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Literature Review on the Use of Nucleic Acid-Based Logic Gates for the Detection of Human Diseases

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LITERATURE REVIEW ON THE USE OF NUCLEIC ACID-BASED LOGIC GATES FOR THE DETECTION OF HUMAN DISEASES

by

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ABSTRACT

Conventional methods for diagnosis of human disease are, at times, limited in different regards including time requirement, either experimental or data processing, sensitivity, and selectivity. It is then that a Point of Care Criteria, which considers the true utility and usefulness of the device, is employed to propose new diagnostic devices capable of overcoming the aforementioned shortcomings of conventional tools. Nucleic acid, characterized for its predictable base-pairing nature, is considered to be a highly-selective, yet greatly modifiable device. Its behavior is then described through Boolean Logic, where “true” or “false” outputs are mathematically described as “1” and “0”, respectively. This mathematical approach is then referred to as Logic Gates, where outputs can be predicted based on satisfied environmental conditions. The mechanisms, capable of exhibiting Logic Gate behavior, are described.

DEDICATIONS

For the love of my life, Karina Pabón Calderón, who's love and support allowed for the completion of this investigation. Thank you for being there always.

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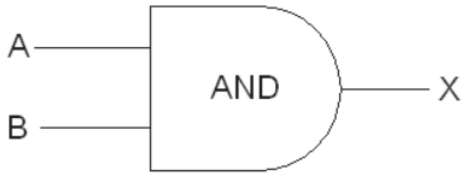
CHAPTER 1: INTRODUCTION

1.1 Boolean Logic

George Boole was an English mathematician who carried out his career in finding a way to translate qualitative values, yes or no, true or false, into quantitative values such as 0's and 1's. As such a branch of computational and logic-based mathematics was named after him and used in computational analysis involving Logic Gates. A Logic Gate is a theoretical model to represent a compound, object, or device, that is able to perform analysis on an input and produce an output that can be quantized.

This mathematical process can be represented by Truth Tables. These tables are able to predict the behavior of the device modeled by a specific Logic Gate. Different Logic Gates exist for different analysis, we consider the example of an AND Logic Gates.

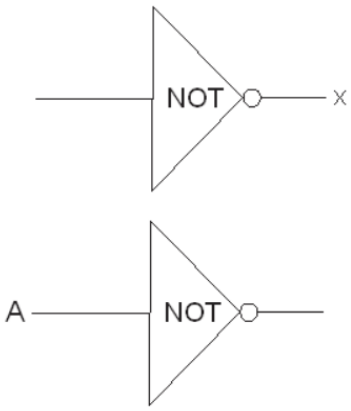


INPUT A	INPUT B	OUTPUT
1	1	1
0	1	0
1	0	0
0	0	0

Figure 1-1: AND Logic Gate representation with its corresponding Truth Table denoting when an Output is and isn't obtained.

In this example, we consider the device is in the presence of either input A, B, or both. As denoted in its Truth Table, if and only if both inputs A and B are present we obtain a true value (1) for the output. If either input A or B is present, but in the absence of the other input, there is

no output value observed. As a blank, it is also tabulated that in the absence of both inputs, no output is observed. This behavior is then tabulated for different Logic Gates to fit the need of the desired analysis. Conventional single-analyte sensors can be modeled as well with Logic Gates, though simple, degenerate Logic Gates such as YES and NOT.



INPUT A	OUTPUT
0	1
1	0

Figure 1-2: NOT Logic Gate representation with its corresponding Truth Table denoting when an Output is and isn't obtained.

1.2 STRUCTURE OF DNA

This type of analysis then requires a device that can analyze an input and only under specific conditions, denoted by the type of Logic Gate it is, produce a measurable, quantitative output. Herein is nucleic acids such DNA and RNA are proposed to fulfil this purpose given its inherent structural and predictable binding characteristics.

