

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

by

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# **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- $\gamma$ test**

#### **Ancillary test**

**Moabs used in the ELISA will only recognise the IFN- $\gamma$  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- $\gamma$ test**

**Wildlife species specific anti-IFN Ab have to be produced or show that the anti-bovine IFN- $\gamma$  Ab is cross-reactive**

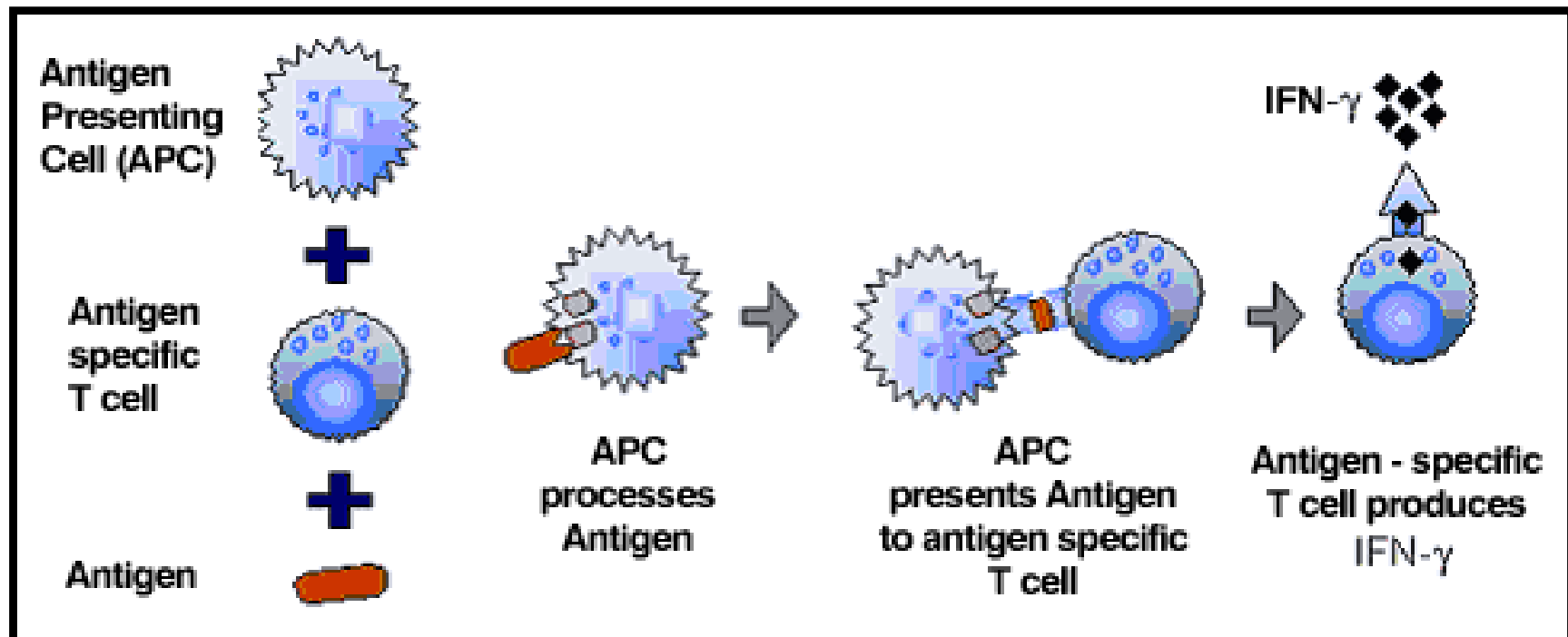
