Received: 13 December 2021 | Revised: 26 January 2022 | Accepted: 27 January 2022

DOI: 10.1002/bies.202100286

BioEssays

PROBLEMS & PARADIGMS

Prospects & Overviews

Potential of the CRISPR-Cas system for improved parasite diagnosis

CRISPR-Cas mediated diagnosis in parasitic infections

Hong You | **Catherine A. Gordon** | **Skye R. MacGregor** | **Pengfei Cai** | **Donald P. McManus**

Immunology Department, QIMR Berghofer Medical Research Institute, Queensland, Australia

Correspondence
Hong You and Donald P. McManus, Immunology Department, QIMR Berghofer Medical Research Institute, Queensland 4006, Australia.
Email: Hong.You@qimrberghofer.edu.au and Don.McManus@qimrberghofer.edu.au

Abstract

CRISPR-Cas technology accelerates development of fast, accurate, and portable diagnostic tools, typified by recent applications in COVID-19 diagnosis. Parasitic helminths cause devastating diseases afflicting 1.5 billion people globally, representing a significant public health and economic burden, especially in developing countries. Currently available diagnostic tests for worm infection are neither sufficiently sensitive nor field-friendly for use in low-endemic or resource-poor settings, leading to underestimation of true prevalence rates. Mass drug administration programs are unsustainable long-term, and diagnostic tools – required to be rapid, specific, sensitive, cost-effective, and user-friendly without specialized equipment and expertise – are urgently needed for rapid mapping of helminthic diseases and monitoring control programs. We describe the key features of the CRISPR-Cas12/13 system and emphasise its potential for the development of effective tools for the diagnosis of parasitic and other neglected tropical diseases (NTDs), a key recommendation of the NTDs 2021–2030 roadmap released by the World Health Organization.

KEYWORDS
CRISPR-Cas12/13 technology, diagnosis of parasitic infections, field-friendly, neglected tropical diseases

INTRODUCTION

The recurrent need for sensitive, accurate, and field-friendly tools for the diagnosis and surveillance of parasitic infections

Parasitic helminths mainly impact low-income communities in developing countries in the tropics and subtropics, causing devastating chronic diseases and significantly impacting on human health and economic development. Distressingly, when the global disease burdens of the most prevalent helminthiases and other neglected tropical diseases (NTDs) are combined, the disability they cause is greater than that of HIV/AIDS, tuberculosis or malaria.[1] Asia and sub-Saharan Africa

Abbreviations: AIOD-CRISPR, All-In-One Dual CRISPR-Cas12a; CARMEN, Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids; cfDNA, cell-free DNA; CLAP, Cas12a-assisted RT-LAMP/AuNP; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; crRNA, CRISPR RNA; DENV, dengue virus; DETECTR, DNA endonuclease-targeted CRISPR trans reporter; dsDNA, double-stranded DNA; FDA, Food and Drug Administration; FLASH-NGS, Finding Low Abundance Sequences by Hybridization-Next Generation Sequencing; HBV, hepatitis B; HPV, human papillomavirus; LAMP, loop-mediated isothermal amplification; miRNA, microRNAs; miSHERLOCK, minimally instrumented SHERLOCK; NTD, neglected tropical disease; POC, point-of-care; RPA, recombinase polymerase amplification; RT, reverse transcription; SATORI, CRISPR-based amplification-free digital RNA detection; SHERLOCK, Specific High Sensitivity Enzymatic Reporter UnLOCKing; SHINE, Streamlined Highlighting of Infections to Navigate Epidemics; ssRNA, single-stranded RNA; STOP, SHERLOCK Testing in One Pot; ZIKV, Zika virus

 © 2022 Wiley Periodicals LLC

remain helminth hot spots harboring the highest infection rates, with circa 90% of the world's parasitic worm infestations occurring in these regions. Indeed, the majority of NTDs recognized by the World Health Organization (WHO) are caused by parasites, which have gained wide-scale attention due to the severe morbidity and mortality caused during infection and the concerted efforts undertaken for their control and elimination. The availability of accurate and effective diagnostics was a key recommendation of the new roadmap for NTDs 2021–2030 recently released by the WHO.[2] As part of this, WHO are seeking expert consultation on diagnostics, with a particular focus on Point-of-care (POC) methods of diagnosis.[3] POC testing facilitates rapid results in situ, enabling control programs to target only infected subjects for drug treatment and providing insight into pathogen transmission dynamics and disease burden. The benefits of accurate diagnosis include reduced drug costs, increased acceptance of treatment, and decreased risk of drug resistance developing. WHO guidelines require that POC tests be rapid, are able to be performed by local personnel with minimal training, and can be battery run if electrical equipment is required.[3] When elimination is on the horizon, it is vital that sensitive diagnostic procedures are available to confirm absence of infection in order to prevent disease re-bounding following drug treatment. Encouragingly, the recently developed CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-based diagnostic platform[4–7] has the potential to fulfill those unmet and urgent needs for parasite detection. The approach is rapid, inexpensive, sensitive, specific, and field-friendly for POC pathogen identification,[5] providing the stepping stone required for a revolutionary new approach to parasite diagnosis.

Traditional diagnostic platforms hinder progress of parasite control efforts

Parasitic infections primarily impact the poorest regions in developing countries, and most parasites cause chronic disease and disabilities which generally do not directly kill their hosts except in the case of malaria. Consequently, there has been relatively limited interest in developing advanced platforms for parasite diagnosis. As reviewed by Amoah et al.,[8] the current diagnosis of schistosomiasis (which infects an estimated 250 million people in 76 countries),[9] monitoring of transmission dynamics and determining the efficacy of drug treatment depends almost entirely on conventional diagnostics.[10–12] These include the stool-based Kato-Katz schistosome egg-detection procedure, miracidial hatching technique, and serological-based assays which lack sensitivity and are inaccurate, labor-intensive, and often unreliable, especially when applied in areas of low schistosomiasis prevalence. The limitations of conventional diagnostics can lead to an underestimation of active *Schistosoma* spp. cases, inaccurate disease transmission rates, and errors in interpreting outcomes of mass drug administration programs, where measures of effectiveness depend on precise monitoring of treatment success (or failure).[13] Given the limitations of the conventional diagnostics currently used, nucleic acid amplification techniques (PCR, nested PCR, PCR-ELISA, quantitative PCR, droplet digital PCR, loop-mediated isothermal amplification [LAMP]) and detection of cell-free DNA (cfDNA)[14] have shown considerable promise in improving diagnostic sensitivity and specificity for parasite identification in humans and other mammalian hosts. However, the use of PCR-based assays in developing countries is constrained by the high cost of PCR reagents, the requirement for expensive laboratory instrumentation (thermal cyclers) for isothermal nucleic acid amplification, which can often provide non-specific amplification resulting in lower detection specificity, and qualified personnel to run the tests and interpret results.

Recent disruptions due to the COVID-19 pandemic, resulting in the cessation of control programs for schistosomiasis and other NTDs as recommended by the WHO,[15] will have a serious impact on disease prevalence, with a reduction in chemotherapy likely to cause rebound infections similar to pre-control levels. The implementation of new alternative molecular diagnostics procedures are urgently needed for better management to control the spread of helminth infections, which are required to be highly specific, sensitive, affordable and technically feasible for application in field laboratories to screen large human and animal cohorts in large-tscale surveys.[16] With the rapidly expanding toolbox for CRISPR-based diagnostics in infectious diseases, the recent advent of the platform stands out with its superiority in rapid, field-friendly,[5] inexpensive processing, providing highly sensitive (similar to PCR-based assays),[17] and specific results, which highlights it potential in solving the challenges of delivering timely and accurate diagnostics for future wide-scale application.

