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Opinion Article

Nucleic Acid Extraction from Biological Samples

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ABOUT THE STUDY

Nucleic Acid Amplification Technology (NAAT) based detection is a major intervention for molecular pathogen diagnosis. However, for NAATs such as real-time quantitative Polymerase Chain Reaction (PCR), the target sample often contains a polymerase inhibitor, so the target nucleic acid must be pre-purified or extracted from the sample of interest. Similarly, screening for genetic disorders depends on the successful extraction of pure patient genomic DNA from clinical samples. However, such extraction techniques traditionally use spin column techniques, which require a high speed centralized centrifuge.

This hinders the potential deployment of NAAT methods such as qPCR or PCR in resource-constrained environments. The development of instrument-less nucleic acid extraction methods, especially those using readily available materials, is of great interest and benefit for NAAT-mediated molecular diagnostic workflows in resource-constrained environments. In this report, we examined a commercially available biomaterial, medicated cotton, to extract E. coli genomic DNA (gDNA) spiked with 30%, 45%, and 60% serum. Extraction was performed completely without equipment using absorbent cotton and sterile toothpicks and was completed in 30 minutes (with chaotropic salt) or 10 minutes (without chaotropic salt). The quality of the extracted DNA was then checked using PCR, followed by agarose gel analysis, qPCR, and sequencing. Our method has shown that high quality DNA extraction can be performed both with and without chaotropic salts, although the quality of the extracted DNA varies.

Nucleic Acid Amplification Tests (NAATs) such as qPCR have remained the primary intervention for the detection and containment of a pathogen-mediated disease outbreak and also for screening genetic diseases, food soiling, and bio warfare prevention. The utility of NAATs is, however, dependent on the quality of nucleic acid templates being used, where the presence of DNA polymerase inhibitors may affect its efficacy as well as accuracy. Silica-based spin-column kits, magnetic particles, or automated extraction-amplification systems could be utilized to extract nucleic acid from the sample of interest.

Despite established sensitivity, automated extraction systems remain expensive with high per sample cost, while silica-based spin-column kits require centralized high-speed centrifuges. Commercial magnetic particles, either silica or carboxyl coated, or pH-modulated charge-switching chitosan-based, are costly. Their in-house preparation, although low cost as shown in Bio-On-the Magnetic Beads (BOMB) initiative or through minimal instrument intensive synthesis, still require precursor chemicals (such as methacrylic acid, triethyl orthosilicate, chitosan, Fe (II) and Fe (III) salts, and alkaline solution), instruments (magnetic stirrer-hot plate), and at least 7-24 h of preparation time. Although felicitous for fast and mostly instrument-free nucleic acid extraction, such magnetic particle preparation is difficult to execute in limited-resource settings.

The requirement of instrument-free methods suitable for limited-resource settings has led to the development and innovation of alternative techniques of nucleic acid extraction. This includes the use of filter paper that enable nucleic acid extraction from various matrices as well as a filter paper fitted device for the same, in-house pre parable spin-column using filter papers, and pipette-actuated magnetic beads among others. While these platforms have greatly advanced the innovations necessary for a limited-resource NAATs detection, neither uses readily available materials or equipment for preparing a nucleic acid extraction system. This would especially be of significant importance in a developing country where items such as filter paper for fabricating a spin column are relatively unavailable.

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