# OBJECTIVE

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



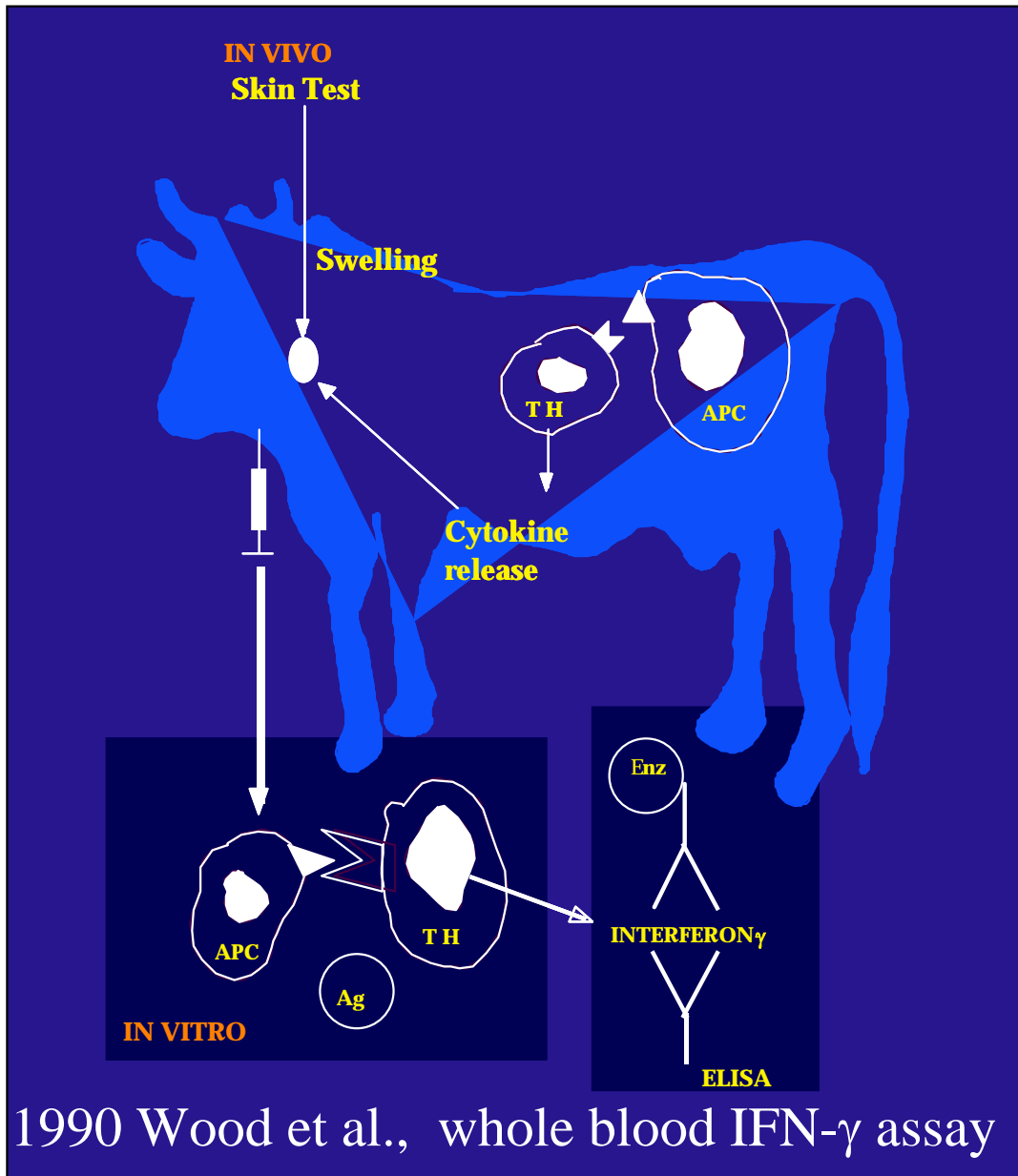
# PRINCIPLE

**IFN- $\gamma$  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- $\gamma$ .**



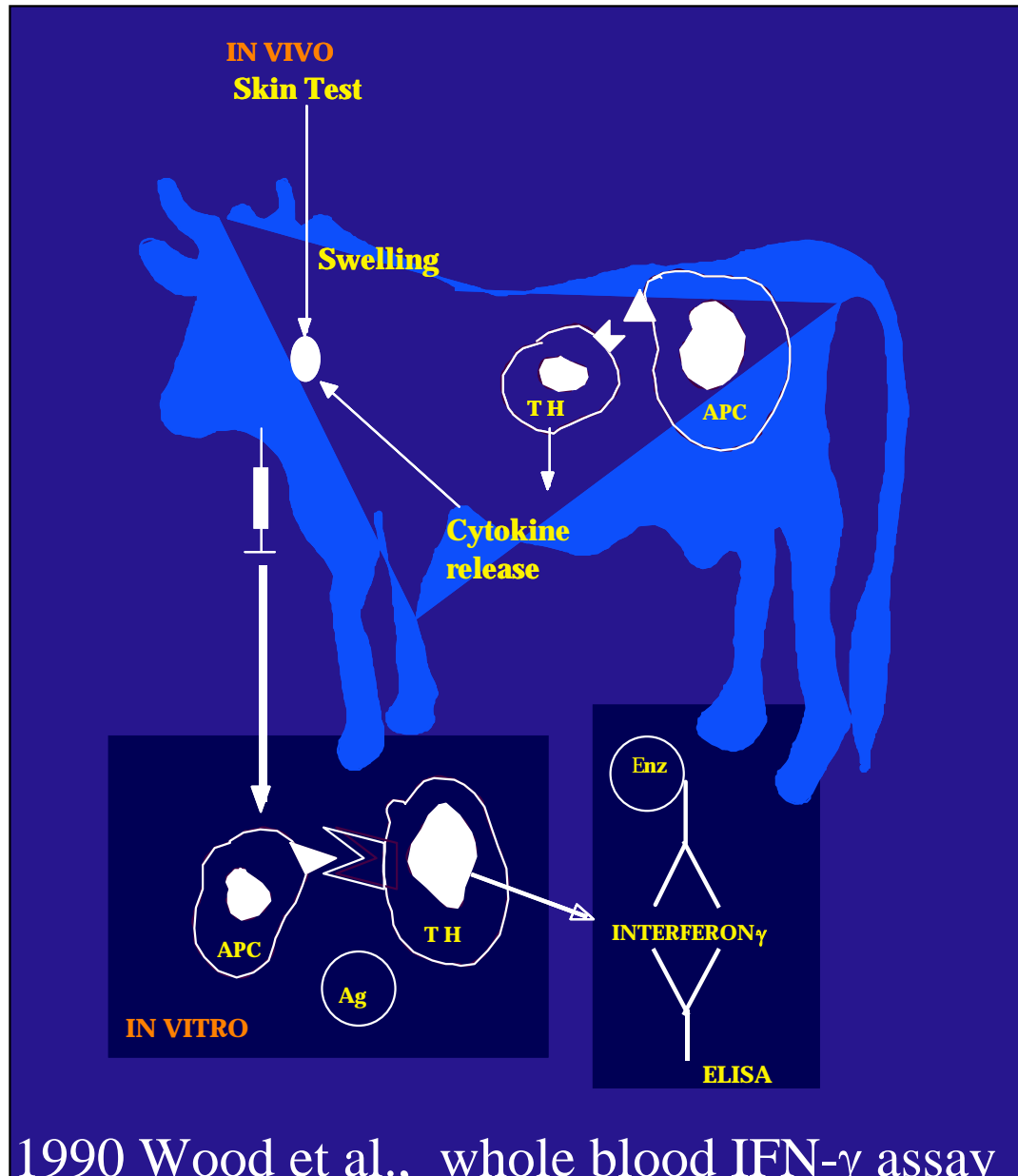


# PRINCIPLE OF IFN- $\gamma$ TEST AS ILLUSTRATED IN CATTLE



- 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- $\gamma$  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- $\gamma$ TEST AS ILLUSTRATED IN CATTLE



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

# **DEVELOPMENT OF AN IFN- $\gamma$ 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 $\alpha$ RhinoIFN- $\gamma$ 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- $\gamma$  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- $\gamma$

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.

