Evaluation of Maxwell 16 for Automated Nucleic Acid Extraction from Whole Blood and Formalin-Fixed Paraffin Embedded (FFPE) Tissue

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Background

Nucleic acid extraction is one of the most technically demanding and labor intensive procedures performed in molecular diagnostic laboratories. Manual sample preparation methods are time consuming and susceptible to contamination, handling errors and variation. Automated nucleic acid extractors are successful in extracting clinical specimens due to their efficient recovery, lack of cross contamination and ease of performance. As a new method or technology becomes available, it is ideal to assess and compare it with the existing methods before being applied to clinical use. The objective of this study was to evaluate the performance of Maxwell 16, (Promega, Madison, WI) in extracting nucleic acid from different specimen types and to compare it to a manual (Qiagen, Valencia, CA) and an automated nucleic acid extraction (Biomerieux, Durham, NC) methods.

Specific Aims

- To assess the quantity and purity of extracted nucleic acid from whole blood and formalin-fixed paraffin embedded tissue (FFPE).
- To compare the ability of these methods to yield high quality extracted nucleic acid as assessed by PCR testing.

Materials and Methods

Whole Blood DNA extraction

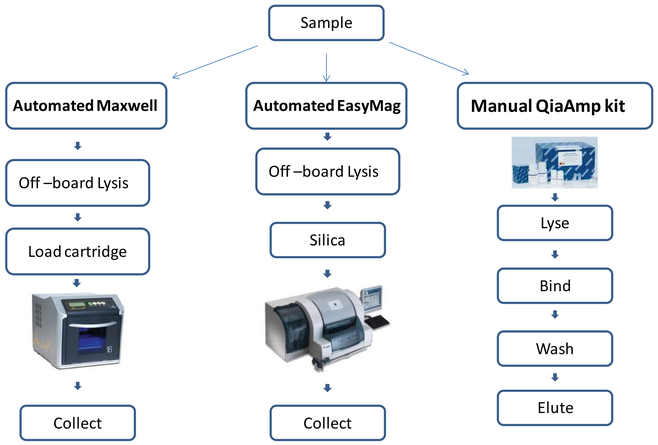


Figure 1: Schematic representation of three different extraction methods employing Maxwell 16 (Promega), EasyMag (Biomerieux) and QiaAmp Blood DNA Kit (Qiagen) for extracting DNA from whole blood sample. **All these methods are silica based.**

Materials and Methods

FFPE tissue DNA and RNA extraction

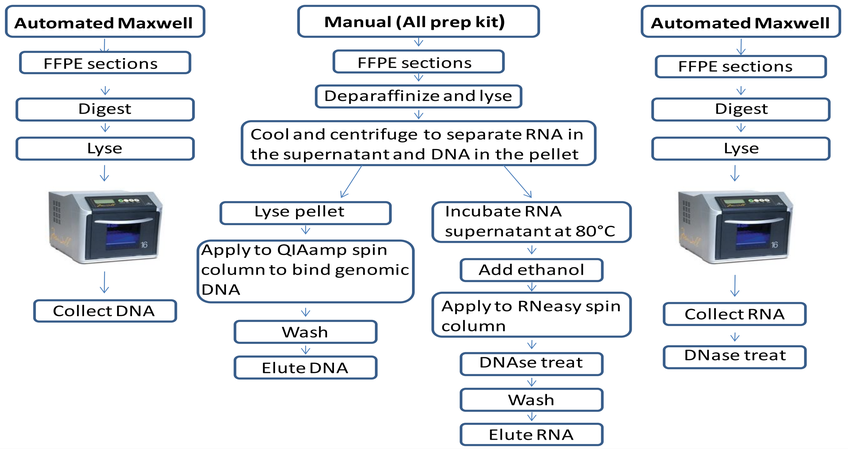
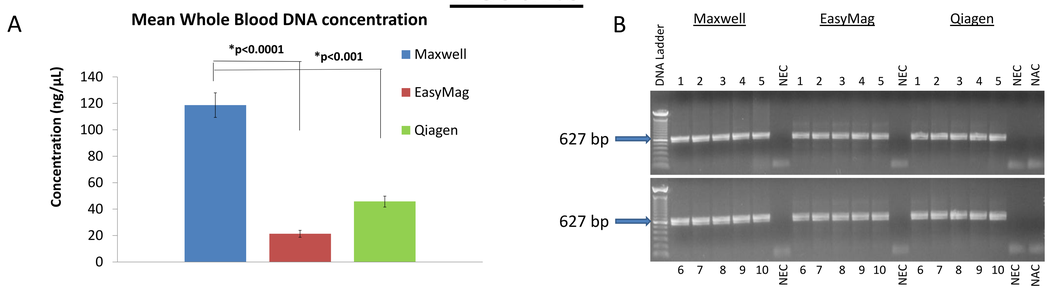


Figure 2: Schematic representation of the two different extraction methods employing Maxwell (Promega) and AllPrep DNA/RNA FFPE kit (Qiagen) for extracting DNA/RNA from FFPE tissue. The starting material for all these methods was two 20µM section of FFPE liver tissue.

