bases were searched using predefined keywords. Table 1 illustrates the search terms and strategy for PubMed which was adapted for the other databases. Additionally grey literature databases GreyLit and OpenGrey were searched and a manual search of the reference lists of relevant publications and reviews was conducted.

Table 1: Search Strategy in PubMed

Search number	Query	Results
4	(("Peptides"[Mesh] OR Peptide*[tiab] OR Epitope*[tiab] AND (humans[Filter])) AND ("Microarray Analysis"[Mesh] OR Microarray*[tiab] OR Biochip*[tiab] OR Chip*[tiab] OR Array*[tiab] AND (humans[Filter]))) AND (Infestation*[tiab] OR Infection*[tiab] OR "Infectious disease*"[tiab] OR "Communicable disease*"[tiab] OR "Contagious disease*"[tiab] OR "Transmissible disease*"[tiab] OR Pathogen[tiab] OR Pathogens[tiab] AND (humans[Filter]))	3,337
3	Infestation*[tiab] OR Infection*[tiab] OR "Infectious disease*"[tiab] OR "Communicable disease*"[tiab] OR "Contagious disease*"[tiab] OR "Transmissible disease*"[tiab] OR Pathogen[tiab] OR Pathogens[tiab]	1,098,661
2	"Microarray Analysis"[Mesh] OR Microarray*[tiab] OR Biochip*[tiab] OR Chip*[tiab] OR Array*[tiab]	178,487
1	"Peptides"[Mesh] OR Peptide*[tiab] OR Epitope*[tiab]	1,720,729

[Tiab] means the title and abstract were searched

Review process and data charting

The retrieved literature were downloaded into Mendeley reference manager, and duplicates were removed. One reviewer (AV) assessed the titles of the studies identified by the search and excluded irrelevant studies. Two reviewers (AV and MK) independently assessed the eligibility of the abstracts and full texts of the retrieved studies to avoid bias. After the articles were selected, data was extracted and recorded in the excel spreadsheet. One author (AV) extracted and recorded the data from each study according to a pretested data extraction excel spreadsheet form (additional file 1) and a second reviewer (HM or MK or TMJ) verified the extracted data. Discrepancies were resolved by consensus and a third evaluator. The extracted data were author, date of publication, DOI, Aim and study domain, geographical location, microorganism or infection, antibody type, epitope prediction/selection, peptide synthesis, microarray printing and key findings.

Methodological quality appraisal and analysis of the evidence

Methodological quality or risk of bias of the included articles was not appraised, which is consistent with guidance on scoping review conduct (19)**.** The narrative synthesis of the results of this review were done in line with the recommendations set out in the PRISMA-ScR (21).

Results and discussion

Identification of potential studies

Electronic searches of seven databases yielded a total of 5929 articles (Pubmed: 3337, Medline (EBCOhost):1223, Cochrane: 17, Web of science: 1232, MedRxiv: 118, Greylit: 0, Open Grey: 2). Additional articles identified through manual searching yielded 11 articles that led to a total of 5940 titles and abstracts eligible for screening. A total of 253 full text articles were screened for eligibility after the removal of duplicate articles and irrelevant articles. Full text screening led to a total of 86 articles (100 studies) that were included in the scoping review. Two records were unable to be obtained in full-text format. A flow chart of the studies identification and selection process is outlined in **Figure 2**.

Figure 2: Flow chart of the studies identification and selection process

Characteristics of the included articles

Characteristics of the included studies are shown in **S2 Table**. There were no articles published before 2001 on the study area and the peer-reviewed literature on the study area has increased considerably in the last few years (**Figure. 3**). Among the articles included, 63 % were published in the last five years (2015-2020) and approximately, 93% have been published in the last decade (2010–2020).

Figure 3: Number of included articles by year (2001-2020)

The included articles, were mainly from Europe 38 (44%) and North America 29 (34%) **Figure. 4**. From South America (Argentina 3 and Brazil 7) and Asia (China 7, Japan 1, and Sri Lanka 1) we included 10 (12%) and 9 (10%) articles respectively. Articles from Europe were divided among several countries, Germany 19, Sweden 6 Switzerland 2, Belgium 2, Denmark 2, Italy 4, Finland 1 Spain 1 and Austria 1. Articles from North America were mostly from USA 29, with one article from Cuba.

Figure 4: Number of articles included in the review by continent

In terms of the pathogens or infectious disease category studies (N=100), most studies were investigating viruses 43% (including SARS-CoV-2, HIV, Ebola) followed by studies investigating bacteria 30% (including TB, Lyme disease, chlamydia trachomatis) and Parasites 25% (Toxoplasma gondi, *S. mansoni, Plasmodium* species). Hitherto enigmatic diseases were investigated in 1 % of the included studies and Fungi (Coccidiodes) was investigated in 2 % of the studies. Two studies investigated health humans' immunosignatures. Out of the 88 peptide microarray serological studies included IgG was the most invested antibody type followed by IgM. The IgG response shows a more specific binding pattern (less noise) than the IgM response, which reflects the higher specificity of IgGs (22). Two studies by Mishara *et al* (2018) (23) and Tokarz *et al* (2020) (24) investigated IgG and IgM profiles in cerebrospinal fluid.

Peptide microarrays

Peptide/epitope identification and prediction

B-cell and T-cell epitopes play a vital role in the development of peptide based vaccines and therapeutics and in the diagnosis of diseases (25,26). In this review, 6 methods were used for the identification and prediction of epitopes. These were computational overlapping peptides sequences, computational permutation scans, published synthetic peptides, computational random peptide sequences, phage display library and *in silico* prediction.

For epitope identification using overlapping peptides, the linear amino acid sequence of a protein is cut into peptides with overlapping sequences (27). This is achieved by shifting a frame of a distinct peptide length of a protein sequence of interest (28).

In a permutation scan, each of the amino acid residues in a known antibody binding peptide is substituted by all amino acids or by one amino acid for example alanine permutation scans (29). Expect for *in silico* prediction methods, computational overlapping peptides sequences, computational permutation scans, computational random peptide sequences and phage display library peptide/epitope prediction methods are costly and time-consuming and demands large resources as they require screening of large arrays of potential epitope candidates. *In silico* prediction methods reduce the burden associated with epitope mapping by decreasing the list of potential epitope candidates for experimental testing(30,31). **Table 2** lists bioinformatics tools for the *in silico* prediction of epitopes on proteins for the studies included in this review. BepiPred 1.0 was the most frequently used software.

Table 2: B-cell epitope prediction software.

Software	Server
MLCE	http://bioinf.uab.es/BEPPE
ABCpred	http://www.imtech.res.in/raghava/abcpred/
BepiPred 1.0	www.cbs.dtu.dk/services/BepiPred/
Epitopia web server	http://epitopia.tau.ac.il/
Antigenic	http://www.bioinformatics.nl/cgi-bin/emboss/antigenic
BCPREDS	http://ailab.ist.psu.edu/bcpred/
Bcepred	http://crdd.osdd.net/raghava/bcepred/bcepred_instructions.html

Peptide microarrays displayed short peptides (ranging 10–20 amino acid residues). Of note, most peptide microarrays displayed peptides with 15 amino acid residues, this length covers 83% of known linear antibody epitopes in the LANL immunology database, including the median length of epitopes (11 amino acids) (32). A few peptide microarrays displayed peptides with 5 and 6 amino acid residues set which are the shortest assumed B-cell epitope lengths (33).

Peptide synthesis

Solid phase peptide synthesis (SPPS), was the method of choice for the production of peptides for most articles, although solution phase synthesis can still be useful for large-scale production of peptides. SPPS can be defined as a process in which a peptide anchored by its C-terminus to an insoluble polymer is assembled by the successive addition of protected amino acids constituting its sequence (34).

Solid phase peptide synthesis, dramatically changed the strategy of peptide synthesis and simplified the tedious and demanding steps of purification associated with solution phase synthesis. SPPS also permitted the development of automation (35).

SPOT synthesis is a special type of SPPS using cellulose as the solid support was used in 38 % of the studies that were included in the review. SPOT synthesis is a robust, rapid, and cost effective method for the simultaneous parallel chemical synthesis of peptides in a miniaturized array (36). SPOT synthesis has several advantages: cellulose is inexpensive and withstands the organic solvents and acids used during peptide synthesis. In addition, cellulose is stable in aqueous solutions and, because it is non-toxic, it is appropriate for screening biological samples. Another advantage of using SPOT synthesis on cellulose is the possibility of modifying the peptide (37). However, SPOT synthesis on porous membranes has its limitations when reducing the spot size to <1 mm and becomes costly and tedious when large numbers of copies of an identical array are required (38).

Peptide laser printing technology offered by PEPperPRINT Inc. (Heidelberg, Germany) (18) was used to produce peptides in 15 % of the included studies. The peptides are produced using a process based on electrostatic deposition and conjugation of dry amino acids, similar to the method used by laser printers.

Peptide microarray synthesis

In general, two methods were used for the synthesis of peptide microarrays: the immobilization of pre-synthesized peptides and *in situ* synthesis of peptides on a solid support. Immobilization of pre-synthesised peptides involved SPOT synthesis, cleavage of solid phase bound peptides from the cellulose support matrix and spotting of the soluble peptides onto various types of planar surfaces for example glass chips using either a contact printer or a non-contact printer which minimizes contamination (39). Common solid phase materials such as functionalized polypropylene and glass were used for SPPS based *in situ* peptide microarrays and cellulose was used for SPOT based *in situ* peptide microarray synthesis (40). The background signal from the *in situ* synthesis method is relatively lower than that produced by immobilizing pre-synthesized peptides because the background surface is selectively inert. However, the quality of peptides from the *in situ* synthesis method is lower than that of the spotting method because the peptide synthesized on a chip cannot be purified. Another problem yet to be solved with all *in situ* systems reported to date is the molecular characterization of the peptides. The lack of direct, *in situ* peptide analysis remains a major roadblock in the development of high-quality peptide arrays (40).

Peptide microarrays are offered by various providers, **Table 3** list the companies and the peptide microarray synthesis method including peptide synthesis and solid phase used by the companies. Peptide microarray providers are not limited to those included in **Table 3**. Of importance, Suzhou Epitope (Suzhou, China) uses polymer coated initiator integrated poly(dimethysiloxane) (iPDMS), as a solid supporting material. With an excellent capacity for preventing or reducing non-specific interactions, iPDMS, is able to provide near zero background for microarray screening. iPDMS can also achieve an extremely low limit of detection (41).

Table 3: Peptide microarrays synthesis companies and the peptide microarray synthesis method including peptide synthesis and solid phase.

Company	**Peptide Synthesis**	**Microarray Synthesis**	**Solid Phase**	**URL**
JPT (Berlin Germany)	SPOT	Non-contact immobilization of pre synthesized peptides	Epoxy functionalized glass slides	https://www.jpt.com/
Nimblegen-Roche	Light directed SPPS	*In situ* light-directed SPPS	Amino-functionalized plastic support/microscope slide	https://sequencing.roche.com/en-us.html
PEPperPRINT (Berlin Germany)	Electrostatic deposition and conjugation of dry amino acids (Peptide laser printing)	*In situ* Peptide Laser Printing	PEPperSlide glass slide	https://www.pepperprint.com/technology/peptide-microarray-analysis/
Schafer-N (Copenhagen, Denmark)	Light directed SPPS	*In situ* light directed SPPS	HD peptide microarrays on Epoxy functionalized glass slides	https://schafer-n.com/
Suzhou Epitope (Suzhou, China)	GL Biochem	Contact immobilization of presynthesized peptides	iPDMS	
Applied Epitope (Tempe, AZ)	Light directed SPPS	*In situ* light directed SPPS	silicon wafer surface/ Functionalized glass slide	https://appliedmicroarrays.com/
Alere Technologies (GMbH, Jena, Germany)		Contact immobilization of presynthesized peptided	Epoxy functionalized glass slides	http://www.alere-technologies.com/
ABIMED peptide arrayer system (MIT Biopolymer facility		*In situ* peptide synthesis	Functionalized cellulose	

Research domains

For the purpose of narrative review, based on the main research objectives, studies were classified into one of the following five research domains: mapping of B-cell linear epitopes, binding assays, immunosignatures characterisation, immunodiagnostics and mapping T-cell epitopes. The largest portion of the studies were related to mapping B-cell linear epitopes 78%, followed by studies on immunosignature characterisation 9%, while 8% reported immunodiagnostics and 6% reported virus and bacterial binding assays. Two studies reported mapping T-cell epitopes.

Mapping B-cell linear epitopes

Antibodies recognize and bind their target protein antigens via surface accessible interaction sites, the linear epitopes or the conformational epitopes (38). High-content peptide microarrays allow linear epitope profiling of entire pathogen proteomes (42). There is great interest in identifying epitopes in antigens for a number of practical reasons (30,43). In the review, characterization of antibody specificities was through identification of epitopes with potential applications in diagnosis of diseases. Epitope mapping identified epitopes useful in monitoring immune responses after chemotherapeutic treatments and vaccinations and for vaccine development. One study used epitope mapping to identify disease aetiology (44). Studies used overlapping peptides for the general epitope mapping and permutation scans or substitution analysis for fine epitope mapping. However it should be noted that mapping of B-cell epitopes using overlapping synthetic peptides permits the elucidation of linear epitopes only (45).

Bacteria, virus and lipopolysaccharides binding assays

Peptides can bind to various targets including bacterial and viral cells and lipopolysaccharides (LPS)(46). In the current review, peptide microarray binding assays were used to uncover the cyclic di-GMP (c-di-GMP) binding site of a *Pseudomonas aeruginosa* protein (PA3740), the Toll-like receptor (TLR) amino acid sequence for bacterial cell binding peptides and random peptide microarrays were used to screen for antimicrobial peptides (AMP).

The rise of multi-drug resistant pathogens is one of the most important global health issues and demands new compounds with novel mechanisms to combat these pathogens(47,48). Drug discovery has not kept pace with the rising multi-drug resistant pathogens partly due to drug cross-resistatance. Short, cationic peptides with antimicrobial activity known as AMPs, are essential to the host defences (48). AMPs are promising alternative to traditional antimicrobial drugs. AMPs are a diverse family of short peptides, between 5 and 50 amino acids in length and most possess an overall net positive charge to their structure (49) that display a broad spectrum killing properties to all pathogens. They are fast acting and have a decreased likelihood to induce pathogenic resistance as compared to traditional antimicrobial drugs and therefore could be next generation antibiotics (49). Screening for AMPs using peptide microarrays is a very convenient tool in the development of these drug candidates (28). In the current review, Svarovsky and Gonzalez-Moa (2011) used fluorescently labelled bacteria and LPS to discover peptide sequences that not only specifically bound to LPS, but incidentally also inhibited bacterial cell growth (50). Betanzos *et al* (2009) using luminescent LPS-quantum dots from O111:B4 and O55:B5 serotypes of *E. coli* revealed that peptides binding to *E .coli* LPS were highly enriched in aromatic and cationic amino acids and most inhibited growth (51). Johnston *et al* (2017) screened a range of pathogens (10 viruses and 11 bacteria) against a library of 10,000 peptides to identify shared and specific pathogen binding peptides that were used for the development of a pathogen binding 100-peptide microarray (52).

TLRs are membrane bound-receptors responsible for recognizing pathogen associated molecular patterns and activation of the immune system. They specifically, recognize LPS, eliciting immune responses against invading bacteria (46). In the current review, a study by Tanaka *et al* (2018) revealed several TLR4 peptides, including GRHIFWRR that demonstrated binding to *Escherichia coli* as well as LPS. These peptides exhibited a high proportion of arginine and lysine residues, positive charge, and low GRAVY value (hydrophilic) (46). Düvel *et al* (2015) using fluorescence labelled c-di-GMP, showed that PA3740 octomer peptides bind c-di-GMP with high affinity and uncovered LKKALKKQTNLR to be a putative c-di- GMP binding motif. (53).

Immunosignatures

There is an increasing awareness that health care must move from post-symptomatic treatment to pre-symptomatic intervention (54). A universal system to diagnose disease, characterize infection or evaluate the response to a vaccine would be useful (55). An ideal system would allow regular monitoring of health status using circulating antibodies to report on health fluctuations. Random peptide microarrays can do this through antibody signatures (54). An immunosignature is a pattern of binding of serum antibodies to an array of thousands of random-sequence peptides in a broad and unbiased fashion (15,56). Immunosignatures are not based on natural peptide sequence, but instead on a representative and diverse chemical space, a fact that simplifies peptide synthesis (57). Antibodies will bind to random peptides under permissive binding conditions. The binding is detected by a fluorescent anti-human secondary antibody. A high-resolution laser scanner provides an intensity value for each peptide (15). Querying immunosignature data using statistical and machine learning the random patterns of antibody peptide interactions can be used to diagnose disease, even many diseases simultaneously (15). In this review, this approach was shown to have diagnostic and prognostic potential for diseases and interrogation of vaccine response.

Immunodiagnostics

Serological assays play a major role in the diagnosis of both past and recent infections (24,58). These assays often based on crude antigen extracts or purified native antigenic proteins or recombinant antigens have constrains. Production of native antigens is limited, and the amounts are difficult to standardize. There is risk of contamination with proteins from organisms used in the production of recombinant antigens. Moreover, some recombinant antigens show lower reactivity than their corresponding native antigens, due to differences in protein folding that can result in altered epitope presentation. To avoid these limitations, several studies have shown that peptide microarrays can be used in serological assays to discriminate infected individuals from healthy individuals (16,24,58–60). Peptide microarray immunoassays were also shown to be capable of simultaneous multiplex diagnosis of different pathogens with a single patient serum sample (16,24,61). However, it is extremely unlikely that a single peptide can distinguish pathogens or strain types reliably (59). To achieve a satisfactory diagnostic sensitivity and a high specificity, it is necessary to use optimized peptide combinations, mimicking reactive epitopes on natural antigens. This strategy improves assay specificity by eliminating non-specific and potentially cross-reactive epitopes.

Targeting a combination of such antigens can enhance assay sensitivity and has been shown to improve the diagnosis of tick-borne diseases (24). To select candidate diagnostic peptide sequences for subsequent analysis, *in silico* predicted B-cell epitopes and previously predicted diagnostic peptides were used in the studies included in the review.

Mapping T-cell epitopes

Rational development and evaluation of peptide based vaccines and therapeutics requires identification and measurement of epitope-specific CD4 and CD8 T-cell responses. Conventional T-cell epitope discovery methods are labour intensive and do not scale well (62). In the current review, two studies (13,62) described the use of peptide microarrays using overlapping peptides, major histocompatibility complexes (MHC) and fluorescent tagged anti-MHC antibodies to map immunodominant T-cell epitopes. This high-throughput identification of T-cell epitopes will provide more information for the design of peptide-based vaccines and for the development of diagnostic reagents, such as MHC peptides.

Strengths and limitations

A clear limitation of conventional peptide microarrays is their restriction to linear protein epitopes, whereas conformational epitope antibody recognition cannot be identified (63). Detection of antibodies recognizing all potential epitopes whether linear, conformational or carbohydrate or LPS is a key requirement to comprehensively profile the humoral immune response (55).

The main advantage of the peptide microarray design is the miniaturisation of antibody-antigen interaction assays, the simultaneous analysis of several peptide sequences and the subsequent reduction in serum volume required from patients since this always represents a limiting factor in serological studies (64). By using peptide microarrays, it is feasible to simultaneously investigate the prevalence of the respective antibody classes in a specific patient and to differentiate the reactivity to all epitopes recognized by the different antibody class. By using different fluorescently labelled secondary antibodies each recognizing a particular antibody class, peptide microarrays permits the detection of different antibody classes within the same microarray (65).

In binding assays, a distinct advantage offered by the peptide microarrays is the immediate visual assessment of all bacterial and viral cells and LPS binding events that enables immediate parallel analysis of all binding peptides. This is useful for selection of orthogonal functional peptides that have different binding targets. A distinct disadvantage, however, is the limited number of potential binding ligands that generally does not allow meaningful selection of consensus sequences or binding motifs (66).

Conclusion

In the review the peptide microarrays were shown to offer a wide range of applications, including, B-cell and T-cell epitope discovery for development of diagnostics and vaccines, serological diagnosis of viruses and bacteria as well as parasitic diseases pathogen and antimicrobial peptides discovery. Their most important was shown to be B-cell epitope mapping or antibody profiling to identify diagnostics and vaccine targets. Immunosignatures identified by random peptide microarrays were shown to be applied in the diagnosis of infections and interrogation of vaccine responses. Analysing the interactions of random peptide microarrays with bacterial and viral cells using binding assays enabled the identification of antimicrobial peptides. Peptide microarray arrays were also used for T-cell linear epitope mapping which may provide more information for the design of peptide-based vaccines and for the development of diagnostic reagents.

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References

1. Afzal H, Zahid K, Ali Q, Sarwar K, Shakoor S, Nasir U, et al. Role of biotechnology in improving human health. J Mol Biomark Diagn. 2016;07(06).

2. Nii-Trebi NI. Emerging and neglected infectious diseases: Insights, advances, and challenges [Internet]. Vol. 2017, Biomed Res. Int. Hindawi Limited; 2017 [cited 2021 Jan 14]. Available from: https://pubmed.ncbi.nlm.nih.gov/28286767/

3. Coronavirus disease (COVID-19) [Internet]. [cited 2021 Jan 14]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019

4. HIV/AIDS [Internet]. [cited 2021 Jan 14]. Available from: https://www.who.int/news-room/fact-sheets/detail/hiv-aids

5. Neglected tropical diseases | Uniting to combat NTDs [Internet]. [cited 2021 Jan 14]. Available from: https://unitingtocombatntds.org/ntds/

6. Heiss K, Heidepriem J, Fischer N, Weber LK, Dahlke C, Jaenisch T, et al. Rapid response to pandemic threats: Immunogenic epitope detection of pandemic pathogens for diagnostics and vaccine development using peptide microarrays. Cite This J Proteome Res [Internet]. 2020;19:4339–54. Available from: https://dx.doi.org/10.1021/acs.jproteome.0c00484

7. Talisuna AO, Okiro EA, Yahaya AA, Stephen M, Bonkoungou B, Musa EO, et al. Spatial and temporal distribution of infectious disease epidemics, disasters and other potential public health emergencies in the World health organisation africa region, 2016-2018. BMC [Internet]. 2020 Jan 15 [cited 2021 Jan 14];16(1):9. Available from: https://globalizationandhealth.biomedcentral.com/articles/10.1186/s12992-019-0540-4

8. Pham P V. Medical biotechnology: Techniques and applications. In: Omics technologies and bio-engineering: Towards improving quality of life. Elsevier Inc.; 2018. p. 449–69.

9. Zandian A, Forsström B, Häggmark-Månberg A, Schwenk JM, Uhlén M, Nilsson P, et al. Whole-proteome peptide microarrays for profiling autoantibody repertoires within multiple sclerosis and narcolepsy. J Proteome Res [Internet]. 2017 Mar 3 [cited 2020 Feb 26];16(3):1300–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28121444

10. Cretich M, Gori A, D'Annessa I, Chiari M, Colombo G. Peptides for infectious diseases: From probe design to diagnostic microarrays. Antibodies [Internet]. 2019 Mar 12 [cited 2021 Jan 14];8(1):23. Available from: https://www.mdpi.com/2073-4468/8/1/23

11. Yun SG, Jang JW, Lee JH, Lim CS, Kim J, Ki Y, et al. Evaluation of novel multiplex antibody kit for human immunodeficiency virus 1/2 and hepatitis C virus using sol-gel based microarray. Biomed Res Int [Internet]. 2015 [cited 2021 Jan 14];2015. Available from: https://pubmed.ncbi.nlm.nih.gov/26457305/

12. Mendes TA de O, Reis Cunha JL, de Almeida Lourdes R, Rodrigues Luiz GF, Lemos LD, dos Santos ARR, et al. Identification of strain-specific B-cell epitopes in *Trypanosoma cruzi* using

genome-scale epitope prediction and high-throughput immunoscreening with peptide arrays. Marques ETA, editor. PLoS Negl Trop Dis [Internet]. 2013 Oct 31 [cited 2021 Jan 14];7(10):e2524. Available from: https://dx.plos.org/10.1371/journal.pntd.0002524

13. Malnati MS, Heltai S, Cosma A, Reitmeir P, Allgayer S, Glashoff RH, et al. A new antigen scanning strategy for monitoring HIV-1 specific T-cell immune responses. J Immunol Methods [Internet]. 2012 Jan 31 [cited 2020 Nov 23];375(1–2):46–56. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/21963950/

14. Sabalza M, Barber CA, Abrams WR, Montagna R, Malamud D. Zika virus specific diagnostic epitope discovery. J Vis Exp [Internet]. 2017 Dec 12 [cited 2020 Nov 24];2017(130). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/29286404/

15. Stafford P, Wrapp D, Johnston SA. General assessment of humoral activity in healthy humans. Mol Cell Proteomics [Internet]. 2016 May 1 [cited 2020 Nov 24];15(5):1610–21. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/26902205/

16. Maksimov P, Zerweck J, Maksimov A, Hotop A, Groß U, Pleyer U, et al. Peptide microarray analysis of *in silico*-predicted epitopes for serological diagnosis of *Toxoplasma gondii* infection in humans. Clin Vaccine Immunol [Internet]. 2012 Jun [cited 2020 Nov 22];19(6):865–74. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/22496494/

17. Lee JS, Song JJ, Deaton R, Kim JW. Assessing the detection capacity of microarrays as bio/nanosensing platforms. Biomed Res Int [Internet]. 2013 [cited 2020 Nov 22];2013. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/24324959/

18. PEPperPRINT: Peptide microarray analysis [Internet]. [cited 2021 Jan 14]. Available from: https://www.pepperprint.com/technology/peptide-microarray-analysis/

19. Peters MDJ, Godfrey CM, Khalil H, McInerney P, Parker D, Soares CB. Guidance for conducting systematic scoping reviews. Int J Evid Based Healthc [Internet]. 2015 Sep 1 [cited 2021 Jan 14];13(3):141–6. Available from: https://pubmed.ncbi.nlm.nih.gov/26134548/

20. Arksey H, O'Malley L. Scoping studies: Towards a methodological framework. Int J Soc Res Methodol Theory Pract [Internet]. 2005 Feb [cited 2021 Jan 14];8(1):19–32. Available from: https://www.tandfonline.com/doi/abs/10.1080/1364557032000119616

21. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA extension for scoping reviews (PRISMA-ScR): Checklist and explanation [Internet]. Vol. 169, Annals of

Internal Medicine. ACP; 2018 [cited 2021 Jan 14]. p. 467–73. Available from: https://pubmed.ncbi.nlm.nih.gov/30178033/

22. Heidepriem J, Krähling V, Dahlke C, Wolf T, Klein F, Addo MM, et al. Epitopes of naturally acquired and vaccine-induced anti-ebola virus glycoprotein antibodies in single amino acid Resolution. Biotechnol J. 2020 Sep 1;15(9).

