Point-of-care diagnostic tools
Selection, evaluation and implementation in resource-constrained settings
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Chapter 1

General introduction
Point-of-care diagnostic tools for use in resource-constrained settings

Diagnostics in the health care system

Diagnosis is defined as the process of determining the cause or condition which explains a person's signs and symptoms. During the diagnostic process, most commonly four types of information-gathering activities are carried out: taking the clinical history and interview, performing a physical exam, obtaining diagnostic testing, and sending the patient for referrals or consultations [1].

Acquiring a clinical history and interviewing a patient includes the documentation of the current concern, past medical history, family history, social history, and other relevant information, such as current medications and dietary supplements [1]. During the physical exam, the patient's demeanour, complexion, posture, level of distress, and other signs are observed. They may contribute to an understanding of the health problem [2], and can help a clinician refining next steps in the diagnostic process [3]. However, traditional “bedside evaluation” skills (history, interview, and physical exam) have received less attention in recent years, due to the increasing emphasis on diagnostic testing, and subsequent growth in this area of medicine [4, 5]. The increase in imaging and laboratory testing in particular has inverted the diagnostic paradigm and bedside evaluation is often bypassed for immediate testing [3].

Laboratory medicine and medical imaging have become a critical feature of standard medical practice over the past 100 years [6, 7]. Diagnostic testing can be divided into in vitro diagnostic (IVD) testing (e.g. clinical chemistry tests, pregnancy tests) and in vivo diagnostics (non-IVDs), such as blood-pressure and temperature measurements, as well as x-ray and ultrasound [8]. An IVD is defined as: a medical device, whether used either alone or in combination, intended by the manufacturer for the in vitro examination of specimens derived from the human body, solely or principally to provide information for diagnostic, monitoring or compatibility purposes [9, 10]. The general process of diagnostic laboratory testing can be described in nine steps: test selection and ordering, sample collection, patient identification, sample transportation, sample preparation, sample analysis, result reporting, result interpretation, and clinical action [11].

Diagnostic testing has become an indispensable tool in clinical practice for the diagnosis and monitoring of diseases, as well as in providing prognosis and predicting treatment response [12, 13]. Diagnostic testing is used alone or in combination with other disease state indicators as first-line clinical decision-making tools and at various stages of disease progression [8]. While diagnostics are most commonly considered to be tools for establishing a diagnosis, their application...
and contribution to quality care and their value across all phases of patient care are much more expansive and often under-recognized [8].

Globally, approximately 60-70% of health care decisions are influenced by diagnostics, but diagnostics constitute only 2-6% of health care spending [8, 14-16]. The global medical device industry has surpassed US$ 350 billion in annual revenue [17]. In 2011, the global market for diagnostics was approximately US$ 43 billion USD of which US$ 16 billion were spent in the US [18]. The clinical diagnostics market is expected to grow at 5-7% p.a. in the near-term with molecular diagnostics, next generation sequencing and point-of-care (POC) diagnostics being the three largest sectors for growth [19].

There are over 40,000 different IVD products available that provide information to doctors and patients on a huge range of conditions [20]. In the USA, the majority of diagnostic testing is conducted within hospitals, either within central laboratories that process many samples daily, or at other sites, particularly the patient’s bedside. Diagnostic testing in the hospital setting represents about 60% of the diagnostic industry’s revenue [21].

**Point-of-care diagnostics**

**Definition**

Point-of-care (POC) testing is defined as diagnostic testing near or at the point of patient care, in contrast to traditional laboratory based testing where collected patient samples must be sent away to a central site for further analysis. Traditional laboratory based testing involves waiting hours or days until results can be delivered to the treating physician. Simplification of technology allows for POC testing which can not only be performed closer to the patient, but is also easier to carry out and often requires only minimal training, e.g. urine dipsticks. In addition, POC testing is much faster and results can be available within in seconds to minutes for some technologies, e.g. glucometer. However, some more complex technologies, which have been simplified for use at POC, e.g. molecular testing such as the Xpert platform, still require a certain level of training and infrastructure and testing takes a few hours.

POC testing allows for optimised treatment decisions, reduced referrals, improved efficiency of care and decreased cost within primary care in western settings [22].
The POC diagnostic market

POC diagnostic tools have become increasingly available in the past 20-25 years. They were originally developed in western settings for glucose testing of diabetes patients and blood gas monitoring in intensive care units, where access to the diagnostic test near the patient or at the bedside could immediately influence patient management [23, 24].

