The exact nature of the cytoplasmic vesicles is currently under investigation as part of a future project, but it appears that nanorods internalize in double-membrane layered early endosome-like compartments away from the nucleus or late endosome-like structures close to the nucleus.

Two-dimensional, confocal fluorescence microscopy images were collected through the use of an LSM 510 confocal laser scan microscope (Carl Zeiss) with a C-Apochromat 63 X/NA 1.2 water-immersion lens in conjunction with an argon ion laser (564 and 488 nm excitations were used for Eu and Tb, respectively). The red and green fluorescence emissions were collected through a 601- and 505-nm long-pass filter for EuPO4·H2O and TbPO4·H2O nanorods, respectively, and are shown in Fig. 1, A and C. A red fluorescence (Fig. 1A) of EuPO4·H2O nanorods and its corresponding phase image (Fig. 1B) are observed in Fig. 1, A and B. Similarly, a green fluorescence (Fig. 1C) of TbPO4·H2O nanorods and its corresponding phase image (Fig. 1D) are shown in Fig. 1, C and D. Furthermore, the fluorescence (Fig. 1, E, G, and I) and corresponding phase image (Fig. 1, F, H, and J) of the control untreated 786-O cells and 786-O cells treated with LnPO4·H2O nanorods are presented in Fig. 1, E–J. There are no LnPO4·H2O nanoparticles observed in the phase image of control untreated 786-O cells in Fig. 1F and no fluorescence (even autofluorescence) in Fig. 1E, which clearly indicates the absence of LnPO4·H2O nanorods. On the other hand, a very nice and clear red fluorescence in Fig. 1G was observed due to the presence of EuPO4·H2O nanorods inside 786-O cells. Similarly, very nice and bright green fluorescence in Fig. 1I indicates the presence of TbPO4·H2O nanorods inside 786-O cells. Overall, there is a significant difference in red (Fig. 1G) and green (Fig. 1I) fluorescence between the untreated control cells (Fig. 1E) and the nanorod-treated cells (Fig. 1, G and I). Similar results were obtained for HUVECs after treatment with LnPO4·H2O nanorods (see Fig. S1-2 in the online Data Supplement). These results demonstrate the internalization of LnPO4·H2O nanorods inside 786-O and HUVECs. One of the most important results was that under the new settings of the confocal microscope, we were able to clearly distinguish the great difference in red and green fluorescence intensities between untreated control cells and nanorod-treated cells, whereas in our earlier study, autofluorescence was present in control untreated cells (5).

Taken together, these results indicate that these fluorescent nanorods can internalize in cells, which in turn can be visualized by microscopy. Therefore, these nanorods offer useful and alternative inorganic fluorescent probes for targeting various molecules in living cells.

Furthermore, we also report the use of inorganic fluorescent EuPO4·H2O, TbPO4·H2O nanorods as a fluorescent label (a novel alternative to conventional organic dyes) in biomedical research. We have shown, by use of confocal microscopy and transmission electron microscopy, internalization of EuPO4·H2O, TbPO4·H2O nanorods into 786-O and HUVECs. These nanorods were observed to localize mainly in the cytoplasm of these cells and did not appear to detrimentally affect cell viability or induce toxicity after internalization.

Finally, our inorganic fluorescent label method is a simple tool for examining the cellular compartments of living cells. Fluorescent nanorods may enable improved detection of malignant tumor cells.

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Development of a Novel, Rapid, and Sensitive Immunochromatographic Strip Assay Specific for West Nile Virus (WNV) IgM and Testing of Its Diagnostic Accuracy in Patients Suspected of WNV Infection, Nisar A. Shaikh, Jun Ge, Yi-Xue Zhao, Paul Walker, and Mike Drebot
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The West Nile virus (WNV), detected in the Western hemisphere in 1999, has since spread rapidly across North America into all 48 continental states of the US, 7 Canadian provinces, many Latin American countries, and...
WNV infection specific IgM are now accepted as a sensitive early marker for Laboratory-based ELISAs designed to detect WNV-specific serum IgM antibodies in human serum by the time WNV-specific serum IgM appears, although no cross-reactivity (10%) with Den-}

WNV infection in a pediatric population have also been reported (7). Many patients remain asymptomatic or have only mild symp-

WNV is transmitted to humans by mosquitoes. The trans-

WNV, a member of the Flaviviridae family, belongs to the Japanese encephalitis serocomplex that includes Japanese encephalitis and St. Louis encephalitis viruses (3). WNV is transmitted to humans by mosquitoes. The trans-

