

Magnetic Nano Particles For Automated PCR Diagnostics

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ABSTRACT

We have developed a magnetic nano particles which can be used for diagnostic . The magnetic nano particles need to be coated with nucleic acid compatible materials allowing the adsorption of DNA , RNA onto it .

Keywords : magnetic nano particles , nucleic acids compatible coating, PCR, automated PCR technique

INTRODUCTION

Magnetic Nano Particles (MNP) capable of extracting nucleic acids

In the diagnostic process using PCR or RT-PCR techniques [1], it is necessary to detect as precisely as possible the category as well as the quantity of virus and bacteria surviving in the patient body . The only way to do that is the effective process of extracting nucleic acids such as DNA or RNA contained in the virus and bacteria . Among the diagnostic techniques using, BOOM process [2] is very well known in terms of multiple capability of detection. This process was discovered by Dr. Rene

Boom [3] at The Medical Academic Institute of Amsterdam University . According to this process, the extraction principle is based on the adsorption of nucleic acids onto the surface of certain kinds of intermediate then the desorption off that surface by some kinds of driving force . The adsorption can be accelerated by a physisorption or chemisorption which causes an adhesion force between nucleic acids and the adhered substrate . The adsorbed nucleic acids

can be desorbed out of the substrate in a chaotropic environment. BOOM process is actually a simple and effective process to isolate the nucleic acids but it does not reach the level of fully automated testing procedure as it must use many step of centrifugation in the nucleic acid isolation step . Many efforts had been made to improve BOOM process for the automated testing target to simplify nucleic acids extraction and isolation , which end up with several products which have been introduced into the medical diagnostic market such as Promega, Quiagen, Viogene [4] but they are still not satisfied for automation diagnostic purposes .

In the present study, we approached magnetic nano particles coated with nucleic acid compatible materials offering much more simple purification effect associated with a magnetic plate . The magnetic plate is used to collect nucleic acids absorbed onto the surface of magnetic nano particles . The magnetic nano particles can be reused many times simply by a combination of pure water wash and magnetic field from magnetic plate . This process exhibits several advantages over the conventional process such as faster, more simple and capable of automatic control and operation . This process can be used in any testing equipments using BOOM principle . So, the magnetic nano particles actually open up a new capability of automated extracting and desorption of nucleic acids and unveils a new ways of automated PCR technique for diagnostic .

In the present study, magnetic nano particles can be selected from a wide range of magnetic materials including ferrites, Co oxide, Ni oxide and even carbon nano tube . First of all, these materials have to form a stable nano format in water or organic solvents where the nucleic acid

compatible materials will be coated . This material is an proprietary products of SHTP LABS and can not be disclosed here yet . However, the precursor needs to be soluble in water or solvent . Then the reaction occurs to form thin film on the surface of the magnetic nano particles . The excess part of the coating materials which does not take part in the coating process is removed by magnetic field . The amount of the coating can be well controlled by reaction temperature which is varied between 45C and 60C . The thick coating film tends to reduce the extraction effect

The purification process can be done just by magnetic field using a high power magnet

PCR TEST PROCEDURE AND RESULTS

DNA Extraction Test

In order to perform the DNA extraction test, several different kinds of home-made magnetic nano particles were mixed with standard DNA solution in a nucleic acid extraction buffer (NAEB). Next, the free DNA molecules were removed from the mixture by washing with Extraction Wash Away Buffer (EWAB) . Finally , the collected DNA by the magnetic nano particles was isolated with highly pure water (miliQ) . The optical density(OD) of DNA is measured at 260nm wavelength by UVVIS spectrophotometer. The OD of the impurities which is protein contamination in DNA is detected at 280nm wavelength . Table 1 shows the DNA extraction results from a number of different magnetic nano particle . It should be noted that any samples showing OD(260nm)/OD280nm) ratio greater than 1.7 should be considered as cleaned and the purification ability of the magnetic nano particles is also higher. According to this table, the magnetic nano particle ID 200428-7,-8,-9, -11 exhibit excellent DNA purification effect

Table 1.

