

Mini-Review

# SHERLOCK and DETECTR: CRISPR-Cas Systems as Potential Rapid Diagnostic Tools for Emerging Infectious Diseases and Cancer-Associated Mutations

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## Abstract:

Sensitive and precise nucleic acid detection is critical for clinical diagnostics and biotechnological advancements. Diagnostic in infectious disease field is very unique from diagnosing any other disease, that is time is of the essence; in outbreaks people die even with each passing hour in some cases, if the correct diagnosis wasn't made; for example Zika in particular is a very challenging virus to diagnose, because it's in very few numbers of copies in the infected person, so it need high sensitive diagnostic approach to spot it, In particular, the advanced tools SHERLOCKv2 and DETECTR, give almost an immediate detection of attomolar amounts of pathogenic nucleic acids with specificity similar to that of PCR but with slight technical settings and that will guide the correct intervention for the patient. SHERLOCKv2 and DETECTR technologies are game changers for our ability to identify infectious disease and rapid detection of tumor DNA or cancer-related viruses with ultra-sensitive tests that don't require a lot of complicated processing to go through. In this paper, we will review cutting-edge infectious disease diagnosis by CRISPR-Cas systems.

**Keywords:** cancer-related viruses; CRISPR-Cas diagnostic tools; DETECTR; infectious disease; SHERLOCKv2

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## 1. Introduction:

Emerging and re-emerging infectious diseases are one of the most intimidating threats to human race, responsible for an immense burden of disabilities and deaths.[1] Pandemics of Spanish flu,[2] swine flu,[3] bird flu,[4] Zika[5] and Ebola virus,[6] deadly

and wide-spread epidemics of SARS and MERS,[7] as well as the ongoing outbreak of COVID-19 epidemic, originated in China in 2019, stroke countries and emerge as the most recent examples of widespread infections reported in this century.[8] Unfortunately, most researches focus on finding cures and vaccines for these diseases instead of giving diagnostic field its well contribution of these researches to help to diagnose it in rapid and precise approach to accelerate the quarantine; in the recent years Discovery of the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR associated protein (Cas) (CRISPR-Cas) revolutionized biology and is already pushing health care systems to the era of precise molecular medicine. Using genetic engineering, CRISPR-Cas systems have been adapted for use in humans and are now being modified and enhanced at an extraordinary pace, enabling precise editing of virtually any DNA or RNA molecule.[9-13]

Rapid detection of nucleic acids is crucial in clinical diagnostics and biotechnology. Kellner *et al.* recently designed a CRISPR-based diagnostic tool that combines nucleic acid pre-amplification with CRISPR–Cas enzymology for specific recognition of desired DNA or RNA sequences. Its termed specific high-sensitivity enzymatic reporter unlocking (SHERLOCK), allows multiplexed, portable, and ultra-sensitive identification of RNA or DNA from clinically applicable samples.[14, 15] Another diagnostic tool we will review, DNA endonuclease-targeted CRISPR trans reporter (DETECTR), a rapid (~30 min), low-cost and accurate CRISPR-Cas12 based lateral flow assay for detection of viral infections.[16]

SHERLOCK & DETECTR diagnostic tools are characterized by sensitivity and specificity comparable to those of traditional PCR, but do not require sophisticated (and therefore expensive) equipment and have a very low estimated cost. Embedding CRISPR-Cas into molecular diagnostics may reform the profile of global diagnostic platform. [15, 16]

In this paper, we review SHERLOCK & DETECTR technologies and describe their properties, functions, and perspectives to become the ultimate diagnostic tools for diagnosing infectious diseases, curbing disease outbreaks and identify cancer-associated mutations.

