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Design Brief

A novel parasitic STI detection system utilizing *Trichomonas vaginalis* specific gene expression*

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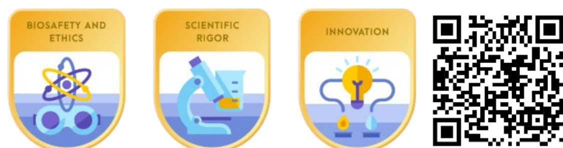
Sexually Transmitted Infections (STIs) are a widespread healthcare concern, affecting 1 in 5 individuals worldwide. Diagnosing STIs is challenging due to their often-asymptomatic development and the limitations of current testing methodologies, which makes an accessible testing method a priority in modern healthcare. Trichomoniasis, caused by the parasite Trichomonas vaginalis, is especially difficult to detect due to its asymptomatic nature and lack of sensitivity in current testing measures. We designed an at-home urinary test for Trichomoniasis, utilizing T. vaginalis transcription factors to trigger a color change in responsive genes through a cell-free transcription/translation reaction. Using the T. vaginalis-specific protein IBP39, we aim to bind it to the DNA initiator/M3 motif for transcription activation. The IBP39 protein should be present and available to transcribe due to lysed T. vaginalis appearing in urine. We will insert the aeBlue chromoprotein past the corresponding DNA initiator/M3 motif; theoretically, if the IBP39 protein is present in the urine, gene transcription will start, signaling a color change to indicate the presence of the IBP39 protein. The genetic construct will be subsequently placed into a test tube along with transcription and translation components. If the solution changes to a blue color, that is an indication of the parasitic infection. In this case, it is strongly recommended to reach out to a doctor for further evaluation and treatment. Offering a private and accessible alternative to conventional testing, this method aims to streamline sexual health screenings, ensuring earlier detection and treatment.

Keywords: Trichomoniasis, STI testing, detection system, gene expression



Sexually Transmitted Infections (STIs) present a significant global healthcare concern, affecting millions of individuals worldwide. Approximately 1 in 5 adults currently carry an STI, fifty percent of which are asymptomatic (Bishop, 2022). STIs encompass a wide range of pathogens, including over 30 different types of bacteria, viruses, and parasitesknown to be transmitted (S*exually Transmitted*, 2023). Among the various types of infections, some are curable, such as syphilis, gonorrhea, chlamydia, and trichomoniasis, while others remain incurable, including hepatitis B, herpes simplex virus, and human papillomavirus (S*exually Transmitted*, 2023). Every day, over 1 million new STI cases are diagnosed, with the World Health Organization's 2020

* *The authors were mentored by Dr. Beth Pethel from Western Reserve Academy and Becky Meyer from Massachusetts Institute of Technology . Please direct correspondence to: pethelb@wra.net.* *This is an Open Access article, which was copyrighted by the authors and published by BioTreks in 2024. It is distributed under the terms of the* Creative Commons Attribution License, *which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited.*

estimate reporting 374 million new infections per year, the most common being trichomoniasis with 156 million; however, due to testing limitations across multiple mediums, the actual number of STI cases is likely much higher (*Sexually Transmitted*, 2023).

Diagnosing STIs presents unique challenges, as they frequently manifest without symptoms or present as common infections such as colds. Laboratories typically rely on blood, urine, vaginal exams, or anatomical samples like skin tissue or lymph node biopsies for testing (*How Does*, n.d.). Even with such invasive samples, the main infectious agents involved in STIs are difficult to culture, or highly sensitive to transport and storage conditions, so techniques that only detect viable microorganisms can give false negative results (Rubio-Sánchez et al., 2023). Addressing these complexities in STI diagnosis calls for continued research and advancements in testing methodologies to ensure accurate detection.

A 2016 national survey of youth aged 15–25 years reported that only 16.6% of female respondents and 6.6% of male respondents had received STI screening within the past year (Gogineni et al., 2021). While at-home testing kits have made STI testing more accessible and less invasive, they are not without their limitations. For instance, even though these tests allow individuals to collect samples in the privacy of their own homes, the samples still need to be sent to a laboratory for analysis, which involves packaging and mailing the samples, followed by the laboratory processing and analyzing the samples (Boskey, 2023). Moreover, the effectiveness of these tests can depend on the type of STI being tested for. Urine tests are primarily used to detect bacterial STIs such as chlamydia and gonorrhea where it may be more difficult to find urine testing for other STIs (Boskey, 2023).

