HW5: In-Vitro Diagnostics Clinical Needs, Methods Comparison and Engineering Mechanisms Between Standard-of-Care and Point-of-Care for Infectious Diseases

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Abstract

A comparison of clinical needs, methods and engineering mechanisms were completed between the standard of care (SOC) and Point of care (POC) diagnostics for infectious diseases. Infectious diseases are one of the leading deaths in developing countries such as Africa and Regions of Asia. Malaria, Tuberculosis and HIV/AIDS were selected for the assessment and the findings were a shared clinical needs and benefits in developing 'better, faster and cheaper' POC. ELISA and LFA were compared in detail in their methods and engineering mechanisms in detecting infectious diseases to achieve high sensitivity and quicker results response time. In the case of LFAs, sensitivity remains an issue since it is difficult to detect small amounts of antigens. Engineering approaches to modify the nanoparticles and time scale of LFAs increase test sensitivity by increasing reaction rates and keeping flow rates from being too high.
1 Introduction

Infectious Diseases are caused by bacteria, viruses, fungi or parasites [14]. These are communicable diseases either from one person to another, bites from insects or animals or ingesting of contaminated food or water [14]. Infectious diseases are #9 for 1.6% of total U.S. YLD (Years Lived with Disability) and #10 for 2.6% of Global YLD [24]. However, infectious diseases are the #1 cause of death in Sub-Saharan Africa and Various Regions of Asia [25]. These countries have low-income, limited resource, high population and overall lack of health care and infrastructure that promotes infectious diseases to wide spread easily and not get proper treatments. This leads to millions of people dying every year and transmission and survival of the disease not only locally but worldwide compromising the overall health state of the world.

Considering the limitations of resource limited countries (RLC), it is essential to come up with a medical and engineering solutions to reduce and eliminate the epidemic nature of infectious diseases. One of the ways to achieve such impact is having high sensitive, easy to use, less maintenance and cost-effective in-vitro diagnostics testing that is suitable for RLC setting [23]. There are two types of in-vitro diagnostics; one is laboratory based microscopy and immunosorbent assay (ELISA) detection methods for antibodies, antigen or proteins, referred to as Standard of Care (SOC). The second type is rapid diagnostic testing based on lateral flow assay (LFA) color band detection methods on an oral swab strip, finger pricks for blood or urine markers, referred to as Point-of-Care (POC).

On the following sections, the clinical needs of three infectious diseases (Malaria, Tuberculosis and HIV/AIDS) are discussed followed by the current diagnostic methods and results assessment utilized by these three infectious diseases. The mechanisms of SOC and POC will be discussed in the subsequent sections, which includes a discussion on the engineering principles and newly emerging technologies such as nanodiagnostics impact in improving POC in particular.

2 Clinical Needs

Out of the long list of infectious diseases, Malaria, Tuberculosis and HIV/AIDS were selected to assess their clinical needs for in-vitro diagnostics especially for their POC needs. All of them have similar themes of clinical needs with some specific needs based on the diseases severity and characteristics. Table 1 below attempts to summaries the clinical needs findings as it pertains to POC.

<table>
<thead>
<tr>
<th>Clinical Needs</th>
<th>Malaria</th>
<th>Tuberculosis</th>
<th>HIV/AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shared Needs</strong></td>
<td></td>
<td>1) Early Detection</td>
<td>4) Biomarkers detectable immediately after infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) High Sensitivity with a strong bio-marker</td>
<td>5) Easy to use home based POC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) Quicker Results Response Time</td>
<td>6) Easy to use home based POC</td>
</tr>
<tr>
<td><strong>Specific Needs</strong></td>
<td>4) Biomarkers detectable immediately after infection</td>
<td>4) Multiplexing to detect multiple drug resistance along with the bacteria</td>
<td>5) Consolidated multiplexing testing</td>
</tr>
</tbody>
</table>

Table 1: Clinical Needs Summary
The shared benefits for developing POC for these diseases lies in early detection resulting in reduced transmission, improved treatment outcomes and reduced health care costs [20]. Especially, in resource limited countries (Africa and parts of Asia), early detection of the diseases will directly impact in saving millions of lives every year, increase life expectancy and quality of life. The following sections will discuss brief background on the infectious diseases, disease type characteristics and highlights the unmet clinical needs.

2.1 Malaria

Malaria is caused by plasmodium parasites through the bites of infected female Anopheles mosquitoes called ”malaria vectors” [9]. 92% of malaria cases and 93% of malaria deaths happen in Sub-Saharan Africa [9]. Two out of the five parasite species that cause malaria in humans, P. falciparum and P.vivax are the deadliest. After the first symptoms such as fever, headache and chills, especially if the P. falciparum is not treated within 24 hours, the malaria can progress and often can lead to death [9].