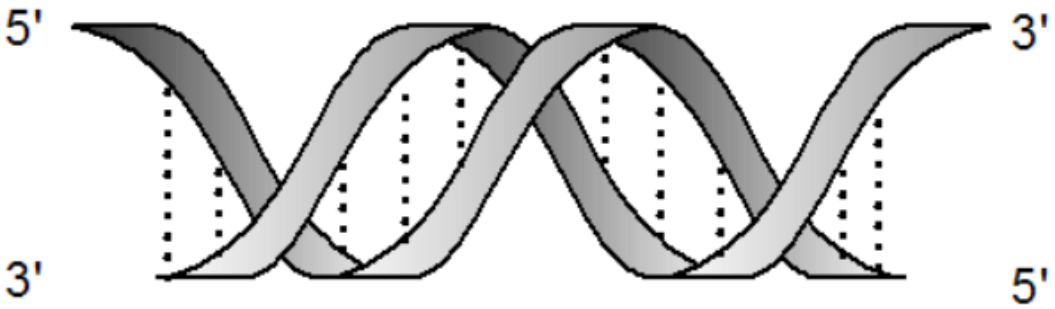


Figure 1-3: Generic DNA helix with anti-parallel strand with hydrogen bonding, denoted by dotted lines, between nucleotide bases.

These biomolecule exhibits hydrogen-bonding between its nucleotides which are called Adenine, Thymine, Cytosine, and Guanine. These bases have functional groups that allow for weak intermolecular forces that stabilize the whole structure. However, the energy associated for the attraction between these nucleotides is not responsible for the overall stability. The bulk of the energy, corresponding to the stability of the compound belongs the stacking interaction between neighboring bases. These interactions are considered for their contribution to stability, however they are also important for the applied design of these compounds in order to perform analysis through Logic Gates. In order for the molecule to interact with analytes, these stacking interactions must be considered to determine the favorability of the association and dissociation of the input-Logic Gate complex. This is a requisite for specific analysis since the output may be dependent on this dissociation.

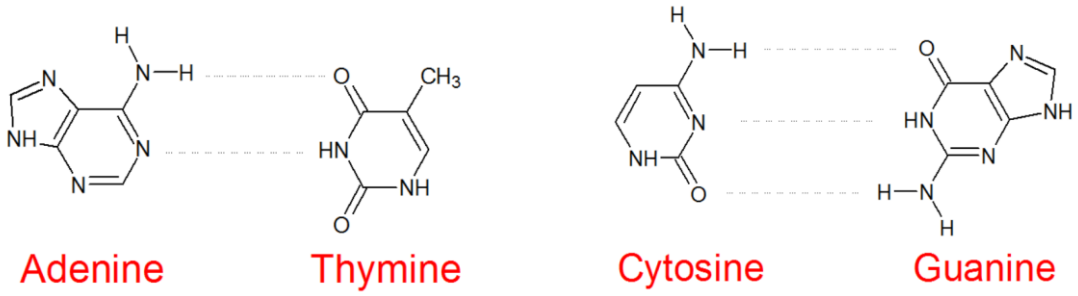


Figure 1-4: Nucleotide base-pairing following Chargaff Base-Pairing Rules

Given this hybridization pattern, we are able to design DNA sequence that is complementary to a target analyte that is also a nucleic acid. This is then useful to be able to bind, for example, foreign DNA so that we may identify a bacterial organism. If a Logic Gate is designed to perform this function and is only able to produce an output in the presence of a target, bacterial DNA strand, we are then able to confidently claim this strand is presence. Part of the novelty of this type of analysis belongs to the capacity of DNA to perform this hybridization in low concentrations such as micro, nano, femto, and even attomolar concentrations. Obtaining DNA with a specific sequence has now also become accessible through methods such as PCR and DNA Printing. If the instrument is unavailable to the lab, it may also be acquired commercially.

We may also consider the capacity for DNA to store genetic information that is specific to one organism. These then can serve as a target strand for identifying genetic mutations that might confer new properties for a specific function. Bacterial organisms such as *Micotuberculosis*, has a specific gene that is responsible for the synthesis of the beta subunit in the RNA Polymerase enzyme. This gene is the target of many antibiotics since it is present in multiple bacteria. Conventionally, drugs such as rifampin would be prescribed to target this gene and end the biological life of the organism. However, mutations have been identified, which

confer resistance to this type of medicine, which then becomes more difficult to target. Furthermore, if treated with common antibiotics, unaware of the mutation, the colonies that are susceptible to this drug will be killed, however the drug-resistant strain will remain and ultimately reproduce, yielding large colonies that are drug resistant. During this time, the patient may become more ill and may be in danger for more serious complications. As such, the necessity for a sensor, in this example, that can analyze the environment and identify such a mutation is present, will be ideal for the efficient and timely diagnosis of a disease.