The virus itself is usually no longer detectable by the time WNV-specific serum IgM appears, although both IgM and IgG may persist for more than a year (12). Laboratory-based ELISAs designed to detect WNV-specific IgM are now accepted as a sensitive early marker for WNV infection (13–15). However, current ELISA assays (e.g., Focus Technologies West Nile Virus IgM Capture ELISA, PanBio) are multistep procedures that require 5–6 h to perform, instrumentation to read the tests, and subsequent calculations to interpret the test results. We describe the development of a novel and a rapid WNV IgM strip test (RapidWN™) that requires minimum sam-

Both the ELISA and the strip format assay use the same principle and the same WNV antigen (suckling mouse brain WNV protein E or recombinantly produced WNV protein E fraction) and flavivirus-specific monoclonal antibody 6B6C-1. The RapidWN uses solid-phase immuno-

The test uses a fragment of recombinant WNV envelope protein (antigen) that was expressed in Esche-

Antibody 6B6C-1, provided by the CDC as a hybridoma cell line, was cultur-

Streptavidin was recombinantly produced and immobilized onto a nitrocellulose (Sartorius) mem-

The reactant concentrations are adjusted and optimized by analysis of calibrators, made from pooled WNV-positive sera, so that the test should produce a positive signal at WNV IgM Index value ≥1.1 of an commercially available Focus Technologies West Nile Virus IgM Capture ELISA device (comparator device) and negative results below that number. Exam-

Visible pinkish-purple horizontal bands appear in the test area if the concentration of the WNV IgM antibodies in the human serum sample is above the cutoff concentra-

RapidWN test precision studies were performed using a reproducibility panel made of different clinical serum specimens at 3 study sites during a 3-day period. Each day a new lot was used and a different operator was used. This 15-member reproducibility panel (blinded) consisted of 6 clinical specimens with a mean index value 25% above the cutoff, 6 clinical specimens with a mean index value 15% below the cutoff, and 3 clinical specimens with a mean index value 6 times the cutoff of the comparator device. All sites and operators produced the expected result for all panel members on every day of testing. Near-cutoff positive and negative samples also produced expected results when tested by 3 operators on the same lot for 10 consecutive days.

No significant interferences were found with common serum analytes such as human serum albumin, bilirubin, hemoglobin, or triglycerides at 2 to 3 times the normal expected values. No cross-reactivity with Japanese, Saint Louis, California, or Eastern equine encephalitis viruses was observed, but some cross-reactivity (10%) with Dengue virus was not.

Reactivity with autoantibodies, such as antinuclear antibodies and rheumatoid factor, is common to all immunoassays (16), and the RapidWN test is not an exception. Care has been taken to block such activities at heterophile antibody titers commonly encountered in a population, but rare samples with higher titers may affect the test.

Clinical specificity of the RapidWN test was assessed on prospective specimens (n = 346) after institutional review board approval at 3 study sites from endemic regions of the US and Canada. Samples were from presumably healthy individuals (n = 67) or patients with non-WNV ailments (n = 279), including 61 febrile pa-
tients. The study group was 47% men and 53% women. There was 99% agreement between methods, with few incidences of heterophile antibody interferences (Table 1).

Studies of diagnostic accuracy were conducted following institutional review board approval at 3 sites on randomized blinded retrospective samples from patients with clinical symptomology consistent with WNV infection. Results presented in Table 1 showed that the RapidWN test produced 100% agreement of serological sensitivity for acute, CDC WNV IgM and IgG ELISA-positive and plaque neutralization reduction test (PRNT)-confirmed samples. There was >97% agreement with other positive samples (CDC WNV IgM and IgG ELISA positive and PRNT confirmed) and 96% agreement with the CDC WNV ELISA-negative specimens. Similar results were obtained when comparison was made with the comparator device. The RapidWN test produced 98% agreement with the WNV-negative and 95% agreement with WNV-positive specimens. The apparent decrease in serological sensitivity is partially due to the fact that ELISA-based devices may produce equivocal results for some samples (index values between 0.9 and 1.09 of the comparator device), whereas a strip test may show positive/negative results within the 10% limit of the cutoff values.

The RapidWN test can also be used on plasma samples. The device produced similar results on 63 cohort plasma samples. The positive test results are suggestive of WNV infection as the IgM levels are at or above the established cutoff level. Invalid results indicate that the test must be repeated. rWNV, recombinant WNV antigen; Mab, monoclonal antibody.