Trichomoniasis, a sexually transmitted infection caused by the parasite *Trichomonas vaginalis*, is especially difficult to diagnose and can lead to intense side effects (*Trichomoniasis*, 2021). These side effects include genital redness, burning, and itching, pain with urination or sex, and discomfort over the lower stomach area. For women, a vaginal swab test is commonly used for testing purposes, where the sample is screened for parasites under a microscope. (*Trichomoniasis*, 2022). Men may experience a delay of days to weeks between sampling and diagnosis due to the need for a urethral swab culture, which can take up to two weeks to process. (Bano, n.d.). While Trichomoniasis is found in urine, it cannot be detected with common at-home testing kits due to its parasitic origin. Parasites often have complex life cycles, remain asymptomatic, or they will develop mechanisms to evade the host immune response, all of which renders at-home testing kits unsuitable for Trichomoniasis detection. (Lawing et al., 2000).

To provide a convenient and private testing solution, this project conveys a design of an at-home urinary strip test for Trichomoniasis. This approach leverages gene transcription by the incorporation of a promoter activated by specific transcription factors found only in *T. vaginalis*. It will use the *T. vaginalis* specific protein, IBP39, which will bind to the DNA initiator element/M3 motif to activate transcription. When the *T. vaginalis* transcription factors are present and gene transcription is initiated, a color change will indicate infection, as colorants generally become visible following the activation of a promoter (Yang et al., 2021). With these genetic alterations, this test is engineered to alter its hue upon detecting proteins associated with *T. vaginalis* in urine. To signal a color change, the aeBlue

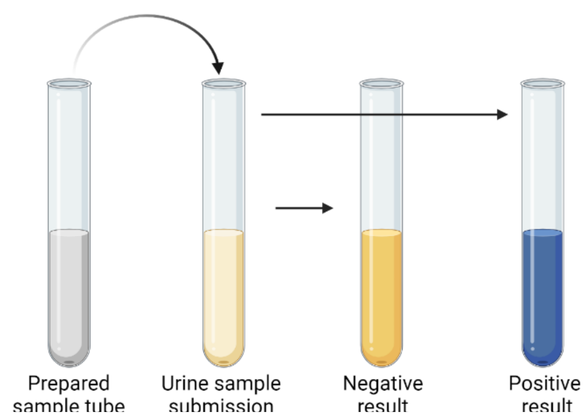


Figure 1. Illustration of the proposed testing system, with a representation of the anticipated outcome and a depiction of the process involved (Made with BioRender.com).

chromoprotein is inserted past the initiator element/M3 motif. The genetic construct will be placed into a test tube along with other transcriptional and translational components. When the *T. vaginalis* transcription factors are present and gene transcription is initiated, the media will turn into a blue color upon detection of the parasitic infection. Figure 1 depicts this process step by step, focusing on the color-changing perspective. This project aims to offer patients a new accessible method for sexual health screenings, providing an alternative to invasive and time-consuming testing methods. With the confidence of privacy and the facilitation of earlier diagnosis, this testing system has potential as a more streamlined path to treatment.

Systems level

As a solution, the detection system is designed to respond to the presence of the IPB39 protein, a biomarker exclusive to Trichomoniasis (Liston et al., 2001). Gene transcription will be utilized to detect IBP39 using protein-specific promoters. Upon the collection of a urine sample, the user will transfer the specimen into a specialized test tube pre-loaded with transcription and translation components, and our DNA construct that is necessary for detection (Figure 2)

In the absence of the IBP39 protein, the solution will remain a yellow color, indicating a negative result. However, if the IBP39 protein is present, gene transcription will activate the aeBlue chromoprotein. The expression of this chromoprotein, characterized by its ability to absorb light and emit a deep blue color, signifies a positive detection (Tsang, 2020). The transition of the solution from yellow to bright blue provides a visually discernible indication of the IBP39 protein's presence, suggesting a diagnosis of Trichomoniasis. In a separate test tube, we will replace the initiator element/M3 motif with a constitutive promoter. This will serve as a positive control for our cell-free system. In this study, we will employ lyophilization to preserve the integrity of our designed system, ensuring long-term stability and facilitating convenient storage options.

