CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)
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Project Number
S1301

Project Title
Determining the Presence of Chlamydophila pneumoniae in the San Joaquin Valley via Development of a Real Time PCR

Abstract

Objectives/Goals
This research project sought to develop a more accurate and reliable real time PCR test for the detection of Chlamydophila pneumoniae via an internal control. This project sought to determine the epidemiological presence of the organism in the San Joaquin Valley.

Methods/Materials
As of April 3rd, 191 patient samples from Children#s Hospital of Central California were used to determine the accuracy, sensitivity, and reproducibility of this novel PCR test. The samples were pre-extracted with the Qiagen DNA mini kit.
Development of the PCR diagnostic method involved unique creation of a master mix containing the primers, probes and internal control. Dilution of primers and probe concentrations for optimal detection were first tested. Secondly, the most specific internal control was developed using Human epithelial cells-2 to culture C. pneumoniae. These cultures were extracted, tested and inserted as a competitive internal control in the master mix.

Results
This study was successful in the development of a specific and reliable Real Time PCR test. Sensitivity was 1.0 x 10E-5 parts of genomic C. pneumoniae DNA and reproducibility, shown by positive and negative controls, was 100%. This study was successful in establishing the first highly specific and accurate competitive internal control (diluted to 2.5 x 10E-8) for the detection of this specific organism. 191 valley specific samples were tested of which 1.8% were positive and 83.7% were negative for C. pneumoniae. The internal control detected an inhibitor in 14.65% of all patient samples.

Conclusions/Discussion
The competitive internal control accuracy narrowed patient sample turnover rates (possible inhibitor present, causing different detection methods to be used) to 14.65% vs. 60% by the CDC, giving this test a 43.7% more accurate first trial sample determinant.
Epidemiologically, results compared similarly with other U.S. areas. The population tested, mainly 5-15 years of age, had a low prevalence. However, 62.5% infants tested worldwide for C. pneumoniae are positive. This population lacks developed immune systems, leaving them susceptible to illness and cause false negatives on the standard detection method, antibody level testing. C. pneumoniae#s ability to manifest into greater human ailments if undiagnosed is cause for retaining this study#s real time PCR test for its significantly faster, more reliable, and cost effective qualities.

Summary Statement
Chlamydophila pneumoniae is an atypical pneumoniae which leads to significant other health problems given manifestation time; this study#s results suggest the developed PCR may overcome detection hindrances posed by previous methods.

Help Received
Thanks to Alwyn Briones and Keith Zucker for lab space and purchase of materials.