POC testing is the fastest growing segment of the diagnostic industry and accounts for 25% of the total revenue of the laboratory industry [25-28]. The global market for point-of-care diagnostics was valued at US$13.8 billion in 2011, 15.4 billion in 2015, and is expected to increase to US$16.5 billion in 2016 [29, 30]. The five-year compound annual growth rate (CAGR) is forecasted at 3.7 percent.

The current market for POC diagnostics can be broken down into 10 segments: glucose monitoring, blood chemistry and electrolyte, pregnancy and fertility, cardiac markers, drugs and alcohol, infectious disease, cholesterol, haemoglobin/haemostasis, urine chemistry and tumour marker [30].

Blood glucose testing POC meters accounted for the largest share at over 40% in 2015. This segment is relatively more saturated in terms of market potential when compared to the other POC segments and thus is not likely to witness any sudden growth spurts [29]. Blood chemistry and electrolyte, the second-largest segment, totalled nearly US$2.3 billion in 2011, and after increasing at a CAGR of 4.8 percent, the segment is expected to reach nearly US$2.9 billion in 2016. The fastest-growing segment, cardiac markers, is projected to increase at a CAGR of 14.4 percent, rising from US$1 billion in 2011 to US$2 billion in 2016 [30]. The infectious disease and tumour marker segments have the next highest projected growth rates, with both expected to increase at CAGRs of more than 10 percent [30].

The USA represents the largest sector of POC testing (40-50% of the global market), followed by Europe (~25%) and Japan (~10%); emerging geographies (e.g., Brazil, India, China) are relatively small but growing fast [19]. The Asia Pacific region in particular is expected to be the most attractive regional market space, owing to the presence of high unmet medical needs and constantly improving healthcare infrastructure, growing medical awareness, and rise in per capita income levels in developing economies including India and China [29].

The wider availability of POC testing has revolutionized the continuum of the patient care process by providing laboratory results rapidly and efficiently at the patients’ bedside in hospitals, in physicians’ office laboratories, outpatient clinics, emergency rooms, and intensive-critical-care units. The trend toward greater POC testing is driven by the faster diagnostic benefits it provides [29].
Point-of-care diagnostics in low and middle income countries

Patients in low and middle income countries suffer from a wide range of diseases, including diseases that also occur in the developed world, but are in addition affected by a wider range of infectious diseases such as malaria, tuberculosis (TB) and other neglected tropical diseases, which kill over 10 million patients every year [31].

In resource-constrained settings (RCS), the impact of POC testing is potentially even greater, because the alternative to a POC test may be no diagnostic support at all, as laboratory infrastructure is often poor [32]. The unmet laboratory needs for assays to address communicable diseases such as HIV, TB and malaria appear to have assisted in catalysing the POC diagnostics industry as a whole [25]. However, some characteristics of POC tests for these markets are different than those for the developed world, such as the need for tests that do not require stable electricity, etc. The WHO has developed the ASSURED criteria as a benchmark to decide if a diagnostic test addresses the required needs in RCS: Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users [33].

In the early 1990s, the first POC tools for use in RCS became commercially available: lateral-flow immunoassays (LFIs), often called rapid diagnostic tests (RDTs), for the diagnosis of malaria [34-38]. Today the use of RDTs for malaria diagnosis is well-established and has replaced microscopy in many settings. Now more than 100 different malaria RDTs are commercially available, giving more choice, but also making it more difficult for the end-user to pick the ‘right’ one [39].

Besides malaria POC diagnostic tools, other infectious diseases such as HIV, Hepatitis B virus (HBV), Syphilis, Hepatitis C virus (HCV) and dengue, can be detected with POC tools. These tests also use the principle of either antibody- or antigen-detection.

Efforts have also been made to develop POC testing based on more sophisticated technologies. In HIV, limited access to CD4 cell counters for immune status monitoring, combined with high rates of loss-to-follow up, and poor treatment retention catalysed strong advocacy and development efforts for further growth in POC testing by the WHO, UNITAID, the Bill and Melinda Gates Foundation, the Clinton Foundation, PEPFAR and the African Society of Laboratory Medicine [25, 41]. This has led to the development of POC CD4 cell counters for monitoring the immune status in HIV patients and in succession also to the development of simplified assays for viral load (VL) testing [42]. Similarly, in the area of TB, the development of a simplified real-time polymerase chain reaction (PCR) analyser, i.e. GeneXpert (Cepheid, USA) has revolutionized TB diagnosis and resistance testing [43, 44].
Besides these successes in the development of POC diagnostic tools for RCS, the overall situation is worrisome. Although funding for research and development (R&D) of new diagnostics for the developing world has doubled from US$62 to US$118 million between 2007 and 2011 [45], less than 5% of annual spending on diagnostics R&D is allocated for neglected diseases [31].