CRISPR opens up a new era for parasite diagnosis

CRISPR together with CRISPR-associated (Cas) proteins (including Cas9, 12, and 13) has accelerated a revolution in genetic engineering and is proving invaluable in our understanding of the molecular mechanisms of disease development, drug resistance, and single-cell biology.[18] CRISPR-Cas9 technology has been utilized for genomic functional studies in free-living and parasitic nematodes (*Caenorhabditis elegans*,[19–21] *Pristionchus pacificus*,[22,23] *Auanema rhodensis* and *A. freiburgensis*,[24] *Strongyloides stercoralis*, *Brugia malayi*, and *Strongyloides ratti*),[25–27] and digenetic trematodes (*Opisthorchis viverrini*[28] and *Schistosoma mansoni*).[29–31] Furthermore, CRISPR-Cas12/13 diagnostics platforms have shown considerable promise in the nucleic acid detection of infections caused by viruses (especially COVID-19)[32] and cancer over the past 5 years, reinforcing the value of extending the CRISPR-Cas mediated platform for the diagnosis of parasitic infections. Recently, successful CRISPR-mediated tests have been developed for the detection of malaria,[33] *Cryptosporidium parvum*[34,35] and *Enterocytozoon hepatopenaei*.[36] In this article, we provide a comprehensive introduction to the CRISPR-Cas diagnostics platform focusing on the CRISPR associated proteins Cas12 and Cas13, considering publications over the period 2017–2021. Then we discuss the potential advantages of CRISPR-Cas-based diagnosis with the aim of applying this new technology to develop, for broad application, unique parasite diagnostic tools that are rapid, specific, sensitive, cost-effective, user

friendly, not requiring specialized equipment or expertise, and can be used in resource-poor communities in developing countries.

THE CRISPR-CAS-BASED DIAGNOSTIC PLATFORM

CRISPR-based technology provides a powerful genetic approach for interrogating genomes and defining the function of key genes in various organisms by triggering specific and heritable genome editing. Native CRISPR-Cas systems exist in many bacteria, presenting an adaptive immune response against sequence-specific degradation from an invading pathogen's genome.[37] Thus, the CRISPR-associated (Cas) immune system has been applied in molecular biology to target and cleave specific nucleic acid sequences, which have been further reconfigured for use in genome editing, drug development, gene expression regulation, the prevention and treatment of cancers, the treatment of genetic and infectious diseases, and in developing novel diagnostics.[37] Cas9 acts as a "molecular scissors" to cut DNA at a location specified by a guide RNA, leading to precise edits of the genome.[38] CRISPR-Cas9 technology has taken the life science community by storm with its widespread use in numerous laboratories worldwide since its inception, allowing researchers to alter genes to identify and potentially eliminate pathogens and diseases and create hardier and more resistant plants, among other important applications.[38] It is important to recount that Emmanuelle Charpentier and Jennifer A. Doudna were awarded the Nobel Prize in Chemistry in 2020 for their outstanding contributions in the development of the CRISPR-Cas9 method for genome editing.

Several Cas proteins (including Cas12a, Cas13, and Cas14) present nonspecific trans-cleavage activity after the site-specific binding of target nucleic acids, a unique feature that when exploited allows for novel applications as a diagnostic tool.[4] Recently, CRISPR-Cas biosensing systems reliant on the collateral cleavage activity of Cas12 and Cas13 have been developed for the detection of various targets, including bacteria, viruses, cancer mutations, and other diseases.[39] A series of articles published in the journal *Science*[4–7] revolutionized the field of molecular diagnostics for infectious diseases using CRISPR-Cas12/13 as a diagnostics tool. These reports demonstrated that these CRISPR-based diagnostic assays allow for ultra-sensitive, specific, multiplexed detection of nucleic acids incorporating a method with the speed, cost-efficiency, and portability required of a POC test. It is clear that with its exceptional detection features, the CRISPR-Cas12/13 system has the potential to become a major player as one of the next-generation diagnostic biosensing platforms.

Since the 2019 global coronavirus outbreak, the dramatically increased requirements for testing for SARS-CoV-2/COVID-19 in a variety of settings and the limitations of conventional diagnostic procedures, presenting high risks for cross contamination and transmission,[40] have driven rapid and substantial progress in the use of CRISPR-Cas12/Cas13 based diagnostic procedures.[41,42] A CRISPR-Cas based platform, termed Specific High Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK), has been established,[5,43] which allows for multiplexed, portable, and ultra-sensitive molecular detection of RNA or DNA from clinically relevant samples. The SHERLOCK assays with recombinase-mediated polymerase pre-amplification of DNA or RNA, and subsequent Cas13- or Cas12-mediated detection[39] via fluorescent and colorimetric readouts can provide results in less than 1 hour with minimum contamination.[44]

The mechanism of the CRISPR-Cas12/Cas13 based diagnostic tests utilizes the collateral nonspecific enzymatic cleavage activities of these proteins.[39] Cas12/Cas13 are guided by CRISPR RNA (crRNA) to cleave next to a specific DNA (for Cas12) or RNA (for Cas13) target sequence. The collateral nonspecific enzymatic cleavage activities of these proteins are then exploited, with off-target cleavage of fluorescent reporter ssDNA (for Cas12) or single-stranded RNA (ssRNA, for Cas13) sensors generating a signal that indicates the presence of the target sequence (Figure 1). CRISPR-Cas12/13-based diagnosis is especially suited for nucleic acid detection under field conditions as it can be combined with isothermal amplification which is optimized for use at a single temperature, amplifying target nucleic acids with a similar level of sensitivity as a PCR-based assay, without the need for a thermal cycler. Isothermal amplification methods include LAMP which amplifies at a constant temperature between 60 and 65°C, and recombinase polymerase amplification (RPA), which is optimal between 37 and 42°C.

CRISPR-Cas13 technology (Cas13-SHERLOCK)

The CRISPR-Cas13 system has been employed as a rapid RNA or DNA detection method with attomolar (aM, 10^{-18} mol/L) sensitivity and single-base mismatch specificity through SHERLOCK, using isothermal RPA followed by in vitro transcription and Cas13 detection.[5] The CRISPR-Cas13 system is mainly comprised of the programmable single-effector RNA-guided RNase Cas13 and 64–66 nt crRNA, which guide the protein to a specific recognition site on target RNA. In Cas13-SHERLOCK, guided by the crRNA, the Cas13 protein binds and cuts the targeted RNA sequence followed by non-specific cleavage of other RNA molecules. Specificity is achieved by crRNA–guild pairing and sensitivity is conferred via signal amplification of collateral cleavage activity by Cas13 on RNA reporters activated during the reaction. The signal, indicative of the positive identification of target RNA/DNA, can be captured on a colorimetric lateral-flow strip (by biotin–fluorescein RNA reporters) or visualized by a fluorescence signal readout (by molecular beacon fluorescent reporters).

Gootenberg et al.[5] first successfully established the Cas13-SHERLOCK in 2017 (Figure 2), utilizing RPA for amplification and Cas13 collateral cutting ability for signal reporting. The same research group (Gootenberg et al.[4]) later developed SHERLOCK version 2 (SHERLOCKv2) with lateral-flow readout and improved the quantitative measurement of input as low as 2 aM and increased (3.5-fold) the signal sensitivity by combining Cas13 with Csm6, an auxiliary CRISPR-associated enzyme. Abudayyeh et al.[45] then created a modified version of the portable SHERLOCK platform in 2019 to detect and quantitate targeted multiple plant genes in 15 minutes. The Cas13-based SHERLOCK platform used in instrument-free assays was

