WHO Evidence Review Group on Malaria Diagnosis in Low Transmission Settings

WHO Headquarters, Geneva,
16-18 December 2013

Why this meeting?

In recent years, the application of nucleic acid amplification (NAA)-based diagnostic tools to detect malaria in the context of epidemiological surveys and in research endeavours has increased significantly.

Many different assays are available with a superior diagnostic performance to microscopy and rapid diagnostic tests.

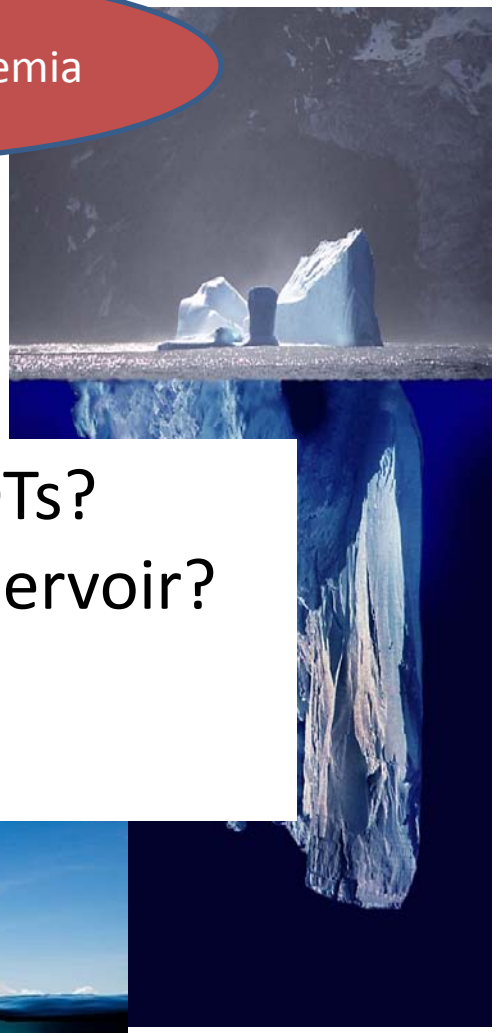
In order to develop recommendations on the role of molecular diagnostic tests for malaria in low transmission areas and to address operational questions raised by national health authorities, WHO/GMP convened a meeting of experts to review the available evidence.

ERG on malaria diagnosis in low transmission settings

Questions from low malaria transmission/pre-elimination countries (Gaps in evidence)

- What are the recommended tests to detect asymptomatic infections in population surveys, active case detection, screening, case management
- What is the gold standard diagnostic test in elimination settings.
- Considering the limitations of microscopy and RDTs what are the recommended diagnostic tools to be used at the community level in areas targeted for elimination.
- What is the role of PCR in elimination settings for surveillance and case management and what are the requirements for QA.
- What are the most sensitive assays to detect gametocytes in research studies.
- What is the best screening tool for detecting asymptomatic carriers at airports and borders.
- Can current serological tests (ELISA) assist in differentiating recent versus old infection
- What are the best diagnostic tools to confirm interruption of transmission and for certification of malaria elimination
- What is the role of current serological techniques for malaria diagnosis (relevance in P. vivax infections) .
- What resources and tools are required to sustain diagnosis capacity in low transmission settings and/or in areas at risk of reintroduction

Symptomatic parasitemia



Who are we missing with microscopy and RDTs?
What factors influence the asymptomatic reservoir?
What is its contribution to transmission ?
When and how to target it ?

Asymptomatic parasitemia

Objectives

The specific objectives of the consultation were to:

1. Review current knowledge on the contribution of sub-microscopic parasitaemia to transmission, particularly in areas with low transmission.

2. Review the diagnostic performance, technical and resource requirements of available nucleic acid amplification (NAA) methods for diagnosing low-density infections with sexual and asexual malaria parasitesand to recommend the most suitable methods of diagnosis for use in population surveys and active case investigations.

Objectives (Cont…)

3. Review requirements to ensure quality for NAA methods and build capacity to support programmatic interventions in pre-elimination and elimination settings.
4. Review and suggest revisions to current WHO recommendations for malaria diagnostic approaches in low transmission settings.
5. Review the malaria diagnostic R&D pipeline and reach consensus on preferred product characteristics of new diagnostic tools to meet public health needs for malaria elimination.