23. Mishra N, Caciula A, Price A, Thakkar R, Ng J, Chauhan L V., et al. Diagnosis of Zika virus infection by peptide array and enzyme-linked immunosorbent assay. MBio. 2018 Mar 1;9(2).

24. Tokarz R, Mishra N, Tagliafierro T, Sameroff S, Caciula A, Chauhan L, et al. A multiplex serologic platform for diagnosis of tick-borne diseases. Sci Rep [Internet]. 2018 Dec 1 [cited 2020 Nov 23];8(1). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/29453420/

25. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins Struct Funct Bioinforma [Internet]. 2006 Aug 7 [cited 2021 Feb 9];65(1):40–8. Available from: http://doi.wiley.com/10.1002/prot.21078

26. Li Pira G, Ivaldi F, Bottone L, Manca F. High throughput functional microdissection of pathogen-specific T-cell immunity using antigen and lymphocyte arrays. J Immunol Methods [Internet]. 2007 Sep 30 [cited 2021 Feb 9];326(1–2):22–32. Available from: https://pubmed.ncbi.nlm.nih.gov/17673252/

27. Weber LK, Palermo A, Kügler J, Armant O, Isse A, Rentschler S, et al. Single amino acid fingerprinting of the human antibody repertoire with high density peptide arrays. J Immunol Methods [Internet]. 2017 Apr 1 [cited 2020 Nov 24];443:45–54. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/28167275/

28. Winkler DFH, Campbell WD. The spot technique the spot technique: Synthesis and screening of peptide macroarrays on cellulose membranes. Methods Mol Biol. 2008;494:47–70.

29. Lagatie O, Van Dorst B, Stuyver LJ. Identification of three immunodominant motifs with atypical isotype profile scattered over the *Onchocerca volvulus* proteome. PLoS Negl Trop Dis [Internet]. 2017 Jan 26 [cited 2020 Nov 23];11(1). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/28125577/

30. Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA. Fundamentals and methods for T- and B-cell epitope prediction. Vol. 2017, J. Immunol. Res. Hindawi Limited; 2017.

31. Sanchez-Lockhart M, Reyes DS, Gonzalez JC, Garcia KY, Villa EC, Pfeffer BP, et al. Qualitative profiling of the humoral immune response elicited by rVSV-ΔG-EBOV-GP using a systems serology assay, domain programmable arrays. Cell Rep [Internet]. 2018 Jul 24 [cited 2021 Feb 9];24(4):1050-1059.e5. Available from: https://doi.org/10.1016/j.celrep.2018.06.077

32. Stephenson KE, Neubauer GH, Reimer U, Pawlowski N, Knaute T, Zerweck J, et al. Quantification of the epitope diversity of HIV-1-specific binding antibodies by peptide microarrays for global HIV-1 vaccine development. J Immunol Methods. 2015 Jan 1;416:105–23.

33. Jaenisch T, Heiss K, Fischer N, Geiger C, Bischoff FR, Moldenhauer G, et al. High-density peptide arrays help to identify linear immunogenic B-cell epitopes in individuals naturally exposed to malaria infection. Mol Cell Proteomics [Internet]. 2019 [cited 2020 Nov 24];18(4):642–56. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/30630936/

34. Al-Warhi TI, Al-Hazimi HMA, El-Faham A. Recent development in peptide coupling reagents. Vol. 16, J. Saudi Chem. Soc. Elsevier; 2012. p. 97–116.

35. Amblard M, Fehrentz JA, Martinez J, Subra G. Methods and protocols of modern solid phase peptide synthesis [Internet]. Vol. 33, Mol. Biotechnol. Springer; 2006 [cited 2021 Jan 14]. p. 239–54. Available from: https://link.springer.com/article/10.1385/MB:33:3:239

36. Fraczyk J, Walczak M, Kaminski ZJ. New methodology for automated SPOT synthesis of peptides on cellulose using 1,3,5-triazine derivatives as linkers and as coupling reagents. J Pept Sci [Internet]. 2018 Feb 1 [cited 2021 Jan 14];24(2):e3063. Available from: http://doi.wiley.com/10.1002/psc.3063

37. Winkler DFH, Hilpert K, Brandt O, Hancock REW. Synthesis of peptide arrays using SPOT-technology and the CelluSpots-method. Methods Mol Biol [Internet]. 2009 [cited 2021 Jan 14];570:157–74. Available from: https://pubmed.ncbi.nlm.nih.gov/19649591/

38. Beutling U, Frank R. Epitope analysis using synthetic peptide repertoires prepared by SPOT synthesis technology in: antibody engineering [Internet]. Berlin, Heidelberg: Springer Berlin Heidelberg; 2010 [cited 2021 Jan 14]. p. 537–71. Available from: http://link.springer.com/10.1007/978-3-642-01144-3_35

39. Dikmans A, Beutling U, Schmeisser E, Thiele S, Frank R. SC2: A novel process for

manufacturing multipurpose high-density chemical microarrays. QSAR Comb Sci [Internet]. 2006 Nov [cited 2021 Jan 14];25(11):1069–80. Available from: http://doi.wiley.com/10.1002/qsar.200640130

40. F.H. Winkler D. Chemistry of SPOT synthesis for the preparation of peptide macroarrays on cellulose membranes. Mini Rev Org Chem [Internet]. 2011 Mar 28 [cited 2021 Jan 14];8(2):114–20. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1570-193X&volume=8&issue=2&spage=114

41. Huang M, Ma Q, Liu X, Li B, Ma H. Initiator integrated poly(dimethysiloxane)-based microarray as a tool for revealing the relationship between nonspecific interactions and irreproducibility. Anal Chem [Internet]. 2015 Jul 21 [cited 2021 Jan 14];87(14):7085–91. Available from: https://pubs.acs.org/doi/abs/10.1021/acs.analchem.5b00694

42. Pérez-Bercoff L, Valentini D, Gaseitsiwe S, Mahdavifar S, Schutkowski M, Poiret T, et al. Whole CMV proteome pattern recognition analysis after HSCT identifies unique epitope targets associated with the CMV status. PLoS One [Internet]. 2014 Apr 16 [cited 2020 Nov 22];9(4). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/24740411/

43. Carmona SJ, Nielsen M, Schafer-Nielsen C, Mucci J, Altcheh J, Balouz V, et al. Towards high-throughput immunomics for infectious diseases: Use of next-generation peptide microarrays for rapid discovery and mapping of antigenic determinants. Mol Cell Proteomics [Internet]. 2015 Jul 1 [cited 2020 Nov 22];14(7):1871–84. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/25922409/

44. Ferrara G, Valentini D, Rao M, Wahlström J, Grunewald J, Larsson LO, et al. Humoral immune profiling of mycobacterial antigen recognition in sarcoidosis and Löfgren's syndrome using high-content peptide microarrays. Vol. 56, IJID. Elsevier B.V.; 2017. p. 167–75.

45. Torréns I, Reyes O, Ojalvo AG, Seralena A, Chinea G, Cruz LJ, et al. Mapping of the antigenic regions of streptokinase in humans after streptokinase therapy. Biochem Biophys Res Commun [Internet]. 1999 May 27 [cited 2021 Feb 9];259(1):162–8. Available from: https://pubmed.ncbi.nlm.nih.gov/10334933/

46. Tanaka M, Harlisa IH, Takahashi Y, Ikhsan NA, Okochi M. Screening of bacteria-binding peptides and one-pot ZnO surface modification for bacterial cell entrapment. RSC Adv. 2018;8(16):8795–9.

47. Bluhm MEC, Knappe D, Hoffmann R. Structure-activity relationship study using peptide arrays to optimize Api137 for an increased antimicrobial activity against *Pseudomonas aeruginosa*. Eur J Med Chem [Internet]. 2015 Oct 20 [cited 2020 Nov 22];103:574–82. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/26408816/

48. Fjell CD, Jenssen H, Hilpert K, Cheung WA, Panté N, Hancock REW, et al. Identification of novel antibacterial peptides by chemoinformatics and machine learning. J Med Chem. 2009 Apr 9;52(7):2006–15.

49. López-Pérez PM, Grimsey E, Bourne L, Mikut R, Hilpert K. Screening and optimizing antimicrobial peptides by using SPOT-synthesis [Internet]. Vol. 5, Frontiers in Chemistry. Frontiers Media S. A; 2017 [cited 2021 Jan 14]. p. 25. Available from: www.frontiersin.org

50. Svarovsky SA, Gonzalez-Moa MJ. High-throughput platform for rapid deployment of antimicrobial agents. ACS Comb Sci. 2011 Nov 14;13(6):634–8.

51. Betanzos CM, Gonzalez-Moa MJ, Boltz KW, Vander Werf BD, Johnston SA, Svarovsky SA. Bacterial glycoprofiling by using random sequence peptide microarrays. ChemBioChem [Internet]. 2009 Mar 23 [cited 2021 Jan 14];10(5):877–88. Available from: http://doi.wiley.com/10.1002/cbic.200800716

52. Johnston SA, Domenyuk V, Gupta N, Batista MT, Lainson JC, Zhao ZG, et al. A simple platform for the rapid development of antimicrobials. Sci Rep. 2017 Dec 1;7(1).

53. Düvel J, Bense S, Möller S, Bertinetti D, Schwede F, Morr M, et al. Application of synthetic peptide arrays to uncover cyclic di-GMP binding motifs. J Bacteriol. 2016;198(1):138–46.

54. Legutki JB, Zhao ZG, Greving M, Woodbury N, Johnston SA, Stafford P. Scalable high-density peptide arrays for comprehensive health monitoring. Nat Commun. 2014 Sep 3;5.

55. Legutki JB, Magee DM, Stafford P, Johnston SA. A general method for characterization of humoral immunity induced by a vaccine or infection. Vaccine [Internet]. 2010 Jun 17 [cited 2020 Nov 23];28(28):4529–37. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/20450869/

56. Navalkar KA, Johnston SA, Woodbury N, Galgiani JN, Magee DM, Chicacz Z, et al. Application of immunosignatures for diagnosis of valley fever. Clin Vaccine Immunol [Internet]. 2014 [cited 2020 Nov 23];21(8):1169–77. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/24964807/

57. Singh S, Stafford P, Schlauch KA, Tillett RR, Gollery M, Johnston SA, et al. Humoral immunity profiling of subjects with myalgic encephalomyelitis using a random peptide microarray differentiates cases from controls with high specificity and sensitivity. Mol Neurobiol [Internet]. 2018 Jan 1 [cited 2020 Nov 24];55(1):633–41. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/27981498/

58. Rizwan M, Rönnberg B, Cistjakovs M, Lundkvist Å, Pipkorn R, Blomberg J. Serology in the Digital Age: Using Long Synthetic Peptides Created from Nucleic Acid Sequences as Antigens in Microarrays. Microarrays. 2016 Aug 10;5(3):22.

59. Arranz-Solís D, Cordeiro C, Young LH, Dardé ML, Commodaro AG, Grigg ME, et al. Serotyping of toxoplasma gondii infection using peptide membrane arrays. Front Cell Infect Microbiol [Internet]. 2019 Nov 29 [cited 2020 Nov 24];9. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/31850240/

60. Bergamaschi G, Fassi EMA, Romanato A, D'Annessa I, Odinolfi MT, Brambilla D, et al. Computational analysis of dengue virus envelope protein (E) reveals an epitope with flavivirus immunodiagnostic potential in peptide microarrays. Int J Mol Sci [Internet]. 2019 Apr 2 [cited 2020 Nov 23];20(8). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/31003530/

61. Sachse K, Rahman KS, Schnee C, Müller E, Peisker M, Schumacher T, et al. A novel synthetic peptide microarray assay detects *Chlamydia* species-specific antibodies in animal and human sera. Sci Rep [Internet]. 2018 Dec 1 [cited 2020 Nov 24];8(1). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/29549361/

62. Haj AK, Breitbach ME, Baker DA, Mohns MS, Moreno GK, Wilson NA, et al. High-throughput identification of MHC class I binding peptides using an ultradense peptide array. J Immunol. 2020 Mar 15;204(6):1689–96.

63. Loeffler FF, Pfeil J, Heiss K. High-density peptide arrays for malaria vaccine development. In: methods in molecular biology [Internet]. Humana Press Inc.; 2016 [cited 2021 Jan 14]. p. 569–82. Available from: https://link.springer.com/protocol/10.1007/978-1-4939-3387-7_32

64. Fernández L, Bleda MJ, Gómara MJ, Haro I. Design and application of GB virus C (GBV-C) peptide microarrays for diagnosis of GBV-C/HIV-1 co-infection. Anal Bioanal Chem [Internet]. 2013 [cited 2020 Nov 23];405(12):3973–82. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/23232955/

65. The distribution and functions of immunoglobulin isotypes - Immunobiology - NCBI Bookshelf [Internet]. [cited 2021 Jan 14]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27162/

66. Svarovsky SA, Gonzalez-Moa MJ. High-throughput platform for rapid deployment of antimicrobial agents. ACS Comb Sci [Internet]. 2011 Nov 14 [cited 2021 Jan 14];13(6):634–8. Available from: https://pubs.acs.org/doi/abs/10.1021/co200088c

Supporting information.

S1 Table. PRISMA Extension for Scoping Reviews guidelines checklist.

S2 Table. General characteristics of the studies included in the scoping review.

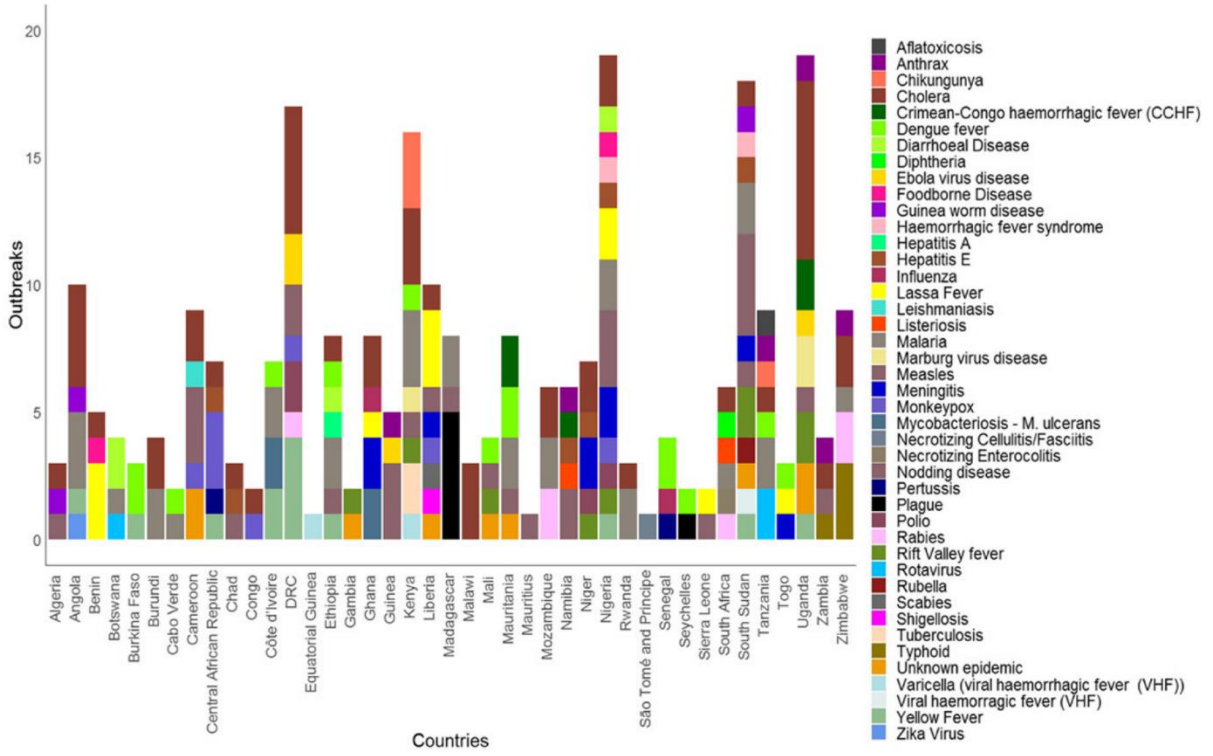


Figure. 1: Infectious disease epidemics in the WHO African region, that occurred during the period 2016 to 2018.

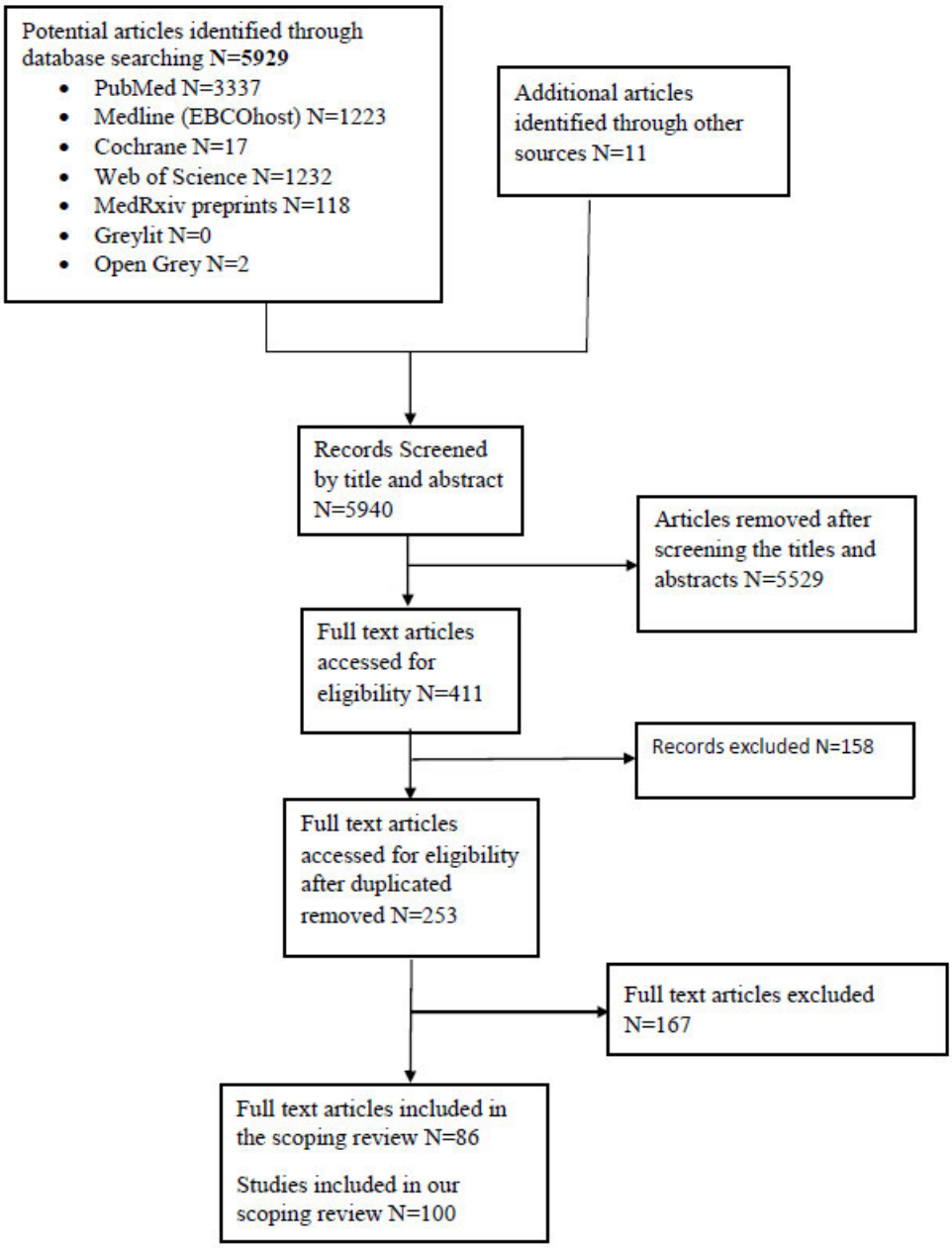


Figure 2: Flow chart of the studies identification and selection process

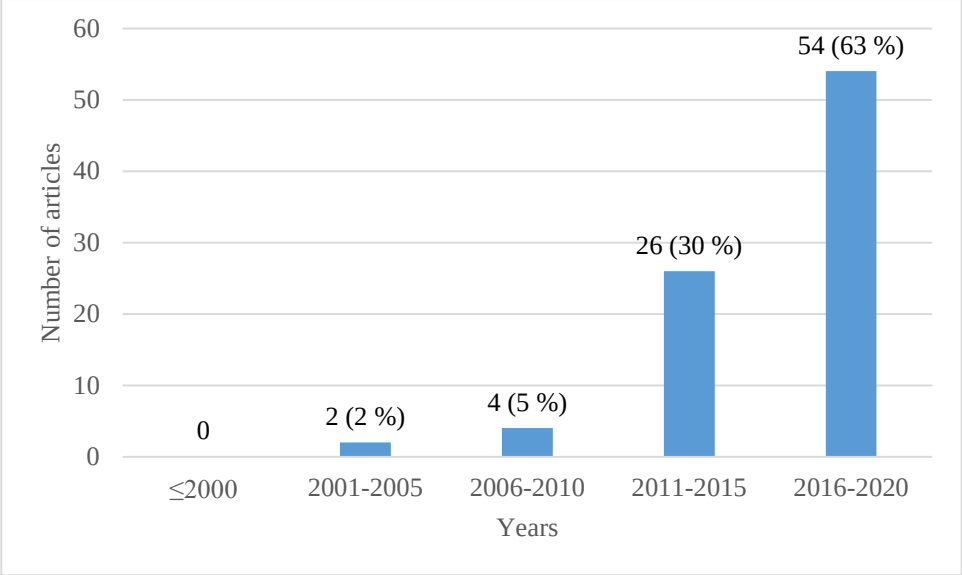


Figure 3: Number of included articles by year (2001-2020)

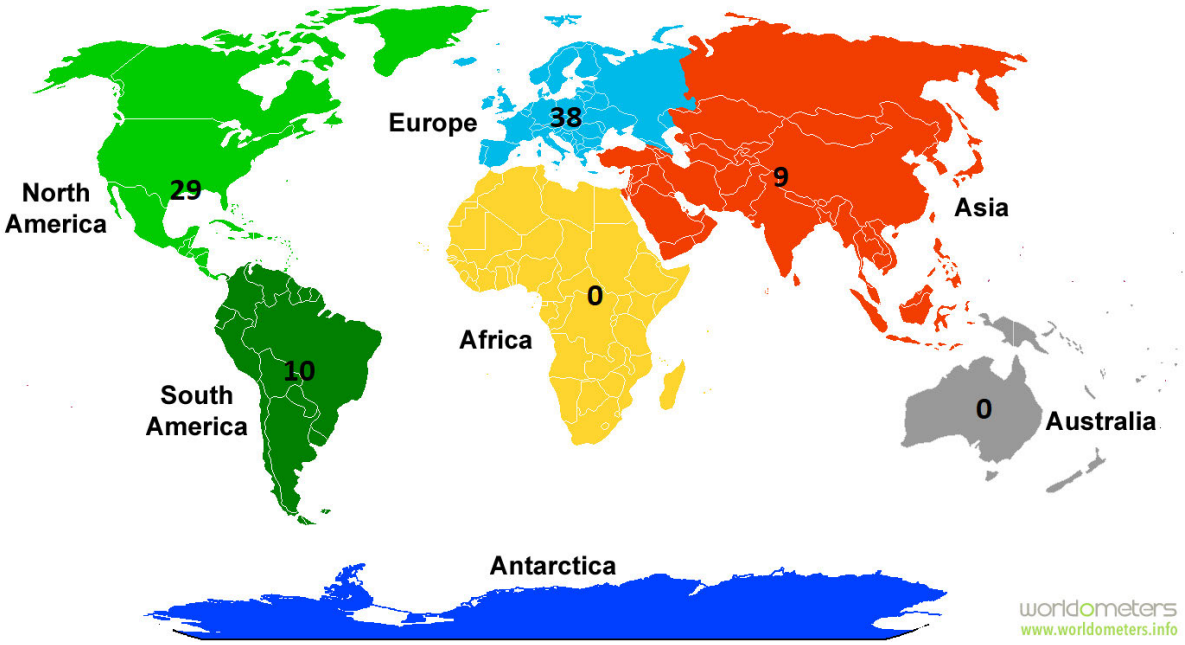


Figure 4: Number of articles included in the review by continent

CHAPTER 3

GENERAL METHODOLOGY

The current chapter describes design of the peptide microarrays and the practical methods shared by chapter 4 and chapter 5.

