Malaria

Nieuwe ontwikkelingen in sneldiagnostiek

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Doel van malariadiagnostiek

1)Wel / geen malaria infectie

2)Determinatie *Plasmodium* soort

optimale behandeling

3)Bij *P. falciparum* (en *P. knowlesi*) infectie

bepaling parasitaemie (ernst van de infectie)

aanwezigheid schizonten

(keuze orale of intraveneuze behandeling)

Methoden voor malaria diagnostiek

- Dikke druppel en uitstrijk (gouden standaard)
- Moleculaire (snel)diagnostiek

Screening (rapid diagnostic test)

- Antigeen sneltesten
- Moleculaire sneltest (LAMP test Meridian)

- Nieuwe sneldiagnostiek

Sensitiviteit, specificiteit & beoordelingstijd

Test	Sensitiviteit	Spec.	Tijd	Expertise
Dikke Druppel	5 -10 par/µl	goed	45+20 min	hoog
QBC	0.5-1 par/ µl	excel*	10+5 min	hoog
Antigeen	50-200 par/µl	goed	5+10 min	laag
LAMP	<1 par /µl	excel *	10+50 min	laag

* Geen species determinatie

Malaria antigeen sneltesten

- Aantonen antigenen van *Plasmodium sp.*
- Makkelijk in gebruik
- Snel resultaat (ca. 5+10 minuten)
- Goedkoop

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Malaria Journal

RESEARCH Open Access

High proportions of *pfhrp2* gene deletion and performance of HRP2-based rapid diagnostic test in *Plasmodium falciparum* field isolates of Odisha

Pallabi Pati[1], Gunanidhi Dhangadamajhi[2], Madhusmita Bal[3] and Manoranjan Ranjit[1*]

Abstract

Background: With the documentation of cases of *falciparum* malaria negative by rapid diagnostic tests (RDT), though at low frequency from natural isolates in a small pocket of Odisha, it became absolutely necessary to investigate the status of HRP-2 based RDT throughout the state and in different seasons of the year.

Methods: Suspected individuals were screened for malaria infection by microscopy and RDT in 25/30 districts of Odisha, India. Discrepancies in results were confirmed by PCR. False negative RDT samples for *Plasmodium falciparum* mono-infection were evaluated for detection of HRP2 antigen in ELISA and genotyped for *pfhrp2*, *pfhrp3* and their flanking genes. Multiplicity of infection was ascertained based on *msp1* and *msp2* genotyping and parasitaemia level was determined by microscopy.

Results: Of the total 1058 patients suspected for malaria, 384 were microscopically confirmed for *P. falciparum* mono-infection and RDT failure was observed in 58 samples at varying proportion in different regions of the state. The failure in detection was due to undetectable level of HRP-2. Although most of these samples were screened during rainy season (45/345), significantly high proportion (9/17) of RDT negative samples were obtained during the summer compared to rainy season (P = 0.0002; OR = 7.5). PCR genotyping of *pfhrp2* and *pfhrp3* in RDT negative samples showed 38/58 (65.5) samples to be *pfhrp2* negative and 24/58 (41.4) to be *pfhrp3* negative including dual negative in 17/58 (29.3). Most of the RDT negative samples (39/58) were with single genotype infection and high proportions of *pfhrp2* deletion (7/9) was observed in summer. No difference in parasitaemia level was observed between RDT positive and RDT negative patients.

Conclusion: High prevalence of parasites with *pfhrp2* deletion including dual deletions (*pfhrp2* and *pfhrp3*) is a serious cause of concern, as these patients could not be given a correct diagnosis and treatment. Therefore, HRP2-based RDT for diagnosing *P. falciparum* infection in Odisha is non-reliable and must be performed in addition to or replaced by other appropriate diagnostic tools for clinical management of the disease.

