

Development of an ELISA for the detection of Interferon-gamma (IFN γ) as a diagnostic tool for tuberculosis in black (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*)

by

¹Darshana Morar, ¹Jacques Godfroid, ²Edwin Tijhaar, ²Aurel S. Negrea, ³Anita L. Michel and ²Victor PMG Rutten

1. University of Pretoria, Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, South Africa
2. Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Utrecht, The Netherlands
3. Agricultural Research Centre, Onderstepoort Veterinary Institute, TB Laboratory, Onderstepoort, South Africa



INTRODUCTION

- **What is BTB?**
- **Symptoms of BTB – symptoms vary between the different species**
- **Effect of BTB on the cattle industry**
- **Occurrence of BTB in other animal populations**



**BTB in different
animal species**



African Buffalo (*Syncerus caffer*)



BTB in Rhinoceros

Reported cases

- **Rhinoceros' Rhinorrhea: Cause of an outbreak of infection due to Airborne *Mycobacterium bovis* in Zookeepers – Dalovisio et al. 1992 (New Orleans, USA)**
- **Epizootic of *Mycobacterium bovis* in a zoologic park – Stetter et al. 1995 (New Orleans, USA)**

Diagnosis in Cattle

TB Skin Test

Reference test

In live cattle TB is diagnosed in the field with the TB skin test

IFN- γ test

Ancillary test

Moabs used in the ELISA will only recognise the IFN- γ of a limited number of ruminant species

Diagnosis in Wildlife

TB Skin Test

- 1. Unknown Specificity and Sensitivity**
- 2. No Validation**
- 3. It requires 2 immobilizations**

IFN- γ test

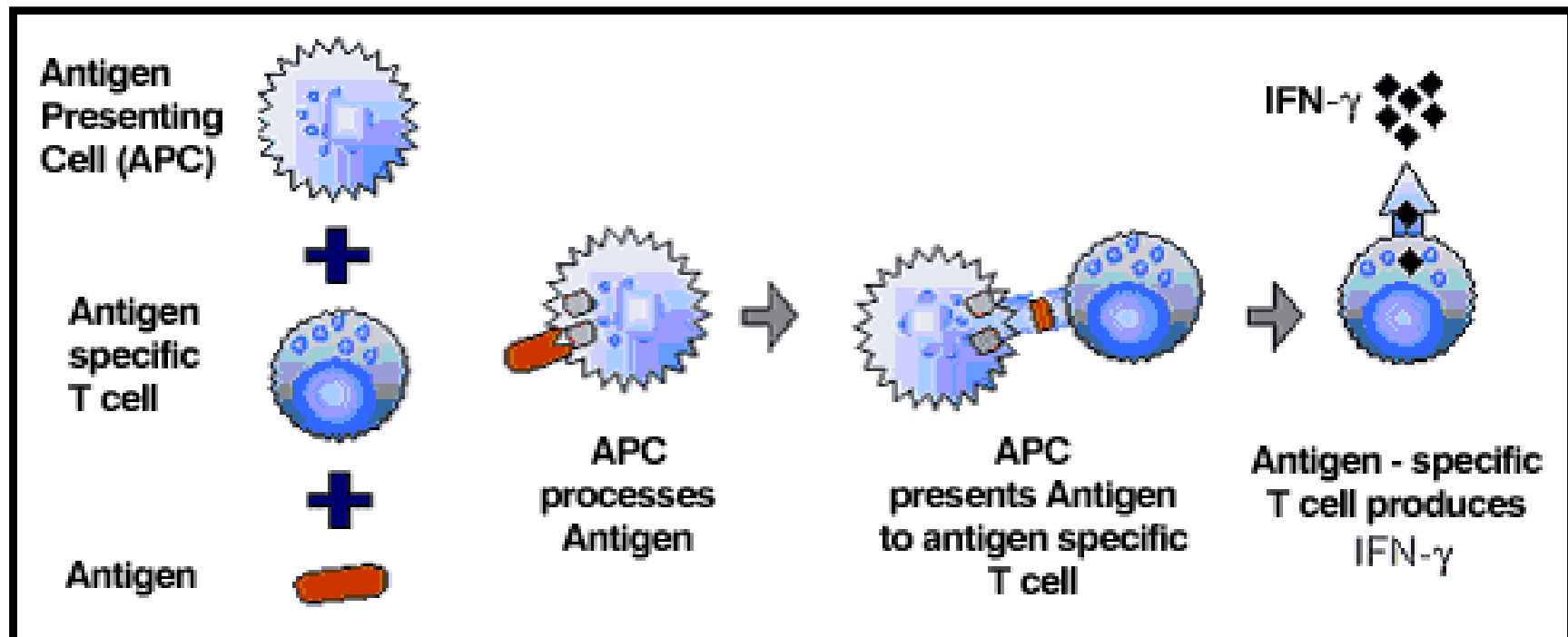
Wildlife species specific anti-IFN Ab have to be produced or show that the anti-bovine IFN- γ Ab is cross-reactive