CHAPTER 2: NUCLEIC ACIDS AS LOGIC GATES

Because of these characteristics, DNA is a suitable device to perform Logic Gate analysis. We consider, however, the different ways DNA has been able to be used to diagnose diseases through different mechanisms. Toe-Hold Mediated Strand Displacement (THMSD) is one of the most common methods for DNA Logic Gates. This method is based on the capacity for DNA to hybridize with specific complementary sequences that may be of interest.

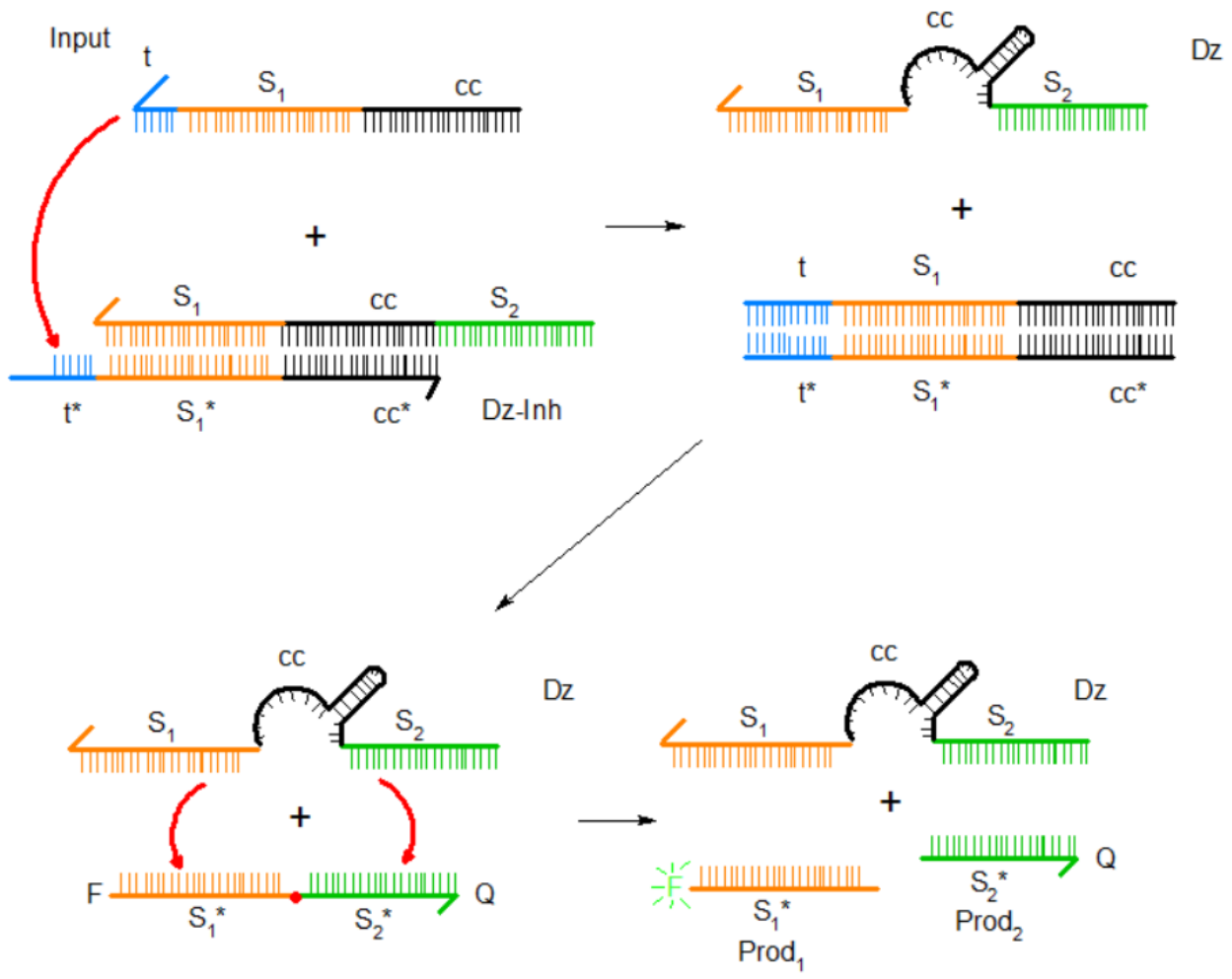


Figure 2-1: THMSD Mechanism: An input strand containing regions t, S_1, and cc in the presence of a DNAzyme-Inhibitor complex. Region t is complementary to region t on the inhibitor portion of the Dz-Inh complex. This region is referred to the toe-hold site. Hybridization between the t and t* regions displaces the bound S_1 and cc regions of the DNAzyme strand of the Dz-Inh complex and releases the strand. This release DNAzyme regains catalytic properties and, in the presence of a fluorescent compound, it will cleave its complementary strands and separate the quencher molecule from the fluorophore and induces a fluorescent output that is measurable with a fluorimeter instrument.*