Keywords: *Plasmodium falciparum*, HRP-2, RDT, *pfhrp2*, *pfhrp3*, Genotyping, Odisha, Season

Malaria antigeen sneltesten

- Aantonen antigenen van *Plasmodium sp.*
- Makkelijk in gebruik
- Snel resultaat (ca. 5+10 minuten)
- Goedkoop

→ zeer veel gebruikte test
(ook in endemische gebieden)

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Molecular surveillance of *pfhrp2* and *pfhrp3* genes deletion in *Plasmodium falciparum* isolates and the implications for rapid diagnostic tests in Nigeria

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[b] Department of Pharmacy Technician Studies, Bayelsa State College of Health Technology, Nigeria
[c] Institute of Tropical Medicine, University of Tübingen, Germany
[d] Department of Pediatrics, Stanford University School of Medicine, Stanford, USA
[e] Department of Biomedical Sciences, College of Health Sciences and Technology, Rochester Institute of Technology, Rochester, NY, USA
[f] Institute for Advanced Medical Research and Training, University College Hospital Ibadan, Nigeria
[g] Faculty of Medicine, Duy Tan University, Da Nang, Vietnam
[h] Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology, Osogbo, Nigeria

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ABSTRACT

Prompt diagnosis and appropriate treatment of malaria remain the hallmark for reducing malaria-related mortality in high transmission areas. *Plasmodium falciparum* histidine-rich protein2 (*Pf*HRP2) based rapid diagnostic tests (RDT) play a vital role in prompt and accurate malaria diagnosis. However, *pfhrp2* gene deletion threatens the RDT test sensitivity. This study reports the presence of *pfhrp2* and *pfhrp3* genes deletion among parasite isolates in Nigeria. Febrile children were screened using histidine-rich protein (HRP2) specific RDT (SD-Bioline RDT) and microscopy for *P. falciparum* infections. All RDT negative samples were re-evaluated by polymerase chain reaction (PCR). The presence of parasite in RDT false negative cases and randomly selected RDT positive cases were validated using PCRs targeting glutamate-rich protein (*glurp*) and merozoite surface proteins (*msp-1* and *msp-2*). Thereafter, exon 2 of *pfhrp2* and *pfhrp3* were amplified, and Sanger sequenced. A total of 511 febrile children were enrolled out of which 309 (61%) were positive by RDT. The presence of *pfhrp2* and *pfhrp3* genes were analyzed in 66 PCR positive samples comprising of 31 RDT false-negative and 35 RDT true positive randomly selected samples. The *pfhrp2* and *pfhrp3* genes failed to amplify in 17% (11/66) and 6% (4/66) samples, respectively. Seven of the eleven samples had only *pfhrp2* deletion while four had both *pfhrp2* and *pfhrp3* deletions. The absence of the *pfhrp2* gene may be responsible for the seven RDT false negative cases observed. Three RDT positive cases lacked *pfhrp2* whereas *pfhrp3* was absent in only four RDT false negative cases. The *pfhrp2* and *pfhrp3* amino acid repeat sequences were highly diverse. The *P. falciparum* isolates lacking *pfhrp2* and *pfhrp3* genes may be circulating and contributing to RDT false negativity in Nigeria. More studies in larger population and seasonally defined cases will be needed to determine the extent of *pfhrp2/3* genes deletion in different geographical areas of Nigeria.

Nigeria:
5-15% Pf is
HRP-2 negatief

Conclusie malaria antigeen sneltesten

Voordelen:
- eenvoudig uitvoerbaar
- snel resultaat

Nadelen
- geen (volledige) soort determinatie
- fout positief (bv na behandeling, reuma-factoren)
- fout negatief (bv lage parasitaemie & HRP2 deletie mutanten)

Malaria antigeen sneltesten worden minder sensitief:

- Kans op fout negatieve antigeentesten steeds groter
- Aanpassing 'screenings assay' voor malaria noodzakelijk!

