These polymers are semicrystalline and possess mechanical properties that compare well with those of polylactide.

Furthermore, the authors found that by blending two enantiomerically pure polymers of opposite stereochemistry in a 1:1 stoichiometric ratio, superior materials could be obtained. Because of a phenomenon known as stereocomplexation (*13*), the materials that result from the simple blending process have melting temperatures that are more than 75°C higher than those of either constituent polymer, potentially enabling use in high-temperature applications.

Plastics will continue to be critical for addressing the continuing demands of our society. New polymeric materials will, for example, be needed for energy generation and storage, to address healthcare needs, for food conservation, and for providing clean water. The circular materials economy will require implementation of an appropriate infrastructure to underpin collection and sorting of plastic that has reached the end of its first life before it reaches the environment (*4*). Beyond the design of new materials, this will require collaboration across scientific and nonscientific disciplines as well as political and public will to ensure success.

Studies such as that of Zhu *et al.*, in which disposed plastics can be infinitely recycled without deleterious effects on their properties, can lead to a world in which plastics at the end of their life are not considered as waste but as raw materials to generate high-value products and virgin plastics. This will both incentivize recycling and encourage sustainability by reducing the requirement for new monomer feedstocks. Current chemical recycling processes are expensive and energetically unfavorable, and further advances in monomer and polymer development and catalyst design are required to facilitate the implementation of economically viable sustainable polymers (*14*). ■

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VIROLOGY

Next-generation diagnostics with CRISPR

CRISPR-Cas biology promises rapid, accurate, and portable diagnostic tools

By **Daniel S. Chertow**

Rapid and accurate identification of infectious diseases is essential to optimize clinical care and guide infection control and public health interventions to limit disease spread both in highly specialized medical centers and remote health care settings. The ideal diagnostic test would be inexpensive, accurate, and provide a result rapidly, allowing for point-of-care use on multiple specimen types without need for technical expertise, ancillary equipment, or power. Such a test for highly pathogenic viruses that emerge in remote settings but might spread globally (for example, Ebola virus and Middle East respiratory syndrome coronavirus) would aid in early case detection and isolation, limiting disease spread and facilitating timely care (*1*). The sentinel discovery that prokaryotes (bacteria and archaea) have heritable adaptive immunity mediated through CRISPR and CRISPR-associated (Cas) proteins has led to transformative advances in molecular biology, most notably in gene editing (*2*). On pages 436, 444, and 439 of this issue, Chen *et al.* (*3*), Myhrvold *et al.* (*4*), and Gootenberg *et al.* (*5*), respectively, highlight how evolving insights into CRISPR-Cas biology are also revolutionizing the field of molecular diagnostics for infectious diseases, through detection of Zika virus (ZIKV), Dengue virus (DENV), and human papillomavirus (HPV) in human samples, and noninfectious diseases, such as detection of gene mutations in circulating cell-free DNA from lung cancer patients.

Prokaryotes store genetic elements from infectious agents (phages, plasmids, or transposons) in genomic loci called CRISPR arrays as memories for adaptive immunity. Cas proteins facilitate adaptive immunity through the processes of adaptation, CRISPR RNA (crRNA) generation, and interference (*6*). During adaptation, foreign genetic material is processed and selected for integration into the CRISPR array, providing a recall element during recurrent infection. Pre-crRNA is transcribed as a long precursor and processed into mature form as crRNA to guide Cas proteins to cleave complementary sequences of foreign elements (interference) to degrade and eliminate those elements. By uncovering the structural and functional components of these diverse systems, new tools, including those applicable to molecular diagnostics, are emerging (see the figure).

Chen *et al.* report the discovery that when CRISPR-Cas12a proteins cleave double-stranded DNA (dsDNA) in a sequence-specific manner, they induce robust nonspecific single-stranded DNA (ssDNA) trans-cleavage. The authors apply this observation to develop a rapid and accurate test to detect carcinoma-associated HPV types 16 and 18 from clinical specimens. HPV dsDNA is extracted from anal swabs and amplified through isothermal preamplification by recombinase polymerase amplification (RPA) (*7*), a method that is rapid and does not require specialized equipment. A Cas12a-crRNA complex binds to and cleaves target HPV dsDNA, which activates trans-cleavage of ssDNA. A fluorescent reporter coupled to ssDNA generates a fluorescent signal upon cleavage. This new approach, called DNA endonuclease-targeted CRISPR trans reporter (DETECTR), offers a promising platform for rapid and accurate detection of cervical cancer–associated HPV subtypes that, consistent with World Health Organization recommendations, might augment screening programs worldwide (*8*).

> ***"Future work will expand upon the range of diagnostic applications for infectious and noninfectious diseases..."***

Myhrvold *et al.* introduce a new approach to release and protect from degradation viral nucleic acids from clinical specimens, bypassing the need for nucleic acid extraction in molecular diagnostics. This method,

