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Validation & Optimization of CAPTURE™: Rapid Diagnosis of Pathogens Causing Urinary Tract Infections

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Cover Page Footnote

This project would not have been possible without the support and advisement of many people. Acknowledged here are all who contributed to this project. Thank you to the research advisor, Dr. Louise O'Keefe, PhD, CRNP. Thank you to GeneCapture, Inc. with Paula Koelle, Senior Scientist, Peggy Sammon, CEO, Dr. Krishnan Chittur, CTO, Savannah Dewberry, Zac McGee, and Carter Wright. Thank you to MicroArrays, Inc. Thank you to HudsonAlpha Institute for Biotechnology BioTrain Internship. Thank you to the University of Alabama in Huntsville Faculty & Staff Clinic with Amber McPhail, MSN, CRNP. Thank you to the University of Alabama in Huntsville Honors College.

Validation & Optimization of CAPTURE™:

Rapid Diagnosis of Pathogens Causing Urinary Tract Infections

Elizabeth Marie Richards Gates
College of Nursing

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Abstract

Background – The number of bacterial strains resistant to antibiotics is growing exponentially. Antibiotics are often prescribed more than needed due to the delay period in diagnosing the pathogen and giving treatment. This delay often pushes prescribers to give a broad-spectrum antibiotic or pushes them to make the patient wait for the proper treatment, in turn allowing the bacterium to potentially mutate. This creates a need for a more rapid, easily used, and cost effective method of identifying pathogens. The objective of this study is to validate the CAPTURE™ method and its ability to identify pathogens, reduce the mechanical processing time, and optimize the sample preparation and lysis protocols.

Methods – The methods for this project included several steps: captor design, sample acquisition, sample prep, sample testing using the CAPTURE™ method, assay analysis, sample comparison, and optimization. Urine samples were gathered from patients with suspected urinary tract infections at the University of Alabama in Huntsville's Faculty & Staff Clinic. Each patient consented to give two urine samples, one sample was sent to the facility's contracted lab for routine identification. The second sample was sent to GeneCapture and considered discarded and de-identified. Institutional Review Board approval was received and is on file from the University of Alabama in Huntsville.

Results – To determine the accuracy of the CAPTURE™ assay pathogen identification, the results from the contracted lab were compared to the results of the CAPTURE™ assay. The results showed that the CAPTURE™ method has the ability to identify pathogens from the lysates.

I. Introduction

The purpose of this research study is to test the validity and reliability of the CAPTURE™ method. The CAPTURE™ was created by GeneCapture, Inc., a start-up company in the Biotechnology field. The CAPTURE™ (Confirming Active Pathogens Through Unamplified RNA Expression) method uses a molecular diagnostic technique to determine a genetic match for the pathogen using DNA captors that will identify the pathogen in less than one hour for minimal cost. CAPTURE™'s purpose is to rapidly diagnose bacterial and viral pathogens. The need for a more rapid diagnostic testing method continues to grow exponentially as antibiotic resistance increases, strains mutate, and cost of healthcare rises.

To begin the study, a partnership was cultivated between GeneCapture, Inc. and the University of Alabama in Huntsville's Faculty & Staff Clinic, a division of the College of Nursing. Institutional Review Board approval was received from The University of Alabama in Huntsville (Appendix A). Patients that presented symptoms of a urinary tract infection (the phrase urinary tract infection will be further noted as UTI in this text) were recruited to participate in the study and consented to give two urine samples (Appendix B, 1) (Appendix B, 2), one to be run through the CAPTURE™ method and the other sent for a culture and sensitivity to the contracted laboratory for usual diagnostic tests and treatment.

The anticipated outcome of this research is the verification of CAPTURE™, through comparative results, to correctly identify these common urinary tract pathogens. These findings may advance further into other infectious pathogens and may be useful in correctly and rapidly diagnosing common to serious infections in clinics and areas with no access to laboratory facilities.

II. Background

PCR

Polymerase chain reaction (denoted as PCR through the rest of the text) is a technique used in many laboratories and development companies worldwide. This technique was invented in the 1980s and has been making many advances in recent decades. PCR allows a particular DNA region, selected by the researcher, to be targeted and copied ("Polymerase Chain Reaction", 2017). Generally, a researcher would want multiple copies of a specific region to analyze it for certain characteristics or functions or pathogen identification (Järvinen, 2009).