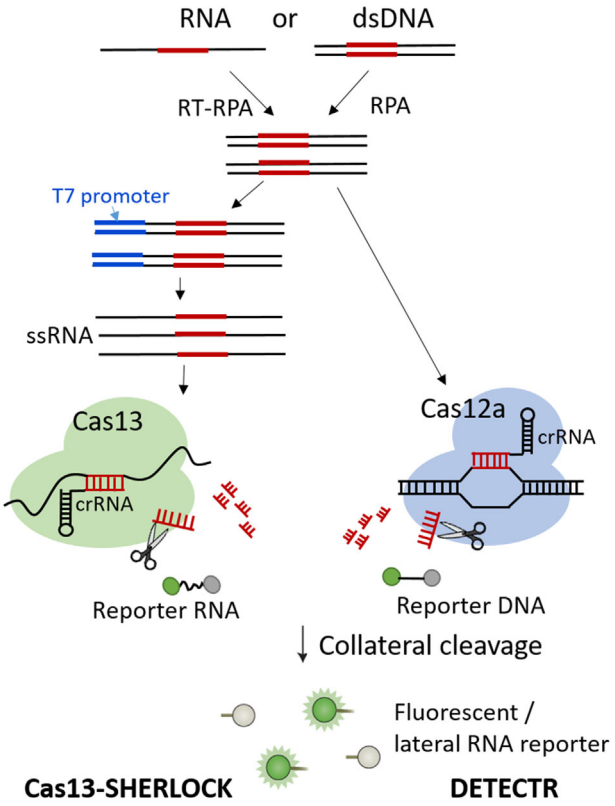


FIGURE 1 Cas complex activation and collateral activity: RT-RPA and RPA-coupled CRISPR-Cas13/Cas12a detection of a target sequence. Cas13 and Cas12a are able to detect the presence of RNA and DNA target sequences, respectively.[80,81] Nucleic acids are amplified as double-stranded DNA (dsDNA) using RT-RPA (for RNA templates) or RPA (for DNA templates). For Cas13 detection (Cas13-SHERLOCK), RPA-amplified dsDNA is transcribed to single-stranded RNA (ssRNA) via T7 transcription. Cas13-crRNA complexes bind to complementary target sequences on the ssRNA, thereby activating and triggering collateral cleavage of fluorescent RNA sensors.[4,7] With Cas12 detection (DETECTR), Cas12 responds selectively to DNA-RPA-amplified dsDNA can be directly detected by Cas12-crRNA complexes without the need for a T7 transcription step, with activation triggering collateral cleavage of a fluorescent ssDNA sensor. Reporter RNA/ssDNA sensors emit fluorescence only upon cleavage by an activated CRISPR-Cas13/Cas12 complex. CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; DETECTR, DNA endonuclease-targeted CRISPR trans reporter; ds, double stranded; RPA, recombinase polymerase amplification; RT, reverse transcription; SHERLOCK, Specific High Sensitivity Enzymatic Reporter UnLOCKing; ss, single-stranded

developed (2018) to successfully detect Zika virus (ZIKV) and dengue virus (DENV)[7] in patient samples at concentrations as low as 1 copy per microliter.

With the outbreak of COVID-19, Zhang et al.[46] extended the SHERLOCK platform for SARS-CoV-2 detection by developing a dipstick method that provided an accurate readout in <1 hour without requiring elaborate instrumentation. Arizti-Sanz et al.[44] developed Streamlined Highlighting of Infections to Navigate Epidemics (SHINE) whose results can be read out in 50 minutes through a sealed tube where the amplification reaction occurs, reducing contamination risk. Additional reports reaffirmed the value of the CRISPR-Cas13-based assay with 90%–95% sensitivity and 99%–100% specificity with detection being rapid (1–2 hours), inexpensive ($0.05 per test), and portable.[44,47] These features provide the basis to expand this field-friendly approach to substantially improve the speed and accuracy of infectious disease diagnosis. To facilitate easier use of the CRISPR-Cas13 system, Fozouni et al.[48] developed an amplification-free CRISPR-Cas13a assay using a mobile phone-based microscope rather than specialized laboratory equipment. To further improve the sensitivity and specificity of the CRISPR-Cas13 system, Shinoda et al.[49] developed a "CRISPR-based amplification-free digital RNA detection (SATORI)" platform; this combines CRISPR-Cas13-based RNA detection and microchamber-array technology in a diagnostic procedure that is able to detect ssRNA targets in 5 minutes with a sensitivity of ~10 fM (10^{-15} mol/L) and has high specificity.[49] Additionally, to establish a powerful platform to test many samples, while simultaneously testing for many pathogens, Ackerman et al.[50] developed Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids (CARMEN) incorporating a microwell array to pair droplets of amplified samples with droplets containing CRISPR-Cas13 detection reagents. This system allows the testing of >4500 crRNA-target pairs on a single array. This scalable, highly multiplexed CRISPR-based nucleic acid detection procedure shifts diagnostic and monitoring efforts from targeted testing of high-priority samples to extensive testing of large sample sets, resulting in major benefits to patients and public health.[50]

CRISPR-Cas13 has also been utilized for early detection and monitoring of cancer markers from clinical patient samples, for identification of drug-resistance mechanisms, and the discovery of novel therapeutic targets via degrading and manipulating cancer-associated transcripts with high efficiency and specificity.[51] A CRISPR-Cas13 based assay has also been developed to detect opportunistic infections post-transplantation and for the monitoring of transplant rejection[52] in order to improve long-term patient outcomes. Several CRISPR-based biosensing platforms have also been developed to detect specific microRNAs (miRNA), by combining LAMP/RPA with reverse transcriptase and other modifications to amplify sequences from miRNA.[53] These platforms combine the advantages of enzyme-assisted signal amplification and enzyme-free amplification biosensing technologies with a CRISPR mediated system for miRNA detection, providing high sensitivity and precision, with fewer limitations in diagnostic by utilizing efficient sensors at a low cost with a simple miniaturized readout.[54] Of note, the CRISPR-Cas13 system has also been used for the localization and dynamic imaging of RNA in live cells to understand their functional biology.[55]

This evidence highlights the advantages of using the CRISPR-Cas13 based system as a field-applicable diagnostic tool, and its potential to be adapted for the detection of other infectious diseases.