3.1 Peptide selection

The immunogenic peptides (B-cell epitopes) were retrieved from the PEPperPRINT and TIBA infectious disease epitope microarray databases. For novel peptides, a literature search for pathogen proteins was conducted in PubMed. The search focused on proteins found on the surface of the pathogens and secretory or excretory proteins. Protein sequences were then obtained either from NCBI (https://www.ncbi.nlm.nih.gov/) and Uniprot (https://www.uniprot.org/) protein databases. The prediction of linear B-cell epitopes on selected protein sequences was done using a bioinformatics tool ABCpred (45). The NCBI Protein BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi select Protein BLAST) bioinformatics tool was then used to select the peptides with the least cross-reactive. Predicted peptides with the ABCpred highest rank and with the least cross-reactivity with peptides from other human pathogens or proteins were selected for the study.

3.2 Peptide Microarray Design and Layout

The peptide microarray was customer designed to include three to five 9aa-18aa peptides derived from each pathogen generated in a laser-printer based approach by PEPperPRINT GmbH (Heidelberg, Germany) (https://www.pepperprint.com/). The peptide microarray contained 16 identical sub-arrays (copies) with 260 peptide positions on each sub-array. The peptides were printed with random distribution across each sub-array. Each sub-array was framed by flag anti-polio (KEVPALTAVETGAT, 3 spots) and flag anti-HA (hemagglutinin glycoprotein of influenza virus) (YPYDVPDYAG, 3 spots) as a quality control measurement. See **Figure 3.1** for details on peptide microarray layout.

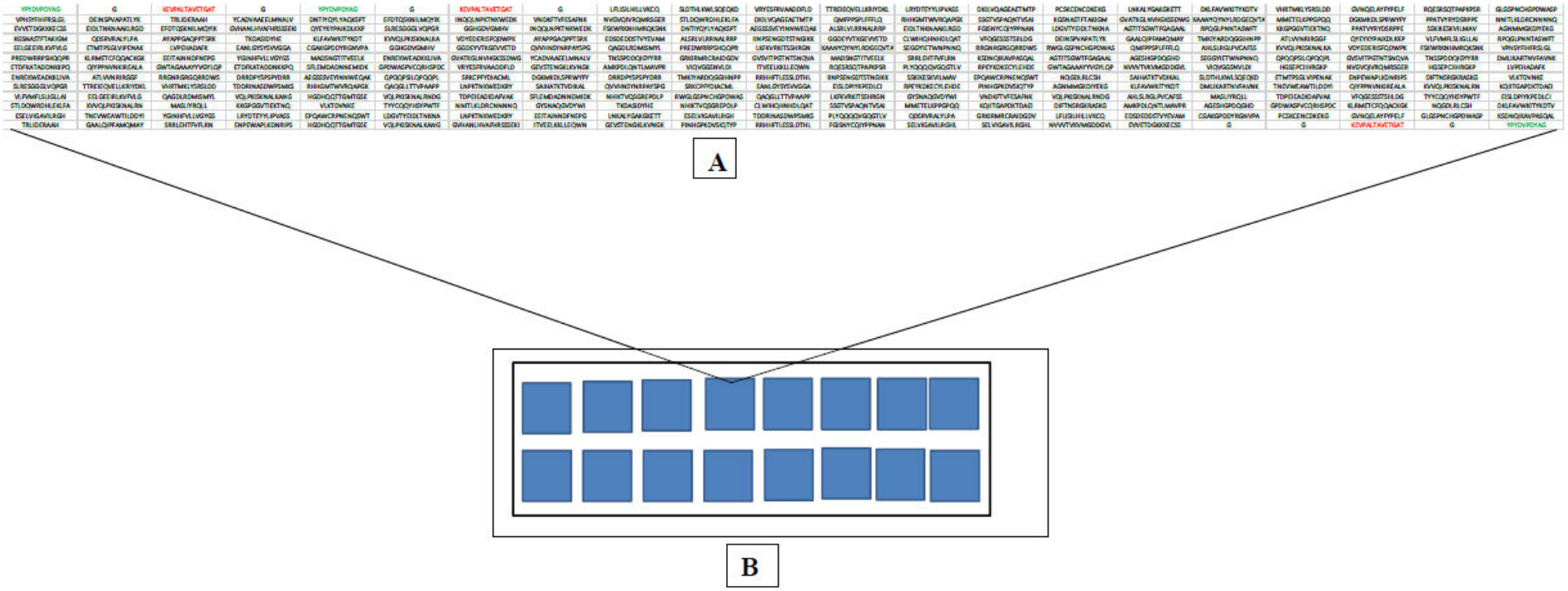


Figure 3.1: Peptide microarray chip design and layout. (**A**) Microarray peptide content, in black are 130 selected peptides printed with random distribution in duplicate to give 260 spots and in green and red are 3 HA and 3 Polio internal control spots. **(B)** Peptide microarray layout with 16 identical array copies (subarrays).

3.3 Peptide Microarrays Immunoassays

The immunoassays were performed in 3/16-well PEPperCHIP® Incubation Trays (PEPperPRINT GmbH, Germany), which allowed for the subdivision of the peptide microarray substrate glass slide into 16 separate incubation wells for each slide. The immunoassays consisted of two steps: the pre-incubation step for identifying false positive signals by binding of the fluorescently labelled secondary antibody followed by the main incubation with serum and the secondary antibodies. Each step involved pre-swelling of the peptide microarray with washing buffer (PBS, pH 7.4 with 0.05% Tween 20) for 10 minutes, followed by incubation with blocking buffer (Rockland blocking buffer MB-070) for 30 minutes. Initially, the peptide microarrays were incubated with secondary antibodies [Goat anti-human IgG (Fc) DyLight680 (0.1 µg/ml) and goat anti-human IgM (µ chain) DyLight800 (0.2 µg/ml)] and control antibodies [mouse monoclonal anti-HA DyLight800 (0.5 µg/ml)] diluted in incubation buffer (washing buffer with 10% blocking buffer) at room temperature for 45 minutes. In the main step the microarrays were incubated with serum or plasma diluted 1:250 in incubation buffer for 16 h at 4°C and 140 rpm orbital shaking followed by incubation with the secondary antibodies. After each incubation step the microarrays were washed three times with washing buffer for 10 seconds. The microarrays were scanned using an LI-COR Odyssey Imaging System; scanning offset 0.65 mm, resolution 21 µm, scanning intensities of 7/7 (red = 680 nm / green = 800 nm). To ensure that all microarrays were responding correctly, all steps were repeated with the Cy3-conjugated anti-influenza virus hemagglutin (HA) control antibody and Cy3-conjugated anti-polio control antibodies (PEPperPRINT GmbH, Germany).

Prior data quantification, all the peptide microarray scans were visually assessed for possible assay or microarray artifacts which would affect the experimental outcome. If required, the assays with individual samples were repeated on new peptide microarrays. For technical reasons, read-out for repeated samples was carried out with an Innopsys InnoScan 710-IR microarray scanner with matching scanner settings (PEPperPRINT GmbH, Germany).

3.4 Image analysis and spot intensity quantification

Quantification of spot intensities was based on 16-bit gray scale tiff files. Microarray image analysis was done with PepSlide® Analyzer (SICASYS Software GmbH; Heidelberg, Germany) and resulted in raw data CSV files for each sample (green = 800 nm = IgM staining, red = 680 nm = IgG staining). A PEPperPRINT software algorithm calculated averaged median foreground intensities (foreground-background signal) and spot-to-spot deviations of spot duplicates, and assembled the outcome in summary files. For duplicate spots, a maximum spot-to-spot deviation of 50% was tolerated, otherwise the corresponding intensity value was zeroed.

3.5 Statistical analysis

The data set used for statistical analysis of the peptide microarray results and for generation of all heat map presentations were based on fluorescence intensity. Duplicate fluorescence values were averaged in Microsoft excel 2013. Bar graphs were also drawn using Microsoft excel 2013. Heat maps were generated online using a Morpheus heat map widget (https://software.broadinstitute.org/morpheus/).

Descriptive statistics was used to summarize the data. Frequency distributions of numerical data was examined for normality and means (SD), or medians (IQR) used as appropriate. The Kolmogorov Smirnov normality test was used to determine data distribution. The Mann-Whitney U test was used to measure non-parametric data (median and inter-quartile range) in SPSS 16.0. Area under the ROC curve (AUC) was measured for each peptide using STATA/MP16.0. P-values lower than 0.05 were considered statistically significant.

3.6 Antibody Reactivity and Discrimination of Infection by Detection of Immunodominant Epitopes.

A positive peptide response was defined as fluorescence intensity ≥ 500 fluorescence intensity units (FU) for both IgG and IgM against each peptide (46). Immunodominant peptides were identified using color-coded heat maps and bar graphs reflecting obtained fluorescence signal intensities. The ability of peptides to distinguish between the infected and uninfected groups was statistically evaluated using the Mann-Whitney-U test. Diagnostic accuracy of the peptides was evaluated by ROC curve analysis and AUC was calculated to assess the overall diagnostic performance (**Table 1**) of peptide.

Table 1**:** Area under the ROC curve and the diagnostic performance of each peptide.

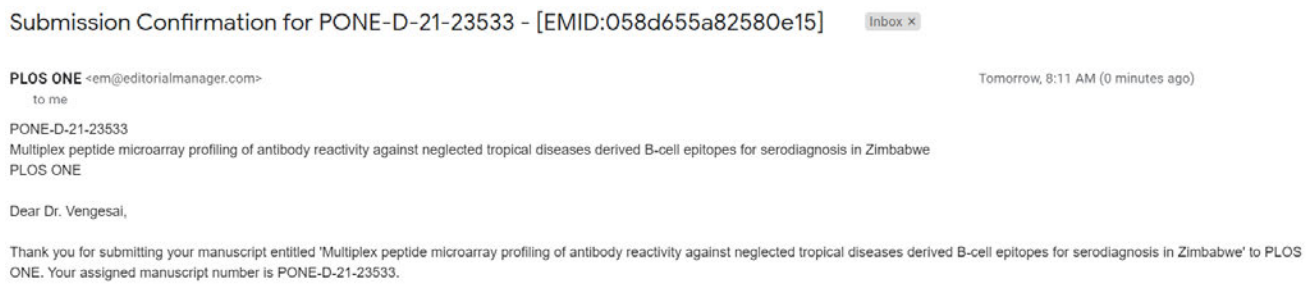
AUC	**Quality of peptide/diagnostic performance**
1	Perfectly accurate test
0.9-1	Outstanding
0.8-0.9	excellent
0.7-0.8	Fair/acceptable
0.6-0.7	Poor
0.5-0.6	Fail
0	perfectly inaccurate test

CHAPTER 4

MULTIPLEX PEPTIDE MICROARRAY PROFILING OF ANTIBODY REACTIVITY AGAINST NEGLECTED TROPICAL DISEASES DERIVED B-CELL EPITOPES FOR SERODIAGNOSIS IN ZIMBABWE.

The following manuscript aimed to develop and evaluate a multiplex peptide microarray for the simultaneous serological detection of *S. mansoni*, *S. haematobium*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Necator americanus*, lymphatic filariasis (*Wuchereria bancroftii*) and trachoma (Chlamydia *trachomatis*) in people living in NTDs endemic areas in Zimbabwe".

This chapter was submitted to PLOS ONE manuscript number PONE-D-21-23533.



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Multiplex peptide microarray profiling of antibody reactivity against neglected tropical diseases derived B-cell epitopes for serodiagnosis in Zimbabwe.

Short title: Peptide microarray of NTDs derived B-cell epitopes

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Abstract

Introduction

Peptides (B-cell epitopes) have broad applications in disease diagnosis and surveillance of pathogen exposure. In this framework, we designed and produced a peptide microarray for the integrated surveillance of neglected tropical diseases. The peptide microarray was evaluated against peptides derived from *Ascaris lumbricoides*, *Necator americanus*, *Schistosoma haematobium*, *Schistosoma mansoni*, *Trichuris trichiura*, *Bacillus anthracis, Mycobacterium leprae, Wuchereria bancrofti*, *Rabies lyssavirus, Chlamydia trachomatis* and *Trypanosoma brucei.*

Methods

S. haematobium was diagnosed using the urine filtration technique. *S. mansoni*, *A. lumbricoides*, *N. americanus* and *T. trichuria* were diagnosed using the Kato Katz and formal ether concentration techniques. Immunogenic peptides were retrieved from the Tackling Infection to Benefit Africa infectious diseases epitope microarray. Further peptides were predicted using ABCpred. IgG and IgM reactivity against the derived peptides were evaluated using peptide microarray multiplex immunoassays. Positive response was defined as fluorescence intensity ≥ 500 fluorescence units. Immunodominant peptides were identified using color-coded heat maps and bar graphs reflecting the obtained fluorescence signal intensities. Receiver Operating Characteristic (ROC) analysis and Mann-Whitney-U test were performed to determine the diagnostic validity of the peptides.

Results

Species-specific responses with at least one peptide derived from each NTD pathogen were observed. The reactive peptides included; for *S. haematobium*, XP_035588858.1-206-220 and XP_035588858.1-206-220 immunodominant for IgG and IgM respectively, for *S.mansoni*, P20287.1-58-72 immunodominant for both antibodies and for *T. trichuria*, CDW52482.1-326-340 immunodominant for IgG and CDW57769.1-2017-2031 and CDW57769.1-1518-1532 immunodominant for IgM. According to ROC analysis most of the peptides selected were inaccurate; with AUC < 0.5. Some peptides had AUC values ranging from 0.5 to 0.5875 for both IgM and IgG suggesting no discrimination.

Conclusion

Species-specific sero-reactivity was indicative of exposure to the different NTDs parasites antigens. Peptide microarrays may be a valuable tool for integrated NTDs surveillance and for the multiplex screening of exposure in endemic areas.

Keywords: B-cell epitopes, epitope prediction, peptide microarrays, diagnostics, neglected tropical diseases, IgG and IgM

Introduction

Neglected Tropical Diseases (NTDs) are a group of 20 bacterial, parasitic and viral chronic infectious diseases that affect over 1.7 billion people globally and are particularly endemic to the tropical and subtropical regions (1,2). They include, schistosomiasis (mainly caused by *Schistosoma haematobium*, *Schistosoma mansoni and Schistosoma japonicum*), soil-transmitted helminthiasis (STH) (commonly caused by *Ascaris lumbricoides, Necator americanus and Trichuris trichuris*), lymphatic filariasis (caused by *Wuchereria bancrofti),* blinding trachoma (caused by *Chlamydia trachomatis*), leprosy (caused by *Mycobacterium leprae*) and Human African trypanosomiasis (caused *Trypanosoma brucei*) (1–4). NTDs also include zoonotic diseases such as rabies (caused by *Rabies lyssavirus*) and anthrax (caused by *Bacillus anthracis*) (5).

NTDs are intimately related to poverty and they tend to cluster in the same poor populations (6,7). Zimbabwe is located in the Southern region of Africa and is endemic to four of the most common NTDs; Schistosomiasis, STH, lymphatic filariasis and trachoma. In 2016 nearly 10 million Zimbabweans required preventative chemotherapy for at least 1 NTD (8) NTD mapping results showed that of the 63 districts in Zimbabwe, 56 are endemic for schistosomiasis, 47 are endemic for STH and 39 for *lymphatic filariasis* (9,10).

Engels and Savioli suggested that there is need for an integrated approach to eradicate NTDs (7). Advances in serological multiplex immunoassays have created enormous potential for large-scale, integrated NTDs surveillance (11). The parallel detection of antibodies has a wide range of potential applications in the diagnosis and surveillance of NTDs as well as in epitope mapping studies, therapeutics and vaccines development (12,13). Peptide microarrays provide rapid and high-throughput immunoassay platforms for the simultaneous identification of B-cell epitopes derived from different NTDs parasites. B-cell epitopes have broad applications in the development of peptide based vaccines, in NTDs diagnosis and surveillance of pathogen exposure (14).

In this framework, we designed and produced a peptide microarray in a laser-printer based approach and validated the microarray using human serum and plasma samples from three rural districts in Zimbabwe. The peptide microarray carried a panel of fifty-one, 9 -18 amino acids B-cell epitopes derived from *A. lumbricoides, N. americanus, S. haematobium, S. mansoni, T. trichiura, B. anthracis, M. Leprae, W. bancrofti*, *Rabies lyssavirus, C. trachomatis* and *T. brucei*. Peptide microarray immunoassays were also established to evaluate the diagnostic performance of peptides derived from *A. lumbricoides, N. americanus, S. haematobium, S. mansoni* and *T. trichiura*.

Materials and Methods

Ethical approval

Ethical approval was obtained from the Medical Research Council of Zimbabwe (MCRZ/A/2571 and MRCZ/A/2443). Permission to conduct the study in the districts was granted by the Provincial Medical Directors, District Medical Officers, councillors and village head-men. Participants provided written consent prior to recruitment. Parents and guardians provided written consent for children after the children had given their assent. Prior to recruitment study objectives were explained to the participants, parents and guardians in both Shona and English languages.

Study Population and Area

Serum and plasma samples were obtained from villagers living in the NTDs endemic areas who agreed to provide urine and stool specimens for parasitological examinations. The villagers were from Shamva, Murewa and Makoni rural districts in Zimbabwe. Shamva and Murewa rural districts are located in Mashonaland Central province (31°40′0" E longitude and 17°10′0" S latitude) and Mashonaland East province (17°38′49″S latitude and 31°46′39″E longitude) in north eastern Zimbabwe respectively, whilst, Makoni district is located in Manicaland province in eastern Zimbabwe (18°32'09.2"S latitude and 32°07'18.9"E longitude).

Parasitological examination

Urine and stool specimens were collected between 10:00 am and 14:00 pm for optimal egg passage necessary for diagnosis of schistosomiasis and STH. The samples were placed in wooden boxes away from sunlight until they were processed and examined. *S. haematobium* was diagnosed by the microscopic examination of urine for parasites eggs using the urine filtration technique. The technique was repeated for three consecutive days in order to avoid misdiagnosis due to day-to-day variation in egg excretion (15). Stool samples were examined for the ova of *T. trichiura, N. americanus, A. lumbricoides* and *S. mansoni* using the Kato-Katz technique and the formal ether concentration technique (16,17) Participants were classified as infected if at least one parasitic egg was detected. Participants who tested positive for schistosomiasis and STH were referred to the nearest health centres for treatment. It is noteworthy that no parasitology diagnosis was conducted for *B. anthracis, M. Leprae, W. bancroftii, R. lyssavirus, C. trachomatis* and *T. brucei.*

Peptide selection

The immunogenic peptides (B-cell epitopes) were retrieved from the PEPperPRINT and TIBA infectious disease epitope microarray databases. For novel peptides, a literature search for pathogen proteins was conducted in PubMed. The search focused on proteins found on the surface of the pathogens and secretory or excretory proteins. Protein sequences were then obtained either from NCBI (https://www.ncbi.nlm.nih.gov/) and Uniprot (https://www.uniprot.org/) protein databases. The prediction of linear B-cell epitopes on selected protein sequences was done using a bioinformatics tool ABCpred (18). The NCBI Protein BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi select Protein BLAST) bioinformatics tool was then used to select the peptides with the least cross-reactive. Predicted peptides with the ABCpred highest rank and with the least cross-reactivity with peptides from other human pathogens or proteins were selected for the study.

Peptide Microarray Design and Layout

The peptide microarray was customer designed to include three to five 9aa-18aa peptides derived from each pathogen and was generated in a laser-printer based approach by PEPperPRINT GmbH (Heidelberg, Germany) (https://www.pepperprint.com/). The peptide microarray contained 16 identical sub-arrays (copies) with 260 peptide positions on each sub-array. Fifty-one duplicate NTDs peptides (details given in **Table 1**) were printed with random distribution across each sub-array. Each sub-array was framed by flag anti-polio (KEVPALTAVETGAT, 3 spots) and flag anti-HA (hemagglutinin glycoprotein of influenza virus) (YPYDVPDYAG, 3 spots) as a quality control measurement. See **Figure 1** for details on peptide microarray layout.

Table 1: TIBA infectious disease epitope microarray and ABCpred selected B-cell epitopes

Peptide name	Linear sequance	Source organism	Source Molecule/ protein	Peptide prediction and selection
CAA60047.1-**553-568**	TMKIYARDQGGIHNPP	*Ascaris lumbricoides*	Cytoplasmic intermediate filament protein	ABC PRED
ACJ03763.1-**50-64**	TDPEIEADIDAFVAK	*Ascaris lumbricoides*	Ag2	TIBA infectious disease epitope microarray
ACJ03764.1-**3852-38-52**	KQIITGAPDKTDAEI	*Ascaris lumbricoides*	Ag1	TIBA infectious disease epitope microarray
AAD13652.1-**133-147**	RRHHFTLESSLDTHL	*Ascaris lumbricoides*	Aba-1 Allergen, Partial	TIBA infectious disease epitope microarray
AAD13652.1-**142-156**	SLDTHLKWLSQEQKD	*Ascaris lumbricoides*	Aba-1 Allergen Partial	TIBA infectious disease epitope microarray
WP_151277871.1-**342-358**	GVATKGLNVHGKSSDWG	*Bacillus anthracis*	anthrax toxin edema factor	TIBA infectious disease epitope microarray
WP_040119992.1-**736-358**	IINPSENGDTSTNGIKK	*Bacillus anthracis*	Chain A Anthrax Protective Antigen	TIBA infectious disease epitope microarray
WP_001022096.1-**35-44**	GGHGDVGMHV	*Bacillus anthracis*	Lethal Factor	TIBA infectious disease epitope microarray
WP_151277871.1-**307-316**	LVPEHADAFK	*Bacillus anthracis*	Edema Factor	TIBA infectious disease epitope microarray
P0C0Z7.2-**260-270**	ATLVVNRIRGGF	*Chlamydia Trachomatis*	60 Kda Chaperonin (Protein Cpn60) (Groel Protein) (57 Kda Chlamydial Hypersensitivity Antigen) (Heat Shock Protein 60) (Hsp60)	TIBA infectious disease epitope microarray
P19542.1-**261-270**	TKDASIDYHE	*Chlamydia Trachomatis*	Major Outer Membrane Porin, Serovar L1 Precursor (Momp)	TIBA infectious disease epitope microarray
AAO67542.1-**291-305**	LKFKVRKITSSHRGN	*Chlamydia Trachomatis*	Outer Membrane Protein Porb	TIBA infectious disease epitope microarray
AAA23156.1-**381-390**	TRLIDERAAH	*Chlamydia Trachomatis*	Major Outer Membrane Protein	TIBA infectious disease epitope microarray
AAA23156.1-**73-81**	VLKTDVNKE	*Chlamydia Trachomatis*	Major Outer Membrane Protein	TIBA infectious disease epitope microarray
CAA43269.1-**311-325**	AMKPDLQNTLMAVPR	*Mycobacterium Leprae*	Antigen 85-B Precursor	TIBA infectious disease epitope microarray
WP_010907696.1-**11-25**	DKILVQAGEAETMTP	*Mycobacterium Leprae*	co-chaperone GroES	TIBA infectious disease epitope microarray
WP_010907618.1-**27-41**	LDGVTYEIDLTNKNA	*Mycobacterium Leprae*	Protein Lsr2 Precursor	TIBA infectious disease epitope microarray
CAA37572.1-**10-24**	EIDLTNKNAAKLRGD	*Mycobacterium Leprae*	Lsr2	TIBA infectious disease epitope microarray
WP_010907696.1-**1-15**	ETMTPSGLVIPENAK	*Mycobacterium Leprae*	co-chaperone GroES	TIBA infectious disease epitope microarray
CAC00543.1-**123-135**	SRKCPFYDIACML	*Necator Americanus*	Necepsin Ii	TIBA infectious disease epitope microarray
AAP41952.1-**180-192**	AGNMMGKDIYEKG	*Necator americanus*	Secreted Protein Asp-2	TIBA infectious disease epitope microarray
AAP41952.1-**194-206**	PCSKCENCDKEKG	*Necator americanus*	Secreted Protein Asp-2	TIBA infectious disease epitope microarray
AHC94315.1-**209-222**	DIFTNSRGKRASKG	*Rabies lyssavirus*	Glycoprotein G Precursor	ABC PRED
AAD10459.1-**312-325**	VPHSYFIHFRSLGL	*Rabies lyssavirus*	ribonucleoprotein (RNP)	ABC PRED
BAJ04981.1-**12-26**	QYEYKYPAIKDLKKP	*Rabies lyssavirus*	Nucleoprotein	TIBA infectious disease epitope microarray
QEJ74712.1-**99-113**	NVGVQIVRQMRSGER	*Rabies lyssavirus*	Phosphoprotein	TIBA infectious disease epitope microarray
XP_012799745.1-**16-30**	SFLEMDADNNEMIDK	*Schistosoma haematobium*	putative 22.6 kDa tegument antigen	TIBA infectious disease epitope microarray
XP_035587815.1-**269-283**	EISLDPIYKPEDLCI	*Schistosoma haematobium*	putative heat shock protein hsp16	TIBA infectious disease epitope microarray
XP_035588858.1-**206-220**	EDSDEDDSTVYEVAM	*Schistosoma haematobium*	putative cleavage and polyadenylation specificity factor	TIBA infectious disease epitope microarray
XP_012797374.1-**78-92**	NHIKTVQSGREPDLP	*Schistosoma haematobium*	Antigen Sm21.7	TIBA infectious disease epitope microarray
AAZ29530.1-**25-29**	PINHGPKDVSIQTYP	*Schistosoma haematobium*	Tegumental Protein Sh13	TIBA infectious disease epitope microarray
P09792.1-**29-43**	VDYEDERISFQDWPK	*Schistosoma mansoni*	Glutathione S-Transferase Class-Mu 28 Kda Isozyme	TIBA infectious disease epitope microarray
P20287.1-**58-72**	GEVSTENGKLKVNGK	*Schistosoma mansoni*	Glyceraldehyde-3-Phosphate Dehydrogenase (Gapdh) (Major Larval Surface Antigen) (P-37)	TIBA infectious disease epitope microarray

AAA29900.1-**145-159**	CGAKGPDDYRGNVPA	*Schistosoma mansoni*	23 Kda Integral Membrane Protein (Sm23)	TIBA infectious disease epitope microarray
P09841.3-**6-20**	LFLISILHILLVKCQ	*Schistosoma mansoni*	Hemoglobinase Precursor (Antigen Sm32)	TIBA infectious disease epitope microarray
AAA29903.1-**222-237**	KSDNQIKAVPASQAL	*Schistosoma mansoni*	Major Egg Antigen	TIBA infectious disease epitope microarray
CDW57769.1-**1518-1532**	VRYESFRVAADDFLD	*Trichuris trichiura*	Parp And Wgr And Ank 2 Domain Containing Protein	TIBA infectious disease epitope microarray
CDW57769.1-**659-673**	DMLIKARTNVFAVNK	*Trichuris trichiura*	Parp And Wgr And Ank 2 Domain Containing Protein	TIBA infectious disease epitope microarray
CDW57769.1-**834-848**	STLDQWRDHLEKLFA	*Trichuris trichiura*	Parp And Wgr And Ank 2 Domain Containing Protein	TIBA infectious disease epitope microarray
CDW52482.1-**326-340**	TNEVWEAWTILDDYI	*Trichuris trichiura*	Wap Domain Containing Protein Slpi-Like	TIBA infectious disease epitope microarray
CDW57769.1-**2017-2031**	RPEYKDKECYLEHDE	*Trichuris trichiura*	Parp And Wgr And Ank 2 Domain Containing Protein	TIBA infectious disease epitope microarray
APD74596.1-**96-110**	ENREKWEADKKLIVA	*Trypanosoma brucei*	Variant Surface Glycoprotein	TIBA infectious disease epitope microarray
CAC33895.1-**78-92**	ETDFKATADDNKKPQ	*Trypanosoma brucei*	VSG protein	TIBA infectious disease epitope microarray
XP_844815.1-**369-382**	SSKIKESKVILMAV	*Trypanosoma brucei gambiense*	64 kDa invariant surface glycoprotein	ABC PRED
CAC33895.1-**163-176**	LNKALYGAKGKETT	*Trypanosoma brucei gambiense*	variant surface glycoprotein LiTat 1.3	ABC PRED
XP_011774209.1-**132-145**	SAIHATKTVDIKAL	*Trypanosoma brucei gambiense DAL972*	mitogen-activated protein kinase 5	ABC PRED
AAC35355.1-**55-68**	EVVETDGKKKECSS	*Wuchereria bancroftii*	Abundant Larval Transcript-2 Protein	ABC PRED
AAC35355.1-**46-60**	GGDEYVTKGEVVETD	*Wuchereria bancroftii*	Abundant Larval Transcript-2 Protein	TIBA infectious disease epitope microarray
AAC35355.1-**77-91**	EPQAWCRPNENQSWT	*Wuchereria bancroftii*	Abundant Larval Transcript-2 Protein	TIBA infectious disease epitope microarray
AAC70783.1-**177-191**	FSKWRKNHMRQKSNK	*Wuchereria bancroftii*	Sxp Antigen	TIBA infectious disease epitope microarray
ADP24698.1-**29-43**	TDDRINASDWPSMKS	*Wuchereria bancroftii*	Glutathione S-Transferase pi class	TIBA infectious disease epitope microarray

The peptide name consisted of the protein/antigen accession number followed by the amino acids positions of the peptide in the protein.