Alternatieve RDT malaria (1)

Endemische gebieden:

Antigeentesten:
- combinatie van meerdere *P. falciparum* antigenen
- bv HRP2 + PfLDH
- in ontwikkeling (nog niet beschikbaar op Westerse markt)

Alternatieve RDT malaria (2)

Endemische gebieden:
Innovatieve methoden: bijvoorbeeld
- LED based fluorescent microscopy
- magneto-optic device detecting malaria pigment

Portable Device Developed for Early-Stage Malaria Detection

By Labmedica International staff writers
Posted on 13 Jun 2018

Over 216 million people were infected with malaria in 2016, and 445,000 individuals died from the disease. The key to solving this health crisis is early-stage diagnosis when malaria therapeutics are most effective.

There are two standard ways of diagnosing malaria, yet both have limitations. The first involves taking a blood sample from a person and looking at it underneath a microscope for red blood cells that have been infected with the malaria parasite. Another method are the rapid diagnostic tests.

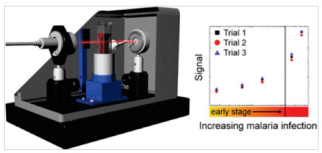


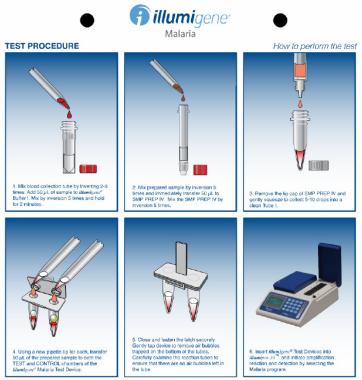
Image: The portable optical diagnostics system (PODS) prototype for diagnosing malaria (Photo courtesy of University of Southern California).

Bioengineers at the University of Southern California (Los Angeles, CA, USA) have developed a portable, magneto-optic technology for early stage malaria diagnosis based on the detection of the malaria pigment, hemozoin. The portable optical diagnostics system (PODS) prototype detects a byproduct generated by all species of the malaria parasite, as such; it is a rapid screening for all malaria strains. Because the amount of hemozoin in the blood is directly related to how far the malaria infection has progressed, it is an ideal indicator of infection.

Malaria PCR (LAMP)

Loop mediated isothermal amplification (LAMP)

- commerciële assay (bv Meridian Illumina)
- analyse tijd ca. 1-2 uur
- wel/geen *Plasmodium* aanwezig



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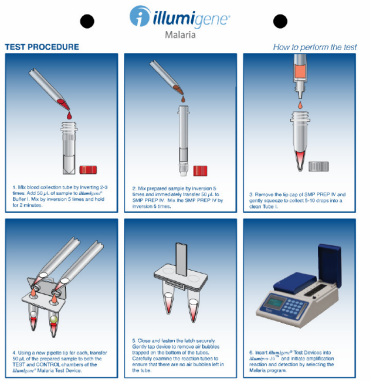


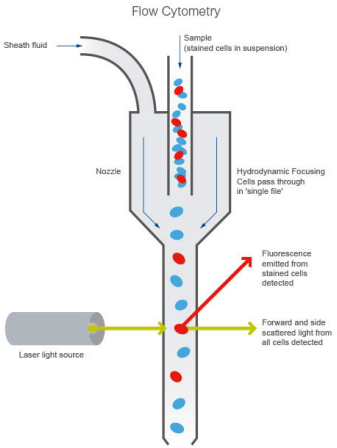
Voordelen:
- Hoge sensitiviteit en specificiteit
- Eenvoudig

Nadelen:
- Relatief langzaam voor screening
- Kostbaar
- Geen species determinatie
- Geen parasitaemie