Furthermore, there is a serious inequity in the allocation of diagnostics R&D funding, with two diseases, tuberculosis and HIV/AIDS, receiving by far the highest percentage of diagnostics R&D funding from every investment sector and almost every year to date [45].

For the most part, funding for neglected-diseases diagnostics relies heavily on the public sector: governments have provided half to two-thirds of diagnostic R&D funding. Philanthropic organisations have been the second largest overall contributors to diagnostic R&D over the past five years, with the Gates Foundation providing 97% of philanthropic funding. Industry has been a relative non-player in diagnostic development for neglected diseases, with reported industry funding ranging from a modest US$ 5 to US$ 8 million per year [45].

**Point-of-care technologies**

**Lateral-flow immunoassays**

LFIs or RDTs are immunochromatographic assays. They are simple to use and do not require any additional specialized or costly equipment. A common example for a LFI is a pregnancy test or malaria RDT. LFIs detect the presence (or absence) of a target analyte in a sample. Most commonly a sandwich assay principle is used. A LFI is built on a series of pads which can transport fluids. The first pad is the sample pad, which holds the sample containing the target analyte. From there the sample (and depending on the test also buffer), migrates to the second pad, the conjugate pad.

The conjugate pad contains antibodies against the target analyte which are conjugated to e.g. gold nanoparticles which are red in colour or latex particles which are often blue in colour. Alternatively, fluorescent or magnetic particles can also be used as conjugate but these require an electronic reader for the test interpretation.

The target analyte binds to the conjugate particles and migrates further on a nitrocellulose membrane. The sample-conjugate mix migrates further on this membrane until it reaches the ‘capture’ area.
The capture area usually contains at least two areas or lines and is visible as stripes or bands. The first line, the control line, captures any particle and indicates that the migration worked well. This line appears regardless of whether or not the target analyte is present. The second line contains a specific capture molecule and binds only to the target analyte-conjugate mix. At the end of the test is a last pad that simply collects excess fluid (Figure 1).

Most LFIs operate on a qualitative basis only. However, some tests LFIs have been developed to determine the target analyte also semi-quantitatively, such as the thyroid stimulating hormone (TSH) evaluated in this thesis.

For quantitative determination of the target analyte lateral flow readers have been developed to measure the intensity of the line as a proxy for its quantitative concentration.

**Figure 1. Lateral Flow Assay principle.**
(Source: http://www.cytodiagnostics.com/store/pc/catalog/lateral_flow_assay.jpg)
The main advantages of LFIs are that they are easy to perform and interpret, and that results are available within minutes. LFIs require only little or no sample preparation time. In addition, they do not require refrigeration which is of particular importance during transport and storage especially in RCS.

One of the disadvantages is however, that sensitivity is not sufficiently high for some disease diagnosis and that cross-reaction to similar targets has been reported with some analyte targets.

**Handheld and benchtop POC analysers**
Quantitative assays that are traditionally run in large analysers have been simplified to integrate the biological process, signal detection and display interface in a small device that can be used at bedside or near the patient. These include handheld and benchtop POC analysers for clinical chemistry, as well as CD4 count analysers. The Pima CD4 analyser (Alere, Germany) uses cartridges containing dried fluorescent labelled monoclonal antibodies against CD3 and CD4 which dissolve into the sample once the cartridge is filled with blood. Then, the marked CD3 and CD4 cells migrate along the detection channel for image capture. The cells that contain signals from both, CD3 and CD4, are automatically detected. These signals are then correlated against the volume of the detection channel to finally display the absolute CD4 count [45].

POC analysers for clinical chemistry analytes such as glucometers and creatinine meters use reagent strips. The analyte of the blood mixes with the reagent on the test strip that produces an electric current [46]. The amount of current produced correlates with the concentration of the target analyte in the blood e.g. creatinine.

**Simplified molecular amplification assays**
Several simplified molecular amplification platforms have been developed recently, that integrate sample purification, nucleic acid amplification and detection in a single-use disposable cartridge. These tests are considered as a POC or near-patient test because they do not need to be performed in a high-level molecular laboratory by highly skilled laboratory personnel, but and can be performed by minimally trained staff in decentralized health structures. However, these platforms still requires a minimal laboratory capacity.