![Fig. 1. Arrangement of different components in RapidWN strip test design and examples of positive (both bands present), negative (test band absent), and invalid (no control band) test results.](image-url)

The positive test results are suggestive of WNV infection as the IgM levels are at or above the established cutoff level. Invalid results indicate that the test must be repeated. rWNV, recombinant WNV antigen; Mab, monoclonal antibody.

### Table 1. Serological sensitivity of RapidWNTM WNV IgM test with other ELISA-based devices.

<table>
<thead>
<tr>
<th>Specimen type (test devices)</th>
<th>n</th>
<th>% agreement (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-flavivirus IgM serum specimens (comparison with Focus Technologies West Nile Virus IgM Capture ELISA)</td>
<td>346</td>
<td>99 (96.5%–99.9%)</td>
</tr>
<tr>
<td>WNV WNV-suspected serum specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute infection (plaque reduction neutralization test confirmed)</td>
<td>24</td>
<td>100 (88%–100%)</td>
</tr>
<tr>
<td>CDC WNV IgM/IgG ELISA positives</td>
<td>64</td>
<td>97 (85.7%–99.7%)</td>
</tr>
<tr>
<td>CDC WNV IgM/IgG ELISA negatives</td>
<td>80</td>
<td>96 (84.5%–99.7%)</td>
</tr>
<tr>
<td>Focus WNV IgM ELISA negatives</td>
<td>151</td>
<td>98 (86.6%–99.5%)</td>
</tr>
<tr>
<td>Focus WNV IgM ELISA positives</td>
<td>145</td>
<td>95 (83.8%–99.4%)</td>
</tr>
</tbody>
</table>
and serum samples when tested concomitantly. This test can also be equally performed on cerebrospinal fluid samples (unpublished results).

The efficacy of ELISA-based or microsphere immunosassays for detecting WNV antibodies has been well documented and provided results confirmed by PRNT (12–14, 17, 18). The RapidWN strip test described here also showed comparable results with the ELISA-based IgM tests and with the PRNT. However, the advantages of the strip test over currently marketed ELISA-based tests include rapid result generation (15 min), ease of use, room temperature stability, and visually readable results. In addition, the strip test can be used for a single sample or multiple samples. Hence, the cost-benefit ratio of a rapid strip test may be better than that of traditional ELISA devices because of decreased labor time, equipment costs, and transport times for samples and test results. Other commonly used immunochromatographic strip tests that generate results visibly have been clinically validated for patients suspected of myocardial ischemia (19). The data presented here show that the RapidWN strip test can be effectively used as a rapid visible test for patients suspected of WNV infections.

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The LiMA Technology: Measurement of ATP on a Nucleic Acid Testing Platform, Sharon Banin, Stuart Wilson, and Christopher Stanley’ (ISEAO Technologies Ltd., London, United Kingdom; * address correspondence to this author at: ISEAO Technologies Ltd., 2 Royal College St., London, NW1 0NH, United Kingdom; fax 44 2076912036, e-mail chris.stanley@iseao.co.uk)

Detection of bacterial growth is used in the diagnosis and treatment of infectious disease, blood screening, food safety, product quality assurance, and life science research. The measurement of intracellular ATP content has long been the standard for rapid bacterial growth and viability measurement (1–3). The current methods used to detect ATP include luminescence generated by the enzyme system firefly luciferase/luciferin. The chemistry involved in this process is simple and can be used with a wide range of luminescence equipment, from handheld devices to sophisticated, laboratory-based instruments for high-throughput applications (4).

We have developed an alternative, quantitative assay method for ATP based on a nucleic acid testing (NAT) format. During the past decade NAT has become the method of choice for bacterial identification (5). NAT hardware, such as thermal cyclers, isothermal instruments, and real-time/kinetic product detection systems, are now commonplace laboratory equipment. The new method, LiMA (Ligase Mediated ATP Amplification Assay) (6), uses DNA ligase, an ATP-requiring enzyme (7), to join 2 oligonucleotides in a nicked-DNA substrate and create a template that can be amplified in a DNA amplification reaction (Fig. 1). Before the LiMA process, the ligase is treated with pyrophosphate to ensure that all the enzyme molecules are in the deadenylated form. Thus the ligase is inactive until it binds a molecule of ATP, which leads to the loss of the pyrophosphate moiety from ATP and the formation of a covalent enzyme—AMP intermediate linked to a lysine side-chain in the enzyme. In this reaction the enzyme becomes charged by an ATP