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called HUDSON (heating unextracted diagnostic samples to obliterate nucleases), is a process of heat and chemical reduction that inactivates the high amounts of ribonucleases (RNases) found in body fluids and then lyses viral particles by disrupting the viral envelope, thereby releasing nucleic acids into solution. The authors combine HUDSON with SHERLOCK (specific high-sensitivity enzymatic reporter unlocking), a Cas13-based nucleic acid detection platform described previously (*9*). Similar to DETECTR, SHERLOCK combines RPA and an RNA-guided Cas13 that induces collateral cleavage of nucleic acids. In SHERLOCK, RNA coupled to a fluorescent reporter is cleaved, producing a fluorescent signal that is amplified though enzymatic activity, which enhances test sensitivity. By combining HUDSON and SHERLOCK, the authors develop a sensitive and specific diagnostic platform to detect the flaviviruses DENV and ZIKV directly from body fluids (urine, saliva, serum, plasma, and whole blood), with limited sample preparation or equipment, that provides a result within 1 to 2 hours. DENV and ZIKV cocirculate in many areas of Central and South America and have similar clinical presentation (*10*). ZIKV infection during pregnancy predisposes to severe congenital anomalies and is sexually transmissible, emphasizing the need for accurate diagnosis among pregnant women and their sexual partners (*11*). HUDSON combined with SHERLOCK reliably differentiates between DENV, ZIKV, and another flavivirus, yellow fever virus (YFV), which cocirculate and cause severe parallel epidemics in Brazil (*10*). The platform can also reliably distinguish between four closely related DENV serotypes and can detect single-nucleotide polymorphisms (SNPs) among ZIKV isolates. This could be applied to detect SNPs that confer antimicrobial resistance [for example, among *Mycobacterium tuberculosis* (TB) isolates] or enhance pathogen virulence or transmissibility [for example, among highly pathogenic avian influenza A (H5N1) viruses], allowing for better tracking of emerging pathogens. Finally, the authors show that the fluorescent readout of SHERLOCK can be replaced with a visual readout on a paper test-strip, suitable for point-of-care field application.

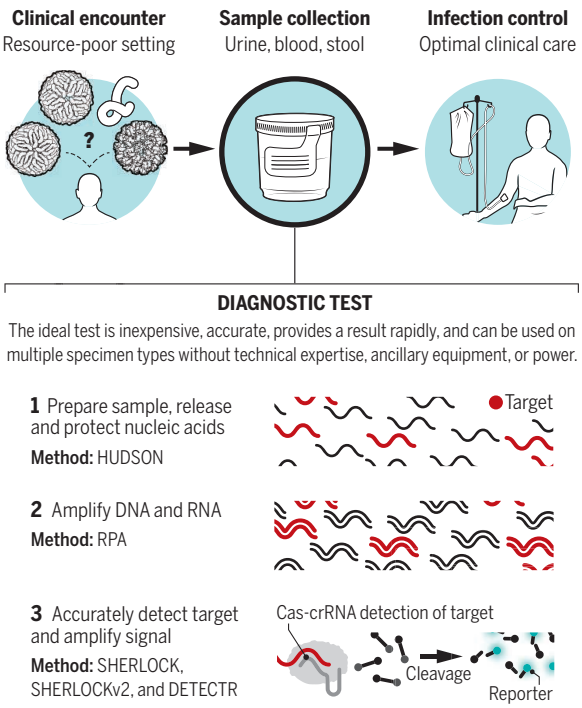
Gootenberg *et al.* introduce SHERLOCK version 2 (v2). This improved assay allows for detection of three ssRNA targets and one dsDNA target in a single reaction. The authors biochemically characterize 17 CRISPR-Cas13a and -Cas13b enzymes, selecting three with distinct cleavage preferences, that when combined with a Cas12a enzyme and RPA accurately detect ZIKV ssRNA, synthetic ssRNA, DENV ssRNA, and synthetic dsDNA by visual readout in less than 90 minutes. A potential application of multitarget RNA and DNA detection by SHERLOCKv2 could be a rapid and accurate diagnostic test for pneumonia pathogens. DNA and RNA viruses alone or in combination with bacterial infection cause pneumonia, a leading killer of children worldwide (*12*). An accurate and affordable point-of-care diagnostic for pneumonia would allow for early and targeted use of antibiotics in remote settings.

Another feature of SHERLOCKv2 is quantitative and sensitive target detection. This could be applied to a portable and accurate test to monitor viral load among HIV patients receiving antiviral therapy in resource-limited settings, substantially improving global HIV care (*13*). SHERLOCKv2 was also used to detect mutations in cell-free DNA from the blood of non–small cell lung cancer patients by fluorescence- and lateral flow–based readouts, further expanding the potential applications to liquid biopsy. Finally, in proof-of-concept in vitro experiments, the authors successfully apply SHERLOCKv2 both as a gene-editing therapeutic that corrects a gene mutation that predisposes to colon cancer, and as a diagnostic to concurrently determine the proportion of genes successfully edited.

These emerging diagnostic tools will by necessity be compared to standard diagnostics to ensure sensitivity and specificity and will need to be field-tested to guarantee performance in patient care settings, as environmental conditions and end-user application might affect performance. Proven assays, if affordable, promise to improve care in resource-limited settings where undifferentiated febrile illness is the norm and where gaps or delays in diagnosis, targeted care, and infection control contribute to infectious disease mortality and spread. For example, TB results in an estimated 1.3 million deaths annually, the leading cause from a single infectious agent, and most deaths could be prevented with early diagnosis and treatment (*14*). Assays might be expanded to provide insight into pathogen resistance patterns to guide antimicrobial therapy, molecular correlates of pathogen viability to guide infection control, and compatibility with additional specimen types such as stool, respiratory secretions, and cerebrospinal fluid, that when integrated with clinical judgment might for example, differentiate etiology of enteritis, pneumonia, and meningitis. Future work will expand upon the range of diagnostic applications for infectious and noninfectious diseases in the clinic, laboratory, and field where assay accuracy, reliability, simplicity, speed, flexibility, and cost will determine the scope of impact. ■

Application of CRISPR diagnostics

Next-generation diagnostics applying CRISPR-Cas biology will facilitate early disease detection and intervention.



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