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Article

Comparison of DNA Concentration and Purity of Animal Blood Extracted Using Different DNA Extraction Kits

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***Abstract*—The efficiency of DNA extraction from whole blood using appropriate method is very important for molecular analysis. Therefore, the aim of this study was to compare the purity and concentration of DNA extraction method from bovine (Bos taurus), chicken (Gallus gallus), and porcine (Sus scrofa) blood. The DNA of blood samples was extracted using three types of kit, namely InnuPREP Blood DNA Mini Kit, Wizard Genomic DNA Purification Kit, and QIAamp DNA Blood Mini Kit. The results showed that blood DNA extracted using QIAamp DNA Blood Mini Kit was found to be the most effective and consistently produced high concentrated and pure DNA for three animal samples. The purity of DNA ranged from 1.73 ± 0.05 Å to 1.94 ± 0.21 Å and the range of blood DNA concentration extracted using the QIAamp DNA were between 13.73 ± 2.11 and 25.01 ± 2.08 ng/µl. However, the blood DNA of porcine was not successfully extracted using InnuPREP Blood DNA Mini Kit and Wizard® Genomic DNA Purification Kit. These results were very crucial for the subsequent use of amplification using polymerase chain reaction (PCR) and to facilitate accurate detection in further analysis.**

***Keywords*— DNA extraction methods; blood DNA; Animal blood**

I. INTRODUCTION

Blood have been widely used in human culture, especially in the Western region in manufacturing blood sausage, blood pudding, and many exotic cuisines [1]. Indeed, the application of blood and its derivatives in food industry provide many benefits due to its functionality to food products such as can increase protein level, enhance water binding capacity, and as emulsifying capacity to enhance the properties of foods [2]. Furthermore, blood and its derivatives can be easily obtained from slaughtered animals and can be used in food products as stabilizers, clarifiers, as well as provide unique nutritional value [3].

Although the blood has been highly processed to be used in food industry as a food additive, it is impermissible in Islam because the transformation of blood to another derivative of physical or chemical process mean was considered as "istiḥālah fāsidah" [4]. The application of blood in food products is impermissible for Muslims and other religion to consume as stated in Al-Quran verse in Sūrat al-Baqarah verse 173; Sūrat Al-Ma'idah verse 3; Sūrat Al-An'am verse 145; and Sūrat An-Nahl verse 115. According to Islamic perspective, blood is prohibited to be consumed by the Muslims as it is considered to be filthy and harmful. Scientific evidence also proved that the application of whole blood in the food industry is restricted due to the cellular fraction is thought to have a higher microbial load [5].

It is crucial for Muslims to be aware and for researchers to find the best method to detect and prove the presence blood and its derivatives in food products. In fact, detection of the blood using polymerase chain reaction or PCR assays is proven to be specific and sensitive for amplification of genetic sequences [6]. However, extraction of DNA from blood samples is the main important preliminary step for PCR assay. A good quality and pure genomic DNA from blood samples play an important role [7] for successful of PCR amplification with high sensitivity and efficiency. Thus, the aim of this study was to compare the purity and concentration of genomic DNA extracted from bovine (*Bos taurus*), chicken (*Gallus gallus*), and porcine (*Sus scrofa*) blood using three types of kit which were InnuPREP Blood DNA Mini Kit, Wizard® Genomic DNA Purification Kit, and QIAamp DNA Blood Mini Kit.

II. MATERIALS AND METHODS

A. Blood Samples

Bovine blood samples were collected at an abattoir in Senawang, chicken blood samples were collected at abattoir in Pedas, and porcine blood samples were collected at an abattoir in Kempas, Johor. All animal samples were inspected and qualified by veterinarians to accomplish the

health requirement standard before proceeding to slaughtering stage. This study did not involve endangered or protected species. Fresh blood was collected in Falcon tube containing 6% ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and was kept on ice (4°C) during transportation and stored at -20°C before proceeding with the analysis.

B. DNA Extraction

The blood samples were prepared for DNA extraction by thawing the samples at room temperature before being injected inside microcentrifuge tubes. The total genomic DNAs of blood samples were extracted using InnuPREP Blood DNA Mini Kit (Analytik Jena, Germany), Wizard® Genomic DNA Purification Kit (Promega, USA), and QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. All methods and extraction kits tested are following the extraction protocols recommended by manufacturers without modification. The CTAB method is following the Shams et al. 2011[8] with modification.

C. DNA Purity and Concentration

The purity and concentration of genomic DNA were determined using spectrophotometric analysis (Implen® Nanophotometer P330, Germany) with Lid 10 setting with a volume of 1 µL genomic DNA by measuring absorbance (Å) at Å260 and Å280. The purity measurement of extracted DNA was determined based on the ratio of Å260/ Å280, and value of ratio between 1.8 and 2.0 indicates that the absorption in the UV range was due to nucleic acids. However, if the ratio is lower than 1.8, it indicates the presence of proteins or other UV absorbers.

