CRISPR/ Diagnostics: A portable lab for everyone

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Professor Kevin J. Zwezdaryk and Chandler H. Monk discuss CRISPR and diagnostics, focusing on the development of a portable lab accessible to everyone

The COVID pandemic highlighted the importance of simple, safe, easy-to-use, and reliable diagnostic platforms. Family members of all ages used a simple sample collection method to provide an easily understandable result – whether they had or did not have COVID. At-home antigen detection tests positively altered public health measures on how individuals and families approached infectious disease diagnosis and surveillance. So why have at-home detection devices not become common tools in our homes?

Antigen-based diagnostics are designed to provide a yes/no answer to the infection status of an individual by measuring indirect markers of disease. These diagnostics are limited due to sensitivity (false negative or positive). Nucleic acid detection methods exhibit superior sensitivity, reproducibility, and specificity. These assays are limited due to the need for specialized equipment and expertise, extended time to results, and sample handling requirements.

Due to these limitations, traditional nucleic acid tests are often excluded as a point-of-care diagnostic solution. Isothermal techniques have been explored to eliminate the need for equipment and reduce the wait time for diagnosis. An ideal point-of-care diagnostic would combine the sensitivity and specificity of a nucleic acid test with the simple workflow and short assay times described by rapid diagnostic approaches.

Critically, the assay must have a simplistic workflow, involving few steps, to enable untrained individuals to reliably use the test from sample collection to results. An innovative technology that can be exploited for expanded testing parameters under diverse clinical, field, or at-home settings is needed.

Clustered Regularly Interspaced Short Palindromic Repeats

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a bacterial adaptive immune system that functions to eliminate foreign genetic material. CRISPR-associated protein (Cas) is an RNA-guided DNA nuclease. The CRISPR-Cas system is divided into two classes, six types, and numerous subtypes based on protein composition and function. These systems have been repurposed to provide novel tools in gene editing (CRISPR-Cas9) and pathogen detection. The list of applications for these tools will likely expand as scientists continue to understand and optimize the mechanisms permitting CRISPR-Cas systems to function.

CRISPR-Cas12a

CRISPR-Cas12a has recently been repurposed as a detection platform. Cas12a identifies T-rich protospacer adjacent motifs (PAM), creating gaps at this site. This initiates the CRISPR RNA (crRNA), an RNA molecule, to guide the CRISPR protein to the target sequence.

Following the binding of the gRNA, a conformational change results in Cas12a protein activation. This permits the double-stranded DNA to be cut. Both DNA strands are initially cut (termed cis-cleavage). Secondary cuts are non-specific and target single- stranded DNA (termed trans-cleavage).

Through this series of steps, bacteria can identify and destroy foreign DNA. Scientists have made two minor modifications to this system, enabling a sensitive pathogen detection assay. The CRISPR crRNA recognition sequences are modified to recognize genetic material for the pathogen of interest. A detection signal, such as a fluorescent marker, is added to the CRISPR-Cas12a system to enable real-time or end-point measurements that provide information on the presence of targeted DNA in the sample. The potential of this system is enormous. It lays the groundwork for providing a highly sensitive, specific, and cheap assay for the detection of any pathogen in the future.

Using the search term "CRISPR-Cas12a" in the National Library of Medicine's PubMed repository, 20 peer-reviewed manuscripts on CRISPR-Cas12a were published in 2018. In 2024, there are 506. Numerous biotech startup companies provide gene editing services based on CRISPR technology, but CRISPR-based diagnostic startups are lagging behind. In the years since the first diagnostic article on CRISPR-Cas12, over 1,500 scientific articles have been published. So why do we not see more of this innovative approach clinically or commercially?

CRISPR-Cas12a alone can rigorously identify pathogens, but it is limited in detecting low quantities of a pathogen. It first requires amplification of the pathogen targets. The standard assay for viral detection remains reverse transcriptase polymerase chain reaction (RT-PCR). This assay has been used with confidence for decades. CRISPR-Cas12a, as a diagnostic, is, in essence, a modified form of PCR. Using traditional PCR methods as a pre-amplification step and then integrating with CRISPR-Cas12a can result in enhanced sensitivity ranging from 10-100x versus PCR alone. This approach remains dependent on technical expertise and expensive equipment.

Innovation in the field

To overcome these hurdles, innovation is being driven through strategies to eliminate the need for PCR-associated amplification. Two approaches are being pursued. The first is the integration of isothermal techniques like recombinant polymerase amplification (RPA) or Loop-mediated isothermal amplification (LAMP).

The DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) system, uses RPA to induce amplification before detection of target nucleic acids. A combined RPA and CRISPR-Cas12a system were used to detect human papillomavirus. It showed a twofold increase versus the multistep CRISPR-Cas12a assay.

Further innovation has shown the potential of combining phage-mediated and rolling circle amplification (RCA) approaches with CRISPR-Cas12a systems. This approach allows the amplification of target material to occur at or near room temperature. This is a key step that must be overcome to bring more powerful infectious disease detection systems to households.

A limited number of teams have reported on the ability to mix a sample with RCA-CRISPR-Cas12a reagents in a collection tube and obtain results in under an hour. All of these systems demonstrate the synergistic effect of integrating well-established isothermal technology with novel CRISPR-Cas systems to overcome the current hurdles in developing a point-of-care assay that exhibits equal or superior sensitivity.

The second approach is an amplification-free method to detect nucleic acids. This integrates current technology with the CRISPR-Cas12a system, resulting in an enhanced detection system through synergistic mechanisms. CRISPR-Cas12a has been used in combination with catalytic enzymes, nanomaterials, rational-designed oligonucleotides, and alternative biosensors or detection platforms to detect pathogens. Despite promising results, the performance of these systems does not significantly surpass the current standard of care, presenting challenges to overcome before commercialization.

How close are CRISPR-Cas12a point-of-care assays?

How close are we to CRISPR-Cas12a point-of-care assays? To be a point-of-care diagnostic, it should meet the World Health Organization REASSURED guidelines Real time connectivity, Ease of sample collection (Affordable, Sensitive, Specific, User-Friendly, Robust and rapid, Equipment-free, and Deliverable to all people who need the test). CRISPR-Cas12a systems adhere to these guidelines. CRISPR-Cas12a proteins can remain stable under diverse environmental conditions. Data interpretation or readouts using this system are not as simple as the antigen-based tests. Novel strategies are being pursued, including repurposing smart devices as an assay detection and interpretation tool. This would permit instant data analysis and results for users and could be combined with electronic medicine approaches, enabling communication between individuals at home and a primary care physician.

Beyond an infectious disease platform, CRISPR-Cas12a can be adapted for use as a surveillance tool for emerging or endemic pathogens, and as a rapid diagnostic for use in the agriculture industry. If researchers can overcome small but critical hurdles, this platform can revolutionize how the healthcare system, public health officials, and individuals approach diagnosis and surveillance of infectious diseases.

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