## ***2. Detection of nucleic acids by CRISPR-Cas:***

Rapid nucleic acid detection is an important part of many applications in human health and biotechnology, including the identifying of infectious diseases, agricultural pathogens, or circulating DNA or RNA associated with disease.[17-19] Standard methods to amplify nucleic acids for detection (such as PCR) are effective but require instrumentation that is not portable, precluding their deployment in the field [20, 21]. CRISPR-Cas-based approaches are being tested to treat hereditary, infectious, and many

other diseases.[22-24] To date, a number of CRISPR-Cas-based approaches to detect and diagnose infectious and non-infectious diseases (ex: Cancers) had been developed.[25-27] CRISPR-based technologies spread further into the area of molecular diagnostics and may replace PCR in many applications in the near future.[28-31] (**Table 1**)

**Table 1:** Some types of CRISPR-Cas diagnostic tools, their applications and characteristics:

Type of CRISPR system	Method	Protein	Target	Amplification	Detection	Model organism	Reported sensitivity
Type VI	SHERLOCK	Cas13a	DNA, RNA	RPA	Fluorescence	Viruses, bacteria, SNPs	$2 \times 10^{-18}$ M
Type V Type VI Type III	SHERLOCKv2	Cas13, Cas12a, Csm6	DNA, RNA	RPA	Fluorescence, lateral flow assay	Viruses, bacteria, SNPs	$8 \times 10^{-21}$ M
Type V	DETECTR	Cas12a	DNA	RPA	Fluorescence	HPV 16/18	$\approx 10^{-18}$ - $10^{-17}$ M

In 2016, CRISPR-Cas systems were first developed to identify nucleic acids for molecular diagnostics.[32] Demand for instrument-free nucleic acid detection technologies has driven the development of multiple techniques for isothermal amplification.[33, 34] However, common approaches for isothermal amplification, such as recombinase polymerase amplification (RPA)[35], require optimization and cannot typically discriminate between single-base-pair differences in target sequences, a distinction that can have important consequences for pathogenicity[36-38]. Recently, enzymes from CRISPR-Cas systems have been adapted for the specific, rapid, sensitive, and portable detecting of nucleic acids.[30, 39, 40] A large set of different CRISPR-based methods used to detect nucleic acids has been recently described. Early technologies utilized the canonical Cas9 protein of type II CRISPR-Cas systems [41] or its modified nucleolytically null, or dead, Cas9 (dCas9) protein [42]. A huge leap toward developing CRISPR-based molecular diagnostics was the discovery of protein collateral activity of Cas12 and Cas13. To date, both the Cas13 and Cas12 protein families of CRISPR systems have been shown to have collateral activity, making them useful for nucleic acid detection applications.[16, 43, 44] The key differences between the Cas13 and Cas12a enzymes are shown in (**Table 2**)

**Table 2:** The main differences between the Cas13 and Cas12a enzymes:

	Cas12a	Cas13
PAM required	Yes	No
PAM identity	TTTV	Not applicable
Cleavage	Single staggered cut	Many cleavage sites

Target type	ssDNA, dsDNA	ssRNA only
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Many of the Cas13 subtypes and orthologs have different preferences, cleaving at specific dinucleotide motifs [45]. In addition, Cas13 subtypes differ in size, DR sequence, and crRNA structure. Although Cas13 has a PAM-like sequence motif called the protospacer flanking site (PFS) that restricts activity to only certain target sites, there are a number of very active Cas13 orthologs, such as LwaCas13a, that show no PFS. Lack of a PFS is a distinguishing feature of these orthologs that enables them to target any possible sequence or mutation. Cas12a has weak collateral activity, enabling nucleic acid detection with low sensitivity [16, 45] When combined with pre-amplification, Cas12a-mediated detection can detect 2 aM concentrations.[45, 46]