OBJECTIVE

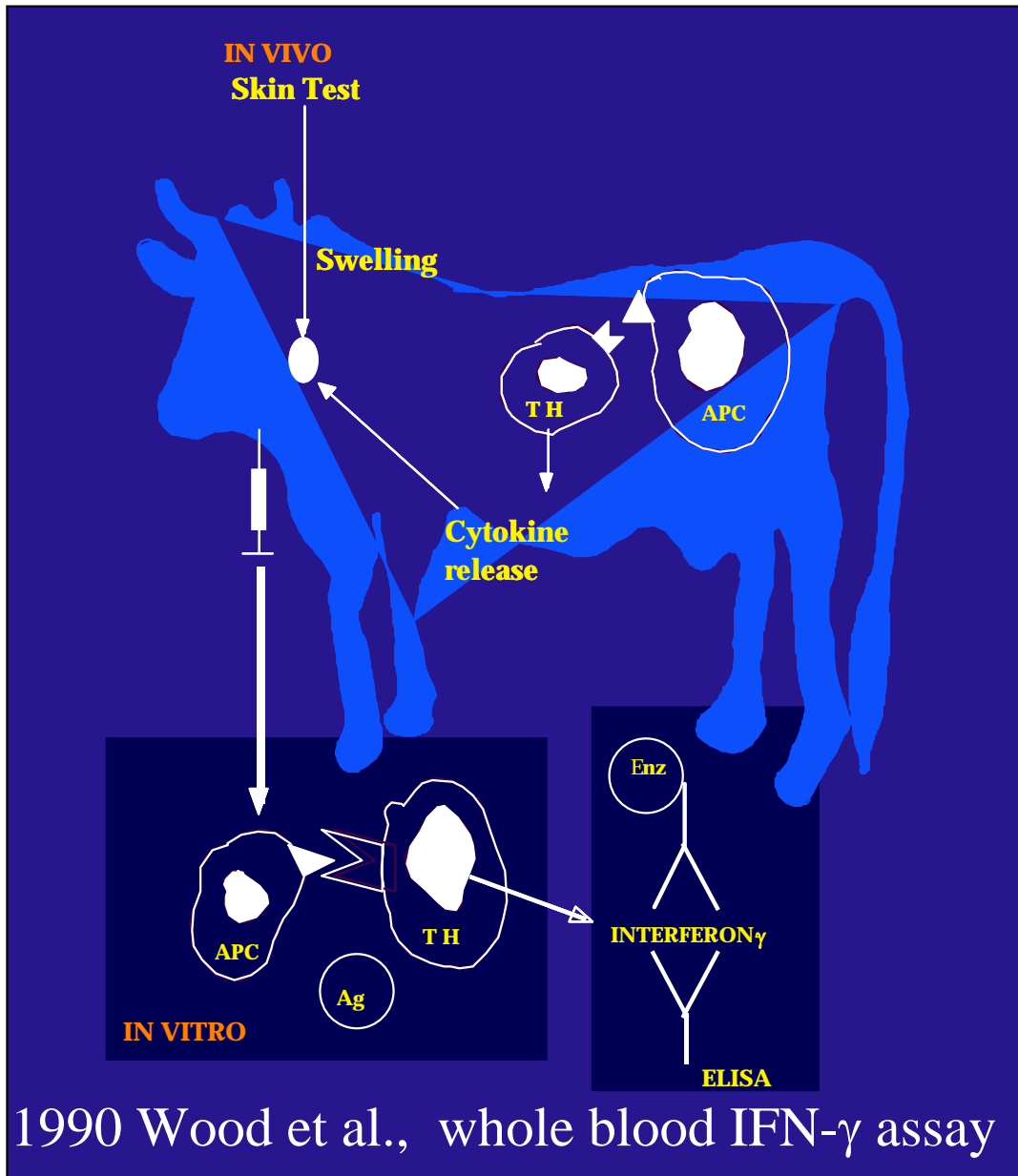
Designing a DIAGNOSTIC TEST that will prove valuable in detecting possible TB infection in rhinoceros using the cytokine IFN- γ as an indicator of *M. bovis* infection.

PRINCIPLE

IFN- γ is a type II interferon, a cytokine produced mainly by Th1 cells & cytotoxic T-cells. In response to a mycobacterial infection, antigen specific Th1 and cytotoxic T-cells are induced. When these cells encounter their specific mycobacterial antigen again, they will respond by producing IFN- γ .