Nieuwe hemo-analyzer

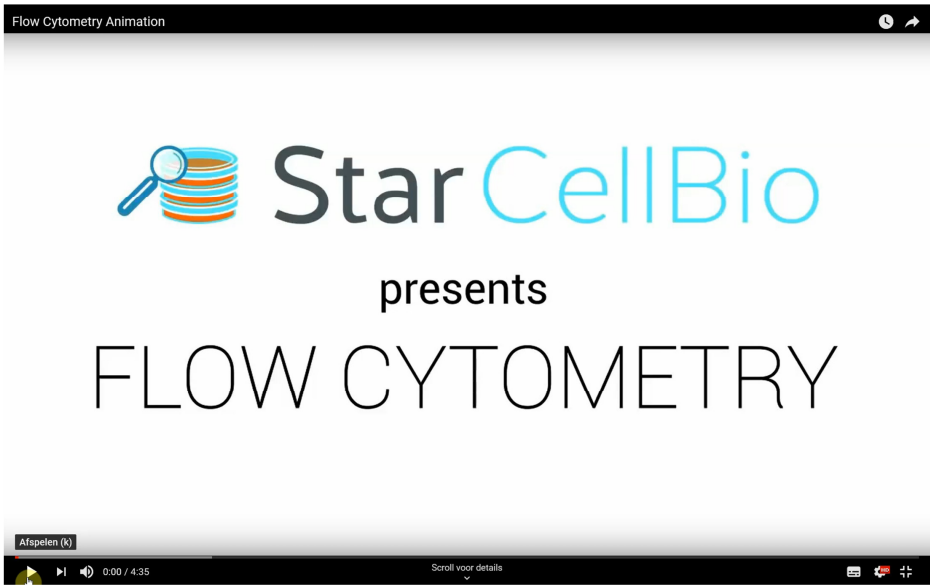
1e laboratorium in Europa voor klinische validatie

Principe detectie malaria XN-31

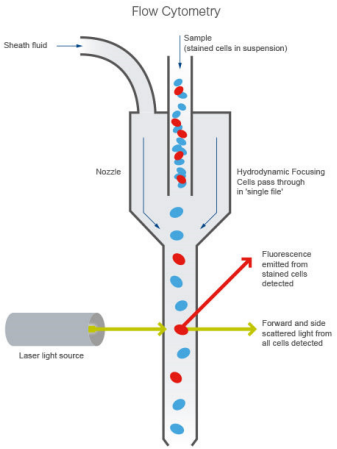


Hemo-analyzer

Klassiek (XN-20):
- Forward – side scatter:
- Bloedcel determinatie
  (RBC en leukocyten differentiatie)



Principe detectie malaria XN-31



Hemo-analyzer

Klassiek:

- Forward – side scatter:
- Bloedcel determinatie
  - (RBC en leukocyten differentiatie)

Nieuw:

