

Malaria serology

- Comparison of three commercial ELISA assays
- Serological follow-up CHMI

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Malaria serology

- Detects malaria exposure, not active malaria infection
- Does not differentiate between plasmodium subspecies
- Screening of blood donors (transfusion-induced malaria)
- Part of workup when testing for tropical splenomegaly syndrome
- Additional marker in case of uncertainty about previous malaria infection

Aims

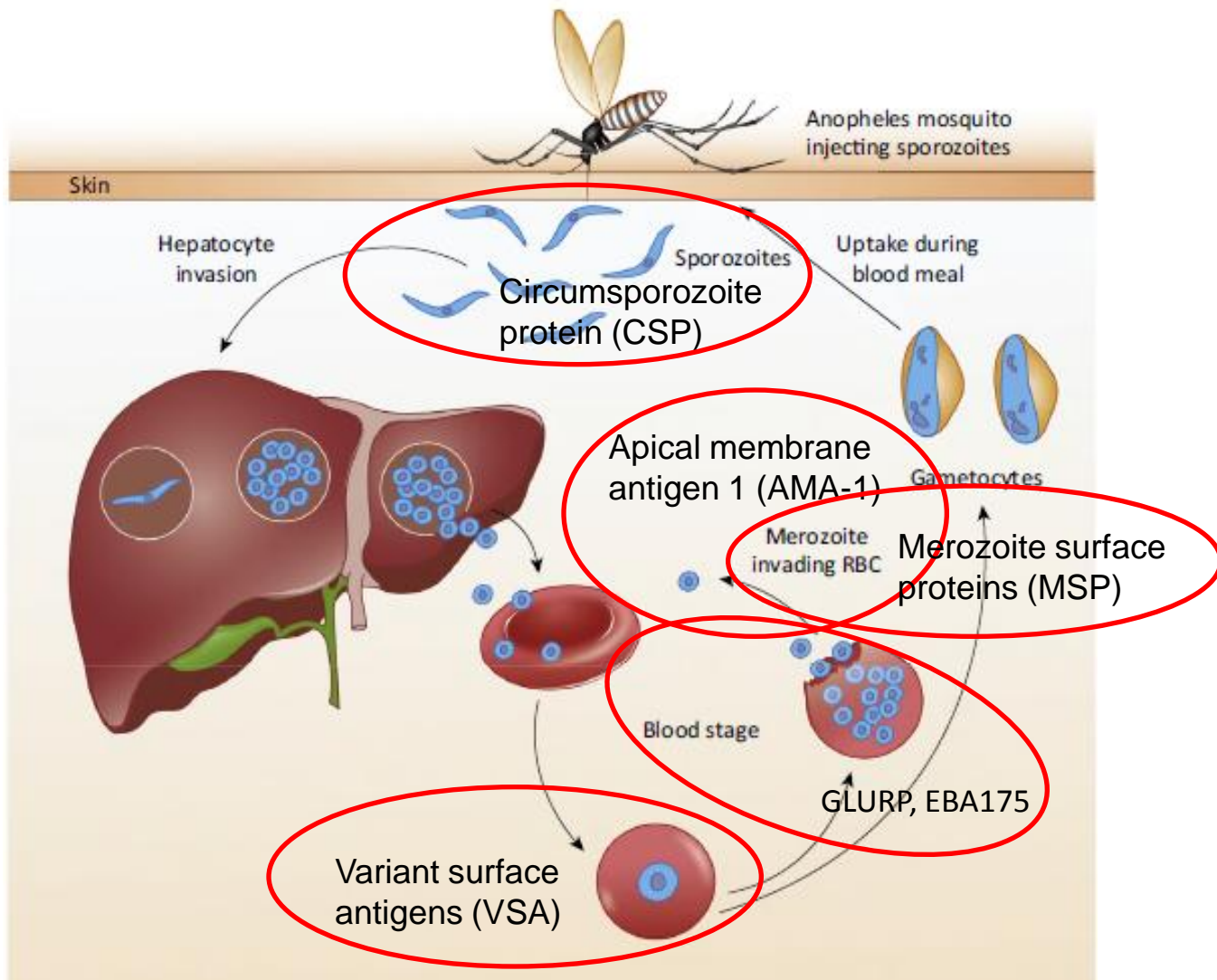


State-of-the-art malaria diagnostics, also serology

1. To compare 3 commercial malaria serological assays in order to establish which one shows the best performance in patient care.
2. To compare the performance of 3 commercial assays and 1 in house assay in an experimental setting where individuals were infected and treated very early during the development of parasitemia.

