CHAPTER 1

INTRODUCTION

1.1 Demand and challenge for point-of-care (POC) diagnostics in limited-resource settings

Diagnostics, defined as the process of attempting to determine or identify a possible disease, is a necessary step before treatment can be started. It plays an important role in global healthcare, in the sense that it does not only provide information for doctors to administer appropriate and timely care to patients, but also provides crucial surveillance data for public health strategies. The quality of a diagnostic test determines the accuracy of the test result and directly influences treatment decisions. Lack of access to high quality diagnostic tests or to even low quality diagnostic tests remains a huge problem in modern society, especially for the developing world or limited-resource settings.

The heavy burden of ill health caused by infectious diseases in the developing world is well recognized, where it accounts for over half of all deaths in children[1]. Malaria, acute respiratory infections, enteric infections, tuberculosis and HIV kill more than 10 million people each year, 95% of whom live in developing countries[1]. This number would have been smaller if early and reliable diagnostics were offered to patients at the initial stage of disease development and prompt treatments were adopted. However, people in the developing world usually have limited access to diagnostic platforms in centralized laboratories due to constraints in physical location or financial conditions. Clinical symptoms and local prevalence of a certain disease could be used occasionally as a rationale for beginning treatment, but it does not apply when diseases have no symptoms or have similar symptoms to other diseases[2]. As a result, the demand for easy-to-use, low-cost, transportable diagnostic platforms is a growing yet unmet need.

The criteria for an ideal diagnostic test for limited-resource settings has been set by the World Health Organization (WHO) Sexually Transmitted Diseases Diagnostics Initiative with the term

"ASSURED test", which stands for: Affordable by those at risk of infection, Sensitive (few false negatives), Specific (few false positives), User-friendly (simple to perform and requiring minimal training), Rapid (to enable treatment at the first visit), Robust (does not require refrigerated storage), Equipment-free, and Delivered to those who need it[3]. These criteria have captured the features of a good POC diagnostic test, by emphasizing both the availability and flexibility of such a test, as well as its accuracy. As is often the case, an assay with low complexity and cost, such as a lateral flow strip pregnancy test, does not offer as much and as accurate information as a sophisticated test, such as an assay involving quantitative polymerase chain reaction (qPCR). However, the complexity and cost of sophisticated tests is usually high, preventing them from being used as POC tests. How to reduce the complexity and cost without sacrificing the accuracy of the test is the first issue to consider before developing a new POC diagnostic test.

If categorized based on the target, POC diagnostic tests include protein assays, nucleic acid assays, human cell assays, metabolite and small molecule assays, and drug and food safety tests[4]. Examples include the prostate-specific antigen (PSA) test, C-creative protein (CRP) test, troponin I test for protein targets[4-5]; tests for human immunodeficiency virus (HIV), influenza, Methicillin-resistant Staphylococcus aureus (MRSA), and tuberculosis (TB) for nucleic acid targets[6]; and tests for cancer, inflammatory response and hematological parameters for whole cell assays[7]. Although some of the tests above have satisfied some criteria of an ASSURED test, most of them are still labor intensive or not cost-effective, especially quantitative assays.

Compared with regular detection assays, which only confirm the presence or absence of a target, quantitative assays often require higher complexity in assay design to measure the amount of target. The standard method for quantification is to monitor the progress of a reaction, the kinetics of which correlate to the amount of input target. As a result, a detection instrument with certain resolution is needed to measure the output signal, which sometimes is not easy to integrate into a

POC platform and cumbersome to operate by untrained users. One way to eliminate the need of such an instrument is to "translate" analog information into end-point digital information.

1.2 The concept of analog-to-digital conversion: two definitions

The definition of "digital" can be two-fold, as explained in a review by our group[8]: "the tendency to switch between discrete states in response to specific triggers", which is often described by "a sharp sigmoidal change between two states over a narrow range of input" (e.g., "off" or "on"); and the isolation of single entity from others, which enables the analysis of this target independently, unlike in a "bulk" format, where the overall output is influenced by interactions between molecules or reactions.

As such, the concept of "analog-to-digital" conversion also has two definitions: converting a system with a continuous signal intensity change to a system with only two distinct states, and converting a system with all targets present in the same environment to a system with each target separated from others and confined within its own environment. As distinguishing two discrete states has much lower requirements for resolution than resolving continuous intensity, the conversion from analog-to-digital for the first definition could facilitate the simplification of readout systems, which will be demonstrated in the next chapter with an enzyme-linked immunosorbent assay (ELISA) system. For the second definition, the amount of input target is directly reflected in the digitized pattern at the endpoint, eliminating the need for real-time monitoring of bulk reaction progress. The remainder of this dissertation focuses on the use of this concept in the quantification of nucleic acids and the identification of nucleic acid sequences.