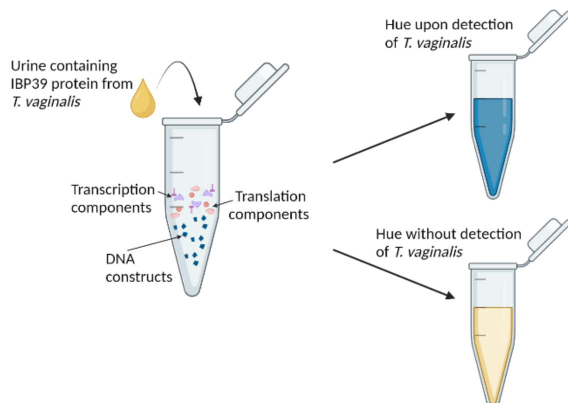


Figure 2. Illustration of the testing system with its essential components such as transcription and translation components, a DNA construct, the urine sample, and additionally, the anticipated outcome (Made with BioRender.com).

Device level

To create our testing concept, we will include a DNA construct containing the necessary components to initiate gene transcription and indicate a reaction. In this DNA construct, we will insert the promoter sequence that initiates gene transcription upon detection of the IPB39 protein. In addition, we will include transcription and translation factors adjacent to a TXTL kit to amplify the IBP39 protein and prepare the sample for transcription. When the urine containing the IBP39 comes in contact with the solutions of the testing kit, the promoter sequence will recognize the protein and initiate transcription. With the inclusion of the aeBlue chromoprotein, which is instrumental for turning the system into a bright blue color, the system will indicate detection.

Parts level

In the proposed experimental framework, the system will employ transcription and translation modules that are analogs to the constituents present in cell-free TXTL systems. Specifically, the system is designed to leverage the components and amplify the expression of the IBP39 protein through the use of ribosomes, amino acids, nucleotides, and RNA polymerase. This addition mirrors

the biochemical environment necessary for efficient protein synthesis, which facilitates its optimal preparation for testing (Garamella et al., 2019).

To facilitate the transcriptional activation of the IBP39 gene, our experimental design needs the M3 motif alongside the transcription initiation region (Inr) specific to *T. vaginalis*. Notably, within the genomic architecture of *T. vaginalis*, the Inr element stands as the sole well-characterized core promoter element (Lau et al., 2006) However, the interaction between the highly conserved Inr element and the M3 motif facilitates the transcriptional regulation of the IBP39 protein gene (Smith et al., 2011). We will also include the U4 terminator associated with IBP39 to maximize transcription and, ultimately, maximize results (Simoes-Barbosa et al., 2008).

In this construct, the aeBlue chromoprotein, used for its photoreceptive properties and emission of a deep blue color under ambient light, serves as a transcriptional signal (Lundin, 2012). It is placed downstream of the promoter sequence, enabling its expression upon transcriptional initiation. This configuration exploits the aeBlue chromoprotein's stability for prolonged signal detection, simplifying the construct while enhancing observational utility.

Safety

The safety of protein purification and use of TXTL kits must be considered in laboratory experimentation, as safety protocols must be strictly followed. All procedures should utilize kits provided by reputable biotechnology companies, ensuring that they comply with biosafety level 1 standards. Researchers should adhere to established guidelines for handling equipment and biological samples, including urine samples (Cell-Free Protein, 2008). This involves wearing appropriate personal protective equipment (PPE) such as lab coats, goggles, and gloves to minimize the risk of exposure to potentially hazardous materials, such as the assays used for experimentation (*Personal Protective*, n.d.).

In our experimentation, handling urine samples classifies as BSL2. Similarly, the experimental use of a *T. vaginalis* lysate will also be Biosafety Level 2 (*Tissue Culture*, n.d.) since *T. vaginalis* is classified as a Biosafety Level 2 organism (*Trichomonas Vaginalis*, n.d.). While handling solutions under Biosafety Level 2, special safety equipment such as biosafety cabinets and appropriate PPE must be used. In addition, scientists must practice proper containment, decontamination, and disposal methods to minimize the risk of exposure. (*Basics of Biosafety*, n.d.)

