Multicolor rapid diagnostics for infectious disease

Kimberly Hamad-Schifferli, Chunwan Yen, Helena de Puig, José Goméz-Marquéz, Irene Bosch, and Lee Gehrke

An assay based on silver nanoparticles of different colors provides rapid diagnosis for multiple diseases.

Recent epidemic outbreaks have highlighted the need for a rapid point-of-care assay that can provide a diagnosis to enable treatment, proper quarantining, and disease surveillance. One promising diagnostic is the lateral flow test, i.e., the same type of assay used in pregnancy tests. It is simply a paper strip to which you add a biological fluid. Two colored stripes show up if it is positive, one if it is negative. These assays are attractive for diagnostics because they are inexpensive, easy to use, and do not require special reagents or experts to run them. Sample-to-answer can be reached in minutes, and can be read out by eye. Because the sample wicks through the strip by capillary action, it does not need power to run, which makes the assays highly portable and deployable.

One shortcoming of these assays is that they can test only for a single disease at a time. However, at point of care, it is critical to be able to test for multiple diseases all at once to enable proper quarantining and treatment. For example, with the current Zika outbreak, Zika virus co-circulates with other diseases such as dengue and chikungunya because they are spread by the same mosquitoes. In addition, the initial symptoms of all three diseases are highly similar and nonspecific. Thus, a point-of-care assay that can screen for all of these simultaneously would enable a more rapid response for proper treatment.

We describe a multiplexed assay developed in the lab by using silver nanoparticles of different colors. Here, the assay shows a different color test line depending on the biomarker that is present: see Figure 1(a). We synthesized silver nanoparticles to be of different sizes in the ~30–50nm size range: see Figure 1(b). The different sizes have different colors that are distinguishable by eye both in solution and on paper: see Figure 1(c). We attached the silver nanoparticles to different antibodies. We attached orange nanoparticles to antibodies that can bind to the

Figure 1. (a) Multiplexed diagnosis of yellow fever (YFV), Ebola (ZEBOV), and dengue (DENV) by silver nanoparticles of different colors used in sandwich immunoassays. (b) Silver nanoparticles synthesized (transmission electron microscopy images). Left to right: Yellow nanoparticle ‘seeds’ (used to grow the larger nanoparticles), orange nanoparticles, red nanoparticles, green nanoparticles. (c) Vials of the silver nanoparticles synthesized and solutions of the samples dried down on paper. (d) Assay strip for a multicolor multiplexed immunoassay. Control line: Positive control band that confirms flow.

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biomarker for yellow fever. Red nanoparticles were attached to antibodies for the biomarker for Ebola virus. Finally, we attached green nanoparticles to antibodies for dengue virus. Consequently, if biomarkers for yellow fever were present (non-structural protein 1, NS1), the stripe appearing at the test line would be orange in color. If the Ebola biomarker (glycoprotein) was present, it would be red. If dengue NS1 was present, it would be green. If all three were present, the test line would be brown. In other words, the color of the readout can tell you what disease biomarker is present.

The three nanoparticle-antibody conjugates were all put into a single lateral flow strip and exposed to the three biomarkers (yellow fever virus NS1, Ebola virus glycoprotein, and dengue virus NS1) in human serum. The resulting test lines were distinguishable by color, both by eye and by analyzing a camera image—see Figure 1(d)—enabling multiplexed detection. The assay readout was complete within minutes. We observed no cross-reactivity between the biomarkers. In addition, the limit of detection of the assay was determined to be \(\sim 150 \text{ng/ml}\). The estimated cost of the device is approximately $5–$10 per lane.

In conclusion, we have demonstrated a multiplexed rapid diagnostic that can differentiate between yellow fever, Ebola, and dengue virus biomarkers by color. Readout of the test line color can be by eye or by analysis of an image, such as one from a mobile phone. This multiplexed readout can be used for differential diagnosis to help with quarantining, disease surveillance, and treatment.

Currently we are working on many aspects of the device to be able to use it in the field. This includes improving the limit of detection and approaches for device stability and storage. In addition, we are testing the assay on patient samples, where the biological fluids are more complex and testing environments less controlled, and for diseases that are co-circulating. Finally, we are developing a mobile phone app that can provide a diagnosis and also map the results with time and geolocation information.

Author Information

Kimberly Hamad-Schifferli
University of Massachusetts Boston
Boston, MA

Kimberly Hamad-Schifferli is an associate professor in the Department of Engineering at the University of Massachusetts Boston, and a visiting scientist at the Massachusetts Institute of Technology (MIT). She was a faculty member at MIT in the Departments of Mechanical Engineering and Biological Engineering from 2002 to 2012, and a staff member at MIT Lincoln Laboratory from 2012 to 2015.

Chunwan Yen, Helena de Puig, José Goméz-Marquéz, Irene Bosch, and Lee Gehrke
MIT
Cambridge, MA

References