Is CRISPR-Cas12a the future in fast and accurate pathogen detection?

- Early and fast detection of pathogens is essential for controlling the spread of disease.
- The currently used methods have several limitations, including standardisation and high cost.
- Clustered regularly interspaced short palindromic repeats (CRISPR-Cas12a), discovered a few years ago, is a method that detects even small levels of pathogens.
- Professor Kevin J Zwezdaryk and a team of dedicated researchers at the Tulane University School of Medicine, USA, are working on a cost-effective, CRISPR-Cas12a-based pathogen detection tool aiming to upgrade patient care.

Pathogens are microorganisms, including viruses, bacteria, and fungi, which invade our bodies and make us sick. Many of these pathogens cause transmittable diseases; therefore, their early and accurate detection is essential not only for choosing effective treatments, but also for controlling the spread of disease and preventing pandemics. On top of this, modern medical practices, such as organ transplantations and new targeted treatments for cancers, have led to a growing number of people with weak immune systems whose lives depend on early and accurate diagnosis of infections.

The most sensitive methods of diagnosing pathogens, such as real-time quantitative polymerase chain reaction or RT-qPCR, are based on the detection of the microorganism's genetic material – their DNA or RNA. Unfortunately, such methods often require expensive equipment, specific environmental circumstances, and trained personnel. In an effort to address the above limitations, Professor Kevin Zwezdaryk and his team at Tulane University School of Medicine, USA, have been working on the development of a novel pathogen detection system based on a specific type of clustered regularly interspaced short palindromic repeats (CRISPR), called CRISPR-Cas12a.

Borrowing from the microcosm

CRISPR is a method that scientists use to perform gene editing by identifying a specific section of a gene, cutting it off, and replacing it accordingly. This enables them to rectify faulty genes that cause defects and diseases in humans and other organisms. The CRISPR method is based on the defence system of bacteria against their invading viruses, and uses enzymes called CRISPR-associated proteins or Cas proteins which function as molecular scissors that cut out viral DNA.

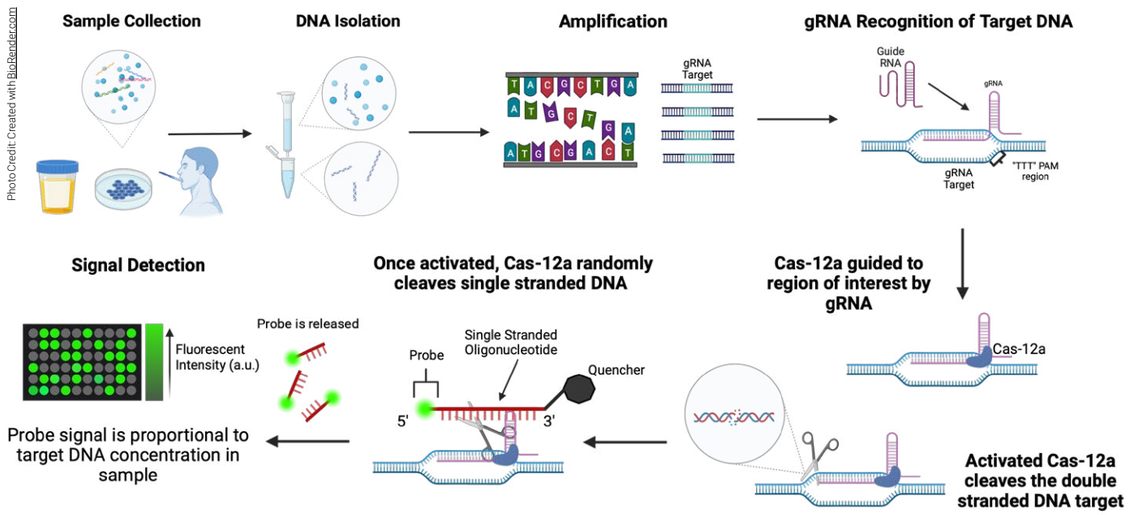
The most popular Cas protein is Cas9. Cas9 utilises a guide RNA (gRNA) that binds to the target DNA sequence, which is then cut off by the enzyme. Since its discovery, this two-component system has been revolutionising medical research. Not only does it offer new treatment options for inherited diseases, but it also improves animal husbandry and agriculture by helping create new and improved breeds. Basic applications of Cas9 include base-editing, RNA tracking, and fluorescent imaging.

