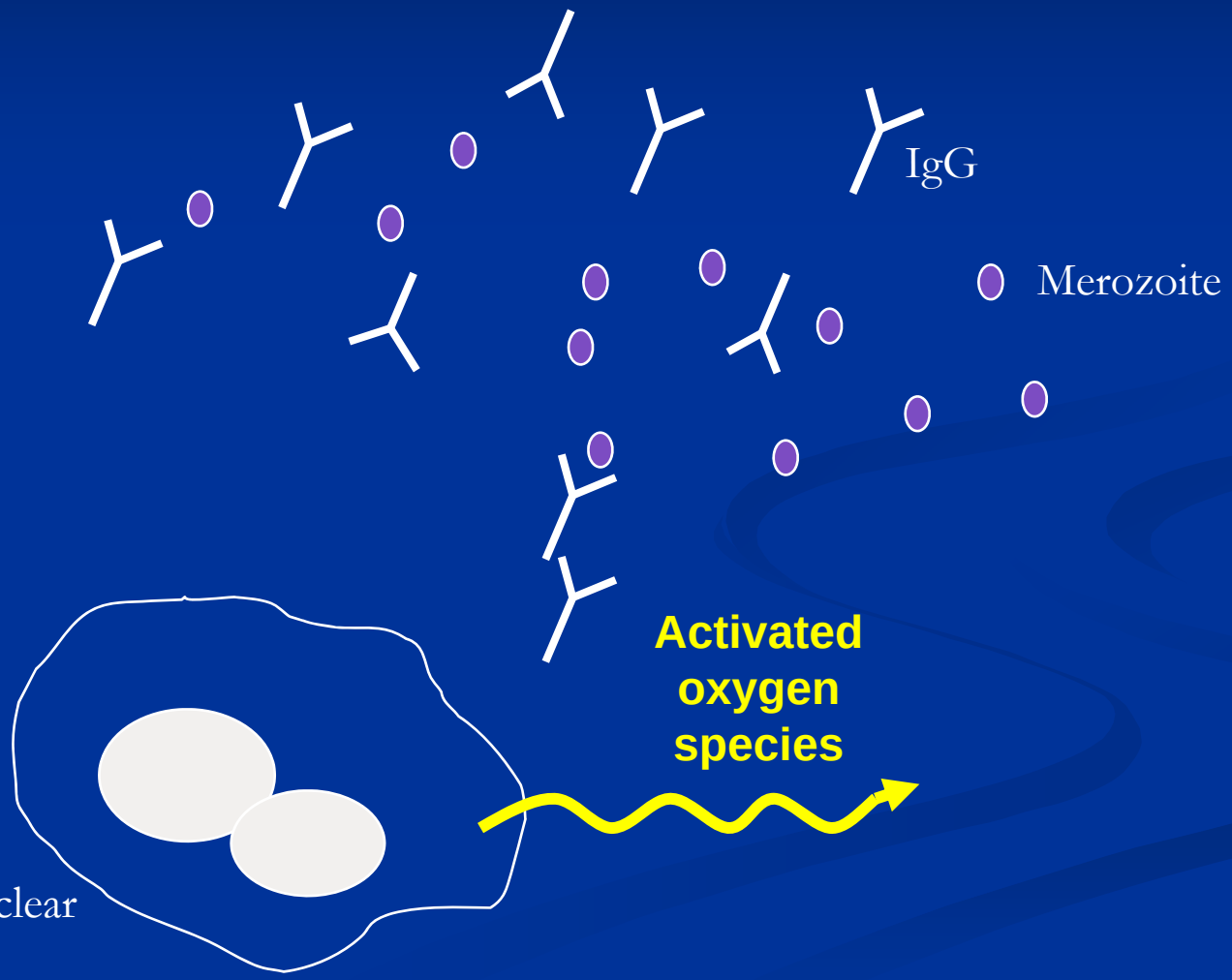
Fifth EDCTP forum – Tanzania 2009

The ADRB assay: a new tool, correlated with clinical protection from *Plasmodium falciparum* malaria, to test malaria vaccine.