The GeneXpert system (Xpert, Cepheid, USA) is the first, and currently the most commonly used example of a simplified molecular assay. The Xpert system can detect a number of targets such as *Mycobacterium tuberculosis* and resistance to rifampicin (MTB/RIF), which was examined in this thesis, but also H1N1, Ebola, HIV, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* amongst others [47].
Regulations of diagnostics

Regulations of in vitro diagnostic medical devices are intended to ensure quality and safety whilst allowing timely access to new products. Generally, a National Regulatory Authority (NRA) ensures the effectiveness of the regulatory system, including the impartial review of new products [48]. This is the case for most high-income countries but less so in developing countries [48].

EU regulatory environment / CE marking

In the European Union the In Vitro Diagnostic Medical Devices Directive (98/79/EC) (“the Directive”) was formally adopted in October 1998, became operational in June 2000 and was updated in 2011 [49, 50].

The Directive introduced common regulatory requirements dealing specifically with the safety, quality and performance of in vitro diagnostic medical devices, thereby aiming to bring them in line with other medical devices. This so-called CE marking (“Conformité Européenne”) is the manufacturer’s declaration that the IVD meets the requirements of the EC Directive. Prior to marketing an IVD, a manufacturer must fulfil four requirements:

1. Assign the IVD to a risk class
2. Ensure that the IVD meets essential requirements (annex I of the Directive)
3. Follow conformity assessment according to article 9 of the Directive
4. Depending on the IVD category, a Notified Body (NB) within the EU has to be involved in the conformity assessment. This may require documentation and auditing for conformance to the Quality Management System (QMS) certification (ISO: 13485) [51].

Risk classes for IVDs in order of increasing perceived risk according to the EU Directive are:

- General IVDs, i.e. all IVDs other than those covered by Annex II and IVDs for self-testing;
- IVDs for self-testing (a device intended by the manufacturer to be able to be used by lay persons in the home environment) excluding self-test devices covered in Annex II;
- IVDs in Annex II List B of the Directive which, amongst others, includes reagents and products for rubella, toxoplasmosis and phenylketonuria as well as devices for self-testing for blood sugar;
- IVDs in Annex II List A of the Directive which includes reagents and products for HIV, Hepatitis B, C and D, and for determining ABO systems and anti-kell.

Device approval in each European country is overseen by a governmental body called a Competent Authority [52]. For the group of ‘general IVDs’ the manufacturer self-declares conformity according to the provisions of Annex III of the Directive.
This includes ISO 13485 certification [51] and having a QMS in place to evaluate the IVD’s performance with appropriate procedures for corrective actions if required. No external review of the documentation or audit of the manufacturing facility is carried out routinely for this group.

Approval for more complex devices are directly handled by NBs, independent companies that specialize in evaluating products for CE mark and who are designated by Competent Authorities [52]. For approval by a NB, devices are subject to performance and reliability testing linked to the risks of their intended use [52].

Unfortunately, there are only few demands on the performance of diagnostic tests by the EU Directive, specifically for the group of general IVDs. In addition, the secrecy clause (Article 19) of the EU Directive even hampers that evidence regarding performance of the testing (or lack thereof) can be placed in the public domain.

**US Food and Drug Administration (FDA)**

In 1976 the FDA was granted authority to regulate medical devices including IVDs and to ascertain safety and effectiveness before marketing [53]. The FDA also uses a risk classification system, which ranges from class I to III [54].

- **Class I** IVDs are the class with lowest risk level and general controls of this class are considered sufficient to ensure safety and effectiveness [55].
- **Class II** IVDs, modest risk, have in addition to general controls further special controls, which are required to provide safety and effectiveness assurance. These include performance standards, post-market surveillance, patient registries, special labelling requirements, and premarket data requirements, i.e. premarket notification, also called 510(k), amongst others.
- If a manufacturer can show that its device is ‘substantially equivalent’ to an already cleared device, additional clinical data are usually not required, although requirements for performance standards and post-marketing surveillance may be imposed [52]. A class II IVD can only be cleared by the FDA but not be approved.
- **Class III** IVDs, high risk, are those to be used to sustain human life or prevent impairment of human health. For these, general and special controls are not considered sufficient. Bench testing and clinical data must be provided to ensure safety and effectiveness in the pre-market approval (PMA) process. The end result is that the device is approved by the FDA.

The US and EU systems for regulations of medical devices have been criticised widely [56-59]. The FDA has been criticised for their cumbersome requirements, delay in approval and quality problems in pre-market submissions, and for being expensive [52, 57, 60, 61]. It has also been mentioned that premarketing procedures may not be comprehensive enough and may be particularly dangerous for devices.
that have already been cleared by the FDA on the basis of substantial similarity to an already marketed device [62].