Process

A series of presentations were made during the first two days of the meeting under five themes:

1. **Malaria epidemiology in low transmission settings.**
2. **Current molecular diagnostic techniques for malaria.**
3. **Quality assurance of molecular diagnostic technologies for malaria.**
4. **Field applications of molecular diagnostic technologies and serology for malaria.**
5. **Future malaria diagnostic technologies for low transmission settings.**

Process (cont…)

When possible, presenters were requested to present the evidence as a systematic review, highlighting any growing consensus and remaining evidence gaps.
Finally, the experts were divided into three working groups to discuss specific questions around three themes:

- **Malaria epidemiology in low transmission settings.**
- **Current molecular diagnostic techniques for malaria.**
- **Field applications of molecular diagnostics for malaria.**

Recommendation 1

Quality assured RDT and microscopy are the primary diagnostic tools for the confirmation and management of suspected clinical malaria in all epidemiological situations, including areas of low transmission, due to their high diagnostic performance in detecting clinical malaria, their wide availability and relatively low cost. Similarly, RDT and microscopy are appropriate tools for routine malaria surveillance (of clinical cases) in the majority of malaria-endemic settings.

Recommendation 2

Generally, the use of more sensitive diagnostic tools should be considered only in low transmission settings where there is already widespread implementation of malaria diagnostic testing and treatment and low parasite prevalence rates (e.g. < 10%). Use of nucleic acid amplification (NAA)-based methods should not divert resources away from malaria prevention and control interventions and strengthening of the health care services, including the surveillance system.

Recommendation 3

Submicroscopic *Plasmodium falciparum* and *P. vivax* infections are common in low as well as high transmission settings. A number of nucleic acid amplification techniques are available and are more sensitive in detection of malaria compared to RDTs and microscopy. The use of NAA methods by malaria programs should be considered for surveys aimed at mapping prevalence of malaria, including submicroscopic infections, and to increase the power of surveys at low transmission intensity.

Recommendation 4

The majority of infections with asexual parasites have gametocytes detectable by molecular amplification methods. All malaria infections (microscopic and submicroscopic) should be considered as potentially infectious and able to contribute to ongoing transmission. There is no operational need for routine detection of gametocytes in malaria surveys. For research applications, nucleic acid sequence-based amplification (i.e. QT-NASBA or real time qPCR) are the recommended gametocyte detection tools.

Recommendation 5

Common standards for nucleic acid based assays should be developed, including use of the WHO International Standard for P. falciparum DNA NAT assays and development of standards for other Plasmodium species, particularly P. vivax should be undertaken. A standard operating procedure should be developed which defines methods for sample collection, extraction, and the recommended equivalent quantity of blood to be added to the assay.
Development of an international, external quality assurance system is strongly recommended to ensure that data obtained from nucleic acid amplification assays are reliable and comparable.