For PCR to function, a DNA polymerase enzyme is required to make new strands of DNA. Taq polymerase is the most common DNA polymerase used for PCR. This particular polymerase is derived from a heat-tolerant bacterium, making it ideal in PCR. PCR uses high temperatures at many points in the process to denature the template DNA ("Polymerase Chain Reaction", 2017). For the polymerase to work, a primer is needed. Two primers are used in a PCR experiment; these primers are short pieces of single-stranded DNA. Each primer is tasked with surrounding the target region of DNA and binding to opposite strands of the template DNA, leaving the target region unbound ("Polymerase Chain Reaction", 2014). When the strands are cooled, the primers are allowed to bind to the template. Once they are bound, the temperature is increased allowing synthesis of new DNA.

This process occurs 25-35 times in a single PCR experiment. Each experiment takes between two and five hours. The time will depend on the length of the DNA sequence that is targeted and being copied. ("Polymerase Chain Reaction", 2014) After the reaction is complete, gel electrophoresis is often used to visualize the reaction. This process takes between 1 and (Järvinen, 2009) 1.5 hours ("Agarose Gel Electrophoresis", n.d.). PCR can also be analyzed on a microarray (Järvinen, 2009). Often, when analyzing a PCR reaction, a researcher may find unexpected amplifications. A limitation of PCR is its need for sample purity. If a contaminant is involved in the reaction, the contaminant may be copied as well, skewing the results (Brookman-Amissah, 2014). Extra steps to prevent contamination between samples must be taken.

CAPTURE™

Confirming Active Pathogens Through Unamplified RNA Expression (the phrase will be further noted as CAPTURE™ in this text) is a method developed by GeneCapture, Inc. to be used as a rapid diagnostic tool. CAPTURE™ uses a stem-loop captor to identify pathogens. The stem is a specific sequence that does not change. The loop is a unique sequence from the pathogen(s) that the specific panel is trying to identify.

The CapLab is the machine, designed by GeneCapture, to carry out the CAPTURE™ process. A lysate from a sample (in this case from urine) was placed in a cartridge along with buffers and targets. The sample is run across an array of appropriate stem-loop captors. If the sequence of the sample finds a match in one of the loops, it will bind. Once the sample binds, the loop is forced to open and an oligonucleotide binds to the hanging stem. This oligo acts as the detector, producing the signal needed to analyze (Boeteng, 2013). (See Figure 1)

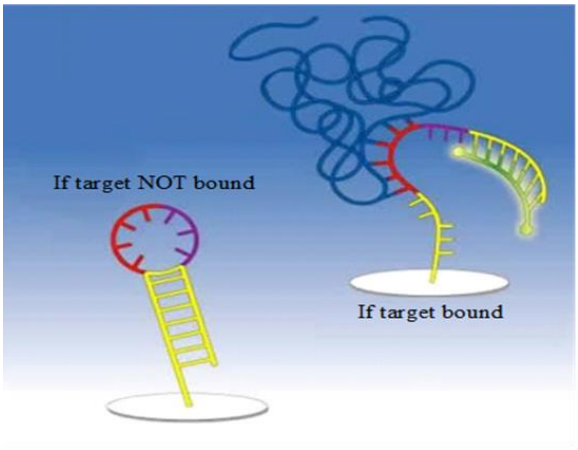

Figure 1: *Pathogenic nucleic acids pass over the captors on the microarray and bind to their complement in the loop region; this binding forces the stem to open (the captors only remain open if the correct target has bound). The captors that remain open then bind to a universal detector. The microarray is washed stringently to remove any mismatched or unbound nucleotides* (Koelle, 2014.)

After this process, the array is cooled back down to allow the loops with no binding to close. The microarray can then be analyzed for the specific pathogen (Boeteng, 2013). (See Figure 2)

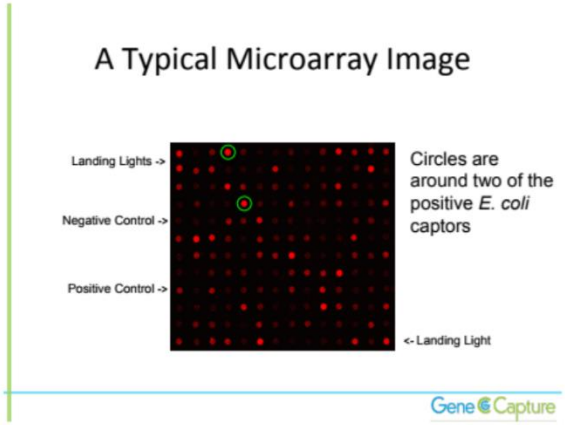

Figure 2: *Microarray. Identifies which captors bound to the sample. The fluorescent detector shows the signal from each bound captor. The 'landing lights' allow the person analyzing to orient the direction of the array and to correctly identify which captors bound.* (Koelle, 2014.)

