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The rapid-simple-ESBL test in *Escherichia coli* and *Klebsiella pneumoniae*

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Background: In order to the growing frequency of extended-spectrum beta-lactamases (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*, treatment of such infections requires rapid and reliable detection of this enzyme.This study aims to develop the rapid-simple-ESBL detection in these organisms by comparing with the double-disc synergy test/combined disc test.

Methods: One hundred and forty-six isolates ofE. coliand 150 isolates ofK. pneumoniaeisolated from the clinical specimens were included in this study. The rapid-simple-ESBL (RS-ESBL) test was performed in phenol red glucose broth containing ceftazidime(CAZ) or cefotaxime(CTX) (2 μg/ml) with or without clavulanic acid in microtiter plate. And the incubation period of this test was 4 hours. All of them were also tested for ESBL producer by double-disc synergy test/combined disc test.

Results: Among 96 ESBL-producing *E. coli* isolates, 94(97.9%) and 95(99.0%) were identified as ESBL-positive by CAZ RS-ESBL and CTX RS-ESBL, respectively when compared with double-disc synergy test/combined disc test. Of 100 ESBL-producingK. pneumoniae, 97(97.0%) were ESBL-positive by both CAZ RS-ESBL and CTX RS-ESBL tests. All of the 100 non-ESBL producers were negative by CTX RS-ESBL test.

Conclusion: The RS-ESBL test was proved to be a reliable, simple and rapid method for detecting ESBL in *E. coli* and *K. pneumoniae.*

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A method for increasing viral detection in human and wildlife samples with low nucleic acid content

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Background: Detection of pathogens in the CSF of viral encephalitis (VE) patients and in excreta from wildlife is often limsequencing using high throughput sequencer (such as Roche 454 junior) is often necessary for these, as well as wildlife, samples. Yet outcomes from such diagnostic approaches are not always successful.

Methods: Total nucleic acids were amplified using Whole Genome (WGA) and Whole Transcriptome Amplification (WTA) techniques prior to PCR detection or high throughput sequencing. Rabies virus (RV) and herpes simplex virus 2 (HSV2) from cell culture supernatant were used as template for optimization. A total of 89 archived encephalitis specimens collected from 2006 to 2011 with indeterminate (32) or positive (57) result for one of herpesvirus 1-5 were subjected to WGA, followed by duplex real-time PCR for herpesvirus family (HHV1-8). Two clinical samples collected from wildlife were used in high throughput sequencing, saliva from *Rousettus leschenaulti* and urine from *Pteropus lylei.* The wildlife sampleswere amplified via WTA and run using high throughput sequencing on the Roche 454 Jr. sequencer. Analysis was performed by using BLASTX algorithm searching against non-redundant protein database (NR) and viral genome database.

Results: 1,718,835 fold increase in RV and 1,313,514 fold increase in HSV2 were achieved. Twelve previously indeterminate and 3 previously negative specimens were found positive for herpes virus after WGA. Eight samples previously found positive for one virus also tested positive for another. The analyzed data of bat saliva from the high throughput sequencing revealed reads matching simian retrovirus, simian adenovirus, and simian cytomegalovirus with approximately 70-90% identity. *Choristoneura occidentalis* granulovirus was also abundantly detected in bat urine with more than 300 mapped reads at >90% identity criteria.

Conclusion: Our results demonstrate the utility of using WTA and WGA for detecting viruses in human and wildlife samples, using both conventional PCR and next-generation sequencing post-processing. Our limited study suggests that these methods may be more broadly applicable to aid in pathogen discovery and more accurate diagnosis in archived clinical samples of similar quality collected prospectively.

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Comparison of different processing methods of blood cultures – impact of MALDI-TOF MS and direct antimicrobial susceptibility testing (AST) on accuracy and time to result

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Background: Bloodstream infections are an important cause of morbidity and mortality. Early and adequate antimicrobial therapy is closely correlated with clinical outcome. Fast and accurate diagnostic methods are an important part in guiding treatment for bloodstream infections.

Methods: This study was conducted to assess the impact of direct processing of positive blood cultures (BC) on accuracy of and time to result using matrix-assisted laser desorption/ionisation

time-of-flight mass spectrometry (MALDI-TOF MS) and automated AST directly from positive BCs. A total of 100 positive BCs were processed using different workflows: conventional culture and AST, MALDI-TOF and AST from subcultures and MALDI-TOF and AST directly from BC. All BCs were processed with all algorithms.