Peptide Microarrays Immunoassays

The immunoassays were performed in 3/16-well PEPperCHIP® Incubation Trays (PEPperPRINT GmbH, Germany), which allowed for the subdivision of the peptide microarray substrate glass slide into 16 separate incubation wells for each slide. The immunoassays consisted of two steps: the pre-incubation step for identifying false positive signals by binding of the fluorescently labelled secondary antibody followed by the main incubation with serum and the secondary antibodies.

Each step involved pre-swelling of the peptide microarray with washing buffer (PBS, pH 7.4 with 0.05% Tween 20) for 10 minutes, followed by incubation with blocking buffer (Rockland blocking buffer MB-070) for 30 minutes.

Initially the peptide microarrays were incubated with secondary antibodies [Goat anti-human IgG (Fc) DyLight680 (0.1 µg/ml) and goat anti-human IgM (µ chain) DyLight800 (0.2 µg/ml)] and control antibodies [Mouse monoclonal anti-HA DyLight800 (0.5 µg/ml)] diluted in incubation buffer (washing buffer with 10% blocking buffer) at room temperature for 45 minutes. In the main step the microarrays were incubated with serum or plasma diluted 1:250 in incubation buffer for 16 h at 4°C and 140 rpm orbital shaking followed by incubation with the secondary antibodies. After each incubation step the microarrays were washed three times with washing buffer for 10 seconds. The microarray were scanned using an LI-COR Odyssey Imaging System; scanning offset 0.65 mm, resolution 21 µm, scanning intensities of 7/7 (red = 680 nm / green = 800 nm). To ensure that all microarrays were responding correctly, all steps were repeated with the Cy3-conjugated anti-HA control antibody and Cy3-conjugated anti-polio control antibodies.

Prior data quantification, all the peptide microarray scans were visually assessed for possible assay or microarray artifacts which would affect the experimental outcome. If required, the assays with individual samples were repeated on new peptide microarrays. For technical reasons, read-out for repeated samples was carried out with an Innopsys InnoScan 710-IR microarray scanner with matching scanner settings.

Image analysis and spot intensity quantification

Quantification of spot intensities was based on 16-bit gray scale tiff files. Microarray image analysis was done with PepSlide® Analyzer ((SICASYS Software GmbH; Heidelberg, Germany) and resulted in raw data CSV files for each sample (green = 800 nm = IgM staining, red = 680 nm = IgG staining). A PEPperPRINT software algorithm calculated averaged median foreground intensities (foreground-background signal) and spot-to-spot deviations of spot duplicates, and assembled the outcome in summary files. For duplicate spots a maximum spot-to-spot deviation of 50% was tolerated, otherwise the corresponding intensity value was zeroed.

Statistical Analysis

Age was expressed as median and interquartile range (IQR). The data set used for statistical analysis of the peptide microarray results and for generation of all heat map presentations were based on fluorescence intensity. Duplicate fluorescence values were averaged in Microsoft excel 2013. Bar graphs were also drawn using Microsoft excel 2013.

Heat maps were generated online using Morpheus heat map widget (https://software.broadinstitute.org/morpheus/). Non-parametric statistical methods were used for data analysis. Univariate comparisons of two independent groups were done using the Mann-Whitney-U test in SPSS 16.0. Area under the ROC curve (AUC) was measured for each peptide using STATA/MP16.0. P-values lower than 0.05 were considered statistically significant.

Antibody Reactivity and Discrimination of Infection by Detection of Immunodominant Epitopes.

A positive peptide response was defined as fluorescence intensity ≥ 500 Relative Fluorescent Units (RFU) for both IgG and IgM against each peptide (19). Immunodominant peptides were identified using color-coded heat maps and bar graphs reflecting obtained fluorescence signal intensities. The ability of peptides to distinguish between the infected and uninfected groups was statistically evaluated using the Mann-Whitney-U test. Diagnostic accuracy of the peptides was evaluated by ROC curve analysis and AUC was calculated to assess the overall diagnostic performance (**Table 2**) of peptide.

Table 2: Area under the ROC curve and the diagnostic performance of each peptide.

AUC	**Quality of peptide/diagnostic performance**
1	Perfectly accurate test
0.9-1	Outstanding
0.8-0.9	excellent
0.7-0.8	Fair/acceptable
0.6-0.7	Poor
0.5-0.6	Fail
0	perfectly inaccurate test

Results

Demography and parasitology data

The study consisted of 170 participants of which 49.1% were males, of all age groups with a median age of 11 (interquartile range 5.25-30.00). Madziwa had 74.7% (127) of the participants while the participants in Murewa and Makoni were 17.1% (29) and 8.2% (14) respectively. Among the villagers included in the study, 4 were infected with *A. lumbricoides*, 14 with *N. americanus*, 61 with *S. haematobium*, 44 with *S. mansoni* and 6 with *T. trichuria* whilst 60 were uninfected and were used as controls during analysis.

Discrimination of *Ascaris lumbricoides* infection

Ascaris lumbricoides derived peptides were reactive with IgG except peptide CAA60047.1-553-568 (TMKIYARDQGGIHNPP) which did not react with neither *Ascaris lumbricoides* infected nor uninfected samples. Peptide ACJ03764.1-3852-38-52 (KQIITGAPDKTDAEI) gave the highest response with a fluorescence intensity of 13563.25 RFU with sera from the *Ascaris lumbricoides* infected group. For IgM, all *Ascaris lumbricoides* derived peptides were reactive with at least one sera from either the *Ascaris lumbricoides* infected or uninfected group. In contrast with IgG peptide ACJ03763.1-50-64 (TDPEIEADIDIAFVAK) gave the highest fluorescence intensity 2806.5 RFU. Looking at the heat maps for *Ascaris lumbricoides* derived peptide there was no immunodominant peptide (**supplementary File S1**). None of the peptides showed a clear discrimination between the *Ascaris Lumbricoides* infected and uninfected group (**Table 3)**.

Table 3: Diagnostic performance of selected peptides

Pathogen	Peptide name	Peptide	IgM Median		IgG Median	
			AUC	p-value	AUC	p-value
Ascaris lumbricoides	ACJ03764.1-3852-38-52	KQIITGAPDKTDAEI	0.4428224	0.847	0.3746959	0.667
	AAD13652.1-133-147	RRHHFTLESSLDTHL	**0.5136032**	0.655	**0.540146**	0.173
	AAD13652.1-142-156	SLDTHLKWLSQEQKD	0.4190998	0.571	0.3527981	0.712
	ACJ03763.1-50-64	TDPEIEADIDAFVAK	**0.5346715**	0.270	**0.5851582**	**0.061**
	CAA60047.1-553-568	TMKIYARDQGGIHNPP	0.4890511	0.438	0.080292	0.361
Necator americanus	AAP41952.1-180-192	AGNMMGKDIYEKG	0.2278912	0.34	**0.7278912**	0.218
	CAC00543.1-123-135	SRKCPFYDIACML	0.00000	0.826	0.00000	0.686
	AAP41952.1-194-206	PCSKCENCDKEKG	0.4115646	0.335	0	0.653
S. haematobium	XP_035588858.1-206-220	EDSDEDDSTVYEVAM	**0.5110029**	0.811	**0.5777417**	**0.037**
	XP_035587815.1-269-283	EISLDPIYKPEDLCI	0.485318	0.765	**0.5435057**	0.135
	XP_012797374.1-78-92	NHIKTVQSGREPDLP	0.3930375	0.572	0.3919553	0.340
	AAZ29530.1-25-29	PINHGPKDVSIQTYP	**0.5373107**	0.219	0.4440795	0.58
	XP_012799745.1-16-30	SFLEMDADNNEMIDK	0.4098124	0.306	0.4451659	0.954
S. mansoni	P20287.1-58-72	GEVSTENGKLKVNGK	0.2385246	0.215	0.1920765	0.001
	AAA29903.1-222-237	KSDNQIKAVPASQAL	0.1948087	0.124	0.1295082	**0.062**
	P09841.3-6-20	LFLISILHILLVKCQ	0.2928962	0.739	0.097541	0.389
	AAA29900.1-145-159	CGAKGPDDYRGNVPA	0.3770489	0.215	0.423224	0.001
	P09792.1-29-43	VDYEDERISFQDWPK	0.3612022	0.020	0.3800546	0.052
Trichuris trichuria	CDW57769.1-659-673	DMLIKARTNVFAVNK	0.3888889	**0.007**	0.3569444	0.236
	CDW57769.1-2017-2031	RPEYKDKECYLEHDE	**0.5347222**	0.792	0.3722222	0.343
	CDW57769.1-834-848	STLDQWRDHLEKLFA	**0.5875**	0.304	0.1722222	0.069
	CDW52482.1-326-340	TNEVWEAWTILDDYI	0.4601399	0.744	**0.5482517**	0.710
	CDW57769.1-1518-1532	VRYESFRVAADDFLD	0.4569444	0.744	0.525	0.833

Discrimination of *Necator americanus* infection

Peptide AAP41952.1-180-192 (AGNMMGKDIYEKG) was the only reactive peptide for IgM reacting with 6 samples from the negative control group with highest response being 2112. 5 RFU. For IgG no reactivity was observed with fluorescence intensity less than 400 RFU for all the *Necator americanus* derived peptide in the *Necator americanus* infected and uninfected groups (**Supplementary File S1)**. Like the heat maps for *Ascaris lumbricoides* derived peptides there was no immunodominant peptide for the *Necator americanus* peptides and none of the peptides showed a clear discrimination between the *Necator americanus* infected and uninfected groups **(Table 3)**.

Discrimination of *S. haematobium* infection

S. haematobium derived peptides were all reactive with IgG with high fluorescence intensities observed in the infected group compared to the uninfected group across all the peptides. Peptide XP_012799745.1-16-30 (SFLEMDADNNEEMIDK) gave the highest response 8576 RFU and by observing the heat maps XP_035588858.1-206-220 (EDSDEDDSTVYEVAM) appeared to be the immunodominant peptide for the *S. haematobium* derived peptides. Peptide XP_035588858.1-206-220 showed discrimination between the *S. haematobium* infected and uninfected group $p<0.037$, however it had an AUC of 0.5777417 (**Figure 3**). Likewise all *S. haematobium* derived peptides reacted with IgM with high fluorescence intensities observed in the infected group compared to the uninfected group across all peptides. Peptide XP_035588858.1-206-220 (EDSDEDDSTVYEVAM) was observed to be the immunodominant peptide and it gave the highest response of 12610 RFU. However, none of the peptides showed a clear discrimination between infected and uninfected groups including XP_035588858.1-206-220 (**Table 3**).

Figure 2: Heat maps and bar graphs generated from peptide microarray data for *S. haematobium*. (A) Heat maps generated from peptide microarray. Samples were arranged in rows and infection status shown on the left key. Peptides shown by their protein accession number and sequence position are arranged in columns. (B) Bar graphs representing the peptide reactivity for each serum and plasma in both the infected and uninfected groups in the study.

Discrimination of *S. mansoni* infection

Peptide microarray technology exhibited levels of IgM reactivity against peptides derived from *S. mansoni* antigens for both *S. mansoni* infected and uninfected groups, with the exception of AAA29903.1-222-237 which did not react with the *S. mansoni* uninfected group. For IgG the technology exhibited reactivity for three peptides; P09792.1-29-43 (VDYEDERISFQDWPK) (reacting with 3 samples from the uninfected group), P20287.1-58-72 (GEVSTENGKLKVNGK) (reacting with 1 sample from the uninfected group) and AAA29903.1-222-237 (KSDNQIKAVPASQAL) (reacting with 1 sample from the infected group) with RFU values of 1482.25, 1144 and 532.5, respectively. Examination of the heat maps revealed that peptide P20287.1-58-72 (GEVSTENGKLKVNGK) was the immunodominant peptide for both IgG and IgM (**Supplementary File S1)**. However none of the peptides showed a clear discrimination between *S. mansoni* infected and the uninfected groups **(Table 3).**

Discrimination of *Trichuris trichuria* infection

Peptide CDW57769.1-659-673 (DNLIKARTNVFAVNK) was the only *Trichuris trichuria* derived peptide that was not reactive with IgG and peptide CDW52482.1-326-340 (TNEVWEAWTILDDYI) gave the highest RFU value of 8572 with sera from the *Trichuris trichuria* uninfected group. For IgM, all the peptides showed immunoreactivity with fluorescence intensities above than 500 RFU for all the peptide in the *Trichuris trichuria* infected and uninfected groups. Visual inspection of the heat maps showed that peptide CDW52482.1-326-340 was immunodominant for IgG and peptide CDW57769.1-2017-2031 (RPEYKDKECYLEHDE) and peptide CDW57769.1-1518-1532 (VRYESFRVAADDFLD) were immunodominant for IgM (**Supplementary File S1)**. None of the peptides showed a clear discrimination between the *Trichuris trichuria* infected and uninfected group (**Table 3**).

Antibody Reactivity Against Peptides Derived from *Bacillus anthracis* Proteins.

Peptide microarray technology showed IgG reactivity against two peptides derived from *Bacillus anthracis* antigens, WP_001022096.1-35-44 (GGHGDVGMHV) and WP_040119992.1-736-358 (IINPSENGDTSTNGIKK) with RFU values of 1034 and 665.25, respectively. For IgM all the peptides were responsive and WP_001022096.1-35-44 gave the highest fluorescence intensity value of 2502.5 RFU. The heat maps indicated that peptide WP_001022096.1-35-44 appeared to be immunodominant for pathogens (**Supplementary File S2).**

Antibody Reactivity Against Peptides Derived from *Mycobacterium leprae* Proteins.

Peptide microarray technology showed that all *Mycobacterium leprae* derived peptides were responsive with at least one plasma or serum sample for both IgG and IgM. Peptide WP_010907696.1-11-25 (DKILVQAGEAETMTP) gave the highest fluorescence intensity for both IgG (2869 RFU) and IgM (7803.5 RFU). The heat maps indicated that peptide CAA37572.1-10-24 (EIDLTNKNAAKLRGD) was immunodominant for both IgG and IgM (**Supplementary File S2)**.

Antibody Reactivity Against Peptides Derived from *Wuchereria bancrofti* Proteins.

Peptide microarray technology showed that both IgG and IgM were reactive with *Wuchereria bancrofti* derived peptides except for AAC35355.1-55-68 (EVVETDGKKKECSS) which had a fluorescent intensity of 494 RFU for IgG. Peptide AAC35355.1-46-60 (GGDEYVTKGEVVETD) gave the highest fluorescence intensity for both IgG (6680.75 RFU) and IgM (3111 RFU). The heat maps indicate that peptide AAC35355.1-46-60 was also immunodominant for both IgG and IgM (**Supplementary File S2)**.

Antibody Reactivity Against Peptides Derived from *Rabies Lyssavirus* Proteins.

Peptide microarray technology showed that only one *Rabies lyssavirus* peptide BAJ04981.1-12-26 (QYEYKYPAIKDLKKP) was reactive with IgG with a fluorescence intensity of 1048 RFU. For IgM all peptides were reactive and QEJ74712.1-99-113 (NVGVQIVRQMRSGER) gave the highest fluorescence intensity (1872 RFU). The heat maps indicate that peptide QEJ74712.1-99-113 was also immunodominant for IgM (**Supplementary File S2)**.

Antibody Reactivity Against Peptides Derived from *Chlamydia Trachomatis* Proteins.

Peptide microarray technology exhibited levels of IgM reactivity against all peptides derived from *Chlymadia trachomatis*. For IgG the technology exhibited reactivity against three peptides; P19542.1-261-270 (TKDASIDYHE), AAA23156.1-73-81 (VLKTDVNKE) and AAA23156.1-381-390 (TRLIDERAAH) with RFU values of 598.25, 1324.5 and 908.75 respectively. Peptide P19542.1-261-270 exhibited the highest response for IgM with fluorescence intensity of 5808 RFU. Peptide P19542.1-261-270 was also immunodominant for both IgG and IgM compared to the other peptides (**Supplementary File S2)**.

Antibody Reactivity Against Peptides Derived from *Trypanosoma Brucei* Proteins.

Peptide microarray technology showed that only one *Trypanosoma brucei* derived peptide XP_011774209.1-132-145 (SAIHATKTVDIKAL) was reactive with IgG with a fluorescence intensity of 1629.5 RFU. For IgM the technology exhibited reactivity against three peptides; XP_844815.1-369-382 (SSKIKESKVILMAV) (reacting with one serum sample), APD74596.1-96-110 (ENREKWEADKKLIVA) (reacting with 5 serum samples) and CAC33895.1-78-92 (ETDFKATADDNKKPQ) with RFU values of 2069.5, 1266.25 and 822.25 respectively. Peptide XP_844815.1-369-382 exhibited the highest response for IgM with fluorescence intensity of 2069.5 RFU. Peptide XP_844815.1-369-382 was also immunodominant for both IgG and IgM compared to the other peptides (**Supplementary File S2)**.

Discussion

In a time of fierce competition for global health funding, surveillance of a single infection is difficult to defend as a priority (20). Accurate surveillance of multiple-infections enable public health officials to identify opportunities for implementation of integrated control programs (21). NTD multiplex peptide microarray tools, could represent an integrated surveillance platform that could be used for monitoring exposure and for disease diagnosis. With this background, B-cell linear epitopes derived from the antigenic proteins of *Ascaris lumbricoides*, *Necator americanus*, *S. haematobium*, *S. mansoni*, *Trichuris trichiura*, *Bacillus anthracis*, *Mycobacterium leprae*, *Wuchereria bancrofti*, *Rabies lyssavirus*, *Chlamydia trachomatis* and *Trypanosoma brucei* were selected for peptide microarray technology. Plasma and serum samples from NTDs endemic areas were evaluated on the peptide microarray to detect infection-specific IgG and IgM antibodies as described in the experimental section.

Serological tests or antibody test may be useful in showing exposure to infections in NTDs low transmission areas (22). IgM antibodies are reliable early indicators of recent exposure and can aid in disease diagnosis. IgG, by contrast, maybe indicators of prior infections which may be resolved or are still resolving, and of protective immunity (may persist for many years offering protection against reinfection) (23,24). NTD species-specific sero-reactivity with both IgG and IgM was indicative of exposure to the different NTDs parasites antigens in Murewa, Makoni and shamva rural districts.

Several immunodominant regions were identified in color-coded heatmaps reflecting obtained signal intensities. As a typical finding of peptide microarray immunoassays, the signal distribution was highly skewed as was described by Hecker and colleagues (25); many peptides were detected with high signals for several samples, with no obvious difference between the infected and uninfected groups. The results were in agreement with Odegaard and Hsieh, 2014 who demonstrated that exposure is universal in schistosomiasis endemic areas as defined by the ubiquity of schistosomiasis specific antibodies (26).

Comparative studies between IgM and IgG antibody detection in endemic areas have showed significant differences in their diagnostic capabilities, demonstrating a higher IgM detection in a low endemic setting, without extensive knowledge of the particular infective conditions in studied individuals (27). In the present study AUC was chosen to summarize the overall diagnostic accuracy of IgG and IgM for *Ascaris lumbricoides*, *Necator americanus*, *S. haematobium, S. mansoni* and *Trichuris trichiura* derived peptides. AUC was chosen because it is a classification-threshold-invariant that measures the quality of model predictions irrespective of classification threshold. AUC takes values from 0 to 1, where a value of 0 indicates a perfectly inaccurate test and a value of 1 reflects a perfectly accurate test. According to AUC values most of the peptides selected were inaccurate (that is they falsely diagnosed villagers infected or uninfected with disease) with AUC values less than 0.5. Two peptides CAC00543.1-123-135 and AAP41952.1-194-206 were perfectly inaccurate (100 % wrong) in predicting villagers with disease and those without disease. Some peptides had AUC values ranging from 0.5 to 0.5875 for both IgM and IgG suggesting no discrimination (that is no ability to diagnose villagers with and without the disease or condition based on the peptides) (28,29).

Justifications for the lack of discrimination between the infected and uninfected groups by the peptides selected in the study are multi-fold. Just like most serological tests peptide microarray assays do not allow for differentiation of acute or previous infections nor discrimination between persisting antibodies and reinfection. Several studies have demonstrated that antibody detection test may not differentiate between active, prior infection or re-infection because antibodies may persist for many months to years after successful treatment in most of the NTDs (30,31). Hinz and colleagues (2017) suggested that remaining or increasing antibody level limits the usefulness of serological tests. In some individuals negative or intermediate results may be misleading due to a low level of antibody response and late or absent seroconversion or an age-dependent decreasing antibody response in people from endemic regions, resulting from cumulative exposure to schistosomes (31). In this study, the serum and plasma specimens were tested in parallel with the reference serology methods with the peptide microarrays. The diagnostic accuracy of the selected peptides were based on a comparison with existing reference assays.

Commonly used diagnostic methods for schistosomiasis and STH have low sensitivity for the detection of light infections; and many light infections are missed due to absence of eggs in urine and stool specimens (32–35). Compared to parasitological diagnosis, serology provides more sensitive tools for the diagnosis of helminths, especially in infections with low intensity (31). The above mentioned points may have resulted in the failure of the peptides in diagnosing schistosomiasis and STHs.

Limitation

Peptide microarray technology excludes non-protein epitopes for example carbohydrates, glycolipids and fatty acids which also serve as biologically relevant targets for humoral immune responses (36,37). Studies have shown that sera of individuals infected with helminth such as *S. haematobium* and *S. mansoni* contain IgG and IgM antibodies against defined carbohydrate epitopes (38,39).This meant that the peptide microarray technology could not detect antibodies that bind non-protein epitopes.

Conclusion

Using a peptide microarray containing fifty-one B-cell epitopes derived from *A. lumbricoides, N. americanus, S. haematobium, S. mansoni, T. trichiura, B. anthracis, M. Leprae, W. bancrofti, Rabies lyssavirus, C. trachomatis* and *T. brucei*, this novel study demonstrates that more individuals of the Shamva, Murewa and Makoni rural districts in Zimbabwe were exposed to schistosomiasis and STHs than the expected infection prevalence. Species-specific sero-reactivity was indicative of exposure to the different parasitic antigens in the study population. Multiplex peptide microarrays are a valuable tool for integrated NTDs surveillance and for the screening of exposure in areas of low endemicity.

