Automated purification of mycoplasma DNA from respiratory samples

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Mycoplasma DNA was purified from 4 different kinds of respiratory research samples using the BioRobot® M48 workstation and the MagAttract® DNA Mini M48 Kit with the QIAGEN Supplementary Protocol "Purification of bacterial DNA from primary samples using the MagAttract DNA Mini M48 Kit". DNA purified from nasopharyngeal swabs, nasopharyngeal secretions, sputum, and tracheal secretions performed well in real-time PCR, and showed identical results when compared to DNA purified by an established manual method.

Mycoplasmas are small, atypical bacteria with flexible cell walls and can be very difficult to detect and identify using traditional culture-based techniques. *Mycoplasma pneumoniae* is the pathogen most commonly associated with primary atypical pneumonia in humans, and is a common cause of both community and hospital acquired lung infections in children and immunocompromised individuals. Infection is often asymptomatic but can result in life-threatening pneumonia or long-term sequalae. In order to ensure accurate life science and clinical research and effective development of diagnostic tests, a rapid and standardized method of processing a range of respiratory research sample types is required.

Table 1. Comparison of Manual and Automated DNA Purification for Quantitative Amplification of *M. pneumoniae* DNA by Real-Time PCR

		Real-time PCR analysis*		
Sample number	Sample type†	Manual DNA purification and in-house PCR: *M. pneumoniae*	Automated DNA purification and real-time PCR: *M. pneumoniae*	Automated DNA purification and real-time PCR: control amplicon
1	NG-swab	+	+	+
2	NG-swab	+	+	+
3	NG-swab	+	+	+
4	NG-swab	+	+	+
5	NG-swab	+	+	+
6	NG-swab	+	+	+
7	NG-fluid	+	+	+
8	Sputum	+	+	+
9	Sputum	–	–	+
10	ET-fluid	–	–	+
11	Positive control	+	+	+
12	Negative control	–	–	+

* + = amplicon detected; – = amplicon not detected.

† NG-swab = Nasopharyngeal swab, NG-fluid = Nasopharyngeal fluid, ET-fluid = Endotracheal fluid.

Material and methods

Four different respiratory sample types (Table 1) were processed. Nasopharyngeal secretions, sputum, and tracheal secretions were processed directly. Nasopharyngeal swabs were incubated in 1x PBS for 30 minutes at room temperature. Samples (200 µl) were processed according to the Bact_200ul protocol using the MagAttract DNA Mini M48 Kit and the App. Package, M48, Infectious Disease. DNA was eluted in 100 µl water.

M. pneumoniae DNA was amplified using 5 µl purified DNA as template in 25 µl real-time PCR spiked with internal positive control (IPC) DNA. A *M. pneumoniae*-specific probe (FAM™) and a probe specific for the internal amplification control (JOE™) were used to detect and identify amplified DNA. A true negative test result is confirmed by a positive control-amplification. Negative results in both test and control amplifications indicate the presence of PCR inhibitors.

Results

Real-time PCR results (Figures 1 and 2) show that automated DNA purification using the BioRobot M48 workstation results in high-performance DNA that is well-suited for sensitive real-time PCR. *M. pneumoniae*-specific amplicons were detected in all positive samples, but not in negative and control samples (Table 1), indicating both cross-contamination–free purification and that purified DNA was not degraded. Internal positive controls were detected in all samples and showed very similar C_T profiles (Figure 2), indicating that purified DNA was free of PCR inhibitors (Figure 1).

