Prospects for DNA-Based Tests to Detect Malaria Infections

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DNA-Based Tests for the Detection of Malaria Parasites

- **DNA-based tests are the mainstay of blood donor screening method to detect infections with HIV, HCV, and West Nile Virus**
- **Why such a blood screening test is not available for malaria?**
- **A large number of publications in malaria–**
  - **Routine diagnosis**
  - **Monitor the efficacy of drugs**
  - **Genotyping**
  - **Vaccine efficacy**

Challenges and Considerations

- **Malaria parasites are highly infectious – 10 blood form *P. vivax* parasites are sufficient to cause a virulent infection**
- **Window period in non-immune travelers**
- **Minimum parasite burden in asymptomatic donors is not known and, therefore the absolute required assay sensitivity is difficult to predict**
- **The number of parasites that survive between the time of donation to transfusion is not known**
- **How to detect to a few parasites potentially present in a unit of blood?**

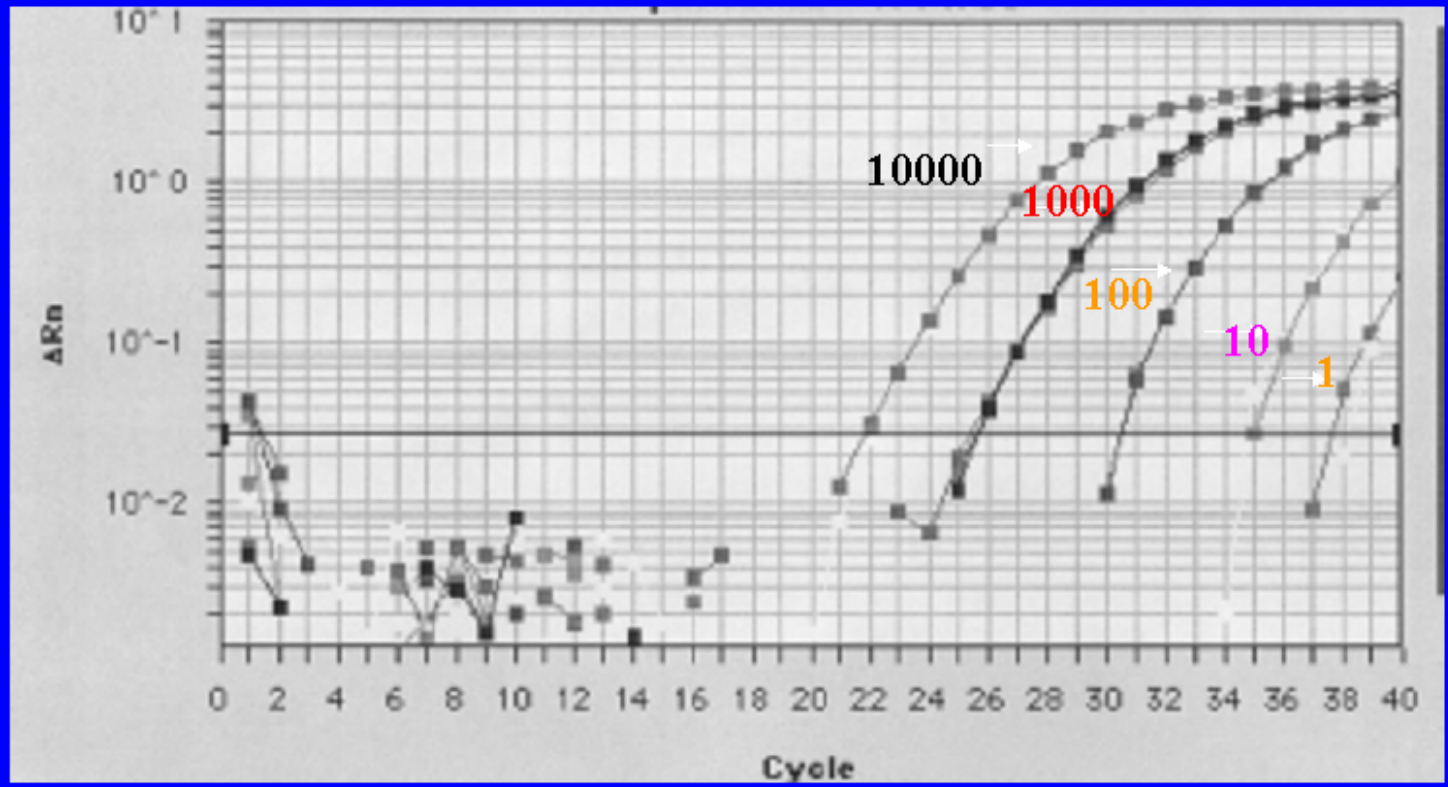
DNA-Based Tests for the Detection of Malaria Parasites

