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http://dx.doi.org/10.1016/j.ijid.2012.05.536

Type: Poster Presentation

Final Abstract Number: 55.026
Session: Diagnostics
Date: Saturday, June 16, 2012
Time: 12:45-14:15
Room: Poster & Exhibition Area

Human African trypanosomiasis diagnosis in first-line health services of endemic countries, a systematic review

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Background: In response to a decrease in incidence of human African Trypanosomiasis (HAT) observed in recent years, large-scale population screening campaigns are gradually being replaced by passive case detection in fixed health centres.

Methods: We conducted a systematic review of the literature between 1970 and 2011 on the performance of diagnostic tools for HAT for use in such first-line health facilities in endemic countries. Diagnostic tests were rated based on the ASSURED criteria (affordability, sensitivity, specificity, user friendliness, robustness, equipment-free and deliverable to those in need).

Results: Our search retrieved 16 different screening and diagnostic methods for HAT. The thermostable format of the CATT test came out as the most suitable screening test at health center level.

Conclusion: Lateral flow antibody detection tests are being developed and could become alternative screening tests in the near future. Confirmation of diagnosis still depends on visualizing the parasite. All currently available confirmation tests are either technically demanding and/or lack sensitivity and thus rather inappropriate for use at health center level. Novel applications of molecular tests may have potential for use at district hospital level.

http://dx.doi.org/10.1016/j.ijid.2012.05.537

Type: Poster Presentation

Final Abstract Number: 55.027
Session: Diagnostics
Date: Saturday, June 16, 2012
Time: 12:45-14:15
Room: Poster & Exhibition Area

Molecular epidemiology of 3 household transmissions of Salmonella Typhi in Kelantan, Malaysia

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Background: Typhoid fever, a systemic febrile illness in humans is caused by *Salmonella enterica* serovar Typhi (*Salmonella* Typhi). It remains a devastating disease in developing countries especially in South-Central and South-East Asia. This disease is endemic in The genetic of *Salmonella* Typhi is highly homogenous, therefore hindered the development of suitable typing methods to differentiate the isolates for epidemiological and phylogenetic purposes.

Methods: In this study, we evaluated the used of multiplex polymerase chain reaction (PCR)-variable-number tandem repeats (VNTRs) method for molecular typing of *Salmonella* Typhi isolates from 3 household transmissions occurred in Kelantan from year 2002 to 2009. Primers were based on published primers of 2 polymorphic loci (TR1 and TR2) in the CT18 of *Salmonella* Typhi. The VNTR results were compared to those obtained by pulsed field gel electrophoresis (PFGE) using *Xba*I restriction enzyme.

Results: Multiplex PCR of 2 VNTRs used in this study showed a high level of discrimination in distinguishing between epidemiologically unlinked *Salmonella* Typhi isolates in one of the household transmission case that could not be discriminated by PFGE.

Conclusion: This multiplex PCR-VNTRs method offers a simple, rapid, reproducible and high discrimination power of molecular typing. Therefore, it could be used as a typing tool in discriminating the genetic relationships among very closely related *Salmonella* Typhi isolates.

http://dx.doi.org/10.1016/j.ijid.2012.05.538

Type: Poster Presentation

Final Abstract Number: 55.028
Session: Diagnostics
Date: Saturday, June 16, 2012
Time: 12:45-14:15
Room: Poster & Exhibition Area

Evaluation of a Typhoid IgM flow assay for the diagnosis of typhoid fever in Cambodian children using a Bayesian modelling approach assuming an imperfect gold standard

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Background: Rapid, reliable point-of-care tests are needed to diagnose typhoid fever (TF) in febrile children attending hospital in endemic areas.

Methods: Unselected children admitted to Angkor Hospital for Children (AHC) with documented fever (≥38°C) were investigated for TF. A Typhoid IgM flow assay (RDT) on admission was compared with blood culture (BC), the current gold standard, real-time PCR of blood, admission diagnosis of typhoid and discharge clinical diagnosis by experienced paediatricians. The RDT measures IgM antibodies against a Typhi-specific lipopolysaccharide antigen with intensity results graded between 1+ and 4+. Estimates of the sensitivities and specificities of the various tests were determined by traditional methods and by Bayesian mathematical modelling using an imperfect gold standard model (MICE).

Results: During 2009 and 2010, 500 children were studied. The median age of the children was 2.7 years (range 1 month-16 years; IQR 1.0-8.0) and the median duration of illness prior to admission was 3 days (range 0-30 days; IQR 2-6). There were 83 (16.6%) children with a clinical admission diagnosis of suspected TF and 44 (8.8%) with a discharge diagnosis of TF: 31 laboratory confirmed (10 positive BC and PCR; 13 positive BC and negative PCR; 8 negative BC and positive PCR) and 13 on clinical assessment alone. The sensitivity of the RDT at ≥2+ when compared to a positive BC and/or PCR was 19/31 (61.3%, 95%CI 42.2-78.2) with a specificity of 454/469 (96.8%, 95%CI 94.8-98.2). The sensitivity of the RDT at 2+ or more compared to a discharge diagnosis of TF was 24/44 (54.5%, 95%CI 38.8-69.6) with a specificity of 446/456 (97.8%, 95%CI 96.0-98.9). Using the model to estimate the data assuming only that BC was 100% specific and that BC and PCR were correlated, the RDT sensitivity was estimated at 80.1% (95% credible values 61.7-92.4%) with a specificity estimate of 98.0% (95% credible values 96.0-99.6%).

