Improved elution of DNA from Whatman FTA® cards using prepGEM®/ forensicGEM® Storage Card extraction kits

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Introduction
Whatman FTA® Cards (GE Healthcare) are a popular choice for storing biological material. The cards allow tissue samples and DNA to be stored at room temperature and they are now the standard for diagnostic, R&D and forensic organisations.

The cards have a cellulose-paper base that has been impregnated with a surfactant, chelating agent, buffer and free radical trap [1]. On application of biological material, cells lyse and the DNA becomes tightly associated with the paper. Because of this strong association, the sample is generally analysed while bound to the paper. The preserving agents are inhibitory to most downstream reactions and must be removed by a series of washes.

However, in situations where it is preferable to elute the DNA from the paper (for example with qPCR), a number of chemical treatments are available. These include using alkali [2] or alternatively methanol to ‘fix’ the DNA [3,4]. The intention of the latter method is to replace aqueous wash steps with methanol which can be removed by evaporation rather than pipetting. Although it is unclear how methanol evaporation removes the inhibitory preserving agents, the method does seem to work and it reduces the number of steps that are difficult to automate.

As an alternative to these methods, ZyGEM manufactures formulations within the prepGEM® and forensicGEM® ranges of DNA extraction products. These have been designed for extracting either blood or buccal swab smears from FTA cards. This application note compares the ZyGEM methods with other elution methods and scores for yield, efficiency, and factors such as time, potential for contamination and the number of tips required for each extraction.

Methods
Preparation of cards and punches

**Blood**: 40 µl of fresh blood from a healthy individual was spotted onto FTA cards. The cards were allowed to dry and were stored for 3 days. Two 1 mm² punches were used for each extraction.

**Saliva**: For each FTA card, a cotton medical swab (Cultiplast®, LP Italiana SPA) was used. The swabs were rubbed vigorously on the inside of the cheek for 30 seconds and then were rolled onto FTA cards applying pressure to squeeze out as much of the liquid as possible. Cards were allowed to dry and were stored for 3 days. Two 1 mm² punches were used for each extraction.

DNA extraction

A total of six different method variants were tested:

A. Whatman alkali method  
B. Modified Whatman method with treatment at 65°C  
C. Methanol method of Johanson et al. [4]  
D. Methanol method of Johanson et al. with proteinase K  
E. ZyGEM recommended method for blood  
F. ZyGEM recommended method for saliva

<table>
<thead>
<tr>
<th>Whatman extraction methods A and B</th>
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<tr>
<td>1. The punches were washed 4 times as follows:</td>
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<td>a. 200 µl of FTA Wash solution was added to the punches and incubated for 5 minutes at room temperature with occasional mixing.</td>
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<td>b. The liquid was removed with a pipette.</td>
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<td>2. The punches were then washed 3 times as follows:</td>
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<td>a. 200 µl of TE buffer was added (10 mM Tris pH 8.0, 1 mM EDTA) and again the tubes were incubated for 5 mins at room temperature with occasional mixing.</td>
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<td>b. The liquid was removed with a pipette.</td>
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<td>3. All the buffer was drained and 35 µl of alkaline incubation buffer added (0.1 N NaOH, 0.3 mM EDTA, pH 13.0).</td>
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<td>4. The samples were incubated at room temperature (Method A) or 65°C (Method B) for 5 mins.</td>
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<td>5. 65 µl of neutralising solution (0.1 M Tris-HCl, pH 7.0) was added and the samples vortexed 5 times to mix.</td>
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<td>6. Tubes were incubated for 10 mins at room temperature.</td>
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<td>7. The tubes were then ‘flash’ vortexed 10 times.</td>
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<td>8. Punches were removed making sure to extract the maximum amount of eluate.</td>
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<th>Methanol extraction methods C and D</th>
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<tr>
<td>1. The punches were overlaid with methanol as follows:</td>
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<td>a. 4 drops were added by pipette onto the punches and allowed to air-dry at room temperature for 20 mins.</td>
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<td>b. The process was repeated twice more incubating at 37°C for 40 mins each.</td>
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<td>2. Once dry, 50 µl of 1x PCR II buffer (Applied Biosystems) was added (Method C) or 50 µl of 1x PCR II buffer containing proteinase K at 50 µg per ml (Method D).</td>
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<td>3. The tubes were incubated at 60°C for 30 mins, 99°C for 10 mins and then held at 4°C.</td>
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<td>4. Punches were left in the extract.</td>
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**forensicGEM® / prepGEM® features:**