The THMSD mechanism is sensitive and selective. Sensitivity is reported through the Limit of Detection (LOD) parameter. This parameter describes what concentration of an analyte must be present in order to, in the case of sensors, detect and obtain an output. For THMSD, reported LOD's were in the nano (10^{-09}) and femtomolar (10^{-12}) regions. This sensitivity is noteworthy when compared to a conventional diagnostic tool with LODs higher than the

aforementioned. Furthermore, we consider the reaction time for this reaction, which was reported to be, on average, 75 minutes. These run-time is also important as conventional diagnostic tools could take up to days or even weeks to return the results of the test. However, we observe that compared to other mechanisms, 75 minutes is lengthy.

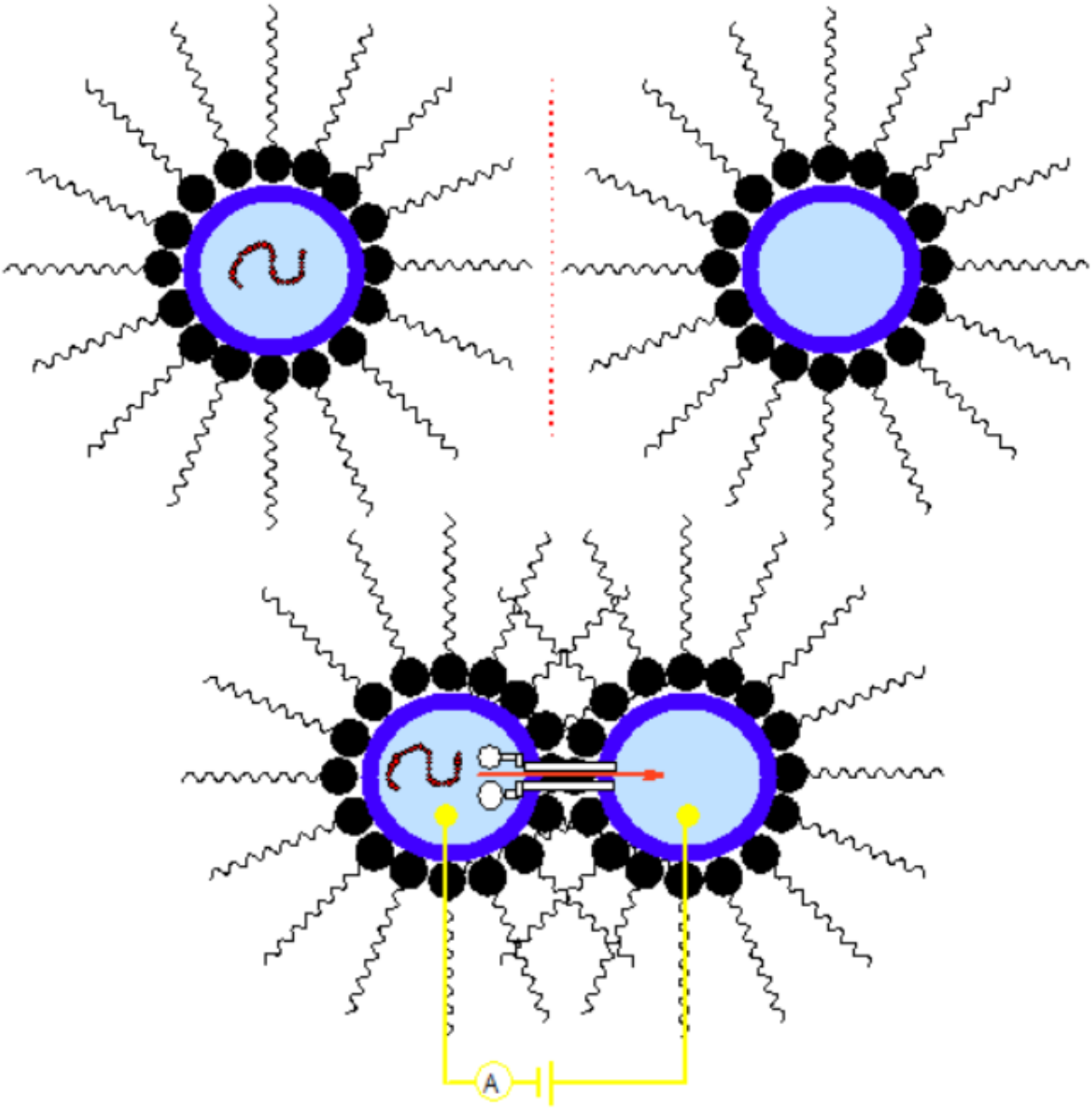


Figure 2-2: Nanopore mechanism for Logic Gate Analysis: In a lipid rich environment, micelles, containing the buffer solution which contains the nucleic acid logic gate, will aggregate with another micelle and will form a nanopore through which content between the micelles can be exchanged through a nanopore. Voltage leads are connected to both micelles and in the absence of analyte, a continuous signal is observed. Only in the presence of analyte will the DNA strand be able to travel through the nanopore and interrupt the circuit, which would be observed as a large peak in the plot of voltage over time.

The reported nanopore technology has been found to be the fastest method for diagnosing diseases through Logic Gate analysis. The basis for this method lies on the use of a nanopore.

The diameter of this nanopore is approximately 2nm. This would impede the travel of the output strand when bound with its complementary strand. This method involves THMSD in order for the output strand to be released and travel through the pore and interrupt the signal from the voltage leads. This mechanism has been reported to obtain output values in 10 minutes. This is considerably faster than conventional methods for diagnosis of diseases. However, the obtained speed comes at the cost of sensitivity. Compared to exclusive THMSD devices, the LOD of micromolar range for the nanopores is much lower, though, in some cases, still more sensitive than conventional devices.