CAPTURE™ looks at expressed RNA rather than rDNA. It has the ability to target hundreds of pathogens in a single assay (Pusey, Chittur, 2007). Captors do have the potential to cross-react with other species but statistical cluster analyses are completed to counter this possibility. For more information regarding the specific binding processes and hybridization, refer to Novel stem-loop probe DNA arrays: Detection of a specific acetotrophic 16s ribosomal RNA signatures, 2012, Jonas Boateng, Robert Zahorchak, Joel Peek, & Krishnan Chittur.

PCR & CAPTURE™ Comparison

	PCR	CAPTURE™
Disease Coverage	Looks at one pathogen at a time	Targets hundreds of pathogens in a single assay
Specificity	Sample purity is key, contaminates are often amplified or duplicated	Crude lysates from any matrix (urine, blood, saliva)
Cost	>$100/test	~$20/test
Sample Turn-Around-Time	few hours to days	Currently 1 hour, goal <30 minutes
Primer Binding	rDNA	Expressed RNA

In this table, the CAPTURE™ diagnostic is compared to multiplex PCR

UTIs

Many factors, lifestyles, and diagnoses such as an active sexual life, menopause, diabetes, difficulty emptying the bladder, or obstruction may lead to UTIs. All of these factors influence the high occurrence rate of UTIs, especially among women. Research has shown that 40 to 60 percent of women will have at least one urinary tract infection in their lives (Stapleton, 2017). Male and female UTIs add up to approximately 150 million cases worldwide per year related with an estimated $6 billion dollar healthcare expenditure (Baldato, 2016). Due to the rate of occurrence, ease of access, and the need for rapid identification, this study chose to collect and analyze suspected UTI samples.

The current method for identifying pathogens in human urine samples is a lengthy process, usually taking two to three days to complete. First, a clean catch midstream void is collected and then a dipstick urinalysis is performed, ending with a quantitative urine culture completed by a lab (Baldato, 2016). Due to this delay in a specific diagnosis of the infecting pathogen, a broad-spectrum antibiotic is often given (Zeeman, 2007). If the culture results differ from the original speculation and the antibiotic given does not hinder the pathogen identified as the infectant, the patient has received an unnecessary treatment that could later lead to antibiotic resistance (Ventola, 2017). Additionally, if the pathogen is not identified rapidly, the pathogen has the potential to mutate and move, causing acute pyelonephritis (kidney infection) (“Urinary Tract Infections”, 2017).

Antibiotic Resistance

Antibiotic resistance is the innate or acquired ability of bacteria to resist the effects of antimicrobial agents. Acquired resistance is of more concern due to the possibility of rendering currently effective drugs, ineffective (Burchum, 2016). Over time, bacteria may become less sensitive to an antibiotic or may lose all sensitivity (Burchum, 2016). Antibiotic resistance is increasing due to overuse and misuse of antibiotics (“Get Smart”, 2014), extended hospitalization (Burchum, 2016), and antibiotic use among food sources i.e. giving animals antibiotics (“Get Smart”, 2014). Research studies have shown that 30 to 50 percent of antibiotics given were incorrect for the infection or the duration of the treatment (Ventola, 2017).

Antibiotics are only effective against bacteria, not viruses or fungi. These agents are not effective against common colds, gastrointestinal viruses, the flu, most sinus infections, or ear infections (“Get Smart”, 2014); however, they are often prescribed for these diagnoses due to delayed diagnostic testing to identify the specific infecting pathogen, patient knowledge deficit, and improper provider prescriptions.

III. Methods

Captor Design

Captor design is crucial to the functionality of the CAPTURE™ method. The purpose of the captor design process is to identify a sequence unique to each pathogen with little to no crossover with other species. Some pathogens fall in the same phylum or genus but

still need to be differentiated to come to a proper diagnosis. There is still the potential of crossover between species; therefore, statistical cluster analyses were completed to account for the potential interactions. GeneCapture owns a proprietary software program that utilizes uploaded genomic information of the most highly expressed genes to seek out sequences that are common to an individual pathogen but also retain unique areas that set it apart from other any others. The sequences are then attached to the 'universal stem' and placed on the array. Though the captor design process looks for a unique sequence for each pathogen, it still must take into account the constantly changing nucleic acids in each organism. For this reason, multiple captors are designed for each pathogen and placed on the array; up to 5 captors will be used for one pathogen. Positive and negative controls are included on each array.