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References

1. Neglected tropical diseases [Internet]. [cited 2021 May 24]. Available from: https://www.who.int/news-room/q-a-detail/neglected-tropical-diseases

2. CDC - Neglected tropical diseases - Diseases [Internet]. [cited 2021 May 24]. Available from: https://www.cdc.gov/globalhealth/ntd/diseases/index.html

3. Mitra AK, Mawson AR. Neglected tropical diseases: Epidemiology and global burden [Internet]. Vol. 2, Tropical medicine and infectious disease. MDPI AG; 2017 [cited 2021 Jun 13]. p. 36. Available from: www.mdpi.com/journal/tropicalmed

4. Da Silva Santos L, Wolff H, Chappuis F, Albajar-Viñas P, Vitoria M, Tran NT, et al. Coinfections between persistent parasitic neglected tropical diseases and viral infections among prisoners from Sub-Saharan Africa and Latin America. J Trop Med. 2018;2018.

5. WHO | Neglected zoonotic diseases [Internet]. [cited 2021 May 24]. Available from: https://www.who.int/neglected_diseases/zoonoses/infections_more/en/

6. Engels D, Zhou XN. Neglected tropical diseases: An effective global response to local poverty-related disease priorities [Internet]. Vol. 9, Infectious Diseases of Poverty. BioMed Central Ltd.; 2020 [cited 2021 May 24]. p. 1–9. Available from: https://doi.org/10.1186/s40249-020-0630-9

7. Engels D, Savioli L. Reconsidering the underestimated burden caused by neglected tropical diseases. Trends Parasitol [Internet]. 2006 Aug [cited 2021 May 24];22(8):363–6. Available from: https://pubmed.ncbi.nlm.nih.gov/16798088/

8. Zimbabwe | ESPEN [Internet]. [cited 2021 May 24]. Available from: https://espen.afro.who.int/countries/zimbabwe

9. Midzi N, Mduluza T, Chimbari MJ, Tshuma C, Charimari L, Mhlanga G, et al. Distribution of schistosomiasis and soil transmitted helminthiasis in Zimbabwe: Towards a national plan of action for control and elimination. Kabatereine NB, editor. PLoS Negl Trop Dis [Internet]. 2014 Aug 14 [cited 2020 Mar 3];8(8):e3014. Available from: http://dx.plos.org/10.1371/journal.pntd.0003014

10. Phiri I, Manangazira P, Macleod CK, Mduluza T, Dhobbie T, Chaora SG, et al. The burden of and risk factors for trachoma in selected districts of Zimbabwe: Results of 16 population-based prevalence surveys. Ophthalmic Epidemiol [Internet]. 2018 Dec 28 [cited 2020 Mar 3];25(sup1):181–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28532208

11. Arnold BF, der Laan MJ va., Hubbard AE, Steel C, Kubofcik J, Hamlin KL, et al. Measuring changes in transmission of neglected tropical diseases, malaria, and enteric pathogens from quantitative antibody levels. PLoS Negl Trop Dis [Internet]. 2017 Feb 7 [cited 2021 Jun

13];11(5):1–20. Available from: https://doi.org/10.1101/106708

12. Sachse K, Rahman KS, Schnee C, Müller E, Peisker M, Schumacher T, et al. A novel synthetic peptide microarray assay detects Chlamydia species-specific antibodies in animal and human sera. Sci Rep [Internet]. 2018 Dec 1 [cited 2021 May 24];8(1):4701. Available from: www.nature.com/scientificreports/

13. Duburcq X, Olivier C, Desmet R, Halasa M, Carion O, Grandidier B, et al. Polypeptide semicarbazide glass slide Microarrays: Characterization and comparison with amine slides in serodetection studies. Bioconjug Chem [Internet]. 2004 Mar [cited 2021 May 24];15(2):317–25. Available from: https://pubs.acs.org/doi/abs/10.1021/bc034118r

14. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins Struct Funct Bioinforma [Internet]. 2006 Aug 7 [cited 2021 Feb 9];65(1):40–8. Available from: http://doi.wiley.com/10.1002/prot.21078

15. Mott KE, Baltes R, Bambagha J, Baldassini B. Field studies of a reusable polyamide filter for detection of *schistosoma haematobium* eggs by urine filtration. Tropenmed Parasitol [Internet]. 1982 Dec 1 [cited 2020 Aug 4];33(4):227–8. Available from: https://europepmc.org/article/med/7164164

16. George S, Geldhof P, Albonico M, Ame SM, Bethony JM, Engels D, et al. Molecular speciation of soil-transmitted helminths egg isolates collected during six drug efficacy trials in endemic countries. Trans R Soc Trop Med Hyg. 2016;110(11):657.

17. Knopp S, Becker SL, Ingram KJ, Keiser J, Utzinger J. Diagnosis and treatment of schistosomiasis in children in the era of intensified control [Internet]. Vol. 11, Expert Review of Anti-Infective Therapy. Expert Rev Anti Infect Ther; 2013 [cited 2020 Aug 4]. p. 1237–58. Available from: https://pubmed.ncbi.nlm.nih.gov/24127662/

18. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins Struct Funct Genet [Internet]. 2006 Oct 1 [cited 2021 May 24];65(1):40–8. Available from: www.imtech.res.in/raghava/bcepred/

19. Schwarz T, Heiss K, Mahendran Y, Casilag F, Kurth F, Sander LE, et al. SARS-CoV-2 proteome-wide analysis revealed significant epitope signatures in COVID-19 patients. Front Immunol [Internet]. 2021 Mar 23 [cited 2021 May 24];12:765. Available from: www.frontiersin.org

20. Lammie PJ, Moss DM, Brook Goodhew E, Hamlin K, Krolewiecki A, West SK, et al. Development of a new platform for neglected tropical disease surveillance. Int J Parasitol. 2012 Aug 1;42(9):797–800.

21. Njenga SM, Kanyi HM, Arnold BF, Matendechero SH, Onsongo JK, Won KY, et al. Integrated cross-sectional multiplex serosurveillance of IgG antibody responses to parasitic diseases and vaccines in coastal Kenya. Am J Trop Med Hyg [Internet]. 2020 Nov 25 [cited 2021 Jun 13];102(1):164–76. Available from: https://osf.io/taknp

22. Eyayu T, Zeleke AJ, Worku L. Current status and future prospects of protein vaccine candidates against *Schistosoma mansoni* infection. Vol. 11, Parasite Epidemiol. Control. Elsevier Ltd; 2020. p. e00176.

23. Mardekian SK, Roberts AL. Diagnostic options and challenges for dengue and Chikungunya viruses. Vol. 2015, BioMed Research International. Hindawi Limited; 2015.

24. Nagar PK, Savargaonkar D, Anvikar AR. Detection of Dengue Virus-Specific IgM and IgG Antibodies through peptide sequences of envelope and NS1 proteins for serological identification. J Immunol Res. 2020;2020.

25. Hecker M, Fitzner B, Wendt M, Lorenz P, Flechtner K, Steinbeck F, et al. High-density peptide microarray analysis of IgG autoantibody reactivities in serum and cerebrospinal fluid of multiple sclerosis patients. Mol Cell Proteomics [Internet]. 2016 Apr 1 [cited 2021 May 24];15(4):1360–80. Available from: https://pubmed.ncbi.nlm.nih.gov/26831522/

26. Odegaard JI, Hsieh MH. Immune responses to *Schistosoma haematobium* infection [Internet]. Vol. 36, Parasite Immunol. Blackwell Publishing Ltd; 2014 [cited 2021 May 24]. p. 428–38. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/pim.12084

27. Cosenza M, Barrios E, Felibertt P, Castillo-Corujo A, Ochoa G, Velasquez E, et al. IgM and IgG responses in *Schistosoma mansoni*-infected mice using egg and worm antigens: Does response vary with parasitic burden and phase of infection? Exp Parasitol. 2017 Aug 1;179:36–42.

28. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. Vol. 5, Journal of Thoracic Oncology. 2010.

29. Malnati MS, Heltai S, Cosma A, Reitmeir P, Allgayer S, Glashoff RH, et al. A new antigen scanning strategy for monitoring HIV-1 specific T-cell immune responses. J Immunol Methods

[Internet]. 2012 Jan 31 [cited 2020 Nov 23];375(1–2):46–56. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/21963950/

30. Martelli G, Di Girolamo C, Zammarchi L, Angheben A, Morandi M, Tais S, et al. Seroprevalence of five neglected parasitic diseases among immigrants accessing five infectious and tropical diseases units in Italy: a cross-sectional study. Clin Microbiol Infect [Internet]. 2017 May 1 [cited 2021 Jun 13];23(5):335.e1-335.e5. Available from: http://dx.doi.org/10.1016/j.cmi.2017.02.024

31. Hinz R, Schwarz NG, Hahn A, Frickmann H. Serological approaches for the diagnosis of schistosomiasis – A review. Vol. 31, Molecular and Cellular Probes. Academic Press; 2017. p. 2–21.

32. Knopp S, Corstjens PLAM, Koukounari A, Cercamondi CI, Ame SM, Ali SM, et al. Sensitivity and specificity of a urine circulating anodic antigen test for the diagnosis of *Schistosoma haematobium* in low endemic settings. PLoS Negl Trop Dis [Internet]. 2015 May 14 [cited 2021 May 24];9(5):e0003752. Available from: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0003752

33. Fenta A, Hailu T, Alemu M, Nibret E, Amor A, Munshea A. Evaluating the performance of diagnostic methods for soil transmitted helminths in the Amhara national regional state, northwest Ethiopia. BMC Infect Dis [Internet]. 2020 Dec 1 [cited 2021 May 24];20(1):1–8. Available from: https://doi.org/10.1186/s12879-020-05533-2

34. Nikolay B, Brooker SJ, Pullan RL. Sensitivity of diagnostic tests for human soil-transmitted helminth infections: A meta-analysis in the absence of a true gold standard. Int J Parasitol. 2014 Oct 1;44(11):765–74.

35. Glinz D, Silué KD, Knopp S, Lohourignon LK, Yao KP, Steinmann P, et al. Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. PLoS Negl Trop Dis [Internet]. 2010 Jul [cited 2021 May 24];4(7):e754. Available from: www.plosntds.org

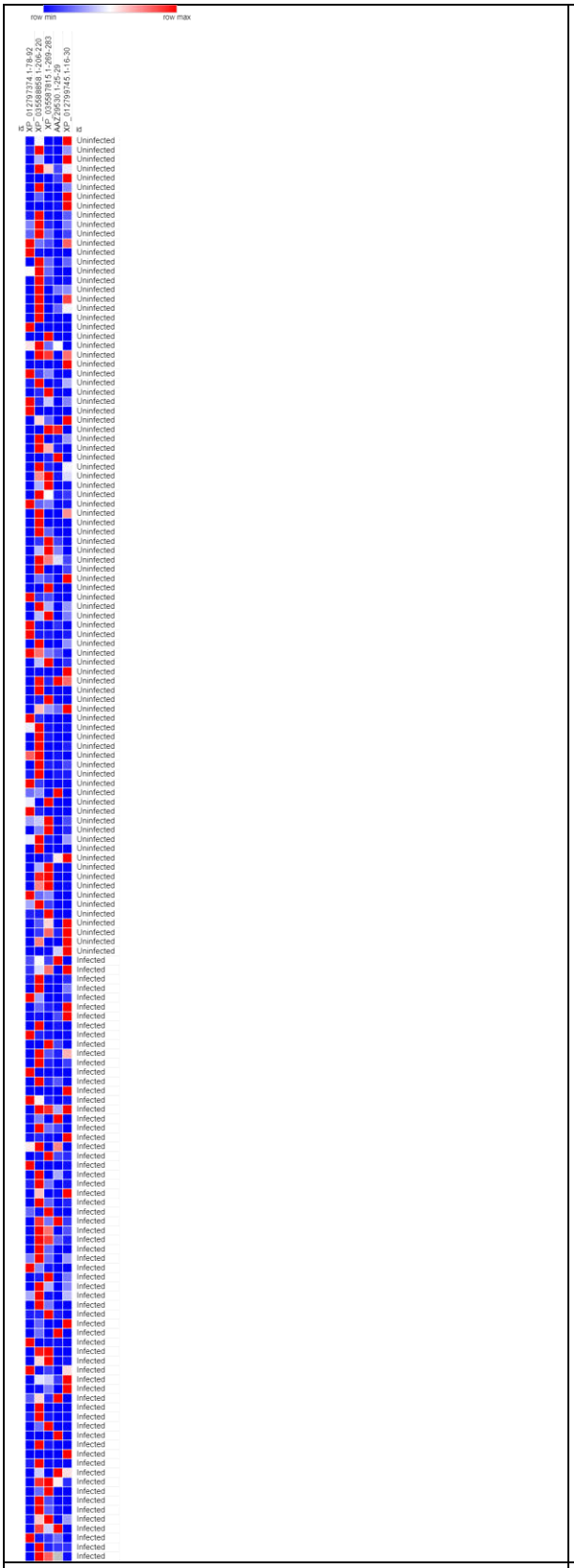
36. Heidepriem J, Krähling V, Dahlke C, Wolf T, Klein F, Addo MM, et al. Epitopes of naturally acquired and vaccine-induced anti-Ebola virus glycoprotein antibodies in single amino Acid Resolution. Biotechnol J. 2020 Sep 1;15(9).

37. Gaseitsiwe S, Valentini D, Mahdavifar S, Magalhaes I, Hoft DF, Zerweck J, et al. Pattern

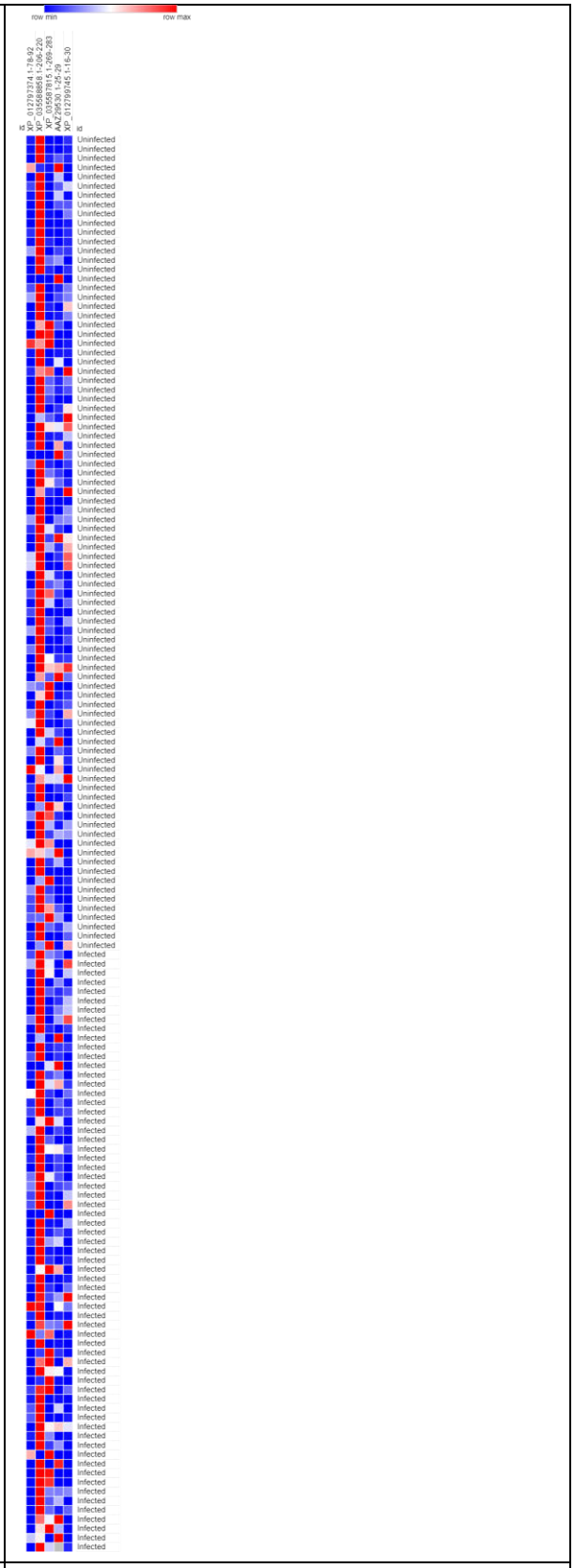
recognition in pulmonary tuberculosis defined by high content peptide microarray chip analysis representing 61 proteins from *M. tuberculosis*. PLoS One [Internet]. 2008 Dec 9 [cited 2020 Nov 24];3(12). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/19065269/

38. Michelle Yang YY, Van Diepen A, Brzezicka K, Reichardt NC, Hokke CH. Glycan microarray-assisted identification of IgG subclass targets in schistosomiasis. Front Immunol. 2018 Oct 9;9(OCT):2331.

39. Van Remoortere A, Van Dam GJ, Hokke CH, Van den Eijnden DH, Van Die I, Deelder AM. Profiles of immunoglobulin M (IgM) and IgG antibodies against defined carbohydrate epitopes in sera of *Schistosoma*-infected individuals determined by surface plasmon resonance. Infect Immun [Internet]. 2001 [cited 2021 Jun 13];69(4):2396–401. Available from: https://pubmed.ncbi.nlm.nih.gov/11254599/



S. haematobium IgG heat map



S. haematobium IgM heat map

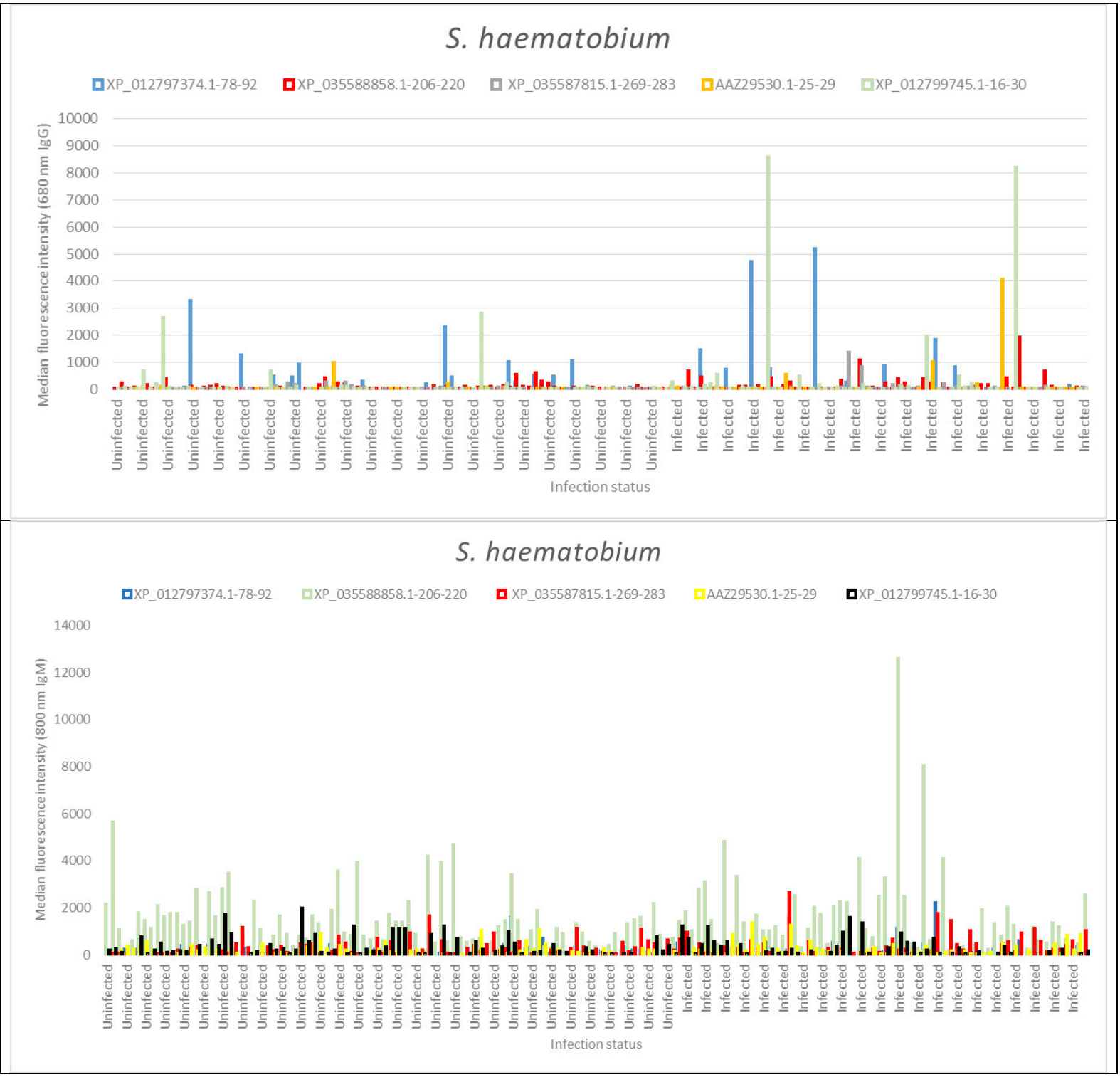


Figure 2: Heat maps and bar graphs generated from peptide microarray data for *S. haematobium*. (A) Heat maps generated from peptide microarray. Samples were arranged in rows and infection status shown on the left key. Peptides shown by their protein accession number and sequence position are arranged in columns. (B) Bar graphs representing the peptide reactivity for each serum and plasma in both the infected and uninfected groups in the study.

CHAPTER 5

PEPTIDE MICROARRAY ANALYSIS OF *IN SILICO* PREDICTED B-CELL EPITOPES IN SAR-COV-2 SEROPOSITIVE HUMANS IN ZIMBABWE.

This chapter consists of a research manuscript which aimed to discover linear B-cell epitopes for SARS-CoV-2 in seropositive humans using *in silico* predicted B-cell epitopes and peptide microarray technology.

This chapter was submitted to A European Journal Tropical Medicine & International Health (TMIH).

Submission Confirmation for Peptide Microarray Analysis of in-Silico Predicted B-Cell Epitopes in SARS-CoV-2 Seropositive Humans in Zimbabwe. Inbox ×

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Peptide microarray analysis of *in silico* predicted B-cell epitopes in SAR-CoV-2 seropositive humans in Zimbabwe.

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Abstract

Introduction

Prediction of immunogenic B-cell epitopes using bioinformatics *in silico* approach complemented by high throughout peptide microarray validation is a powerful method for the quick selection of potential epitopes. A workflow for profiling B-cell epitopes derived from SARS-CoV-2 structural proteins using an *in silico* approach and peptide microarray immunoassay using SARS-CoV-2 seropositive sera from healthcare workers that reside in Zimbabwe is herein reported.

Methodology

SARS-CoV-2 seropositivity was detected using two rapid chromatographic immunoassays (Wuhan UN science Biotechnology Companies UNICOV-40 test kit and the Standard-Q Covid-19 IgM/IgG Duo antibody test from SD Biosensor). Sera in the control (SARS-CoV-2 negative) group was tested using rapid chromatographic immunoassays and RT-PCR. Immunogenic peptides mimicking B-cell linear epitopes were predicted *in silico* using ABCpred. IgG and IgM antibodies against the SARS-CoV-2 spike protein, membrane glycoprotein and nucleocapsid derived peptides were measured in sera using peptide microarray immunoassay.

Results

Healthcare workers included in the study were RT-PCR negative for SARS-CoV-2. Using rapid chromatographic immunoassays, 10 were SARS-CoV-2 IgM positive and 6 were SARS-CoV-2 IgG positive. From a total of 10 peptides contained in the microarray, 4 (QTH34388.1-1-14, QRU89900.1-41-54, QTN64908.1-136-149 and QLL35955.1-22-35) showed reactivity against IgG with at least a single sera from a SARS CoV-2 sero-positive health care worker. Four peptides (QRU89900.1-41-54, QSM17284.1-76-89, QTN64908.1-136-149 and QPK73947.1-8-21) also showed reactivity against IgM. The reactive peptides were derived from the membrane glycoprotein and nucleocapsid protein.

Conclusion

In silico peptide prediction and peptide microarray immunoassay validation may provide a powerful platform for the discovery of SARS-CoV-2 B-cell epitopes.

Keywords: SARS-CoV-2, B-cell epitopes, epitope prediction, peptide microarrays, antibodies

Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV-2) is the source of the current corona virus disease 2019 (COVID-19) pandemic. COVID-19 has had devastating consequences that have significantly impacted the economy, education, social interactions and public health (1,2). According to Africa CDC, on the 13th of July 2021 there were a total of 6,027,574 cases and 153,549 deaths since the start of the COVID-19 pandemic (3). Zimbabwe a country in Southern Africa had 73,271 confirmed COVID-19 cases and 2,274 deaths as of July 13 2021 (4,5).

Given the ongoing SARS-CoV-2 pandemic, discovery of T-cell and B-cell epitopes corresponding to the immune system's antibody response against SARS-COV-2 is an urgent global health priority. SARS-CoV-2 epitopes are important for the development and monitoring of vaccines, development of effective and safe therapeutics and diagnostic tests (1,2,6,7). Indeed PEPperPRINT GmbH (https://www.pepperprint.com/) and JPT ((https://www.jpt.com/indications/sarscov2) produced peptide microarrays for SARS-CoV-2 T-cell and B-cell epitope discovery. PEPperPRINT produced the peptide microarrays by translating the entire SARS-CoV-2 proteome into overlapping peptides and printing them onto PEPperSlide glass slides.

Experimental techniques including overlapping peptides and phage display library, for the comprehensive mapping of B-cell epitopes are time consuming and expensive even for a single target protein (8). In contrast, *in silico* B-cell epitope prediction bioinformatics techniques are a manageable alternative that allow for virtual cost-effective, scans in the search for immunodominant epitopes with serological diagnostic potential (9–11). *In silico* B-cell epitope prediction coupled with peptide microarray technology provides a quick way for the discovery of B-cell epitopes (11).