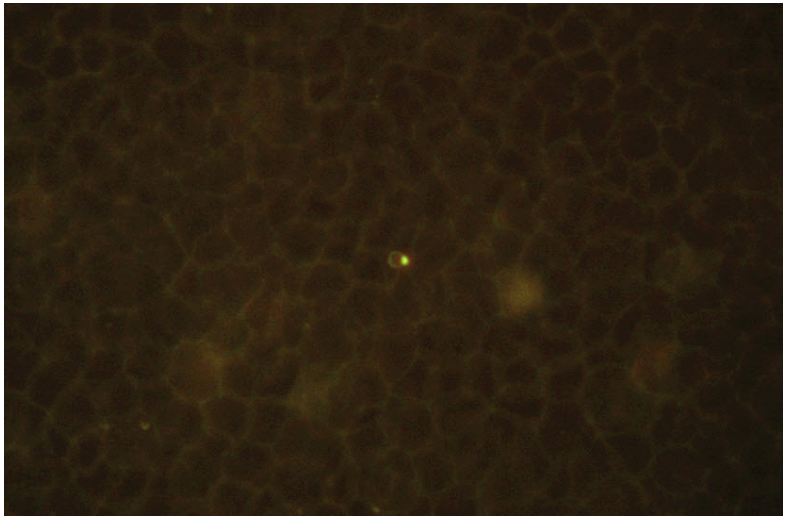
Klassieke bloed differentiatie

+

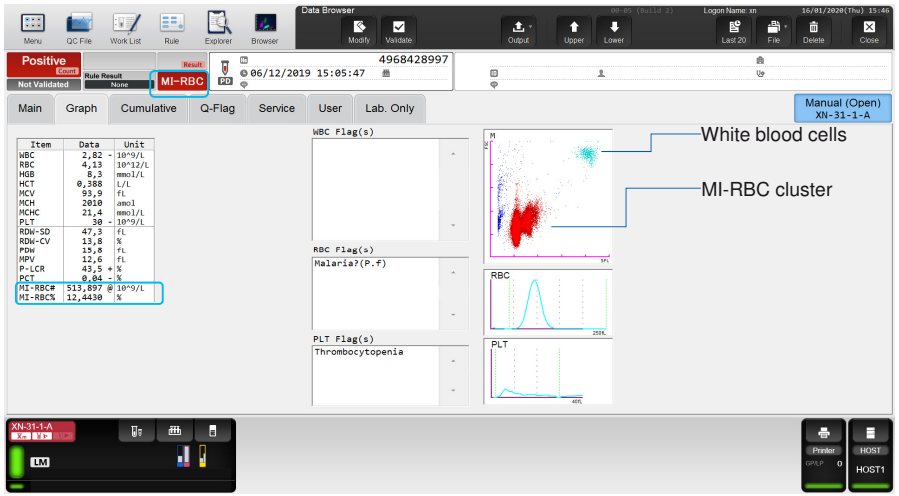
Fluorescente probe die bindt aan nucleïnezuren
(DNA en RNA)

→ RBC met DNA ???

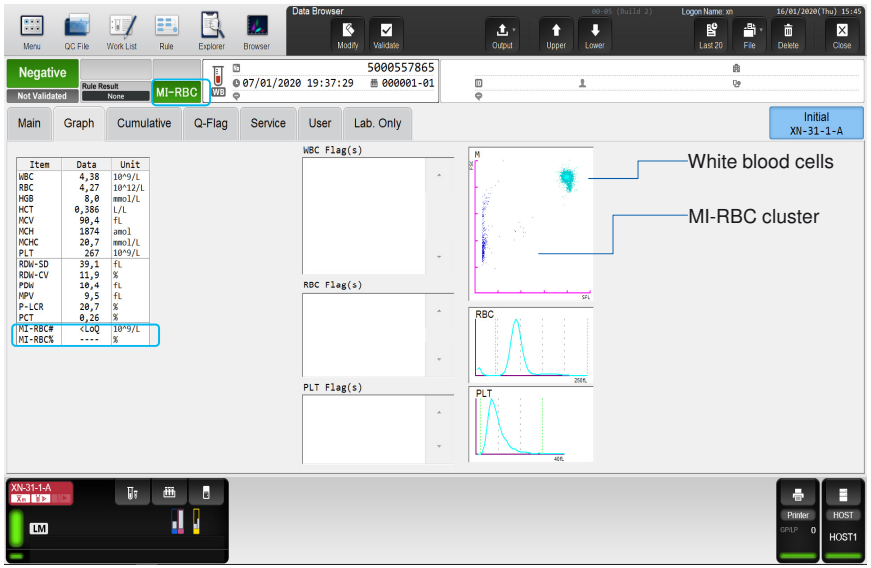
Fluorescentie van *Plasmodium* parasiet in QBC



