Case study: in vitro diagnostics for malaria control

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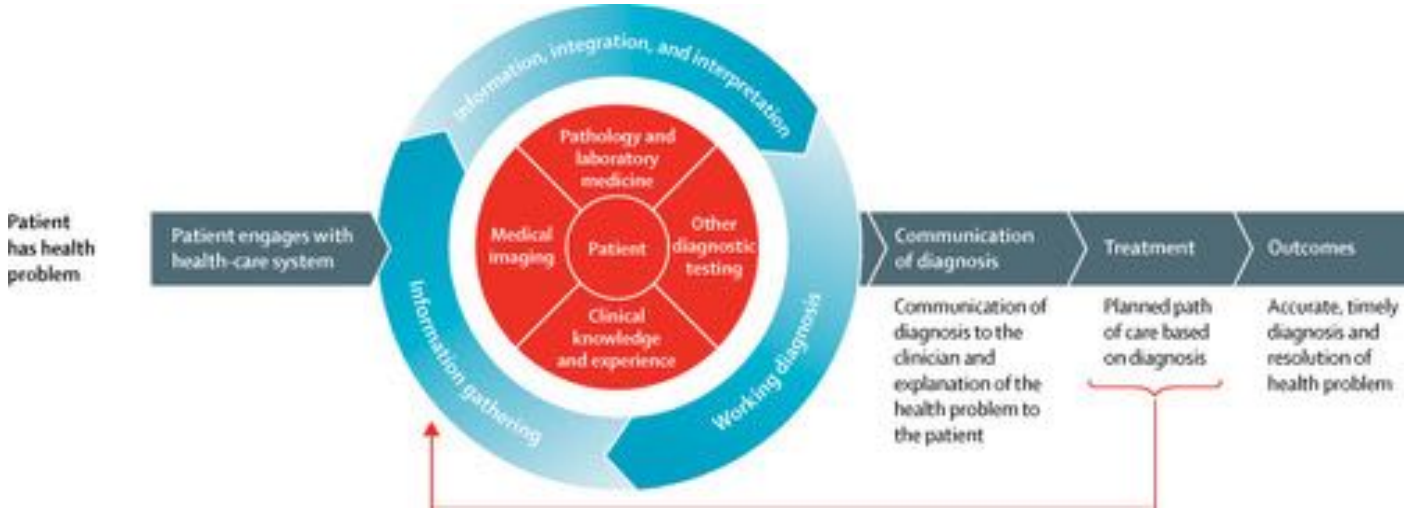
Overview

1. In vitro diagnostics (IVD) for poverty-associated diseases
    - Role of diagnosis in health prevention and care
    - Levels of health care and laboratory services in LMIC
    - Point-Of-Care IVD in limited resource settings
2. Malaria
    - Infection and disease
    - Global health burden and control strategies
    - Present and future of IVD tools
3. From the concept to the IVD product and its use
    - Product development partnerships
    - IVD performance in context: implementation research
    - Laboratory quality management system

Diagnosis in health prevention and care

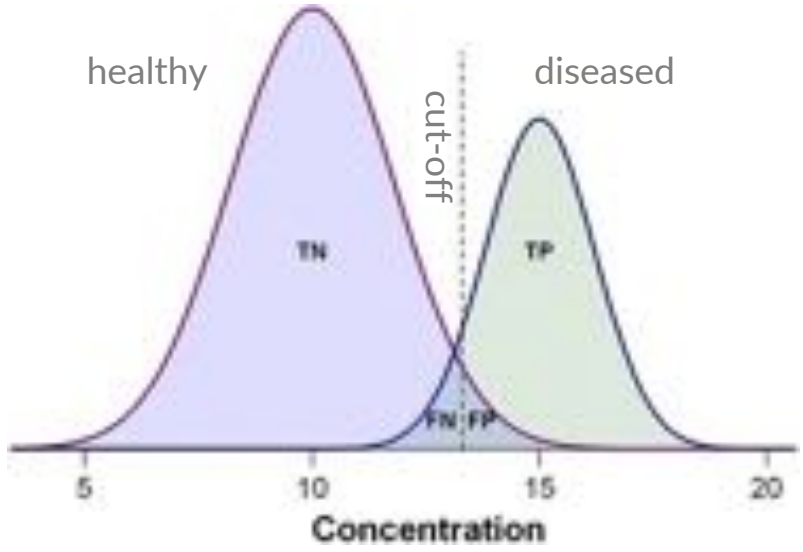


- Plays a central role in health care services and public health interventions
- Process of classification of a subject into categories (disease +/-) that allow medical/public health decisions to be made
- Accurate and timely diagnosis results in correct treatment and better health outcome for individuals and communities
- Can be based on interviews, physical examinations, imaging and laboratory investigations
- In vitro diagnostic (IVD) tests measure analytes in biological samples that are considered markers of disease or *biomarkers*

Wilson et al. *The Lancet* 2018

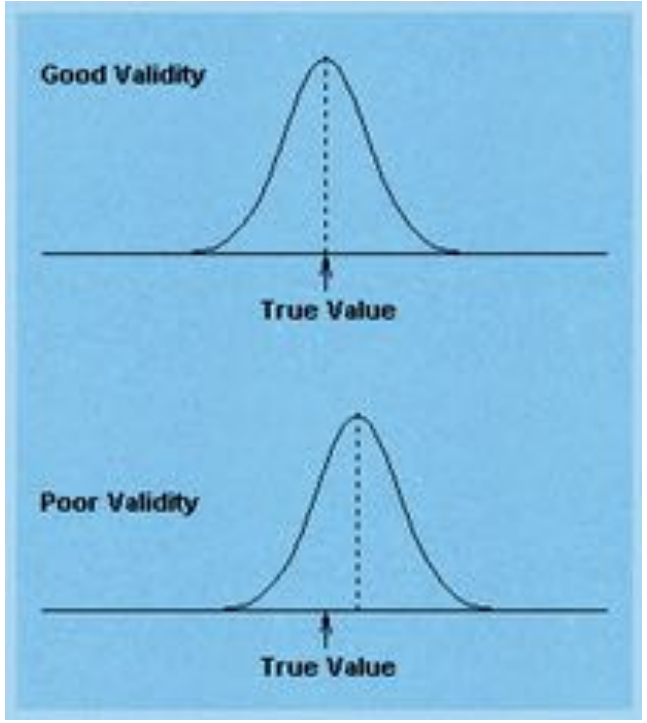
Accuracy of diagnosis

- The result of any IVD test has a certain degree of inaccuracy
- There are several potential sources of inaccuracy, or error in measurement
  - biological variation
  - procedure variation
  - observer variation
- When chosing and applying an IVD test for a given purpose/target population it is important to be aware of the sources and size of error measurement
- The accuracy of an IVD test can be evaluated assessing its validity and reliability



Validity of a diagnostic test

- Validity means the extent to which a test measures the true value of the biomarker
- Assessed by comparing the test results with those of the best available test or *gold standard*
- For categorical outcomes (e.g. malaria infection +/-) it is measured by sensitivity and specificity
- Sensitivity: proportion of true positives identified by the test
- Specificity: proportion of true negatives identified by the test

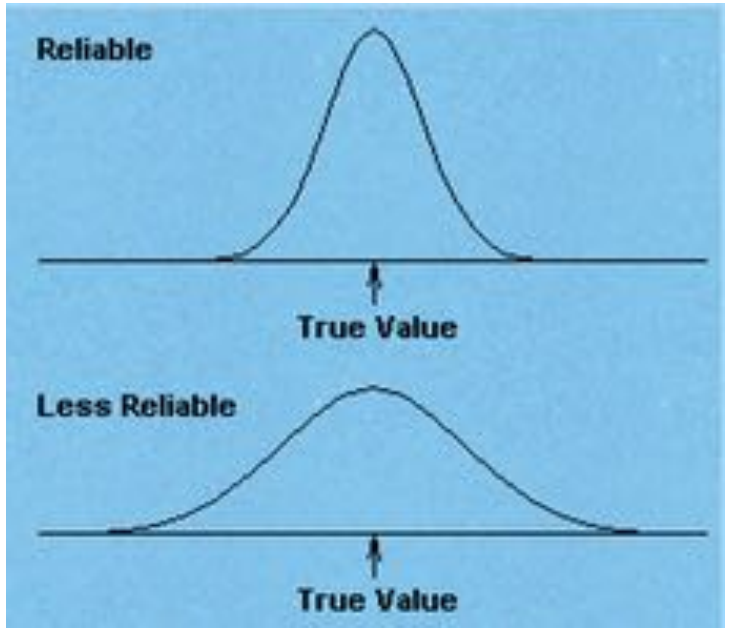


Distribution of test results is centered on the true value

Distribution of test results is NOT centered on the true value

Reliability of a diagnostic test

- Also called *repeatability*, means the extent to which a test will produce the same results if it is repeated
- Intra-observer reliability: if the same observer repeats the test on the same subject two or more times (test-retest), how close the results will be?
- Inter-observer reliability: if two or more different observers repeats the test on the same subject, how close the results will be?
- For categorical outcomes it is measured by the Kappa statistics: the proportion of agreement between tests results beyond that expected by chance