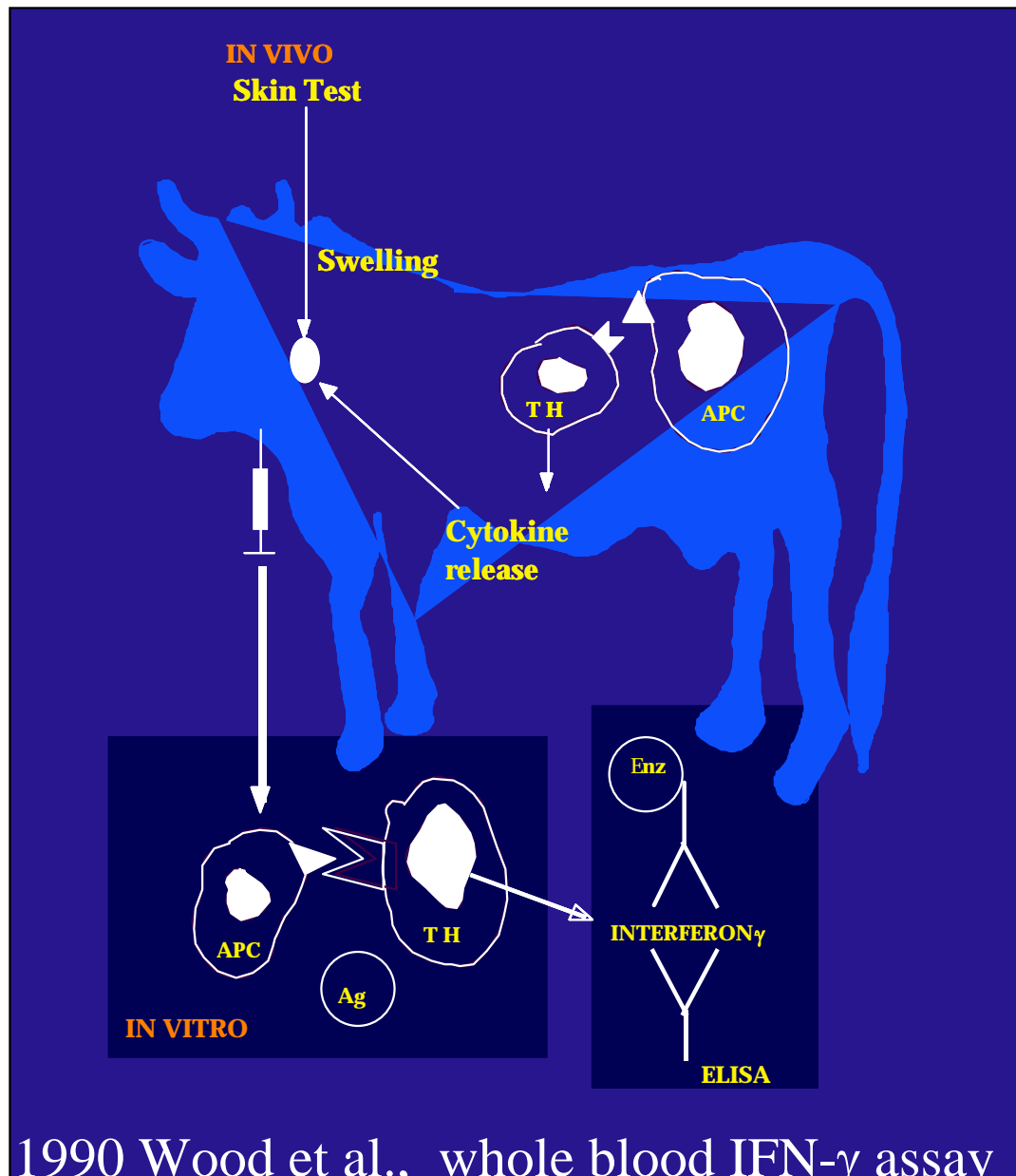


PRINCIPLE OF IFN- γ TEST AS ILLUSTRATED IN CATTLE



- Animals infected with *M. bovis* have lymphocytes in their blood that can recognise specific mycobacterial antigens present in bovine tuberculin purified protein derivatives (PPD's)
- During this recognition process the cytokine IFN- γ is generated and secreted by the bodies immune system
- This forms the basis of the test that was developed and patented by the CSIRO Australia

PRINCIPLE OF IFN- γ TEST AS ILLUSTRATED IN CATTLE



- Lymphocytes in whole blood cultures are exposed to tuberculin PPD antigens and the production of IFN- γ from the stimulated T-cells is detected using a monoclonal antibody based sandwich immunoassay (EIA)
- Lymphocytes from uninfected cattle do not produce IFN- γ and hence IFN- γ detection correlates with infection