In our testing model, patients should perform the test in a clean environment with minimal contaminants. Safety protocol and instructions will be provided with a test to ensure proper use. Patients should wash their hands before and after using the testing system and use gloves ("Review Safety,"

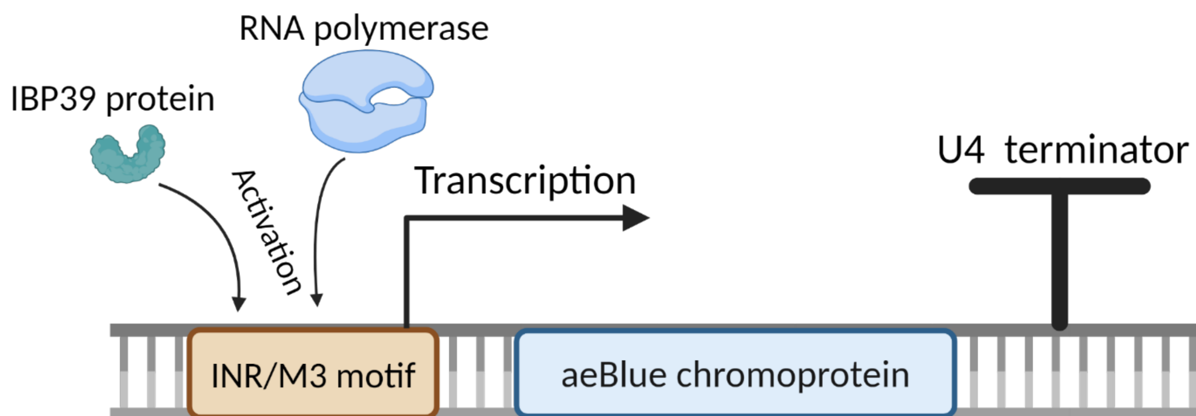


Figure 3. Schematic representation of the DNA construct comprising the promoter sequence with the aeBlue chromoprotein. The figure illustrates the interaction between the IBP39 protein and RNA polymerase (Made with BioRender.com).

2006).

Discussions

If our experimentation proves successful, a new method towards rapid STI testing would be introduced, opening the possibility to explore its application for detecting various other infections. To further extend our research, we aim to broaden the scope of our investigation by incorporating the detection of additional STIs utilizing our model in a separate system. To establish this extension, we need to identify an associated protein for a targeted STI, along with a corresponding promoter or promoter sequence. Incorporating these new components will enable gene transcription to be triggered upon interaction with the specific protein associated with the additional STI.

For instance, the Major Outer Membrane Protein (MOMP), which is typically linked with *Chlamydia trachomatis*, can be detected in genital swabs of patients with Chlamydia (Wen et al., 2016). If we adopt a DNA construct similar to our previous design, we could utilize the specific *ompA* promoter (named P3) associated with MOMP to initiate transcription upon its detection (Cong et al., 2016). We could also incorporate a color-changing element such as chromoprotein eforRed, which naturally displays a red hue when expressed (Sun, 2011). This serves as an example of how our testing construct could be applied to other infections.

If this approach proves successful, we can explore the possibility of integrating multiple models into one testing kit. This integration may involve employing different color-altering genes to signify the detection of various STIs within a single test tube. This expansion not only addresses the pressing need for accessible and user-friendly diagnostic tools but also contributes to the advancement of public health initiatives aimed at combating the spread of STIs. By pursuing these goals, we endeavor to enhance the overall effectiveness and inclusivity of STI detection methods. Accessible testing like this will contribute to better healthcare outcomes for individuals worldwide, especially in geographical areas where testing may be particularly limited.

Next Steps

Our primary objective is to identify the most efficient method for detecting Trichomoniasis, ensuring that the construct we develop is both feasible and reliable. To evaluate the accuracy of our system, we will obtain our specifically designed DNA construct and integrate it with TXTL components to comprise our testing kit. Our evaluation will progress through several stages. Initially, we will assess the effectiveness of our test on concentrated IBP39 protein through protein purification. We will use a similar concentration of IBP39 to its concentration in urine for this purpose. Following this, we will obtain a cell lysate from *T. vaginalis* and utilize the purified lysate as a sample for our construct. Once we establish the viability of our test at this stage, we will proceed to test the lysate at various concentrations resembling those found in urine samples. If successful, it is possible to move on to human testing using both positive and control urine samples. This step will ensure that our system's performance remains unaffected by other components commonly found in urine, validating its use for practical application. If the use of the blue chromoprotein does not provide sufficient color change when IBP39 is present, we will consider switching to the GFP (Green Fluorescent Protein).

Author contributions

M.Z. is the founder of the project. S.Y. and M.Z. conducted the preliminary research, found and studied the proteins and promoters, and are fully and equally responsible for the construct design. M.Z. studied the safety of the project in terms of experimentation and home testing. S.Y. is the main editor and script writer of the paper. S.Y. also researched the current methods and explored the benefits of our construct across other diseases. M.Z. is the main artist for the project and led the effort to create a video. S.Y. contributed the next steps and discussion.

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