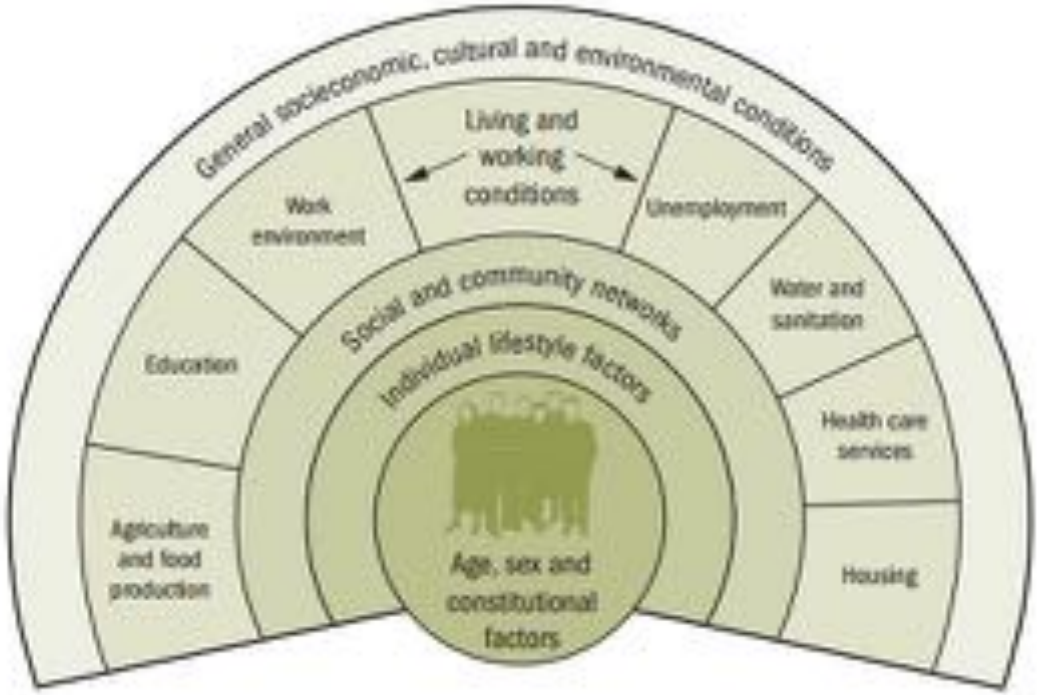


Distribution of test results shows LITTLE variation around the true value

Distribution of test results shows LARGE variation around the true value

Levels of health prevention and care
Determinants of health

- Determinants of health act at multiple levels of individuals and communities life
- Public health interventions aim to eliminate/reduce the causes, onset, complication or recurrence of disease, and also concern multiple levels



Public health is the "*art and science of health protection, prevention and promotion*" (Acheson, WHO, 1998)

Levels of health prevention and care

Health care services

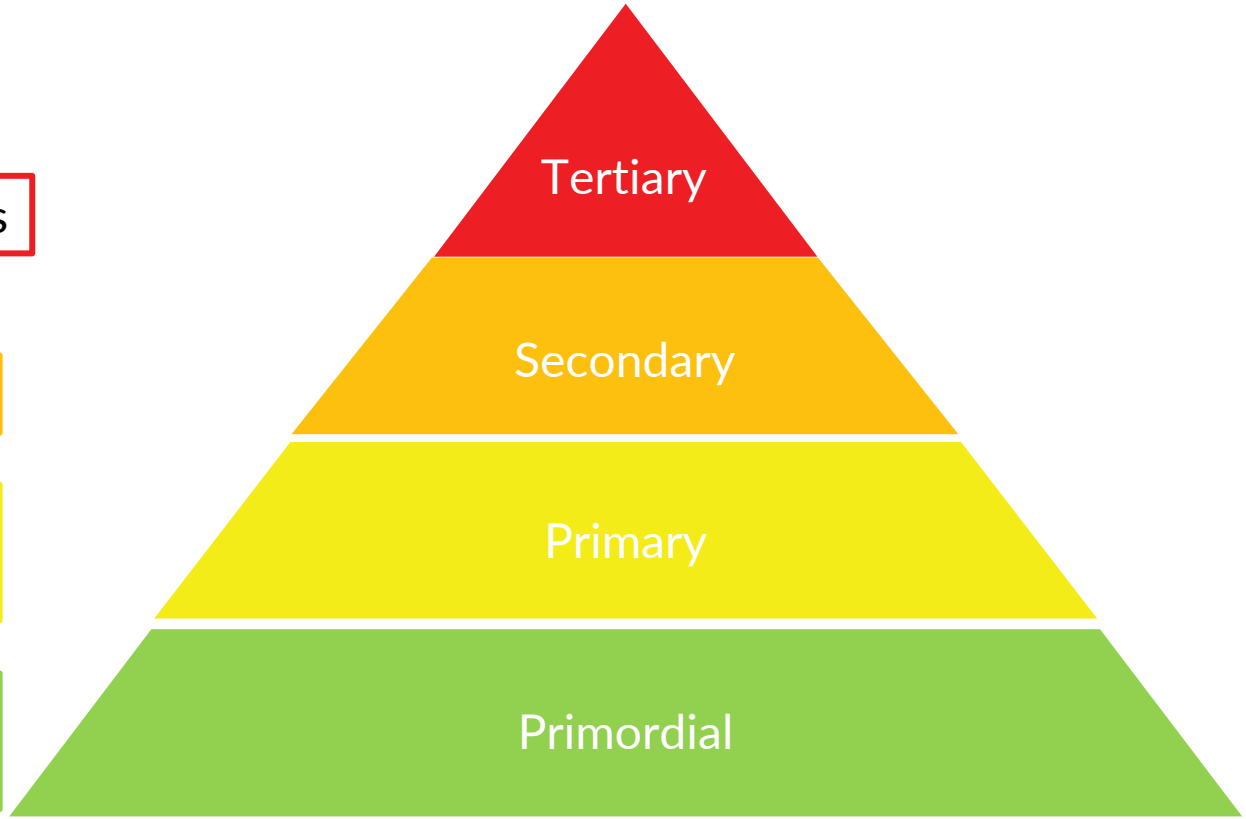
Population target

Individuals with illness

High risk groups

High risk groups
General population

General population
Society at large



Aim of intervention

Prevent disease burden
E.g. prognosis,specialist care

Prevent disease progression
E.g. early diagnosis&treatment

Prevent disease occurrence
E.g. screening, vaccination

Prevent causes of disease
E.g. environmental policies

Levels of health prevention and care

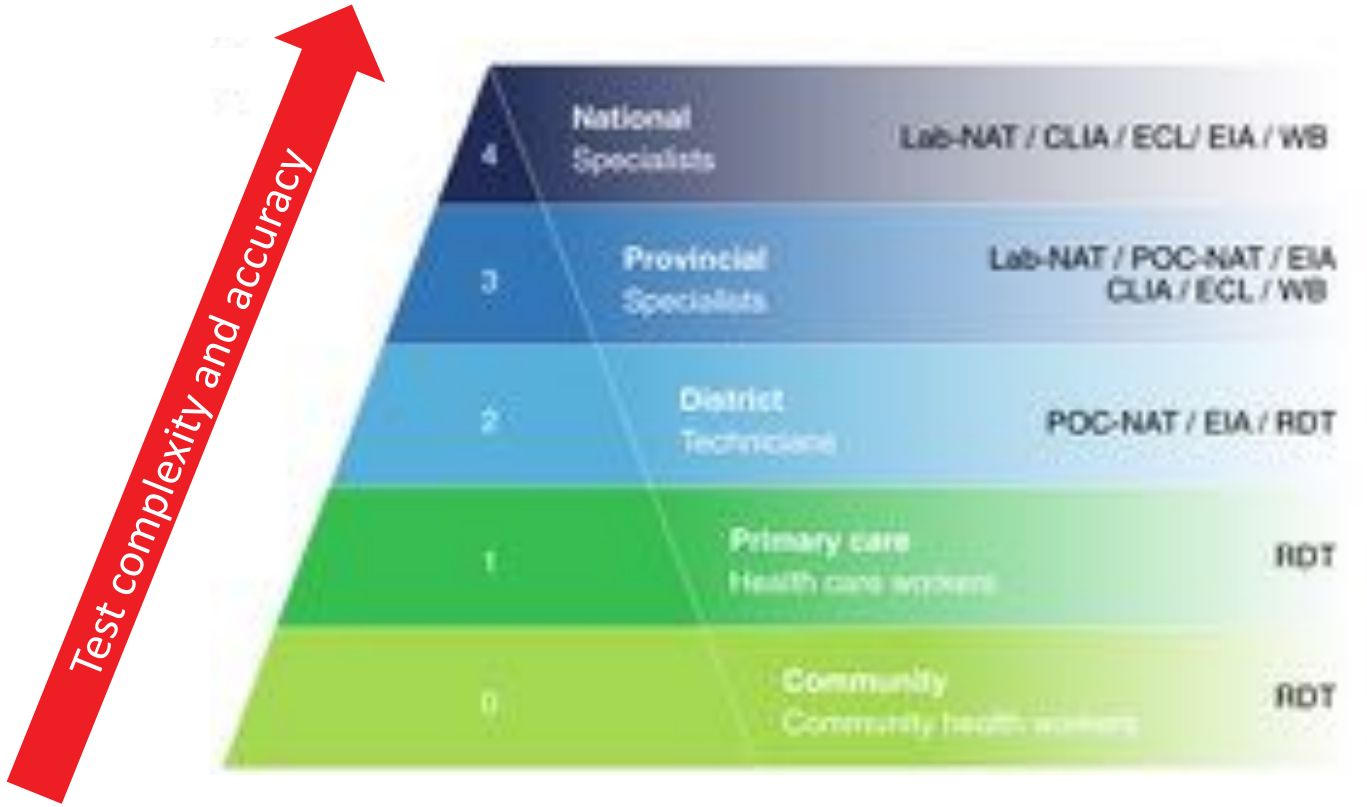
Example LMIC: Burkina Faso

Level of health care service	Distribution (2020)	Services
Community health workers	2-4 per village	Health education, Integrated Management of Childhood Ilnesses
Primary care facilities	2041 (1 every 1000 residents)	Immunisation, outpatient consultations, basic maternal and infant care, diagnosis and treatment of common and mild diseases, wound dressing, essential prophylactic drugs and nutritional supplements
District medical centres	71	Outpatient/inpatient consultation in general medicine, paediatrics, gynaecology-obstetrics and orthodontics, laboratory services, pharmacy and surgery
Regional hospitals	9	Specialist care in all branches of medicine and surgery
National hospitals	6	Surveillance and clinical research

