

Who or what is SHERLOCK?

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ABSTRACT

Background

Polymerase chain reaction (PCR) is the most commonly used method for detecting nucleic acids. However, PCR requires specialized and expensive equipment, as well as specially trained personnel. Recently, new innovative diagnostic methods have been developed to detect nucleic acids using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene editing technology.

Objective

This manuscript reviews the newly emerging diagnostic methods that exploit the CRISPR technology.

Results

The programmable endonuclease properties of CRISPR have been harnessed for use in diagnostic testing.

Specific High-sensitivity Enzymatic Reporter un-LOCKing (SHERLOCK) and DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) are diagnostic tools that can be used to detect specific RNA/DNA at low attomolar concentrations. Heating Unextracted Diagnostic Samples to Obliterate Nuclease (HUDSON), is a process of heat and chemical reduction that allows for direct detection of nucleotides in body fluids.

HUDSON and SHERLOCK can be combined to detect RNA/DNA directly from urine, saliva, serum, plasma, and whole blood with limited sample preparation or equipment with results in 1 to 2 hours. In addition, a lateral flow readout has been developed to facilitate assay detection.

Conclusions:

Potential uses of this emerging technology are numerous due to the analytical sensitivity and specificity, simplicity, speed, and flexibility.



The ability to measure nucleic acids with highly sensitive methods is important for a number of applications such as: environmental monitoring, food safety, and detection of biological threats. Ultra-sensitive methods also have utility in clinical diagnostics for early detection of infectious diseases, testing the blood supply, and screening for cancer.

The most commonly used method for detecting nucleic acids is polymerase chain reaction (PCR). PCR is capable of routinely detecting <100 copies of nucleic acid per sample, which is in the low attomolar to zeptomolar range. PCR, however, requires specialized and expensive equipment, as well as specially trained personnel. For laboratories without this equipment or personnel, sending samples to a specialized laboratory takes time. In addition, in some cases, such as certain viral infections, the viral load is so low that even PCR is not sensitive enough.

Recently, new innovative diagnostic methods have been developed to detect nucleic acids using the CRISPR gene editing technology. In order to understand these new diagnostic methods, it is important to understand CRISPR.

CRISPR stands for “Clustered Regularly Interspaced Short Palindromic Repeats”. (1) This is a naturally occurring genome editing tool that is part of the bacterial immune system used to

fight against invading viruses. This system has been harnessed by scientists to modify genes in living cells.

Bacteria store genetic elements from infectious agents in genomic loci called CRISPR arrays as memories for adaptive immunity. In a very simplified explanation, this immune system works as follows. When bacteria senses the presence of viral DNA it produces a unique RNA, which matches that of the invading virus. This RNA forms a complex with a protein enzyme called CAS9 (CRISPR associated protein 9). CAS9 is an endonuclease (an enzyme that cuts DNA). The endonuclease is guided by the RNA to its DNA target. When the bacterial guide RNA finds its match DNA the Cas9 enzyme cuts the DNA in a specific location. Hence, Cas 9 is referred to as a “programmable endonuclease” because one can program it to cut a specific DNA by providing a unique RNA.

Over the past few years, researchers have realized that they can harness this system to cut, not just viral DNA, but any DNA at a specific location by changing the guide RNA to match the target of interest. In addition, this can be done, not just in a test tube, but in the nucleus of a living cell. This technology has enabled scientists to do incredible things such as edit the human genome to correct naturally occurring mutations.

Recently, this CRISPR technology has been harnessed for use as a diagnostic test. In 2017, a group of scientists reported the development of a technology called SHERLOCK which stands for: “Specific High-sensitivity Enzymatic Reporter unLOCKing”. (2) Their goal was to develop a method to rapidly detect nucleic acids with high sensitivity, single base specificity, on a portable platform.