Charlotte Joos [1,2] , Laurence Marrama [3] , Hannah E.J. Polson [2] , Aïssatou Touré [1] , Ronald Perraut [1] & Shirley Longacre [2] .

(1) Unité d'Immunologie, Institut Pasteur de Dakar, Sénégal
(2) Laboratoire de Vaccinologie Parasitaire, Institut Pasteur, Paris, France
(3) Unité d'Epidémiologie, Institut Pasteur de Dakar, Sénégal

The Antibody Dependant Respiratory Burst (ADRB)



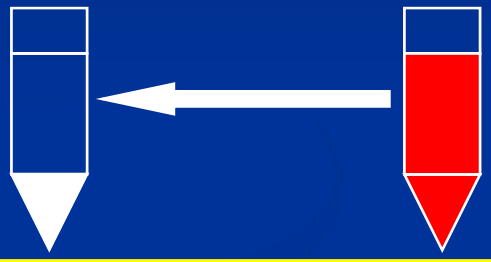
The ADRB detected by chemiluminescence (CL) - Methodology

Merozoites : 40 µL
+
Sera or IgG : 10 µL

Opsonization 37 C

2 simultaneous step 1 ≈ 1H30

PNN purification



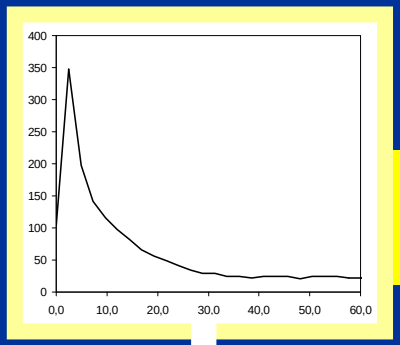
Neutrophil :
100 µL/well (5.10^5 PNN)

Step 2 : inject Few seconds

isoluminol:
100 µL (2 µg)

Automated reading

Step 3 1H



Results in Excel

cjoos@pasteur.sn

Standardization of results for reproducibility

- Positive standard (HIS) in the first and last wells
- ADRB index determination:

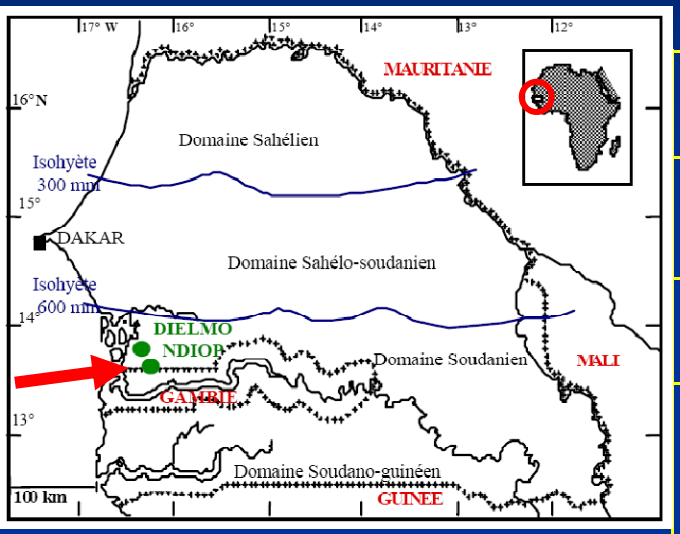
1000 * (Max rlu tested sera) / (Max rlu (average) HIS)

- Induce good inter-trials reproducibility: ≈ 80%
- No responder in ADRB assay : ADRB < 250 units