Ministère de la Santée, Burkina Faso, Annuaire statistique 2020

Levels of health prevention and care

Laboratory services in LMIC

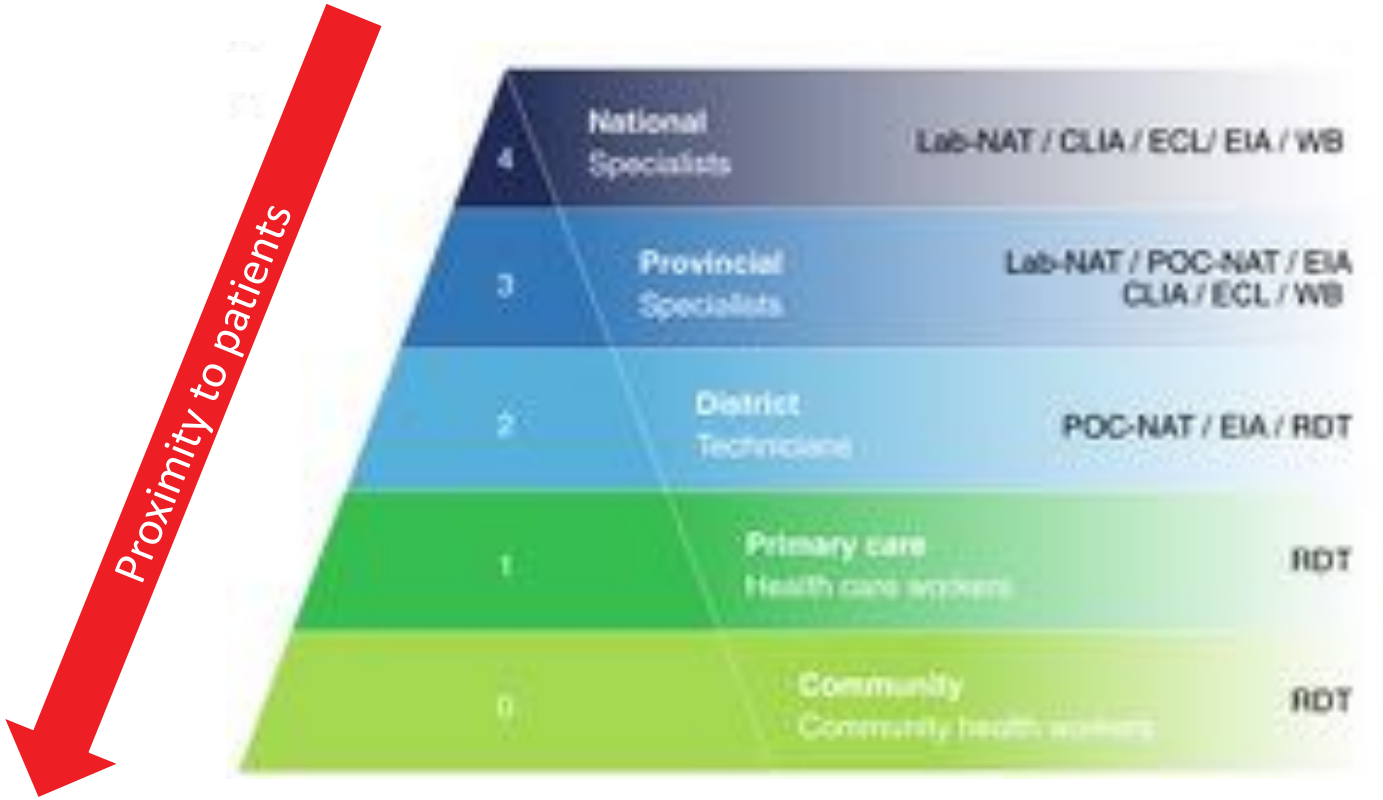


NAT: Nucleic Acid Test;
CLIA/ECL/EIA/WB: Immuno Assays
RDT: Rapid Diagnostic Test
Lab: laboratory
POC: point-of-care

Land et al. *Nature Microbiology* 2019

Levels of health prevention and care

Laboratory services in LMIC



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Levels of health prevention and care

Laboratory services in LMIC

Laboratory of Primary Health Care Centre in Mundri, South Sudan. The laboratory technician is reporting the result of a malaria Rapid Diagnostic Test.

DOCTORS WITH AFRICA CUAMM

The Republic of South Sudan

UNIVERSITÀ DI PISA

ITALIAN AGENCY FOR DEVELOPMENT COOPERATION

THE GLOBAL FUND

IVD for rural and remote areas

- In 2003 the WHO defined a set of criteria for the ideal IVD test that can be used in resource-limited settings to guide treatment and clinical management decisions

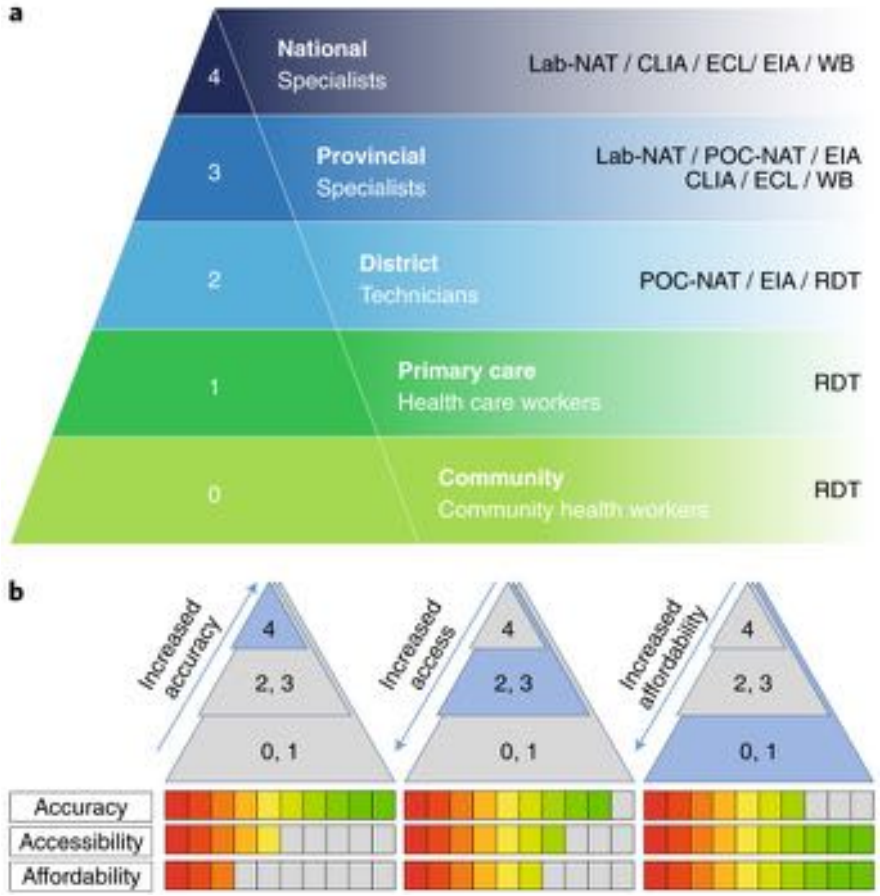
  ASSURED: Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, Deliverable to end-users

- New criteria have been proposed in 2019 by Land and colleagues

  RE-ASSURED: Real-time connectivity, Easy of specimen collection and environmental friendliness

