Stability of Bordetella pertussis and Bordetella parapertussis in the ESwab Transport System for Culture and PCR

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Introduction (cont)

Revised Abstract

Objectives
Pertussis (whooping cough) is caused by Bordetella pertussis and Bordetella parapertussis, with several states in the USA reporting an increased incidence. Laboratory detection methods include culture, direct fluorescent assay, and PCR methods. Successful culture and PCR detection require proper specimen collection and transport. This study examined the effectiveness of the ESwab system (consisting of liquid Amies transport medium and a flocked nasopharyngeal swab, Copan Diagnostics, Inc.) for maintenance of viability of B. pertussis and B. parapertussis for culture and preservation of nuclear material for detection by PCR.

Methods
Eight Bordetella isolates were tested, including 5 B. pertussis isolates (ATCC 9340 and 8467 strains, and 3 recent clinical isolates) and 3 B. parapertussis isolates (ATCC 15237 strain and 2 recent clinical isolates). Test methods were based on CSLI M40 guidelines. Three Bordetella saline suspensions (108, 109, and 1010 CFU/mL) were prepared. Each suspension was used to inoculate ESwabs in triplicate. The inoculated ESwabs were stored refrigerated (2-8°C) for up to 96 hours prior to plating. Bacteria from the ESwabs were cultured on Regan Lowe agar plates at 24, 48, and 96 hours of refrigerated storage post-incubation; plates were then incubated at 37-4°C in ambient air for a minimum of 4 days. The numbers of colonies at 24, 48, and 96 hours were compared to the 0 hour count to determine percent recovery (viability). Real-time PCR, using primers targeting the IS481 gene of B. pertussis and the IS1001 gene of B. parapertussis, was performed on 24-hour and 96-hour inoculum in the ESwab.

Results
Bordetella was isolated from all of the ESwabs after 96 hours of refrigerated storage. Percent recovery ranged from 27.2% to 96.3% for B. pertussis, and from 32.7% to 74.5% for B. parapertussis, and was similar across the three inoculum densities for all isolates tested. PCR detected B. pertussis and B. parapertussis in all of the 24- and 96-hour ESwabs, regardless of initial inoculum concentration (104 or 106).

Conclusion
The ESwab maintains sufficient viability of B. pertussis and B. parapertussis to permit detection in bacteria cultures and preserves DNA integrity for PCR detection, even after 96 hours of refrigerated storage.

Bacteria viability
All tests for bacterial viability were modified from the quantitative elution method described in CSLI M40 A. Modifications included:

Infection with Bordetella pertussis, the bacterium that causes pertussis (whooping cough), is spread person-to-person transmission via aerosolized respiratory droplets or by direct contact with respiratory secretions. Pertussis manifests with mild upper respiratory symptoms that begin 7-10 days (range 6-21 days) after exposure, followed by a severe lingering cough that becomes paroxysmal and can last for weeks or even months. Coughing paroxysms in frequency and are often followed by vomiting. A similar, milder disease is caused by B. parapertussis.


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