For a non-immune individual, it takes up to 10-15 days to start having the first symptoms [9]. Although, the best option is prevention by using insecticide-treated mosquito nets and indoor residual spraying, it might not be possible to prevent or even be aware of the presence of the mosquitoes in most cases. Therefore, there is a clinical need of early detection and treatment of malaria to reduce disease and prevent deaths. Early detection would be possible by increasing sensitivity and specificity to detect low level parasites that hasn’t developed to first symptom [10].

According to World Heath Organization (WHO), the total funding for malaria control/elimination is near US3.1billion in 2017 [9], which shows the seriousness of the diseases and the need for a cost-effective and value-adding diagnosis for early detection. A parasite-based diagnostic testing is recommended to confirm the parasitological within 30minutes or less before administrating treatment [9].

Figure 1 below show vector control, preventive vaccines, diagnostics, drug treatment at various stages of Malaria disease progression. Knowing the different stages of the progression helps with formulating an analyte to detect the infection before the first symptoms appears after 10-15 days.
Malaria seems to have a well advanced disease state understanding and robust RDT within 30 minutes or less results. The one area with an unmet need on the diagnostics is finding an early detectable biomarker with in the window of the first 10-15 days to expedite the early detection process and reduce transmission.
2.2 Tuberculosis

Tuberculosis (TB) is one of the top 10 causes of death worldwide and it is caused by bacteria called Mycobacterium Tuberculosis that usually attack the lungs \([9, 11]\). TB is highly contagious through air when a person coughs, sneezes or talks. 72\% of the TB cases are in Africa followed by India cases of 27\% \([12]\).

There are two types of TB, first latent TB that is inactive form of the disease and is not transmittable, however, 5-15\% population with latent TB have a chance of falling ill with active TB. The second type is active TB disease which may begin with a mild symptoms of cough, fever, night sweats and weight loss for months. Due to its slow progress of illness, people can delay care and result in transmitting the disease to other people \([9]\). If not treated properly, 45\% of HIV-negative people with TB and nearly all HIV-positive people die because of the TB \([9]\).

The commonly used rapid diagnostic test (Xpert MTB/RIF) has been in use since 2010, which detects TB and resistance to the treatment drug \([9]\). The diagnosis takes 2 hours and it is a recommended initial test by WHO. One of the clinical challenges with TB is diagnosing multi-drug resistance especially drug-resistant TB since it is complex and expensive \([9]\). Therefore, the clinical needs for TB diagnosis is in the ability to detect multiple drug resistance technology, cost-effect and faster Point of Care (POC) type of diagnostics.

Figure 2 below shows the research need for TB Assay that is mainly focused on understanding the assay and accurately detecting it. One of the main need for TB is the ability to multiplex by detecting not only the bacteria’s antigen/antibodies but also by detecting how much the person is drug resistant to various TB treatments.
LAM Stands for Lipoarabinomannan, it is an antigen from the cell wall lipopolysaccharide. TB can initially be confused with colds and flu symptoms that misleads people from seeking treatment quickly. In addition, the current POC for TB does that 2 hours and need to complete multiple tests for the bacteria and drug resistance. All these inconveniences may not encourage people to seek diagnosis and treatment until the TB has progressed. Easy to use home based POC could encourage people to take the test more readily than the current situation.

2.3 HIV/AIDS

The Human Immunodeficiency Virus (HIV) targets the immune system and weakens people’s defence system against infections and other disease types [9]. Gradually, the virus cause the infected individuals to become immunodeficient and make the individual vulnerable to a wide range of diseases [9]. The progressed stage of HIV infection is Acquired Immunodeficiency Syndrome (AIDS). In the past two decades, 38% of HIV related deaths reduced because of the lifelong antiretroviral therapy (ART); currently 59% of adults and 52% of children receive ART. Two Thirds of the population who lives with HIV is in Africa.

The rapid diagnostics tests (RDT) such as serological tests detect the presence or absence of antibodies or antigen in response to the virus [9]. Therefore, HIV is not directly detected directly. Furthermore, it takes up to 28 days to develop the antibodies that can be detected by the RDT and it is the highest time to transmit the virus to other individuals [9].

The clinical need for HIV diagnosis is a search for effective analyte with antigen or RNA to for an early detection of HIV [14]. Furthermore, considering only 75% of the people with
HIV know their status, more access to cheaper and easy to use POC is needed to diagnose more people who do not know their status yet [9]. The other clinical need is for infants born to mothers living with HIV who needs virological testing (faster by detecting the virus or part of it directly) instead of serological testing (takes time to detect the antibodies/antigen) [14].