- PCR-amplification (primary and nested-PCR)
- TaqMan assay, Real-time PCR
- Microarray test

DNA-Based tests for the Detection of Malaria Parasites

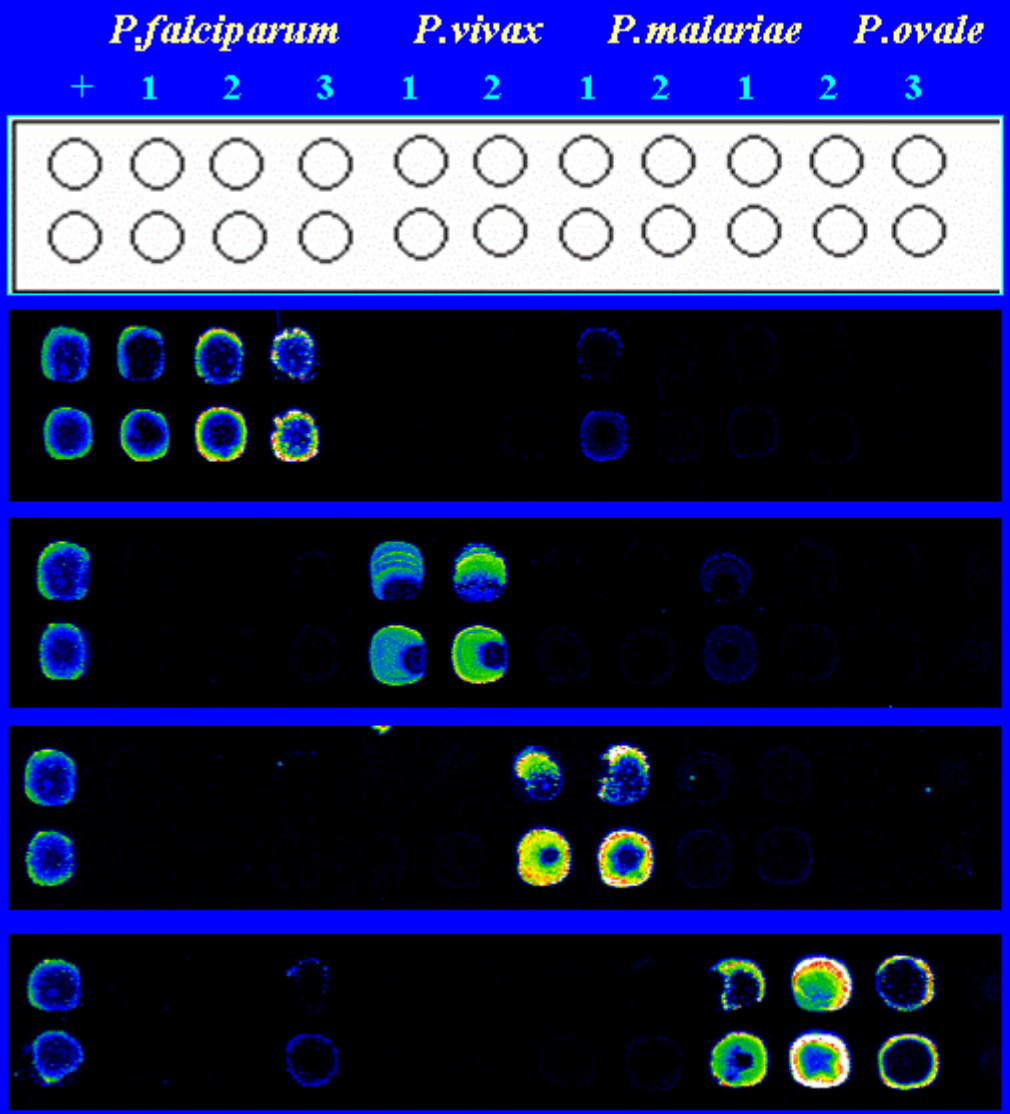
- 18 S rRNA gene is the primary target
- First malaria rRNA gene isolated by Tom McCutchan in 1983
- RNA based diagnosis of *P. falciparum.* McCutchan group. Lancet 1989
- 7-8 copies of the gene per genome
- Contains both conserved and semi-conserved regions allowing Plasmodium species identification