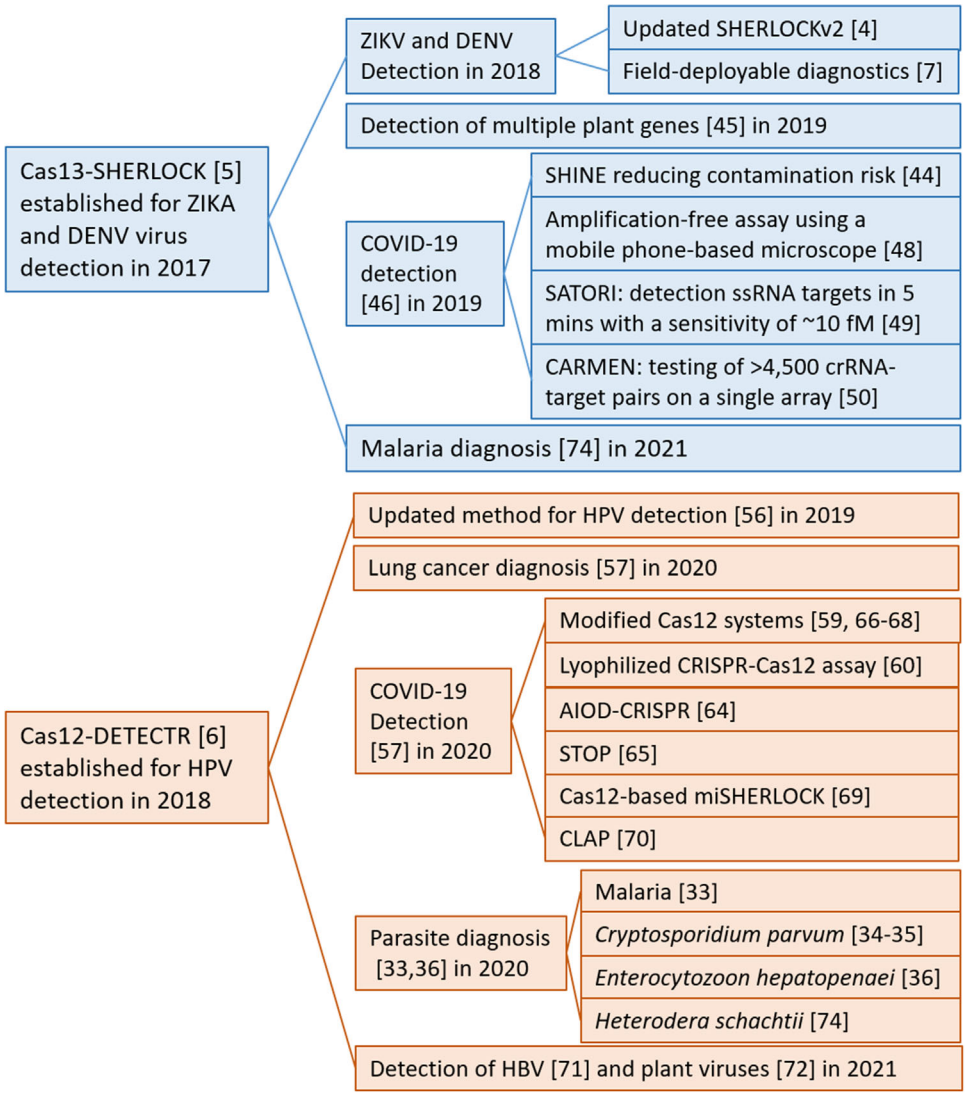


FIGURE 2 Development of the CRISPR-Cas12/13 based platform for the diagnosis of different diseases. Cas13-SHERLOCK was first developed in 2017 for the detection of Zika and Dengue viruses. This diagnostic tool was then used to detect plant genes and then COVID-19 immediately after its outbreak in 2019. The method was recently (2021) applied for malaria diagnosis, currently the only report where Cas13-SHERLOCK has been employed to detect a parasitic disease. Cas12-DETECTR was firstly developed in 2018 for detection of HPV and it was then modified in different versions for the detection of lung cancer and COVID in 2020. Since 2020 the Cas12-based method has been applied as an accurate and field-friendly diagnostic tool for malaria, *Cryptosporidium parvum*, *Enterocytozoon hepatopenaei*, and *Heterodera schachtii*. The approach was adapted for the detection of HBV and plant viruses in 2021. AIOD-CRISPR, All-In-One Dual CRISPR-Cas12a; CARMEN, Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids; CLAP, Cas12a-assisted RT-LAMP/AuNP; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; DENV, dengue virus; DETECTR, DNA endonuclease-targeted CRISPR trans reporter; HBV, hepatitis B; HPV, human papillomavirus; miSHERLOCK, minimally instrumented SHERLOCK; SATORI, CRISPR-based amplification-free digital RNA detection; SHINE, Streamlined Highlighting of Infections to Navigate Epidemics; STOP, SHERLOCK Testing in One Pot; ZIKV, Zika virus

CRISPR-Cas12-based technology (DETECTR)

By combining Cas12a ssDNase activation with isothermal amplification, Chen et al.[6] created an approach termed DNA endonuclease-targeted CRISPR trans reporter (DETECTR) (Figure 2), achieving attomolar sensitivity for DNA detection of human papillomavirus (HPV) in patient anal swab samples. The CRISPR-Cas12a system was then further advanced as CRISPR-Cas12a-POC to detect circulating HPV DNA in plasma incorporating a paper-strip test capable of providing immediate results, achieving a limit of detection (0.24 fM) similar to that obtained by PCR.[56] The CRISPR-Cas12a system was also utilized to detect endogenous DNA mutations of targeted genes in plasma and tissue specimens of lung cancer patients.[57]

Broughton et al.[58] in 2020 reported on utilizing CRISPR–Cas12-based DETECTR for detection of SARS-CoV-2. This assay combines LAMP with simultaneous reverse transcription (RT–LAMP) of

extracted RNA samples,[59] followed by Cas12 detection of predefined target sequences with single nucleotide target specificity and cleavage of a reporter molecule. The DETECTR assay features an easy-to-use reporting format with readout on a lateral flow strip within 30–40 minutes, though it has slightly reduced sensitivity compared with SARS-CoV-2 detection by PCR. A lyophilized CRISPR-Cas12 kit assay for SARS-CoV-2 detection (CASPR Biotech, USA) based on RT-LAMP and CRISPR-Cas12 was also developed, with an analytical sensitivity for testing of 7.5 copies/μL (within the range of commercial RT-qPCR 100–1000 copies/mL).[60] The CRISPR-Cas12a detection system provides a practical alternative for SARS-CoV-2 diagnosis in terms of detection robustness and operational cost.[6,61–63]

Aiming for more accurate and faster detection of SARS-CoV-2, Ding et al.[64] established the All-In-One Dual CRISPR-Cas12a (AIOD-CRISPR) test and Joung et al.[65] created a CRISPR-Cas12b-based test called SHERLOCK Testing in One Pot (STOP). Ramachandran et al.[66] developed electric field-driven microfluidics to control and accelerate this CRISPR assay by cofocusing Cas12-gRNA, reporters and target within a microfluidic chip[67] whereas Garcia-Venzor et al.[68] also reported fast detection of SARS-CoV-2 from patient samples based on LAMP and CRISPR-Cas12, showing high specificity at an affordable cost. Very recently, a Cas12-based minimally instrumented SHERLOCK (miSHERLOCK)[69] platform was established by introducing a unique instrument-free approach integrating a RNA paper-capture method compatible with RPA and Cas12a detection, thereby improving assay sensitivity 2- to 20-fold.[69] This system has been extended to identify specific mutations of SARS-CoV-2.[69] The CRISPR-Cas12a-based RT-LAMP assay developed by Zhang et al.,[70] named as Cas12a-assisted RT-LAMP/AuNP (CLAP) incorporates gold nanoparticle (AuNP) for visual colorimetric readout due to its strong molar absorption coefficient integrated AuNP. Under optimal conditions, the CLAP system exhibited superior specificity and was able to specifically detect down to 4 copies/μL of SARS-CoV-2 RNA in 40 minutes, with results visualized directly by the naked eye.[70]

The advantages of using a LAMP-Cas12a diagnostics system were further supported by a report of a rapid and accurate POC assay for hepatitis B (HBV), achieving a limit of HBV detection of 1 copy/μL using a fluorescent readout or lateral flow test strips within 13 and 20 minutes, respectively, showing 100% sensitivity and comparable specificity with qPCR.[71] The LAMP coupled CRISPR-Cas12a module was also extended for the sensitive detection of plant DNA viruses providing easy-to-interpret visual readouts in ~1 hour using a simple, low-cost fluorescence visualizer, making it suitable for POC applications.[72]

Overall, the successful development of field-applicable CRISPR-Cas12 based diagnostics highlights the potential of extending this novel technology to detect other infectious diseases, including parasitic infections. Currently, both DETECTR and SHERLOCK CRISPR-based COVID-19 diagnostics have been authorized for emergency use by the Food and Drug Administration (FDA).[32]

DEPLOYMENT OF THE CRISPR-CAS SYSTEM FOR THE DIAGNOSIS OF PARASITE INFECTION

Supported by the ever expanding numbers of pre-clinical and clinical studies which have shown the utility of CRISPR-Cas12/13-based diagnosis for viral diseases (especially SARS-CoV-2) and cancer, the CRISPR-mediated approach has been extended for the ultrasensitive detection of the single-celled parasites including *Cryptosporidium parvum*,[34,35] *Enterocytozoon hepatopenaei*,[36] malaria,[33,73] and a plant-parasitic nematode – *Heterodera schachtii*.[74]

Cryptosporidium parvum

C. parvum is an enteric unicellular parasite causing zoonotic cryptosporidiosis in humans and domestic/wild animals, and represents a major threat to public health globally.[75] A lack of suitable large-scale screening of drinking and environmental water for detecting low numbers of the contaminating microbe has impeded progress in controlling cryptosporidiosis. An ultrasensitive CRISPR-Cas12a-powered immunosensing approach has been recently developed for *Cryptosporidium* detection by linking antibody-based recognition with CRISPR-Cas12a-based fluorescent signal amplification through an antibody-DNA conjugate.[34] This method is able to detect whole 4 μm size oocysts with a range of 6.25–1600 oocysts/mL at a maximum sensitivity of a single oocyst per sample.[34] These ultrasensitive CRISPR-Cas-based biosensing assays have potential for both water quality assessment and whole pathogen detection. A similar approach has been employed to detect nucleic acid of *C. parvum* from human and cattle fecal samples with high flexibility, sensitivity, and specificity.[35] The procedure was able to detect a single copy of a cloned *C. parvum* 60-kDa glycoprotein gene, 10 oocysts/g clinical fecal sample, without requiring genomic DNA extraction and no cross-reactivity occurred with other species of *C. parvum* or other enteric parasitic protozoa.