| Magnetic nano particle sample ID | Detected DNA concentration (Extracted DNA concentration) | OD 260nm /OD280 nm) |
|----------------------------------|--|---------------------|
| 200428-95-5 | 4.8 (9.6ug/ml) | 1.672 |
| 200428-95-7 | 4.9 (9.8ug/ml) | 1.707 |
| 200428-95-8 | 12.8 (25.6ug/ml) | 1.766 |
| 200428-95-9 | 18.0 (36.0ug/ml) | 1.722 |
| 200428-95-10 | 44.4 (88.8ug/ml) | 1.699 |
| 200428-95-11 | 22.3 (44.6ug/ml) | 1.746 |

Nucleic Acid Extraction from Patient Blood

Next, we selected the patient blood containing Hepathetis B (HBV-DNA) and C (HCV-RNA) which had been tested and confirmed to test out the diagnostic capability of the magnetic nano particles in the present study . The same magnetic nano particles which had been used in the DNA extraction test above described were mixed with patient blood and DNA or RNA existing in the blood cell, have been isolated with the same process above mentioned for DNA extraction test . The number of extracted DNA or RNA was magnified by real time PCR as described in Fig. 1

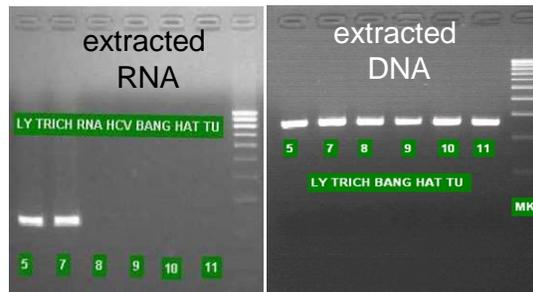


Fig. 1 Effect of magnetic nano particles on the extraction of DNA and RNA

The extraction results are shown on Fig 2 in which one can recognize that all of the magnetic nano particle ID 200428-95-5, -7, -8, -9, -10, -11 can extract DNA while only two the magnetic nano particle ID -5 and -7 can extract RNA . It should be noted that DNA exists in Hepathetis B and RNA exist in Hepathetis C

Table 2 Quantitative results of PCR test of HBV-DNA detection

| Magnetic nano particle ID | The amount of DNA detected in 10ul extraction liquid when used HBV-TQPCR mix | The amount of HBV-DNA detected in 1ml blood cell |
|---------------------------|--|--|
| 200428-95-5 | 4.33 x 10 ⁴ | 4.33 x 10 ⁶ |
| 200428-95-7 | 8.24 x 10 ³ | 8.24 x 10 ⁵ |
| 200428-95-8 | 2.72 x 10 ⁴ | 2.72 x 10 ⁶ |
| 200428-95-9 | 6.12 x 10 ⁴ | 6.12 x 10 ⁶ |
| 200428-95-10 | 5.30 x 10 ⁴ | 5.30 x 10 ⁶ |
| 200428-95-11 | 1.51 x 10 ⁴ | 1.51 x 10 ⁶ |

It should be noted that HBV-TQPCR is the HBV detection test with real time PCR using Taqman probe(TQ=tagman)

Table3 Test result with HCV-RNA

| Blood sample ID | Total amount of HCV-RNA confirmed in advance in blood sample | Total amount of HCV-RNA detected by two magnetic nano particles ID 200428-95-5 and -6 | |
|-----------------|--|---|----------------------------|
| | | 200428-95-5 | 200428-95-7 |
| 23039 | 3.33 x 10 ³ /ml | 2.06 x 10 ³ /ml | 3.06 x 10 ³ /ml |
| 23044 | 2.08 x 10 ³ /ml | 8.85 x 10 ³ /ml | 7.46 x 10 ³ /ml |
| 23045 | 1.01 x 10 ⁴ /ml | 1.15 x 10 ³ /ml | 1.45 x 10 ⁴ /ml |
| 23046 | 1.03 x 10 ⁴ /ml | 8.26 x 10 ³ /ml | 1.30 x 10 ⁴ /ml |
| 23049 | 1.06 x 10 ³ /ml | 1.20 x 10 ³ /ml | 2.80 x 10 ³ /ml |

CONCLUSION

Magnetic nano particles coated with nucleic acid compatible agent has successfully tested more than 157 blood samples, shown significant improvement in diagnostic technique in terms of better efficiency, more reliable, faster output results . The technique looks promising for full automation PCR

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