1.3 Digitization can be realized on microfluidic platforms, using SlipChip as an example

Microfluidics, the manipulation of small volumes of liquid, provides a convenient tool for studying system at the single-target level. Many ways of achieving digitalization of targets have been reported such as moving droplets in micro channels[9], static droplets in compartments

controlled by valves[10], magnetic beads[11] and other delicate structures to capture single targets[12]. The high resolution and the ability to increase local concentration of generated product or secretion grants microfluidic devices significant advantages over other platforms.

SlipChip is a static droplet manipulation platform designed to operate multiple reactions simultaneously[13]. The SlipChip is composed of two pieces etched with ducts and wells (Figure 1). The two pieces are in close contact and sealed with lubricating oil. Through relative movement (slipping), the relative position of features on different pieces changes, resulting in the connecting and disconnecting of wells. In the “loading” position, fluid moves from the inlet through ducts and wells to the outlet; in the “isolating” position, the fluid stream is broken into discrete droplets inside each well; and in the “mixing” position, some droplets mix with other droplets pairwise when wells overlap. Reagents and samples can be loaded into different sets of wells, and kept separated until the device is slipped. SlipChip has been used to perform protein crystallization[14], immunoassays[15] and digital PCR[16].

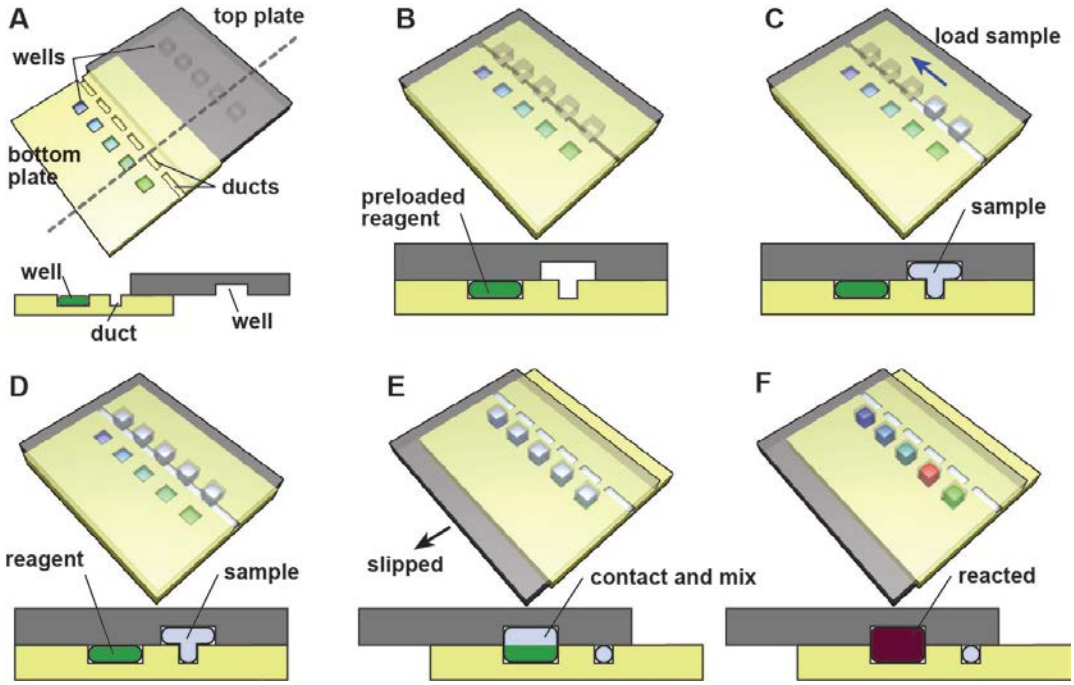

Figure 1 Schematic overview of fluid handling on SlipChip. (A) Off-set position of SlipChip, composed of a top plate and bottom plate, which are both etched with wells and ducts. (B) Assembled SlipChip at the "loading" position, where wells and ducts are connected. (C-D) The sample is loaded from inlet through the connected wells and ducts to the outlet. (E) Slipping-induced liquid stream to be broken into separate droplets and merged with preloaded reagent. (F) Reactions are triggered and different results are generated. Reproduced from reference 13 with permission of Royal Society of Chemistry.

Most of my graduate research involved the use of SlipChip microfluidic devices for the isolation and confinement of single molecules. In Chapter 2 of this dissertation the first definition of analog-to-digital conversion is described using the example of a threshold-enabled cystatin C quantification assay with visual readout on SlipChip. After the completion of that project, the focus of my research switched from protein to nucleic acid targets, and applied different nucleic acid amplification chemistries at the single-molecule level in a digital format for viral load quantification, which is described in Chapter 3 and 4. In these two chapters, strategies to allow large dynamic range and to eliminate thermal cycling are introduced to satisfy the requirements of limited-resource settings. To fully evaluate the performance of isothermal amplification chemistry, I tested the two major challenges for digital assays—efficiency and robustness, which are described in Chapter 4 and 5. Lastly, I expanded my research beyond viral load quantification

to genotyping, and developed a novel one-step isothermal assay for Hepatitis C Virus (HCV) genotyping, described in Chapter 6.