Results



- Figure 3: **Quantity and quality assessment of extracted DNA from whole blood samples** A) DNA concentration obtained by Maxwell was significantly greater than either EasyMag (p<*0.0001) or QiaAmp Blood DNA kit, Qiagen (p<*0.001), n=10, for all methods. Mean DNA purity (260/280) ratio was 1.84, 1.58 and 1.84 for Maxwell, EasyMag and QiaAmp Blood DNA kit, respectively. B) β-actin PCR and Agarose gel electrophoresis demonstrate the amplification product of the extracted DNA from all methods. The numbers 1-10 represent 10 different whole blood DNA extracts from three different methods.

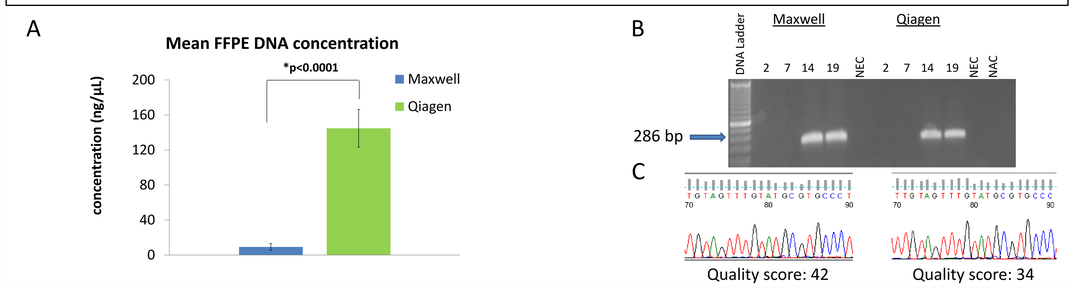


Figure 4: **Quantity and quality assessment of extracted DNA from FFPE tissues.** A) FFPE DNA concentration obtained by AllPrep FFPE DNA/RNA Kit, Qiagen was significantly greater than that by Maxwell (p<*0.0001). FFPE Mean DNA purity (260/280 ratio) was 1.9 and 2.0 for Maxwell and Qiagen, respectively, n=19 for both methods. B) CDKL5 PCR and Agarose gel electrophoresis for exon 3 show amplification of 2/4 extracts from both methods. C) CDKL5 exon 3 Sequencing of the PCR product shown in Figure 4B demonstrates that the extracted DNA from both methods could be used for downstream applications.

Results

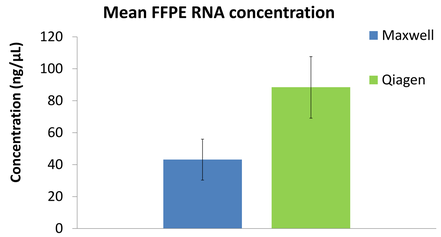


Figure 5: **Quantitative assessment of extracted RNA from FFPE.** No significant difference in the FFPE RNA yield between Maxwell (Promega) and AllPrep FFPE DNA/RNA Kit (Qiagen) was noted (p=0.098), n=19 for both methods. FFPE Mean RNA purity (260/280 ratio) was 1.4 and 2.2 for Maxwell and Qiagen, respectively.

- Maxwell FFPE RNA had no detectable GAPDH in all samples
- All prep FFPE DNA/RNA kit (Qiagen) had detectable GAPDH in 9 of 19 samples
- Of the 9 positive Qiagen FFPE RNA extracts, 5 amplified between 31-35 threshold cycles and 4 amplified between 36-39 threshold cycles

NOTE: Maxwell, did not have a specific FFPE RNA kit at the time of this study. This protocol was designed using the Tissue LEV kit from Promega, along with reagents from the FFPE DNA kit

Conclusions

- Maxwell 16, Promega efficiently extracts DNA from whole blood, is semi-automated and allows for relatively high throughput processing of samples
- **Whole Blood DNA** : Maxwell performs better than either EasyMag or QiaAmp Blood DNA Kit in terms of quantity of DNA extracted. All three extraction methods (Maxwell 16, EasyMag and QiaAmp Blood DNA kit) performed well in terms of amplification and purity
- **FFPE DNA**: Manual method using AllPrep FFPE DNA/RNA kit performs better than Maxwell. However, unsuccessful PCR amplification of 2/4 representative extracts from both methods suggest DNA degradation or inhibitors
- **FFPE RNA**: Both AllPrep FFPE DNA/RNA kit and Maxwell perform similarly in terms of quantity. However, the Maxwell FFPE RNA extraction method requires further optimization due to unsuccessful amplification of RNA extracts

Future studies

- Optimization of the FFPE Maxwell and manual RNA extraction methods
- Expand assessment to other specimens (e.g., fresh-frozen tissue)

References

- AllPrep DNA/RNA FFPE Handbook (80234) and QiaAmp DNA Blood Mini Kit Handbook (51104), Qiagen
- Maxwell 16 LEV Blood DNA Kit technical manual (TM333), FFPE Tissue LEV DNA Purification Kit technical manual (TB382) and Maxwell 16 Tissue LEV Total RNA Purification Kit technical manual (TB367), Promega

Acknowledgements

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