Development of ELISAs for the detection of interferon-gamma in rhinoceroses and elephants as diagnostic tools for *Mycobacterium bovis* and *Mycobacterium tuberculosis* infections

BY

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List of Abbreviations

a	alanine
aa	amino acid
A	ampicillin
ABTS	2, 2'-azino-bis(3-ehtylbenzthiazoline-6-sulphonic acid)
AfEpIFN-γ	African elephant interferon-gamma
Ag85	antigen 85
AG	ampicillin and glucose
AK	ampicillin and kanamycin
AMI	antibody-mediated immunity
AsEpIFN-γ	Asian elephant interferon-gamma
AUCC	Animal Use and Care Committee
BCG	Bacille Calmette-Guérin
bps	base pairs
BSA	bovine serum albumin
BTB	bovine tuberculosis
cDNA	complementary DNA
CDR	complementarity determining region
CFP-10	culture filtrate protein
CMI	cell mediated immunity
Con A	concanavalin A
CSF	colony stimulating factor
DAB	diaminobenzidine
DIVA	differentiating between infected and vaccinated animals
DR	direct repeat
DTH	delayed type hypersensitivity
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EIA	enzyme immuno-assay
ELISA	enzyme-linked immunosorbent assay
ESAT-6	early secreted antigenic target-6
ETR	exact tandem repeats
FCS	foetal clone serum

G	glucose
GW	Gateway
h	hour / s
HiP	Hluhluwe-iMfolozi Park
HIV	human immunodeficiency virus
HRP	horse radish peroxidase
HT	hypoxanthine thymidine
ICGA	immuno-chromatographic assay
IUCN	International Union for Conservation of Nature
IDT	intradermal test
IFN-γ	interferon-gamma
IgG	immunoglobulin G
IGRA	interferon-gamma release assay
IL	interleukin
IgY	yolk immunoglobulin
IgY^{uu}	IgY produced at Utrecht University
IgY^{up}	IgY produced at University of Pretoria
IPTG	isopropyl-β-D-1-thiogalactopyranoside
ip	intraperitoneal
IS	insertion sequence
IMAC	immobilized metal affinity chromatography
K	kanamycin
KNP	Kruger National Park
LB	Luria broth
LBAA	latex bead agglutination assay
LTBI	latent tuberculosis infection
MAPIA	multi-antigen print immuno-assay
MBCF	*Mycobacterium bovis* culture filtrate
MDR-TB	Multi-drug resistant-tuberculosis
min	minute / s
MHC	major histocompatibility complex
MPB/T	major secreted immunogenic protein
MP	fat-free milk powder

MIRU	mycobacterial interspersed repetitive units
MTBC	*Mycobacterium tuberculosis* complex
MW	moleclular weight
NCBI	National Center for Biotechnology Information
NDSB 201	non detergent sulfobetaines
NK	natural killer cell
nt	nucleotide
OD	optical density
OIE	Organisation Mondiale de la Santé Animale / World Organisation for Animal Health
OPD	*ortho*-phenylenediamine dihydrochloride
OVI	Onderstepoort Veterinary Institute
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGRS	polymorphic (GC)-rich sequences
POD	peroxidase
PPD	purified protein derivative
QFT	QuantiFERON®
REA	restriction enzyme analysis
rEpIFN-γ	recombinant elephant interferon-gamma
rEqIFN-γ	recombinant equine interferon-gamma
RFLP	restriction fragment length polymorphism
rMoGMCSF	recombinant mouse granulocyte macrophage colony stimulating factor
RNA	ribonucleic acid
rRhIFN-γ	recombinant rhinoceros interferon-gamma
rpm	revolutions per minute
RT	Rapid Test
RT-PCR	reverse transcriptase-PCR
s	second / s
scFv	single chain variable fragment
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis

SICTT	single intradermal comparative tuberculin test
SIT	single intradermal test
SOE	splice overlap extension
TB	tuberculosis
TEA	triethylamine
Th1	T-helper cell that participates in CMI
Th2	T-helper cell that participates in AMI
TMB	tetramethylbenzidine
TNF	tumour necrosis factor
TST	tuberculin skin test
2xTY	tryptone yeast medium
U	units (unit of enzyme)
UP	University of Pretoria
USDA	United States Department of Agriculture
UU	Utrecht University
v	valine
V_H	variable part of the heavy chain
V_L	variable part of the light chain
VNTR	variable number of tandem repeats
WHO	World Health Organisation

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Thesis Summary

Development of ELISAs for the detection of interferon-gamma in rhinoceroses and elephants as diagnostic tools for *Mycobacterium bovis* and *Mycobacterium tuberculosis* infections

by

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Promoter: Prof. J. Godfroid
Co-Promoters: Prof. V. PMG. Rutten and Dr. J. Fehrsen

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Bovine tuberculosis, caused by *Mycobacterium bovis*, has been reported in many wildlife species. In addition, it has been reported that *Mycobacterium tuberculosis* causes tuberculosis mainly in Asian elephants (*Elephas maximus*). The disease cannot be diagnosed clinically in its early stages since clinical signs only appear during the later stages of the infection. For early detection diagnosis has to be performed using cell mediated immune based techniques. For cattle, validated tests include the *in vivo* intradermal skin test (IDT) and the *in vitro* interferon-gamma (IFN-γ) based test. The IDT has not been validated for use in wildlife. In addition, this test would not be suitable for use in rhinoceroses and elephants due to their skin anatomy and the fact that animals have to be captured and immobilized twice. Bovigam™, proven to be very effective in detecting *M. bovis* infections in cattle, is used as an ancillary test but this enzyme-linked immunosorbent assay (ELISA) only recognizes the IFN-γ of cattle and of a limited number of other ruminant species. Therefore, anti-IFN-γ antibodies for different wildlife species have to be produced in order to make use of an IFN-γ test for the diagnosis of (bovine) tuberculosis in wildlife.

This thesis presents the results of a series of studies aimed towards the development of an IFN-γ capture ELISA for the early detection of *M. bovis* and *M. tuberculosis* infections, and the detection of infectious animals (shedders) in wildlife species. The first set of studies led to the production of monoclonal and polyclonal antibodies against recombinant white

rhinoceros IFN-γ (rRhIFN-γ) in mice and chickens respectively. One monoclonal antibody, 1H11 (and its subclone 1D11), was identified as a suitable antibody for the capture of both rRhIFN-γ and native RhIFN-γ, using polyclonal IgY as a detecting antibody (Chapter 2). To increase the number of IFN-γ specific antibodies to RhIFN-γ, the phage-displayed technique was utilized in the second study (Chapter 3). An immune phage-display library targeted against rRhIFN-γ was constructed. The library was panned against both rRhIFN-γ and recombinant Asian elephant IFN-γ (rAsEpIFN-γ). The antibodies, single chain variable fragments (scFvs), generated in this study (Chapter 3) were used as capture antibodies and 1D11 or IgY as detecting antibodies in an ELISA for the detection of rRhIFN-γ and rAsEpIFN-γ. The capture ELISAs proved to be most effective in detecting rRhIFN-γ. Recombinant AsEpIFN-γ could only be detected with the scFv/IgY ELISA format. In the third study (Chapter 4) efforts were concentrated at producing monoclonal antibodies in mice against rAsEpIFN-γ. Six monoclonal antibodies were identified. Three were specific to rAsEpIFN-γ and three cross-reacted with recombinant equine IFN-γ (rEqIFN-γ). These antibodies along with polyclonal IgY were used in different capture ELISAs to determine which one would provide the optimal results in detecting rAsEpIFN-γ. Results indicated detection of rAsEpIFN-γ was best achieved when a cross-reactive antibody was used as a capture antibody and a specific antibody was used as a detecting antibody.

Altogether, these results document the detection of rRhIFN-γ and rAsEpIFN-γ in different capture ELISAs. Therefore, these ELISAs provide the first steps towards the development of suitable diagnostic tools for the detection of *M. bovis* and *M. tuberculosis* infections in wildlife species.

"You are never given a wish without also being given the power to make it come true. You may have to work for it, however." (Bach 1977)