Globally, over 300,000 women die from cervical cancer each year; 90% of these deaths occur in low- and middle-income countries (LMICs). Virtually all cases of cervical cancer are caused by persistent infection with high-risk types of human papillomavirus (HPV). In May 2018, Dr. Tedros Adhanom Ghebreyesus, Director-General of the World Health Organization, announced a global call to eliminate cervical cancer and underscored the need to ensure that all girls are vaccinated against HPV and that every woman over 30 is screened and treated for precancerous lesions. Standard methods to screen for high-risk HPV require costly equipment, trained personnel, and advanced infrastructure—resources that are not available in many LMICs. Similar challenges exist for detection of many viral illnesses. In this issue of ACS Central Science, Zamani and colleagues describe a novel, low-cost electrochemical sensor for viral detection that can potentially be manufactured and used in low-resource settings. They demonstrate sensitive and specific detection of HPV 16 and 18, the two most common high-risk HPV types. Notably, the authors demonstrate detection of HPV in patient samples. They also show the platform can be readily adapted to detect other viruses, including detection of a plasmid containing the HIV genetic sequence.

As shown in Figure 1, the electrochemical sensor is used to detect viral genetic material in a three-step process. Traditional methods are first used to extract viral genetic material, which is then amplified using loop-mediated isothermal amplification (LAMP)—an isothermal method that is feasible to implement in low-resource settings. LAMP products are added to a CRISPR-based recognition system that is activated in the presence of target amplicon, circumventing the challenge of nonspecific amplification associated with LAMP. The Cas12a reaction mix is deposited on gold electrodes that are functionalized with methylene blue (MB) tagged oligonucleotides (Figure 2). The gRNA of Cas12a is engineered so that specific loci in each viral genome of interest activate the endonuclease and cause it to cleave the MB-tagged oligonucleotide, thereby resulting in an electrochemical signal. Electrochemical signal from MB is measured before and after treatment with the Cas12a enzyme to detect whether the virus is present.

The three-electrode biosensor (Figure 2) is fabricated using a simple process that does not require costly equipment. The three-electrode biosensor (Figure 2) is fabricated using a simple process that does not require costly equipment. Gold leaf (24K) is applied to a self-adhesive sheet and cut to make peelable gold stickers. These sticker electrodes are placed on transparency sheets with wax-printed sample wells. The reference electrode and the leads of the counter...
and working electrodes are painted with conductive silver paint to glue aluminum foil contacts to the leads of all three electrodes. The electrodes are then functionalized for biosensing with MB-tagged oligonucleotides. Each electrode uses only $0.16 worth of gold and costs around $0.50 to fabricate. In comparison, similar commercial electrodes cost around $4/electrode.

The authors demonstrate the sensor’s use of three Cas12a assays to specifically detect DNA targets containing the HIV gag gene, HPV 16 E7 gene, and HPV 18 E7 gene. To increase test sensitivity, the authors used LAMP to amplify target DNA prior to CRISPR-based recognition and detection with the sensor. After a 20 min LAMP reaction, a lower limit of detection of $1.2 \times 10^4$ copies of an HPV 18 plasmid was demonstrated. Finally, eight clinical samples were tested in triplicate for HPV 18 and yielded a sensitivity of 100% and a specificity of 89%. DNA was extracted from cervical swabs using laboratory methods and LAMP is performed on a thermal cycler. All five PCR-positive samples were detected as positive using the electrochemical sensor; however, one biological replicate of one PCR-negative sample had positive results on both the electrochemical sensor and gel electrophoresis.

The electrochemical detection platform described here has significant potential to improve accessibility for viral diagnostics in low-resource settings. The platform is applicable to any nucleic acid target. Postamplification CRISPR-based recognition addresses issues with nonspecific amplification that can limit the utility of LAMP for clinical diagnostics. Finally, there is potential to reduce the cost of test consumables and hardware. The paper reports that each electrode costs only $0.50 with a $2.30 per test cost for the LAMP and CRISPR components, plus $2,000 for the hand-held potentiostat used for signal detection; although not used by the authors, low-cost heaters are readily available to power the LAMP reaction. In contrast, other commercially available point-of-care tests cost between $5 and $20 per test and $20,000 or more for instrumentation.

More work needs to be done to translate this electrochemical viral detection strategy for use in low-resource settings. First, the sample-to-answer workflow requires incorporation of point-of-care friendly techniques for nucleic acid extraction and LAMP. Paper-based sample processing could permit equipment-free nucleic acid extraction, while heating techniques such as wireless resistive heaters or chemical heaters could be used to perform LAMP. Another challenge associated with this technique is the binary result, which limits information about viral load that could reveal the stage of disease progression. Furthermore, future iterations of this work must also confront integration of workflow, as each user step requires additional labor and introduces potential for contamination. Finally, this sensor must be validated for its ability to detect clinically relevant levels of other viral targets, including HIV.

Figure 1. Workflow of electrochemical detection strategy. Viral genetic material is extracted from a sample and amplified using LAMP. The resulting amplicons activate the Cas12a endonuclease, which cleaves an MB-tagged oligonucleotide, resulting in a decreased electrochemical signal indicating a positive result.

Figure 2. Assembled final electrode. Reproduced from ref 2. Copyright 2021 The Authors. Published by American Chemical Society.
clinical use will also require validation with a greater number of clinical samples to confirm that anomalies in the results, such as the PCR-negative sample detected as positive on both the sensor and gel electrophoresis, are not commonplace.

These necessary next steps in the development and translation of this platform, however, do not detract from its significance in the field of global health and point-of-care diagnostics. The electrochemical strategy described here has tremendous potential to increase access to timely diagnosis of viral illness for patients in low-resource settings by providing a low-cost, minimal-equipment, highly sensitive, and specific alternative to standard nucleic acid detection methods.

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