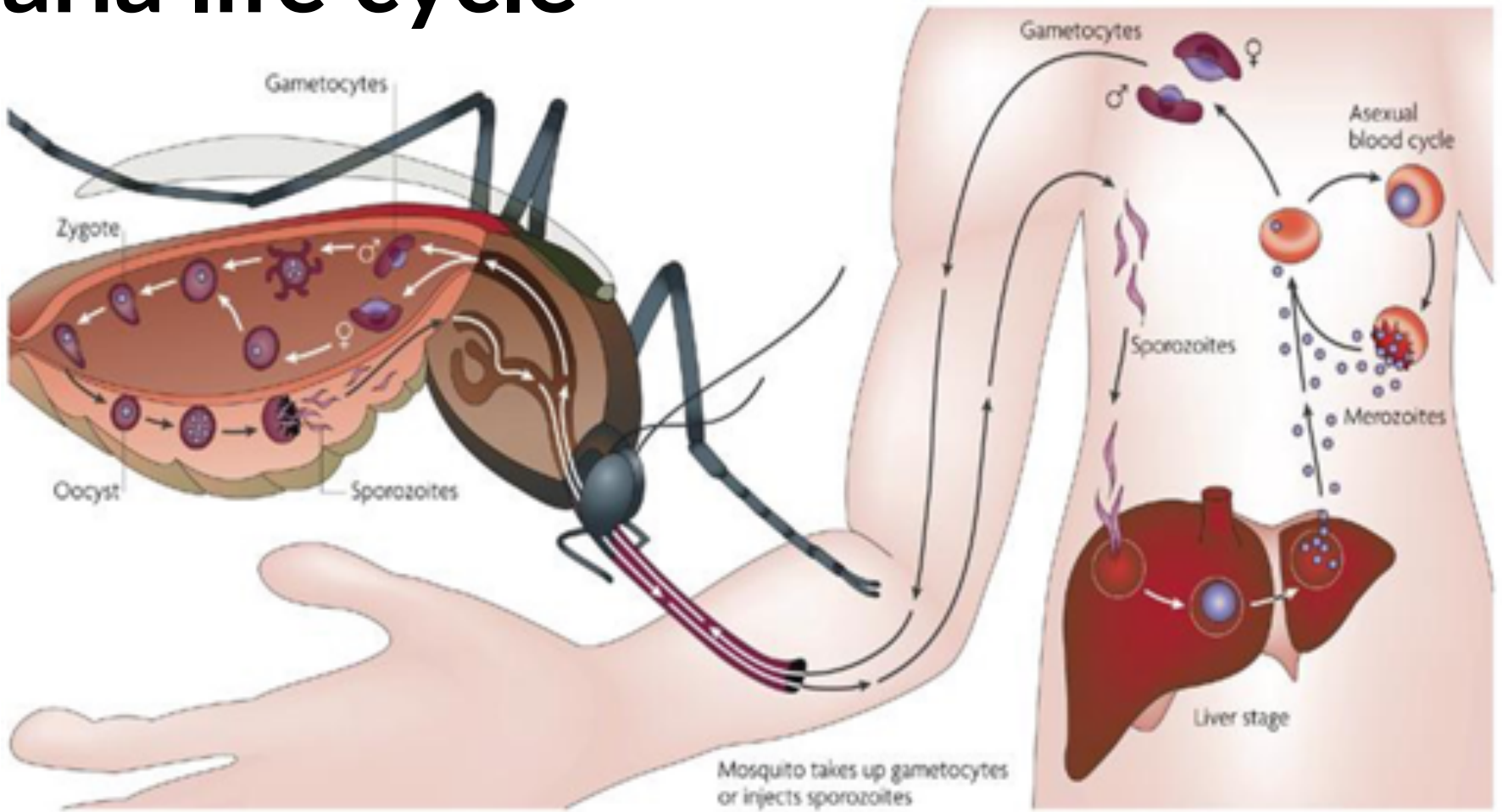
- A trade-off exists between accuracy, accessibility and affordability of tests employed at different levels of the laboratory system in LMIC

- Innovation is key for such trade-off to move towards better standards for the management of poverty related diseases at the Point-Of-Care (POC)



Land et al. *Nature Microbiology* 2019

Malaria life cycle



Parasite: *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, *P. knowlesi*
Vector: *Anopheles gambiae*, *An. funestus*, *An. arabiensis*

Malaria disease

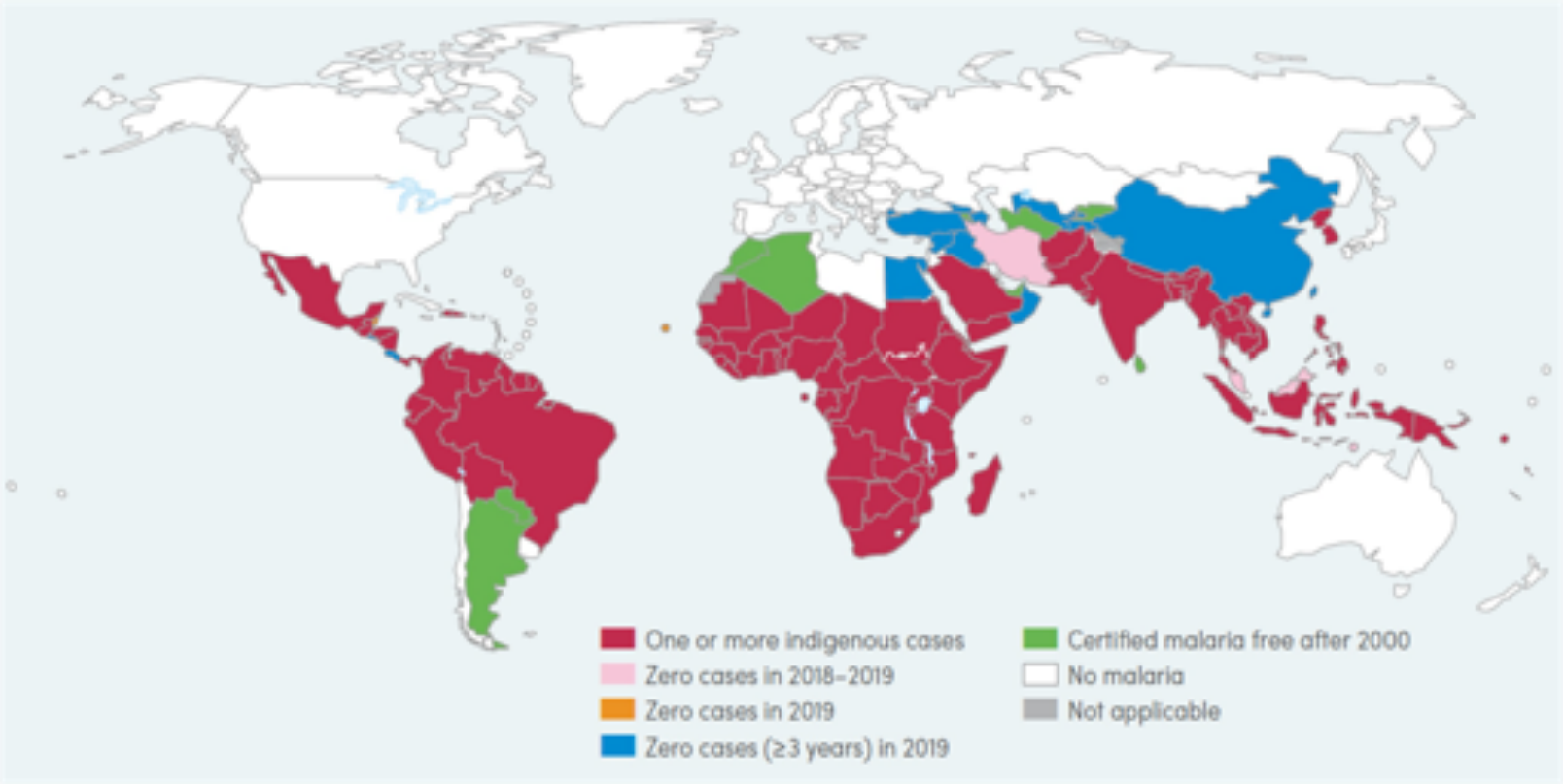
<u>Uncomplicated</u>

- All species
- Fever, chill, headache, exhaustion, arthromyalgia…

<u>Severe life-threatening disease</u>

- Mostly *P. falciparum*
- Children: severe anaemia, impaired consciousness and respiratory distress
- Pregnant women: abortion, premature birth, low birth weight, newborn mortality, maternal aneamia

The global health burden of malaria



WHO, *World Malaria Report 2021*

241 million clinical cases, 627000 deaths in 87 countries in 2020

Malaria control strategies and tools

Prevention

Insecticide Treated Nets

Chemoprevention

(Vaccination)

Diagnosis

Microscopy

Rapid antigen tests

(Molecular assays)

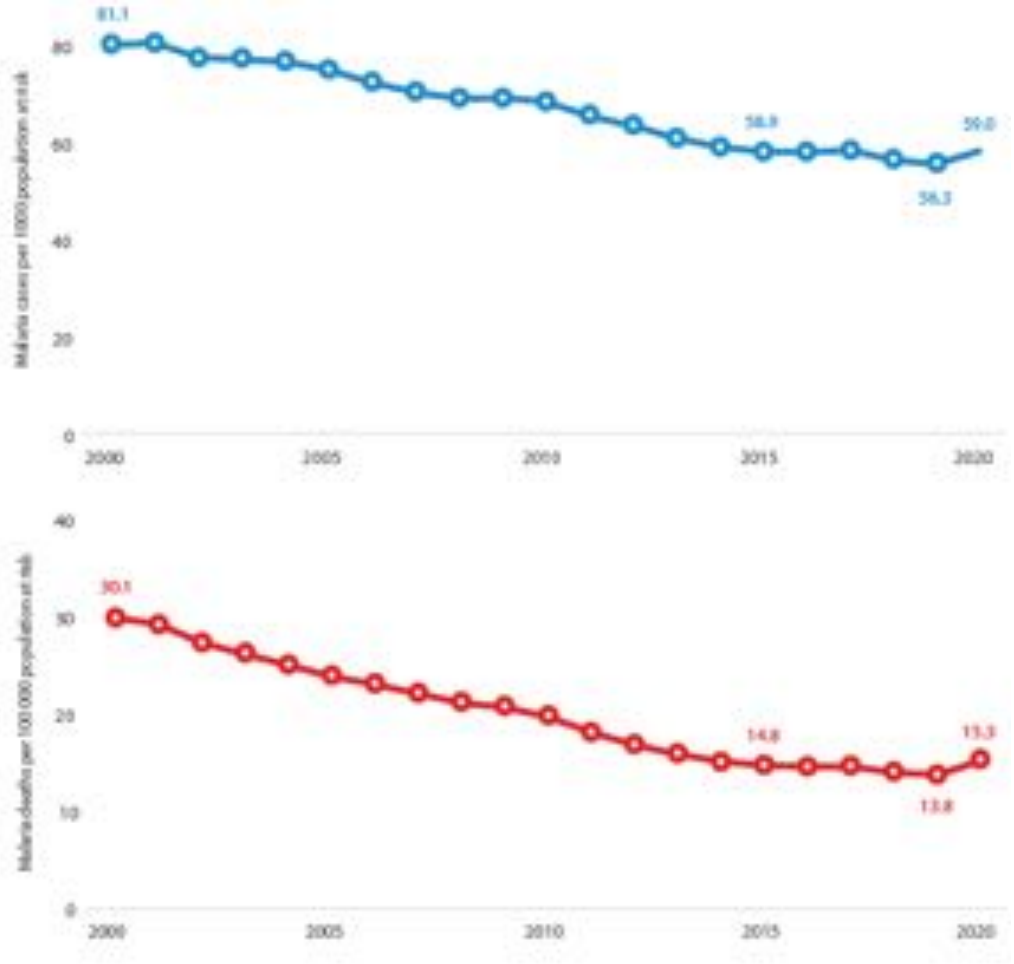
Treatment

Uncomplicated malaria

Severe malaria

(Reservoir)

