Short Communication

A Handy Field-Portable ELISA System for Rapid Onsite Diagnosis of Infectious Diseases

Harpal Singh1,4, Kazuhiro Morioka2, Masayuki Shimojima4, Le Van An6, Hizuru Nakajima2, Akihide Hemmi2, Katsumi Uchiyama2, Shih Keng Loong7, Sazaly AbuBakar7, Ming Yang1, and Masami Sugamata3,6*

1Department of Intelligent Mechanical Systems, Graduate School of System Design; 2Department of Applied Chemistry, Graduate School of Urban Environmental Sciences; 3Department of Hygiene and Public Health, Graduate Schools of Human Health Sciences, Tokyo Metropolitan University, Tokyo; 4Department of Virology 1, National Institute of Infectious Diseases, Tokyo; 5Mebius Advanced Technology Ltd., Tokyo, Japan; 6Department of Microbiology, Hue University of Medicine and Pharmacy, Hue City, Vietnam; and 7Tropical Infectious Diseases Research & Education Center (TIDREC), University of Malaya, Kuala Lumpur, Malaysia (where this work was carried out)

SUMMARY: Enzyme-linked immunosorbent assays (ELISAs) are considered the gold standard for the detection of various immunological reactions and can be used for the detection of infectious diseases during outbreaks or in the care of individual patients. To be useful in the timely implementation of prevention and control measures against infectious diseases, a diagnostic modality should be rapid, accurate, and affordable. In the current study, we demonstrate the efficiency (90% less time and volume consumption compared with those of a standard 96-well ELISA), detection capability, and ease of operation of a field-portable, battery-operated ELISA system, approximately the size of a cellular phone (12 × 6 × 5.5 cm), in the serological diagnosis of measles and rubella viruses that has the potential for onsite testing such as during disease outbreaks.

The development of rapid and accurate laboratory capabilities for the identification of pathogens is important in the implementation of effective prevention and control strategies against infectious diseases. Although much progress has been made in diagnostic testing such as that for the care of individual patients or for the rapid detection of pathogens during disease outbreaks, many of these testing modalities require skilled operation and complex instrumentation, which has limited their use for onsite testing (1–4).

To encourage the use and implementation of onsite diagnostic testing, especially for pathogen identification in the field during infectious disease outbreaks, it is crucial that the tests be simple to use, rapid (i.e., short time to complete), cost-effective (e.g., requires less reagent and sample volumes), field-portable (e.g., does not require complex instrumentation or power sources) and have the ability to detect multiple pathogens simultaneously (5,6). Although numerous tests such as point-of-care (POC) (5,7) and lab-on-a-chip (LOC) tests (8,9) have been developed to meet these needs, they may still require complex manipulation, expensive instrumentation, and technical skills to operate (7). More importantly, the development of laboratory capabilities that support the surveillance of infectious diseases in remote locations and hard to reach populations, where sample collection and testing can be carried out away from centralized testing facilities, is critical to allow medical and other public health measures to be readily implemented (4).

In the current study, we report the preliminary evaluation and validation of a recently developed, easy-to-handle field-portable ELISA system that consists of a fabricated ‘9-well microplate’ and a portable, battery-operated fluorescence detection system. As proof-of-concept for the detection of infectious diseases, this portable ELISA system was utilized to detect antibodies against measles and rubella viruses from human serum samples.

The ELISA vessel used in this study, called herein the ‘9-well microplate’ was fabricated using polydimethylsiloxane (PDMS) as previously described (10). The 9-well microplate was 30 mm in length and width, 5 mm in height and consisted of 9 wells with a well diameter of 3.5 mm, an inside depth of 4 mm, and a maximum total volume capacity of 40 µL per well (Fig. 1). The portable, battery-operated, fluorescence detection system consisted of a fluorescence (in arbitrary units, a.u.) reader instilled with 9 light-emitting diodes (LEDs) and an inbuilt stage to accommodate the 9-well microplate (Fig. 1). The overall dimensions of this system were 12 cm in length, 6 cm in width, and 5.5 cm in height, and it weighed approximately 310 g. The portable reader was equipped with a USB 2.0 port for the transmission of a.u. data to a laptop personal computer.