Assays

| Brand | Name | Antigen | Antibody | Input | Qual/Q uant | Result | Controls |
|--------------------------------------|--|---|---------------------|-------|----------------|-------------------------------------|--|
| Trinity Biotech IBL | Lab21 Malaria Total antibody EIA | Mix of P.f and P.v recombinant antigens | IgG, IgA, IgM | 50ul | Semi-Q | Ab Index = OD sample /cut-off | 4 per run (3 NC, 1 PC) |
| Euroimmuun | Anti- plasmodium Elisa IgG | Whole cell antigens of all 5 P. species | IgG | 10ul | Kwal | Ratio | 3 per run (Cal, PC, NC) |
| NovaTec / ApaDia | NovaLisa | Mix of P.f and P.v recombinant CSP and MSP1 from P. f. and P. v. | IgG IgM | 10ul | Kwal | Cut-off | 5 per run (blanc, NC, 2 cut-off, PC) |
| Home made Elisa | Whole parasite Elisa | P. f. whole parasite | | | | | |



Patient panel (n=83)

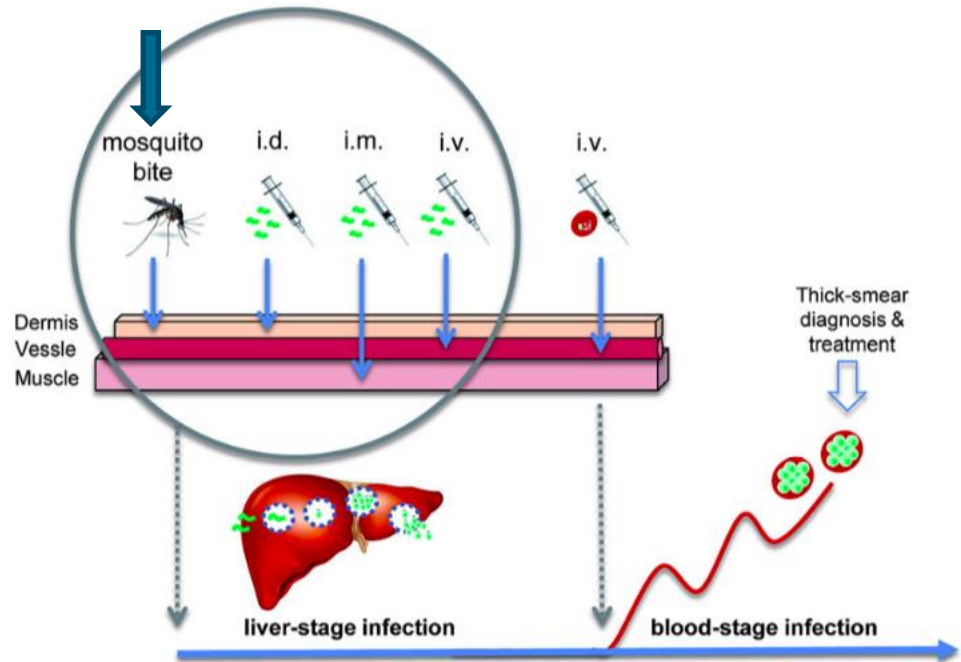
Reconvalescent sera of

- *P. falciparum*, n = 15
- *P. ovale*, n = 5
- *P. vivax*, n = 10
- *P. malariae*, n = 4
- *P. knowlesi* (simian) , n=3