Results of control



In the period 2000-2020:

- 1.7 billion clinical cases averted
- 10.6 million deaths averted
- Significant decrease in both incidence of clinical cases (27%) and mortality (51%) by 2015
- Declined has stalled in 2015-19
- Increase in 2020 compared to 2019 due to Covid19 partial disruption of malaria preventive and care services

WHO, *World Malaria Report 2021*

Current diagnostic tools

Based on detection of *Plasmodium* parasites or its molecules in peripheral blood (finger prick/venous sampling)

Microscopy

detection of parasites
species identification and parasite count
gold standard
LOD: 5-100 par/µl

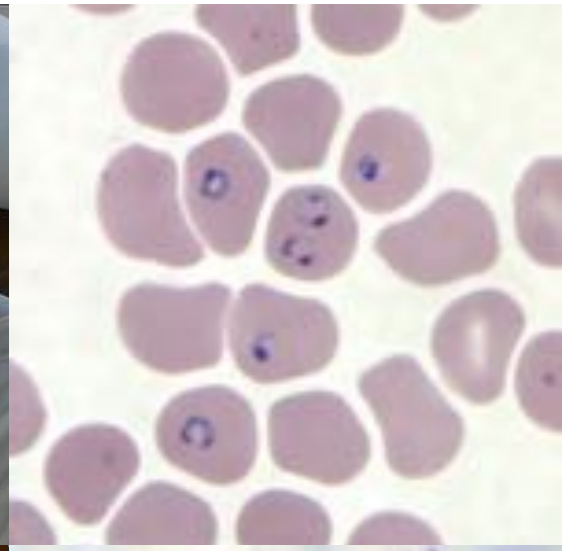
Rapid Diagnostic Test

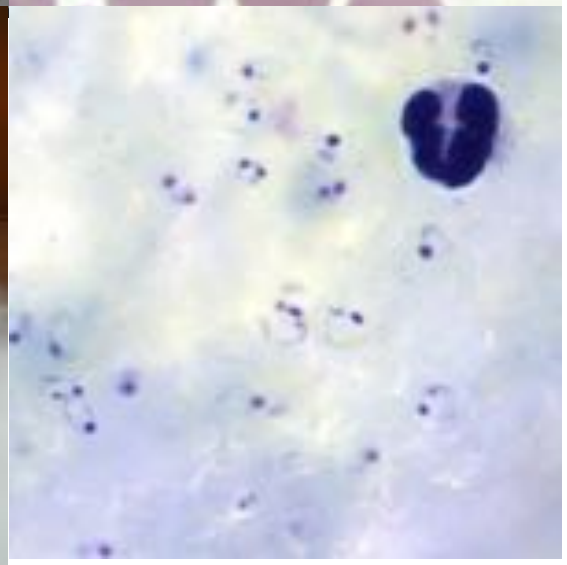
detection of parasite proteins
(Pf-HRP and Pan-LDH)
qualitative result
LOD: 100-200 par/µl

Molecular Assays

detection of parasite DNA
LAMP, PCR and Next Generation Sequencing
LOD: 0.01-5 par/µl

Current diagnostic tools





Microscopy

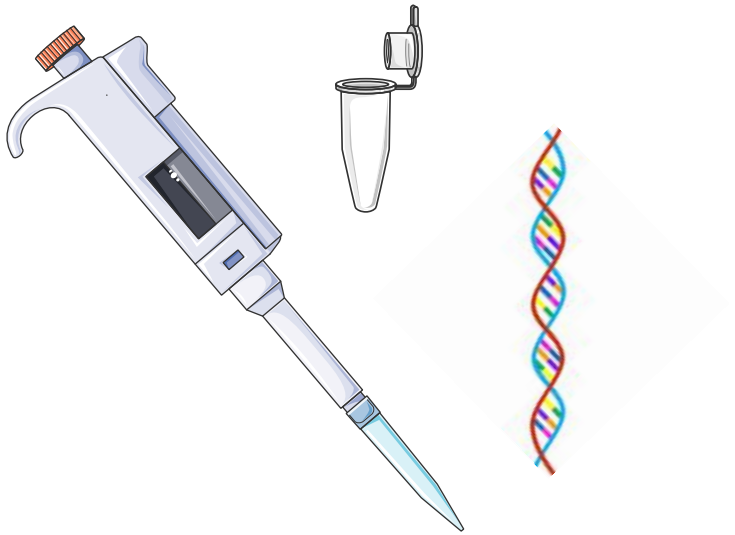
- *Gold standard*
- Detection of plasmodium parasites
- Allows species and stage identification, and quantification of parasites per µl of blood
- Preparation of thin and thick blood films (BF)
- Giemsa staining
- Optical microscopy examination (1000X in oil)
- Theoretical limit of detection (LOD) 4 par/µl
- Actual range 5-100 par/µl, depending on the operator
- Lenghty diagnosis (1-4 hours)
- Requires laboratory equipment and reagents, and specifically trained personnel

Current diagnostic tools

Malaria Rapid Diagnostic Tests (mRDTs)

- Detection of plasmodium proteins (Pan-LDH, Pf-HRP)
- Immunocromatographic lateral flow tests
- Equipment-free, user-friendly
- Rapid diagnosis (15-30 min)
- Recommended for diagnosis at community/primary health care level
- Optimal sensitivity for *Plasmodium falciparum* at parasite densities ≥100 par/µl
- Variable/suboptimal sensitivities for different species/lower parasite densities
- Sensitivity threatened by *P. falciparum* HRP-negative clones
- Recently, ultasensitive mRDT have been developed for PfHRP detection (2 par/µl)

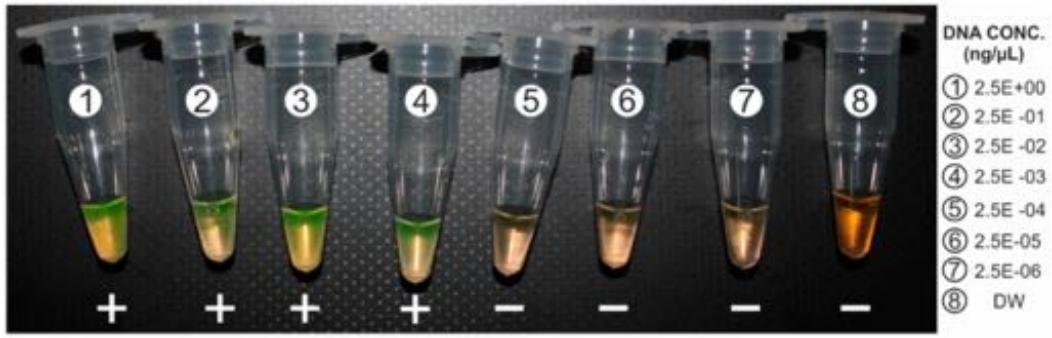
Current diagnostic tools



Molecular methods

- Detection of parasite DNA (RNA)
- Amplification of specific genome sequences
- Variety of methods based on PCR, LAMP, Next Generation Sequencing
- Lower limit of detection than both mRDT and microscopy, range 0.01-5 par/µl depending on the procedure
- Allow detection of low parasitemias
- Require laboratory equipment and reagents, and specifically trained personnel
- Costlier than both mRTD and microscopy

Current diagnostic tools



Loop-mediated AMPlification *vs* Polymerase Chain Reaction

- Use 2-3 primer pairs → greater specificity
- Isothermal reaction → does not require thermocycler
- Loop primers accelerate the reaction → 40 minutes
- Amplification leads to production of $Mg_2P_2O_7$ → thorbidity
- Detection via photometry or by naked eye when coupled with a dye (e.g. malachite green)
- Minimal pre-amplification steps (e.g. simplified DNA extraction)
- Cheaper, faster, easier
- Potential for adaptation to POC testing