IgG opsonized on merozoite induce the ADRB response

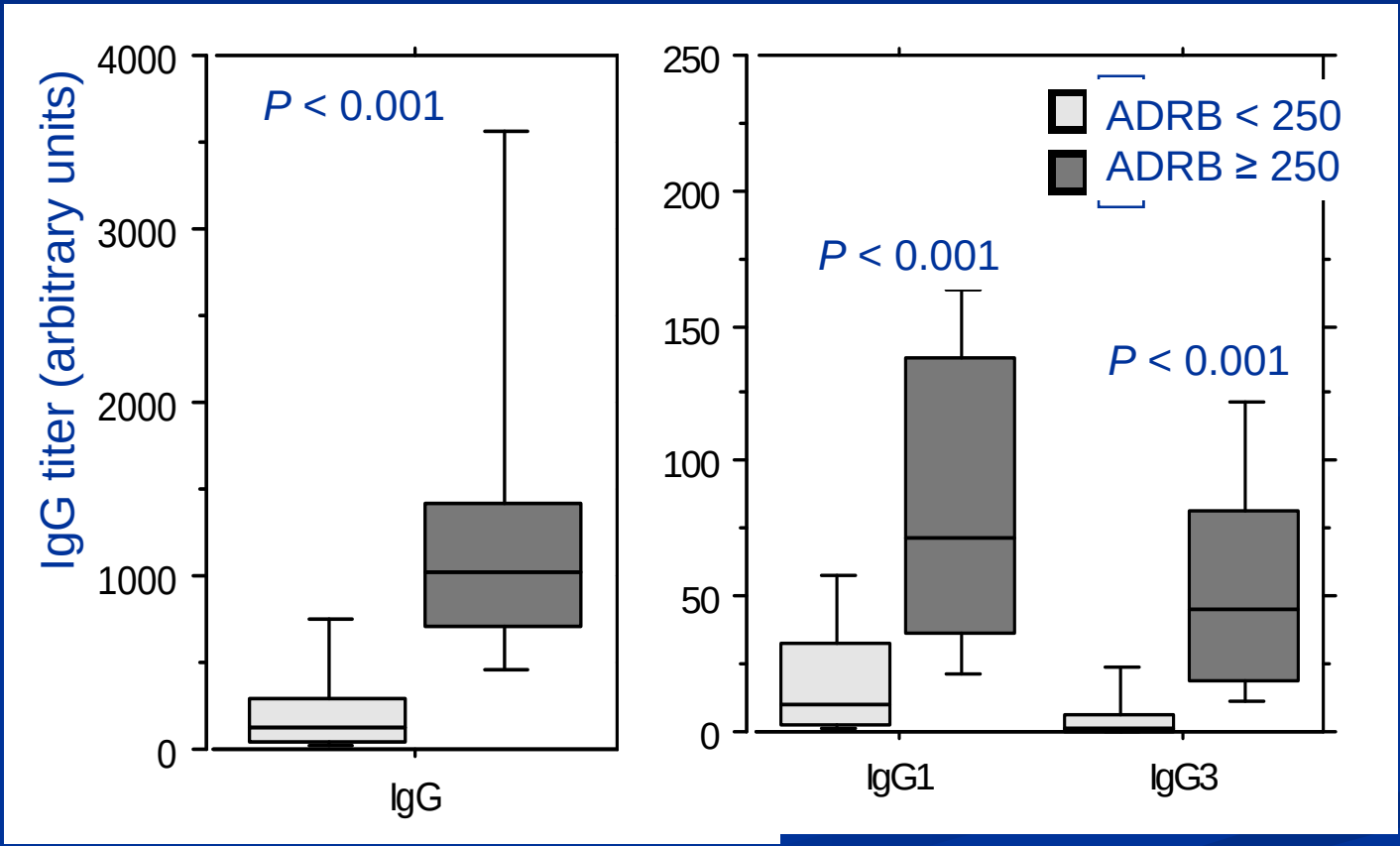
- Merozoite or sera alone : no signal
- No effect of Complement
- Total IgG depleted sera: lost of signal

Two study sites with two endemic profiles



	NDIOP	DIELMO
ENDEMICITY	Mesoendemic	Holoendemic
TRANSMISSION	Seasonal	Perennial
INFECTIVE BITES	≈ 20 / inhabitant / year	≈ 1 / inhabitant / DAY
MORE SENSIBLE AGE GROUP	< 15 years RR = 13.1	< 6 years RR = 87.5
IMMUNE AGE GROUP	≥ 30 years RR = 1	≥ 15 years RR = 1