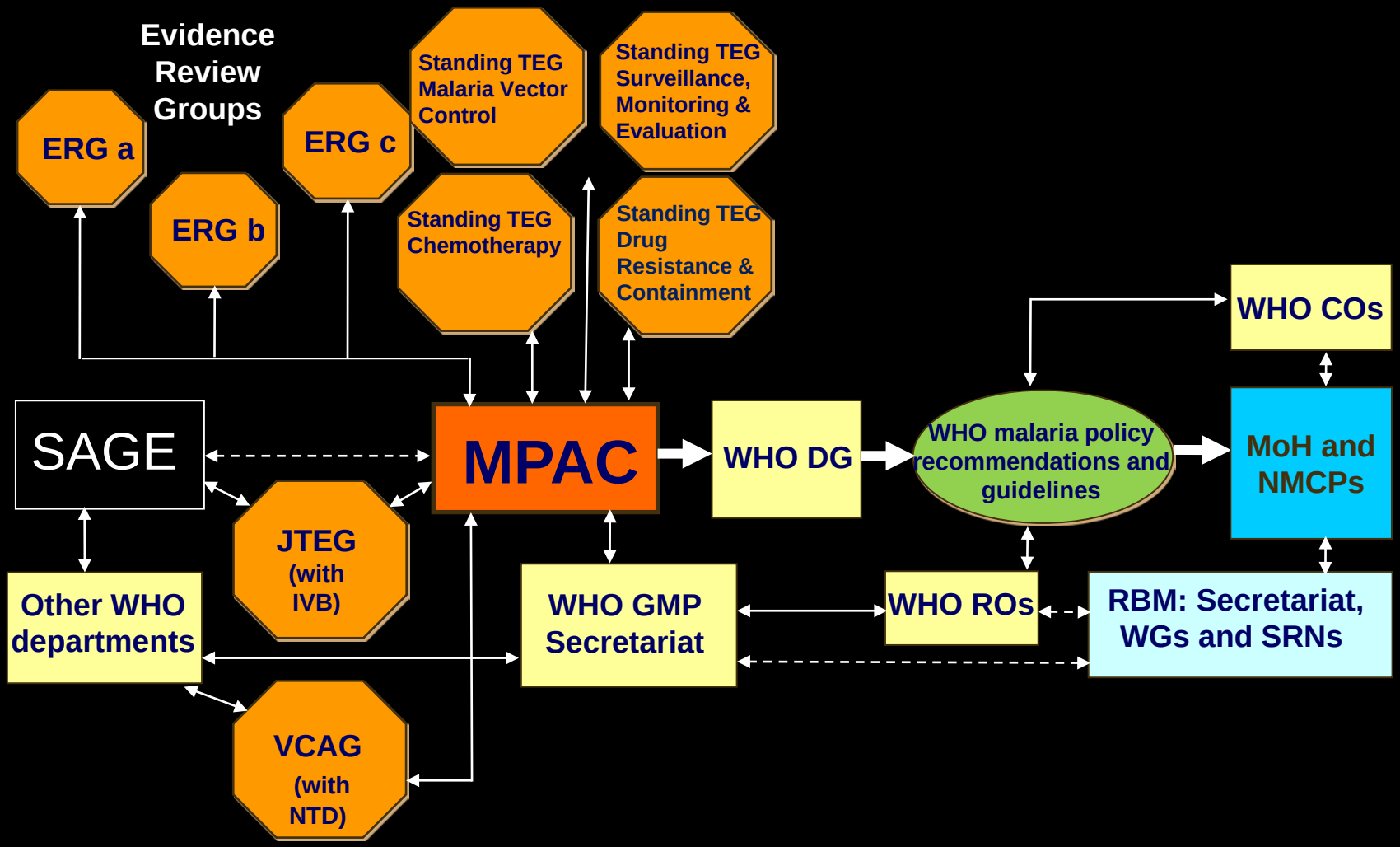
Recommendation 6

There is a need for standardisation of reagents (antigens and controls), assay methodologies and analysis for malaria serology. Until that becomes available there is a limited role for serological assays in the routine operational monitoring of transmission in elimination settings, but it may still have a role for epidemiological surveys in certain elimination settings.

Recommendation 7

There is a need for more research to understand better the contribution of submicroscopic infections in malaria transmission in low endemic settings and to identify which diagnostic strategies and NAA-based diagnostic techniques are most cost-effective in accelerating malaria elimination, compared to conventional malaria elimination methods. Additionally, markers to identify recent malaria infections, and diagnostic tools that detect *P. vivax* hypnozoites are needed.

WHO policy setting process for malaria



The following preferred product characteristics for new technologies were discussed at the meeting:

- **An ability to detect parasitaemia of ≤2 parasites/µl.**
- **Need for a sample volume of not more than 50µl blood.**
- **An assay that is not instrument specific.**
- **Flexibility in power supply.**
- **An ability to detect malaria parasites at genus level and then conduct species differentiation on positive samples.**

Results should ideally be available within 16 hours (same working day or early on the following day for patients providing samples just before closing hours), with a maximum waiting time of 24 hours for results.

The assay should allow processing of 48 samples/person/platform/day.

Reagents should be stable at 4°C for a minimum of one year, and at room temperature for a minimum of six months.