Current diagnostic tools

Feature	Microscopy	mRDTs	qPCR	LAMP
Detection of	Parasites	Proteins	DNA	DNA
LOD (par/µl)	5-100	100-200	0.01-5	0.01-5
Species id	Yes	Pf/Pv/Pan	Yes	Pf/non-Pf
Stage id	Yes	No	No	No
Parasite count	Yes	No	Semiquantitative	No
Expertise	+++	+	+++	++
Equipment	Microscope	None	Real Time PCR	Isothermal block
Materials and reagents	Slides, ethanol, meethanol, giemsa, buffered water	ICT test	Primers and probes, Taq polymerase, dNTPs	Primers and probes, Bsm polymerase, dNTPs
Time to results	>3 hours	15 minutes	>3 hours	45 minutes
Cost	+	+	+++	++
Health care level in LMICs	Secondary and above	Community, primary	National, uncommon	National, uncommon

Diagnosis and disease management

- In malaria endemic countries fever (T>37.5°C) in children <5 years old can be associated with malaria, acute respiratory infections, gastroenteritis, tiphoid, urinary infection, occult bacteremia
- Diagnostic algorithms and tests must be employed to predict successful treatment, improve health outcomes, reduce the prevalence of severe disease and death and maintain drug effectiveness
- WHO/UNICEF recommends parasitological confirmation (BF/RDT) before malaria treatment and provide guidelines for managing fever at the different levels of health care

WHO Fever management in peripheral health care settings 2013

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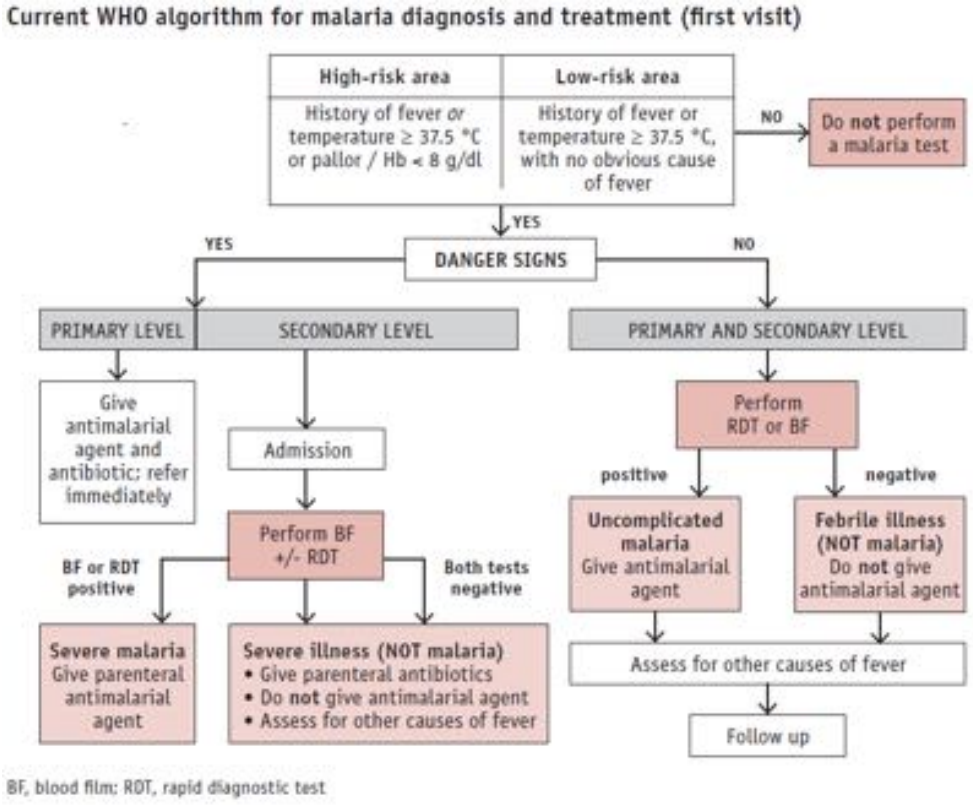
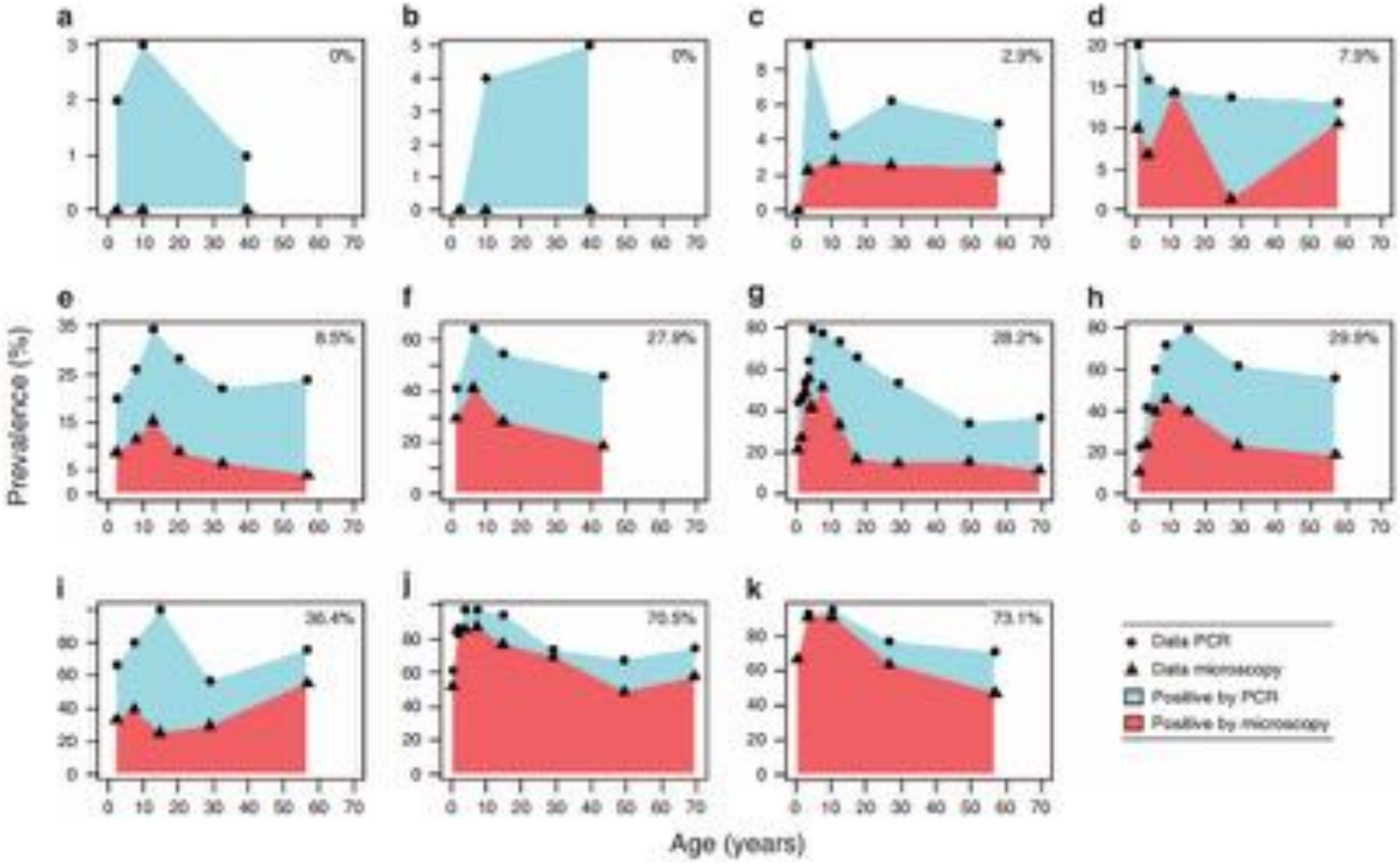


WHO Fever management in peripheral health care settings 2013

Diagnosis and disease control/elimination



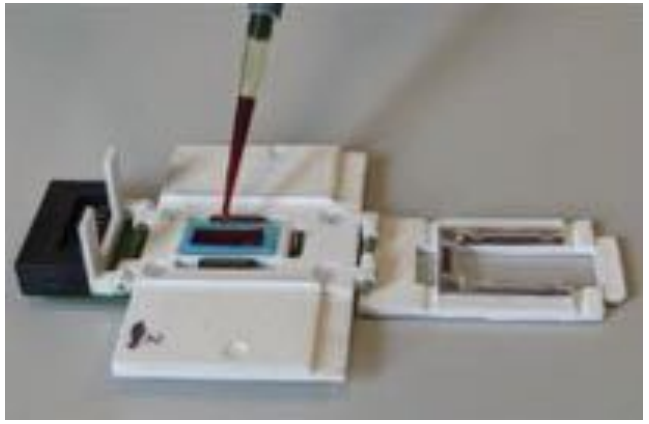
- The choice of the most appropriate IVD test depends the epidemiological context and public health priorities
- Frequency of infections with low parasite densities below the limit of detection of microscopy/RDT is greatest at lowest levels of malaria transmission
- Such infections do rarely cause severe disease but contribute to transmission

Okell et al. *Nature Communications* 2012

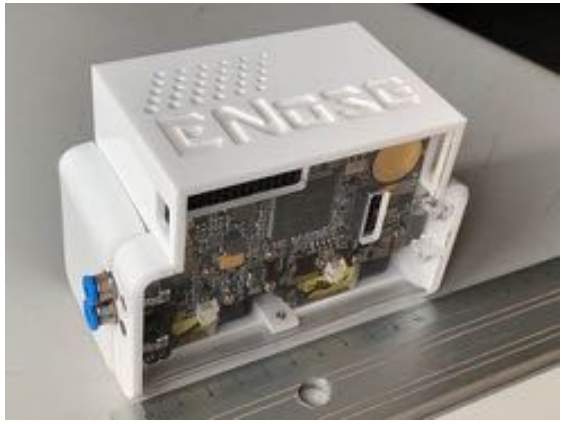
Development of new diagnostic tools

POC microscopy
detection of parasites

Tmek
detection of parasite haemozoin



electronic Nose
detection of Volatile Organic Compounds (VOCs)