The critical question for HIV is how early can it be detected. Hurt et. al. [15] outlines this time for detection will depend on 1) the target being detected, 2) when the target is present following infection, 3) the concentration of target in the specimen, 4) the volume of specimen tested, and 5) the test’s lower limit of target detection.

Figure 3 below shows how the virological testing (HIV RNA) has much faster detection time (approximately 10 days) compared to commonly used serological testing (Antibody/antigen, which takes up to 30 days). For HIV/AIDS, the ability to detect earlier with the most effective and accurate bio-marker is important. Currently, the detection time for HIV is increasing as we move from detecting proteins (greater than 30 days) to detecting antibodies (Approx. 30 days) to antigens (approx. 20 days) to virus(approx. 10 days).
Given the lack of cure and highly transmissions nature of HIV/AIDS, a lot of good advancement has been made in the last 30-35 years. Although, the number of HIV infected people has decreased and due to the use of ART sustained, there are 25% of infected but unaware people that can be an agent in continuing the transmission. This rises the unmet need of easy to use home-based POC for resource limited countries would be essential. Once a person is diagnosed positive, multiple level of repetitive diagnostic testing is executed to complete the diagnosis. This calls a need to consolidate the multiple testing to a single or reduced step but highly sensitive POC.
2.4 Diagnostic Methods Comparison: SOC vs. POC Discussion

What we observe from the three infectious diseases discussed above (i.e., Malaria, Tuberculosis and HIV/AIDS) the traditional diagnostic methods of microscopy is used hand in hand with the Rapid Diagnostics Testing (RDT). The common drawbacks in the standard care of microscopy-related diagnosis is the need of significant laboratory testing time, laboratory infrastructure and trained staff and relatively expensive because of testing time, personnel training and equipment maintenance [21]. On the other hand, the microscopy-related diagnosis tend to have high sensitivity, accuracy of results and ability to handle multiple testing at once (multiplexing).

In parallel, the common benefit in RDT diagnosis is the cost-effectiveness and quicker response time leading to early detection and faster treatment options. In the meantime, this POC can be less sensitive in detection, may not be widely accessible in resource-limited countries and result in false negative results that can be misleading in taking the early treatment options.

Table 2 below compares the diagnostic methods between SOC and POC for Malaria, Tuberculosis and HIV/AIDS. The findings confirm most of the common themes of pros and cons, however, there are some contrary results shown such as lower sensitivity for Malaria SOC but high sensitivity for TB POC. Also Malaria’s SOC and POC has little to no cost difference. The main reason for the contrary results could be due to the disease severity level and the resource-limited nature of the countries where these diseases are being treated.

<table>
<thead>
<tr>
<th>SOC/Standard of Care</th>
<th>Malaria</th>
<th>Tuberculosis (TB)</th>
<th>HIV/AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>POC/Point of Care</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diagnosis Methods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test Time</td>
<td>Clinical Microscopy</td>
<td>One Step Malaria (PF/IV viral) W/B Rapicard InstaTest</td>
<td>Xpert MTB/RIF</td>
</tr>
<tr>
<td></td>
<td>several days</td>
<td>≤ 30 mins</td>
<td>≤ 2 hrs</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Results Signal Report Method</td>
<td>Present of parasite in blood Color Band</td>
<td>Stained glass slide under a microscope</td>
<td>Automated GeneXpert Machine to read out results</td>
</tr>
<tr>
<td>Cost</td>
<td>Inexpensive</td>
<td>Inexpensive</td>
<td>Inexpensive</td>
</tr>
<tr>
<td>Reference</td>
<td>Wongprichanabai et al.</td>
<td>WHO Website</td>
<td>CDC Website</td>
</tr>
</tbody>
</table>

Table 2: Diagnostic Methods Comparison

For instance Malaria, where 92% of the people with the disease lives in Africa, the type of the SOC and POC is going to be greatly compromised of quality and standard because of the resource-limited nature of the African countries. SOC for Malaria does not have the specificity to picking up Malaria parasites only so it does pick up other types of regional parasites [19]. As some literature [20, 22, 23] pointed out the POC design criteria for resource-limited countries (RLC) would have to be different than developed countries. Besides cost, which is not the sole problem of RLC, the ease of use and less maintenance are also critical needs for accessibility and usability of POC in RLC. [23].

In the meantime, the search for novel and advanced analyze and detection modalities are also important as extensively discussed on Kim et al. [20]. For example, the HIV/AIDS POC is always looking for the most effective biomarker to detect the virus present as early as possible as depicted on Figure 3 in HIV/AIDS Section above.
The promise of nanoparticles to improve analytical sensitivity of diagnostic assays [20] might be at its early technology development stage, however, can impact POC all around the world by promoting high sensitivity for early detection of infectious diseases while maintaining cost effective and easy to use. The following sections will discuss further into the mechanisms and engineering analysis of the two most common diagnostic methods of SOC (ELISA) and POC (LFA).