The EU system has been criticised for failing to gather meaningful data and a lack of transparency upon which approval is determined and has been in the spotlight for conflicts of interest in its evaluation process and general regulatory failure [59, 60, 63].

**WHO Pre-Qualification of In Vitro Diagnostics**

The WHO includes a regulatory system functioning in one of the main core building blocks of health systems: access to medical products, vaccines, and technologies of assured quality, safety, and efficacy [64]. In order to enforce access to quality assured IVDs, the WHO established the WHO Pre-Qualification (WHO-PQ) programme for IVDs in 2010.

The WHO-PQ programme considers only products with high individual or public health risk to be eligible for WHO-PQ; these products would be class C or D according to the classification scheme of the Global Harmonization Task Force (GHTF) – see below [65].

WHO-PQ programme focuses on sustainability of IVDs for resource-constrained settings with priority on IVDs for HIV/AIDS, malaria and hepatitis B and C. Recently, the WHO-PQ portfolio expanded to other areas as well: glucose-6-phosphate dehydrogenase (G6PD) deficiency, screening tests for human papillomavirus (HPV), and emergency assessments in outbreak situations such as Ebola and Zika, amongst others (http://www.who.int/diagnostics_laboratory/evaluations/en/).

The WHO-PQ process includes three main components:
- Review of the application and dossier
- Laboratory evaluation of the product
- Inspection of the manufacturing site.

**Global Harmonization Task Force (GHTF) and International Medical Device Regulators Forum (IMDRF)**

The GHTF was founded in 1992 with members from the USA, Canada, Japan and Australia and the EU in order to respond to the growing need for international harmonization in the regulation of medical devices. The GHTF developed a risk classification scheme (Class A-D) considering personal and public health risks [9, 10]. For class A, manufacturers are required to provide less substantial submission of dossiers whereas class D requires stringent conformity assessments, including performance in a clinical setting that is representative of the intended use [9, 10].
The International Medical Device Regulators Forum (IMDRF) was conceived in February 2011 and continued the work of the disbanded GHTF. IMDRF also includes members from China, Russia and Brazil. The WHO and the Life Science Innovation Forum of the Asia-Pacific Economic Community are official observers. The Asian Harmonization Working Party (AHWP) and the Pan American Health Organization (PAHO) are affiliate organizations.

**Situation in low- and middle-income countries**

A number of harmonisation efforts for regulation of medical devices have also been made in low- and middle-income countries. Similar to the GHTF, several groups were formed to harmonise regulation of medical devices in different regions, such as the Asian Harmonization Party (AHWP), the pan-African Harmonization Working Party (PAHWP) or the Latin American IVD Association (ALADDIV).

However, these groups only give recommendations. Ultimately, countries have their own unique set of requirements [48] and often access to good quality devices is not guaranteed [66]. Most countries in sub-Saharan Africa lack the means to ensure appropriate regulatory control [67, 68]. The consequences are use of poor quality products, and the presence of falsified and mislabelled products [69]. The lack of regulatory authorities and quality assurance in laboratories in RCS makes it difficult to trace quality problems with IVDs. On the other hand where controls occur, they can act as a barrier to products entering the country [68].

Some countries, such as South Africa, Brazil, Argentina and others, have started to request evidence of EU (CE mark) or USA (FDA compliance) [70] quality labels of imported products, in the absence of national requirements [71]. However, the significance of the CE mark and FDA compliance is limited for some IVDs, particularly those that are considered as low risk [69].
Rationale of this thesis

Access to diagnostics and treatment for HIV, TB and malaria in low-income countries for the public sector, is provided by national Ministries of Health who procure most of their diagnostics and drugs through the Global Fund [72]. In order to be eligible for procurement through the Global Fund to fight AIDS, TB and Malaria (GFATM), a diagnostic device or test must be pre-qualified by the WHO or be on the list of diagnostics that has been approved by an Expert Review Panel (ERPD) [73]. This mechanism ensures that only good quality and safe diagnostic products are used.

