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7.13^7 copies/mL (median 1.33^5 copies/mL). Two PAND PCR positive and 4 negative samples were sent to a reference laboratory for confirmation. In one case, the result was discordant. Of 16 samples tested with the serotype specific RT-PCR, 13 (81%) could be attributed to a subtype: 7 to subtype I, 2 to subtype II, 2 to subtype III and 2 to subtype IV.
Conclusion: Our results show that a combination of serological and RT-PCR are required for rapid and reliable dengue virus diagnostics at all stages of infection.

O487 RespiFinder-kit: simultaneous detection of 15 atypical viruses commonly involved in respiratory tract infections

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Objective: Acute respiratory tract infection is the most widespread type of acute infection in adults and children. The number of pathogens involved is numerous. The objective is the development of a multiparameter assay which enables the detection of all pathogens commonly involved in respiratory tract infections. The RespiFinder test currently involves influenza A, B and H5N1 virus, respiratory syncytial virus A and B, parainfluenza virus type 1, 2, 3 and 4, coronavirus 229E, OC43 and NL63, rhinovirus, human metapneumovirus and adenovirus (subgenera A, B, C, D, E and F). The aim was to obtain the same specificity and sensitivity as singleplex QPCR and to complete detection and differentiation within 6 hours.
Method: The RespiFinder-kit is based on a new multiplex PCR technology which enables simultaneous amplification of up to 40 fragments. Detection of a virus is dependant on two specific probes. These two probes hybridise adjacently to each other. After ligation of the specific probes, all targets are amplified with one universal primerset. The amplified fragments can be discriminated by size fractionation. A competitive internal RNA control is added to each sample allowing discrimination between a true negative sample and a negative sample due to a PCR failure. Prior to the multiplex amplification, viral RNA is converted into cDNA using an one step RT-PCR followed by a limited number of PCR cycles.
Results: The RespiFinder-kit was compared with singleplex QPCR assays as well as conventional culturing procedures. Sensitivity of the assay was compared with QPCR using serial dilutions of virus cultures. The same sensitivity was obtained with the RespiFinder-kit as with QPCR. Clinical samples were tested with all three approaches. This showed increased sensitivity of the two nucleic acid based tests over the conventional diagnostic procedures. The results with the RespiFinder-kit and the QPCR assay showed a high degree of correlation.
Conclusions: We showed that the RespiFinder-kit enables simultaneous detection of 15 viruses commonly involved in respiratory tract infections within 6 hours and with the same sensitivity and specificity as singleplex QPCR reactions.

O488 High prevalence of *Legionella pneumophila* in severe community-acquired pneumonia, as determined by a commercially available PCR assay

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Objectives: *Legionella pneumophila* is one of the main causative agents of severe community acquired pneumonia (CAP). When legionellosis occurs, it can be fatal if diagnosis and treatment are not promptly established, particularly in elderly and immunocompromised patients. Therefore, rapid detection and diagnostic methods are needed to improve the outcome of infected patients and to reduce unnecessary antibiotic therapy. The main goals of this work were:

- To assess the use of a new PCR-based method to support *L. pneumophila* diagnosis in patients with severe CAP, as compared to current laboratory methods.
- To determine the prevalence of *L. pneumophila* in severe CAP, requiring admission in intensive care unit (ICU).

Methods: A total of 95 clinical specimens (BAL, blood and urine) from 39 patients admitted in the intensive care unit (ICU) with severe CAP, were collected from December 2004 to October 2006. The presence of *Legionella pneumophila* was assayed simultaneously by the antigen urine test (Binax®), by microbiological culture in selective charcoal medium and by conventional PCR using a commercially available kit (Legiofast, Microbial SL).
Results: PCR analysis of *Legionella pneumophila* was positive in 21 patients (56.4%) whereas only 9 (23.1%) resulted positive when the enzyme immunoassay technique was used. No positive results were obtained by plate culture. No false negatives were obtained with the PCR kit. Moreover, all positives with Legiofast were in accordance with clinical parameters of *Legionella* infection.
Conclusions: The PCR assay used in this work enables a rapid and sensitive diagnosis using as low as 200 μl of urine. Prevalence of *L. pneumophila* is significantly higher when analysed by PCR as compared to the urine antigen test ($P=0.01$), suggesting that the prevalence of this pathogen in severe CAP is higher than suspected so far. Since urine antigen test is restricted to serogroup 1 of *L. pneumophila*, the fact that the PCR kit detects serogroups 1 to 15 may help to explain this difference. The use of this PCR as a complement of conventional techniques is recommended to improve the detection of *L. pneumophila* in clinical laboratories.

O489 Cost-effective method for differentiation between *Salmonella* species and other members of the Enterobacteriacae referred to the national *Salmonella* reference laboratory in England and Wales

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Objectives: Traditional methods for isolation and identification of *Salmonella* species require a series of biochemical and serological tests. Our purpose was to determine the cost effectiveness of incorporating molecular testing for differentiation between *S. enterica*, *S. bongori* and other members of the Enterobacteriacae. The latter are commonly referred to our laboratory for confirmation as "probable salmonellas" or *Salmonella* cultures are received contaminated with other organisms. These require selective isolation prior to identification. This in turn requires time and expense, including special media, additional exposure to microbes and more technologist time to perform subcultures. As many laboratories face budgetary constraints including loss of personnel, we propose a simple, cost-effective method of confirming *Salmonella* sp. prior to typing.
Method: We tested over 350 known Enterobacteriacae with a simple PCR assay. On the basis of their initial biochemistry, e.g. atypical results, we also tested 250 "probable salmonella" isolates referred to us for typing. Oligonucleotide primers were prepared according to the sequence of the chromosomal invA gene and the PCR assay was performed from a boiled cell template. An internal amplification control (IAC) was included to indicate possible PCR inhibitors. The trial now aims to evaluate 6-month resource use and costs of both PCR and traditional methods.
Results: The primers were specific as no amplification products were obtained for over 150 isolates of non-*Salmonella* Enterobacteriacae covering 20 species. In contrast, we were able to detect the target amplicon from a wide range of *Salmonella* strains and sub-groups. All 96 serovars (211 isolates) tested were shown to carry the invA gene. Most of the "probable *Salmonella* spp." did not produce a PCR product. Further work confirmed them as non-salmonellas. For the isolates that did demonstrate the presence of the invA gene, further testing found that these were salmonella cultures contaminated with other organisms. Current cost savings already demonstrated by using the PCR assay were largely attributable to the time taken by the technologist to perform all the traditional tests before producing a final customer report.
Conclusion: This assay provides a cost-effective rapid means of distinguishing between routine specimens that have been misidentified as *Salmonella* strains and those that do actually require further selection and typing.