3 Enzyme-linked Immunosorbent Assay: ELISA

ELISA (acronym for enzyme-linked immunosorbent assay) is a technique for antigen detection. It is commonly used to measure antibodies, antigens, proteins, and glycoproteins in biological samples. Given that the methods are highly reproducible and inexpensive it is a common choice for large scale sample testing. Other benefits of using ELISA are clear; we direct the reader to the chart in Figure (6) for a further reference. ELISA methods work on the principle that enzymes are capable of reacting with substrates multiple times before decaying. Thus, a weak signal from an antigen can be enhanced. Parting from an immobilized target, enzyme-labeled antibodies are added and cultured so that they form an antibody-antigen complex. Later, an enzyme substrate is added and the enzyme product is quantified (see Figure 5). Although many variations of Elisa exist (see Figure 4), such as direct, indirect, competitive, sandwich, among others, the steps involved are analogous. Broadly the relevant stages involved are [5]:

1. Adsorption of antigen or antibody to the plastic solid phase
2. Addition of the test sample and subsequent reagents
3. Incubation of reactants
4. Separation of bound and free reactants by washing
5. Addition of enzyme-labeled reagent
6. Addition of enzyme detection system (color development)
7. Visual or spectrophotometric reading of the assay
Figure 4: Different ELISA formats. Taken from [6]

Figure 5: Direct ELISA method. Taken from [4]
Depending on the test to be performed, ELISA methods might not be adequate. Its sensitivity is in the femto- to nano-molar range. Moreover quantification of results might require specialized instruments which might be expensive depending on their sensitivity. Then, specific antigen or antibodies reagents, and long hours of cultivation might be a barrier for particular samples [7].

3.1 Mathematical Model and governing equations

We proceed to describe the governing equation in a 3-Dimensional space, as it is the case in micro-plate well assays. For simplicity we assume that the well has a cylindrical geometry with diameter equal to 3mm, and height equal to 10 mm. We denote by Ω the inside of the well, by ∂Ω the boundaries, and by Γ the bottom of the well (See figure 7).
Here, we focus on the direct assay technique (see Figure 5). Thus, parting from an assay with an established concentration of antigens at $\Gamma$, we describe the two steps:

1. addition enzyme-labeled antibodies, and
2. addition of enzyme substrates.

We will assume that the intermediate steps are conducted in such a way that, the steady state of process 1 is independent from step 2 above.

### 3.1.1 Step 1: Addition of enzyme-labeled antibodies

Let $[P]$ denote the concentration of (without loss of generality) proteins of interest to be detected; $[A]$ the concentration of enzyme labeled antibodies; and $[E]$ the functional enzyme complex $AP$. Now, consider the chemical process:

$$A + P \xrightarrow{k_1} E.$$  \hspace{1cm} (1)

We presume this reaction can only take place near the surface $\Gamma$. Specifically we fix $\ell = 10\text{nm}$ to be the minimum distance from $\Gamma$ at which the reaction takes place. Then, the general form of the reaction diffusion process is given by

$$\frac{\partial}{\partial t}[A] = D_\Gamma \Delta_\Gamma [A] - k_1 \ell [A][P] + k_2 [E] \quad \in \Gamma$$  \hspace{1cm} (2)

$$\frac{\partial}{\partial t}[E] = D_\Gamma \Delta_\Gamma [E] + k_1 \ell [A][P] - k_2 [E] \quad \in \Gamma$$  \hspace{1cm} (3)

$$\frac{\partial}{\partial t}[P] = D_\Omega \Delta_\Omega [P] \quad \in \Omega$$  \hspace{1cm} (4)
where \( D_i \) is the diffusion coefficient in the respective domain, and \( \Delta = \nabla^2 \) is the Laplace-Beltrami operator in the appropriate domain. To the above we impose the following boundary conditions

\[
D_\Omega \frac{\partial}{\partial n} [P] = - k_1 \ell [A][P] + k_2 [E] \quad \in \Gamma \tag{5}
\]

\[
\frac{\partial}{\partial n} [P] = 0 \quad \in \partial \Omega \setminus \Gamma \tag{6}
\]

\[
\frac{\partial}{\partial n} [A] = \frac{\partial}{\partial n} [E] = 0 \quad \in \partial \Gamma \tag{7}
\]

where \( \partial n \) is to be taken as the outward normal vector of the respective domain.

