

Research Article

# Application of BioRobot M48 to forensic DNA extraction

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## Abstract

The development of a nucleic acid extraction method based on magnetic separation has opened up possibilities for automation of DNA extraction. The BioRobot M48 is one of robotic stations applicable to automated DNA extraction in forensics. However, each new method should be thoroughly validated before application to routine casework. Our aim was to compare the effectiveness of the currently utilized organic/Microcon 100 based extraction procedure and magnetic extraction with BioRobot M48. The DNA concentration of DNA extracts obtained from different kinds of typical forensic material was evaluated followed by amplification with the SGM Plus or Identifiler kit and capillary electrophoresis using ABI 3100 Avant. We can conclude that BioRobot M48 is a very effective instrument for DNA extraction from most specimens and can be successfully applied in forensic laboratories.

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## 1. Introduction

DNA extraction is a crucial step in forensic examination of biological traces [1]. The organic method is considered as a most efficient and reliable technique for DNA extraction and is widely used for DNA isolation from different sources of material analysed in forensic science. On the other hand the organic method is also found to be offensive to health, very laborious and not suitable for automation. The BioRobot M48 instrument, which implements a magnetic based nucleic acid extraction method is an interesting alternative in the genetic examination of biological samples. It can be applied to different kind of specimens and significantly improving the throughput simultaneously minimises the danger of contamination [2–6]. In forensic science, before any new method is applied to the routine casework, it must be subjected to validation experiments. Our aim was to compare between the effectiveness of the organic/Microcon 100 based extraction procedure (which has been successfully used for many years by our laboratory) and magnetic extraction with BioRobot M48.

## 2. Materials and methods

The study included three types of samples: (1) liquid blood dilutions 1:10 and 1:100; (2) typical forensic samples (blood-stains, semen stains, saliva stains, hair roots); (3) heavily degraded samples and problematic specimens (rotten blood, old soft tissues, paraffin embedded tissues, bone material). All samples were extracted using both phenol-chloroform (organic) extraction followed by Microcon 100 concentration and magnetic DNA extraction using BioRobot M48 according to the protocols recommended by the manufacturer (Qiagen). DNA concentration was measured with a fluorimetric method using Fluoroscan Ascent FL (Labsystems). Real time PCR and Quantifiler kit (Applied Biosystems) were used for evaluation of DNA concentration in analysed bone samples. Samples were amplified using the SGM Plus or Identifiler multiplex kit. PCR products were then analysed on ABI 3100 Avant genetic analyser (Applied Biosystems).

## 3. Results

### 3.1. Sensitivity test

Two undiluted liquid blood samples (50  $\mu$ l) and their dilutions 1:10 and 1:100 (also 50  $\mu$ l) were subjected to

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examinations. In the case of diluted blood samples slightly better results of STR profiling were noted for the magnetic extraction protocol.

### 3.2. Typical biological traces

The experiment included 76 regular biological traces which are usually analysed in forensic laboratories like bloodstains, semen stains and saliva stains deposited on different substrates, and hair bulbs. DNA concentrations obtained for adequate samples were significantly lower in the case of the magnetic DNA extraction but this did not affect the ultimate STR profiling. It was also noted that samples with dye color present after the preincubation step were clear after isolation on BioRobot M48. The same samples after organic extraction were still colored. The results indicated that magnetic extraction protocol was more efficient in removing dyes (which are potential inhibitors), than the standard phenol-chloroform method.

### 3.3. Heavily degraded samples

This experiment included 37 highly degraded samples—rotten blood, soft tissues, bones and paraffin embedded tissues. In this experiment better profiling results were obtained for samples extracted with the organic method.

## 4. Modifications of standard magnetic extraction protocol

### 4.1. Pre-incubation time extension

Samples of 50  $\mu$ l of liquid blood and 0.25 cm<sup>2</sup> of a bloodstain were subjected to analysis with the incubation step recommended by the manufacturer (15 min) and the extended incubation time (1 h, 2 h, 3 h and 24 h). It was found that an increase in DNA concentration was correlated with time extension. For all samples full DNA profiles were obtained. We further addressed this issue and chose 5 bloodstains extracted with BioRobot M48 for which partial SGM profiles were obtained using the recommended 15 min incubation step and reanalysed them with extended 24 h incubation time. The modified protocol enabled full STR profiles in all samples.

### 4.2. Magnetic extraction as a tool for additional inhibitor removal

The magnetic DNA extraction is very efficient in removing inhibitors of the PCR reaction. We combined the phenol-chloroform extraction protocol used for bone material with the magnetic method which was used as a final purification step. Real time PCR showed that inhibitors of the PCR reaction present after the standard phenol-chloroform protocol were efficiently removed by additional magnetic purification. This modification applied to 11 bone samples improved the DNA profiling results in six cases. Further experiments are necessary to confirm this initial finding.

## 5. Conclusions

1. Application of BioRobot M48 enables extraction of 48 samples from typical forensic sample in less than 3 h and thus can significantly increase laboratory throughput.
2. Magnetic extraction protocol implemented in BioRobot M48 may be successfully applied to the extraction of DNA from samples containing tiny amounts of DNA.
3. Analysis of regular biological traces was equally robust using the phenol-chloroform protocol and magnetic extraction and thus BioRobot M48 can be used as a good alternative for analysis of forensic biological traces.
4. In the case of heavily degraded samples better results are obtained using the standard organic method followed by Microcon/Centricon 100 concentration.
5. It was found that extension of the 15 min incubation step which is recommended in the manufacturer's protocols in the case of DNA extraction from biological traces may increase the concentration of the DNA extract and this improve profiling results.
6. As the magnetic DNA extraction is very efficient in removing inhibitors it can be applied for this purpose as an additional step in the phenol-chloroform DNA extraction procedure used for bone material.

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## Conflict of interest

None.

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