Seronegative sera

- Dutch children 1-2 years, n=11
- Cross reactive samples
 - Rheumatoid factor, n=5
 - Toxoplasma (IgM+ & IgG+) , n=10
 - Mycoplasma, n=10
 - Leishmania, n=5
 - EBV (IgM+ & IgG+) n=10

CHMI (n=10)



Seropositivity of commercial assays of microscopic confirmed clinical malaria samples.

| | Trinity N (%) | Novatec N (%) | Euroimmuun N (%) |
|---------------------------------|--------------------------|--------------------------|-----------------------------|
| <i>P. falciparum</i> | | | |
| - First week n=7 | 1 (14) | 2 (29) | 1 (14) |
| - Rest n=8 | 6 (75) | 4 (50) | 4 (50) |
| <i>P. ovale (n=5)</i> | 2 (40) | 4 (80) | 5 (100) |
| <i>P. vivax (n=10)</i> | 9 (90) | 9 (90) | 9 (90) |
| <i>P. malariae (n=4)</i> | 4 (100) | 4 (100) | 4 (100) |
| <i>P. knowlesi (n=3)</i> | 1 (33) | 1 (33) | 1 (33) |
| | | | |
| Negatives | 0/7 (0) | 1/9 (11) | 1/11 (9) |

Pooled samples from endemic positive *P. falciparum* patients;

→ 100% positivity for all assays

Specificity

| n pos (n equiv) | N total | Trinity | Novatec | Euroimmuun |
|---------------------------|---------|---------|---------|------------|
| Rheumatoid Factor | 5 | 0 | 0 (1) | 0 (1) |
| <i>Toxoplasmosis</i> | 10 | 0 | 0 | 0 |
| <i>Mycoplasma spp.</i> | 10 | 0 | 0 | 1 |
| <i>Leishmania spp.</i> | 5 | 0 | 0 | 0 (1) |
| <i>Epstein Barr virus</i> | 10 | 0 | 0 | 0 |

Dilution

| P.falciparum verdund | | | | | | |
|----------------------|---------|--|---------|--|-----------|--|
| Monsternr: | Trinity | | Novatec | | Eurolmmun | |
| 12-6 onv | 19,49 | | 6,512 | | 7,456 | |
| 12-6 1:2 | 19,49 | | 6,512 | | 7,456 | |
| 12-6 1:5 | 19,49 | | 4,797 | | 6,624 | |
| 12-6 1:10 | 19,49 | | 3,421 | | 5,128 | |
| 12-6 1:50 | 19,49 | | 1,142 | | 1,887 | |
| 12-6 1:50 | 20,735 | | 1,23 | | 1,927 | |
| 12-6 1:100 | 18,76 | | 0,704 | | 1,04 | |
| 12-6 1:200 | 15,224 | | 0,431 | | 0,589 | |
| p07002146 (d5) onv | 19,49 | | 4,056 | | 7,199 | |
| p07002146 (d5) 1:2 | 19,49 | | 2,728 | | 6,271 | |
| p07002146 (d5) 1:5 | 19,49 | | 1,928 | | 4,301 | |
| p07002146 (d5) 1:10 | 19,49 | | 1,366 | | 3,052 | |
| p07002146 (d5) 1:50 | 10,769 | | 0,478 | | 1,113 | |
| p07002146 (d5) 1:50 | 6,407 | | 0,413 | | 0,81 | |
| p07002146 (d5) 1:100 | 4,424 | | 0,308 | | 0,5 | |
| p07002146 (d5) 1:200 | 2,741 | | 0,213 | | 0,312 | |

Trinity uses 5x more input
(50 ul vs 10 ul)

Trinity is the most sensitive assay
for *P. falciparum*.