DEVELOPMENT OF AN IFN- γ ELISA IN RHINO

- Based on this principle a diagnostic test has been developed for rhinos**
- In this test WB and or PBMCs' are isolated and stimulated with *M.bovis* specific antigens and the subsequent production of IFN-g by specific T-helper cells will be determined by an IFN-g specific ELISA**
- The basis of this ELISA is 2 monoclonal antibodies (M1 & M36) specific for IFN-g of rhinos**

EXPERIMENTS AND METHODOLOGY [1]

Generation of α RhinoIFN- γ antibodies

As a first step towards an *in vitro* diagnostic test for BTB in rhinoceros the following steps were followed:

The gene of interest was

- **Cloned**
- **Sequenced**
- **Expression of purified proteins**
- **Immunisation of mice / chickens**
- **Production of monoclonal & polyclonal antibodies**
- **Set up of IFN- γ ELISA**

EXPERIMENTS AND METHODOLOGY [2]

BCG Vaccination of 2 white rhinos



- WHY?** In order to show that rhinos are able to produce $\text{IFN}\gamma$ after a BTB antigen recall, they have to be sensitized, hence BCG vaccination

- HOW?** Blood in heparin and EDTA tubes was collected and tested in our ELISA system to determine the presence of $\text{IFN}\gamma$

EXPERIMENTS AND METHODOLOGY [3]

1. To determine if the test can detect recombinant and native IFN- γ

2. To determine if the test can detect BCG Vaccinated Rhino

Antigens Used:

Bovine PPD – Antigen of interest

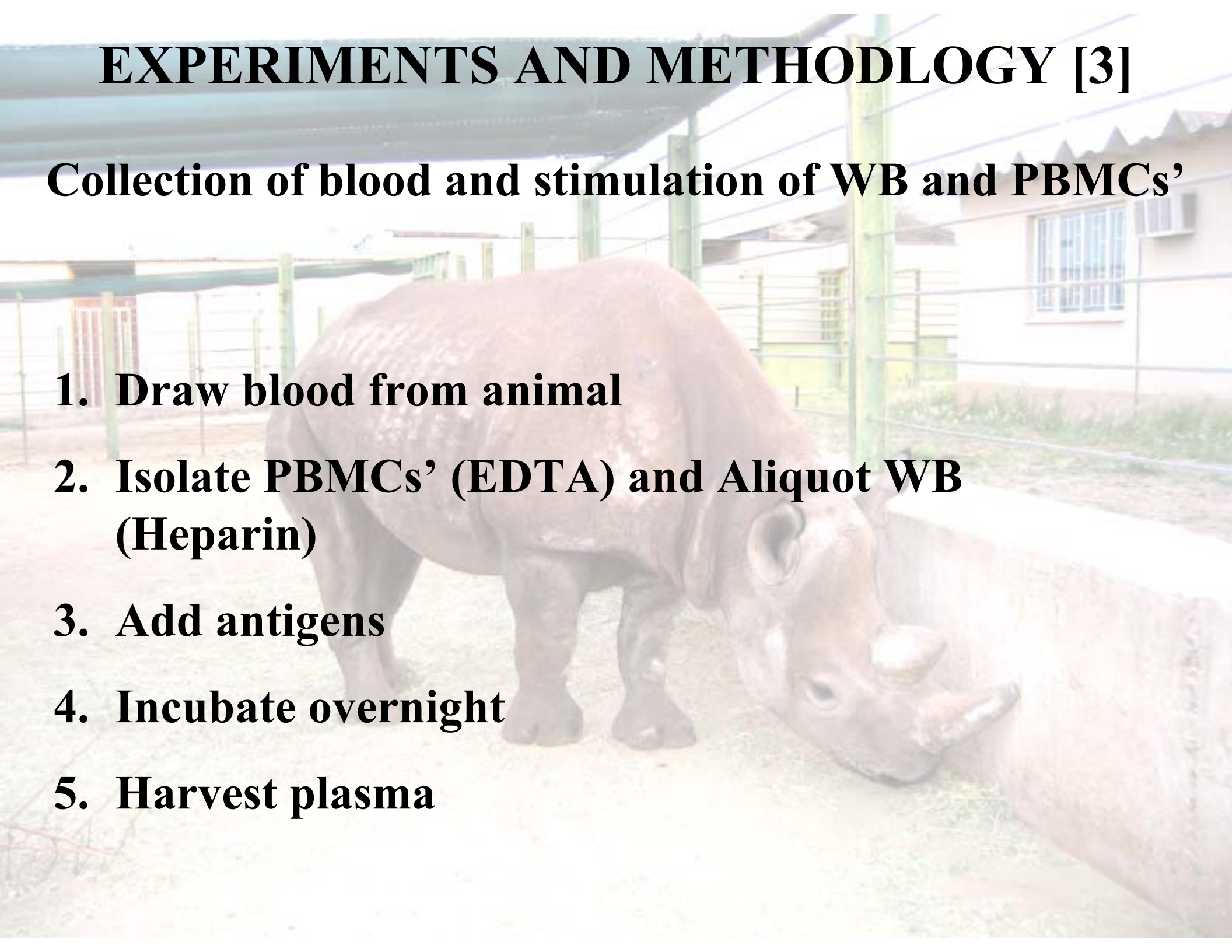
ESAT6 – Mycobacterial antigen that is not present in BCG vaccinated animals and therefore would serve as a negative control.