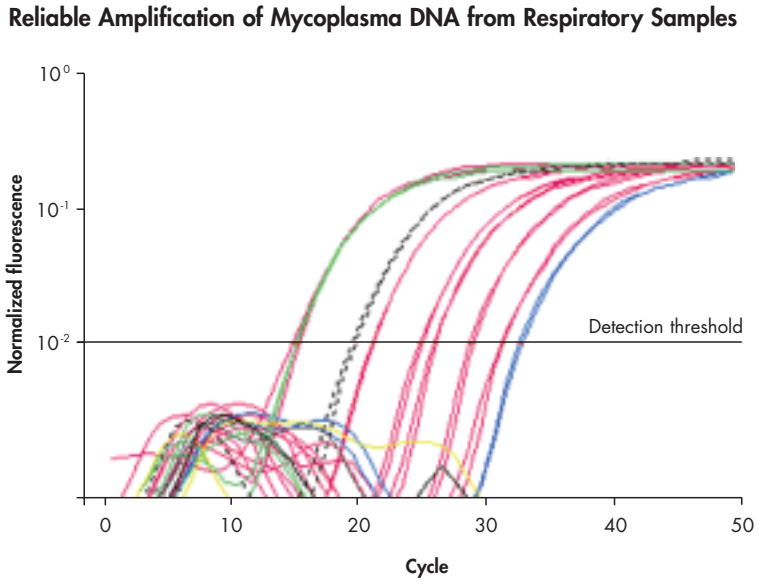

Figure 1 Real-time PCR of *M. pneumoniae* DNA using purified DNA representative of purifications from 3 different types of respiratory sample containing *M. pneumoniae*. **Red**: 6 x nasopharyngeal swab; **Blue**: nasopharyngeal fluid; **Green**: sputum; **Yellow**: endotracheal fluid; **Broken lines**: 2 x positive control samples isolated with Bact_200ul protocol on the BioRobot M48 workstation; **Gray**: negative controls.

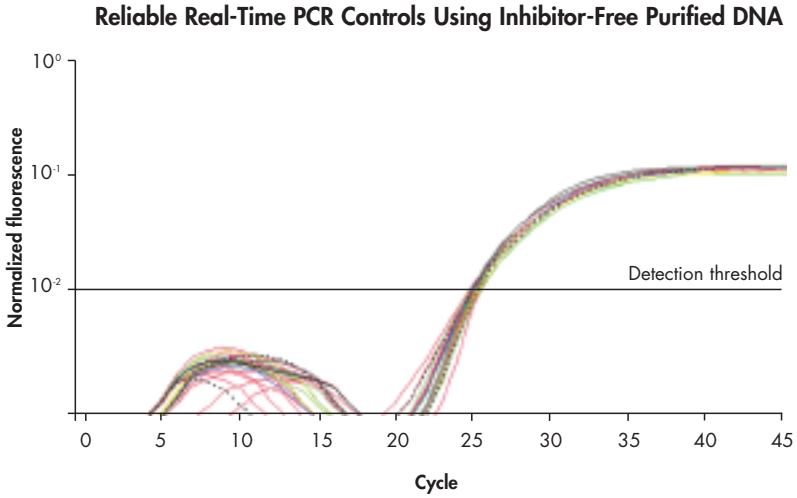

Figure 2 Internal positive control DNA was amplified efficiently from all samples and no significant variation was observed in C_T values, indicating purified DNA was consistently free from PCR inhibitors. **Red**: nasopharyngeal swab samples; **Blue**: nasopharyngeal fluid; **Yellow**: endotracheal fluid; **Green**: sputum; **Gray**: negative controls; **Broken lines**: positive control isolated with Bact_200ul protocol on the BioRobot M48 workstation.

Conclusions

High performance *M. pneumoniae* DNA was purified from a range of respiratory research samples, using the Bact_200ul protocol on the BioRobot M48 workstation, in combination with the MagAttract DNA Mini M48 Kit and the App. Package, M48, Infectious Disease. Using this DNA in sensitive downstream applications, such as quantitative real-time PCR, allows:

- **Standardized amplification of *M. pneumoniae* DNA** — from respiratory samples and swabs
- **Rapid purification** — with no enrichment culture
- **Low CVs and reliable controls** — using inhibitor-free DNA

Ordering Information

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

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