Molecular Detection of a Plurality of Pathogens in UTM-RT

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AMENDED ABSTRACT

Background/Aims: Successful laboratory diagnosis for infectious diseases require the transport of pathogens in a cellular form from the site of collection to the laboratory in a sterile manner. Molecular detection techniques do not require that the organisms remain viable, only that the DNA remains intact. Cultures transmitted to the laboratory were analyzed by PCR over a period of five days postincubation (p.i.) in UTM-RT.

Methods: Molecular Contagious Virus (MCV) was identified by reverse PCR and confirmed by Phylogenetic Comparing in clinical specimens. All other pathogens were purchased from ATCC as ready-to-use pellets. A total of 28 pathogens were evaluated. The detection of the virus was confirmed by Cytochrome oxidase, RNA polymerase, and phosphatase markers.

Results: A standard curve was produced for PCR amplification (Ct). The 50% difference between the Ct values indicated a 1:5 dilution. The Ct values increased over time for all pathogens and were analyzed by a one-way ANOVA. The pathogens were grouped by genus: Bacterial, Viral, or Fungal. The results show that the C. glabrata strain was the most resistant to the protocol, while the viruses were the most susceptible.

Conclusions/Discussion: The results suggest that the transport medium, UTM-RT, is an effective method for the transport of a wide variety of pathogens.

RESULTS

The results from the pathogens stability experiments from Day 0 through Day 5 in post-inoculation were observed. The conventional PCR and real-time PCR reactions were presented as gel photographs and bar graphs, respectively. The HPV conventional PCR reaction was recorded by a real-time PCR for HPV analysis. Each real-time PCR reaction contained three concentrations of a positive vector control and one negative control (NOC). The NOC consisted of the substitution of water for DNA. The vector controls were designed to contain the amplification of each mutation. The results were used to generate a standard curve from 0.015 to 50,000 copies/ml. The amplification curve passed a threshold level and input concentration. TheRotor-Gene software (version 6.0) was used to generate an automated threshold and to calculate a copy number/dilution/week of each time point.

CONCLUSIONS

Twenty-five known bacterial, viral, and fungal pathogens relevant to OBGYN-related diagnostic tests were spiked into UT-RT and evaluated for up to five days post-incubation. PCR was performed at 4°C intervals (Day 0 through Day 5) and total DNA (500 µl) was extracted and assayed for the presence of each pathogen using conventional PCR (CPRD) or real-time PCR assay (all others). Samples were amplified by real-time PCR and conventional PCR for five days post-inoculation.

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