Sample Acquisition

Samples were gathered from patients with suspected UTIs that presented to the University of Alabama in Huntsville's Faculty & Staff Clinic. The staff of the clinic asked the patients if they would give two samples, one for regular lab testing and one to be given to GeneCapture for testing. Patients were made aware that none of their personal information would be given to GeneCapture or be used for this study. The samples were considered discarded and de-identified. Samples were kept in a refrigerator at the clinic until time of acquisition. The samples were then transported, by a GeneCapture staff member, to the lab in a marked biohazard container.

Sample Prep

To prepare each sample received from the clinic, a specific series of manual steps were followed. In this process a lysis buffer was used to break open the cells of the pathogen and fragment the RNA. (The specific process and lysis buffer have been excluded from this text for proprietary reasons). After the steps were completed, a sample of the lysate was plated to confirm sterility. In addition, through dilution plating, a portion of each original urine sample was used to confirm and compare growth and identity of the pathogen with the results received from the clinic's contracted laboratory.

Sample Testing

The lysate along with the buffers (the specific buffers have been excluded from this text for proprietary reasons) are paced in a cartridge designed specifically for the CapLab. The machine uses a simple pump, like that used in a common aquarium, to move a succession of fluids (the lysate and buffers) across the array. As the fluid moves across the array, the target (lysate) binds to the corresponding captors. A rinse is then completed to wash away any excess target. The fluorescent detector will then bind to the open captors and another rinse will be completed. A final rinse is completed to stabilize duplex formation before scanning.

Assay Analysis

The future of the CapLab will allow the assay analysis and, therefore the identification of the pathogen, to be completed within the CapLab itself. (See Figure 3).

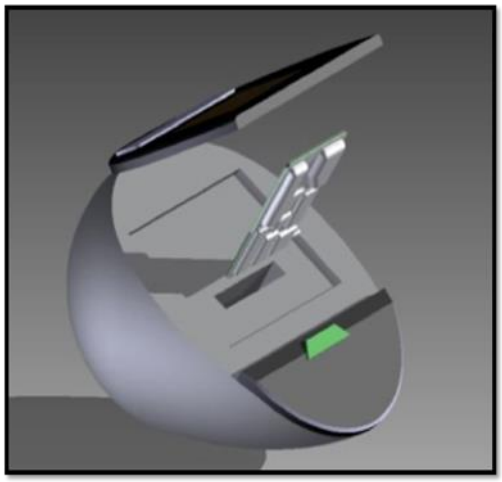

Figure 3: *Vision for future CapLab design.*

During this study a GenePix 4200b Scanner (Molecular Devices, Inc, Sunnyvale, CA) was used to scan the resulting microarrays containing the now closed captors and bound florescence. (See Figure 2). With the map of the placement of each captor, the pathogen was identified from the signal on the array.

Comparison

Once the assay was analyzed and the infecting pathogen determined by the CAPTURE™ method, the results were compared to the results given by the University's contracted lab. The results from the lab took 2-3 days to receive.

Optimization

After each sample was tested and analyzed, the process was scrutinized for areas that needed improvement due to overlap, lack of specificity, and chemical change. During the time that this particular study was completed, the lysis protocol was updated several times and captors were redesigned.

IV. Results

Sample	+/-	ID	+/-	ID
UAH001	(+)	Group B Strep	(-)	
UAH002	(+)	E. coli	(-)	
UAH003	(-)	Mixed flora	(-)	
UAH004	(-)	No growth	(-)	
UAH005	(+)	E. coli	(+)	E. coli
UAH006	(-)	No growth	(-)	
UAH007	(-)	No growth	(-)	
UAH008	(-)	No growth	(-)	
UAH009	(-)	No growth	(-)	
UAH010	(-)	Mixed flora	(-)	
UAH011	(+)	Group B Strep	(-)	
UAH012	(-)	Mixed flora	(-)	
UAH013	(+)	E. coli	(+)	E. coli
UAH014	(+)	K. pneumoniae	(+)	K. pneumoniae
UAH015	(-)	No growth	(-)	
UAH016	(-)	Mixed flora	(-)	
UAH017	(-)	No growth	(-)	
UAH018	(-)	No growth	(-)	
UAH019	(+)	E. coli	(+)	E. coli

Shown above, the results from the contracted lab are compared to the results of the CAPTURE™ assay. When a negative result was received from the CapLab and not the laboratory, the captors as well as the lysis protocol were analyzed for areas that needed optimization. Some captors had to be redesigned to have a higher specificity and less overlap with other species. The lysis protocol had to be adjusted to account for samples that yielded very small amounts of lysate.