However, when it comes to selecting diagnostic tools for use in RCS also other factors must also be taken into consideration. The performance evaluations carried out by the WHO-PQ programme are performed under ideal conditions with well-trained staff at reference laboratories, and reflect the maximum possible accuracy of a test. Evaluations at reference level can only be a first step when it comes to assessing and selecting a diagnostic tool for use. It is also of importance to generate evidence of the accuracy of a POC test at the location of its end-use, as a number of factors impact on the ultimate performance of the POC test. On-site evaluations examine the diagnostic accuracy of a product under real-life conditions putting more emphasis on ease-of-use, implications of transport and storage, geographical variations of the pathogen, presence of local co-infections which may cause cross-reactivity etc. This knowledge is important for countries and policy makers to make rational choices, but also for manufacturers to improve their products. Furthermore, other factors, such as the shelf-life of the product, lead-time for deliveries, cost, and additional materials required but not provided with the test kit etc. also influence procurement decisions.

Guidance on the selection, evaluation and implementation of IVDs for use in RCS is scarce. The policy makers are left carrying out complex market reviews, searching peer-reviewed literature on previous evaluations of diagnostic products, or looking for guidance from the WHO Often in the end leaving more questions than answers, the selection of safe and efficient diagnostic products is a cumbersome process, due to limited information being available.
Outline of the thesis

The main objective of this research was the selection, evaluation and implementation monitoring of POC diagnostic tools for use in RCS.

Chapter 2 gives an overview of commonly used diagnostic tools in resource-constrained settings, and outlines challenges and gaps in the diagnosis of common infectious diseases, and the monitoring of side-effects of treatments.

On the basis of the main objective stated above, several research questions were developed for various diagnostic areas:

Malaria diagnosis

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes [74]. Almost half the world’s population, about 3.2 billion people, are at risk of malaria [74]. Malaria is most commonly diagnosed by microscopy or the use of malaria RDTs. In the early 1990s the first malaria RDTs emerged on the market [34-38]. Today more than 100 different malaria RDTs are commercially available. Since 2008, the WHO/FIND/CDC/TDR have carried out yearly evaluations of malaria RDTs [75]. In the last round of evaluations in 2015 (Round 6), 41 products from 22 manufacturers were evaluated [39]. These comprehensive evaluations were carried out at the CDC on cultured and wild-type preserved specimens. The WHO uses these evaluations together with a dossier review and an inspection of the production site to pre-qualify malaria tests [65]. Although pre-qualification is currently not a requirement for WHO procurement [39], it will be starting at the end of 2017 [76]. The evaluations and systems described above provide an excellent overview and information on malaria RDTs.

The SD Bioline test (05FK60) is one of best performing malaria RDTs according to the WHO/FIND/CDC product testing evaluations that can differentiate between P. falciparum and non-falciparum infections, in order to a) diagnose a malaria infection and b) streamline treatment decision. The WHO/FIND/CDC product testing evaluations are conducted under ideal conditions and reflect the maximum possible accuracy of an RDT; as such they do not replace on-site evaluations. As a consequence, clinical staffs in the field often raise concerns about the quality of RDT in their setting. To assess the on-site performance of one of the most commonly used malaria RDTs globally, the following research question was formed:
Chapter 3 describes the parallel testing of RDT with malaria microscopy at two clinics in Rakhine state, Myanmar, for a period of 14 months, as a programmatic response to doubts and concerns of medical and paramedical staff on malaria RDTs.

**HIV diagnosis, monitoring of disease progression and treatment side-effects**

Approximately, 36.7 (34.0–39.8) million people were living with HIV at the end of 2015 with 2.1 (1.8–2.4) million people who became newly infected in 2015 globally [77].

**HIV diagnosis**

HIV infection is often diagnosed through RDTs, which detect the presence or absence of HIV antibodies. Most often these tests provide same day test results; essential for same day diagnosis and early treatment and care [77]. The diagnostic accuracy, i.e. sensitivity and specificity, of HIV RDTs is of utmost importance. The WHO currently recommends the use of 2-3 HIV tests in high prevalence settings (≥5%) in serial order to diagnose HIV, and 3 HIV tests in low prevalence settings (<5%) as a baseline strategy [78].

Similarly to malaria RDTs, the WHO examines the operational characteristics and performance of HIV RDTs under ideal conditions [79-81] and uses these results together with inspection results to pre-qualify HIV RDTs for use.

It has been suggested that the accuracy of HIV RDTs differs by geographical location and studies have postulated differing reasons for such discrepancies [82-88]. Therefore it has been recommended to check the diagnostic accuracy in target populations and develop site specific diagnostic algorithms [78]. However, these time-consuming and costly, and are often not carried out on a regular basis, if at all. Therefore the following research questions were formed:

1.a) Is the performance of the HRP-II/pan-pLDH combo test from SD Bioline (05FK60) at project site compared to microscopy similar to performance in ideal conditions reported in the WHO/FIND assessment?