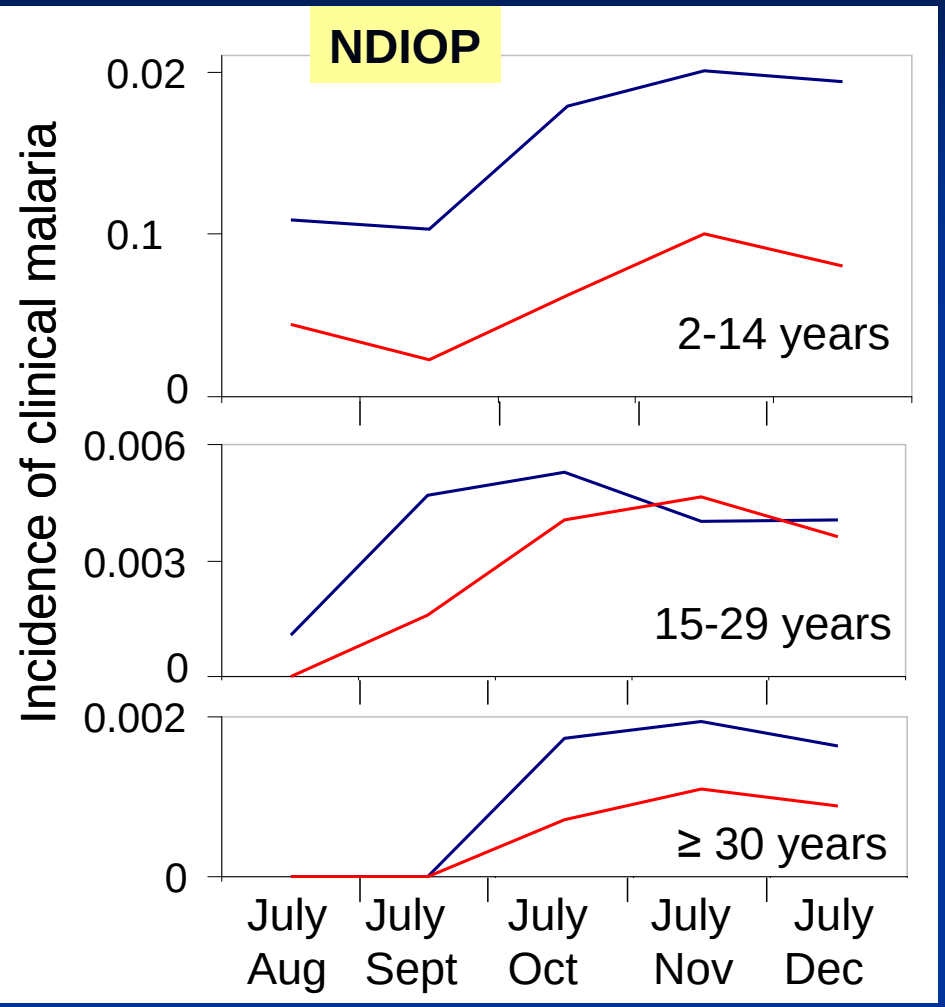
IgG1 and IgG3 opsonized on merozoite induce the ADRB response