Instead of using the Cas9 endonuclease, SHERLOCK uses a related, but different protein, Cas13a. Cas13a binds and cleaves RNA rather than DNA substrates. After Cas13a cleaves its

target RNA, it adopts an enzymatically “active” state rather than reverting to inactive state, like Cas9. Cas13a then binds and cleaves additional RNAs regardless of homology. This is referred to as “collateral cleavage”. It is this property of Cas13a that opens up the possibility of using Cas13a as a diagnostic tool.

SHERLOCK works by amplifying RNA (or DNA with a reverse transcriptase) using recombinase polymerase amplification (RPA) which is an isothermal nucleic acid amplification. Isothermal amplification does not require specialized instrumentation, as it uses a single temperature. The amplified nucleotides are combined with the Cas13a nuclease, a guide RNA that matches the nucleic acid sequence of interest, and a short nucleotide sequence that is coupled to a fluorescent reporter and a quencher. If the target sequence is present in the pool of amplified nucleotides, the non-specific RNase activity of Cas13a becomes activated and the RNA reporter will be cleaved resulting in activation of the fluorophore. Therefore, the fluorescent signal is used as an indicator to determine whether the target sequence is present in the original pool of nucleotides. Hence the name “Specific high-sensitivity enzymatic reporter unlocking”.

The authors demonstrated that SHERLOCK could distinguish between Zika virus (ZIKV) and dengue virus (DENV) in clinical isolates (serum or urine) where concentrations can be as low as 2000 copies/mL (3.2aM). They were also able to distinguish between several pathogenic bacterial strains, genotype human DNA, and identify mutations in cell-free tumor DNA. (2) One year later, in an issue of science, three papers were published that further advanced field of molecular diagnostics using CRISPR technology. (3-5) A group from Jennifer Doudna’s group at the University of California Berkley reported on a detection system similar to SHERLOCK that they termed DETECTR for: “DNA Endonuclease Targeted CRISPR Trans Reporter”. Their system

utilizes Cas12a which cleaves double stranded DNA and activates non-specific cleavage of single stranded DNA. They use the system to detect carcinoma associated HPV from clinical specimens also using RPA with attomolar sensitivity.

In another paper in the same issue of Science, Myyhrvold *et al* reported on a new method to release and protect from degradation viral nucleic acid’s from clinical specimens, thereby bypassing the need for nucleic acid extraction. (4) This method, called Hudson for: “Heating Unextracted Diagnostic Samples to Obliterate Nuclease”, is a process of heat and chemical reduction that inactivates the high amount of ribonucleases found in body fluids and then lyses viral particles by disrupting the viral envelope, thereby releasing nucleic acid’s into solution. The authors combined HUDSON and SHERLOCK to detect ZIKV and DENV directly from urine, saliva, serum, plasma, and whole blood with limited sample preparation or equipment with results in 1 to 2 hours.

In the third paper, Gootenberg *et al* advanced this technology even further with SHERLOCKv2. (5) The authors describe the following four advances to SHERLOCK:

1. four-channel multiplexing;
2. quantitative measurement as low as 2 aM;
3. 3.5-fold increase in signal sensitivity;
4. development of a lateral flow readout.

The flexibility of this revolutionary new technology has enormous potential. Proposed uses have included:

- Rapid detection of pneumonia pathogens (viral and bacterial) in one assay
- Monitoring viral load in HIV patients receiving therapy in resource limited areas
- Liquid biopsy to detect mutations in cell free DNA

- Rapid TB results before patient is lost to follow-up
- Rapid detection of pathogen resistance genes
- CRISPR to gene - edit mutations - SHERLOCK to determine proportion of genes successfully edited

In conclusion, the programmable endonuclease properties of CRISPR have been harnessed for use in diagnostic testing.

SHERLOCK and DETECTR are diagnostic tools that can be used to detect specific RNA/DNA.

HUDSON pairs with SHERLOCK for direct detection of nucleotides in body fluids.

Potential uses of this technology are numerous due to the analytical sensitivity and specificity, simplicity, speed, and flexibility.

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