For evaluation purposes, an IgG ELISA for the detection of antibodies against measles and rubella viruses was performed using the 9-well microplate, and the
results were validated by a standard 96-well ELISA. Serum samples from patients who visited the Outpatient Department of the University Malaya Medical Center, Kuala Lumpur, Malaysia and the Hue University Hospital, Hue City, Vietnam in July 2014 were collected under informed consent. All protocols were approved by the Medical Ethics Committee of the University Malaya Medical Center and the Research and Ethical Committee of the Hue University of Medicine and Pharmacy for the use of human subjects. There were no criteria established for the selection of patients recruited in the current study as our aim was to demonstrate the detection capability and efficiency of the portable ELISA system in comparison to those of a standard 96-well ELISA. Similarly, clinical and vaccination histories were also not collected.

A total of 14 serum samples were collected including 7 Malaysia (serum sample No. 1 to 7) and 7 from Vietnam (serum sample No. 8 to 14) for the measles and rubella virus IgG ELISA. The IgG ELISA screening protocol, reagent volumes, and incubation times for the 9-well microplate and the 96-well ELISA used in this study are summarized in Table 1. Positive and negative cut-off values for the assays were determined from the mean of 2 independent tests (per plate) using measles and rubella virus hemagglutination antigen-positive and -negative reference antisera (Denka-Seiken, Tokyo, Japan).

The total time required to complete the 9-well microplate ELISA (2.5 h) was approximately 90% less than that for the 96-well ELISA (16 h), which includes the time required for antigen coating. Additionally, the total volume of reagents consumed in the 9-well microplate ELISA (0.63 mL) was 90% less than that of the 96-well ELISA (4.2 mL). The enzyme-mediated signal amplification achieved by the 9-well microplate

| Step                  | 9-well microplate ELISA | 96-well ELISA |
|-----------------------|-------------------------|---------------|
|                       | Incubation time (min)   | Volume (μL)   | Incubation time (min) | Volume (μL) |
| Coating               | 30                      | 30            | Overnight             | 100         |
| Wash (PBS-T)          | —                       | 40 × 3        | —                     | 300 × 3     |
| Blocking (PBS-T-M)    | 30                      | 30            | 60                    | 200         |
| Wash (PBS-T)          | —                       | 40 × 3        | —                     | 300 × 3     |
| Primary antibody      | 30                      | 30            | 60                    | 100         |
| Wash (PBS-T)          | —                       | 40 × 3        | —                     | 300 × 3     |
| Secondary antibody    | 30                      | 30            | 60                    | 100         |
| Wash (PBS-T)          | —                       | 40 × 3        | —                     | 300 × 3     |
| Substrate             | 30                      | 30            | 30                    | 100         |
| Estimated total       | 150                     | 630           | ~960                  | 4,200       |
| Measurement           | a.u.                    | OD_{ave}      |                        |             |

PBS, phosphate buffered saline; PBS-T, 0.05% Tween-20/PBS; × 3, wash 3 times; PBS-T-M, 5% skimmed milk in 0.05% Tween-20/PBS; a.u., arbitrary unit; OD_{ave}, optical density at 405 nm/reference at 490 nm.

1) Predetermined optimal quantity 1:4 and 1:2 for measles and rubella virus hemagglutination antigen (HA) in sodium carbonate buffer, respectively.
2) Human serum samples, HA-positive and -negative reference antisera for measles and rubella viruses, respectively; dilution (1:100) in PBS-T-M.
3) Goat anti-human IgG-horseradish peroxidase conjugated (Thermo-Fisher, Waltham, MA, USA); dilution (1:1,000) in PBS-T-M.
4) 9-well microplate ELISA, Amplex Red (Thermo-Fisher); 96-well ELISA, ABTS (Roche, Mannheim, Germany).
5) 9-well microplate ELISA, a.u. measured using portable battery-operated fluorescence reader; 96-well ELISA, OD_{ave} measured using Model 680 Microplate Reader (Bio-Rad, Hercules, CA, USA).
ELISA was capable of being quantified using the portable fluorescence intensity reader, which was about the size of a cellular phone ($12 \times 6 \times 5.5$ cm), and did not require complex, bulky instrumentation or electricity. As proof-of-concept, the measles virus IgG ELISA results obtained from the serum samples showed similar detection capabilities between 2 systems, which was exemplified by the similar antibody titer levels observed (Fig. 2A and B). Additionally, the IgG ELISA for rubella virus exhibited similar results between 2 systems with the exception of 1 sample (sample No. 14), which showed the presence of antibodies in the portable ELISA and absence of antibodies in the 96-well ELISA (Fig. 2C and D). The results obtained for this sample were consistent with following multiple testing. Techniques for protein immobilization or surface modification of the 9-well microplate should be addressed in the future to reduce potential interference from nonspecific binding. Additionally, complete clinical and vaccination histories may have been useful in determining the possible reasons for this disagreement.