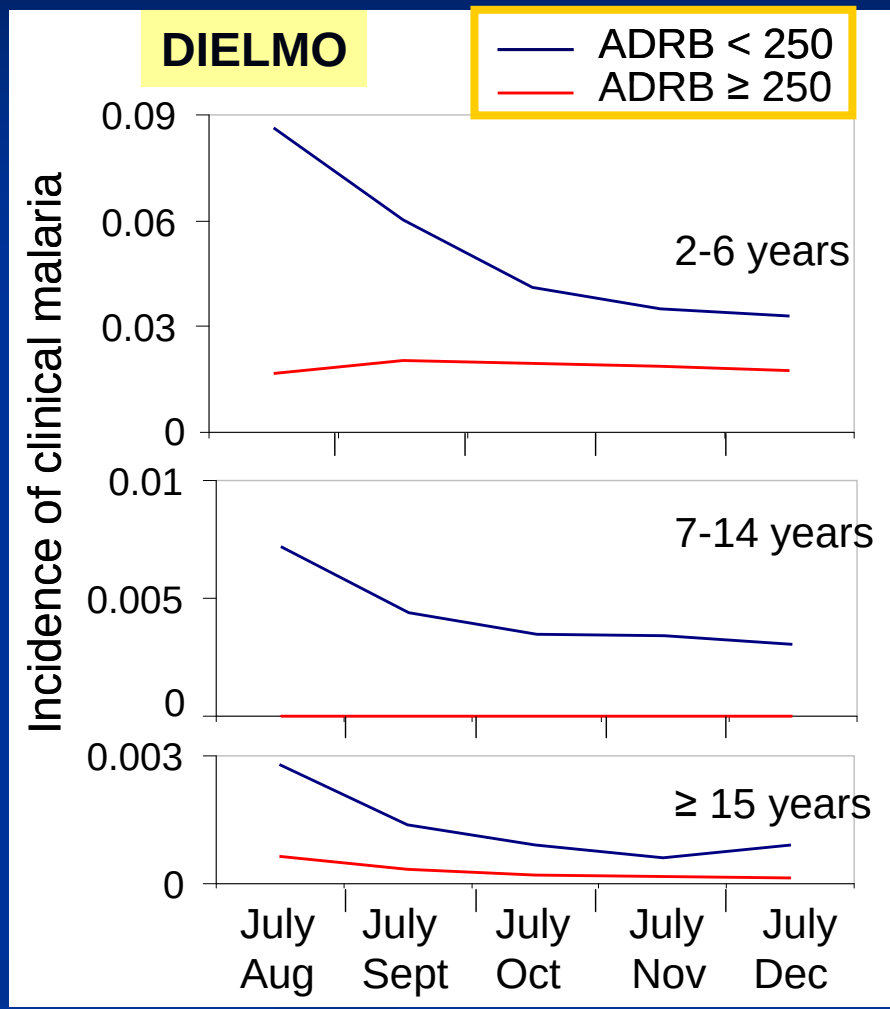


88 aparasitemic Ndiop residents tested

cjoos@pasteur.sn

Significant correlations between ADRB and clinical protection





RR=1.8 if ADRB<250 (5.5months) *P* = 0.036
(in multivariate model: ADRB + age)

114 inhabitants of each village tested

RR=17.5 if ADRB<250 (5.5months) *P* = 0.006
(in multivariate model: ADRB + age + Hb)

2 ways to study effect of Ab anti-MSP1p19 in the ADRB assay

- **USED OF ANTI-MSP1p19 DEPLETED SERA**
  1. Antigenic (Bv) catching of specific Ab
  2. His-tag catching of complex Ag-Ab

  Efficacy : 99%

  Lack of Ab anti-BvMSP1p19

- **USED OF ISOGENIC MEROZOITE CLONES**
  - D10-PcMEGF :