Several *in silico* B-cell epitope prediction bioinformatic databases (**Table 1)** are available (8), in which computational strategies guides the selection of candidate epitopes for peptide microarray immunoassay validation (12). Against this background, the present study aimed to discover linear B-cell epitopes derived SARS-CoV-2 spike protein, membrane glycoprotein and nucleocapsid protein using the described ABCpred (11) *in silico* B-cell epitope prediction and peptide microarray immunoassay. The predicted peptides were validated against well characterized SARS-CoV-2 seropositive sera from health workers in Zimbabwe.

Table 1: *In silico* B-cell epitope prediction software (8).

Software	Server
MLCE	http://bioinf.uab.es/BEPPE
ABCpred	http://www.imtech.res.in/raghava/abcpred/
BepiPred 1.0	www.cbs.dtu.dk/services/BepiPred/
Epitopia web server	http://epitopia.tau.ac.il/
Antigenic	http://www.bioinformatics.nl/cgi-bin/emboss/antigenic
BCPREDS	http://ailab.ist.psu.edu/bcpred/
Bcepred	http://crdd.osdd.net/raghava/bcepred/bcepred_instructions.html

Materials and Methods

Ethical approval and Study population

Ethical approval for this study was obtained from the Medical Research Council of Zimbabwe (MCRZ/A/2571/ and MRCZ/A2443/). Healthcare workers (cleaners, security officers, nurses, administrators and doctors) were recruited into the study from 24 health facilities in Bulawayo, Zimbabwe (20.1457˚ S, 28.5873˚ E) in June 2020. Prior to recruitment the study objectives were fully explained to the healthcare workers who then gave their written consent to participate in the study.

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Antibody testing/ Serological test

Five millilitres of venous blood was collected from each worker. The blood was then separated into serum samples within 24hrs of collection by centrifugation at 3000g for 15 minutes. The serum was used to detect SARS-CoV-2 antibodies (IgM and IgG) against the spike protein, nucleocapsid protein and membrane protein using two rapid immunoassay kits (Wuhan UNscience Biotechnology Companies UNICOV-40 test kit and the nationally recommended Standard-Q Covid-19 IgM/IgG Duo antibody test from SD Biosensor and Standard-Q Covid-19 IgM/IgG Duo antibody test from SD Biosensor).

SARS-CoV-2 Real-Time reverse transcriptase (RT)-PCR-diagnosis

Clinical specialists collected nasopharyngeal swabs according to WHO and CDC protocols (https://www.who.int/docs/default source/coronaviruse/whoinhouseassays.pdf). RNA was then extracted from these swabs using the using the respiratory sample RNA isolation kit. Diagnosis of SARS-CoV-2 virus was performed using Real-Time reverse transcriptase (RT)-PCR as described by Rusakaniko *et al* (2021). The nucleocapsid protein gene and the virus open reading frame1ab (ORF1ab) gene were amplified simultaneously as recommended by WHO. An internal control (RNasep) gene was used together with negative and positive samples in the assay (13).

Peptide selection

SARS-CoV-2 spike protein, nucleocapsid protein membrane glycoprotein sequences were obtained from the NCBI protein database (https://www.ncbi.nlm.nih.gov/). The bioinformatics tool ABCpred was used for the *in silico* prediction of the SARS-CoV-2 B-cell epitopes on the selected protein sequences. The peptides that had the least cross-reactivity were selected using the NCBI Protein BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi select Protein BLAST). Peptides that had the highest ABCpred rank and the least cross-reactivity with peptides from other human pathogens or proteins were selected for inclusion on the peptide microarrays.

Peptide microarray design and layout

Peptide microarray design

The peptide microarray was designed to contain 9aa-18aa peptides that were obtained from a variety of pathogens and printed in a laser-printer technique by PEPperPRINT GmbH (Heidelberg, Germany) (https://www.pepperprint.com/). Each sub-array on the peptide microarray contained 260 peptide positions. The microarray had 16 sub-arrays (copies) that were framed by flag anti-polio (KEVPALTAVETGAT, 3 spots) and flag anti-HA (hemagglutinin glycoprotein of influenza virus) (YPYDVPDYAG, 3 spots) as a quality control measurement. Ten SARS-CoV-2 structural proteins derived peptides (14aa and 16aa) (details given in **Table 2**) were printed with random distribution across each sub-array.

Table 2: ABCpred predicted B-cell linear epitopes

Peptide name	Source Protein	Peptide Sequence	Peptide length
PDB: 7KRQ_A-879-894	Chain A, spike glycoprotein	AGTITSGWTFGAGAAL	16
PDB: 7KRQ_A-257-272	Chain, A spike glycoprotein	GWTAGAAAYYVGYLQP	16
QPK73947.1-8-21	membrane glycoprotein	ITVEELKKLLEQWN	14
PDB: 7LX5_B-686-701	Chain B, spike glycoprotein	GVSVITPGTNTSNQVA	16
QSM17284.1-76-89	nucleocapsid protein	TNSSPDDQIGYYRR	14
QLL35955.1-22-35	nucleocapsid protein	DGKMKDLSPRWYFY	14
QTH34388.1-1-14	membrane glycoprotein	MADSNGTITVEELK	14
QTN64908.1-135-148	membrane glycoprotein	ESELVIGAVILRGH	14
QRU89900.1-41-54	nucleocapsid protein	RPQGLPNNTASWFT	14
QTN64908.1-136-149	membrane glycoprotein	SELVIGAVILRGHL	14

The peptide name consisted of the protein/antigen accession number followed by the amino acids positions of the peptide in the protein

Peptide microarrays immunoassays

PEPperCHIP® Incubation Trays were used for the immune assays. Identification of false positive signals was done during the pre-incubation step by binding of the fluorescently labelled secondary antibody. The serum was then incubated with the secondary antibodies. The washing buffer (PBS, pH 7.4 with 0.05% Tween 20) was used at each step for pre-swelling for 10 minutes. The next incubation step involved the use of the blocking buffer (Rockland blocking buffer MB-070) for 30 minutes. Goat anti-human IgG (Fc) DyLight680 (0.1 µg/ml), goat anti-human IgM [(µ chain) DyLight800 (0.2 µg/ml)] and control antibodies (Mouse monoclonal anti-HA DyLight800 (0.5 µg/ml) were used during incubation initially. These secondary antibodies were diluted in the incubation buffer (washing buffer with 10% blocking buffer) for 45 minutes at room temperature. The microarrays were then incubated with plasma or serum that was diluted using the ratio of 1:250 in incubation buffer for 16 h. The microarrays were then stored at 4°C and put on a shaker at140 rpm. The secondary antibodies were then used during the incubation step.

The microarrays were washed three times with washing buffer for 10 seconds and scanned using an LI-COR Odyssey Imaging System; scanning offset 0.65 mm, resolution 21 µm, scanning intensities of 7/7 (red = 680 nm / green = 800 nm). To ensure that all microarrays were responding correctly, all steps were repeated with the Cy3-conjugated anti-HA control antibody and Cy3-conjugated anti-polio control antibodies

Prior data quantification, all the peptide microarray scans were visually evaluated for possible assay or microarray artifacts which would affect the experimental outcome. If required, the assays with individual samples were repeated on new peptide microarrays. For technical reasons, read-out for repeated samples was carried out with an Innopsys InnoScan 710-IR microarray scanner with matching scanner settings.

Image analysis and spot intensity quantification

Microarray image analysis was done using PepSlide® Analyzer ((SICASYS Software GmbH; Heidelberg, Germany) and resulted in raw data CSV files for each sample (green = 800 nm = IgM staining, red = 680 nm = IgG staining).

Quantification of spot intensities was based on 16-bit gray scale tiff files. Averaged median foreground intensities (foreground-background signal), spot-to-spot deviations of spot duplicates were calculated using a PEPperPRINT software algorithm and assembled the outcome in summary files. For duplicate spots a maximum spot-to-spot deviation of 50% was tolerated, otherwise the corresponding intensity value was regarded as artefact and was zeroed.

Statistical analysis

The data set used for statistical analysis of the peptide microarray results were based on fluorescence intensity. Bar graphs were created in Microsoft excel 2013. Heat maps were generated online using Morpheus heat map widget (https://software.broadinstitute.org/morpheus/). Non-parametric statistical methods were used for data analysis with p-values lower than 0.05 considered statistically significant. Age was expressed as median and interquartile range (IQR). Univariate comparisons of two independent groups were done using the Mann-Whitney-U test in SPSS 16.0. Area under the ROC curve (AUC) was measured for each peptide using STATA/MP16.0.

Antibody reactivity and discrimination between the infected and uninfected groups by detection of immunodominant epitopes.

A positive peptide response was defined as fluorescence intensity ≥ 500 FU (fluorescence intensity units) for both IgG and IgM against each peptide (14). Color-coded heat maps and bar graphs reflecting obtained fluorescence signal intensities represented immunodominant peptides. The ability of peptides to distinguish between the seropositive and seronegative groups was statistically evaluated using the Mann-Whitney-U test. ROC curve analysis assessed diagnostic accuracy of the peptides and AUC assessed the overall diagnostic performance (**Table 3**) of peptide.

Table 3: Area under the ROC curve and the diagnostic performance of each peptide.

AUC	Quality of peptide/diagnostic performance
1	Perfectly accurate test
0.9-1	Outstanding
0.8-0.9	Excellent
0.7-0.8	Fair/acceptable
0.6-0.7	Poor
0.5-0.6	Fail
0	Perfectly inaccurate test

Results

Demographic and clinical characteristics

Forty-nine Zimbabwean health care workers [14.3% (7) males and 85.7% (42) females] with the age range of 20 to 64 years (median age: 38.9; IQR: 29-23) from Bulawayo Matebeleland Province were recruited in the study. The cohort of health workers were of two different health facilities [87.8% (43) hospital and 12.2% (6) clinic] and comprised of 53.1% (26) nurses , 2% (1) doctor, 16.3% (8) nurse aides, 16.3% (8) student nurses, 8.2% (4) general hand and 4.1% (2) clerks. Through questionnaires of travel history, 12.2% (6) travelled outside the country in the past five months and 18.4% (9) had contact with inbound individuals in the past four months. Four workers who did not have demographic data were also included in the study. All individuals were tested negative for SARS-CoV-2 using RT-PCR. Using rapid chromatographic immunoassays, 10 were SARS-CoV-2 IgM positive and 6 were SARS-CoV-2 IgG positive.

SARS-CoV-2 B-cell epitope profiling

Ten SARS-CoV-2 ABCpred *in silico* predicted peptides were screened on a peptide microarray platform. Seven peptides that reacted above the cut-off (500 FU) were detected (**Figure 1**). For IgG four were detected; QTH34388.1-1-14 derived from the membrane glycoprotein (reactive with one seropositive worker and one seronegative worker), QRU89900.1-41-54 derived from the nucleocapsid protein (reactive with one seronegative worker), QTN64908.1-136-149 derived from membrane glycoprotein (reactive with one seronegative worker) and QLL35955.1-22-35 derived from nucleocapsid protein (reactive with one seropositive worker). With respect to IgM, four for peptides were detected; QSM17284.1-76-89 derived from nucleocapsid protein (reactive with one seronegative worker), QPK73947.1-8-21 derived from membrane glycoprotein (reactive with one seropositive worker), QRU89900.1-41-54 (reactive with one seronegative worker) and QTN64908.1-136-149 (reactive with one seronegative worker). None of these peptides was singularly detected in more than one of the seropositive samples tested. None of the epitopes showed discrimination between the SARS-CoV-2 seropositive group and seronegative group **(Table 4).** None of the detected peptide was immunodominant for neither IgG nor IgM as shown by the heatmaps **(Figure 1).**

Figure 1: Heat maps and bar graphs generated from peptide microarray technology data. A. Heat maps generated from peptide microarray technology. Samples were arranged in rows and infection status shown on the left key. Peptides shown by their protein accession number and sequence position are arranged in columns. The false-colored rainbow color from blue to red corresponds to the signals of antibody binding from low to high, respectively. (B) Bar graphs representing the peptides reactivity for each serum and plasma in the study (IgG above and IgM below).

Table 4: Diagnostic performance of SARS CoV-2 ABCpred derived peptides

Peptide	IgG		IgM	
	AUC	p-value	AUC	p-value
QRU89900.1-41-54	0	0.087	0	0.054
QTH34388.1-1-14	0.1398176	0.71	0	0.352
QSM17284.1-76-89	0.1428571	0.604	0.0785714	0.367
PDB: 7LX5_B-686-701	0.1428571	0.894	0	0.518
PDB: 7KRQ_A-879-894	0	0.496	0	0.966
PDB: 7KRQ_A-257-272	0	0.7	0.1857143	0.425
QLL35955.1-22-35	0.4012158	0.181	0	0.118
QTN64908.1-135-148	0.2613982	0.62	0.1857143	0.903
QPK73947.1-8-21	0.2613982	0.394	0	0.352
QTN64908.1-136-149	0	0.496	0.1880952	0.183

Discussion

Peptide microarray technology is an ideal tool to decipher epitope-specific B-cell immune responses toward the proteome of an emerging pathogen such as SARS-CoV-2. The technology enable simultaneous analysis of peptides in a fast and cost-effective way for applications, such as epitope discovery (15). SARS-CoV-2 have relatively few numbers of proteins, classified as either structural or non-structural. Among structural proteins, the spike glycoprotein, the nucleocapsid protein, the membrane glycoproteins and the envelope protein are the major ones (1). With this background, the study focused on the spike protein, nucleocapsid protein and the membrane glycoprotein and peptide microarray immunoassays. Ten SARS-CoV-2 peptides were predicted *in silico* with ABCpred. Following epitope prediction, peptide microarrays were generated in a laser-printer based approach by PEPperPRINT and evaluated with SARS-CoV-2 seropositive and seronegative sera.

One of the principal condition in antibody testing is to ensure that there is limited cross-reactivity with antibodies developed to other antigens (16). Antibody tests for SARS-CoV-2 infection are impeded by immunological cross-reactivity among the human coronaviruses. The SARS-CoV-1 and SARS-CoV-2 genomes are highly similar. SARS-CoV-2 has ~30 kb positive-sense single-stranded RNA genome which shares ~80% sequence identity with that of SARS-CoV-1 (2,17). Consequently, many of the proteins found in SARS-CoV-2 (NC_045512.2) are also found in SARS-CoV-1 (AY515512.1 or NC_004718.3) with 77.1% of the protein sequences shared in their proteomes (18).

In the present study, predicted peptides with the ABCpred highest rank and with the least cross-reactivity with peptides from other human pathogens or proteins were selected for inclusion on the peptide microarrays to mitigate this limitation.

Several SARS-CoV-2 studies have reported antibody reactivity against the spike protein, nucleocapsid protein and the membrane protein with binding mainly occurring on the spike protein and nucleocapsid protein, indicating that these two proteins are immunodominant (2,7,19,20). However we detected only the nucleocapsid protein and the membrane glycoproteins antibody reactivity suggesting possible early infection as it has been postulated that antibody to the nucleocapsid protein is more sensitive than the spike protein antibody for detecting early SARS-CoV-2 infection (21).

Limitations

The spike glycoprotein is transcribed into 1273 aa, envelope protein into 76 aa, membrane protein into 220 aa to 260 aa, and nucleocapsid protein into 419 aa (22). In the present study, only three 16 aa non overlapping peptides covering approximately 4 % of protein sequence were selected for the spike protein, only three 14 aa non overlapping peptides covering approximately 10 % of protein sequence were selected for the nucleocapsid protein and with respect to membrane glycoprotein four 14 aa non overlapping peptides covering approximately 20 % of protein sequence were selected. The implication for such selection is that potential immunogenic peptides may be missed and we recommend including all predicted peptides in future studies.

In silico prediction of B-cell epitopes is still an active biotechnology research field and a number of servers show improved performance, however, their prediction accuracies are still not satisfactory. ABCpred server predict B cell epitopes in an antigen sequence with 65.93 % accuracy using artificial recurrent neural network (machine based technique) (10). Current B-cell epitope predictors are based on epitopes derived from heterogeneous experimental conditions including many cases in which laboratory animals were immunized with relatively large doses of highly purified antigens. Unfortunately, it has been reported that humoral immune responses against the same antigen differ between species and members of the same species. Significant variability in individual B-cell epitope reactivity has been reported in tuberculosis and toxoplasmosis (12).

Conclusion

This study identified five B-cell epitopes derived from SARS-CoV-2 structural proteins. *In silico* prediction of B-cell epitopes coupled with peptide microarray technology may provide a powerful platform for the discovery of SARS-CoV-2 structural proteins B-cell epitopes.

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DECLARATIONS

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that they have no competing interests.

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Authors' contributions

A.V and T.M selected the peptides and designed the peptide microarray. PEPperPRINT printed the peptide microarray and conducted microarray immunoassays. A.V. analyzed data. All authors read and approved the manuscript.

References

1. Farrera-Soler L, Daguer JP, Barluenga S, Vadas O, Cohen P, Pagano S, et al. Identification of immunodominant linear epitopes from SARS-CoV-2 patient plasma. PLoS One [Internet]. 2020

Sep 1 [cited 2021 Jun 14]; 15(9 september):e0238089. Available from: https://doi.org/10.1371/journal.pone.0238089

2. Wang H, Wu X, Zhang X, Hou X, Liang T, Wang D, et al. SARS-CoV-2 Proteome microarray for mapping COVID-19 antibody interactions at amino acid resolution. ACS Cent Sci [Internet]. 2020 Dec 23 [cited 2021 Jun 14];6(12):2238–49. Available from: https://dx.doi.org/10.1021/acscentsci.0c00742

3. Coronavirus disease 2019 (COVID-19) – Africa CDC [Internet]. [cited 2021 Jun 14]. Available from: https://africacdc.org/covid-19/

4. COVID-19 Information | U.S. Embassy in Zimbabwe [Internet]. [cited 2021 Jun 14]. Available from: https://zw.usembassy.gov/covid-19-information-2/

5. Zimbabwe COVID: 40,077 cases and 1,635 deaths - Worldometer [Internet]. [cited 2021 Jun 14]. Available from: https://www.worldometers.info/coronavirus/country/zimbabwe/

6. SARS-CoV-2 [Internet]. [cited 2021 Jun 14]. Available from: https://www.jpt.com/indications/sarscov2

7. Poh CM, Carissimo G, Wang B, Amrun SN, Lee CYP, Chee RSL, et al. Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. Nat Commun [Internet]. 2020 Dec 1 [cited 2021 Jun 14];11(1):1–7. Available from: https://doi.org/10.1038/s41467-020-16638-2

8. Vengesai A, Kasambala M, Mutandadzi H, Mduluza-5 Jokonya TL, Mduluza T, Naicker T. Scoping review of the applications of peptide microarrays on the fight against human Short title: Peptide microarrays application 4. bioRxiv [Internet]. 2021 Mar 4 [cited 2021 Jun 14];2021.03.04.433859. Available from: https://doi.org/10.1101/2021.03.04.433859

9. Van Regenmortel MHV. Structural and functional approaches to the study of protein antigenicity [Internet]. Vol. 10, Immunol Today; 1989 [cited 2021 Jun 14]. p. 266–72. Available from: https://pubmed.ncbi.nlm.nih.gov/2478146/

10. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins Struct Funct Genet [Internet]. 2006 Oct 1 [cited 2021 Jun 14];65(1):40–8. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/prot.21078

11. Giacò L, Amicosante M, Fraziano M, Gherardini PF, Ausiello G, Helmer-Citterich M, et al. B-Pred, a structure based B-cell epitopes prediction server. Adv Appl Bioinforma Chem [Internet].

2012 Jul 25 [cited 2021 Jun 14];5(1):11–21. Available from: http://dx.doi.org/10.2147/AABC.S30620

12. Carmona SJ, Sartor PA, Leguizamón MS, Campetella OE, Agüero F. Diagnostic peptide discovery: Prioritization of pathogen diagnostic markers using multiple features. PLoS One [Internet]. 2012 Dec 14 [cited 2021 Jun 14];7(12):50748. Available from: www.plosone.org

13. Rusakaniko S, Sibanda EN, Mduluza T, Tagwireyi P, Dhlamini Z, Ndhlovu CE, et al. SARS-CoV-2 serological testing in frontline health workers in Zimbabwe. PLoS Negl Trop Dis [Internet]. 2021 Mar 1 [cited 2021 Jun 14];15(3):e0009254. Available from: https://doi.org/10.1371/journal.pntd.0009254

14. Schwarz T, Heiss K, Mahendran Y, Casilag F, Kurth F, Sander LE, et al. SARS-CoV-2 proteome-wide analysis revealed significant epitope signatures in COVID-19 patients. Front Immunol [Internet]. 2021 Mar 23 [cited 2021 May 24];12:765. Available from: www.frontiersin.org

15. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2 [Internet]. Vol. 2020, cochrane database of systematic reviews. John Wiley and Sons Ltd; 2020 [cited 2021 Jun 14]. Available from: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013652/full

16. La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM. Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. Vol. 41, Reprod. Biomed. Online. Elsevier Ltd; 2020. p. 483–99.

17. Arya R, Kumari S, Pandey B, Mistry H, Bihani SC, Das A, et al. Structural insights into SARS-CoV-2 proteins. Vol. 433, J. Mol. Biol. Academic Press; 2021. p. 166725.

18. Ceraolo C, Giorgi FM. Genomic variance of the 2019-nCoV coronavirus. J Med Virol [Internet]. 2020 May 1 [cited 2021 Jun 14];92(5):522–8. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jmv.25700

19. Holenya P, Lange PJ, Reimer U, Woltersdorf W, Pan-Terodt T, Glas M, et al. Peptide microarray based analysis of antibody responses to SARS-CoV-2 identifies unique epitopes with potential for diagnostic test development. medRxiv [Internet]. 2020 Nov 27 [cited 2020 Dec 11];2020.11.24.20216663. Available from: https://doi.org/10.1101/2020.11.24.20216663

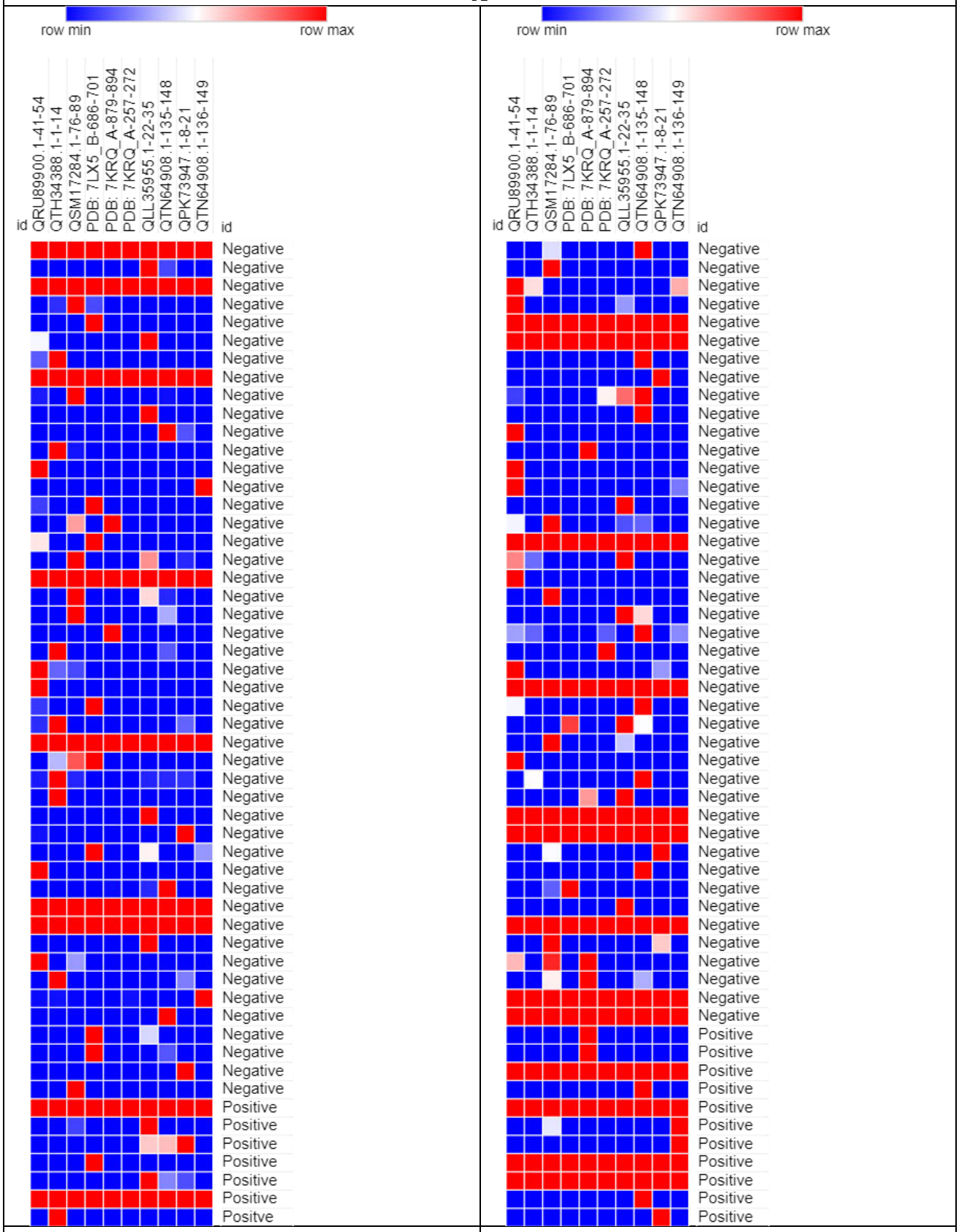
20. Lopandić Z, Protić-Rosić I, Todorović A, Glamočlija S, Gnjatović M, Ćujic D, et al. Igm and igg immunoreactivity of sars-cov-2 recombinant m protein. Int J Mol Sci [Internet]. 2021 May

1 [cited 2021 Jun 14];22(9):4951. Available from: https://doi.org/10.3390/ijms22094951

21. Burbelo PD, Riedo FX, Morishima C, Rawlings S, Smith D, Das S, et al. Detection of nucleocapsid antibody to SARS-CoV-2 is more sensitive than antibody to spike protein in COVID-19 patients. medRxiv. 2020 Apr 24;2020.04.20.20071423.