The CAPTURE™ results for sample UAH002 did not yield E.coli as the culture results did. It was determined that the RNA extraction process for this sample resulted in too little detectable RNA. E. coli was detected in future samples. Culture results for the samples UAH001 and UAH011 found Streptococcus agalactiae or Group B Strep. Further research showed that this particular bacterium can be a cause of UTIs, a result of a kidney stone and can be very harmful during pregnancy. No captors for this bacterium were on the panel used in this study; after discussion, captors for this bacterium were designed and added to a new panel for future studies.

A lab culture of "mixed flora" indicates that only low levels of multiple bacteria were grown but were not an indicator of infection. The mixed flora result was most likely yielded due to a non-sterile urine sample. The urinary tract and urine are sterile unless contaminated by pathogens. Normal flora are bacteria that live on or in a human body at all times. These bacteria are not pathogenic and aid the body in fighting off harmful bacteria. Every person carries normal flora in areas such as the gastrointestinal tract, nose, mouth, skin, and specifically, around the genitals. Using a clean catch method to obtain a urine sample would eliminate this flora from contaminating a sample; however, patients often complete the clean catch method incorrectly, resulting in a contaminated sample. (See figure 4 for steps to gather a proper clean catch sample)

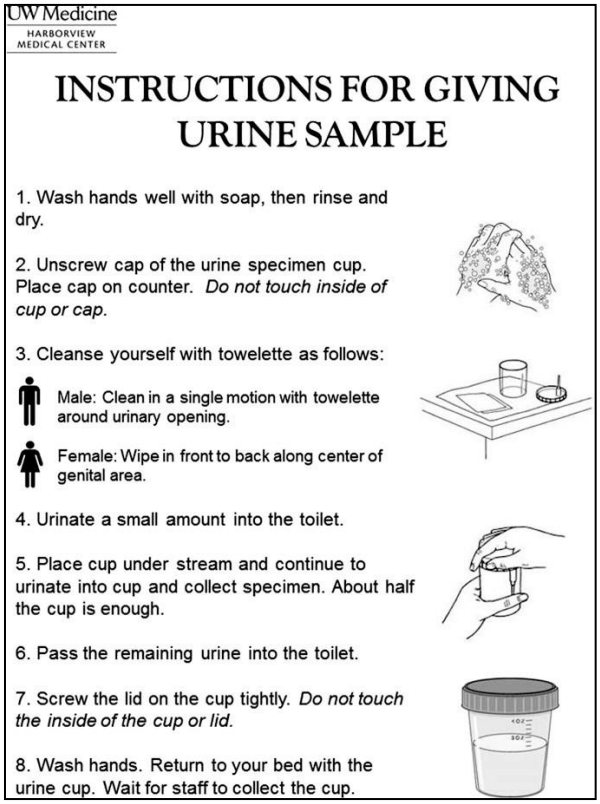

Figure 4: *Steps to properly gather a clean catch urine sample.*

V. Conclusion

This study, which specifically looked at UTI samples to validate and optimize the CAPTURE™ method is an ongoing study and is now being conducted alongside other more in- depth studies at the GeneCapture lab. Through this research study, it was determined that the CAPTURE™ method does have the ability to identify specific pathogens from human samples. It was determined that the CAPTURE™ method can be completed in one hour with the direction of being completed in less time as more steps in the process become a part of the automation of the CapLab. Further optimization of the UTI captor panel is currently underway.

The CAPTURE™ method and this study have many implications for medical and nursing practice. This form of rapid diagnostics allows for determination of the infecting pathogen in a clinic and at the bedside for timely and accurate treatment, thereby reducing the exponential growth in the number antibiotic resistant pathogens. This technology has the potential and accessibility to be used in areas with little to no access to laboratories or medical facilities such as: military camps, disaster areas, and wilderness/remote installments. There are possibilities for use in pandemic emergence and tracking. CAPTURE™ can also be utilized in agriculture to identify plant pathogens reducing crop destruction and increasing food insecurity. There is also potential for cancer detection and treatment monitoring. This research is ongoing and it will be exciting to determine how this technology will improve health outcomes, in the future.

Publication of the expanded validation study is expected in the near future.

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