Verduunningsreeks ovale /vivax

| P.ovale verdund | | | | | | |
|-----------------|---------|---|---------|--|---------------|--|
| Monsternr: | Trinity | | Novatec | | EuroImm un | |
| 2-52 onv | 19,49 | | 1,892 | | 7,456 | |
| 2-52 1:2 | 19,49 | | 1,207 | | 7,362 | |
| 2-52 1:5 | 16,793 | | 0,643 | | 5,148 | |
| 2-52 1:10 | 14,029 | | 0,426 | | 3,956 | |
| 2-52 1:50 | 5,936 | } | 0,183 | | 1,452 | |
| 2-52 1:50 | 3,326 | | 0,189 | | 1,21 | |
| 2-52 1:100 | 2,108 | | 0,154 | | 0,684 | |
| 2-52 1:200 | 1,12 | | 0,141 | | 0,415 | |

Novatec is less sensitive for *P. ovale* compared to Trinity and Euroimmun.

| P.vivax verdund | | | | | | |
|-----------------|---------|--|---------|--|---------------|--|
| Monsternr: | Trinity | | Novatec | | EuroImm un | |
| 11-13 onv | 19,49 | | 6,512 | | 7,456 | |
| 11-13 1:2 | 19,49 | | 6,512 | | 7,456 | |
| 11-13 1:5 | 19,49 | | 6,512 | | 7,456 | |
| 11-13 1:10 | 19,49 | | 6,512 | | 7,456 | |
| 11-13 1:50 | 19,49 | | 5,701 | | 7,456 | |
| 11-13 1:50 | 21,854 | | 5,423 | | 7,893 | |
| 11-13 1:100 | 21,854 | | 4,902 | | 7,893 | |
| 11-13 1:200 | 21,854 | | 3,974 | | 7,614 | |
| 11-13 1:400 | 21,854 | | 2,791 | | 6,056 | |

Sensitivity for *P. vivax* is comparable for all three assays.

Verdunningsreeksen *P. malariae* / *P. knowlesi*

| P.malariae verdund | | | | | | |
|-----------------------|---------|--|---------|--|---------|----|
| Monsternr: | Trinity | | Novatec | | EuroImm | un |
| 3-39 onv | 13,109 | | 5,021 | | 5,685 | |
| 3-39 1:2 | 11,976 | | 3,551 | | 4,244 | |
| 3-39 1:5 | 12,139 | | 1,788 | | 2,519 | |
| 3-39 1:10 | 12,304 | | 1,221 | | 1,558 | |
| 3-39 1:50 | 10,284 | | 0,489 | | 0,476 | |
| 3-39 1:50 EuroIm(10) | 5,16 | | 0,411 | | 1,246 | |
| 3-39 1:100 EuroIm(20) | 3,478 | | 0,258 | | 0,703 | |
| 3-39 1:200 EuroIm(40) | 2,607 | | 0,198 | | 0,396 | |

Trinity is the most sensitive test regarding *P. malariae*.

| P.knowlesi verdund | | | | | | |
|-------------------------|---------|--|---------|--|---------|----|
| Monsternr: | Trinity | | Novatec | | EuroImm | un |
| 5-2-2015 onv | 19,49 | | 2,452 | | 3,439 | |
| 5-2-2015 1:2 | 18,914 | | 1,36 | | 2,546 | |
| 5-2-2015 1:5 | 16,886 | | 0,661 | | 1,192 | |
| 5-2-2015 1:10 | 15,622 | | 0,463 | | 0,784 | |
| 5-2-2015 1:50 | 6,217 | | 0,216 | | 0,19 | |
| 5-2-2015 1:50 (Euro 5) | 5,758 | | 0,161 | | 1,123 | |
| 5-2-2015 1:100(Euro10) | 3,106 | | 0,14 | | 0,629 | |
| 5-2-2015 1:200 (Euro20) | 1,537 | | 0,134 | | 0,37 | |

Trinity is the most sensitive test regarding *P.knowlesi*.

