Japanese Encephalitis
The Future of In-Field Detection

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Abstract
Japanese encephalitis (JE) is a mosquito-borne pathogen belonging to the flavivirus genus which infects approximately 68,000 people annually, causing significant morbidity and mortality. Diagnosis is often based on clinical signs and symptoms which are easily mistaken for other diseases. The introduction of a field based identification system for JE where nucleic acid extraction, amplification and identification are all conducted in-field would increase the efficiency of diagnosis and improve surveillance of JE. Rapid detection could reduce the morbidity and mortality rate of people living in susceptible areas.

Introduction
- Japanese encephalitis is spreading geographically to previously uninfected areas (Fig. 1).
- Japanese encephalitis virus (JEV) is transmitted through a zoonotic cycle between the mosquito vector, mainly Culex species, and the host (Fig. 2).
- There is no approved anti-viral therapy or cure for JE, however, vaccines are in wide scale use.
- We are developing a novel approach to extract, amplify and identify nucleic acid for rapid in-field JEV detection.

Viral transmission:

Mosquito bites the infected pig and picks up the JE virus
Infected mosquito bites the bird passing on the JE virus
Infected mosquito bites a human passing on JEV virus - Dead end host

Host

Figure 1. Zoonotic cycle showing the transmission of JEV

Figure 2. Geographical distribution of JEV

Extraction
Field based extraction of nucleic acid utilizes:
- Air pressure filtration (Fig. 3)
- Nucleic acid binding properties to silica gel membrane (Fig. 4) and
- Chaotropic properties of guanidinium thiocyanate

Amplification
- Nucleic acid amplification occurs isothermally (at a single temperature, 37°C)
- Simplest, portable and robust
- Labelled primers enable lateral flow detection

Identification
- Diagnosis via lateral flow device (Fig. 5)
- Prototype indicates successful detection of labelled JEV nucleic acids (Fig. 6)

Figure 4. Binding of nucleic acid to silica gel matrix. (A) Under high salt conditions, sodium acts as a cation bridge that attracts negatively charged oxygen in the phosphate backbone of nucleic acid to the positively charged silica particles (blue). (B) Under low ionic conditions, once the salt is removed by washing, the nucleic acid molecules detach from the silica and can be eluted in solution

Conclusion
- Silica matrix is a promising medium for field extraction of viral nucleic acids
- Could create low cost, user friendly diagnostic requiring only simple lab equipment
- Since vaccine programs are run in JE-identified areas, rapid JE diagnostics could help identify these areas

Figure 3. Syringe used for air pressure filtration

Figure 5. Milenia Biotech HybriDetect Lateral Flow device

Figure 6. Milenia lateral flow dipsticks showing successful detection of JEV nucleic acids (left), and a negative control result (right)

References

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