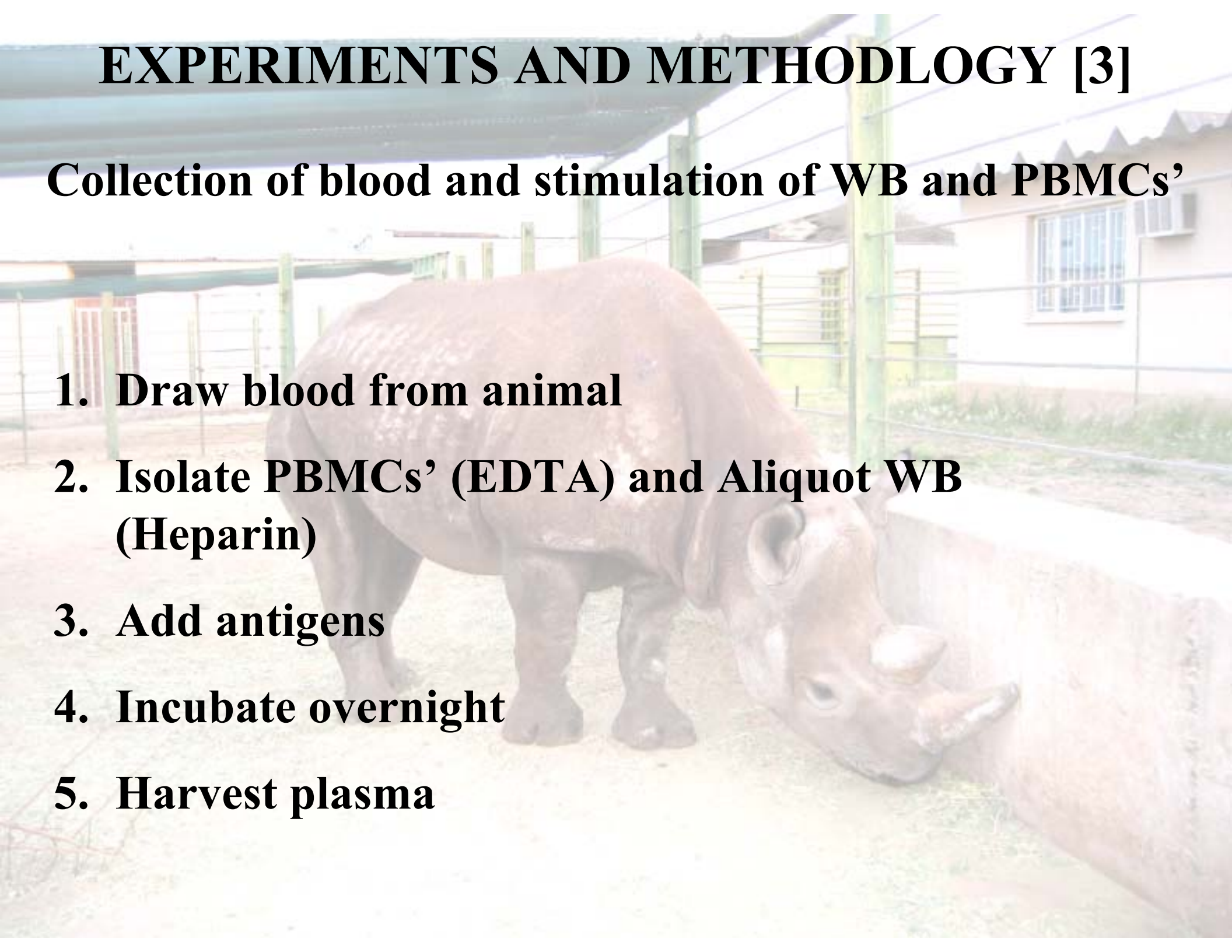
**Concanavalin A (Con A)** – Mitogen used for polyclonal activation and thus would serve as a positive control