### 3. Detection of nucleic acids by CRISPR-Cas type V and VI:

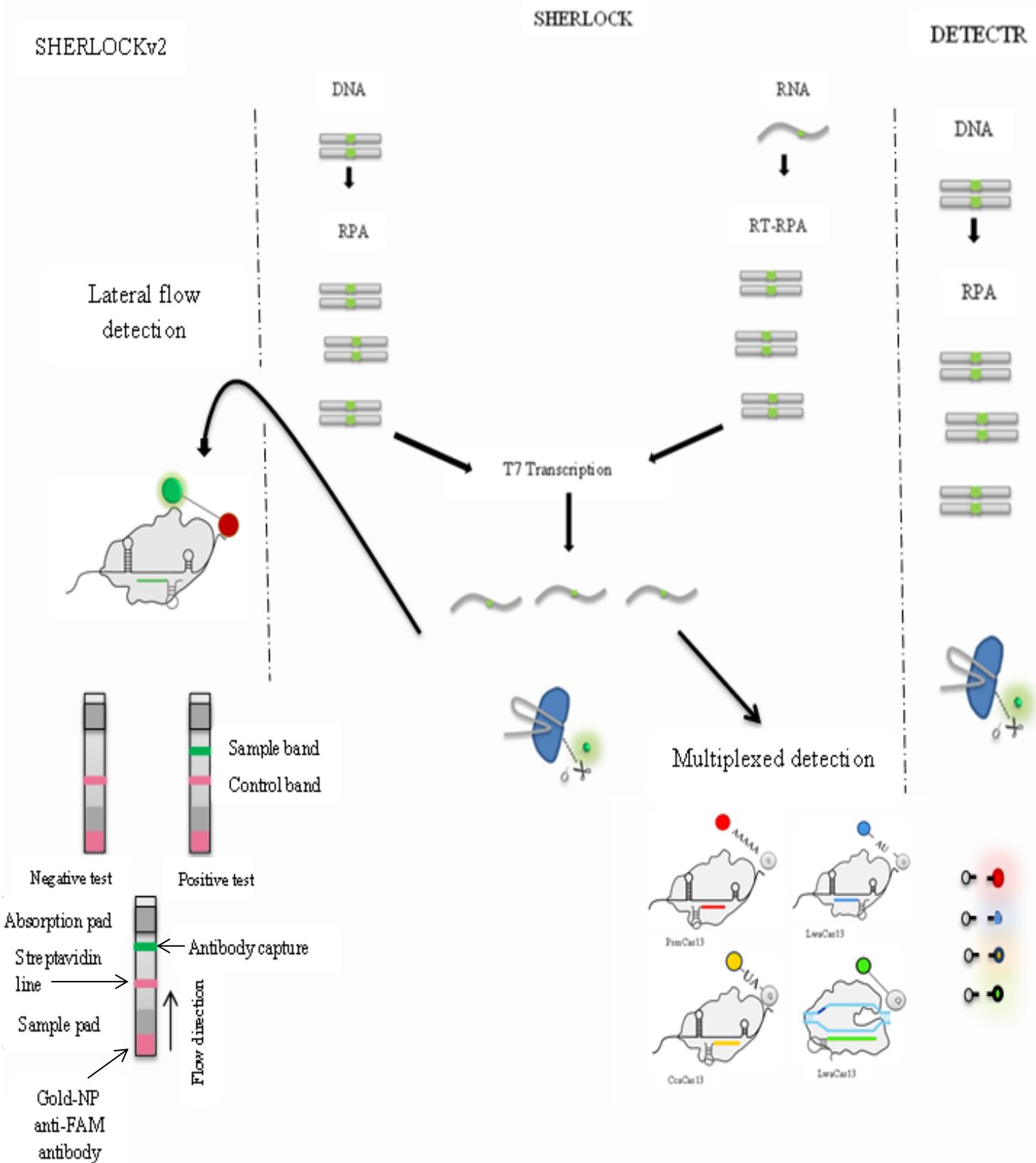
In 2017, Jennifer Doudna's group presented the CRISPR-Cas diagnostic tool named DNA endonuclease-targeted CRISPR trans reporter (DETECTR) [16]. This method depends on collateral activity of Cas12a protein activated after recognition of target RNA by Cas12a. The authors demonstrated that Cas12a protein from *Lachnospiraceae bacterium ND2006* (LbCas12a) exhibits non-specific collateral activity and degrades all adjacent DNA molecules after recognizing target RNA. If the reaction with Cas12a protein and targeting crRNA is complemented by single-stranded DNA-reporters (probes) and then mixed with the biological sample, crRNA-dependent recognition of pathogenic nucleic acids by Cas12a turns on collateral activity that destroys DNA probes. DNA probes are designed similarly to conventional TaqMan probes, in which one end of the reporter is bound by a fluorophore and the opposite is linked to a quencher. Degradation of the DNA probes releases fluorophores and results in stable and strong fluorescent signal detected by a fluorimeter. Additionally, DETECTR has been combined with an isothermal pre-amplification step to enrich target sequences (RPA). RPA enhances analytical sensitivity of the diagnostic test and helps to avoid the need for sophisticated and expensive equipment. Other orthologous proteins from different organisms, AsCas12a (*Acidaminococcus* sp.), FnCas12a (*Franciella novicida*), AaCas12b (*Alycyclobacillus acidoterrestris*) [47] also have collateral activity and can be facilitate to make diagnostic platforms by the same principle as DETECTR. DETECTR was provisionally used to detect HPV and differentiate between HPV16 and HPV18, the most pro-oncogenic types of HPV. In crude DNA extracts, DETECTR identified HPV16 in 25 of 25 cases and HPV18 in 23 of 25 cases, provisionally determined by PCR. Remarkably, the whole DETECTR analysis takes only 1 hour to complete.[16]