Conclusion: Using a Bayesian modelling approach, that assumes an imperfect gold standard, the RDT had a sensitivity of 80% and specificity of 98%. Further studies of this Typhoid IgM Flow assay in other settings are needed.

http://dx.doi.org/10.1016/j.ijid.2012.05.539

Type: Poster Presentation

Final Abstract Number: 55.029
Session: Diagnostics
Date: Saturday, June 16, 2012
Time: 12:45-14:15
Room: Poster & Exhibition Area

Validation of two methods for the detection, identification and quantification of arboviruses in resource-limited settings

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Background: Recent Rift Valley Fever and Dengue outbreaks and the detection of arboviruses in vectors point to the real threat of arboviral disease in Kenya. Current methods for identifying and isolating these viruses include tissue culture, EIAs or conventional PCR. These methods are limited by difficulty in identifying novel or multiple infections, time to diagnosis, assay reliability and reproducibility and the availability and cost of stocking reagents for these rare and episodic diseases. In this study we evaluated whether the Evagreen© dye for real-time PCR and automated cell monitoring systems could address these challenges.

Methods: Chikungunya virus was inoculated into Vero cells and monitored for cytopathic effects (CPE) daily using standard microscopy or in real-time using the xCELLigence® system which detects changes in electrode impedence caused by adherent cells. Reverse-transcribed viral RNA was detected by conventional PCR or by quantitative real-time PCR with Evagreen® dye. A virus neutralization test for Chikungunya using sero-positive human serum was performed using standard plaque assays and the xCELLigence® system. The financial and time investments, quality and reliability of results were determined for each method.

Results: Viral cultures monitored by microscopy required ~ 40 minutes of staff time daily with CPE detected at day 3 and confirmed by a second 3-day passage. On xCELLigence®, CPE was detected by 40hrs with a signature proliferation profile that could be distinguished from cellular anomalies and other virus profiles and required ~5 minutes of staff time daily to monitor. The alternate methods for virus neutralization and antigen detection gave more consistent and reliable results compared to the conventional assays and reduced personnel times by ~50%, assay times by ~ 2X, and minimized reagent use significantly by capturing real-time data electronically.

Conclusion: The xCELLigence® system and real-time PCR using Evagreen® dye adopted for the detection and quantification of viruses and diagnosis of arboviral infections delivered significant gains in timeliness of reporting, quality and quantity of data outputs. In spite of high initial costs, the long-term savings on reagents and human resource are ideal for resource-limited laboratories to achieve sustainable and quality arbovirus diagnostic and research outputs.

http://dx.doi.org/10.1016/j.ijid.2012.05.540

Type: Poster Presentation

Final Abstract Number: 55.030
Session: Diagnostics
Date: Saturday, June 16, 2012
Time: 12:45-14:15
Room: Poster & Exhibition Area

Development of a quantitative pan – pLDH ELISA for the diagnosis of low parasitaemia malaria

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Background: Accurate and timely diagnosis of malaria infection is the key to effective treatment, particularly against a background of rising drug resistance. In recent years lateral flow rapid tests (RDT's) have increasingly been used to supplement traditional microscopy and have achieved widespread acceptance due to their ease of use, accuracy, and speed of diagnosis. However with increased usage has come a greater awareness of their limitations. RDT's targeted against the parasite enzymes lactate dehydrogenase (pLDH) and aldolase have poor sensitivity for parasitaemia levels below 100 parasites/μl while the popular histidine-rich protein (HRP2) assays lack correlation with live parasite numbers and may miss cases where the HRP2 gene has been deleted.

Methods: The assay essentially involved addition of blood samples in different dilutions followed with overlaying of conjugate into the reactant wells. It required an incubation of one hour. After washing, chromogen substrate is added into the wells and the binding of pLDH is visualized by the appearance of colour in 15 min interval.

Results: We report here the development of a quantitative pan-pLDH ELISA which reliably detects low levels of parasites in the blood of patients infected with the major human malarial species(*P. falciparum* 5-10 parasites/μl of blood,*P. vivax* 40-60parasites/μl of blood,*P. ovale* 30 parasites/μl of blood sample).

Conclusion: Availability of a sensitive, high-throughput pan-species malarial assay may prove useful in different settings such as surveillance, blood banking, mapping the spread of malaria in meso- and hypo-endemic areas, as well as in more traditional direct diagnosis and treatment monitoring.

http://dx.doi.org/10.1016/j.ijid.2012.05.541