**Heat Killed BCG** – Additional antigen



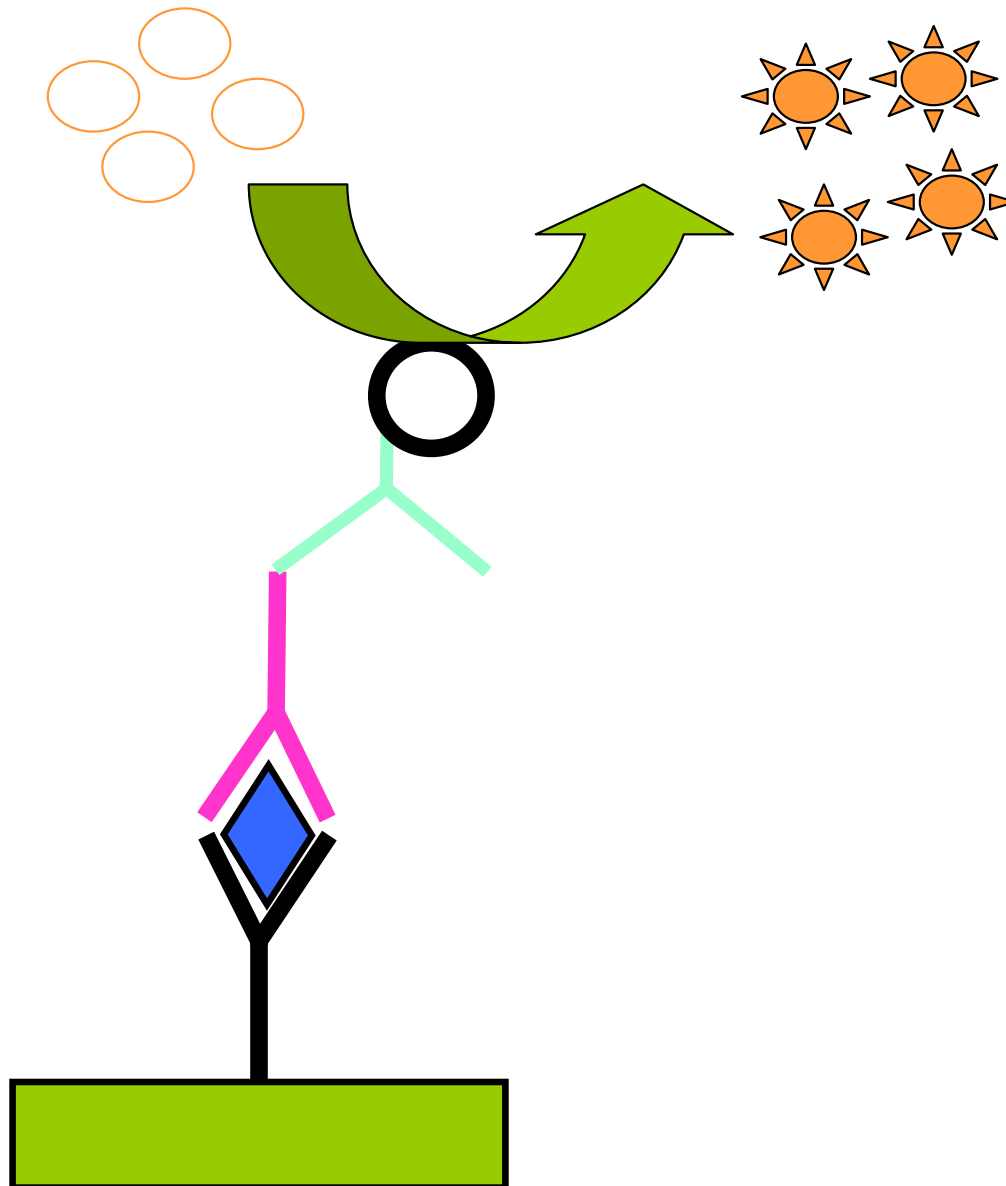
# **EXPERIMENTS AND METHODOLOGY [3]**

## **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 ground. 