

## Comparison of enzymes to lyse streptococcal bacteria prior to automated DNA purification on the BioRobot® M48

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Different enzymes were tested for their suitability to lyse Gram-positive bacteria for DNA purification on the BioRobot M48 followed by quantification using real-time PCR. The BioRobot M48 procedure provided high-quality DNA for real-time PCR with all 3 lysis methods tested.

Streptococci are Gram-positive bacteria associated with a number of diseases. Group A streptococci are often found in the throat or on the skin. People carrying group A streptococcus can be asymptomatic, or infection can lead to a number of relatively mild illnesses, such as “strep throat” or impetigo. In rare cases, group A streptococcus can cause invasive, life-threatening illnesses, including Streptococcal Toxic Shock Syndrome (STSS) and necrotic fasciitis (“flesh-eating bacteria”) (1). Group B streptococci mainly cause illness in newborns, pregnant women, elderly people, and immunocompromised persons. It is the most common cause of life-threatening infection in newborns, with a mortality rate of 5–20% and significant morbidity rates for both mother and infant (2). Standard diagnostic methods for detection of group B streptococcus rely on culture, requiring 18 to 48 hours (3). In contrast, purification of streptococcal DNA on the BioRobot M48 requires approximately 3 hours for 48 samples. Using real-time PCR for detection, samples can easily be processed and results interpreted the same day, allowing faster time to results and earlier clinical intervention.

Efficient lysis is a key factor for purification of bacterial DNA, especially for Gram-positive bacteria, such as *Streptococcus* spp. or potential bioerror agents, such as *B. anthracis* (4). Standard enzymatic lysis methods prior to fully automated DNA purification on the BioRobot M48 workstation include the use of lysozyme and/or lysostaphin. We tested the efficiency of lysis using these lytic enzymes or a commercial, recombinant lysozyme solution.

### Material and methods

Group A streptococci were grown overnight in Todd Hewitt broth, and group B streptococci were grown overnight in LIM broth. All bacteria were clinical isolates, identified by the Microbiology Laboratory at CompuNet Clinical Laboratories. Lysozyme and lysostaphin were obtained from Sigma; Ready-Lyse™ Lysozyme Solution was obtained from Epicentre Biotechnologies. Lysozyme and lysostaphin were reconstituted in RNase-free water at 50 mg/ml and 5 mg/ml, respectively. Ready-Lyse working solution was prepared by diluting the stock solution (250,000 units/μl) in PBS to 1250 units/μl.



200 µl of the overnight culture was centrifuged at 5000 x g for 5 minutes, and the pellet was resuspended in 200 µl PBS. For lysis, 20 µl of lysozyme, 10 µl of lysostaphin plus 20 µl of lysozyme, or 10 µl of Ready-Lyse working solution were added. Incubation was carried out for 30 minutes at 37°C for lysozyme and lysostaphin, or for 30 minutes at room temperature for the Ready-Lyse solution. The lysozyme–lysostaphin lysis was not tested with group A streptococcus.

Bacterial DNA was purified on the BioRobot M48 workstation using the MagAttract® DNA Mini M48 Kit and the fully automated Bact\_200ul protocol on the App. Package, M48, Infectious Disease CD-ROM. Purified DNA was analyzed on an Applied Biosystems 7500 Real-Time PCR System using TaqMan® PCR assays developed at CompuNet.

## Results and discussion

Threshold cycle ( $C_T$ ) values were comparable for all 3 lysis methods, indicating similar yields (Table 1, Figure 1). According to the supplier, use of Ready-Lyse Solution can lead to higher yields since lysozyme is positively charged at neutral pH and can therefore bind and precipitate DNA. This effect was not observed with lysozyme when using the BioRobot M48 procedure. This could be due to the fact that the fully automated procedure has no centrifugation or precipitation steps after addition of lysozyme, and therefore the risk of lysozyme-mediated precipitation of DNA is reduced. Purification on the BioRobot M48 gave high-quality bacterial DNA for real-time PCR with all 3 lysis methods.

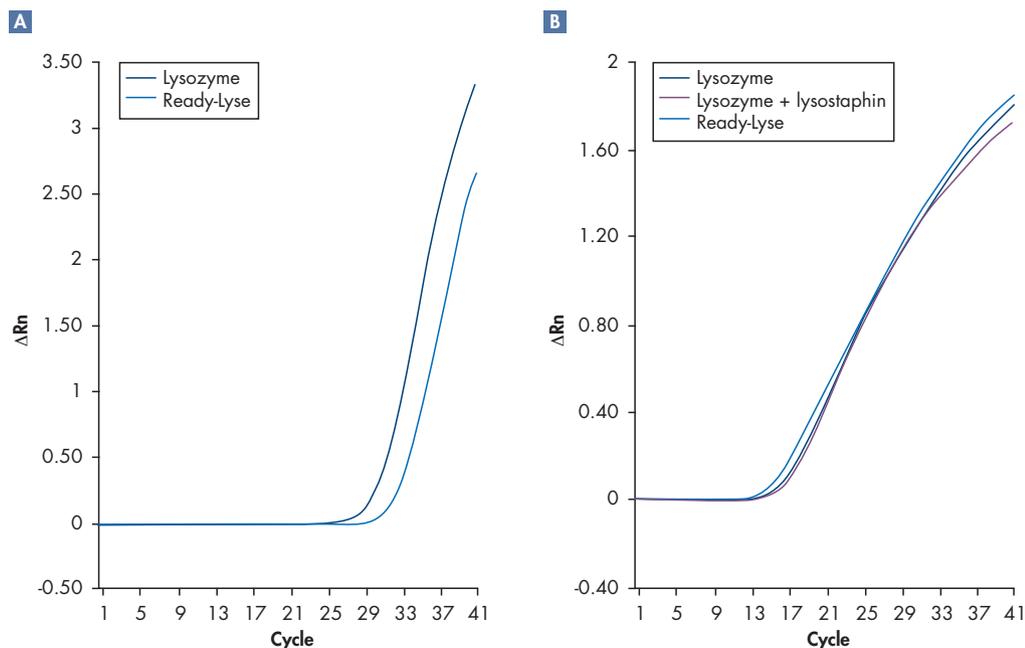
**Table 1.  $C_T$  Values for DNA Purified Following Different Enzymatic Lysis Methods**