Although many advancements have been made to 96-well ELISAs to achieve high specificity and sensitivity, increase automation, and increase ease of use, they are time-consuming, require large volumes of reagents, and lack portability (11,12). Therefore, field-portable diagnostics that are easy to handle, lightweight, and capable of reducing both time and consumption of reagents without compromising on detection accuracy are highly desirable (4). The portable ELISA system described in the current study meets these needs and could thus strengthen surveillance systems by allowing onsite test-
ing and regular serological surveys in rural areas. The generation of valuable disease surveillance information will allow better risk assessments and guide the implementation of public health strategies. In addition, the portable fluorescence detection system with readily available, rechargeable batteries, which allows the quantification of signal amplification using minimal instrumentation and no power sources.

The expansion of this portable ELISA system to include other immunoassay formats (e.g., antigen-capture ELISA, competitive ELISA, or IgM ELISA) broadens its application for the onsite screening and diagnosis of infectious disease agents on par with the detection capability of standard ELISAs but with less time and cost to run. The rapid and accurate identification of causative agents of infectious diseases will not only aid in establishing the etiologies of these diseases but will guide the implementation of public health measures in a timely and effective manner in response to these threats, which are lacking in rural settings. In comparison to other disease-specific testing modalities such as rapid kits or immunochromatographic strips, the portable ELISA system will potentially allow the serologic detection of any infectious diseases in the field as long as the appropriate coating antigens or antibodies are available.

In conclusion, the use of the portable ELISA system described in the current study required less time and reagents compared with a standard 96-well ELISA for the detection of antibody titers against measles and rubella virus. This portable ELISA system and the use of a portable, battery-operated fluorescence detection system without the need for complex instrumentation, skills, or infrastructure indicate that it is a valuable model for the detection of infectious disease pathogens and serological diagnosis of infectious diseases on par with the standard, bench top, 96-well ELISA. The speed, accuracy in detection, and ease of use of this portable ELISA system results in an opportunity for its onsite application for testing, such as in the field.

Acknowledgments We extend our appreciation to the doctors, nurses, and laboratory staffs at the University Malaya Medical Center, Kuala Lumpur, Malaysia and the Hue University Hospital, Hue City, Vietnam who assisted us by collecting blood samples from the volunteers in this study. This study was financially supported by the Tokyo Metropolitan Government High Technology Research Fund. H.S. is supported by the Asian Human Resource Fund of the Tokyo Metropolitan Government, and K.M. is supported by the Japan Society for the Promotion of Science.

Conflict of interest None to declare.

REFERENCES
1. Cantón R. Role of the microbiology laboratory in infectious disease surveillance, alert and response. Clin Microbiol Infect. 2005; 11:3-8.
2. Coker RJ, Hunter BM, Rudge JW, et al. Emerging infectious diseases in Southeast Asia: regional challenges to control. Lancet. 2011;377:599-609.
3. Pitsuwan S. Challenges in infection in ASEAN. Lancet. 2011;377: 619-21.
4. Caliendo AM, Gilbert DN, Ginocchio CC, et al. Better tests, better care: improved diagnostics for infectious diseases. Clin Infect Dis. 2013;57:Suppl 3, S139-70.
5. Yager P, Edwards T, Fu E, et al. Microfluidic diagnostic technologies for global public health. Nature. 2006;442:412-8.
6. Drain PK, Hyle EP, Noubary F, et al. Diagnostic point-of-care tests in resource-limited settings. Lancet Infect Dis. 2014;14: 239-49.
7. Pai NP, Vadnais C, Denkinger C, et al. Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. PLoS Med. 2012;9:e1001306.
8. Sun S, Yang M, Kostov Y, et al. ELISA-LOC: lab-on-a-chip for enzyme-linked immunodetection. Lab Chip. 2010;10:2093-100.
9. Yang M, Sun S, Kostov Y, et al. Lab-on-a-chip for carbon nanotubes based immunoassay detection of Staphylococcal Enterotoxin B (SEB). Lab Chip. 2010;10:1011-7.
10. Xue S, Zeng H, Yang J, et al. A compact immunoassay platform based on a multicapillary glass plate. Sensors (Basel). 2014;14: 9132-44.
11. Lequin RM. Enzyme immunoassay (EIA)/Enzyme-linked immunosorbent assay (ELISA). Clin Chem. 2005;51:2415-8.
12. Chin CD, Laknasasopin T, Cheung YK, et al. Microfluidics-based diagnostics of infectious diseases in the developing world. Nat Med. 2011;17:1015-9.