

PRODUCTION AND EVALUATION OF THE KIT USING MAGNETIC SILICA COATED NANO-IRON BEADS TO EXTRACT THE NUCLEIC ACID FROM DIFFERENT SAMPLES

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Key words: Magnetic beads, Nucleic acid extraction.

BACKGROUND

PCR technique in Vietnam is being applied in many diagnostic laboratories. However, output quality varies between laboratories mainly due to lack of an automatic option, especially in the nucleic acid extraction step. Hence, PCR work results depend not only on the quality of diagnostic kits but also on the skills of technical staffs. Nucleic acid extraction automation is the key for a more robust PCR work, minimizing human errors and enhance PCR result reliability.

AIMS

Production and evaluation of the kit using magnetic silica coated nano-iron beads to extract the nucleic acid from different samples for detecting pathogens using PCR and real-time PCR techniques.

MATERIALS – METHODS

In this study, we designed and manufactured various components for extraction kit, named ^{NK}DNARNAprep MAGBEAD, implementing the KingFisher automatic nucleic acid extraction open system (KingFisher, KingFisher ML, KingFisher Duo, KingFisher Flex). These components include: magnetic silica coated nano iron beads, extraction buffers and binding buffers, washing buffers and elution buffers. We also optimized different extraction protocols for both DNA and RNA from different sample types, such as serum, plasma, autopsy, shrimp post, shrimp tissue, clam tissue, etc. We validated the quality of these components by direct comparison on the same samples with other commercially available extraction kit such as BOOM (for DNA extraction), Trizol-LS (for RNA extraction), alkaline heat-shock extraction (for DNA extraction from crustacean / seafood). Additionally, we also compared DNA / RNA extraction from human pathogens (HBV, HCV and MTB) using automatic extraction versus MagnaPure (Roche).

RESULTS - DISCUSSIONS

The results suggest that our automatic extraction kit (namely ^{NK}DNARNAprep-MAGBEAD): (1) give the same quality / quantity of extracted nucleic acids as MagnaPure (Roche) for DNA/RNA extraction from human pathogens (HBV, HCV, MTB); (2) can be used in the same DNA/RNA extraction five-step protocol for a maximum duration of 45 minutes; (3) is at least 10-times more sensitive than traditional in-house extraction methods; (4) is simplified into two different protocols for fluid and tissue samples; (5) has a 6-month shelf-life at room temperature.

Table 1:

Summary the different samples, the reference extraction method as well as the method of evaluation for the target pathogens and the results of the sensitivity

Samples	Target	Ref. extraction method	Method of evaluation	Results
Sera, plasma	HBV, HCV	MagnaPure (Roche); BOOM (HBV); Trizol-LS (HCV)	Real-time PCR	Equivalent MagnaPure 10x more sensitive than BOOM, Trizol LS
Sputa	MTB	MagnaPure; BOOM	Real-time PCR	Equivalent MagnaPure and BOOM
Shrimp post larvae	WSSV, MBV	Alkaline lysis	PCR	10x more sensitive than alkaline lysis
Genital swab	HPV	Phenol-Chloroform	PCR	10x more sensitive than Phenol-Chloro
Whole blood	β-thalassemia, α-thalassemia	QIAGEN kit	PCR, MLPA	Equivalent QIAGEN kit
Amniotic fluid	Aneuploidy	QIAGEN kit	Aneufast	Equivalent QIAGEN kit
Hair root, nail, dry blood	Paternity	QIAGEN kit	Human identifier	Equivalent QIAGEN kit

CONCLUSION

The newly designed and manufactured automatic DNA/RNA extraction kit powered by KingFisher open extraction system is the key for diagnostic quality that can be robustly applied by clinical diagnostic laboratories to fully utilize PCR techniques without having to implement more expensive closed PCR system. Therefore, a solution for current technical errors in diagnosis is guaranteed

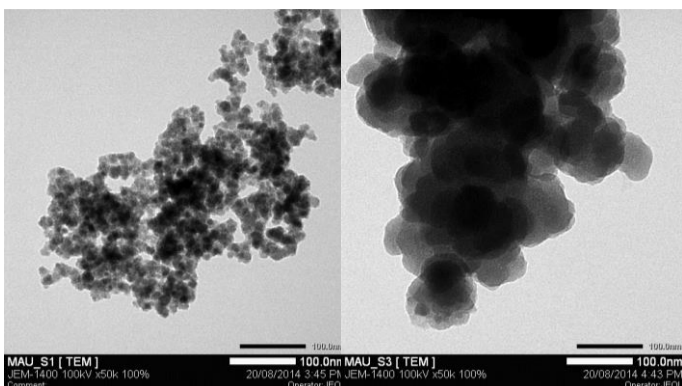


Photo 1: The TEM photo of the nano-iron bead coated with silica (A) MAGBEAD (home-made) versus (B) MAGSI (commercial)



Photo 2: The ^{NK}DNARNAprep MAGBEAD includes magnetic silica coated nano iron beads, extraction buffers, binding buffers, washing buffers and elution buffers.



Photo 3: The ^{NK}DNARNAprep MAGBEAD kit was used for DNA or RNA extraction of different clinical samples and the automatic extraction machine are KingFISHER flex (top), KingFISHER ML (lower left), and KingFISHER Duo (lower right)

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