Streptococcus group	Sample ID number	Threshold cycle ( $C_T$ )		
		Lysozyme	Lysozyme + lysostaphin	Ready-Lyse Solution
A	9	33.17	n.t.	30.98
A	10	18.06	n.t.	17.13
A	15	n.d.	n.t.	38.12
A	16	18.78	n.t.	20.35
A	17	26.90	n.t.	28.14
A	20	30.26	n.t.	30.22
A	22	29.62	n.t.	32.03
A	23	29.83	n.t.	29.77
<b>Mean ± SD</b>	–	26.66 ± 5.91	–	28.34 ± 6.67
B	1	22.12	21.75	19.88
B	2	15.65	15.14	17.48
B	5	22.91	21.81	21.65
B	6	17.56	25.65	18.60
B	9	14.75	16.91	21.41
B	11	16.68	17.19	15.90
B	12	14.47	15.65	16.30
<b>Mean ± SD</b>	–	17.73 ± 3.44	19.16 ± 3.94	18.75 ± 2.33

n.t.: not tested.

n.d.: not detected.

## High-Quality DNA from *Streptococcus* spp. for Real-Time PCR



**Figure 1** Following pretreatment with the indicated lytic enzymes, bacterial DNA was purified using the MagAttract DNA Mini M48 Kit on the BioRobot M48 workstation. DNA was analyzed on the Applied Biosystems 7500 Real-Time PCR System. A representative amplification plot is shown for **A** DNA purified from group A streptococci (sample 22) and **B** DNA purified from group B streptococci (sample 11).

## Conclusions

- Automated purification of streptococcal DNA using MagAttract technology with real-time PCR detection presents substantial time savings compared to standard culture-based methods. Results are available the same day, with DNA purification from 48 samples in only 3 hours, whereas culture requires 18–48 hours.
- Ready-Lyse Solution provides a viable substitute for lysozyme for pretreatment of streptococcus bacteria before DNA purification using automated Bact\_200ul protocols.
- Since lysostaphin is very expensive and did not give any appreciable increase in yields, we do not recommend it for streptococcus. Ready-Lyse Solution is comparable with lysozyme in price.
- Ready-Lyse Solution provided greater ease of use, making it especially well-suited for non-routine users or for use in the field. Lysis takes place at room temperature with no need for heating blocks or water baths. The Ready-Lyse working solution can be stored at 2–8°C for up to 2 weeks whereas lysozyme must be made fresh or used from frozen aliquots that are used once and discarded.
- DNA purification on the BioRobot M48 workstation was robust, providing high-quality bacterial DNA for real-time PCR with all 3 lysis methods.

## References

1. Stevens, D.L. (1995) Streptococcal toxic-shock syndrome: spectrum of disease, pathogenesis, and new concepts in treatment. *Emerging Infectious Diseases* **1**, 69.
2. Prevention of perinatal group B streptococcal disease: a public health perspective. (1996) *MMWR* **45**, 1.
3. Schrag, S., Gorwitz, R., Fultz-Butts, K., and Schuchat, A. (2002) Prevention of perinatal group B streptococcal disease: revised guidelines from CDC. *MMWR* **51**, 1.
4. Zoll, G., Grote, G., Jaske, C., Maatmann, I., and Köhne, S. (2004) Automated purification of anthrax DNA from soil for biodefense applications. *QIAGEN News* **2004**, e24.

## 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, Infectious Disease	Software protocol package for infectious disease applications on the BioRobot M48 workstation	9016145
BioRobot EZ1	Robotic workstation for automated purification of nucleic acids using EZ1 kits, installation, 1-year warranty on parts and labor†	9000705
EZ1 DNA Bacteria Card	Preprogrammed card for EZ1 bacterial DNA purification protocols	9016362
EZ1 DNA Tissue Kit (48)	48 Reagent Cartridges (Tissue), 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes (2.0 ml), 50 Elution Tubes (1.5 ml), Buffer G2, Proteinase K	953034

\* Warranty PLUS 2 (cat. no. 9237714) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

† Warranty PLUS 2 (cat. no. 9237720) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

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The BioRobot M48 workstation, App. Package, M48, Infectious Disease, MagAttract M48 Kits, EZ1 workstation, EZ1 DNA Bacteria Card, and EZ1 DNA Tissue Kit are intended as general-purpose devices. No claim or representation is intended for their use to identify any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of the BioRobot M48 workstation, App. Package, M48, Infectious Disease, MagAttract M48 Kits, EZ1 workstation, EZ1 DNA Bacteria Card, and EZ1 DNA Tissue Kit for any particular use, since their performance characteristics have not been validated for any specific organism. The BioRobot M48 workstation, App. Package, M48, Infectious Disease, MagAttract M48 Kits, EZ1 workstation, EZ1 DNA Bacteria Card, and EZ1 DNA Tissue Kit may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 4,683,202 and 4,683,195 owned by F. Hoffmann-La Roche Ltd.

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