Besides its numerous applications in biotechnology, CRISPR can also be used to detect the DNA or RNA of pathogens accurately and reliably. After detecting the DNA of pathogens, Cas proteins assist in amplifying this information by creating many copies of it. These copies are next detected and counted to calculate the levels of a pathogen in bodily fluids.

New CRISPR in town

Despite its broad use in biotechnology, Cas9 has its limitations – including its large size and its restricted applications in some scientific fields such as pathogen detection. A CRISPR enzyme, called Cas12a, was discovered a few

Cas12a demonstrates enhanced genome editing efficiency, is easier to program for editing many genes simultaneously, and its small size makes it easier to pack and deliver for certain applications.



Step-by-step breakdown of the CRISPR-Cas12a pathogen detection system.

years ago and its unique properties have since captured the attention of researchers around the globe. Cas12a demonstrates enhanced genome editing efficiency, is easier to program for editing many genes simultaneously, and its small size makes it easier to pack and deliver for certain applications.

In addition, researchers have discovered another property of Cas12: CRISPR-Cas12a can recognise specific DNA sequences, bind to them, and create more gRNA. By adding a detection signal (ie, a fluorescent probe), researchers can detect the presence of virus or bacteria. When detected using specialised equipment, they accurately reflect even low levels of the pathogen in a specimen.

Can CRISPR-Cas12a detect CMV?

Cytomegalovirus (CMV) is a common virus that rarely causes problems in healthy individuals. It can, however, cause problems to people with a weakened immune system, including patients undergoing transplant surgery and pregnant women susceptible to passing the virus to their baby. The infection can cause hearing loss and developmental problems in newborns. Despite causing severe defects, in 80% of cases, there is no obvious sign of infection. Suspicion of a congenital infection occurs later in life when the infected baby does not pass hearing or cognitive tests. A rapid, cost-effective test that could screen every newborn baby would allow clinicians to identify infected babies and provide therapy to reduce severe complications.

The common method used for detecting viruses is RT-qPCR. This method is limited due to technical expertise, specialised equipment, and lack of standardisation. To address the above testing limitations, Zwezdaryk and his team have been developing a new CMV rapid detection system based on CRISPR-Cas12a.

The team used human saliva and urine samples that were artificially infected in the lab with strains of CMV and other viruses of the same family (herpesviruses). To amplify the signal of the viral DNA in the specimens, the researchers initially used the polymerase chain reaction method (PCR) and then they applied CRISPR-Cas12a to further amplify, detect, and measure the levels of the viral DNA.

The results of the experiment showed that the method was very sensitive, with viral detection levels found to be lower than 10 infectious units per ml (<10 IU/mL). The method also showed great specificity since it only detected and measured the CMV strains and not the closely related herpesviruses. The method demonstrated similar accuracy and sensitivity when applied in both saliva and urine samples. The total time length of the application was found to be approximately 90 minutes.

Why is CRISPR-Cas12a promising?

The results of the above study show that a pathogen detection system that utilises CRISPR-Cas12a can be 10–100 times more sensitive than the gold standard RT-qPCR techniques. CRISPR-Cas12a can also be used on urine samples. In infants infected with CMV, the virus is found in higher concentrations in urine than saliva or blood, making the method ideal for a non-invasive early detection.

Zwezdaryk and his team are currently developing a technique that will allow CRISPR-Cas12a to be used without the initial PCR amplification. To achieve this, they are planning to substitute PCR with isothermal amplification. This method is compatible with CRISPR-Cas12a and can amplify target DNA sequences at room temperature. This means that CRISPR-Cas12a, if paired with isothermal amplification, could replace the need for expensive equipment, making the assay more cost-efficient.