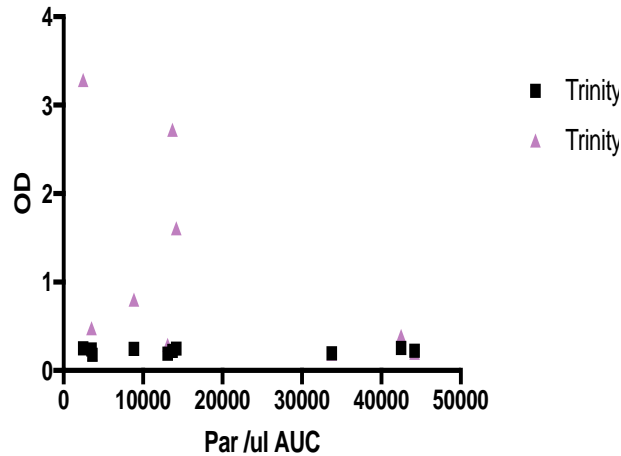
CHMI

Number of reactive samples in experimentally *P. falciparum* infected individuals, before infection and 35 and 140 days after infection.

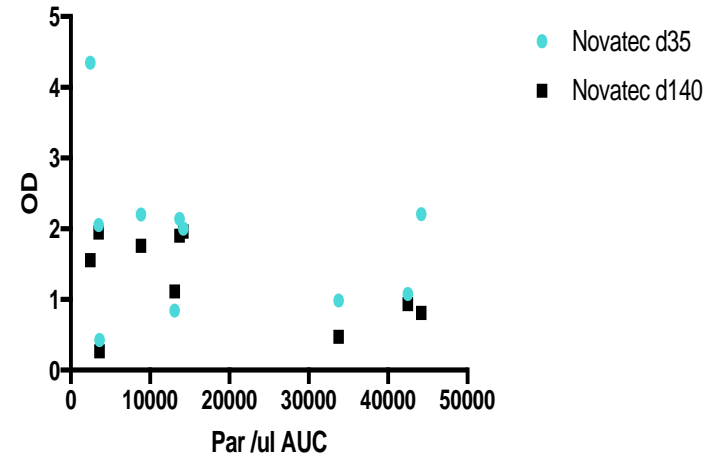
| Assays | N=10 | Positive N (%) |
|---|------|----------------|
| <i>Trinity lab21 Malaria Total antibody EIA (Ig)</i> | | |
| - Before infection | | 0 |
| - 35 days after infection | | 0 |
| - 140 days after infection | | 3 (30) |
| <i>Novatec novaLisa™ Malaria antibody (Ig)</i> | | |
| - Before infection | | 0 |
| - 35 days after infection | | 6 (60) |
| - 140 days after infection | | 6 (60) |
| <i>Euroimmuun anti-Plasmodium Elisa (IgG)</i> | | |
| - Before infection | | 0 |
| - 35 days after infection | | 1 (10) |
| - 140 days after infection | | 4 (40) |
| <i>In house asexual whole parasite ELISA</i> | | |
| - Before infection | | 0 |
| - 35 days after infection | | 9 (90) |
| - 140 days after infection | | 9 (90) |

Serotiter & Parasite exposure

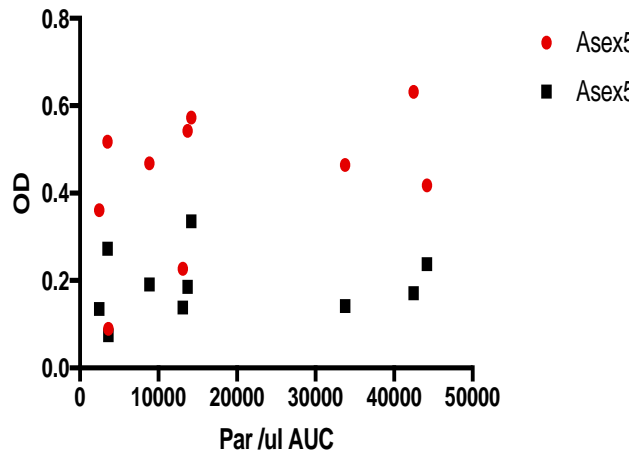
Correlation between OD Trinity and parasite exposure