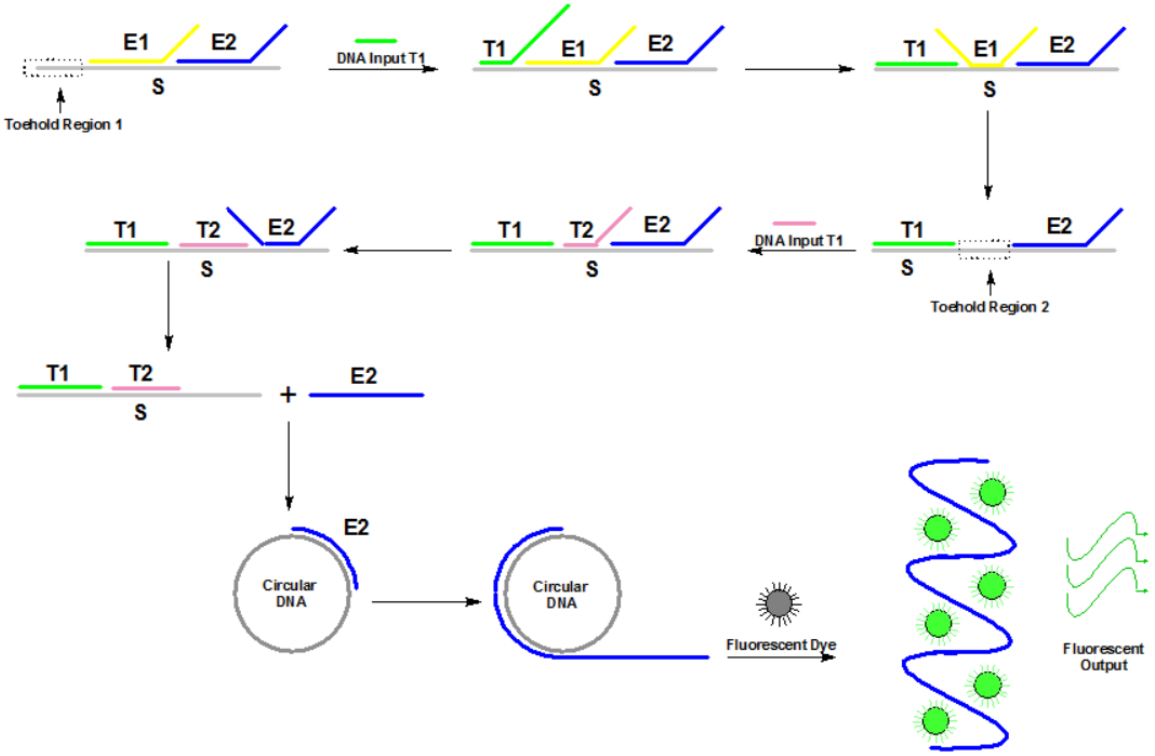


*Figure 2-3: AND Logic Gate output amplified through Rolling Circle Amplification. Strand **S** is hybridized with **E1** and **E2**. In the presence of **T1** strand, **E1** will be displaced and a new Toehold Region 2. This new vacancy allows for **T2** strand to bind and displace **E2**. This strand will only be displaced in the presence of both **T1** and **T2** strands. The output signal can then be quantized through the aid of a polymerase to extend the **E2** sequence. In the presence of fluorescent dies, a fluorescence signal will be emitted.*

We may also consider a third mechanism which can serve as the Logic Gate and also a tool to amplify output signal of a different mechanism, such as THMSD or Nanopores, in order to perform quantitative interpretation. As observed in **Figure 7**, an AND Logic Gate is designed to release output strand **E2** only in the presence of both target analytes, **T1** and **T2**. **E2** is complementary to a specific region of free-floating circular DNA and will hybridize if they come into close proximity. A specific polymerase is then employed to act on the **E2**-Circular DNA complex and elongate the **E2** strand. This elongated **E2** strand will then interact with a fluorescent dye that will ultimately yield our output signal of fluorescence, which can be quantized through the aid of a fluorimeter. This method for analysis is powerful since the signal is being amplified, which enhances the LOD of this system. As previously mentioned the THMSD mechanism has LODs reported in the nano and picomolar range. For RCA, this sensitivity has been reported to reach nano, pico, and attomolar range (10^{-18}). This is noteworthy as an already sensitive device can become six orders of magnitude more sensitive and is still able to preserve a fast experiment time with a reported run-time of 20 minutes.

CHAPTER 3: POINT OF CARE CRITERIA