Overcoming this hurdle would allow the assay to be completed anywhere. Zwezdaryk and his team have completed preliminary tests showing that this is possible. They mixed all the reagents in a single reaction (called a one-pot reaction) and allowed the process to proceed for 2 hours at 37°C. Samples artificially infected with CMV were positive using fluorescent detection methods. CRISPR-Cas12a has so far displayed great sensitivity, accuracy, and a prospect to soon become more cost-effective, making this system an ideal base for the development of a rapid CMV point-of-care CRISPR assay.

> **CRISPR-Cas12a has so far displayed great sensitivity, accuracy, and a prospect to soon become more cost-effective, making this system an ideal base for the development of a rapid CMV point-of-care CRISPR assay.**

Personal response

How could this new pathogen detection method be used in clinical practice?

The CRISPR pathogen detection systems as they currently exist are a minor improvement to standard PCR detection assays. The CRISPR system improves sensitivity and specificity. If we can overcome the technical and biochemical hurdles of a one-pot reaction, then we foresee CRISPR replacing PCR tests. Medical staff would collect a sample, add it to the CRISPR one-pot reaction, and the patient would receive their results a few hours later.

Could the method be applied for detecting other viruses and bacteria? What would the restrictions be in this case?

We have already expanded our system to include detection of other human herpesviruses. We have a pipeline that allows us to identify, optimise, and validate targets specific to different pathogens in approximately 6 weeks. This system can be used to detect any pathogen, including bacteria and fungi.

What future do you envision for CRISPR-Cas12a pathogen detection? Could this method be adapted for an at-home use?

A very ambitious one! COVID at-home tests changed how we approach testing. Our goal is to make a CRISPR-Cas12a test that achieves the same simplicity of an at-home COVID test but provides more information. Others in the field have proposed using smart devices (your phone or tablet) as instruments to measure the results of the CRISPR-Cas12a test.

If you are feeling sick, you would add a swabbed sample to the CRISPR-Cas12a test. Then, mix the tube and place on the counter for 2 hours. When complete, use your phone to image the results. An installed app would analyse the results. You would have the option of sharing the results electronically with your primary care doctor. Your doctor could provide a treatment plan including a prescription. Basically, you can determine why you are sick and pick up the required medication in 3–5 hours.

How are you planning to further test and validate the CMV CRISPR assay for the purposes of screening?

We are validating our assay using two approaches. First, we are comparing our CRIPRS-Cas12a results with a very sensitive form of PCR named digital droplet PCR. This method permits very accurate quantification of the amount of pathogen. Second, we are obtaining clinical samples that have been previously tested using PCR. We are comparing the positive and negative rates between the two approaches to determine how accurate and efficient our system is.

Details

Kevin Zwezdaryk

Chandler Monk

e: kzwezdar@tulane.edu
t: +1 504-988-8678
w: www.zwezdaryklab.tulane.edu

Bio

Kevin J Zwezdaryk is an Assistant Professor of Microbiology and Immunology at Tulane University School of Medicine. His research examines how viruses alter host cell metabolism. His team correlates viral-associated metabolic changes to unhealthy ageing, specifically neurodegeneration. Rapid detection of viruses is an essential tool in age-related infectious disease studies.

Chandler H Monk is a doctoral student in the Bioinnovation Program at Tulane University. This research is part of her dissertation.

References

- Zhao, J, et al (2024) Application of nucleic acid signal amplification in biosensing and bioimaging. *Biomedical Technology*, 5, 14–25.
- Monk, CH, et al, (2023) Development of a CRISPR-Cas12a rapid diagnostic for human cytomegalovirus. *Antiviral research*, 215, 105624.
- Wang, Y, et al, (2023) Application of CRISPR/Cas12a in the rapid detection of pathogens. *Clinica Chimica Acta*, 548, 117520.
- Dronina, J, et al, (2022) Towards application of CRISPR-Cas12a in the design of modern viral DNA detection tools (Review). *Journal of Nanobiotechnology*, 20, 41.

Tulane University