III. RESULTS AND DISCUSSION

Table 1 shows the data of purity and concentration of genomic DNA of bovine, chicken, and porcine. Results found that the highest purity of genomic blood DNA was extracted using QIAamp DNA Blood Mini Kit, which the value was 1.73±0.05, 1.94±0.21, and 1.78±0.34 for bovine, chicken, and porcine, respectively. However, when the genomic DNA of blood was extracted using InnuPREP Blood DNA Mini Kit and Wizard® Genomic DNA Purification Kit, the value was between 0.33 and 0.84. The genomic DNA of porcine blood was not successfully extracted using InnuPREP Blood DNA Mini Kit. All DNA extraction was done in triplicates for each blood in order to control the validity of the results obtain and to rule out experimental bias or some random error.

The concentration of genomic DNA was also good when it was extracted using QIAamp DNA Blood Mini Kit with the ranged between 13.73±2.11 and 25.01±2.08 ng/µl. The concentration of genomic DNA extracted using InnuPREP Blood DNA Mini Kit were 24.96±3.18 ng/µl, and 20.44±2.52 ng/µl for bovine and chicken blood, respectively. However, when the blood samples were extracted using Wizard® Genomic DNA Purification Kit, the concentration of genomic DNA was too low compared to other DNA extraction kits.

Indeed, blood samples are very complex and consist of high levels of proteins such as blood plasma, and hemoglobin which could be strong inhibitors of PCR. Therefore, different protocols of DNA extraction have to be tested to prepare total DNA free from PCR inhibitors [9]. Results found that concentration of genomic DNA extracted using InnuPREP Blood DNA Mini Kit was better than using Wizard® Genomic DNA Purification Kit. The procedure of InnuPREP Blood DNA Mini Kit utilizes spin column-based nucleic acid extraction with silica-based membrane, whereas Wizard® Genomic DNA Purification Kit use a four step process based on principle of salt precipitation of DNA.

QIAamp DNA Blood Mini Kit showed the best performance of extracted genomic DNA compared to other DNA extraction kits. This DNA extraction kit was found the most effective and consistently extracted highly concentrated and pure DNA in all three animal samples. Furthermore, genomic DNA of *Mycobacterium bovis* in bovine tissue obtained the best performance when was extracted using QIAamp DNA Blood Mini Kit, followed by RBC and FTA Elute Micro Card which suggested that the cross-contamination was not observed in the extraction of DNA in the tests performed with this kit [10]. Previously, blood plasma has been successfully extracted from seventeen surimi-based products using this kit [11].

The genomic DNA of porcine was not successfully extracted using InnuPREP Blood DNA Mini Kit and Wizard® Genomic DNA Purification Kit. Indeed, porcine blood by-products are characterized by wide variation in crude protein contents [12]. The color of porcine blood was also the darkest among other types of blood and appear to have more viscosity and suggested to have more component of protein or PCR inhibitors compared to bovine and chicken blood. The spin column of InnuPREP Blood DNA Mini Kit was also clogged with porcine blood when compared with two other blood types.

TABLE 1
THE PURITY AND CONCENTRATION OF GENOMIC DNAs OF BOVINE, CHICKEN, PORCINE EXTRACTED FROM THREE DIFFERENT TYPES OF KITS

Blood Samples	**InnuPREP Blood DNA Mini Kit**	
	Purity of DNA	**Concentration of genomic DNA (ng/µl)**
Bovine	0.83±0.12	24.96±3.18
Chicken	0.67±0.03	20.44±2.52
Porcine	NS	NS
Blood Samples	**QIAamp DNA Blood Mini Kit**	
	Purity of DNA	**Concentration of genomic DNA (ng/µl)**
Bovine	1.73±0.05	25.01±2.08
Chicken	1.94±0.21	18.12±12.76
Porcine	1.78±0.34	13.73±2.11
Blood Samples	**Wizard® Genomic DNA Purification Kit**	
	Purity of DNA	**Concentration of genomic DNA (ng/µl)**
Bovine	0.33±0.51	Too low
Chicken	0.84±0.65	Too low
Porcine	0.33±0.21	<2.0

Notes: Conc. = concentration; NS = Not successful

IV. CONCLUSIONS

In summary, DNA extraction from blood is influenced using different extraction method. Results suggested that genomic DNA extraction from different types of blood were greatly influenced by procedures in the kit due to some require no columns or the like in the centrifugation steps. This is crucial for the preliminary step before amplifying by PCR assay.

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