Enterocytozoon hepatopenaei

E. hepatopenaei (EHP) is a microsporidian pathogen that causes growth retardation of the pacific whiteleg shrimp, *Penaeus vannamei*, leading to severe losses in shrimp productivity and the aquaculture industry in the Australian Pacific region and Asia.[76] To timely manage the parasite and the disease of hepatopancreatic microsporidiosis that it causes, it is crucial to routinely monitor the microsporodian for its timely prevention and management, but field-deployable diagnostic kits for EHP are still scarce. Accordingly, Kanitchinda et al.[36] combined RPA and a CRISPR-Cas12a fluorescence assay by targeting the EHP *ptp2* gene, and detected as few as 50 copies of DNA without cross reactivity with closely related microsporidia. The test can be performed at 37°C within 1 hour and the readout is visualized by

the naked eye with UV/blue-light excitation or by lateral flow detection. This CRISPR-Cas12a based diagnostic is promising for on-site surveillance of EHP and has potential for use as a critical diagnostic tool to help control this disease and in preventing its spread to other areas.

Malaria

Malaria is one of the deadliest diseases in history and 500 million people are estimated to be currently infected with 2.5 billion at risk of exposure to malaria infection in endemic areas.[77] Achieving malaria elimination requires ultrasensitive diagnostics to detect low parasite density infections (<100 parasites per microliter blood). A Malaria Cas12a-based SHERLOCK assay was developed by Lee et al.[33]; this was able to detect an infection of below two parasites per microliter blood, a limit of detection recommended by the WHO, highlighting its sensitivity and diagnostic value as a promising tool well-suited to clinical POC application in resource-limited settings. Furthermore, the Cas13a-based SHERLOCK has also been developed for differentiation of *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*.[73]

The emergence of pathogen drug resistance poses a significant threat to human health. Given emerging drug resistance is becoming a core challenge for malaria control, a next-generation CRISPR-Cas9 diagnostic method, Finding Low Abundance Sequences by Hybridization-Next Generation Sequencing (FLASH-NGS), was developed to detect drug resistance genes in *P. falciparum*.[78] The FLASH technique uses a set of Cas9 guide RNAs designed to cleave gene sequences of interest into fragments which are then utilized in specific hybridization for Illumina sequencing. The FLASH-NGS method goes beyond other CRISPR-based diagnostic tools in that it enables high levels of multiplexing (thousands of targets) and is advanced by the precision and sequence confirmation of next-generation sequencing.[78] The procedure achieved sub-attomolar gene detection with minimal background, highlighting its exquisite ultrasensitive features in identifying drug-resistance associations in malaria parasites and also in the detection of antimicrobial resistance genes in pneumonia-causing gram-positive bacteria.[78]

Heterodera schachtii

H. schachtii is a plant cyst nematode which causes serious economic losses in sugar beet production. Apparently, it is critical and necessary to develop a rapid and field-friendly diagnosis tool for better prevention and control this disease. Yao et al.[74] combined RPA with CRISPR-Cas12a technology to successfully detect plant samples infected with *H. schachtii* within 1 hour by fluorescence. The sensitivity of the system developed was greater than electrophoresis and was similar to that of an RPA/lateral flow dipstick procedure reported in the same study. The method also has high specificity, facilitating rapid, accurate, and direct detection of *H. schachtii* from infected plant tissue, and is potentially useful for monitoring and management of this plant parasitic nematode in the field.

Supported by the recent establishment of CRISPR-mediated tests for the successful detection of these unicellular parasites, continued development and application of the CRISPR-Cas approach for improved diagnosis of parasitic helminths is critical for ongoing control efforts and to achieve future elimination targets.

CONCLUSION AND OUTLOOK

As the COVID-19 pandemic has illustrated, timely and accurate diagnosis is pivotal to effective health care but as noted by the recently published Lancet Commission on diagnostics, diagnostic access and capacity is poor and unbalanced in many parts of the world, with 47% of the global population having little to no access to diagnostics.[79] This mirrors the situation in the field of parasitology where there is an urgent need to develop rapid, specific, sensitive, easily configurable, field-friendly, and cost-effective diagnostic tools to better manage, control, and eventually eliminate the spread of parasitic infections and improve patient outcomes. The emergence of COVID-19 has dramatically accelerated the development of CRISPR-Cas12/13 technology, which presents with many qualities that underpin it as a powerful new diagnostics vehicle. The CRISPR-Cas12/13 approach can provide a method to rapidly screen large cohorts of patients and animals, enabling more effective local targeting of drug treatment to effectively reduce the cost of control efforts. Obtaining accurate and reliable field diagnosis data through this technology would greatly improve the effectiveness of current WHO parasite control programs, help identify drug resistant parasites, and allow the development of powerful rapid POC diagnosis for effective treatment low- and higher-endemic areas. CRISPR-Cas12/13 could thereby play a major role in solving the challenges of delivering timely and accurate diagnostics. With its potential for future wide-scale application, utilizing CRISPR technology for parasite diagnosis represents an effective and feasible public health objective that could reshape health care, improve epidemiological management on a global level, and lead to substantial reductions in the time required to achieve elimination goals.

ACKNOWLEDGMENTS

This research received funding support from a National Health and Medical Research Council (NHMRC) of Australia Investigator Grant to DPM (APP1194462) and QIMR Berghofer Medical Research Institute seed grant (2021) to HY.

AUTHOR CONTRIBUTIONS

Hong You: Conceptualization and writing – original draft preparation; Catherine A. Gordon, Skye R. MacGregor, Pengfei Cai, and Donald P. McManus: Writing – review and editing. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