Development of new diagnostic tools

Newton Nm1

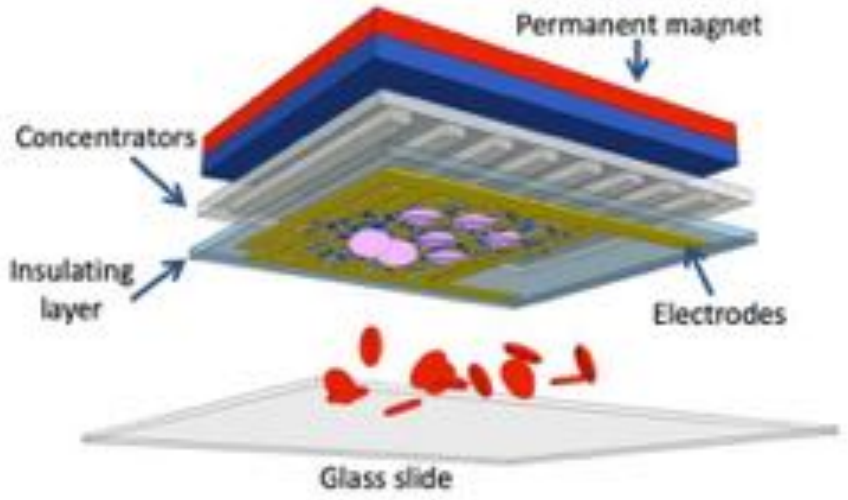
Cyscope

AIscope

POC microscopy

- Ongoing attempts at developing microscopes for use at the Point-Of-Care
- Battery-powered, portable, robust, affordable microscopes
- Netwon Nm1 (Cambridge Optics, United Kingdom)
  - Great stability and portability
- Cyscope (Partec, Germany)
  - Ready-to-use slides with dried-in fluorescent staining reagents
- AIscope (Open Source)
  - Neural Network recognition technology is employed to reach diagnosis
- Determination of LOD and accuracy in the field ongoing

Development of new diagnostic tools

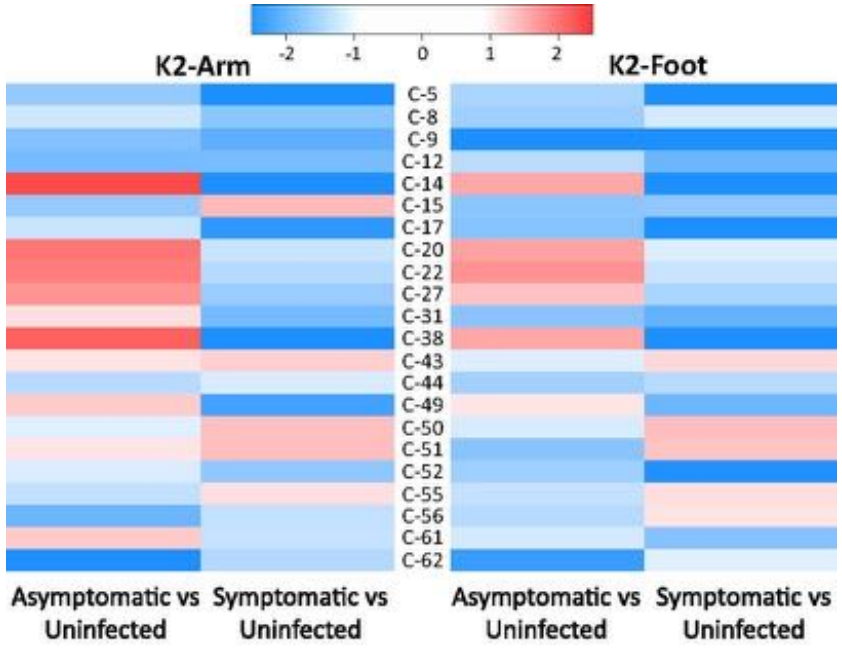


www.tmekdiagnostics.com

Tmek (Polytechnic University of Milan, Italy)

- Detection of haemozoin, a crystal with paramagnetic properties produced by the parasite as a result of haemoglobin digestion
- Magnetic separation of infected-RBC from healthy RBC
- Detection by sensing electrodes
- The signal is displayed on a notebook or smartphone connected to the diagnostic apparatus
- Quantitative result
- Short detection time (10 min)
- Determination of LOD and accuracy in the field ongoing

Development of new diagnostic tools



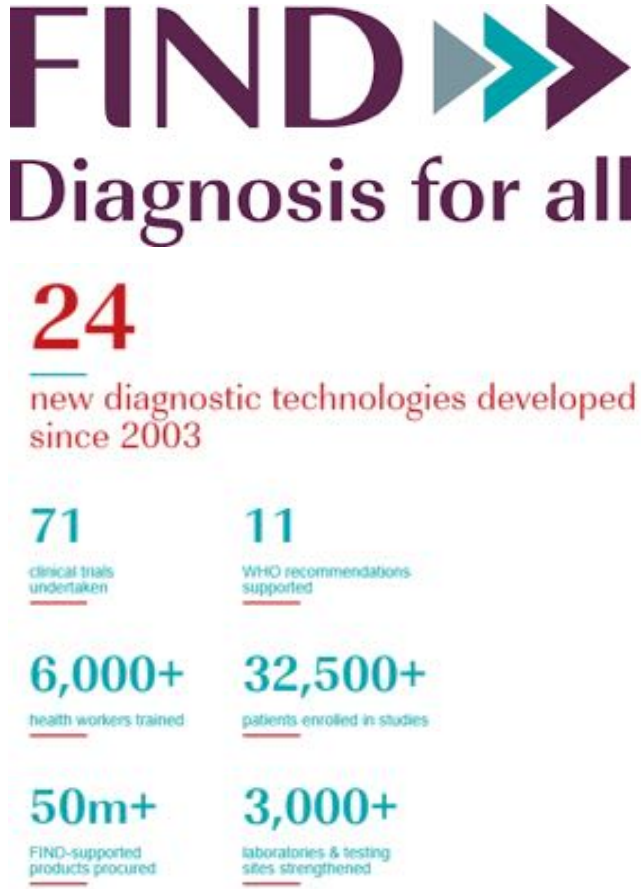
De Moraes et al *PNAS* 2018

eNose

- Detection of Volatile Organic Compounds (VOC)
- Gas sensor arrays
- Observation that malaria infected subjects are more attractive to mosquitoes (e.g. Lacroix et al. Plos Biol 2005)
- Some experimental evidence of emission of malaria-specific VOC profiles in RBC culture supernatant, mice and humans skin/breath (De Moraes et al *PNAS* 2014. Berna et al *J Inf Dis* 2015, De Moraes et al *PNAS* 2018)
- Non-invasive specimen collection
- Biological variation and lack of reference datasets challenge diagnostic accuracy

Product development partnerships

- Product development partnerships (PDP) include partners from Non Governamental Organizations (NGOs), academia, government, industry and funders, to combine the expertise and generate interest for the investment in R&D for poverty related diseases
- Foundation for Innovative New Diagnostics (FIND) is an example of PDP funded in 2003 with the mandate to boost innovation towards IVD for diseases and populations that would be otherwise neglected from the market, and to make IVD testing an integral part of sustainable and resilient health systems
- FIND promotes the development, evaluation and implementation of new diagnostic tools for malaria, HIV and TB, for Neglected Tropical Diseases and recently for Covid19



Target Product Profile

- Definition of a Target Product Profile (TPP) by expert is needed to inform the strategies for the development of new IVD tests
- TTP includes details on intended use, target population and IVD test features (performance and operational characteristics)
- ASSURED criteria are carefully considered when defining TTP for POC tests in LMIC
- Important tool to ensure that that R&D activities are focused on relevant products and designed for the contexts and needs of end-users

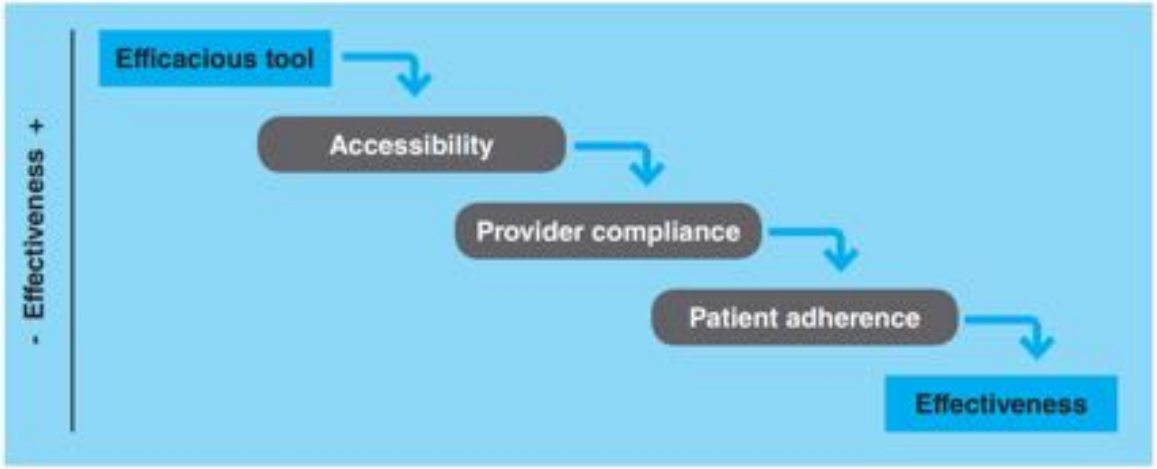
Target Product Profile for a Multiplex Multi-Analyte Febrile Illness Test for use on the MAPDx platform