Training for conduct of the assay should require no more than five working days, with a minimum number of steps involved in conducting the assay

Shipping conditions should not require a temperature of less than 4°C

No hazardous waste should be produced during the assay and there should be no risk of harm for the user

The contamination risk is low.
The assay is affordable

The equipment needed to undertake the assay is portable

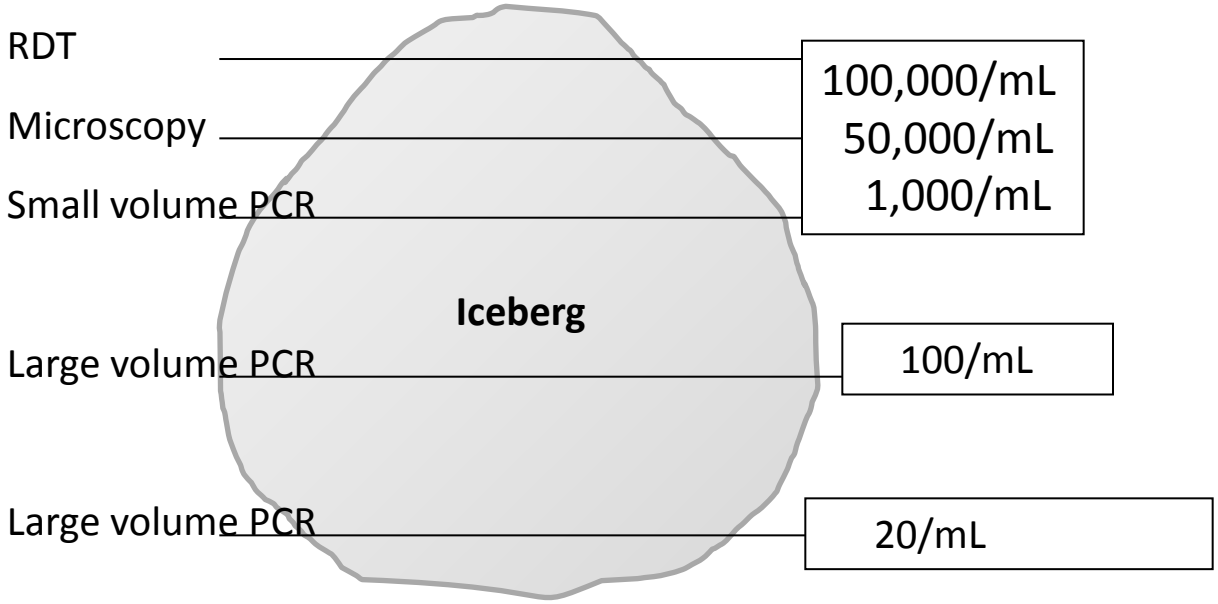
The assay is automated with an objective end-point reading.

Results are simple to interpret.

Desirable network connectivity for data transfer.

The assay specificity for detection of the *Plasmodium* genus is 95% or higher.