ORCID

Hong You https://orcid.org/0000-0002-8790-7793

REFERENCES

1. Hotez, P., & Aksoy, S. (2017). PLOS Neglected Tropical Diseases: Ten years of progress in neglected tropical disease control and elimination … More or less. *PLOS Neglected Tropical Diseases*, *11*(4), e0005355.
2. World Health Organization Team, Control of Neglected Tropical Diseases. (2021). Ending the neglect to attain the Sustainable Development Goals: A road map for neglected tropical diseases 2021–2030. (Eds:Malecel Mwelecele Ntuli) World Health Organization. https://www.who.int/publications/i/item/9789240010352.
3. World Health Orgnization. (2021). Public consultation: Target product profiles for diagnostic tests to meet schistosomiasis and soil-transmitted helminth programme needs.
4. Gootenberg, J. S., Abudayyeh, O. O., Kellner, M. J., Joung, J., Collins, J. J., & Zhang, F. (2018). Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science*, *360*(6387), 439–439.
5. Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., Verdine, V., Donghia, N., Daringer, N. M., Freije, C. A., Myhrvold, C., Bhattacharyya, R. P., Livny, J., Regev, A., Koonin, E. V., Hung, D. T., Sabeti, P. C., Collins, J. J., & Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, *356*(6336), 438–438.
6. Chen, J. S., Ma, E., Harrington, L. B., Da Costa, M., Tian, X., Palefsky, J. M. & Doudna, J. A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, *360*(6387), 436–439.
7. Myhrvold, C., Freije, C. A., Gootenberg, J. S., Abudayyeh, O. O., Metsky, H. C., Durbin, A. F., Kellner, M. J., Tan, A. L., Paul, L. M., Parham, L. A., Garcia, K. F., Barnes, K. G., Chak, B., Mondini, A., Nogueira, M. L., Isern, S., Michael, S. F., Lorenzana, I., Yozwiak, N. L., … Sabeti, P. C. (2018). Field-deployable viral diagnostics using CRISPR-Cas13. *Science*, *360*(6387), 444–448.
8. Amoah, A. S., Hoekstra, P. T., Casacuberta-Partal, M., Coffeng, L. E., Corstjens, P., Greco, B., van Lieshout, L., Lim, M. D., Markwalter, C. F., Odiere, M. R., Reinhard-Rupp, J., Roestenberg, M., Stothard, R., Tchuem Tchuente, L. A., van de Vlas, S. J., & Dam, G. J. (2020). Sensitive diagnostic tools and targeted drug administration strategies are needed to eliminate schistosomiasis. *Lancet Infectious Diseases*, *20*(7), e165–e172.
9. Ross, A. G., Bartley, P. B., Sleigh, A. C., Olds, G. R., Li, Y., Williams, G. M., & McManus, D. P. (2002). Schistosomiasis. *New England Journal of Medicine*, *346*(16), 1212–1212.
10. Gordon, C. A., Kurscheid, J., Williams, G. M., Clements, A. C. A., Li, Y., Zhou, X. N., Utzinger, J., McManus, D. P., & Gray, D. J. (2019). Asian schistosomiasis: Current status and prospects for control leading to elimination. *Tropical Medicine and Infectious Disease*, *4*(1), 40.
11. He, P., Gordon, C. A., Williams, G. M., Li, Y., Wang, Y., Hu, J., Gray, D. J., Ross, A. G., Harn, D., & McManus, D. P. (2018). Real-time PCR diagnosis of *Schistosoma japonicum* in low transmission areas of China. *Infectious Diseases of Poverty*, *7*(1), 8.
12. CF, V. A. N. D., Gordon, C. A., Li, Y., Williams, G. M., Wang, Y., Luo, Z., Gobert, G. N., You, H., McManus, D. P., & Gray, D. J. (2017). Rodents, goats and dogs – their potential roles in the transmission of schistosomiasis in China. *Parasitology*, *144*(12), 1633–1642.
13. Song, J., Liu, C., Bais, S., Mauk, M. G., Bau, H. H., & Greenberg, R. M. (2015). Molecular detection of schistosome infections with a disposable microfluidic cassette. *PLOS Neglected Tropical Diseases*, *9*(12), e0004318.
14. Weerakoon, K. G., & McManus, D. P. (2016). Cell-free DNA as a diagnostic tool for human parasitic infections. *Trends in Parasitology*, *32*(5), 378–378.
15. World Health Organization. (2020). COVID-19: WHO issues interim guidance for implementation of NTD programmes.
16. Nwoko, O. E., Mogaka, J. J. O., & Chimbari, M. J. (2021). Challenges and opportunities presented by current techniques for detecting schistosome infections in intermediate host snails: A scoping review. *International Journal of Environmental Research and Public Health*, *18*(10), 5403.
17. Zhu, X., Wang, X., Li, S., Luo, W., Zhang, X., Wang, C., Chen, Q., Yu, S., Tai, J., & Wang, Y. (2021). Rapid, ultrasensitive, and highly specific diagnosis of COVID-19 by CRISPR-based detection. *ACS Sensors*, *6*(3), 881–881.
18. Yan, F., Wang, W., & Zhang, J. (2019). CRISPR-Cas12 and Cas13: The lesser known siblings of CRISPR-Cas9. *Cell Biology and Toxicology*, *35*(6), 489–489.
19. Friedland, A. E., Tzur, Y. B., Esvelt, K. M., Colaiacovo, M. P., Church, G. M., & Calarco, J. A. (2013). Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. *Nature Methods*, *10*(8), 741–741.
20. Chiu, H., Schwartz, H. T., Antoshechkin, I., & Sternberg, P. W. (2013). Transgene-free genome editing in *Caenorhabditis elegans* using CRISPR-Cas. *Genetics*, *195*(3), 1167–1167.
21. Dickinson, D. J., Ward, J. D., Reiner, D. J., & Goldstein, B. (2013). Engineering the *Caenorhabditis elegans* genome using Cas9-triggered homologous recombination. *Nature Methods*, *10*(10), 1028–1028.
22. Witte, H., Moreno, E., Rodelsperger, C., Kim, J., Kim, J. S., Streit, A., & Sommer, R. J. (2015). Gene inactivation using the CRISPR/Cas9 system in the nematode Pristionchus pacificus. *Development Genes and Evolution*, *225*(1), 55–55.
23. Nakayama, K. I., Ishita, Y., Chihara, T., & Okumura, M. (2020). Screening for CRISPR/Cas9-induced mutations using a co-injection marker in the nematode Pristionchus pacificus. *Development Genes and Evolution*, *230*(3), 257–257.
24. Adams, S., Pathak, P., Shao, H., Lok, J. B., & Pires-daSilva, A. (2019). Liposome-based transfection enhances RNAi and CRISPR-mediated mutagenesis in non-model nematode systems. *Scientific Reports*, *9*(1), 483.
25. Gang, S. S., Castelletto, M. L., Bryant, A. S., Yang, E., Mancuso, N., Lopez, J. B., Pellegrini, M., & Hallem, E. A. (2017). Targeted mutagenesis in a human-parasitic nematode. *PLoS Pathogens*, *13*(10), e1006675.
26. Waaijers, S., Portegijs, V., Kerver, J., Lemmens, B. B., Tijsterman, M., van den Heuvel, S., & Boxem, M. (2013). CRISPR/Cas9-targeted mutagenesis in *Caenorhabditis elegans*. *Genetics*, *195*(3), 1187–1187.
27. Liu, C., Grote, A., Ghedin, E., & Unnasch, T. R. (2020). CRISPR-mediated transfection of Brugia malayi. *PLOS Neglected Tropical Diseases*, *14*(8), e0008627.
28. Arunsan, P., Ittiprasert, W., Smout, M. J., Cochran, C. J., Mann, V. H., Chaiyadet, S., Karinshak, S. E., Sripa, B., Young, N. D., Sotillo, J., Loukas, A., Brindley, P. J., & Laha, T. (2019). Programmed knockout mutation of liver fluke granulin attenuates virulence of infection-induced hepatobiliary morbidity. *Elife*, *8*, e41463.
29. Ittiprasert, W., Mann, V. H., Karinshak, S. E., Coghlan, A., Rinaldi, G., Sankaranarayanan, G., Chaidee, A., Tanno, T., Kumkhaek, C., Prangtaworn, P., Mentink-Kane, M. M., Cochran, C. J., Driguez, P., Holroyd, N., Tracey, A., Rodpai, R., Everts, B., Hokke, C. H., Hoffmann, K. F., … Brindley, P. J. (2019). Programmed genome editing of the omega-1 ribonuclease of the blood fluke, Schistosoma mansoni. *Elife*, *8*, e41337.
30. You, H., Mayer, J. U., Johnston, R. L., Sivakumaran, H., Ranasinghe, S., Rivera, V., Kondrashova, O., Koufariotis, L. T., Du, X., Driguez, P., French, J. D., Waddell, N., Duke, M. G., Ittiprasert, W., Mann, V. H., Brindley, P. J., Jones, M. K., & McManus, D. P. (2021). CRISPR/Cas9-mediated genome editing of *Schistosoma mansoni* acetylcholinesterase. *FASEB Journal*, *35*(1), e21205.
31. Sankaranarayanan, G., Coghlan, A., Driguez, P., Lotkowska, M. E., Sanders, M., Holroyd, N., Tracey, A., Berriman, M., & Rinaldi, G. (2020).

Large CRISPR-Cas-induced deletions in the oxamniquine resistance locus of the human parasite *Schistosoma mansoni*. *Wellcome Open Research*, *5*, 178.