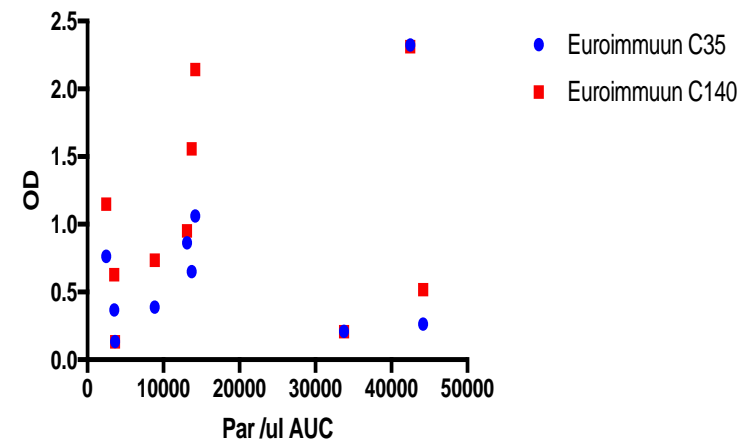
Correlation between OD Novatec and Parasite exposure



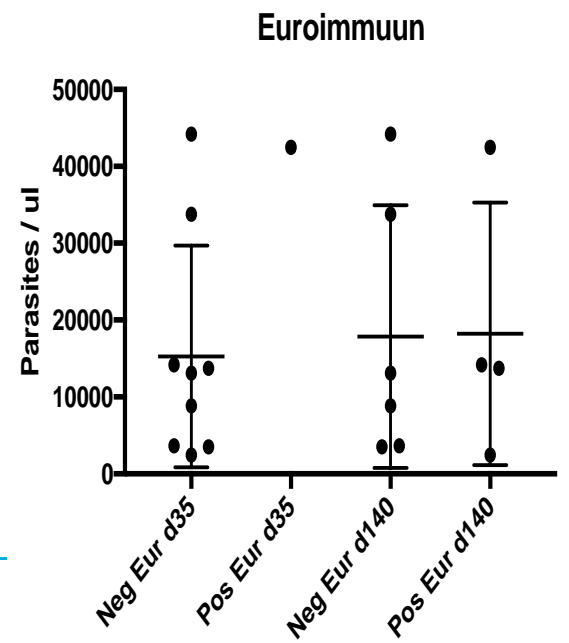
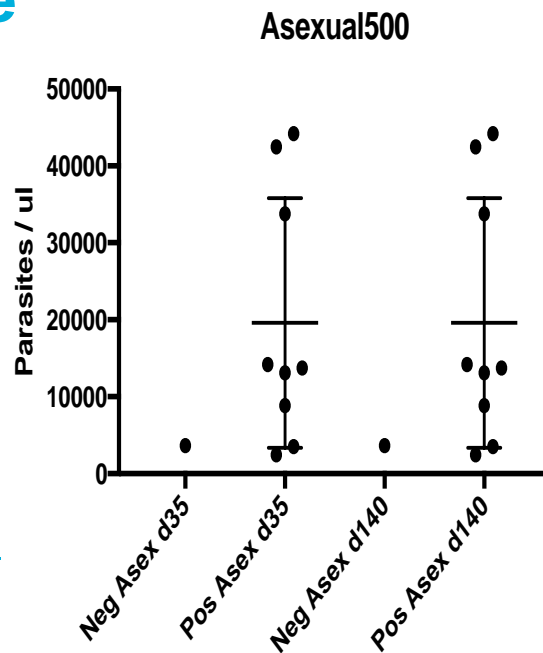
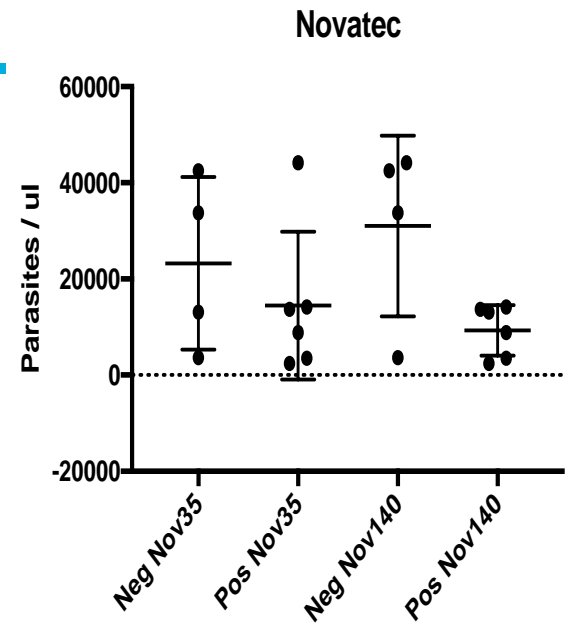
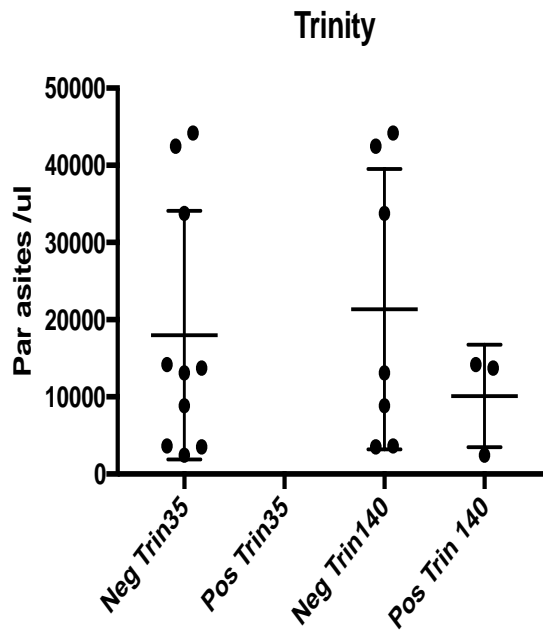
Correlation between OD Asex500 and Parasite exposure