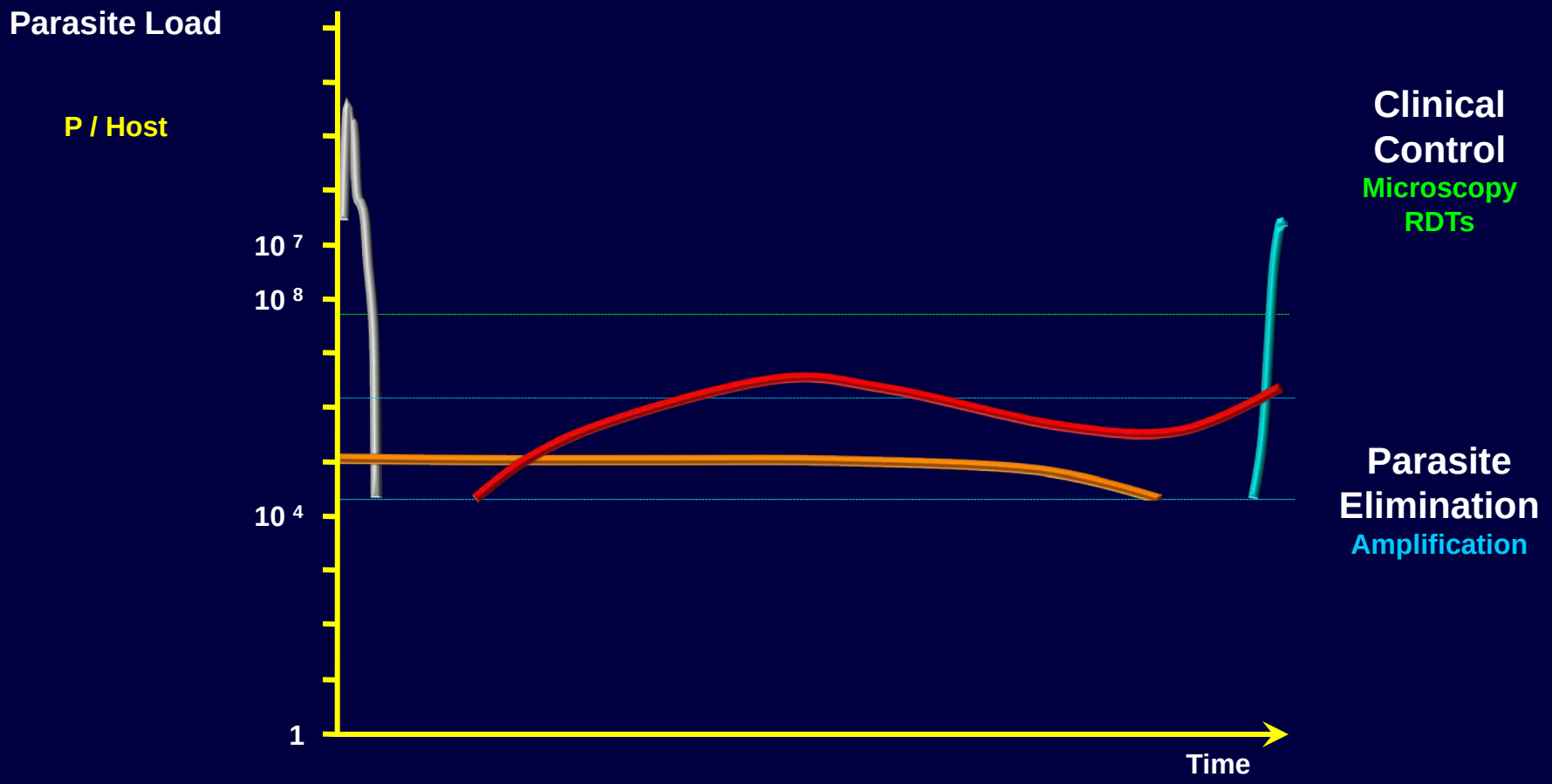
The ultra-sensitive molecular techniques to quantity low density malaria parasitaemias



Effects of different sensitivities of detection on estimated prevalence of malaria

- think of malaria as an iceberg

The Visible & The Invisible



What are the molecular tools currently available for moving closer to the filed?

LAMP method

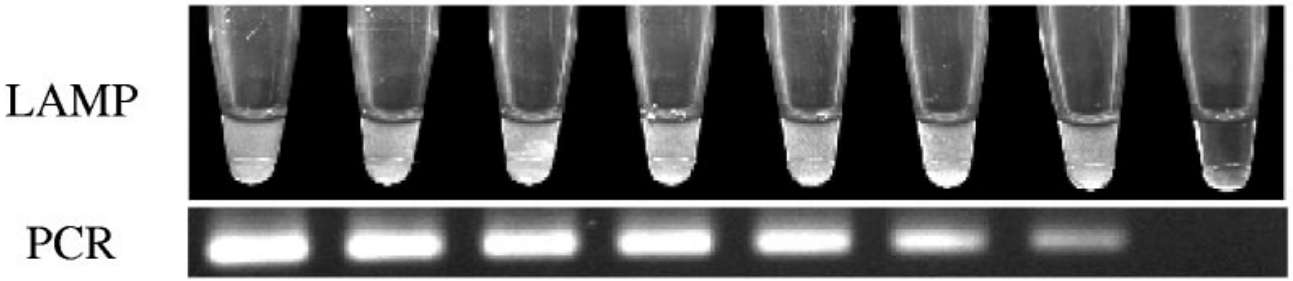
Different versions available

Commercial kit with Find collaboration

RealAmp kit (CDC)

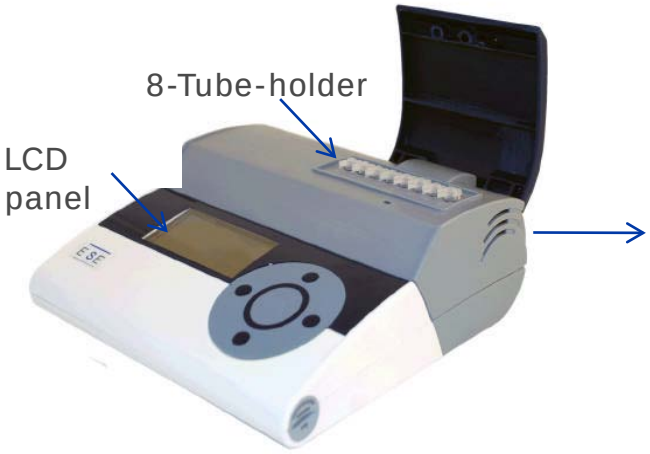
Loop-Mediated Isothermal Amplification (LAMP)