32. Palaz, F., Kalkan, A. K., Tozluyurt, A., & Ozsoz, M. (2021). CRISPR-based tools: Alternative methods for the diagnosis of COVID-19. *Clinical Biochemistry*, *89*, 1–13.
33. Lee, R. A., Puig, H., Nguyen, P. Q., Angenent-Mari, N. M., Donghia, N. M., McGee, J. P., Dvorin, J. D., Klapperich, C. M., Pollock, N. R., & Collins, J. J. (2020). Ultrasensitive CRISPR-based diagnostic for field-applicable detection of Plasmodium species in symptomatic and asymptomatic malaria. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(41), 25722–25731.
34. Li, Y., Deng, F., Hall, T., Vesey, G., & Goldys, E. M. (2021). CRISPR/Cas12a-powered immunosensor suitable for ultra-sensitive whole Cryptosporidium oocyst detection from water samples using a plate reader. *Water Research*, *203*, 117553.
35. Yu, F., Zhang, K., Wang, Y., Li, D., Cui, Z., Huang, J., Zhang, S., Li, X., & Zhang, L. (2021). CRISPR/Cas12a-based on-site diagnostics of *Cryptosporidium parvum* IId-subtype-family from human and cattle fecal samples. *Parasites & Vectors*, *14*(1), 208.
36. Kanitchinda, S., Srisala, J., Suebsing, R., Prachumwat, A., & Chaijarasphong, T. (2020). CRISPR-Cas fluorescent cleavage assay coupled with recombinase polymerase amplification for sensitive and specific detection of *Enterocytozoon hepatopenaei*. *Biotechnology Reports (Amst)*, *27*, e00485.
37. Ghorbani, A., Hadifar, S., Salari, R., Izadpanah, K., Burmistrz, M., Afsharifar, A., Eskandari, M. H., Niazi, A., Denes, C. E., & Neely, G. G. (2021). A short overview of CRISPR-Cas technology and its application in viral disease control. *Transgenic Research*, *30*(3), 221–221.
38. Du, X., McManus, D. P., French, J. D., Jones, M. K., & You, H. (2021). CRISPR/Cas9: A new tool for the study and control of helminth parasites. *BioEssays*, *43*(1), 2000185.
39. Li, Y., Li, S., Wang, J., & Liu, G. (2019). CRISPR/Cas systems towards next-generation biosensing. *Trends in Biotechnology*, *37*(7), 730–730.
40. Aquino-Jarquin, G. (2021). Recent progress on rapid SARS-CoV-2/COVID-19 detection by CRISPR-Cas13-based platforms. *Drug Discovery Today*, *26*(8), 2025–2025.
41. Choi, J. R., Hu, J., Tang, R., Gong, Y., Feng, S., Ren, H., Wen, T., Li, X., Wan Abas, W. A., Pingguan-Murphy, B., & Xu, F. (2016). An integrated paper-based sample-to-answer biosensor for nucleic acid testing at the point of care. *Lab on a Chip*, *16*(3), 611–611.
42. Scheler, O., Glynn, B., & Kurg, A. (2014). Nucleic acid detection technologies and marker molecules in bacterial diagnostics. *Expert Review of Molecular Diagnostics*, *14*(4), 489–489.
43. Kellner, M. J., Koob, J. G., Gootenberg, J. S., Abudayyeh, O. O., & Zhang, F. (2019). SHERLOCK: Nucleic acid detection with CRISPR nucleases. *Nature Protocols*, *14*(10), 2986–3012.
44. Arizti-Sanz, J., Freije, C. A., Stanton, A. C., Petros, B. A., Boehm, C. K., Siddiqui, S., Shaw, B. M., Adams, G., Kosoko-Thoroddsen, T. F., Kemball, M. E., Uwanibe, J. N., Ajogbasile, F. V., Eromon, P. E., Gross, R., Wronka, L., Caviness, K., Hensley, L. E., Bergman, N. H., MacInnis, B. L., … Myhrvold, C. (2020). Streamlined inactivation, amplification, and Cas13-based detection of SARS-CoV-2. *Nature Communications*, *11*(1), 5921.
45. Abudayyeh, O. O., Gootenberg, J. S., Kellner, M. J., & Zhang, F. (2019). Nucleic acid detection of plant genes using CRISPR-Cas13. *CRISPR Journal*, *2*, 165–171.
46. Zhang, F., Abudayyeh, O. A., & Gootenberg, J. S. (2020). A protocol for detection of COVID-19 using CRISPR diagnostics. https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20(updated).pdf.
47. Shihong Gao, D., Zhu, X., & Lu, B. (2021). Development and application of sensitive, specific, and rapid CRISPR-Cas13-based diagnosis. *Journal of Medical Virology*, *93*(7), 4198–4204.
48. Fozouni, P., Son, S., Diaz de Leon Derby, M., Knott, G. J., Gray, C. N., D'Ambrosio, M. V., Zhao, C., Switz, N. A., Kumar, G. R., Stephens, S. I., Boehm, D., Tsou, C. L., Shu, J., Bhuiya, A., Armstrong, M., Harris, A. R., Chen, P. Y., Osterloh, J. M., Meyer-Franke, A., … Ott, M. (2021). Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy. *Cell*, *184*(2), 323–333.
49. Shinoda, H., Taguchi, Y., Nakagawa, R., Makino, A., Okazaki, S., Nakano, M., Muramoto, Y., Takahashi, C., Takahashi, I., Ando, J., Noda, T., Nureki, O., Nishimasu, H., & Watanabe, R. (2021). Amplification-free RNA detection with CRISPR-Cas13. *Communications Biology*, *4*(1), 476.
50. Ackerman, C. M., Myhrvold, C., Thakku, S. G., Freije, C. A., Metsky, H. C., Yang, D. K., Ye, S. H., Boehm, C. K., Kosoko-Thoroddsen, T. F., Kehe, J., Nguyen, T. G., Carter, A., Kulesa, A., Barnes, J. R., Dugan, V. G., Hung, D. T., Blainey, P. C., & Sabeti, P. C. (2020). Massively multiplexed nucleic acid detection with Cas13. *Nature*, *582*(7811), 277–277.
51. Palaz, F., Kalkan, A. K., Can, O., Demir, A. N., Tozluyurt, A., Ozcan, A., & Ozsoz, M. (2021). CRISPR-Cas13 system as a promising and versatile tool for cancer diagnosis, therapy, and research. *ACS Synthetic Biology*, *10*(6), 1245–1245.
52. Kaminski, M. M., Alcantar, M. A., Lape, I. T., Greensmith, R., Huske, A. C., Valeri, J. A., Marty, F. M., Klambt, V., Azzi, J., Akalin, E., Riella, L. V., & Collins, J. J. (2020). A CRISPR-based assay for the detection of opportunistic infections post-transplantation and for the monitoring of transplant rejection. *Nature Biomedical Engineering*, *4*(6), 601–601.
53. Li, C., Li, Z., Jia, H., & Yan, J. (2011). One-step ultrasensitive detection of microRNAs with loop-mediated isothermal amplification (LAMP). *Chemical Communications (Cambridge)*, *47*(9), 2595–2597.
54. Granados-Riveron, J. T., & Aquino-Jarquin, G. (2021). CRISPR/Cas13-based approaches for ultrasensitive and specific detection of microRNAs. *Cells*, *10*(7), 1655.
55. Yang, L. Z., Wang, Y., Li, S. Q., Yao, R. W., Luan, P. F., Wu, H., Carmichael, G. G., & Chen, L. L. (2019). Dynamic imaging of RNA in living cells by CRISPR-Cas13 systems. *Molecullar Cell*, *76*(6), 981–997.
56. Tsou, J. H., Leng, Q., & Jiang, F. (2019). A CRISPR test for detection of circulating nuclei acids. *Translational Oncology*, *12*(12), 1566–1566.
57. Tsou, J. H., Leng, Q., & Jiang, F. (2020). A CRISPR test for rapidly and sensitively detecting circulating EGFR mutations. *Diagnostics (Basel)*, *10*(2), 114.
58. Broughton, J. P., Deng, X., Yu, G., Fasching, C. L., Servellita, V., Singh, J., Miao, X., Streithorst, J. A., Granados, A., Sotomayor-Gonzalez, A., Zorn, K., Gopez, A., Hsu, E., Gu, W., Miller, S., Pan, C. Y., Guevara, H., Wadford, D. A., Chen, J. S., & Chiu, C. Y. (2020). CRISPR-Cas12-based detection of SARS-CoV-2. *Nature Biotechnology*, *38*(7), 870–870.
59. Ali, Z., Aman, R., Mahas, A., Rao, G. S., Tehseen, M., Marsic, T., Salunke, R., Subudhi, A. K., Hala, S. M., Hamdan, S. M., Pain, A., Alofi, F. S., Alsomali, A., Hashem, A. M., Khogeer, A., Almontashiri, N. A. M., Abedalthagafi, M., Hassan, N., & Mahfouz, M. M. (2020). iSCAN: An RT-LAMP-coupled CRISPR-Cas12 module for rapid, sensitive detection of SARS-CoV-2. *Virus Research*, *288*, 198129.
60. Curti, L. A., Primost, I., Valla, S., Alegre, D. I., Perglione, C. O., Repizo, G. D., Lara, J., Parcerisa, I., Palacios, A., Llases, M. E., Rinflerch, A., Barrios, M., Bonnet, F. P., Gimenez, C. A., & Marcone, D. N. (2021). Evaluation of a lyophilized CRISPR-Cas12 assay for a sensitive, specific, and rapid detection of SARS-CoV-2. *Viruses*, *13*(3), 420.
61. Liu, J. J., Orlova, N., Oakes, B. L., Ma, E., Spinner, H. B., Baney, K. L. M., Chuck, J., Tan, D., Knott, G. J., Harrington, L. B., Al-Shayeb, B., Wagner, A., Brotzmann, J., Staahl, B. T., Taylor, K. L., Desmarais, J., Nogales, E., & Doudna, J. A. (2019). CasX enzymes comprise a distinct family of RNA-guided genome editors. *Nature*, *566*(7743), 218–223.
62. Zhang, W. S., Pan, J., Li, F., Zhu, M., Xu, M., Zhu, H., Yu, Y., & Su, G. (2021). Reverse transcription recombinase polymerase amplification coupled with CRISPR-Cas12a for facile and highly sensitive colorimetric SARS-CoV-2 detection. *Analytical Chemistry*, *93*(8), 4126–4126.