### 3.1.2 Step 2: Addition of enzyme substrates

We assume that the species of step 1 have been cultivated and washed as to leave a steady state concentration of enzyme-labeled antibodies at the surface \( \Gamma \). Now, let \([S]\) denote the concentration of the enzyme substrate, \([C]\) the concentration of the complex \( ES \), and let \([S^\ast]\) be the concentration of the enzyme product. A version of enzyme kinetics is given by the chemical reaction

\[
E + S \xrightarrow{\frac{r_1}{r_2}} C \xrightarrow{r_3} E + S^\ast. \tag{8}
\]

Analogous to the derivation for governing equations in step 1, we presume that the substrate and its product diffuse freely on the medium. Moreover, as we did before, the enzymatic reactions are restricted to an area near the boundary \( \Gamma \). Then the governing equations are of the form:

\[
\frac{\partial}{\partial t} [E] = D_\Gamma \Delta_\Gamma [E] - r_1 \ell [E][S] + (r_2 + r_3)[C] \quad \in \Gamma \tag{9}
\]

\[
\frac{\partial}{\partial t} [S] = D_\Omega \Delta_\Omega [S] \quad \in \Omega \tag{10}
\]

\[
\frac{\partial}{\partial t} [C] = D_\Gamma \Delta_\Gamma [C] + r_1 \ell [E][S] - (r_2 + r_3)[C] \quad \in \Gamma \tag{11}
\]

\[
\frac{\partial}{\partial t} [S^\ast] = D_\Omega \Delta_\Omega [S^\ast] \quad \in \Omega \tag{12}
\]

together with the following boundary conditions

\[
D_\Omega \frac{\partial}{\partial n} [S] = - r_1 \ell [E][S] + r_2 [C] \quad \in \Gamma \tag{14}
\]

\[
D_\Omega \frac{\partial}{\partial n} [S^\ast] = r_3 [C] \quad \in \Gamma \tag{15}
\]

\[
\frac{\partial}{\partial n} [S] = \frac{\partial}{\partial n} [S^\ast] = 0 \quad \in \partial \Omega \setminus \Gamma \tag{16}
\]

\[
\frac{\partial}{\partial n} [E] = \frac{\partial}{\partial n} [C] = 0 \quad \in \partial \Gamma \tag{17}
\]
### Table 1. Summary of Apparent Binding Constants from Kinetic Analysis of Nonequilibrium Binding Data

<table>
<thead>
<tr>
<th>Binding System</th>
<th>$k_a$ (M$^{-1}$ s$^{-1}$)</th>
<th>$k_d$ (s$^{-1}$)</th>
<th>$K$ (M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG–protein G</td>
<td>$3.29 \times 10^4$</td>
<td>$2.90 \times 10^{-4}$</td>
<td>$1.13 \times 10^8$</td>
</tr>
<tr>
<td>IgG–protein A</td>
<td>$8.02 \times 10^3$</td>
<td>$2.77 \times 10^{-4}$</td>
<td>$2.90 \times 10^7$</td>
</tr>
</tbody>
</table>

![Figure 8: Taken from [27]](image)

![Figure 9: Diffusion time](image)

#### 3.1.3 Parameters and the Damkohler number

It has been found that at 20°C the diffusion coefficient of human IgG in 10-times-diluted serum is $(4.4 \pm 0.2) \times 10^{-7}$ cm$^2$s$^{-1}$, while the diffusion coefficient of rabbit anti-human IgG in a purified preparation is $(2.9 \pm 0.2) \times 10^{-7}$ cm$^2$s$^{-1}$ [26]. Moreover, binding rates for IgG have been theoretically estimated in a study such as [27]. Their results are summarized in Figure 8. Observe that the diffusion time $(\tau = L^2/2D)$ for a well of 1cm (as in 7) is about 20 days. A plot of the time to diffusion is show in Figure 9. Thus, it is unrealistic to allow the labeled antibodies to diffuse from the top. In fact, the standard protocol for ELISA requires the solutions to be added directly into the wells [28]. Thus we will compute the Damkhohler number as defined in [29] by

$$D_a = kC_0^{n-1}\tau$$

where $k$ is the kinetic constant, $C_0$ is the initial concentration of the reagent, $n$ is the order of the reaction, and $\tau$ is the residence time. For the residence time here we assume is the time it takes the reagent to diffuse a length larger that the reaction domain defined by $\ell = 10$nm. Explicitly, taking $k$ to be the maximum among the kinetic rates provided in 8, we get

$$D_a = kC_0^{n-1}\tau = 5.26 \times 10^{-4}C_0.$$  

Note that the units of $k$ are M$^{-1}$s$^{-1}$. Thus, in order for $D_a$ to be less than 0.01 we require the initial concentration of the reagents to be of the order

$$C_0 < 20M$$

which is reasonable. With this at hand, we know that the kinetics will be the limiting parameters of the reaction. Thus, in the next section, we will proceed by assuming that diffusion in negligible, and we will reduce the system.
3.1.4 Lumped system for captured analyte and steady state analysis