Concanavalin A (Con A) – Mitogen used for polyclonal activation and thus would serve as a positive control

Heat Killed BCG – Additional antigen

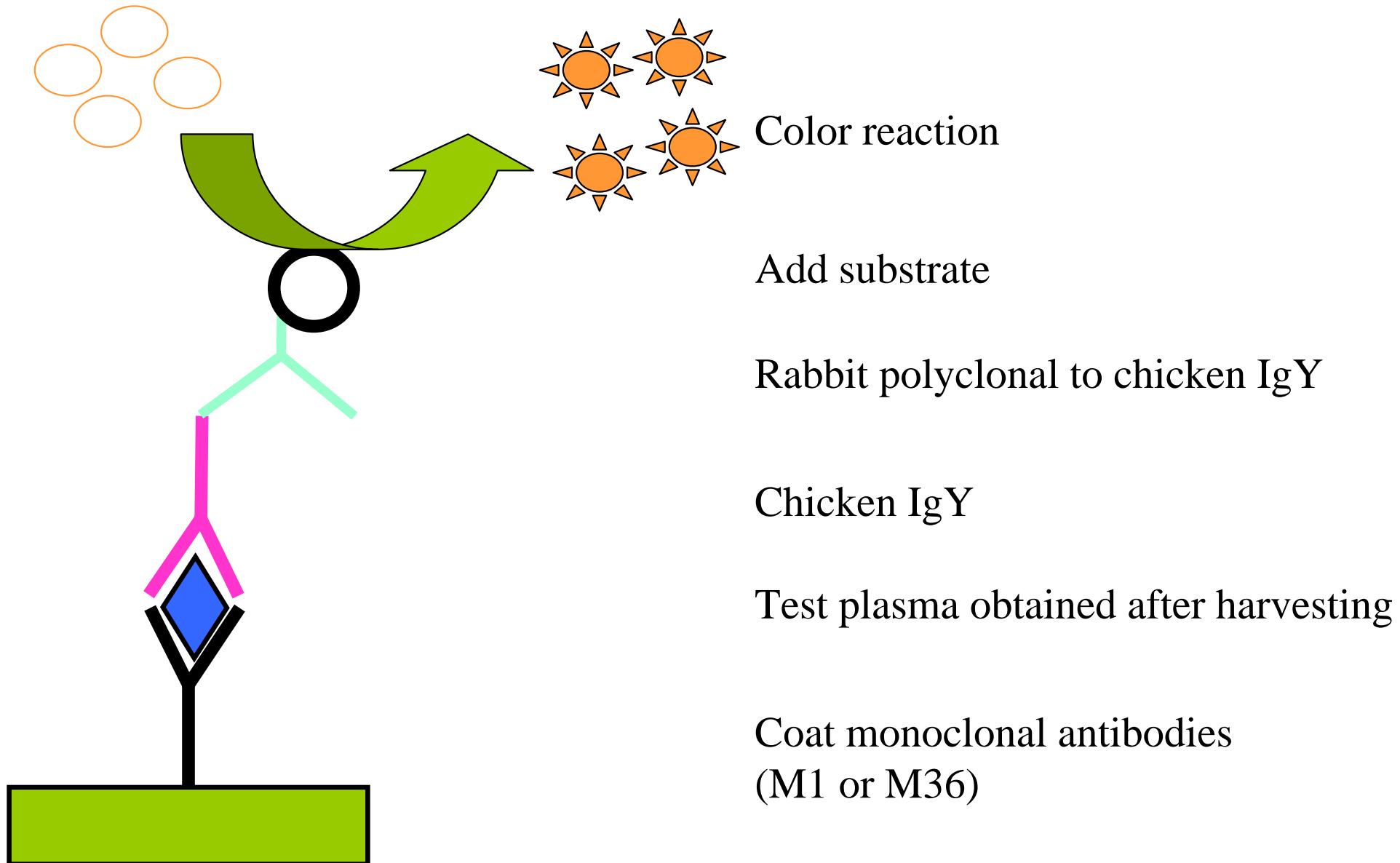
EXPERIMENTS AND METHODOLOGY [3]

Collection of blood and stimulation of WB and PBMCs'

- 1. Draw blood from animal**
 - 2. Isolate PBMCs' (EDTA) and Aliquot WB (Heparin)**
 - 3. Add antigens**
 - 4. Incubate overnight**
 - 5. Harvest plasma**
- 
- A rhinoceros is shown in a fenced enclosure, likely a zoo or research facility. The rhinoceros is dark brown and is standing on a dirt path. In the background, there are green metal fences and a building with windows. The image is slightly faded, serving as a background for the text.