22. Satarker S, Nampoothiri M. Structural proteins in severe acute respiratory syndrome coronavirus-2. Vol. 51, Arch. Med. Res. Elsevier Inc.; 2020. p. 482–91.

A



IgG heat map

IgM heat map

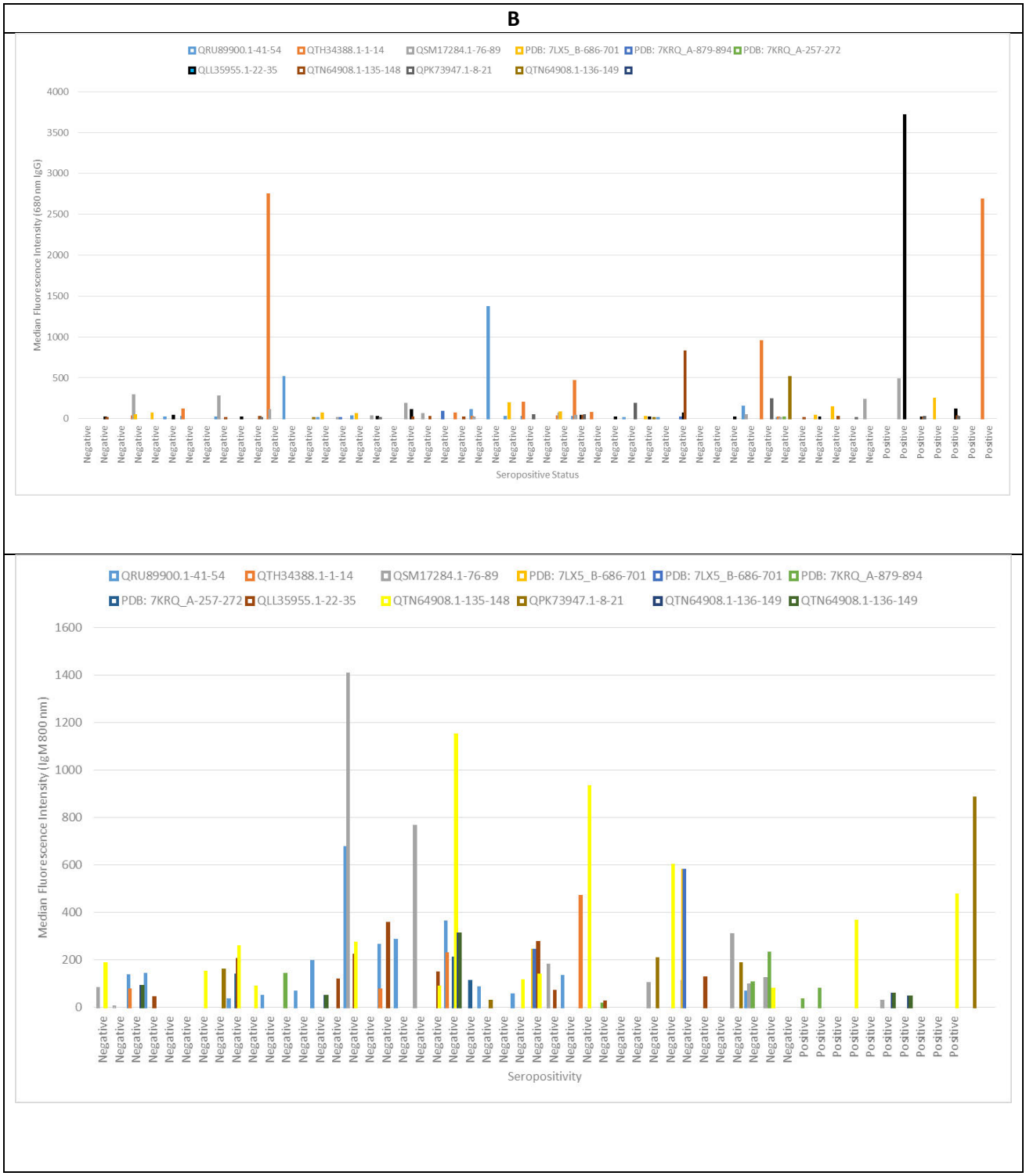


Figure 1: Heat maps and bar graphs generated from peptide microarray technology data. A. Heat maps generated from peptide microarray technology. Samples were arranged in rows and infection status shown on the left key. Peptides shown by their protein accession number and sequence position are arranged in columns. The false-colored rainbow color from blue to red corresponds to the signals of antibody binding from low to high, respectively. (B) Bar graphs representing the peptides reactivity for each serum and plasma in the study (IgG top and IgM bottom).

CHAPTER 6

SYNTHESIS

6.1 Background

The general objective of this study was to select published peptides and predict immunogenic B-cell epitopes (peptides) using bioinformatics softwares and validation of the epitopes using multiplex peptide microarray immunoassays.

Serological assays commonly used for the diagnosis of infectious diseases are relatively inexpensive and easy to perform compared to biochemical tests, cultures or nucleic acid-based methods (47). In cases where faecal specimens are unavailable serology can have a role in diagnosis of STHs and *S. mansoni* (15). However, for sero-diagnosis of human helminthic infections, many tests in use rely on native antigens, either extracted from whole pathogens maintained in laboratory animals, or cultivated *in vitro* to obtain metabolic antigens. These natural antigens are limited in availability and suffer from batch-to-batch variation and their production is laborious. Recombinant antigens used in sero-diagnostic tests require a high degree of purification to avoid cross-reactivity due to contaminants from the expression system (48). The limitations associated with native antigens and recombinant antigens such as unspecific binding and cross-reactivity in serological diagnosis of various diseases, may be resolved by the use of standardized and highly pure synthetic peptide (48). Studies on leishmaniasis, Chagas disease, schistosomiasis, paracoccidioidomycosis, tuberculosis, and, more recently, on cryptococcosis, among others, have shown that this approach has potential for the early diagnosis of disease, thus reducing the morbi-lethality of individuals affected by these infections and ultimately changing their prognosis (49,50).

For improving diagnostic test performance, it is desirable to identify highly specific and highly reactive epitopes from the proteome of the pathogen in question and synthetically produce the corresponding peptide antigens. Synthetic peptides are advantageous for diagnostic applications since they are well defined, easily produced in large amounts, highly pure and often cost-saving if compared to the production of natural antigen in animal models or *in vitro* culture (48).

The background provided in Chapter 1 describes that collections of short synthetic peptides derived from different pathogens can be immobilised on solid planar supports such as microscope glass slides to produce peptide microarrays. Peptides microarrays are further described as large scale high throughput, screening platforms for the simultaneous identification of multiple pathogens from small quantities of serum or plasma and other body fluids (22). These peptide microarrays may be used for the intergrated diagnosis and surveillance of infectious diseases. The main advantage of the peptide microarray design is the miniaturisation of antibody-antigen interaction assays, the simultaneous analysis of several peptide sequences and the subsequent reduction in serum volume required from patients since this always represents a limiting factor in serological studies.

6.2 Scoping review of the applications of peptide microarrays on the fight against human infections.

This thesis investigated the clinical applications of peptide microarrays on the fight against human infections. A scoping review conducted showed that peptide microarrays to offer a wide range of applications, including, B-cell and T-cell epitope discovery, intergrated serological diagnosis and surveillance of infectious diseases and antimicrobial peptides discovery. Their most important application was shown to be B-cell epitope mapping (antibody profiling) to identify diagnostics and vaccine targets.

In this thesis, we also reviewed 6 methods that are used for the identification and prediction of B-cell epitopes. These were computational overlapping peptides sequences, computational permutation scans, published synthetic peptides, computational random peptide sequences, phage display library and *in silico* prediction. In the study we also reviewed that expect for *in silico* prediction methods experimental techniquenes; computational overlapping peptides sequences, computational permutation scans, computational random peptide sequences and phage display library peptide/epitope prediction methods are costly and time-consuming and demands large resources as they require screening of large arrays of potential epitope candidates (51,52). With this background published peptides (see **Table 6.1**) and *in silico* predicted peptides were used for this study. Published peptides were retrieved from the Tackling Infection to Benefit Africa infectious diseases epitope microarray. Novel Immunogenic peptides mimicking B-cell linear epitopes were predicted *in silico* using ABCpred (http://www.imtech.res.in/raghava/abcpred/).

Results from the review showed that three methods are used for the synthesis of peptides, solid phase peptide synthesis (SPPS) (which is the method of choice for the production of peptides for most researchers), SPOT synthesis and peptide laser printing technology offered by PEPperPRINT Inc. (Heidelberg, Germany) (53). This thesis also reviewed that generally, two methods are used for the synthesis of peptide microarrays: the immobilization of pre-synthesized peptides and *in situ* synthesis of peptides on a solid support. Peptide microarrays are offered by various providers including PEPperPRINT Inc. (Heidelberg, Germany) which generated custom peptide microarrays used in the study *in situ* in a laser-printer based approach.

6.3 Multiplex peptide microarray profiling of antibody reactivity against neglected tropical diseases derived B-cell epitopes for serodiagnosis in Zimbabwe.

In a time of fierce competition for global health funding, surveillance of a single infection is difficult to defend as a priority (54). Accurate surveillance of multiple-infections enable public health officials to identify opportunities for implementation of integrated control programs (55). Infectious diseases multiplex peptide microarray tools, could represent an integrated surveillance platform that could be used for monitoring exposure and for disease diagnosis. The above debate led to the following objective of the study, designing and production of a peptide microarray for the integrated surveillance of neglected tropical diseases endemic in Zimbabwe. Sero-reactivity was observed against peptides derived from *A. lumbricoides, N. americanus, S. haematobium, S. mansoni, T. trichiura, B. anthracis, M. leprae, W. bancrofti, R. lyssavirus, C. trachomatis* and *T. brucei*. Results from the thesis showed species-specific responses with at least one peptide derived from each NTD pathogen were observed. The reactive peptides included; for *S. haematobium,* XP_035588858.1-206-220 and XP_035588858.1-206-220 immunodominant for IgG and IgM respectively, for *S.mansoni,* P20287.1-58-72 immunodominant for both antibodies and for *T. trichuria,* CDW52482.1-326-340 immunodominant for IgG and CDW57769.1-2017-2031 and CDW57769.1-1518-1532 immunodominant for IgM. According to ROC analysis most of the peptides selected were inaccurate; with AUC < 0.5. Some peptides had AUC values ranging from 0.5 to 0.5875 for both IgM and IgG suggesting no discrimination.

6.4 Peptide microarray analysis of *in silico* predicted B-cell epitopes in SAR-CoV-2 seropositive humans in Zimbabwe.

Serologic assays detects the presence of antibodies against SARS-CoV-2 structural protein. Depending on the antibody type and antigen target, serologic tests may indicate exposure (non-neutralising antibodies) to SARS-CoV-2 (37). A large number of studies have examined antibody responses to SARS-CoV-2 using overlapping peptides derived from the SARS-CoV-2 structural proteins as antigens to identified reactivitity at the single epitope level. However, as highlighted earlier, experimental techniques including overlapping peptides, for the comprehensive mapping of B-cell epitopes are time consuming and expensive even for SARS-CoV-2 which have relatively few genes. In contrast, *in silico* B-cell epitope prediction bioinformatics techniques are a manageable alternative that allow for virtual cost-effective, scans in the search for immunodominant epitopes (30,31). Also with the fact that *in silico* B-cell epitope prediction coupled with peptide microarray technology provides a quick way for the discovery of B-cell epitopes one of the objective of the study aimed to discover linear B-cell epitopes for SARS-CoV-2 using *in silico* predicted linear B-cell epitopes and multiplex peptide microarray immunoassay.

In this thesis, ten SARS-CoV-2 ABC *in silico* predicted peptides were screened on a peptide microarray platform. Seven peptides that reacted above the cut-off (500 FU) were detected. For IgG four were detected; QTH34388.1-1-14 derived from the membrane glycoprotein, QRU89900.1-41-54 derived from the nucleocapsid protein, QTN64908.1-136-149 derived from membrane glycoprotein and QLL35955.1-22-35 derived from nucleocapsid protein. With respect to IgM, four for peptides were detected; QSM17284.1-76-89 derived from nucleocapsid protein, QPK73947.1-8-21 derived from membrane glycoprotein (reactive with one seropositive worker), QRU89900.1-41-54 and QTN64908.1-136-149. None of these peptides was singularly detected in more than one of the seropositive samples tested. None of the epitopes showed discrimination between the SARS-CoV-2 seropositive group and seronegative group**.** None of the detected peptide was immunodominant for neither IgG nor IgM**.**

6.5 Strengths of peptide microarray technology

The peptide microarray technology used in this study has many advantages, such as reduced laboratory efforts due to parallel multiplex screenings of serum, plasma, cerebrospinal fluid or other body fluids against multiple antigens derived from different pathogen. Minimal demand of sample volumes is also an important feature of peptide microarrays.

6.6 Limitations of peptide microarray technology

The peptide microarray technology used in this study displayed linear peptides and excludes non-protein epitopes for example carbohydrates, glycolipids and fatty acids which also serve as biologically relevant targets for humoral immune responses (56,57). Studies have shown that sera of individuals infected with helminth such as *S. haematobium* and *S. mansoni* contain IgG and IgM antibodies against defined carbohydrate epitopes (58,59).This meant that the peptide microarray technology could not detect antibodies that bind non-protein epitopes. While this does not appear to confound the diagnostic utility, we may miss conformation determinants or non-protein epitopes important in pathogenesis (60).

In silico prediction of B-cell epitopes is still an active biotechnology research field and a number of servers show improved performance, however, their prediction accuracies are still not satisfactory. ABCpred server predict B cell epitopes in an antigen sequence with 65.93 % accuracy using artificial recurrent neural network (machine based technique) (61). Current B-cell epitope predictors are trained on epitopes derived from heterogeneous experimental conditions including many cases in which laboratory animals were immunized with relatively large doses of highly purified antigens.

Unfortunately, it has been reported that humoral immune responses against the same antigen differ between species and members of the same species. Significant variability in individual B-cell epitope reactivity has been reported in tuberculosis and toxoplasmosis (62).

6.7 General Conclusions

This novel study highlights the fact that more individuals were exposed to schistosomiasis and STHs than the expected infection prevalence in Shamva, Murewa and Madziwa rural districts, Zimbabwe (Chapter 4). Sero-reactivity of a peptide derived from each NTD with at least one sample was indicative of the presence of these NTDs in the study population. This study presents seven novel B-cell epitopes for SARS-CoV-2 discovered using *in silico* predicted linear B-cell epitopes and multiplex peptide microarray immunoassay (Chapter 5). Notably, the SARS-CoV-2 reactive peptides were derived from the membrane glycoprotein and nucleocapsid protein. The reactive peptides (ranging from 8aa to 18 aa) discovered in the study are shown in Table 6.1 and most of the peptides were reactive with both IgM and IgG and IgM only compared to IgG only.

This study also demonstrates that multiplex peptide microarrays are a valuable tool for integrated NTDs surveillance and for the multiplex screening of exposure in areas of low endemicity. We conclude that bioinformatics *in silico* peptide prediction and multiplex peptide microarray immunoassay validation is a powerful platform for the simultaneous discovery of SARS-CoV-2 B-cell epitopes.

Table 6.1: Reactive B-cell epitopes

Peptide name	**Peptide Sequence**	**Source organism**	**Prediction/selection**	**Antibody type**
QPK73947.1-8-21	ITVEELKKLLEQWN	SARS-CoV-2	ABC PRED	IgM
QSM17284.1-76-89	TNSSPDDQIGYYRR	SARS-CoV-2	ABC PRED	IgM
QLL35955.1-22-35	DGKMKDLSPRWYFY	SARS-CoV-2	ABC PRED	IgG
QTH34388.1-1-14	MADSNGTITVEELK	SARS-CoV-2	ABC PRED	IgG
QTN64908.1-135-148	ESELVIGAVILRGH	SARS-CoV-2	ABC PRED	IgM
QRU89900.1-41-54	RPQGLPNNTASWFT	SARS-CoV-2	ABC PRED	IgG and IgM
QTN64908.1-136-149	SELVIGAVILRGHL	SARS-CoV-2	ABC PRED	IgG
CAA60047.1-**553-568**	TMKIYARDQGGIHNPP	*Ascaris lumbricoides*	ABC PRED	IgM
ACJ03763.1-**50-64**	TDPEIEADIDAFVAK	*Ascaris lumbricoides*	TIBA infectious disease epitope microarray	IgG and IgM
ACJ03764.1-**3852-38-52**	KQIITGAPDKTDAEI	*Ascaris lumbricoides*	TIBA infectious disease epitope microarray	IgG and IgM
AAD13652.1-**133-147**	RRHHFTLESSLDTHL	*Ascaris lumbricoides*	TIBA infectious disease epitope microarray	IgG and IgM
AAD13652.1-**142-156**	SLDTHLKWLSQEQKD	*Ascaris lumbricoides*	TIBA infectious disease epitope microarray	IgG and IgM
WP_151277871.1-**342-358**	GVATKGLNVHGKSSDWG	*Bacillus anthracis*	TIBA infectious disease epitope microarray	IgM
WP_040119992.1-**736-358**	IINPSENGDTSTNGIKK	*Bacillus anthracis*	TIBA infectious disease epitope microarray	IgG and IgM
WP_001022096.1-**35-44**	GGHGDVGMHV	*Bacillus anthracis*	TIBA infectious disease epitope microarray	IgG and IgM
WP_151277871.1-**307-316**	LVPEHADAFK	*Bacillus anthracis*	TIBA infectious disease epitope microarray	IgM
P0C0Z7.2-**260-270**	ATLVVNRIRGGF	*Chlamydia Trachomatis*	TIBA infectious disease epitope microarray	IgM
P19542.1-**261-270**	TKDASIDYHE	*Chlamydia Trachomatis*	TIBA infectious disease epitope microarray	IgM
AAO67542.1-**291-305**	LKFKVRKITSSHRGN	*Chlamydia Trachomatis*	TIBA infectious disease epitope microarray	IgM
AAA23156.1-**381-390**	TRLIDERAAH	*Chlamydia Trachomatis*	TIBA infectious disease epitope microarray	IgG and IgM
AAA23156.1-**73-81**	VLKTDVNKE	*Chlamydia Trachomatis*	TIBA infectious disease epitope microarray	IgG and IgM
CAA43269.1-**311-325**	AMKPDLQNTLMAVPR	*Mycobacterium Leprae*	TIBA infectious disease epitope microarray	IgG and IgM
WP_010907696.1-**11-25**	DKILVQAGEAETMTP	*Mycobacterium Leprae*	TIBA infectious disease epitope microarray	IgG and IgM
WP_010907618.1-**27-41**	LDGVTYEIDLTNKNA	*Mycobacterium Leprae*	TIBA infectious disease epitope microarray	IgG and IgM
CAA37572.1-**10-24**	EIDLTNKNAAKLRGD	*Mycobacterium Leprae*	TIBA infectious disease epitope microarray	IgG and IgM
WP_010907696.1-**1-15**	ETMTPSGLVIPENAK	*Mycobacterium Leprae*	TIBA infectious disease epitope microarray	IgG and IgM
CAC00543.1-**123-135**	SRKCPFYDIACML	*Necator Americanus*	TIBA infectious disease epitope microarray	
AAP41952.1-**180-192**	AGNMMGKDIYEKG	*Necator americanus*	TIBA infectious disease epitope microarray	

AAP41952.1-**194-206**	PCSKCENCDKEKG	*Necator americanus*	TIBA infectious disease epitope microarray	IgM
AHC94315.1-**209-222**	DIFTNSRGKRASKG	*Rabies lyssavirus*	ABC PRED	IgM
AAD10459.1-**312-325**	VPHSYFIHFRSLGL	*Rabies lyssavirus*	ABC PRED	IgM
BAJ04981.1-**12-26**	QYEYKYPAIKDLKKP	*Rabies lyssavirus*	TIBA infectious disease epitope microarray	IgG and IgM
QEJ74712.1-**99-113**	NVGVQIVRQMRSGER	*Rabies lyssavirus*	TIBA infectious disease epitope microarray	IgM
XP_012799745.1-**16-30**	SFLEMDADNNEMIDK	*Schistosoma haematobium*	TIBA infectious disease epitope microarray	IgG and IgM
XP_035587815.1-**269-283**	EISLDPIYKPEDLCI	*Schistosoma haematobium*	TIBA infectious disease epitope microarray	IgG and IgM
XP_035588858.1-**206-220**	EDSDEDDSTVYEVAM	*Schistosoma haematobium*	TIBA infectious disease epitope microarray	IgG and IgM
XP_012797374.1-**78-92**	NHIKTVQSGREPDLP	*Schistosoma haematobium*	TIBA infectious disease epitope microarray	IgG and IgM
AAZ29530.1-**25-29**	PINHGPKDVSIQTYP	*Schistosoma haematobium*	TIBA infectious disease epitope microarray	IgG and IgM
P09792.1-**29-43**	VDYEDERISFQDWPK	*Schistosoma mansoni*	TIBA infectious disease epitope microarray	IgG and IgM
P20287.1-**58-72**	GEVSTENGKLKVNGK	*Schistosoma mansoni*	TIBA infectious disease epitope microarray	IgG and IgM
AAA29900.1-**145-159**	CGAKGPDDYRGNVPA	*Schistosoma mansoni*	TIBA infectious disease epitope microarray	IgM
P09841.3-**6-20**	LFLISILHILLVKCQ	*Schistosoma mansoni*	TIBA infectious disease epitope microarray	IgM
AAA29903.1-**222-237**	KSDNQIKAVPASQAL	*Schistosoma mansoni*	TIBA infectious disease epitope microarray	IgG and IgM
CDW57769.1-**1518-1532**	VRYESFRVAADDFLD	*Trichuris trichiura*	TIBA infectious disease epitope microarray	IgG and IgM
CDW57769.1-**659-673**	DMLIKARTNVFAVNK	*Trichuris trichiura*	TIBA infectious disease epitope microarray	IgM
CDW57769.1-**834-848**	STLDQWRDHLEKLFA	*Trichuris trichiura*	TIBA infectious disease epitope microarray	IgG and IgM
CDW52482.1-**326-340**	TNEVWEAWTILDDYI	*Trichuris trichiura*	TIBA infectious disease epitope microarray	IgG and IgM
CDW57769.1-**2017-2031**	RPEYKDKECYLEHDE	*Trichuris trichiura*	TIBA infectious disease epitope microarray	IgG and IgM
APD74596.1-**96-110**	ENREKWEADKKLIVA	*Trypanosoma brucei*	TIBA infectious disease epitope microarray	IgM
CAC33895.1-**78-92**	ETDFKATADDNKKPQ	*Trypanosoma brucei*	TIBA infectious disease epitope microarray	IgM
XP_844815.1-**369-382**	SSKIKESKVILMAV	*Trypanosoma brucei gambiense*	ABC PRED	IgM
CAC33895.1-**163-176**	LNKALYGAKGKETT	*Trypanosoma brucei gambiense*	ABC PRED	
XP_011774209.1-**132-145**	SAIHATKTVDIKAL	*Trypanosoma brucei gambiense DAL972*	ABC PRED	IgG
AAC35355.1-**55-68**	EVVETDGKKKECSS	*Wuchereria bancroftii*	ABC PRED	IgM
AAC35355.1-**46-60**	GGDEYVTKGEVVETD	*Wuchereria bancroftii*	TIBA infectious disease epitope microarray	IgG and IgM

AAC35355.1-**77-91**	EPQAWCRPNENQSWT	*Wuchereria bancroftii*	TIBA infectious disease epitope microarray	IgG and IgM
AAC70783.1-**177-191**	FSKWRKNHMRQKSNK	*Wuchereria bancroftii*	TIBA infectious disease epitope microarray	IgG and IgM
ADP24698.1-**29-43**	TDDRINASDWPSMKS	*Wuchereria bancroftii*	TIBA infectious disease epitope microarray	IgG and IgM

The peptide name consisted of the protein/antigen accession number followed by the amino acids positions of the peptide in the protein.

6.8 Recommendations

Seroreactivity of at least one peptide derived from each NTD with at least one sample was indicative of the presence of these NTDs in the study population, however, we would need to include sera from uninfected individual from non-endemic areas with no history of exposure to determine the seroprevalence of the NTDs.

A clear limitation of conventional peptide microarrays is their restriction to linear protein epitopes, whereas conformational epitope antibody recognition cannot be identified (63). Detection of antibodies recognizing all potential epitopes whether linear, conformational or carbohydrate or LPS is a key requirement to comprehensively profile the humoral immune response (64). In future peptide microarray studies we recommend including both linear and conformational protein epitope peptide for evaluation.

We also recommend that the most prominent epitopes, recognizing different sera should be investigated in a substitution analysis to identify the amino acids that are crucial for the binding of the corresponding antibody species the antibody fingerprint. The results of the peptide microarrays should also be confirmed by ELISA using synthetic peptides.

The peptide microarray technology described in this study is too complex and expensive for routine clinical microbiology. We recommend that the peptides discovered be transferred to a wide range of platforms including, enzyme-linked immunosorbent assay, radio-immunoassay, lateral flow, western blot, and bead-based assays, where they may facilitate diagnostics, epidemiology, and vaccinology. Lateral flow assay can be used in field settings in low resource countries.

