

antigen (PGA) with nanoparticle sandwich ELISA, compared to the traditional sandwich ELISA

Results: Sandwich ELISA achieved sensitivity of 93%, specificity of 92.5%, positive predictive value (PPV) of 95.7% and negative predictive value (NPV) of 88%, while nano-sandwich ELISA achieved sensitivity, specificity, PPV and NPV of 95.8%, 95%, 97.2% and 92.6%, respectively.

Conclusion: In conclusion, our research study provides that nano-sandwich ELISA is a well-established reference test for diagnosis of giardiasis.

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Dried cerebrospinal fluid spots for diagnosing Japanese Encephalitis Virus (JEV) infection by Anti-JEV IgM antibody capture enzyme-linked immunosorbent assay: Harnessing the potential of a fully saturated pre-cut filter paper disc

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Background: The use of filter paper as a simple, inexpensive tool for storage and transportation of blood, 'Dried Blood Spots' or Guthrie cards, is well-established. In contrast, there are a paucity of data on dried cerebrospinal fluid (CSF) spots. This has potential applications in low-resource settings, such as Laos, where laboratory facilities for central nervous system (CNS) diagnostics are only available in Vientiane. In Laos, a major cause of CNS infection is the Japanese encephalitis virus (JEV). We aimed to develop a dried CSF spot protocol and evaluate performance using the World Health Organisation recommended anti-JEV IgM antibody capture enzyme-linked immunosorbent assay (JEV MAC-ELISA).

Methods & Materials: Sample volumes, spotting techniques and filter paper type were evaluated using a CSF-substitute of anti-JEV IgM positive serum diluted in Phosphate Buffer Solution to end-limits of detection by JEV MAC-ELISA. The optimised protocol was compared with routine, neat CSF in a pilot, retrospective study of JEV MAC-ELISA on consecutive CSF samples collected 2009-15 from three Laos hospitals.

Results: A conventional protocol, involving eluting one punch in 200 µl PBS, did not detect the end-dilution, nor did multiple punches utilising various spotting techniques. However pre-cut filter paper enabled saturation with five times the volume of CSF-substitute, sufficiently improving sensitivity to detect the end-dilution. 132 CSF samples stored as dried CSF spots for one month at 25-30°C showed 81.6% (65.7-92.3 95%CI) positive agreement, 96.8%

(91.0-99.3 95%CI) negative agreement and a kappa coefficient of 0.81 (0.70-0.92 95%CI) results from neat CSF.

Conclusion: The novel design of pre-cut filter paper saturated with CSF could provide a useful tool for JEV diagnostics in settings with limited laboratory access. It has the potential to improve national JEV surveillance and inform vaccination policies. The saturation of filter paper also has significant implications for use in the wider context of pathogen detection, including dried spots for detecting other analytes in CSF, and other body fluids.

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Development of a rapid point of care immuno-filtration assay for serodiagnosis of cutaneous anthrax in India

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Background: Anthrax, caused by *Bacillus anthracis* is known to occur globally since antiquity. In human, the disease manifests itself in one of three forms: cutaneous, gastrointestinal or pulmonary depending upon the route of spore entry. Cutaneous anthrax is a disease of public health importance also in country like India. Therefore, there is a need to develop an improved, simple, sensitive, specific, user-friendly, field usable, cost-effective and universal detection method for serodiagnosis of anthrax in human and animals.

Methods & Materials: Protective antigen (PA) and lethal factor (LF) genes were cloned and expressed in *E. coli*. The recombinant proteins were purified to homogeneity. A flow through system with nitrocellulose membrane was optimized by coating 1 µg of each protein and 1 µg of rabbit IgG as a control. A 30 µl serum sample was added on to membrane followed by addition of protein A conjugated colloidal gold. The appearance of color on spots was observed for the results.

Results: The detection sensitivity of the assay was found to be 10 µg/ml anti-toxin IgG. The test system was evaluated using anthrax infected sera (n=150) collected from patients presenting typical clinical symptoms of anthrax from anthrax endemic area in India. Sera from apparently healthy human (n=250), vaccinated individuals (n=5) and non-anthrax infected persons (n=30) were also tested. Flow through system was found to be 100% sensitive and specific as compared to 93.65 and 98.44% sensitivity and specificity in ELISA.

Conclusion: The system is very rapid and takes only 2 min. The system is extremely useful for point of care diagnostic assay for clinical samples. This can be used for surveillance of anthrax infection in human as well as animals also.

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