EXPERIMENTS AND METHODOLOGY [3]

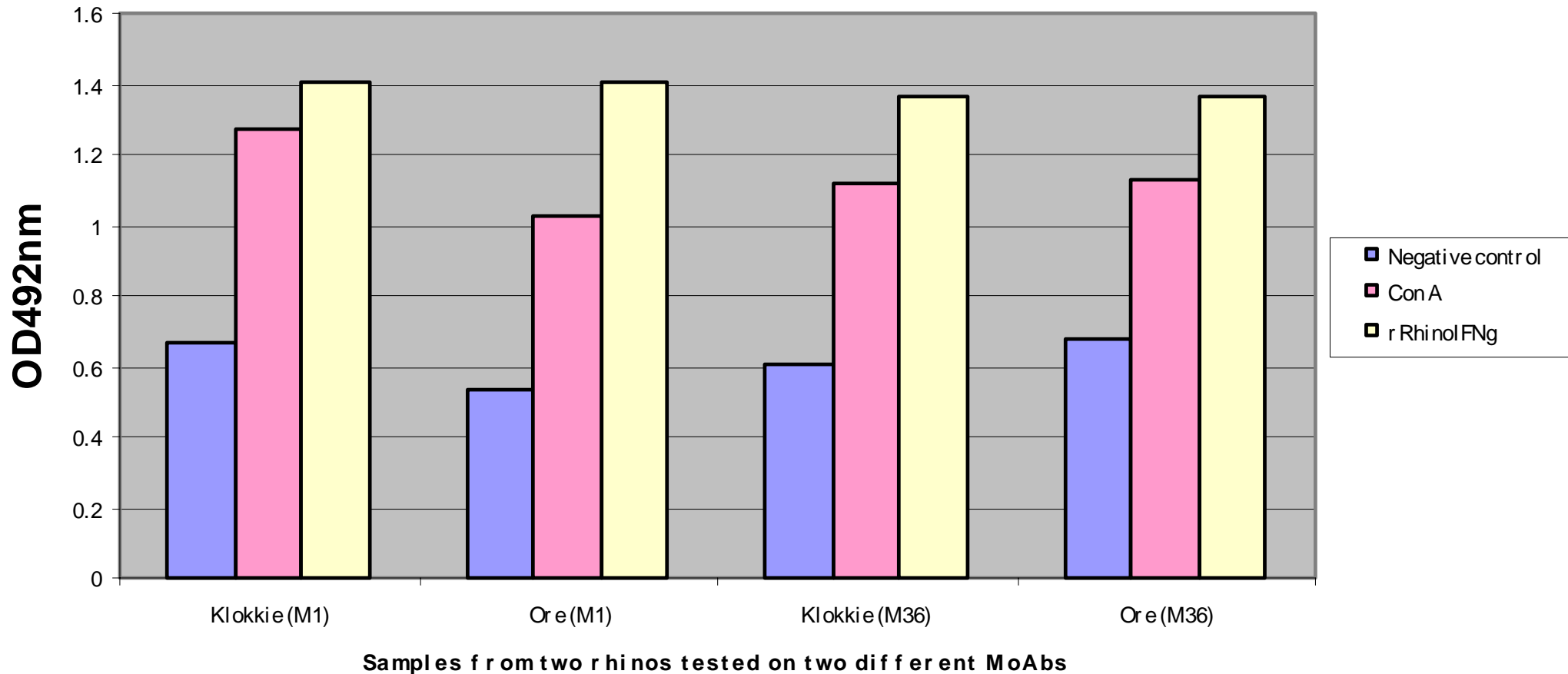
Capture ELISA of recombinant and native Rhinoceros IFN-g



RESULTS [1]

