

Automated DNA purification from nasopharyngeal aspirates for detection of *Bordetella pertussis*

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DNA purification using the BioRobot® M48 workstation and MagAttract® DNA Mini M48 Kit was compared with a manual method. The fully automated procedure on the BioRobot M48 gave comparable results while reducing workload.

High Correlation of C_T Values with Automated and Manual DNA Purification

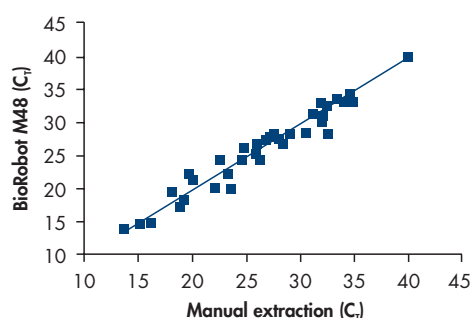


Figure 1 Bacterial DNA was purified from 46 nasopharyngeal aspirates using a manual method (see “Materials and methods”) or automated on the BioRobot M48 workstation. *B. pertussis* DNA was identified using real-time PCR. Comparison of C_T values showed a high correlation ($r^2 = 0.97$).

Table 1. Comparison of *B. pertussis* DNA Detection in Nasopharyngeal Aspirates with Manual DNA Extraction and Automated DNA Purification Methods

Manual extraction	BioRobot M48 automated purification	
	<i>B. pertussis</i> positive	<i>B. pertussis</i> negative
<i>B. pertussis</i> positive	36	0
<i>B. pertussis</i> negative	0	10

Bordetella pertussis causes pertussis, also known as “whooping cough”, a highly contagious respiratory illness that can cause serious complications or death, particularly in infants. Despite widespread childhood vaccination, pertussis has increased significantly in the US since 1990, with an annual average of 9431 cases during 1996–2003 (3.3 per 100,000 population). The majority of cases in the US occur in patients who are either too young to have been vaccinated (35.1% of cases occur in infants <6 months old) or so old that immunity induced by childhood vaccination has waned (60.7% of cases occur in patients ≥7 years old).

Rapid diagnosis of pertussis is essential for patient management and appropriate infection control. The disease can be highly communicable in its early stages, and diagnosis can be delayed or missed if symptoms are atypical or nonspecific. Culture-based diagnosis of pertussis requires 3–12 days and has reduced sensitivity as the infection progresses. PCR provides a rapid and sensitive method for detection of *B. pertussis* in nasopharyngeal aspirates following DNA purification. Manual DNA purification is tedious and labor-intensive, demonstrating the need for a simple, walkaway, automated method. We compared our manual DNA extraction method with fully automated DNA purification on the BioRobot M48 workstation. Compared with the manual method, the fully automated procedure streamlined the workload and provided high-quality DNA with reduced inhibition in downstream analyses.

Materials and methods

Nasopharyngeal washes collected from 46 patients were frozen, thawed, and extracted using both manual DNA extraction and automated DNA purification.

For manual DNA extraction, the specimen was treated with proteinase K for 1 hour at 65°C followed by a 1-hour incubation in a heat block set to 100°C. Automated DNA purification was carried out using the



MagAttract DNA Mini M48 Kit with the fully automated Bact_200ul protocol on the BioRobot M48 workstation. *B. pertussis* DNA was detected by real-time PCR analysis of the insertion sequence 481 (IS481) on the ABI PRISM® 7700 Sequence Detection System.

Results and discussion

Bacterial DNA purified by the manual method and by the fully automated method on the BioRobot M48 workstation identified the same samples as positive or negative for *B. pertussis* DNA (Table 1). Comparison of C_T values from real-time PCR analysis demonstrated a strong correlation between the two methods (Figure 1).

Conclusions

- Automated purification on the BioRobot M48 workstation provided high-quality bacterial DNA for real-time PCR.
- The automated method provided a more streamlined workload compared with the manual method.
- Both automated and manual methods identified the same samples, with a high correlation of C_T values ($r^2 = 0.97$).

Ordering Information

Product	Contents	Cat. no.
BioRobot M48	Robotic workstation for automated purification of nucleic acids using MagAttract M48 kits, computer, installation, 1-year warranty on parts and labor	9000708
MagAttract DNA Mini M48 Kit (192)	For 192 DNA preps: MagAttract Suspension B, Buffers, Proteinase K	953336
App. Package, M48, Inf. Dis. (CD)	Software protocol package for infectious disease applications, v. 2.0, on the BioRobot M48 workstation	9016145

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The PCR process is covered by the foreign counterparts of U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-La Roche AG. 1030469 06/2005 © 2005 QIAGEN, all rights reserved.

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