As discussed in the previous subsection, diffusion plays a minor role in our model. Thus, to analyze the steady state of analytes we will reduce the model in section 3.1.1. To begin, we presume that diffusion gradients are negligible and derive a lumped model for the concentration profiles. We define the mean concentration of $P$ as

$$
\bar{P}(t) = \frac{1}{|\Omega|} \int_{\Omega} P(t, x) \, dx.
$$

Then, taking the derivative with respect to time we get

$$
\frac{d}{dt} \bar{P} = \frac{1}{|\Omega|} \int_{\Omega} \frac{\partial}{\partial t} P(t, x) \, dx = \frac{1}{|\Omega|} \int_{\Omega} D_\Omega \Delta_\Omega P \, dx.
$$

By using the divergence theorem we have

$$
\frac{d}{dt} \bar{P} = \frac{1}{|\Omega|} \int_{\partial\Omega} D_\Omega \nabla P \cdot ds = \frac{1}{|\Omega|} \int_{\Gamma} -k_1 \ell[A][P] + k_2[E] \, ds.
$$

Assuming that the concentrations of $P, A,$ and $E$ are constant across the bottom surface $\Gamma$ we have that

$$
\frac{d}{dt} \bar{P} = \frac{1}{|\Omega|} \int_{\Gamma} (-k_1 \ell[A][P] + k_2[E]) \, ds = \frac{|\Gamma|}{|\Omega|} (-k_1 \ell[A][P] + k_2[E]).
$$

In an analogous manner we deduce mean concentrations for $A$ and $E$. We have

$$
\frac{d}{dt} \bar{A} = \frac{1}{|\Gamma|} \int_{\Gamma} \frac{\partial}{\partial t}[A]
$$

$$
= \frac{1}{|\Gamma|} \int_{\gamma} -k_1 \ell[A][P] + k_2[E] \, ds
$$

$$
= \frac{1}{|\Gamma|} (-k_1 \ell[A][P] + k_2[E]) \int_{\Gamma} \, ds
$$

$$
= - k_1 \ell[A][P] + k_2[E].
$$

Finally, for $E$,

$$
\frac{d}{dt} \bar{E} = \frac{1}{|\Gamma|} \int_{\Gamma} \frac{\partial}{\partial t}[E]
$$

$$
= \frac{1}{|\Gamma|} \int_{\gamma} k_1 \ell[A][P] - k_2[E] \, ds
$$

$$
= \frac{1}{|\Gamma|} (k_1 \ell[A][P] - k_2[E]) \int_{\Gamma} \, ds
$$

$$
= k_1 \ell[A][P] - k_2[E].
$$

16
Thus, our lumped system satisfies

\[
\frac{d}{dt} P = \frac{\Gamma}{\Omega} (-k_1 \ell [A][P] + k_2 [E]) \tag{26}
\]

\[
\frac{d}{dt} A = -k_1 \ell [A][P] + k_2 [E] \tag{27}
\]

\[
\frac{d}{dt} E = k_1 \ell [A][P] - k_2 [E]. \tag{28}
\]

Note that the equations reveal the following conservation laws

\[
C_1 = [A]_0 + [E]_0, \tag{29}
\]

\[
C_2 = \frac{\Gamma}{\Omega} [E]_0 + [P]_0. \tag{30}
\]

Now we proceed to find the analytical solution of the steady state using the parameters shown in Table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>value</th>
<th>units</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Gamma)</td>
<td>(\pi(0.015)^2)</td>
<td>cm²</td>
</tr>
<tr>
<td>(\Omega)</td>
<td>(\pi(0.015)^2)</td>
<td>cm³</td>
</tr>
<tr>
<td>(k_1)</td>
<td>(3.29 \times 10^4)</td>
<td>M⁻¹ s⁻¹</td>
</tr>
<tr>
<td>(k_2)</td>
<td>(2.9 \times 10^{-4})</td>
<td>s⁻¹</td>
</tr>
<tr>
<td>(\ell)</td>
<td>(1 \times 10^{-6})</td>
<td>cm</td>
</tr>
<tr>
<td>([A]_0)</td>
<td>(C_1)</td>
<td>M</td>
</tr>
<tr>
<td>([P]_0)</td>
<td>(C_2)</td>
<td>M</td>
</tr>
<tr>
<td>([E]_0)</td>
<td>0</td>
<td>M</td>
</tr>
</tbody>
</table>