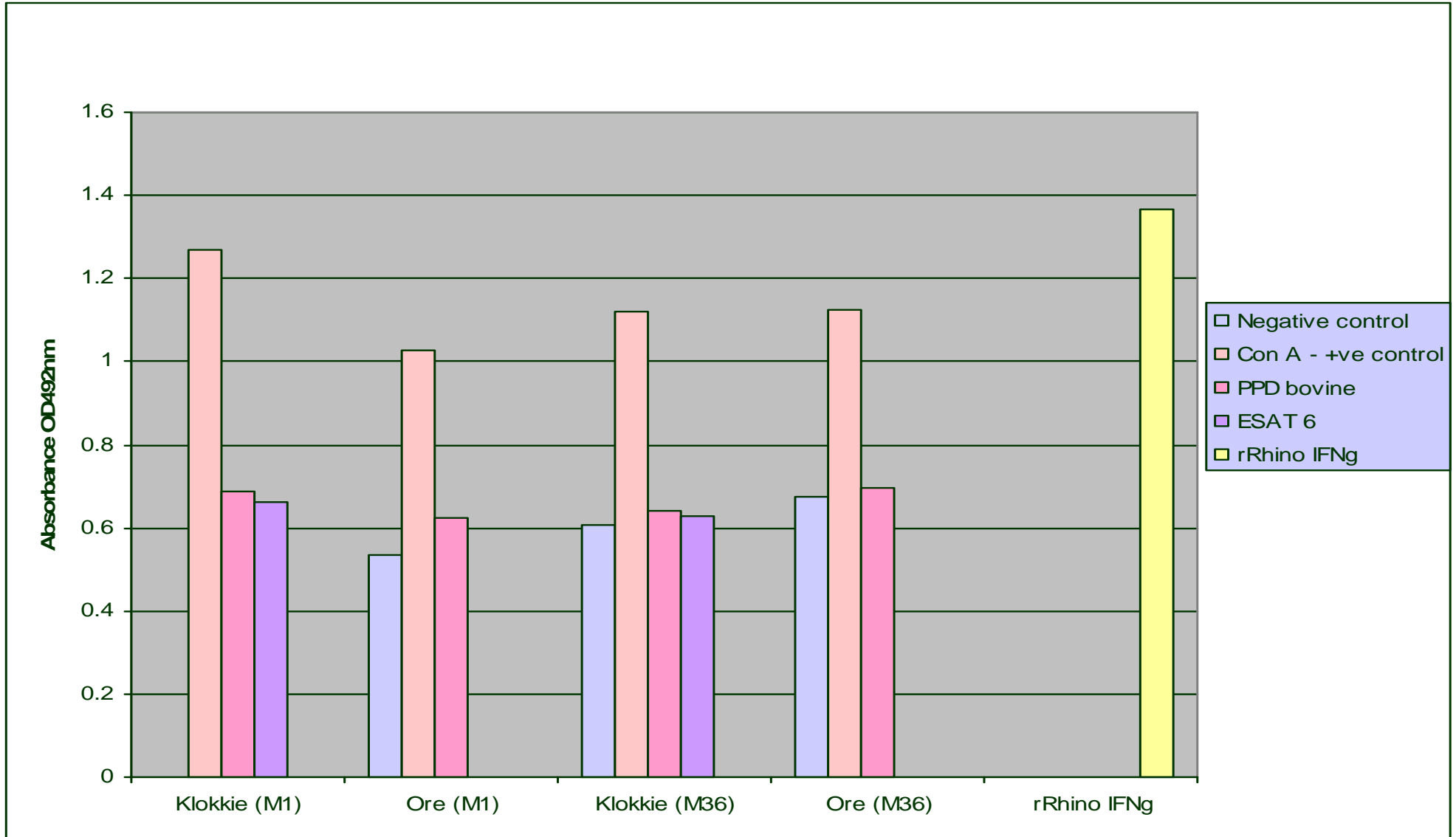
Detection of Recombinant and Native Rhino IFN-g in PBMC's

Detection of Recombinant and Native Rhinoceros IFN-gamma from PBMCs'



RESULTS [2]

Detection of Recombinant and Native Rhino IFN-g in PBMC's



RESULTS

Detection of Recombinant and Native Rhino IFN- γ

- **WB cannot be used (important background noise in the negative control)**
- **Our test is capable of detecting recombinant and native IFN-g in PBMC**
- **No BTB Ag specific IFN-g could be detected – same OD after Bovine PPD, ESAT 6 recall and Negative control.**
- **Most likely hypothesis: Need of a boost vaccination of rhinoceros in order to detect BTB Ag specific IFN-g**