	Characteristic	Minimum Requirement	Optimal Requirement
	Scope of the Platform		
1	**Intended Use**	In the context of infectious diseases, intended for individual patient management for patients presenting with symptoms consistent with severe febrile illness without a known source[1] to test for the presence of markers of current infection by target pathogens	
2	**Description of System**	The system will consist of an instrument[2] designed for use in combination with a self-contained, disposable assay cartridge(s)[3] containing all required reagents to execute a test from sample to result	
3	**Target Use Setting**	Level 2[4] Healthcare Facility (District Hospital or above) defined as having a functioning laboratory with trained personnel, water, electricity with intermittent surges and/or outages, limited climate control, dust, and medical staff onsite; The target use setting does not include mobile testing facilities	Level 1[4] Healthcare Facility with rudimentary staffed/equipped laboratory, inconsistent electricity, including frequent surges and/or outages, no climate control, dust, but trained medical staff on-site for result interpretation and patient management
4	**Target User**	Trained laboratory personnel (e.g., 1-2 year certificates)	Minimally skilled healthcare personnel (e.g. 3-6 months, able to operate an integrated test with minimal additional steps)
5	**Target population**	Adults to children > 6 months of age	Same, plus neonates (including prematures) up to 6 months of age
	Instrument		

https://www.finddx.org/tpps/

Implementation research

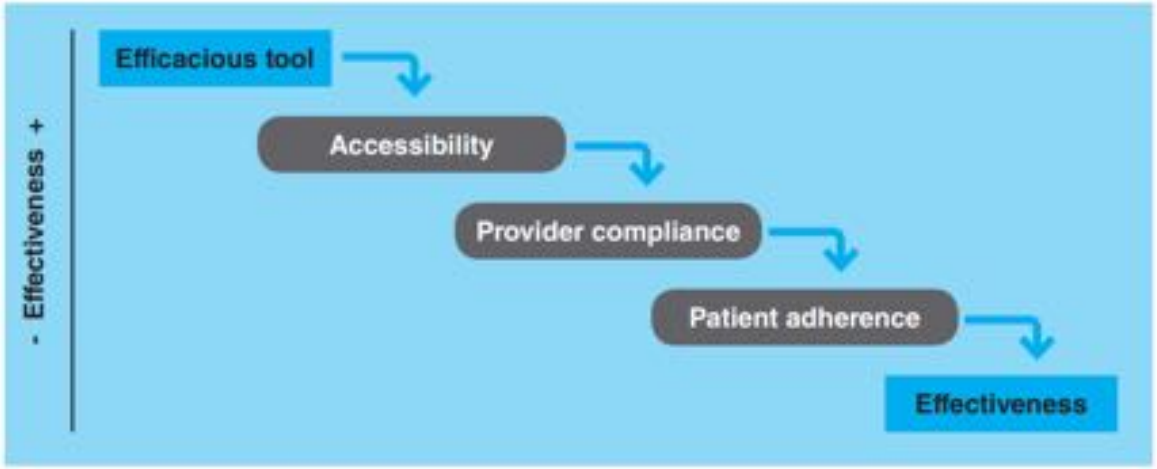
- Performance or effectiveness of an IVD test, i.e. capacity of prompting medical actions that result in individual and/or community benefit, is context dependent
- The predictive value of a test with given sensitivity and specificity varies with the disease prevalence in a geographical area, population or population group
  - The positive predictive value (PPV) of the test, which is the probability that a subject with a positive test result actually has the disease of interest, increases with the prevalence of the disease
  - Conversely the negative predictive (NPV) value of the test, which is the probability that a subject with a negative test result is actually disease-free, decreases with the prevalence of the disease



WHO implementation research toolkit, 2014

Implementation research

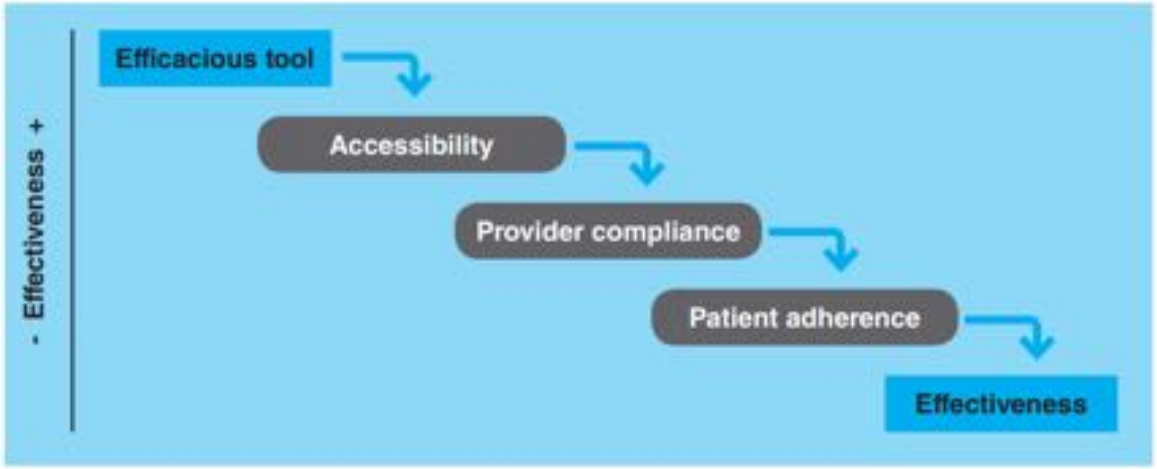
- The actual use of a test depends on its accessibility and acceptability, which are also context-dependent
- Accessibility to a test is a function of the features of the test (e.g. ASSURED criteria) and of the organization of health care/laboratory services
- Use of a test relies on its acceptability by both providers and users
  - Delivery by providers can be affected by workload, lack of incentive/motivation, easiness of the procedure
  - Uptake by users can be affected not only by the test intrinsic invasiveness but also on population attitudes, beliefs and behaviours, risk of marginalisation or lack of treatment



WHO implementation research toolkit, 2014

Implementation research

- Implementation research is fundamental to assess the performance of a test in real-life context, through large field-based interventional studies
- Indicators of performance are often clinical cases/deaths/DALYs averted
- DALY: Disability Adjusted Life Years
- At the same time such studies test strategies for increased feasibility and optimised performance
- It is also important to evaluate the cost-effectiveness of a new IVD compared to the existing one: Incremental Cost-Effectiveness Ratio (ICER)



WHO implementation research toolkit, 2014

Laboratory Quality Management

- The effective use of a diagnostic test depends on the quality management of the laboratory in which it is implemented
- Independently from the complexity of the service provided, every laboratory service should respond to a minimum standard of quality
- The laboratory quality management system (LQMS) is a comprehensive process that aims at the optimization of laboratory testing from sample collection to delivery of laboratory results
- Internal and external quality control
- Standard Operating Procedures (SOPs) and Bench Aids
- Staff training and supervision
- LMQS ensures both delivery of accurate test results and biosafety for laboratory workers and communities

WHO Laboratory Quality Management System Handbook 2011

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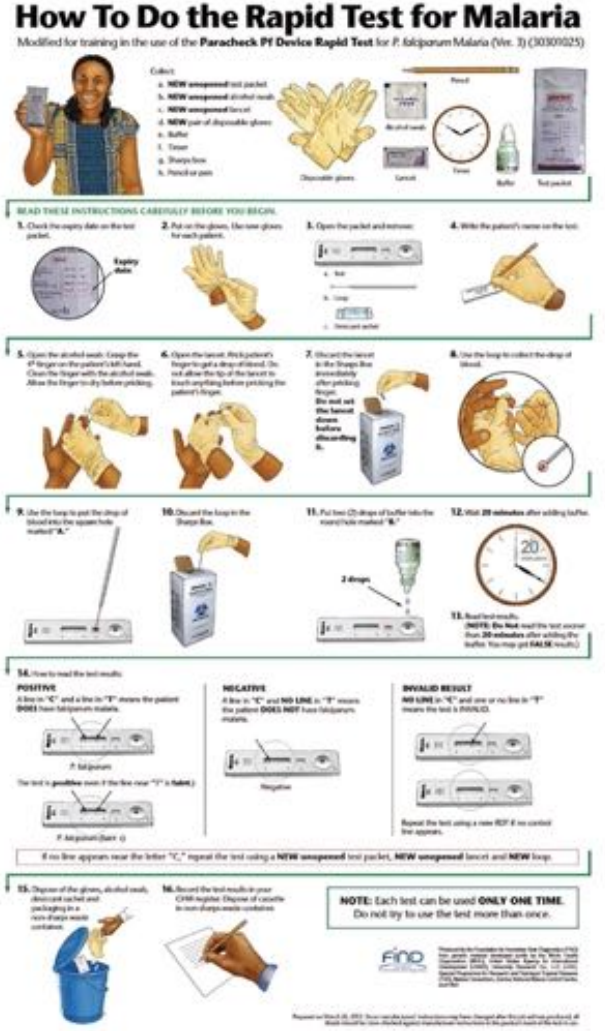

Acknowledgments

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Thank you very much for the attention

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