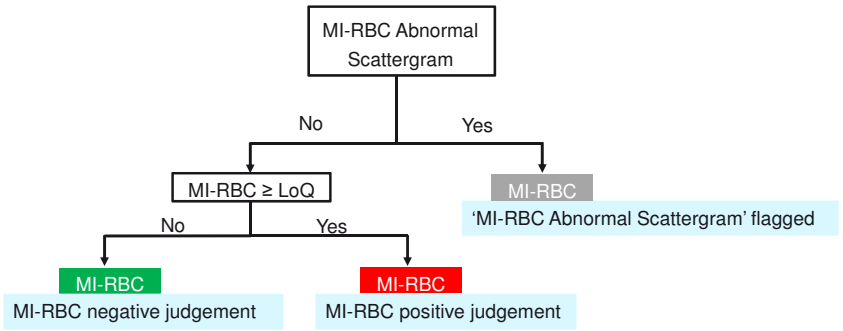
Malaria positive scattergram



Malaria negative scattergram



Uitkomsten XN-31 malaria RDT

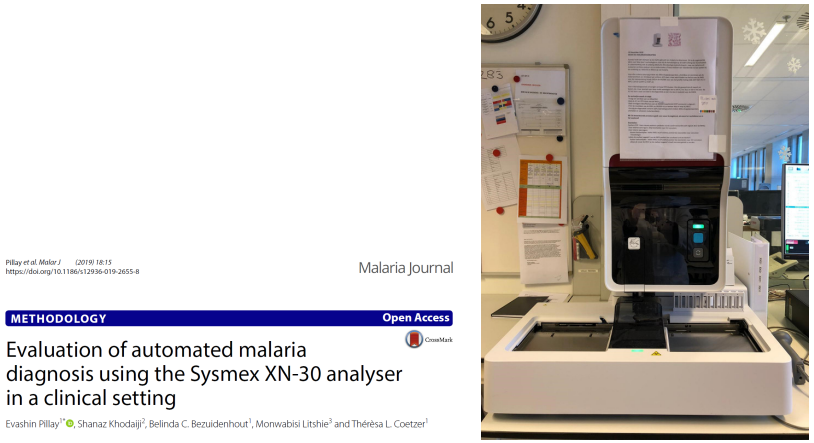


- Huidige detectie limiet (LoQ): 20 geïnfecteerde RBC / 10 µl

→ Theoretische detectielimiet van 2 parasieten / µl

Voorgaande studies XN-31 malaria RDT

Voorgaande studies XN-31 malaria RDT



Pillay et al. Malar J (2019) 18:15
https://doi.org/10.1186/s12936-019-2655-8

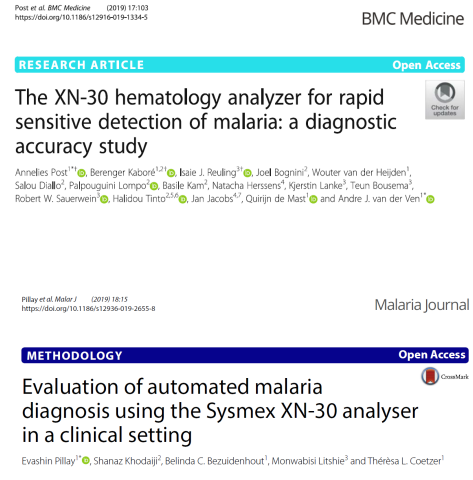
Malaria Journal

METHODOLOGY Open Access

Evaluation of automated malaria diagnosis using the Sysmex XN-30 analyser in a clinical setting

Evashin Pillay[1*], Shanaz Khodaiji[2], Belinda C. Bezuidenhout[1], Monwabisi Litshie[3] and Thérèsa L. Coetzer[1]

Voorgaande studies XN-31 malaria RDT



Post et al. BMC Medicine (2019) 17:103
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BMC Medicine

RESEARCH ARTICLE Open Access

The XN-30 hematology analyzer for rapid sensitive detection of malaria: a diagnostic accuracy study