2.a) How accurate (i.e. sensitivity, specificity and predictive values) are the current HIV diagnostic algorithms at HIV testing and counselling centres?

2.b) How accurately do the most commonly used HIV RDTs and 2 confirmatory tests perform with samples from 6 locations in sub-Saharan Africa?

2.c) Can more accurate diagnostic algorithms be identified for the 6 locations based on findings from research questions 2a & 2b?
Question 2a was answered by assessing the diagnostic accuracy of routinely used RDT-based HIV diagnostic algorithms at 6 HIV testing and counselling (HTC) sites in 5 sub-Saharan African countries, by comparing the HIV status given to clients at local level to the HIV status determination at an AIDS reference laboratory using the state of the art gold standard for HIV diagnosis. The findings are reported in Chapter 7.

To answer research question 2b, a head-to-head evaluation of 8 HIV RDTs and 2 simple confirmatory assays was made. Specimens were collected at these 6 HTC centres over a period of 3 and a half years. The standardized, centralised evaluation of RDTs was conducted at a WHO collaborating centre and internationally recognized AIDS reference laboratory. The results are described in Chapter 8.

To answer question 2c, the results of the evaluation of individual HIV RDTs (chapter 8) were used to simulate HIV diagnostic algorithms combining several RDT, in order to determine if other combinations of RDTs could improve the diagnostic accuracy for HIV diagnosis at the 6 HTC sites. The results are presented in Chapter 9.

**HIV disease progression monitoring**

A biological parameter to assess anti-retroviral treatment (ART) eligibility is the CD4+ T-lymphocytes count. CD4+ counts are also frequently used for ART monitoring, especially when there is no access to viral load testing [40]. Since 2013, the WHO recommends that ART is provided to all individuals with confirmed HIV infection who have CD4 cell counts ≤500 cells/μL and that individuals with CD4 counts ≤350 cells/μL should be given priority to initiate treatment [89]. The introduction of a POC CD4 test is an important tool to improve patient retention and shorten time to initiation on anti-retroviral therapy (ART) [90]. One of the most widely used POC CD4+ analysers, the Alere Pima, was commercially launched in 2010 and has been WHO prequalified [91]. However, end-users have reported a high number of errors and rejected analyses by the Pima CD4 analyser. Therefore, the following research question was formulated:

> 2.d) What are the causes for the high frequency of invalid CD4 results using the Alere Pima CD4 test at project sites?

In order to answer question 2d, an analysis of errors generated by the Pima CD4 POC analyser was carried out with data collected over a 30 months period from 39 sites. The sites represented laboratories, primary health-care clinics and mobile clinics in communities, and users included laboratory technicians, clinicians and lay workers. Findings are reported in Chapter 10.
General introduction

**Treatment side effect monitoring of anti-retroviral drugs**

Creatinine is a parameter that is required to monitor renal function and is important to analyse in patients under treatment with potentially nephrotoxic drugs, such as the anti-HIV drug Tenofovir [92-95]. HIV programmes are often located in decentralized settings where no laboratory is available to carry out blood analysis. The use of potentially nephrotoxic drugs in these settings leads to the demand of POC analysers for appropriate patient management in RCS. The following research question was formulated after a market review of creatinine POC analysers:

2.e) Is the StatSensor Xpress Creatinine analyser’s (Nova Biomedical Cooperation, Waltham, MA, USA) accuracy and precision sufficient for use as a POC analyser?

To address research question 2e, an evaluation of the diagnostic accuracy and precision of the handheld StatSensor Xpress Creatinine analyser was carried out, to assess its potential use in RCS for monitoring kidney function. Measurements of the StatSensor Xpress were compared to a commonly used clinical chemistry analyser (Vitros 5,1FS, Ortho Clinical Diagnostics, Inc, Rochester, USA). Results are documented in Chapter 11.

**Hepatitis C screening**

Approximately 130-150 million people are infected with the Hepatitis C virus (HCV) worldwide, of which most live in Central and East Asia and North Africa [96].

Screening of potential blood donors is an important indication for HCV testing [97, 98]. This is especially true in RCSs as the highest prevalence of transfusion-transmitted infections is found in low-income countries [99]. Worldwide 25 countries are not able to screen all donated blood for one or more infections and only 16% of blood transfusion services are monitored by external quality assessment schemes in low-income countries [99]. In addition, all HIV-positive patients should be assessed for HCV co-infection in order to adjust treatment choice if needed [98].