In 2018, Zhang's and his group presented SHERLOCK, a diagnostic tool based on CRISPR-Cas type VI system [14, 15, 46]. SHERLOCK is based on the same principles as DETECTR, but depend on activity of Cas13 nuclease from *Leptotrichia wadei*. Cas13 specifically recognizes and cleaves only RNA, rather than DNA like Cas12a, thus non-specifically corrupting any adjacent RNA molecules. In vitro transcription of the isolate

enables recognition of DNA targets. Isothermal amplification RPA can be used to enrich target molecules and increase sensitivity. The amplified RNA fragments are mixed with Cas13 protein crRNA and fluorescent RNA probes. If the target molecules are present in the sample, Cas13 recognizes them via crRNA and indiscriminately cleaves (by collateral activity) fluorescent RNA probes, disrupting the interaction between the fluorophore and the quencher. The presence and intensity of the fluorescent signal thus indicate the amount of the target in the biological sample. The authors demonstrated that SHERLOCK detects Zika virus, dengue virus, various pathogenic bacteria, and SNPs in DNA with attomolar sensitivity. All components of the SHERLOCK had a major drawback: it was qualitative, not quantitative, but a year later, the authors presented the second named SHERLOCKv2 [45].

In a clinical or field setting, to differentiate between pathogens that cause similar symptoms, it can be advantageous to test for the presence of multiple sequences at once. Therefore, Zhang group has also combined a multiplex option into SHERLOCKv2. Multiplexing was enabled by the observation that the nonspecific trans-cleavage activities of Cas13 from diverse species exhibited strongly skewed, and different, preferences for certain sequence motifs. For example, LwaCas13a from Lachnospiraceae bacterium NK4A179 has a much stronger preference for rA-rU over rG-rA dinucleotides, while PsmCas13B from Prevotella sp. MA2016 has the opposite preference. Therefore, reporter probes labeled with different fluorophores, each containing a corresponding unique nonspecific cleavage motif, can differentiate activity of the corresponding enzymes, which also have orthogonal guide RNA sequences that can differentiate the multiplex target sites.[48] (**Figure 1**)

SHERLOCKv2 was engineered to produce a visual colorimetric readout on commercial lateral flow strips that do not require any special equipment. (**Figure 1**) In this setting, the presence of the target is determined by visually inspecting the strips with different intensity of staining. SHERLOCKv2 superior to SHERLOCK by that, the whole of SHERLOCKv2 reaction is performed in a single step by directly applying the biological sample to the test strip without purifying and isolating nucleic acids. To conclude, SHERLOCKv2 is a highly sensitive quantitative diagnostic platform suitable for multiplex signal detection and colorimetric detection on lateral flow strips [45]. (**Table 2**)