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



Color reaction

Add substrate

Rabbit polyclonal to chicken IgY

Chicken IgY

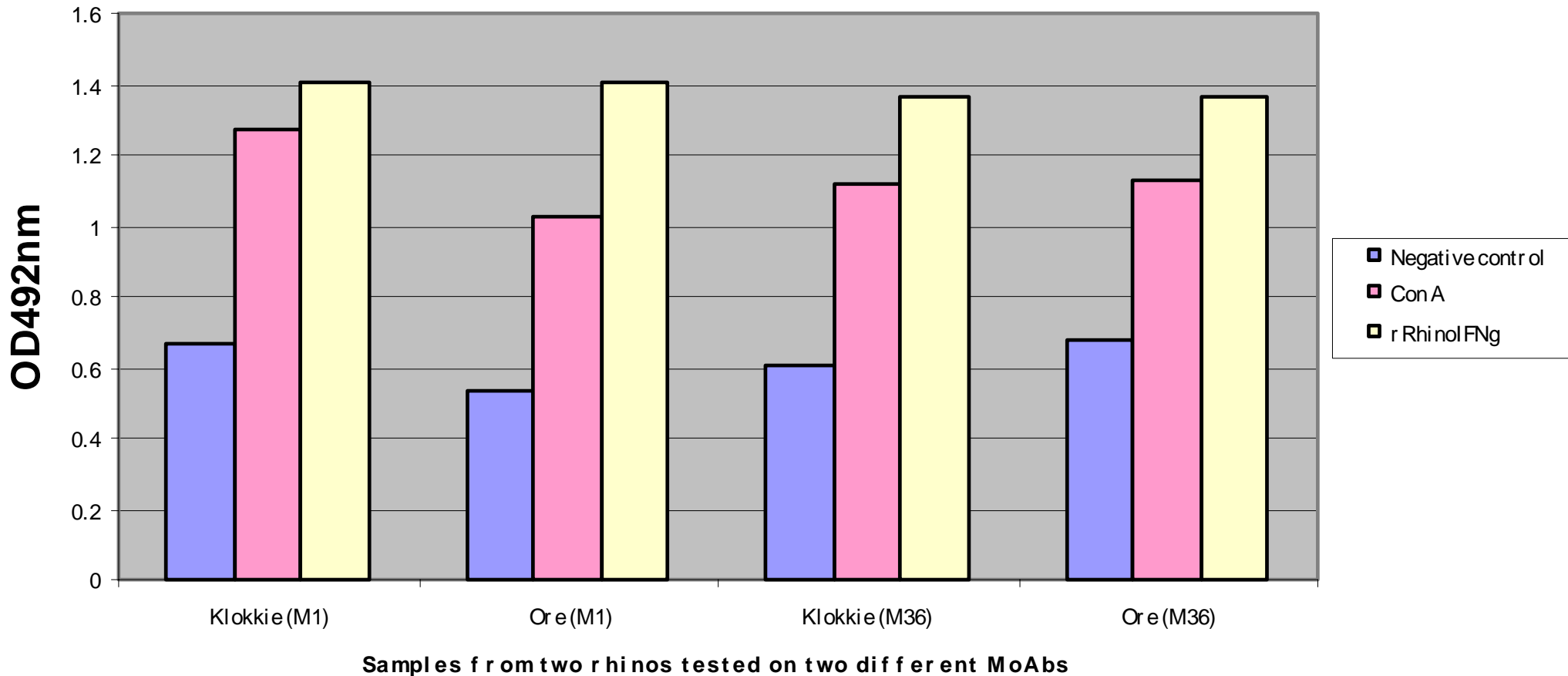
Test plasma obtained after harvesting

Coat monoclonal antibodies  
(M1 or M36)