63. English, M. A., Soenksen, L. R., Gayet, R. V., de Puig, H., Angenent-Mari, N. M., Mao, A. S., Nguyen, P. Q., & Collins, J. J. (2019). Programmable CRISPR-responsive smart materials. *Science*, *365*(6455), 780–780.
64. Ding, X., Yin, K., Li, Z., Lalla, R. V., Ballesteros, E., Sfeir, M. M., & Liu, C. (2020). Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. *Nature Communications*, *11*(1), 4711.
65. Joung, J., Ladha, A., Saito, M., Kim, N. G., Woolley, A. E., Segel, M., Barretto, R. P. J., Ranu, A., Macrae, R. K., Faure, G., Ioannidi, E. I., Krajeski, R. N., Bruneau, R., Huang, M. W., Yu, X. G., Li, J. Z., Walker, B. D., Hung, D. T., Greninger, A. L., … Zhang, F. (2020). Detection of SARS-CoV-2 with SHERLOCK One-Pot testing. *New England Journal of Medicine*, *383*(15), 1492–1492.
66. Ramachandran, A., Huyke, D. A., Sharma, E., Sahoo, M. K., Huang, C., Banaei, N., Pinsky, B. A., & Santiago, J. G. (2020). Electric field-driven microfluidics for rapid CRISPR-based diagnostics and its application to detection of SARS-CoV-2. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(47), 29518–29518.
67. Tsou, J. H., Liu, H., Stass, S. A., & Jiang, F. (2021). Rapid and sensitive detection of SARS-CoV-2 using clustered regularly interspaced short palindromic repeats. *Biomedicines*, *9*(3), 239.
68. Garcia-Venzor, A., Rueda-Zarazua, B., Marquez-Garcia, E., Maldonado, V., Moncada-Morales, A., Olivera, H., Lopez, I., Zuniga, J., & Melendez-Zajgla, J. (2021). SARS-CoV-2 direct detection without RNA isolation with loop-mediated isothermal amplification (LAMP) and CRISPR-Cas12. *Frontiers in Medicine (Lausanne)*, *8*, 627679.
69. de Puig, H., Lee, R. A., Najjar, D., Tan, X., Soeknsen, L. R., Angenent-Mari, N. M., Donghia, N. M., Weckman, N. E., Ory, A., Ng, C. F., Nguyen, P. Q., Mao, A. S., Ferrante, T. C., Lansberry, G., Sallum, H., Niemi, J., & Collins, J. J. (2021). Minimally instrumented SHERLOCK (miSHERLOCK) for CRISPR-based point-of-care diagnosis of SARS-CoV-2 and emerging variants. *Science Advances*, *7*(32), eabh2944.
70. Zhang, Y., Chen, M., Liu, C., Chen, J., Luo, X., Xue, Y., Liang, Q., Zhou, L., Tao, Y., Li, M., Wang, D., Zhou, J., & Wang, J. (2021). Sensitive and rapid on-site detection of SARS-CoV-2 using a gold nanoparticle-based high-throughput platform coupled with CRISPR/Cas12-assisted RT-LAMP. *Sensors & Actuators, B: Chemical*, *345*, 130411.
71. Ding, R., Long, J., Yuan, M., Zheng, X., Shen, Y., Jin, Y., Yang, H., Li, H., Chen, S., & Duan, G. (2021). CRISPR/Cas12-based ultra-sensitive and specific point-of-care detection of HBV. *International Journal of Molecular Sciences*, *22*(9), 4842.
72. Mahas, A., Hassan, N., Aman, R., Marsic, T., Wang, Q., Ali, Z., & Mahfouz, M. M. (2021). LAMP-coupled CRISPR-Cas12a module for rapid and sensitive detection of plant DNA viruses. *Viruses*, *13*(3), 466.
73. Cunningham, C. H., Hennelly, C. M., Lin, J. T., Ubalee, R., Boyce, R. M., Mulogo, E. M., Hathaway, N., Thwai, K. L., Phanzu, F., Kalonji, A., Mwandagalirwa, K., Tshefu, A., Juliano, J. J., & Parr, J. B. (2021). A novel CRISPR-based malaria diagnostic capable of Plasmodium detection, species differentiation, and drug-resistance genotyping. *EBioMedicine*, *68*, 103415.
74. Yao, K., Peng, D., Jiang, C., Zhao, W., Li, G., Huang, W., Kong, L., Gao, H., Zheng, J., & Peng, H. (2021). Rapid and visual detection of Heterodera schachtii using recombinase polymerase amplification combined with Cas12a-mediated technology. *International Journal of Molecular Sciences*, *22*(22), 12577.
75. Leitch, G. J., & He, Q. (2012). Cryptosporidiosis-an overview. *Journal of Biomedical Research*, *25*(1), 1–1.
76. Chaijarasphong, T., Munkongwongsiri, N., Stentiford, G. D., Aldama-Cano, D. J., Thansa, K., Flegel, T. W., Sritunyalucksana, K., & Itsathitphaisarn, O. (2021). The shrimp microsporidian *Enterocytozoon hepatopenaei* (EHP): Biology, pathology, diagnostics and control. *Journal of Invertebrate Pathology*, *186*, 107458.
77. Ashley, E. A., Pyae Phyo, A., & Woodrow, C. J. (2018). Malaria. *Lancet*, *391*(10130), 1608–1621.
78. Quan, J., Langelier, C., Kuchta, A., Batson, J., Teyssier, N., Lyden, A., Caldera, S., McGeever, A., Dimitrov, B., King, R., Wilheim, J., Murphy, M., Ares, L. P., Travisano, K. A., Sit, R., Amato, R., Mumbengegwi, D. R., Smith, J. L., Bennett, A., … Crawford, E. D. (2019). FLASH: A next-generation CRISPR diagnostic for multiplexed detection of antimicrobial resistance sequences. *Nucleic Acids Research*, *47*(14), e83.
79. Fleming, K. A., Horton, S., Wilson, M. L., Atun, R., DeStigter, K., Flanigan, J., Sayed, S., Adam, P., Aguilar, B., Andronikou, S., Boehme, C., Cherniak, W., Cheung, A. N., Dahn, B., Donoso-Bach, L., Douglas, T., Garcia, P., Hussain, S., Iyer, H. S., …Walia, K. (2021). The Lancet Commission on diagnostics: Transforming access to diagnostics. *Lancet*, *398*(10315), 1997–2050.
80. Kaminski, M. M., Abudayyeh, O. O., Gootenberg, J. S., Zhang, F., & Collins, J. J. (2021). CRISPR-based diagnostics. *Nature Biomedical Engineering*, *5*(7), 643–656.
81. Cox, D. B. T., Gootenberg, J. S., Abudayyeh, O. O., Franklin, B., Kellner, M. J., Joung, J., & Zhang, F. (2017). RNA editing with CRISPR-Cas13. *Science*, *358*(6366), 1019–1027.

How to cite this article: You, H., Gordon, C. A., MacGregor, S. R., Cai, P., & McManus, D. P. (2022). Potential of the CRISPR-Cas system for improved parasite diagnosis. *BioEssays*, e2100286. https://doi.org/10.1002/bies.202100286