These key factors of DNA, sensitivity, selectivity, and stability, are imperative for our analysis if we are to consider this technology viable for the diagnosis of human disease. The following questions is then asked: in the face of already existing and accurate diagnostic tools, can nucleic-acid based logic gates replace conventional tools and become a faster, more sensitive standard? For this matter, we resort to the Point of Care (POC) criteria to evaluate a diagnostic tool to determine if it is useful and efficient with specific target standards. POC describes the need for all diagnostic tools to be accessible, easy-to-use, and affordable.

Table 3-1: Summary of POC Parameters for Nucleic-Acid Based-Logic Gates for the Detection of Human Diseases

Mechanism	LOD	Time	Diseases
THMSD	Nanomolar: 10^{-09}M Picomolar: 10^{-12}M	60, 90 minutes	Neurological, Cancer, Bacterial, Viral, miRNA, Genetic
Nanopore	Micromolar: 10^{-06}M Picomolar: 10^{-12}M	10 minutes	
RCA	Picomolar: 10^{-12}M Attomolar: 10^{-18}M	20 minutes	

Since the analysis portion of the technology is already built-in, this device can be used by any individual and can be exported to all locations. Because the Logic Gates can be tuned to detect specific diseases, an assay could be developed, which would take the form of an OR Logic Gate. This device has the capacity of being used any individual with ease. Moreover, the output, which can take the form of a simple fluorescence signal, provides sufficient information to inform someone of a serious condition and seek appropriate medical care well in advance of more serious, end-stage phases of certain diseases. We consider the example of micro RNA molecules (miRNA), who's mere presence is indicative of serious damage or conditions, for

example, in the heart (miRNA-21 presence indicates cardiac hypertrophy). These molecules, which exist in low abundance, must be detected early on so that appropriate and timely medical care can be administered so that the patient has a greater chance of recovery or even survival in life-threatening conditions.