# RESULTS [1]

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

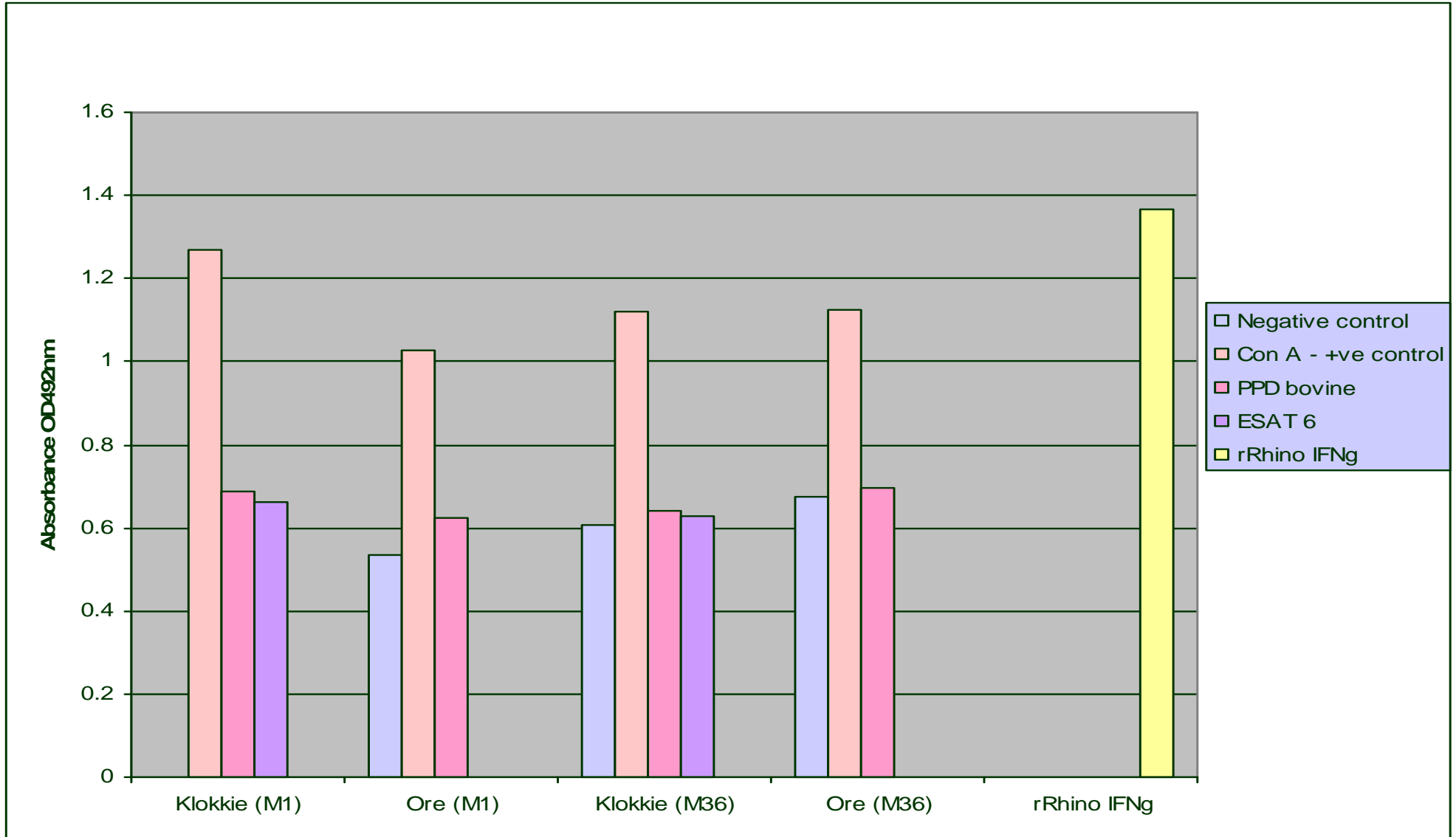
### Detection of Recombinant and Native Rhinoceros IFN-gamma from PBMCs'