Table 3: Parameters used

Our variable of interest \([E]\) reaches steady state at

\[
3.04 \times 10^{-7} \left( \sqrt{(-1.65 \times 10^6 C_1 + 1.65 \times 10^6 C_2 + 149)^2 + 9.8 \times 10^8 C_1} \right. \tag{31}
\]

\[
\left. +1.65 \times 10^6 C_1 + 1.65 \times 10^6 C_2 + 149 \right) \tag{32}
\]

A plot of the steady state concentration of \([E]\) for several choices of \(C_1\) and \(C_2\) is shown in Figure 10.
4 Lateral Flow Assay

Lateral flow assays (LFAs) are simple diagnostic tools that can detect specific biomolecules. They are fast, inexpensive, easy to use, and portable [1]. LFAs have made fast, point-of-care diagnostics a reality for many conditions that would typically require long laboratory testing. Sensitivity remains an issue with LFAs, but this is being addressed by the growing field of thermal contrast techniques [1]. Though they lack sensitivity, LFAs rely on a robust mechanism of action for diagnosis when there is sufficient antigen present.

LFAs consist of four main components: 1) A sample pad, 2) a conjugation pad, 3) a nitrocellulose membrane test region, and 4) an absorbent pad [1,2]. The sample pad is dipped into the specimen of interest and is pulled through the nitrocellulose membrane through capillary action. The conjugation pad contains antibody-coated gold nanoparticles (GNPs). The antibodies are specific to the antigen of interest, which will then bind to the GNP. The next stage of the LFA is where detection occurs. The test line is coated with antibodies that target the antigen on the GNP complex as it flows through the device. The antibodies bind the antigen, thus stopping the GNP complex from progressing through the device. The accumulation of GNPs in the test section signals the presence of the particular antigen of interest. The control strip ensures that the GNPs and antibodies are working correctly. If this line is not visible during the assay, it is unreliable because the nanoparticles could be defective or there was some other malfunction with the device. The absorbent pad is the final destination for the GNPs as they flow through the LFA.
Figure 11: Schematic of a typical lateral flow assay [2]. LFAs are typically composed of a sample pad, a conjugation pad, a test region, and an absorption pad.

Figure 12: LFAs utilize antibody-coated GNP to bind the antigen of interest and subsequently bind to the monoclonal antibodies bound to the surface of the membrane.

Figure 13: When the Peclet number is too large (\( Pe >> 1 \)), GNPs do not have enough time to diffuse to the capture antibody before being carried away by the flow. LFAs with smaller Peclet numbers allow sufficient time for diffusion to the capture antibody for binding.
Figure 14: At high concentrations, LFA test signal experiences a hook effect, resulting in a regression of the signal.

The intensity of the test region visual signal is indicative of the concentration of the analyte. Figure 14 shows a representative curve of the intensity signal as the concentration of the analyte increases. At most concentrations, the intensity follows a fairly predictable pattern-increasing concentration leads to a stronger signal. However, at very high concentrations the signal undergoes a high dose hook effect and the signal begins to decrease. Ideally, all of the GNP complexes would bind to antibodies bound to the test strip region. At high concentrations, the antibodies bound to the test strip are increasingly filled. This makes it more difficult for conjugated antibodies bound to the antigen to bind them. Therefore, due to the high concentration, conjugated antibodies in the fluid phase bind to one another. With the antigen already bonded on both sides, the GNPs pass through the test region without contributing to the signal. The signal will then be lower than expected at high concentrations.

GNP capture is a crucial aspect in determining the sensitivity of LFAs. Poor GNP capture will make for an ineffective LFA. Therefore, GNP capture must be optimized for proper performance of the LFA. Velocity, diffusion, and reaction rates all play a role in GNP capture. These parameters can be defined using the Peclet number (Equation 33) and the Damköhler number (Equation 34).

\[
P_T = \frac{U \cdot R}{D}
\]

\[
Da = \frac{kCR}{D}
\]

\[
L = \frac{V}{\tau}
\]

\[
\sqrt{D \tau} = L_{diff}
\]

The Peclet number is the ratio of convection to diffusion of a system. For LFAs, Peclet numbers less than one, where diffusion dominates convection, are desirable. This corre-
sponds to the low convection region on the left hand side of Figure 15. When diffusion dominates, there is an increase in transit time that allows for the GNP reporters to diffuse away from the streamline and bind to the capture antibodies. In order to decrease the time to make a reading, Pe is usually much larger than one and diffusion becomes the rate limiting step [3]. This can be demonstrated by calculating a residence time as found in Equation 35 and comparing to the diffusion length scale from Equation 36. We used previously reported values of $0.2 \text{ mm/s}$, $10^{-11} \text{ m}^2/\text{s}$, and $2\text{mm}$ for the velocity, diffusivity, and length of the test strip as an example. Applying Equation 35 yields a residence time of 10s. Using this result in Equation 36 yields a diffusion length of $31.6\mu\text{m}$. Within the 10 second time frame, particles under $31.6\mu\text{m}$ would be able to bind to the capture antibody by diffusing across the streamline. This provides a good balance of both speed and signal strength, both of which are important components in the design of LFAs.

