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Pocket lab for the rapid detection of monkeypox virus

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Dear Editor,

Recently, Wilnard Yeong Tze Tan et al. [1] reported the first imported case of monkeypox in Singapore in 2022. The monkeypox outbreak is spreading globally and has been declared a Public Health Emergency of International Concern, the WHO’s highest level of alert. Learned from the COVID-19 pandemic, the containment of infectious disease spread relies on efficient on-site detection [2,3], especially in public transport hubs, communities and source-limited regions.

The current main assay to support the detection of monkeypox is polymerase chain reaction (PCR) [4]. PCR assays require precisely controlled temperature cycling, thermocycler instrumentation, trained technical personnel, specialized laboratory settings and tedious multi-step protocols. Its inability to rapidly screen and on-site detection significantly hinders the global efforts to effectively contain and mitigate the spread of monkeypox. Though some immunosays (e.g. lateral flow strips) could simplify the analysis operation, serologic reactivity between orthopoxviruses is a substantial barrier [5].

Thus, the urgent global demand for rapid monkeypox screening necessitates the development of alternative testing tools suitable for source-limited settings.

To achieve this goal and approach WHO’s ASSURED criteria, we developed a pocket lab to rapidly detect the monkeypox virus (Fig. 1a). It was made to easily fit inside a pocket at less than 500 g (Fig. 1b). With this mini-size, water-resistant and fully-equipped case, all the analysis procedures, from nucleic acid extraction to naked-eyes-readout, were able to be conducted. There were several core gears in this pocket lab (Fig. 1a): 1) Heating block-A: 3D-printed heating block up to 80 °C for 5 min to release viral DNA; 2) Heating block-B: 3D-printed block providing 40 °C-heating for signal amplification; 3) Ready-to-use tubes: tubes containing lyophilized enzymes under the protection of trehalose; 4) Sampling tubes: tubes containing sodium chloride and magnesium acetate solution; 5) Handheld ultraviolet (UV) flashlight; 6) Rehydration buffer: buffer containing primers and fluorescent reporter.

Operationally, only three simple steps were required to analyze a suspected sample: 1) Sample was added into Sampling tube and heated at 80 °C for 5 min to release viral DNA; 2) While waiting for the heating, 20 μL Rehydrate buffer was added into Ready-to-use tube; 3) 10 μL heated sample mixture was transferred into rehydrated Ready-to-use tube, and the tube was placed in Heating block-B at 40 °C for 20 min. Afterwards, under the excitation of handheld UV light, the bright enough fluorescence result was able to be read in a natural light environment. The turn-around time was 25 min, and the reagent cost less than $1/reaction. We spiked pseudotyped monkeypox viral particles (monkeypox viral genes in replication-deficient Ad5 virus) into the human plasma to simulate the complex matrix, such as lesion exudate. By using our pocket lab, as low as ten virus particles were accurately detected (Fig. 1c and d).

In principle (Fig. 1e), we achieved this pocket detection by incorporating two complementary technologies, Recombinase Polymerase Amplification (RPA) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), improving analytical sensitivity and specificity simultaneously [6]. For their ideal compatibility on aspects of reaction temperature and conditions, these two reactions took place in a single tube. The related sequences have been listed in Table 1. The RPA acted as a ‘signal amplifier’ and the CRISPR acted as a ‘signal filter’. The specific sequence of monkeypox viral DNA (B6R or F3L gene) was exponentially amplified by RPA, and the amplicons were scanned by the CRISPR guide RNA (gRNA) and Cas12a enzyme. Only in the presence of the viral target, the CRISPR/Cas12a-mediated trans-cleavage occurred, generating bright fluorescence. Even though some non-specific RPA byproducts formed, they would not trigger the cleavage activity of CRISPR (Fig. 1f vs Fig. 1g).

Our pocket lab possesses the potential to bring monkeypox tests closer to the traveller and communities, and overcome problems with poor laboratory infrastructure and inadequately trained personnel.

Abbreviations: WHO, World Health Organization; COVID-19, Coronavirus disease 2019; PCR, Polymerase Chain Reaction; ASSURED, Affordable, Sensitive, Specific, User-friendly, Rapid&Robust, Equipment-free, Delivered; UV, Ultraviolet; RPA, Recombinase Polymerase Amplification; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; gRNA, guide RNA.

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J.W.: Formal analysis, Methodology, Data curation, Writing - original draft; X.M.: Resources; J.L.: Investigation, Validation, Supervision; B.P.: Conceptualization, Writing - review & editing, Funding acquisition, Project administration.

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Declaration of competing interest

None.

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