# RESULTS [2]

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



# RESULTS

## Detection of Recombinant and Native Rhino IFN- $\gamma$

- **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**

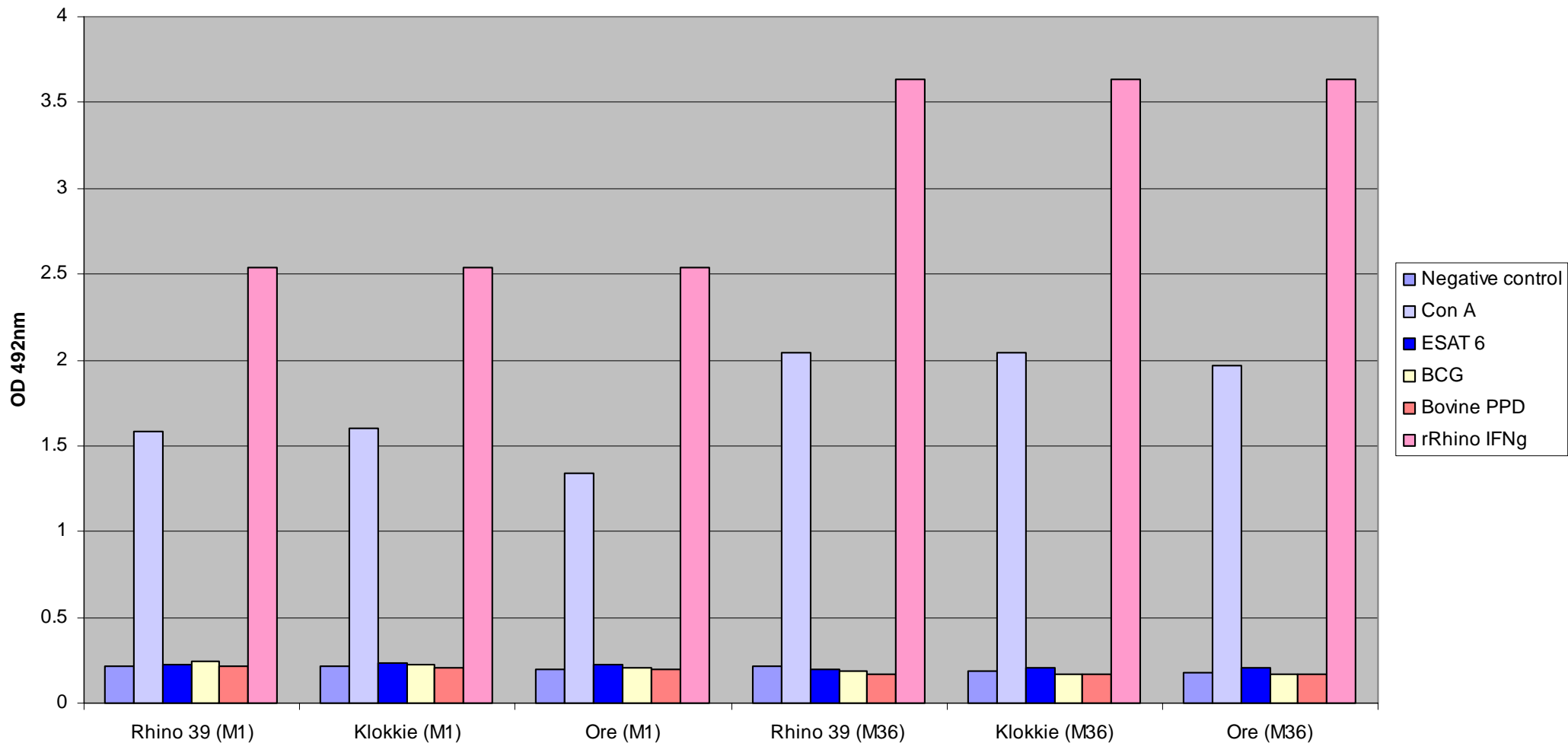
# **Boost Vaccination**

- **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- $\gamma$  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- $\gamma$ After boost vaccination

Detection of Rhinoceros IFN-gamma from PBMCs'





# RESULTS

## Detection of IFN- $\gamma$ 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- $\gamma$  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**

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**THANK YOU!**