Annelies Post[1*], Berenger Kaboré[1,2], Isaie J. Reuling[1], Joel Bognini[2], Wouter van der Heijden[1], Salou Diallo[2], Palpouguini Lompo[2], Basile Kam[4], Natacha Herssens[4], Kjerstin Lanke[3], Teun Bousema[3], Robert W. Sauerwein[1], Halidou Tinto[2,5,6], Jan Jacobs[4,7], Quirijn de Mast[1] and Andre J. van der Ven[1*]

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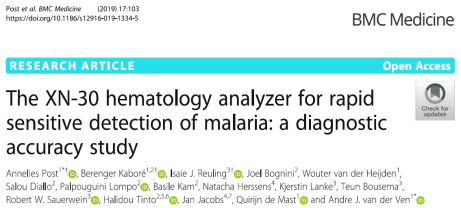

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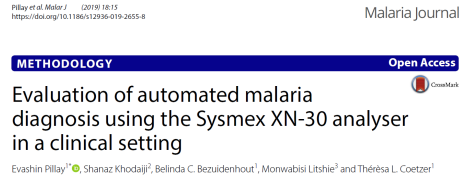

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Voordelen:

Volledig geautomatiseerde analyse & interpretatie

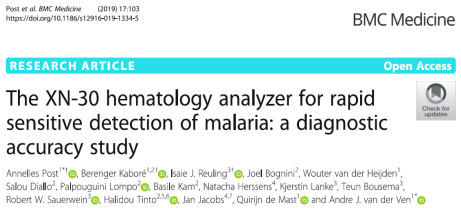
Hoge sensitiviteit
(ca. 2 parasieten/µl)

Hoge specificiteit

Snel (ca. 30 sec. !!)

Indicatie species en parasitaemie

Voorgaande studies XN-31 malaria RDT



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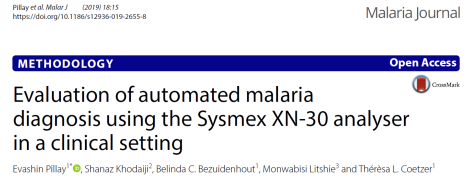

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Voordelen:

Volledig geautomatiseerde analyse & interpretatie

Hoge sensitiviteit
(ca. 2 parasieten/µl)

Hoge specificiteit

Snel (ca. 30 sec. !!)

Indicatie species en parasitaemie

Nadeel

Kostbaar apparaat

Klinische validatie Rotterdam

Specificiteit: reticulocyt afwijkingen
erytroblast afwijkingen
overige erytrocyt en andere bloedcel afwijkingen

Sensitiviteit: Detectie limiet verbetering
nu standaard analyse 10 µl bloed
(vergroting volume sample analyse)

Accuratesse parasitaemie bepaling

Plasmodium species determinatie
P. falciparum vs non-falciparum

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Accuratesse parasitaemie bepaling

Plasmodium species determinatie
P. falciparum vs non-falciparum

*Alle malaria aanvragen worden 24*7 ook in trial verband geanalyseerd*

Resultaten XN-31 studie Rotterdam

Microscopische uitslag (DD, uitstrijk & QBC)	XN-31 neg	XN-31 abnormal	XN-31 POS
Negatief	51	0	0
P. falciparum (+ follow up)	0	0	9
P. ovale	0	0	1
P. malariae (ca. 1 parasiet / 2 dd)	0	1	0

Resultaten XN-31 studie Rotterdam

Microscopische uitslag (DD, uitstrijk & QBC)	XN-31 neg	XN-31 abnormal	XN-31 POS
Negatief	51	0	0
P. falciparum (+ follow up)	0	0	9
P. ovale	0	0	1
P. malariae (ca. 1 parasiet / 2 dd)	0	1	0

Geen fout negatieve resultaten (wel 1x Pm als 'abnormal')
Geen fout positieve resultaten

Correlatie *P. falciparum* parasitaemie is hoog

Dankwoord

Afd. Klinische Chemie
- Tania Khartabil
- Henk Russcher

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- Rob Koelewijn
- Analisten parasitologie

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