CHAPTER 7

REFERENCES

1. ZIMBABWE. Neglected tropical disease treatment report 2017. Uniting to combat neglected tropical diseases.
2. CDC's neglected tropical diseases program [Internet]. [cited 2020 Mar 3]. Available from: www.cdc.gov
3. Neglected tropical diseases can be eliminated in our lifetime: A Call for Action | WHO | Regional Office for Africa [Internet]. [cited 2020 Mar 3]. Available from: https://www.afro.who.int/health-topics/ntds/ntds-can-be-eliminated
4. Hotez PJ, Asojo OA, Adesina AM. Nigeria: "Ground zero" for the high prevalence neglected tropical diseases. PLoS Negl Trop Dis [Internet]. 2012 Jul 31 [cited 2020 Jun 20];6(7):e1600. Available from: https://dx.plos.org/10.1371/journal.pntd.0001600
5. Hotez PJ, Kamath A. Neglected tropical diseases in sub-Saharan Africa: Review of their prevalence, distribution, and disease burden. Vol. 3, PLoS Neglected Tropical Diseases. PLoS Negl Trop Dis; 2009.
6. D E, L S. Reconsidering the underestimated burden caused by neglected tropical diseases. Trends Parasitol. 2006;22(8).
7. Molyneux DH, Hotez PJ, Fenwick A. 'Rapid-impact interventions': How a policy of integrated control for Africa's neglected tropical diseases could benefit the poor. Vol. 2, PLoS Medicine. PLoS Med; 2005. p. 1064–70.
8. Zimbabwe | ESPEN [Internet]. [cited 2020 Mar 3]. Available from: http://espen.afro.who.int/countries/zimbabwe
9. Midzi N, Mduluza T, Chimbari MJ, Tshuma C, Charimari L, Mhlanga G, et al. Distribution of schistosomiasis and soil transmitted helminthiasis in Zimbabwe: Towards a national plan of action for control and elimination. Kabatereine NB, editor. PLoS Negl Trop Dis [Internet]. 2014 Aug 14 [cited 2020 Mar 3];8(8):e3014. Available from: http://dx.plos.org/10.1371/journal.pntd.0003014
10. Phiri I, Manangazira P, Macleod CK, Mduluza T, Dhobbie T, Chaora SG, et al. The Burden of and Risk Factors for Trachoma in Selected Districts of Zimbabwe: Results of 16 Population-Based Prevalence Surveys. Ophthalmic Epidemiol [Internet]. 2018 Dec 28 [cited 2020 Mar 3];25(sup1):181–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28532208
11. Zimbabwe | ESPEN [Internet]. [cited 2021 May 24]. Available from: https://espen.afro.who.int/countries/zimbabwe
12. Bhutta ZA, Sommerfeld J, Lassi ZS, Salam RA, Das JK. Global burden, distribution, and interventions

for infectious diseases of poverty. Infect Dis Poverty [Internet]. 2014 Jul 31 [cited 2020 Jun 5];3(1):21. Available from: https://idpjournal.biomedcentral.com/articles/10.1186/2049-9957-3-21

13. Rydevik G, Innocent GT, McKendrick IJ. Evaluating diagnostic tests with near-perfect specificity: Use of a Hui–walter approach when designing a trial of a DIVA test for bovine tuberculosis. Front Vet Sci [Internet]. 2018 Aug 15 [cited 2020 Feb 26];5(AUG):192. Available from: https://www.frontiersin.org/article/10.3389/fvets.2018.00192/full

14. Utzinger J, Becker SL, Knopp S, Blum J, Neumayr AL, Keiser J, et al. Neglected tropical diseases: Diagnosis, clinical management, treatment and control. Vol. 142, Swiss Medical Weekly. EMH Media; 2012.

15. Khurana S, Sethi S. Laboratory diagnosis of soil transmitted helminthiasis. Trop Parasitol. 2017;7(2):86.

16. WHO | World health organization [Internet]. [cited 2020 Jun 5]. Available from: https://www.who.int/schistosomiasis/epidemiology/table/en/

17. CDC - Trichuriasis - Diagnosis [Internet]. [cited 2020 Jun 5]. Available from: https://www.cdc.gov/parasites/whipworm/diagnosis.html

18. Lymphatic filariasis [Internet]. [cited 2020 Jun 5]. Available from: https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis

19. Lammie P, Solomon A, Secor E, Peeling R. Diagnostic needs for ntd programs. 2011;

20. CDC - Schistosomiasis - Diagnosis [Internet]. [cited 2020 Jun 5]. Available from: https://www.cdc.gov/parasites/schistosomiasis/diagnosis.html

21. Schistosomiasis [Internet]. [cited 2020 Jun 5]. Available from: https://www.who.int/news-room/fact-sheets/detail/schistosomiasis

22. Zandian A, Forsström B, Häggmark-Månberg A, Schwenk JM, Uhlén M, Nilsson P, et al. Whole-proteome peptide microarrays for profiling autoantibody repertoires within multiple sclerosis and narcolepsy. J Proteome Res [Internet]. 2017 Mar 3 [cited 2020 Feb 26];16(3):1300–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28121444

23. El-Manzalawy Y, Dobbs D, Honavar VG. In silico prediction of linear B-cell epitopes on proteins. Methods Mol. Biol. Humana Press Inc.; 2017. p. 255–64.

24. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. The Lancet. Lancet Publishing Group; 2014. p. 2253–64.

25. WHO | Soil-transmitted helminthiases [Internet]. [cited 2020 Jun 5]. Available from: https://www.who.int/gho/neglected_diseases/soil_transmitted_helminthiases/en/

26. CDC - Soil-transmitted helminths [Internet]. [cited 2020 Jun 5]. Available from: https://www.cdc.gov/parasites/sth/index.html

27. Trachoma [Internet]. [cited 2020 Jun 5]. Available from: https://www.who.int/news-room/fact-sheets/detail/trachoma

28. Situation Summary | CDC [Internet]. [cited 2020 May 27]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/summary.html

29. Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. Acta Paediatr [Internet]. 2020 Jun 14 [cited 2020 May 27];109(6):1088–95. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/apa.15270

30. Ren L-L, Wang Y-M, Wu Z-Q, Xiang Z-C, Guo L, Xu T, et al. Identification of a novel coronavirus causing severe pneumonia in human. Chin Med J (Engl) [Internet]. 2020 May 5 [cited 2020 May 28];133(9):1015–24. Available from: http://journals.lww.com/10.1097/CM9.0000000000000722

31. NIH clinical trial of investigational vaccine for COVID-19 begins | National Institutes of Health (NIH) [Internet]. [cited 2020 May 27]. Available from: https://www.nih.gov/news-events/news-releases/nih-clinical-trial-investigational-vaccine-covid-19-begins

32. COVID-19 situation update worldwide, as of 11 November 2020 [Internet]. [cited 2020 Nov 12]. Available from: https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases

33. Coronavirus [Internet]. [cited 2020 Nov 12]. Available from: https://www.who.int/health-topics/coronavirus#tab=tab_1

34. Coronavirus disease 2019 (COVID-19) Situation Report-63 HIGHLIGHTS.

35. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis [Internet]. 2020 Mar 21 [cited 2020 Apr 2]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/32198501

36. Diagnostic testing for SARS-CoV-2 [Internet]. [cited 2020 Nov 18]. Available from: https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2

37. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Clinical infectious diseases ® 2020;XX(XX):1-8. [cited 2020 May 28]; Available from: https://academic.oup.com/cid/advance-article-abstract/doi/10.1093/cid/ciaa310/5810754

38. Kai-Wang To K, Tak-Yin Tsang O, Leung W-S, Raymond Tam A, Wu T-C, Christopher Lung D, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. 2020 [cited 2020 May 31]; Available from: www.thelancet.com/infection

39. Wikramaratna P, Paton RS, Ghafari M, Lourenco J. Estimating false-negative detection rate of SARS-CoV-2 by RT-PCR. medRxiv [Internet]. 2020 Apr 14 [cited 2020 May 28];2020.04.05.20053355. Available from: https://doi.org/10.1101/2020.04.05.20053355

40. Li D, Wang D, Dong J, Wang N, Huang H, Xu H, et al. False-negative results of real-time reverse-transcriptase polymerase chain reaction for severe acute respiratory syndrome coronavirus 2: Role of deep-learning-based ct diagnosis and insights from two cases. Korean J Radiol. 2020 Apr 1;21(4):505–8.

41. Zhang W, Du RH, Li B, Zheng XS, Yang X Lou, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect [Internet]. 2020 Jan 1 [cited 2020 May 28];9(1):386–9. Available from: https://www.tandfonline.com/doi/full/10.1080/22221751.2020.1729071

42. The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks in clinical chemistry and laboratory medicine (CCLM) - Ahead of print [Internet]. [cited 2020 May 28]. Available from: https://www.degruyter.com/view/journals/cclm/ahead-of-print/article-10.1515-cclm-2020-0240/article-10.1515-cclm-2020-0240.xml

43. Qian C, Zhou M, Cheng F, Lin X, Gong Y, Xie X, et al. Development and multicenter performance evaluation of the first fully automated SARS-CoV-2 IgM and IgG immunoassays. medRxiv. 2020 Apr 21;2020.04.16.20067231.

44. SARS-CoV-2 serology tests | COVID testing | COVID serology | COVID-19 molecular tests [Internet]. [cited 2021 Mar 25]. Available from: https://worldwide.promega.com/applications/sars-cov-2-covid-serology-tests-pcr-testing/

45. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins Struct Funct Genet [Internet]. 2006 Oct 1 [cited 2021 May 24];65(1):40–8. Available from: www.imtech.res.in/raghava/bcepred/

46. Schwarz T, Heiss K, Mahendran Y, Casilag F, Kurth F, Sander LE, et al. SARS-CoV-2 proteome-wide analysis revealed significant epitope signatures in COVID-19 patients. Front Immunol [Internet]. 2021 Mar 23 [cited 2021 May 24];12:765. Available from: www.frontiersin.org

47. Bergamaschi G, Fassi EMA, Romanato A, D'Annessa I, Odinolfi MT, Brambilla D, et al. Computational analysis of dengue virus envelope protein (E) reveals an epitope with flavivirus immunodiagnostic potential in peptide microarrays. Int J Mol Sci [Internet]. 2019 Apr 2 [cited 2020 Nov 23];20(8). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/31003530/

48. List C, Qi W, Maag E, Gottstein B, Müller N, Felger I. Serodiagnosis of *Echinococcus spp*. infection: Explorative selection of diagnostic antigens by peptide microarray. PLoS Negl Trop Dis [Internet]. 2010

Aug 3 [cited 2020 Feb 26];4(8):e771. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20689813

49. Noya O, Patarroyo M, Guzman F, de Noya B. Immunodiagnosis of parasitic diseases with synthetic peptides. Curr Protein Pept Sci [Internet]. 2003 Aug 1 [cited 2020 Mar 3];4(4):299–308. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1389-2037&volume=4&issue=4&spage=299

50. Brandão RMS de S, Martins LMS, Monte SJH do. Synthetic peptides as an alternative tool for the diagnosis of cryptococcosis in fungal pathogenicity [Internet]. InTech; 2016 [cited 2020 Feb 26]. Available from: http://www.intechopen.com/books/fungal-pathogenicity/synthetic-peptides-as-an-alternative-tool-for-the-diagnosis-of-cryptococcosis

51. Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA. Fundamentals and methods for T- and B-cell epitope prediction. Vol. 2017, Journal of Immunology Research. Hindawi Limited; 2017.

52. Sanchez-Lockhart M, Reyes DS, Gonzalez JC, Garcia KY, Villa EC, Pfeffer BP, et al. Qualitative profiling of the humoral immune response elicited by rVSV-ΔG-EBOV-GP using a systems serology assay, domain programmable arrays. Cell Rep [Internet]. 2018 Jul 24 [cited 2021 Feb 9];24(4):1050-1059.e5. Available from: https://doi.org/10.1016/j.celrep.2018.06.077

53. PEPperPRINT: Peptide microarray analysis [Internet]. [cited 2021 Jan 14]. Available from: https://www.pepperprint.com/technology/peptide-microarray-analysis/

54. Lammie PJ, Moss DM, Brook Goodhew E, Hamlin K, Krolewiecki A, West SK, et al. Development of a new platform for neglected tropical disease surveillance. Int J Parasitol. 2012 Aug 1;42(9):797–800.

55. Njenga SM, Kanyi HM, Arnold BF, Matendechero SH, Onsongo JK, Won KY, et al. Integrated cross-sectional multiplex serosurveillance of IgG antibody responses to parasitic diseases and vaccines in coastal Kenya. Am J Trop Med Hyg [Internet]. 2020 Nov 25 [cited 2021 Jun 13];102(1):164–76. Available from: https://osf.io/taknp

56. Heidepriem J, Krähling V, Dahlke C, Wolf T, Klein F, Addo MM, et al. Epitopes of naturally acquired and vaccine-induced anti-Ebola virus glycoprotein antibodies in single amino acid resolution. Biotechnol J. 2020 Sep 1;15(9).

57. Gaseitsiwe S, Valentini D, Mahdavifar S, Magalhaes I, Hoft DF, Zerweck J, et al. Pattern recognition in pulmonary tuberculosis defined by high content peptide microarray chip analysis representing 61 proteins from *M. tuberculosis*. PLoS One [Internet]. 2008 Dec 9 [cited 2020 Nov 24];3(12). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/19065269/

58. Michelle Yang YY, Van Diepen A, Brzezicka K, Reichardt NC, Hokke CH. Glycan microarray-assisted identification of IgG subclass targets in schistosomiasis. Front Immunol. 2018 Oct 9;9(OCT):2331.

59. Van Remoortere A, Van Dam GJ, Hokke CH, Van den Eijnden DH, Van Die I, Deelder AM. Profiles of immunoglobulin M (IgM) and IgG antibodies against defined carbohydrate epitopes in sera of *Schistosoma*-infected individuals determined by surface plasmon resonance. Infect Immun [Internet]. 2001 [cited 2021 Jun 13];69(4):2396–401. Available from: https://pubmed.ncbi.nlm.nih.gov/11254599/

60. Tokarz R, Mishra N, Tagliafierro T, Sameroff S, Caciula A, Chauhan L, et al. A multiplex serologic platform for diagnosis of tick-borne diseases. Sci Rep [Internet]. 2018 Dec 1 [cited 2020 Nov 23];8(1). Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/29453420/

61. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins Struct Funct Genet [Internet]. 2006 Oct 1 [cited 2021 Jun 14];65(1):40–8. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/prot.21078

62. Carmona SJ, Sartor PA, Leguizamón MS, Campetella OE, Agüero F. Diagnostic peptide discovery: prioritization of pathogen diagnostic markers using multiple features. PLoS One [Internet]. 2012 Dec 14 [cited 2021 Jun 14];7(12):50748. Available from: www.plosone.org

63. Loeffler FF, Pfeil J, Heiss K. High-density peptide arrays for malaria vaccine development. Methods Mol. Biol. [Internet]. Humana Press Inc.; 2016 [cited 2021 Jan 14]. p. 569–82. Available from: https://link.springer.com/protocol/10.1007/978-1-4939-3387-7_32

64. Legutki JB, Magee DM, Stafford P, Johnston SA. A general method for characterization of humoral immunity induced by a vaccine or infection. Vaccine [Internet]. 2010 Jun 17 [cited 2020 Nov 23];28(28):4529–37. Available from: https://pubmed-ncbi-nlm-nih-gov.ukzn.idm.oclc.org/20450869/

CHAPTER 8

APPENDIX

8.1 S1 Table. PRISMA Extension for Scoping Reviews guidelines checklist.

SECTION	ITEM	PRISMA-ScR CHECKLIST ITEM	REPORTED ON PAGE #
TITLE			
Title	1	Identify the report as a scoping review.	1
ABSTRACT			
Structured summary	2	Provide a structured summary that includes (as applicable): background, objectives, eligibility criteria, sources of evidence, charting methods, results, and conclusions that relate to the review questions and objectives.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known. Explain why the review questions/objectives lend themselves to a scoping review approach.	3 and 4
Objectives	4	Provide an explicit statement of the questions and objectives being addressed with reference to their key elements (e.g., population or participants, concepts, and context) or other relevant key elements used to conceptualize the review questions and/or objectives.	5
METHODS			
Protocol and registration	5	Indicate whether a review protocol exists; state if and where it can be accessed (e.g., a Web address); and if available, provide registration information, including the registration number.	N/A
Eligibility criteria	6	Specify characteristics of the sources of evidence used as eligibility criteria (e.g., years considered, language, and publication status), and provide a rationale.	5 and 6
Information sources*	7	Describe all information sources in the search (e.g., databases with dates of coverage and contact with authors to identify additional sources), as well as the date the most recent search was executed.	6
Search	8	Present the full electronic search strategy for at least 1 database, including any limits used, such that it could be repeated.	6 and 7
Selection of sources of evidence†	9	State the process for selecting sources of evidence (i.e., screening and eligibility) included in the scoping review.	7
Data charting process‡	10	Describe the methods of charting data from the included sources of evidence (e.g., calibrated forms or forms that have been tested by the team before their use, and whether data charting was done independently or in duplicate) and any processes for obtaining and confirming data from investigators.	7
Data items	11	List and define all variables for which data were sought and any assumptions and simplifications made.	Click here to enter text.
Critical appraisal of individual sources of evidence§	12	If done, provide a rationale for conducting a critical appraisal of included sources of evidence; describe the methods used and how this information was used in any data synthesis (if appropriate).	7
Synthesis of results	13	Describe the methods of handling and summarizing the data that were charted.	7
RESULTS			
Selection of sources of evidence	14	Give numbers of sources of evidence screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally using a flow diagram.	8 and figure 2
Characteristics of sources of evidence	15	For each source of evidence, present characteristics for which data were charted and provide the citations.	8 and 9 and S2 Table
Critical appraisal within sources of evidence	16	If done, present data on critical appraisal of included sources of evidence (see item 12).	N/A

SECTION	ITEM	PRISMA-ScR CHECKLIST ITEM	REPORTED ON PAGE #
Results of individual sources of evidence	17	For each included source of evidence, present the relevant data that were charted that relate to the review questions and objectives.	S2 Table
Synthesis of results	18	Summarize and/or present the charting results as they relate to the review questions and objectives.	S2 Table
DISCUSSION			
Summary of evidence	19	Summarize the main results (including an overview of concepts, themes, and types of evidence available), link to the review questions and objectives, and consider the relevance to key groups.	9-18
Limitations	20	Discuss the limitations of the scoping review process.	18 and 19
Conclusions	21	Provide a general interpretation of the results with respect to the review questions and objectives, as well as potential implications and/or next steps.	19
FUNDING			
Funding	22	Describe sources of funding for the included sources of evidence, as well as sources of funding for the scoping review. Describe the role of the funders of the scoping review.	N/A

8.2: Ethical approval by Medical Research Council of Zimbabwe (MRCZ)

Telephone: 791792/791193
Telefax: (263) - 242 - 790715
E-mail: mrcz@mrcz.org.zw
Website: http://www.mrcz.org.zw

Medical Research Council of Zimbabwe
Josiah Tongogara / Mazowe Street
P. O. Box CY 573
Causeway
Harare

APPROVAL

MRCZ/A/2443 **26 February 2019**

Prof T. Mduluza
UZ- Department of Biochemistry
P.O Box MP 167
Mount Pleasant
Harare

RE: Developing and evaluating a comprehensive multiplex peptide array serological diagnostic in Zimbabwe (Lab-on-Chip)

Thank you for the application for review of Research Activity that you submitted to the Medical Research Council of Zimbabwe (MRCZ). Please be advised that the Medical Research Council of Zimbabwe has **reviewed** and **approved** your application to conduct the above titled study.

This approval is based on the review and approval of the following documents that were submitted to MRCZ for review: -

a) Completed MRCZ 101 application form
b) Study Protocol
c) Informed consent forms

- **APPROVAL NUMBER** **: MRCZ/A/2443**

This number should be used on all correspondence, consent forms and documents as appropriate.

- **TYPE OF MEETING** **: EXPEDITED**
- **APPROVAL DATE** **: 26 February 2019**
- **EXPIRATION DATE** **: 25 February 2020**

After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the MRCZ Offices should be submitted three months before the expiration date for continuing review.

•**SERIOUS ADVERSE EVENT REPORTING**: All serious problems having to do with subject safety must be reported to the Institutional Ethical Review Committee (IERC) as well as the MRCZ within 3 working days using standard forms obtainable from the MRCZ Offices or website.

•**MODIFICATIONS**: Prior MRCZ and IERC approval using standard forms obtainable from the MRCZ Offices is required before implementing any changes in the Protocol (including changes in the consent documents).

•**TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the MRCZ using standard forms obtainable from the MRCZ Offices or website.

•**QUESTIONS**: Please contact the MRCZ on Telephone No. (0242) 791792, 791193 or by e-mail on mrcz@mrcz.org.zw

Other

- Please be reminded to send in copies of your research results for our records as well as for Health Research Database.
- You're also encouraged to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.

Yours Faithfully

[redacted]

MRCZ SECRETARIAT
FOR CHAIRPERSON
MEDICAL RESEARCH COUNCIL OF ZIMBABWE

PROMOTING THE ETHICAL CONDUCT OF HEALTH RESEARCH

Telephone: 791193/08644073772
Telefax: (263) - 242 - 790715
E-mail: mrcz@mrcz.org.zw
Website: http://www.mrcz.org.zw

Medical Research Council of Zimbabwe
Josiah Tongogara / Mazowe Street
P. O. Box CY 573
Causeway
Harare

APPROVAL

MRCZ/A/2571

20 December 2019

Arthur Vengesai
UZ - Biochemistry Department
630 Churchill Ave,
P.O. Box MP 167
Mount Pleasant
Harare

RE: Developing and Evaluating a Comprehensive Multiplex Peptide Array Serological Diagnostics and Surveillance of Neglected Tropical Diseases in Zimbabwe

Thank you for the application for review of Research Activity that you submitted to the Medical Research Council of Zimbabwe (MRCZ). Please be advised that the Medical Research Council of Zimbabwe has **reviewed** and **approved** your application to conduct the above titled study.

This approval is based on the review and approval of the following documents that were submitted to MRCZ for review:-

1. Protocol version 1.0 dated November 14, 2019
2. Informed consent form version 2 dated November 14, 2019
3. Data collection tools Version November 14, 2019

- **APPROVAL NUMBER** : MRCZ/A/2571

This number should be used on all correspondence, consent forms and documents as appropriate.

- **TYPE OF MEETING** : Expedited
- **EFFECTIVE APPROVAL DATE** : 18 December, 2019
- **EXPIRATION DATE** : 17 December, 2020

After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the MRCZ Offices should be submitted three months before the expiration date for continuing review.

•**SERIOUS ADVERSE EVENT REPORTING**: All serious problems having to do with subject safety must be reported to the Institutional Ethical Review Committee (IERC) as well as the MRCZ within 3 working days using standard forms obtainable from the MRCZ Offices or website.

•**MODIFICATIONS**: Prior MRCZ and IERC approval using standard forms obtainable from the MRCZ Offices is required before implementing any changes in the Protocol (including changes in the consent documents).

•**TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the MRCZ using standard forms obtainable from the MRCZ Offices or website.

•**QUESTIONS**: Please contact the MRCZ on Telephone No. (0242) 791793 or by e-mail on mrcz@mrcz.org.zw

Other

- Please be reminded to send in copies of your research results for our records as well as for Health Research Database.
- You're also encouraged to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.
- In addition to this approval, all clinical trials involving drugs, devices and biologics (including other studies focusing on registered drugs) require approval of Medicines Control Authority of Zimbabwe (MCAZ) before commencement

[redacted]

MRCZ SECRETARIAT
FOR CHAIRPERSON
MEDICAL RESEARCH COUNCIL OF ZIMBABWE

MEDICAL RESEARCH COUNCIL OF ZIMBABWE
2019 -12- 18
APPROVED
P.O. BOX CY 573 CAUSEWAY, HARARE

PROMOTING THE ETHICAL CONDUCT OF HEALTH RESEARCH

8.3 Manuscripts emanating from the work.

8.3.1 Clinical utility of peptide microarrays in the serodiagnosis of neglected tropical diseases in sub-Saharan Africa: Protocol for a diagnostic test accuracy systematic review. bmjopen-2020-042279.R1

Your submission to BMJ Open has been accepted Inbox ×

BMJ Open <onbehalfof@manuscriptcentral.com> Jul 12, 2021, 3:02 PM (18 hours ago)
to me, vengesaiarthur

COVID-19: A message from BMJ: https://authors.bmj.com/policies/covid-19

12-Jul-2021
bmjopen-2020-042279.R1 - Clinical utility of peptide microarrays in the serodiagnosis of neglected tropical diseases in sub-Saharan Africa: Protocol for a diagnostic test accuracy systematic review

Dear Mr. Vengesai:

We are pleased to accept your article for publication in BMJ Open.

Within 2-3 working days, you will receive an email with payment options and instructions from BMJ's e-commerce partner, Copyright Clearance Center. You will be able to choose either to pay by credit card or invoice. If you are not making the payment yourself, you may forward the email to the person or organisation that will be paying on your behalf. Your article will not be processed by production until you have paid the article processing charge or requested an invoice. For more details on open access publication please visit our Author Hub: https://authors.bmj.com/open-access/.

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8.3.2 A systematic and meta-analysis review on the diagnostic accuracy of antibodies in the serological diagnosis of COVID-19. https://doi.org/10.1186/s13643-021-01689-3.

Vengesai *et al. Systematic Reviews* (2021) 10:155
https://doi.org/10.1186/s13643-021-01689-3

Systematic Reviews

RESEARCH Open Access

A systematic and meta-analysis review on the diagnostic accuracy of antibodies in the serological diagnosis of COVID-19

Arthur Vengesai[1,2*], Herald Midzi[1], Maritha Kasambala[1], Hamlet Mutandadzi[3], Tariro L. Mduluza-Jokonya[1,2], Simbarashe Rusakaniko[3], Francisca Mutapi[4], Thajasvarie Naicker[2] and Takafira Mduluza[1,2]