  P. falciparum genetically modified

  P. chabaudi MSP1p19 EGF domains

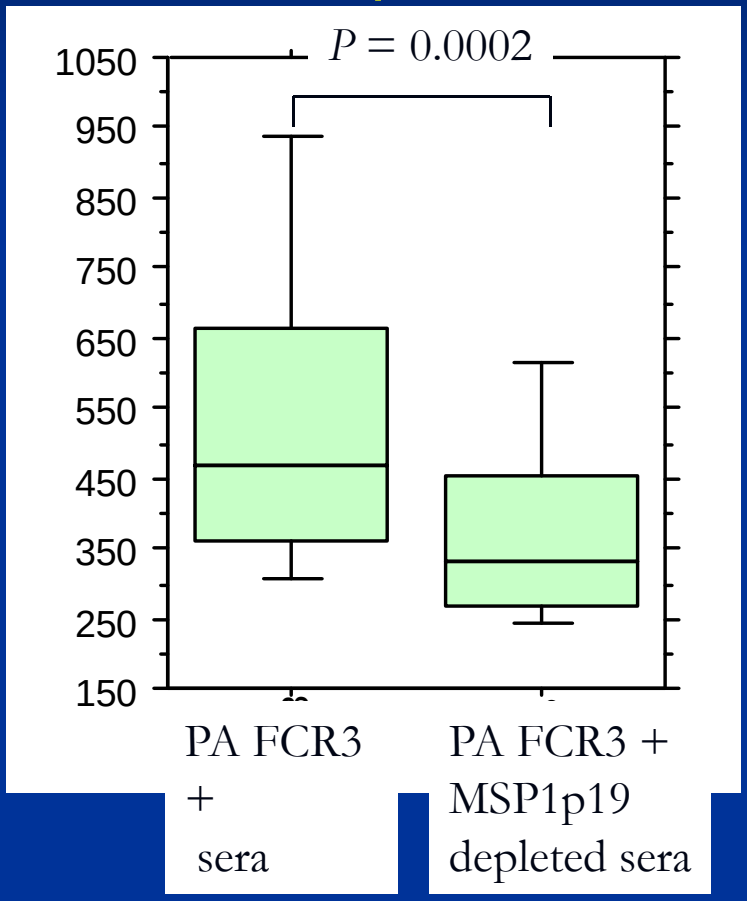
  No cross react

  Mz no recognized by Ab anti-Pf MSP1p19

O'Donnell et al., J. Exp. Med. 2001, 193

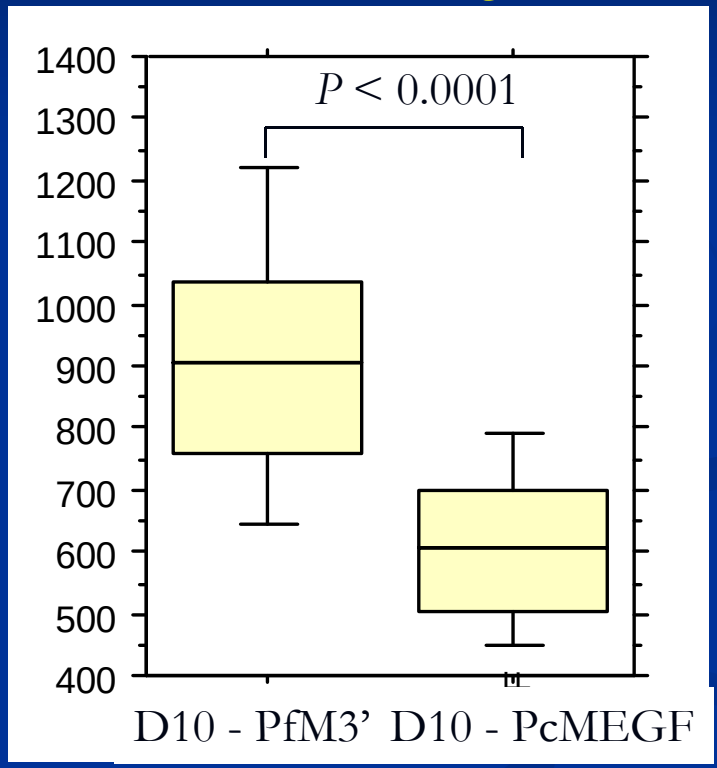
Ab anti-MSP1p19 induce 1/3 of the ADRB response

Sera vs depleted sera



Lack of 29% of ADRB signal

Normal vs transgenic mz



Lack of 32% of ADRB signal

CONCLUSION

- **An antibody functional assay statistically correlated with clinical protection in meso and holoendemic area.**
- **Importance of MSP1p19 vaccine (Bv)**
- **An assay to introduce in preclinical and clinical trials ?**

AUF

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Thank you

UNIVERSITE PIERRE & MARIE CURIE
LA SCIENCE A PARIS

UNIVERSITE CHEIKH ANTA DIOP DAKARENSIS
LUX MEA LEX
SIGILLUM UNIVERSITATIS

INSTITUT PASTEUR

cjoos@pasteur.sn