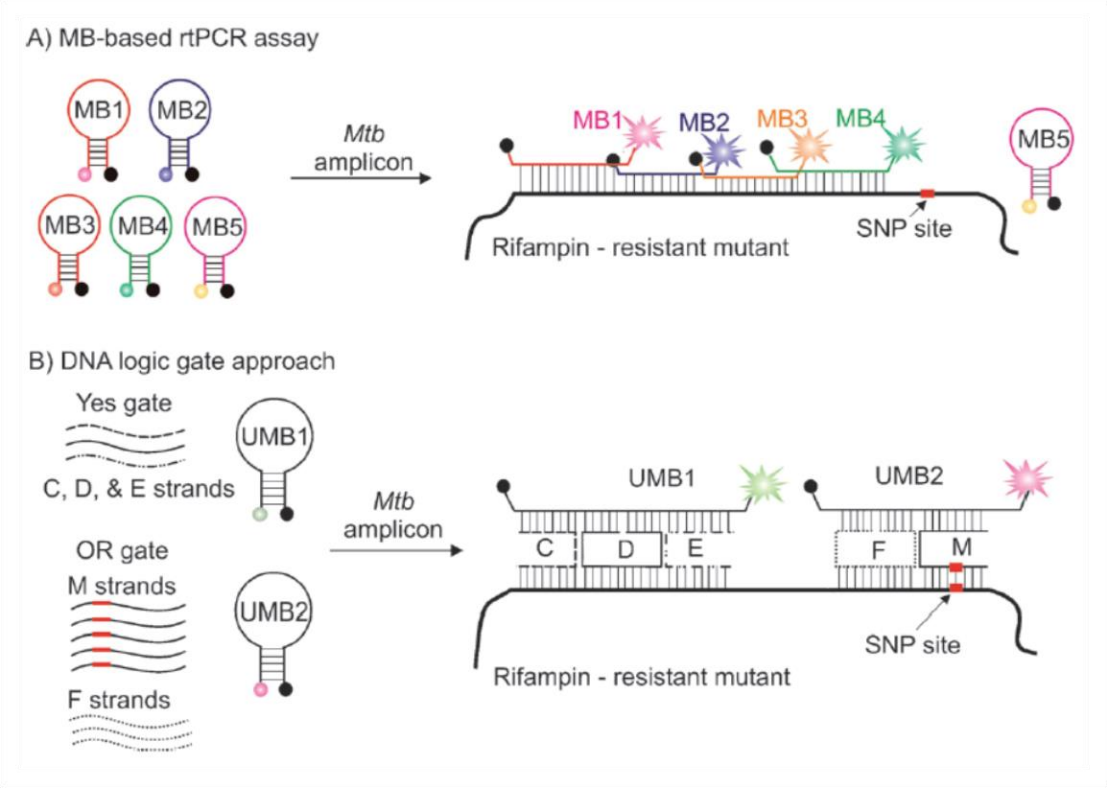


Figure 3-1: (a) Conventional diagnostic tool for rifampin-resistant mycobacterium tuberculosis. This device uses 5 different sensors that look for mutations that confer the drug resistance, these may be called YES Logic Gates, that will produce a fluorescent signal if successfully hybridized. (b) Logic Gate diagnostic tool that uses 2 Logic Gates, YES and OR, where the former identifies the presence of mycobacterium tuberculosis DNA and the latter, with multiple M strands, each unique to a type of Rifampin-resistance, will detect mutations. Both will produce fluorescent output signal with different wavelengths unique to each other.

We consider the example mycobacterium tuberculosis, a pathogen associated with the tuberculosis disease. After a confirmed diagnosis, a patient would be prescribed an antibiotic. A common drug being Rifampin. Rifampin targets the gene that codes the β-subunit of an RNA polymerase. However, mutations in this gene reduce the binding affinity of this antibiotic,

rendering a drug-resistant strain of the bacterium. Currently, there is a diagnostic tool that is able to confirm the mutation of this gene. It uses 5 molecular beacons that function as YES Logic Gates, which produce a fluorescent signal if successfully hybridized with the mutation sequence. This tool is costly as it requires 5 different sequences to be tuned and synthesized. On the other hand, the development of ribozymes, a catalytic RNA molecule, allows for OR Logic Gate analysis. While a conventional tool already exists, and is able to detect multiple mutations that confer rifampin resistance, this ribozyme Logic Gate is based on only 2 molecular beacons, one that functions as a YES Logic Gate to confirm the presence of mycobacterium tuberculosis DNA and a second OR Logic Gate, with multiple M-strands, each unique to a specific mutation sequence, that will confirm a rifampin mutation. This technology is more selective than its conventional counterpart and is able to remove the need for a technician to confirm the diagnosis.

CHAPTER 4: FUTURE APPLICATION

Furthermore, we consider the future of this technology, not only as a powerful diagnostic tool, but also expanding its capabilities and create a drug delivery device that is able to treat diseases as soon as they are diagnosed. This field, called theranostics, is being researched and already prototypes have been developed to deliver, in vitro, drugs to treat diseases after following a Logic Gate circuit.

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