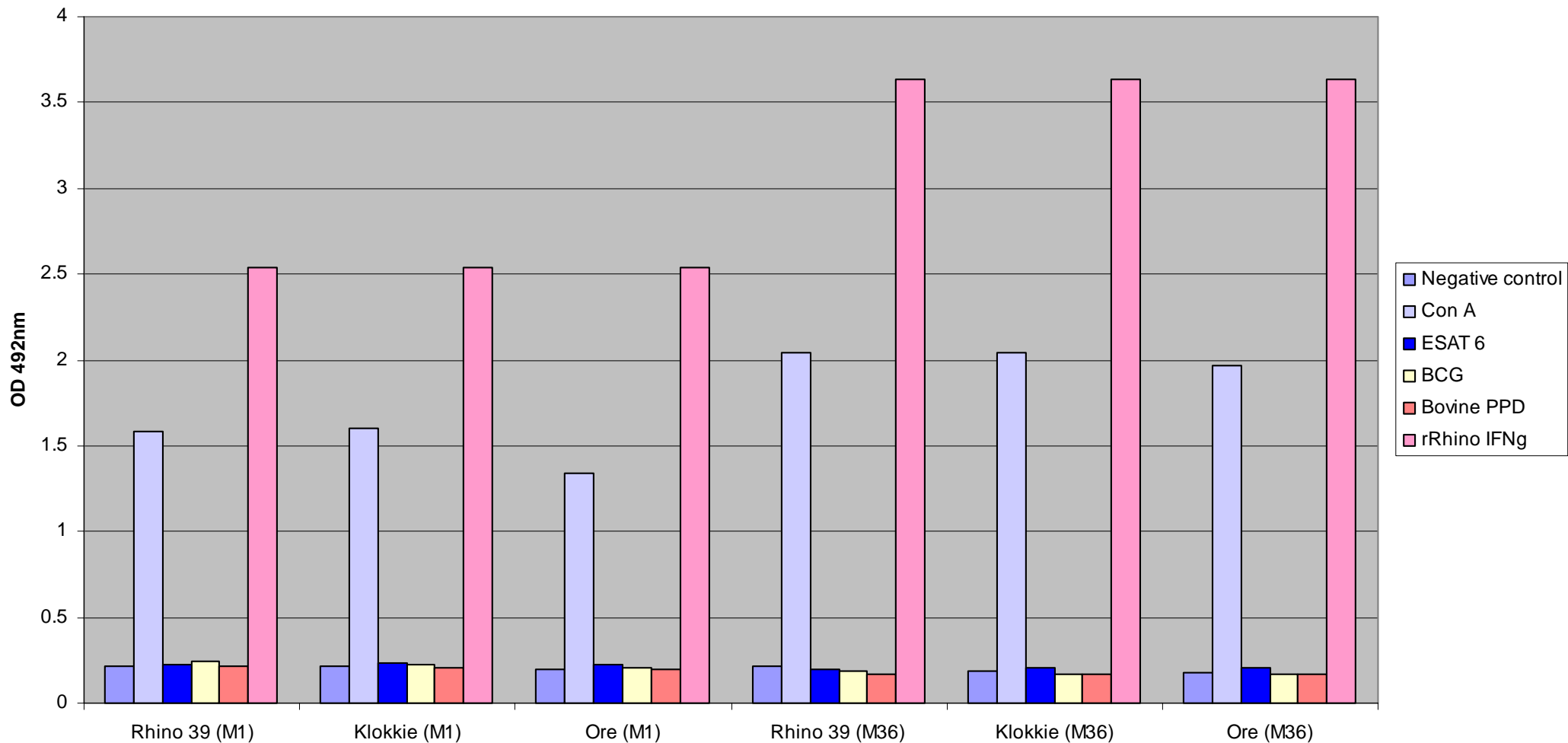
Boost Vaccination

- The rhinos were vaccinated again with BCG vaccine**
- Ten weeks later blood was collected and WB and PBMCs' were isolated and stimulated as previously mentioned**
- The overnight samples were harvested and the plasma or supernatant was used for the detection of IFN- γ in the capture ELISA**
- In addition blood was also collected from a rhino that was not vaccinated with the BCG vaccine (negative control)**

RESULTS [3]

Detection of Recombinant and Native Rhino IFN- γ After boost vaccination

Detection of Rhinoceros IFN-gamma from PBMCs'



RESULTS

Detection of IFN- γ after antigenic recall in BCG boost vaccinated Rhinos

- **WB cannot be used (important background noise in the negative control)**
- **Our test is capable of detecting recombinant and native IFN-g in PBMC**
- **No BTB Ag specific IFN-g could be detected – same OD after Bovine PPD, ESAT 6 recall and Negative control.**
- **Same results as before and after BCG boost vaccination**

CONCLUSION

The Rhinoceros IFN- γ ELISA established for PBMC will enable further development of a whole blood assay, that will be instrumental in diagnosis of BTB in rhinoceros

BCG vaccination (following the protocol used in cattle – dose, route of administration, boost) did not elicit a measurable immune response in adult rhinos

ACKNOWLEDGEMENTS

Staff and colleagues at the Department of Infectious Diseases and Immunology of the Utrecht University in The Netherlands, Veterinary Faculty at the Department of Veterinary Tropical Diseases, University of Pretoria, South Africa, TB Laboratory Onderstepoort Veterinary Institute and SANParks.

THANK YOU!