Detection Limit of the TaqMan Assay for *P. falciparum* Parasites Spiked in Normal Blood



Detection limit - 1 parasite/μL of blood

Microarray Test



Plasmodium DNA oligonucleotide Probes dotting Pattern

Pf ss-DNA

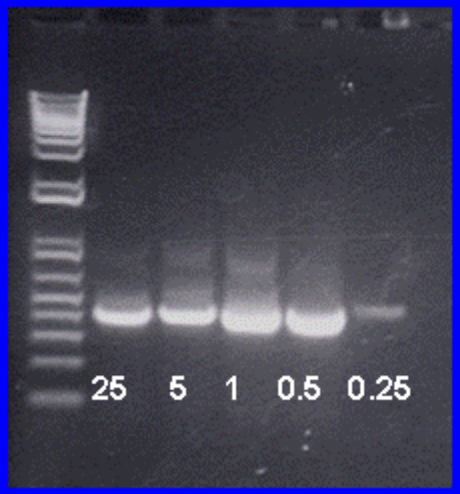
Pv ss-DNA

Pm ss-DNA

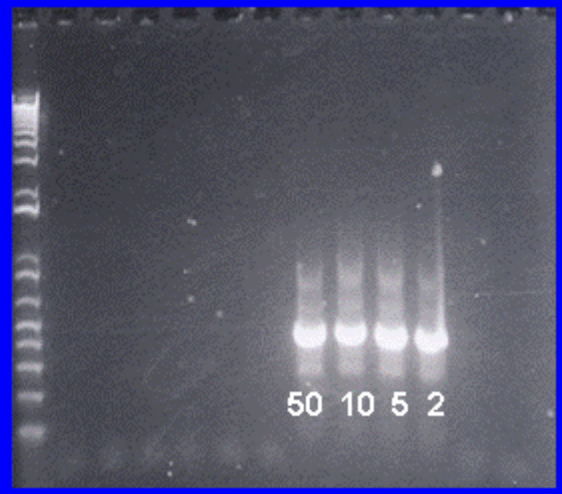
Po ss-DNA

DNA based tests for ring form *Plasmodium* falciparum Detection using 18s rRNA by Nested PCR

Whole blood method



Genomic DNA isolation



Whole Blood Boiling Method

- Detects 0.25 parasite/μl of blood
- Potentially could leave up to 112, 500 parasites undetected in a unit of blood

DNA Isolation QIAamp Blood Kit

- Detects up to 2.0 parasites/ml of blood
- Potentially could leave up to 900 parasites undetected in a unit of blood

DNA-Based Tests for the Detection of Malaria Parasites*

- **Non-radioactive PCR hybridization (whole blood). Drug efficacy trial: 20 parasites/ml. Ciceron et al. JCM 1999**
- **Quantitative real-time PCR (genomic DNA isolation). Vaccine efficacy trial: 20 parasites/ml. Andrews et al. AJTMH 2005**
- **Nest-PCR (genomic DNA isolation). Parasite spiking study: 2 parasites/ml, S Kumar, FDA (unpublished)**

*Data based on a few published reports

Determining the Time of Sporozoite Exposure to Appearance of Blood Form Parasites (window period) by a PCR Test

- **Vaccine efficacy study. Following sporozoite challenge, real-time PCR can detect malaria parasite in the blood up to 5 days before microscopy. Andrews et al. AJTMH 2005**
- **It is reasonable to predict that real-time PCR could be used to detect malaria parasites in the blood of the majority of non-immune travelers two-weeks after departing an endemic area**

Prospects for DNA-Based tests for Blood Donor Screening for Malaria Infections

- **Highly infectious nature of malaria parasites causes a potential risk from a few parasites that could be present in a unit of blood**
- **Highest sensitivity achieved: 2 to 20 parasites/ml**
- **Minimum number of infectious parasites present in a unit of blood: Not known (biggest road block)**
- **Possible solutions:**

  - A technology for parasite concentration

  - An accurate knowledge of the minimum parasite burden in infected donors – that would allow to determine the required assay sensitivity

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