Reaction rate goes hand in hand with convection as a reduced velocity will increase the time for reactions to occur. The Damkohler number, defined by Equation 34, provides a ratio of the reaction rate constant to diffusion. Reaction is the rate limiting step, which means that higher Da numbers are preferable [3]. Larger reaction rates and, thus, larger Damkohler numbers would lead to better binding. This corresponds to the large Damkohler numbers on the right hand side of Figure 16, where reaction rate dominates diffusion. When taking both the Peclet number and the Damkohler number into account, the ideal LFA would have a small Peclet number and a larger Damkohler number. This means that convection is dominated by diffusion, which is dominated by reaction.

Figure 15: Plot of Peclet number (Equation 33) as a function of velocity and diffusivity.
With the importance of GNP capture in mind, there are certain methods that should theoretically maximize the chances of the GNP binding to the membrane. One thing that can be in order to increase the size of the nanoparticle. This has the benefit of increasing the number of surface antibodies on the GNP and making capture more likely [3]. This, coupled with the fact that their absorption and scattering profiles make larger GNPs more readily detectable [3]. As mentioned earlier, slowing down the flow allows the GNPs to have more time to bind to the capture antibodies. A third and final method to maximize binding would be to change the shape of the nanoparticle to a geometry such as a rod with a high surface area to volume ratio. The larger surface area would serve a similar purpose as using a larger particle in that there are more available binding sites for the capture antibody to come into contact with.

Nanoparticle size is a critical parameter in designing LFAs. 30nm is a common size for GNPs used in these types of systems. However, larger particles can help improve the signal as we have already addressed. However, the benefits of increasing the size of the GNP eventually cease to outweigh the costs. Zhan et al [3] found that a functional limit is reached at around 100nm. Due to their larger size, they have a much larger Damkohler number, which means that the reaction rate will be limited by the slower diffusion of the larger particle. Practically, it is also very expensive to make larger and larger nanoparticles as it requires more gold and many more antibodies [3]. Eventually, settling becomes an issue if the size of the GNP gets to be too large. In this case, the GNP will settle within the pores of the membrane before before reaching the test line. It has been estimated that GNPs around 400nm would not follow the streamline, but rather settle before reaching the test strip [3]. This effect lowers the accuracy of the device and makes detection of the analyte more difficult. Therefore, GNPs must be designed in such a way so as to maximize their likelihood of capture through modifications to their size and shape without becoming so large that test accuracy is compromised.
5 Conclusions

Three infectious diseases, Malaria, Tuberculosis and HIV/AIDS have been assessed for their clinical needs and benefits of diagnostic testing especially POC. There are a number of shared clinical needs such as early detection, high sensitivity and quicker results response time. The shared benefits of developing POC are reduced disease transmission, improved treatment outcomes and reduced health care costs. Furthermore, the benefits for resource limited countries quadruples by saving lives, increasing life expectancy and quality of life.

The comparison between SOC and POC diagnostic methods showed currently, utilized together. Unless in some cases, SOC are highly sensitive but takes long time and need trained personnel and infrastructure. On the other hand, POC take less time to get results, are easy to use but lack the sensitivity of SOC.

In our analysis for ELISA assays we proved that the steady state profile of the enzyme-labeled antibody depends only on the kinetics of the chemical reaction. Moreover, we graphically showed that the steady state depends linearly in the initial concentration of trapped antibody and initial concentration of enzyme-labeled antibody. We give reasonable parameters ranges for which the analysis is valid. Moreover, we provided a constructive method for finding the steady state values parting from a lumped assumption.

In our evaluation of lateral flow assays, we demonstrated that small Peclet numbers, where diffusion dominates convection, and large Damköhler numbers, where reaction dominates diffusion, are most effective. In reality, though, these are not always attainable parameters. Peclet numbers are often much higher than one so that the assay can be performed in a matter of minutes. This limitation reduces signal strength due to the speed at which the GNPs are passing by the test strip. Sensitivity remains a significant roadblock to the development and adoption of fast POC diagnostic tests. Thermal contrast utilizing GNPs is one approach that is being developed to increase LFA sensitivity to small amounts of antigen even when visible detection shows a negative result. Fast, POC diagnostic assays have a massive potential to change healthcare. Their speed, affordability, and ease of use streamline diagnostics, resulting in earlier diagnoses and better outcomes.
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