- Isothermal at 60^{o}C-65^{o}C
  - No need for sophisticated thermocycler
- Primers designed to four different locations-theoretically more specific
- Turbidity endpoint reading--*subjective*



REALAMP

A



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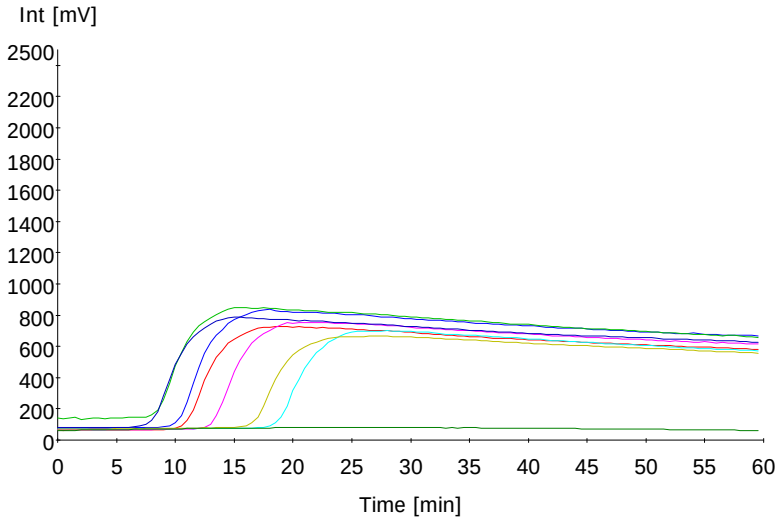
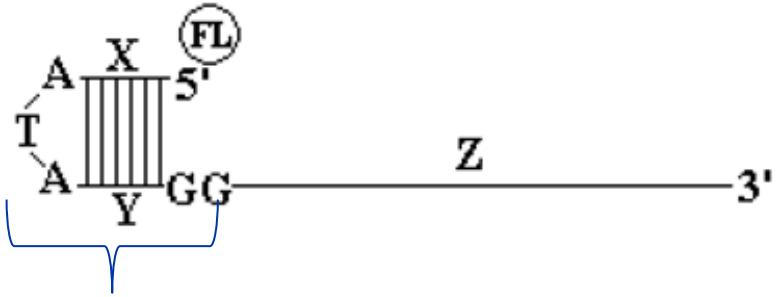
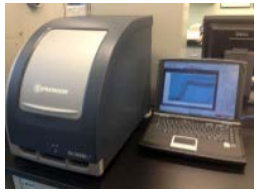


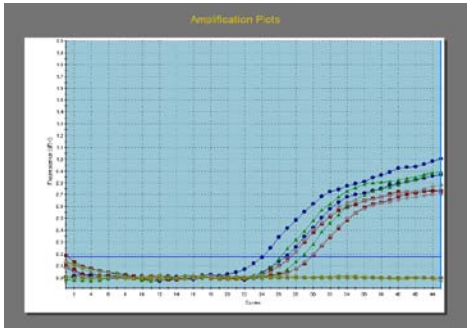
- Equipped with temperature settings and fluorescence detection = RealAmp
- Portable and simple to use equipment
  - Can be battery-operated as stand alone
  - blue-tooth capability

2. Photo-induced Electron Transfer PCR (PET-PCR) for Screening Large Number of Samples



5’ hairpin structure





New method for use in developing country settings

Transferred to Haiti for use in national public health lab

Brazil is validating the use of this technology (Dr. Silvia Di Santi, SUCEN, São Paulo)

Peru is planning to test (Dr. Lescano, NAMRU)

Lucchi NW, Narayanan J, Karell MA, Xayavong M, Kariuki S, et al. (2013) Molecular Diagnosis of Malaria by Photo-Induced Electron Transfer Fluorogenic Primers: PET-PCR. PLoS ONE 8(2): e56677. doi:10.1371/journal.pone.0056677

¡Gracias!