**Figure 1:** Shows SHERLOCK, SHERLOCKv2 and DETECTR assay steps.

#### **4. CRISPR-tools for detection emerging and re-emerging infectious diseases**

As was briefly mentioned, Emerging infectious diseases (EIDs) include infections that are entirely new in a population or that may have existed before in the population but are now gaining rapid and continued spread and/or wide geographical range. [49] Several factors such as immigration of people, human behavioral changes, ecological variations, agricultural practices, host/intermediate factors, animal-human exchanging and microbial genetic changes, all affect infectious disease emergence and spread. [50-53] Most emerging infections originate from a specific population and can spread to a new population or become selectively advantaged that it can lead to the emergence of new strains of the pathogen.[54, 55]

Corona viral infections represent an intimidating threat to the global health, appearing every 10 years with new outbreaks, in December 2019; a new strain has been spread across Wuhan City, China [56]. It was designated as coronavirus disease 2019 (COVID-19) by the WHO [57]. In late January 2020, WHO declared the outbreak a global pandemic with cases in more than 199 countries where the COVID-19 spreading fast outside China, most significantly in United States, Italy and Spain with over 32,239 deaths and 686,032 cases confirmed while 146,400 recovered. PCR assays have been developed for SARS CoV-2 recognition. Due to the rapid spread of the virus, rapid diagnostics is essential for curb the transmission by accelerate the wheel of control guidelines. CRISPR-Cas diagnostic tools would be supportive for effective identification, diagnosis and management of the infection.

#### **5. CRISPR-Cas platforms for detection cancer-associated mutations:**

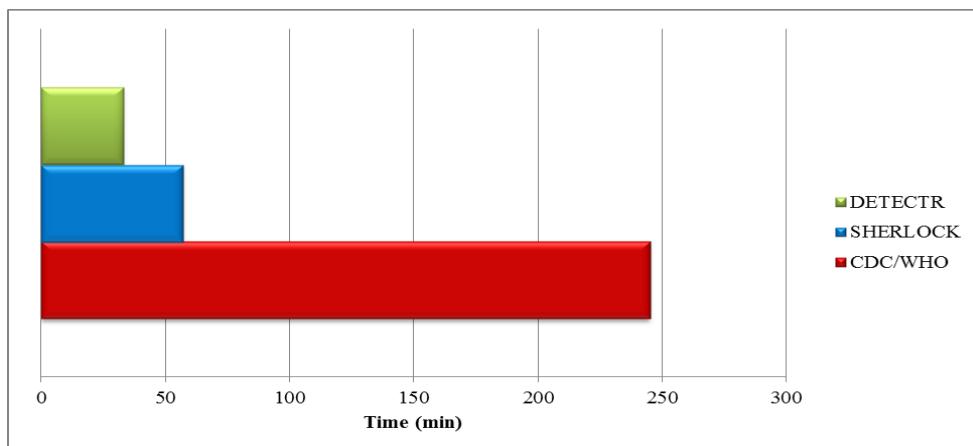
Cancer is a complex disease caused by combinations of cellular genetic mutations and heterogeneous microenvironments.[58] The single-nucleotide specificity of SHERLOCK has been applied to provide genotyping profile of cancer patients by revealing of cancer-associated mutations from circulating cell-free DNA, even in serum or urine samples to low attomolar concentrations reach to 0.1%. In similar cases, the specificity of Cas13 can be boosted by the introduction of a ‘synthetic mismatch’ into the crRNA [45, 46]. The Cas13 enzyme used in SHERLOCK does not necessitate strict sequence partialities at the target site, while Cas12 require a PAM for cleavage. This tolerates a wider target range for SHERLOCK as matched to DETECTR [59].

In simulated cfDNA samples, SHERLOCKv2 can detect two cancer mutations under low allelic fraction with single-base mismatch sensitivity [46]. In addition to in vitro RNA target detection, catalytically inactive LwaCas13 retains its RNA-binding activity such that it can be coupled to a fluorescent probe to enable live cell RNA tracking [60]. This provides an alternative method to recognize and visualize RNA.