- Thermostable enzyme & optimised buffer.
- Simple wash and two-step temperature shift (75°C & 95°C).
- Closed-tube procedure secures against contamination.
- DNA is ready for PCR, qPCR, SNP testing in 20 - 30 mins.
- No overnight incubations or centrifugation required.
- Compatible with PCR or profiling reagents.
ZyGEM extraction method E (blood)
1. 100 µl of water was added to punches. Samples were briefly vortexed and placed at room temperature for 15 minutes.
2. The tubes were vortexed and the liquid removed by pipette.
3. Punches were resuspended in:
   a. 44 µl Water
   b. 5 µl ZyGEM MAGENTA Buffer
   c. 1 µl prepGEM
4. Tubes were placed in a thermal cycler and heated at 75°C for 15 mins followed by 95°C for 15 mins.
5. Tubes were centrifuged at 16,000 x g for 5 mins.
6. The supernatant was decanted into new tubes.

ZyGEM extraction method F (saliva)
1. 100 µl of water was added to punches. Samples were briefly vortexed and placed at room temperature for 15 minutes.
2. The tubes were vortexed and the liquid removed by pipette.
3. Punches were resuspended in:
   a. 44 µl Water
   b. 5 µl ZyGEM SKY Buffer
   c. 1 µl prepGEM
4. Tubes were placed in a thermal cycler and heated at 75°C for 15 mins followed by 95°C for 5 mins.
5. Liquid was decanted into new tubes.

Quantification was carried out using a qPCR with primers specific for the human GAPDH gene. The qPCR reagents used were the Invitrogen Platinum® SYBR® Green qPCR SuperMix-UDG with ROX.

Results and Discussion
A schematic of the methods is shown in Figure 1 and an analysis of the methods and yields of DNA are given in Table 1.

A key factor to consider when working with archived samples is that ideally, only a small amount of the archive material should be consumed. As a result, the amount of sample used in the extraction process will be small and therefore extraction should be efficient and secure from extraneous contamination. Security is particularly important for crime samples or rare material.

In these experiments, the method offered by Whatman [2] repeatedly failed to yield measurable quantities of DNA. Furthermore the method is labour-intensive and requires multiple tube-openings that are not recommended for samples requiring rigorous security.

The methanol method [4] gave good yields of DNA but the procedure is protracted and most importantly, requires long periods of air drying where the tube is open to potential contamination. With over 100 minutes spent with the sample exposed to the environment (or at best a PCR hood) it is unlikely that the method is a viable option for laboratories requiring rigorous containment.

The ZyGEM method out-performed all other methods. Overall, the method:
- Takes less time to perform
- Is simpler and less prone to error
- Requires less technical input releasing staff for other tasks
- Is the method most secure from contamination.

While showing these advantages, the ZyGEM methods minimised the likelihood of contamination, provided high yields of non-inhibitory DNA that performs well in polymerase-based reactions such as the PCR. In addition, it is suited for forensic profiling [5].

![Figure 1](Image.png)

**Figure 1.** (Above) Schematic diagram illustrating the stages in the procedures including when human input is required and when the sample is exposed to potential contamination.

**Table 1.** (Left) Analysis of parameters relating to the six methods including DNA yields and concentrations in the extracts. For each parameter, the method showing the best performance is highlighted in red text.

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<tr>
<td>Blood</td>
<td>55</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>&lt;1</td>
<td>37</td>
</tr>
<tr>
<td>Saliva</td>
<td>37</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>&lt;1</td>
<td>50</td>
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For more information visit: [www.zygem.com](http://www.zygem.com) or email: info@zygem.com