Results: Time to result ranged from 34-96 hours with conventional protocols, whereas results could be obtained in as little as 17 hours with MALDI and AST directly from the positive BC. In 87,5% of all monomicrobial BSI, pathogens were correctly identified to species level directly from the positive BC with higher identification rates in gram-negative pathogens (95%) than in gram-positives (78%). Direct AST results could be obtained in 70% of isolates. When these were compared to AST from overnight culture according to CLSI, results were identical in 84%.

Conclusion: MALDI-TOF and direct AST testing from positive BCs can significantly improve time to result in the lab. Further studies are needed to assess the impact of these measures on clinical outcome.

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Is neuroimaging really helpful in viral encephalitis? Analysis of magnetic resonance imaging of 65 cases with known etiologies

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Background: Magnetic resonance imaging (MRI) has been recognized as important tool in diagnosing etiology of viral encephalitis (VE). Typical MRI patterns suggest the diagnosis and therapeutics such as in the case of herpes simplex encephalitis. The purpose of this study was to analyze MRI patterns in VE patients with known viral etiologies. The study included patients admitted to King Chulalongkorn Memorial and Ramathibodi hospitals from January 2001 to September 2011.

Methods: MRI of VE patients during the non-comatose phase were reviewed and categorized into 5 patterns according to anatomical involvement as focal central, focal peripheral, multifocal central, multifocal peripheral and diffuse or combined patterns. Enhancement patterns as well as other associated findings such as, parenchymal edema, mass effect, hemorrhage, vasculitis were also analyzed.

Results: Brain and spinal MRI studies of 65 VE patients were analyzed. Those associated with Japanese encephalitis (JEV), dengue, and influenza were classified as endemic VE, the remaining as sporadic VE. There were 10 patients with herpes simplex (HSV) type I (15.4%), 9 with type II (13.9%), and 8 with unspecified (12.3%), 12 with varicella zoster (VZV) (18.5%), 7 with JEV (10.8%), 6 each with Epstein-Barr (EBV) (9.2%) and cytomegalovirus (CMV) (9.2%), 5 with dengue (57.7%) and one each with John Cunningham virus (JCV) (1.5%), and influenza B (1.5%). HSV was found as peripheral (13/25), central (7/25) and combined (5/25) patterns. Central (6/10), peripheral (2/10), and combined (2/10) patterns were associated with VZV. EBV presented with central (2/5) or combined (3/5) pattern. JEV had central (4/7) or combined (3/7) patterns. Dengue had central (2/4) or combined (2/4) pattern. Nine of 11 patients had MRI evidence of spinal cord involvement. Three of 9 patients (CMV and HSV) had focal cord lesions. The remaining 6 patients (EBV 3, one each for CMV, HSV and VZV) had extensive cord involvement. Other patterns were not unique to any individual virus.

Conclusion: Although typical MRI abnormalities enable diagnoses, varying imaging patterns in the same etiologic group and the presence of similar features among different groups should alarm clinicians not to rely solely on imaging results.

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Usage of saliva for non-invasive dengue diagnosis

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Background: Dengue fever (DF) is a mosquito-borne viral disease. It occurs in over 100 countries and the most significant epidemics occur in Southeast Asia, the Americas and the Western Pacific. All the four serotypes can cause DF and the more severe and fatal Dengue Haemorrhagic Fever (DHF). The necessity of a venous blood collection in all dengue diagnostic assays can hinder early detection of dengue cases and thus could delay vector control efforts. The Environmental Health Institute (EHI) has developed a saliva based test using antigen capture anti-DENV IgA (ACA) enzyme-linked immunosorbent assay (ELISA) technique. We believe saliva testing can encourage patients to be more receptive towards dengue diagnosis. In our continuing effort to improve DENV diagnostics, we have also optimised our RT-PCR protocol to be able to detect and serotype DENV 1-4 in clinical saliva samples.

Methods: Saliva (and corresponding blood samples) from febrile patients were collected using buccal swabs and kept in Universal Transport Media (UTM). RT-PCR was done on both saliva and serum samples and results from dengue confirmed patients were compared. Virus isolation was also performed using the saliva samples.

Results: We are able to detect and identify the different DENV serotypes in the saliva samples, and saliva serotypes correlated with DENV serotypes in the corresponding serum samples. Virus isolation was also successful in 80% of the samples.

Conclusion: Saliva can also potentially replace blood as the biospecimen of choice for dengue diagnosis without compromising any downstream virus work.

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