Correlation between OD Euroimmuun and Parasite exposure



Seropositivity & Parasite exposure



Conclusions

- In natural *Pf*, *Pv*, *Pm* and *Pk* infections the Trinity assay performs better compared to Euroimmun and Novatec assays.
- In natural *Po* infections the Euroimmun assay performs better compared to Trinity and Novatec assays.
- In *Pf* CHMI the Novatec assay performs significantly better than the Trinity and Euroimmun assays at days 35 and 140 post infection.
- In *Pf* CHMI the in house whole cell *Pf* ELISA shows the best test performance at days 35 and 140 post infection.

Why do natural and experimental malaria infections behave serologically different?

Travellers / patients

- Unknown timing of exposure
- Unknown previous exposure
- Potential co-infections
- Many cycles with blood stage exposure
- High degree of antigenic variation
- Mostly “high” parasitaemia (above detection limit)

CHMI volunteers

- Limited blood-stage exposure (early treatment)
- Relatively low parasitaemia (around detection limit)
- Heterogenous innate immune responses to the malaria parasite*
- Less pronounced inflammation*
- Less induction of counter regulatory pathways*

* Scholzen & Sauerwein. *Parasitology* 2016, 143, 224-35

Impact of malaria exposure on antiparasite cellular and humoral immuneresponses after controlled human malaria infection. Infection & Immunity, 83 (5), 2185-96.

Dutch



Tanzanian sero -



Tanzanian sero+



| | | | |
|-------------------------|----|-----|----|
| Days to positivity | 10 | 12 | 11 |
| Parasitemia | ↓ | ↑ | = |
| Antibody increase | = | ↑ | ↑↑ |
| <i>Pf</i> spec INFgamma | ↑ | (↑) | = |

Additional conclusions

- Experimental and natural *Pf* infections result in a different humoral immune responses.
- Naive and previously exposed *Pf* infections result in different immune responses.
- Difference between the various commercial assays is caused by varying host immune responses to different choices of recombinant antigens and different choice of antibody subtype (IgM, IgA and/or IgG).

Thanks to

- Paul Daemen, Marga Toonen (clinical microbiology lab Radboudumc)
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