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.

Materials and Methods
Whole Blood DNA extraction

Automated Maxwell

1. Collect DNA
2. Lyse
3. Bind
4. Wash
5. Elute DNA
6. Perform PCR

Manual QiaAmp kit

1. Sample
2. Load cartridge
3. Off-board lysis
4. Silica
5. Collect
6. Wash
7. Elute DNA
8. Perform PCR

Automated EasyMag

1. Collect DNA
2. Lyse
3. Bind
4. Wash
5. Elute DNA
6. Perform PCR

Manual (All prep kit)

1. Collect DNA
2. Lyse
3. DNAse treat
4. Deparaffinize and lyse
5. Incubate RNA supernatant at 80°C
6. Collect RNA
7. DNase treat
8. Collect RNA
9. Elute RNA
10. Perform PCR

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=0.298), 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 10 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 throughout 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 (B0234) and QIAamp DNA Blood Mini Kit Handbook (S1104), Qiagen

Maxwell 16 LEV Blood DNA Kit technical manual (TM333), FFPE Tissue LEV DNA Purification Kit technical manual (TB383) and Maxwell 16 Tissue LEV Total RNA Purification Kit technical manual (TB367), Promega

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