### 5. Potential applications of CRISPR diagnostics:

As was briefly mentioned, the ideal diagnostic assay should provide accurate and sensitive identification of the pathogen while being affordable, portable, and able to distinguish different variants of the pathogen. Currently, no such test exists. Developing new tools which come across the requirements of the WHO standard diagnostic test can completely reshape epidemiological surveillance and medical health care system for the majority of infectious and noninfectious diseases in the world.[61, 62]

The most amazing features of DETECTR are the accuracy and speed in providing results in matter of minutes, Broughton *et al.* provide evidence-based comparison between DETECTR, SHERLOCK, and CDC/WHO on 2019 novel Coronavirus SARS-CoV-2 using a CRISPR-based DETECTR Lateral Flow Assay.[63] (**Figure 2**)



**Figure 2:** comparison of SARS-CoV-2 assay workflows for DETECTR, SHERLOCK, and CDC/WHO.

### 6. Advantages and limitations:

SHERLOCK is ultra-sensitive and specific. It is capable of single-molecule detection in 1- $\mu$ L sample volumes (2 aM) of both DNA and RNA targets. In addition, by scaling up the pre-amplification volume, it is possible to achieve single-molecule detection in large sample input volumes (up to 540  $\mu$ L; 8 zM) [45]. SHERLOCK leverages the specificity of Cas13 [43, 46, 60] and Cas12 enzymes [64-66]. SHERLOCK reaction can be lyophilized and used after long storage periods without impacting the sensitivity and specificity of the test [46]. Similar viruses, such as dengue virus and Zika virus, can easily be distinguished by SHERLOCK [46]. The specificity of Cas13 can be enhanced by the introduction of a ‘synthetic mismatch’ into the crRNA [45, 46]. An attractive feature of SHERLOCK is the rapid nature of the assay. Usually, RPA is

performed for 5–10 min as an initial reaction, and part of this solution is transferred to the Cas13 detection reaction as a two-step reaction, which can then detect the target in 5 min.[15] Another advantage of the SHERLOCK platform over other detection platforms (such as TaqMan qPCR) is the low cost of its components. A typical single-plex reaction is approximately \$0.60 [46].

Regardless of its advantages over existing detection technologies, SHERLOCK has several caveats that can make it unacceptable for certain cases. SHERLOCK currently involves the preparation and testing of reaction components, some of which require expertise in protein purification and RNA biology. Moreover, pre-designed assays, including reaction mixtures and RNA/DNA oligonucleotides, are currently not commercially available for SHERLOCK. Existing standard detection technologies may also be more appropriate for applications that do not demand the speed or portability of SHERLOCK, such as oncology assays.[15]

Another potential limitation of SHERLOCK is the multi-step nucleic acid amplification process, which may affect precise target quantification. Although we recently demonstrated the quantitative detection of nucleic acids with SHERLOCK, absolute digital quantification such as in digital droplet PCR is currently not possible, and small differences in target quantity (<2× changes) may not be detected. SHERLOCK may therefore be less useful for precise gene expression profiling.[15]

On the other hand, DETECTR possess unique features, the most important one is the speed (Figure 2); other advantages are that, no heavy equipment's required (portable) and with low mismatch results; It is capable of single-molecule detection in rage of 70-300 copies/µL and allows differentiation of viral subtypes.[63]

**Note:** CRISPR diagnostics tools have not yet been approved by the FDA.

## 5. Conclusion:

SHERLOCK and DETECTR had begun a new era in molecular diagnostics field by providing portable, highly sensitive diagnostic tools, suitable for diagnosing emerging infectious diseases and cancer mutations in a matter of hour. Still, it remains to be resolute: how proficiently these CRISPR-Cas systems can be used in a multiplexed manner to target and detect complex genetic mutations in cancers, with minimal false-positive signals.

Engineered Cas13 and Cas12a enzymes suggest novel approaches for rapid detection of tumor DNA or cancer-related viruses with ultra-sensitive, rapid recognition and can be further enhanced for sensing multiple target DNAs simultaneously; with this evidence, we believe CRISPR-Cas systems are driving a biotechnological revolution.

**Conflicts of Interest:** The authors declare that there are no conflicts of interest regarding the publication of this paper.

**Funding:** The authors received no financial support for the research, authorship, and/or publication of this article.

**Acknowledgement:** The authors acknowledge the Deanship of Scientific Research at University of Bahri for the supportive cooperation.

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