HCV RDTs are simple tools that can be used to screen for HCV infection for any of these indications by detecting antibodies against the virus. Although HCV RDTs are part of the WHO pre-qualification programme, no RDT had been pre-qualified at the start of the study, and the last reports on HCV RDT operational characteristics date back to 2001 [100, 101].

Therefore it was required to first gain knowledge on the optimal performance of
HCV RDTs and the following research question was proposed:

3.a) How accurately (i.e. sensitivity and specificity) do three HCV RDTs perform on panels of stored sera at a reference laboratory?

In order to address this question, three RDTs for the detection of antibodies against hepatitis C virus were assessed for their diagnostic accuracy at an internationally recognized reference laboratory, the Paul-Ehrlich-Institute, Germany. Chapters 4 and 5 describe the findings of these evaluations.

**Diagnosis of tuberculosis infection and resistance to rifampicin and monitoring of treatment side-effects**

**Diagnosis of tuberculosis infection and resistance to rifampicin**

Tuberculosis (TB) remains a major public health problem, as evidenced by the estimated 9 million incident cases yearly, of which 300,000 are multi-drug resistant (MDR). Approximately, 1.5 million people died from TB in 2013 [102].

In 2010, the MTB/RIF (Mycobacterium tuberculosis / rifampicin) test platform (GeneXpert, Cepheid) became available, integrating sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification, and amplicon detection [43]. The 2011 guidelines released by WHO described implementation of Xpert as simple, requiring only minimal staff training, and feasible in diverse settings [103]. The Xpert MTB/RIF test is recommended by WHO as the initial diagnostic test for TB wherever resource was available based on increased performance compared to microscopy [104]. Both studies in ideal and real-life conditions had been conducted, but experience from a large-scale implementation with new testing strategies was missing.

We examined the added value of the Xpert MTB/RIF following 3 implementation strategies:
4. How does the Xpert MTB/RIF improve diagnostic yield compared to microscopy only, using the following 3 strategies?
4.a) As the initial diagnostic test for all presumptive TB cases in parallel with microscopy
4.b) As the initial diagnostic test limited to patients at high risk of MDR-TB and HIV-associated TB
4.c) Used as an add-on test to microscopy in high-risk groups
4.d) Which challenges have been encountered during the large scale implementation of the Xpert platform?

Chapter 6 reports on the diagnostic yield of the Xpert MTB/RIF test using three different strategies in 18 countries, at 33 sites, using 38 Xpert four-module instruments over a 20 month period in which more than 50,000 samples underwent testing.

Treatment side-effect monitoring of second line TB drugs
The WHO recommends that patients on second line TB drugs, such as para-aminosalicylic acid, ethionamide, and prothionamide, are screened for hypothyroidism every 6 months or earlier if symptoms arise [105].

Monitoring thyroid stimulating hormone (TSH) levels in order to screen for hypothyroidism can be extremely challenging in RCSs, as the standard ELISA test requires significant laboratory infrastructure, and is relatively costly. A simple, inexpensive test that could substitute for the ELISA assay in identifying patients who require thyroxin treatment (i.e., those with TSH >10 mIU/mL) would greatly improve the monitoring possibilities of hypothyroidism in RCSs. A market review identified one POC test for with a cut-off of 10 mIU/mL, for which no independent evaluation data were available. This assay was assessed for its diagnostic accuracy at a reference laboratory, to answer the following question:

4.d) What is the diagnostic accuracy (i.e. sensitivity and specificity) of TSH-Check-1 RDT with a cut-off level of ≥10 mIU/mL?

Chapter 12 presents the evaluation results of the TSH-Check-1 RDT (Vedalab, France) with a cut-off of 10 mIU/mL, which is also used for initiating treatment for hypothyroidism in patients taking anti-tuberculosis drugs. Two hundred and fifteen samples, half with a TSH level <10 mIU/mL and half with a TSH level ≥10 mIU/mL when tested with the reference method, were analysed with the TSH-Check-1 RDT at the University Medical Center in Utrecht, Netherlands for this evaluation.
Selection of diagnostic tests: the need for a more pragmatic approach

Chapter 13 outlines steps to consider when selecting diagnostic tests, and highlights the gaps that currently prevent good quality and safe use of diagnostic tools in RCS.

Chapter 14 is the concluding chapter of this thesis. It discusses the main findings and limitations of the work presented in this thesis and provides recommendations for further research to increase appropriate access to POC diagnostic tools in RCS.
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