Evaluation of Potential DNA Sources and Need of DNA Analysis for Personalized Medicine in Microfluidic Device

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Abstract

DNA analysis is one of primary requirement in genetic studies for disease diagnosis, treatment and precaution steps as the characterization of many diseases are linked with genes. Particularly, genetic testing is vital to detect heritable disease or developing infectious disease, and newborn screening to identify genetic disorder that can be treated at early stage. Fundamentally the raw source of DNA such as blood, saliva, tissue section and urine consist of complex matrices with heterogeneous contents caused traditional bio-analytical methods difficult to perform plus time consuming analysis. Thus, DNA extraction from most appropriate biological sample for downstream process to replace conventional method has been widely studied due to its crucial role in clinical diagnostic and genetic analysis. Meanwhile, the growing technology has recognized the importance of microfluidic platform as healthcare point of device especially in addressing the issue needs for modern biological rapid synthesis and analysis by using only small amount of sample and reagent volumes. This paper highlighted the potential DNA sources that can be used for DNA analysis in microfluidic point of care device.

Keywords: *DNA source, DNA extraction, DNA analysis, personalized medicine, microfluidic device.*

1. Introduction

Deoxyribonucleic acid (DNA) is double helix structured molecule that contains biological information which passed from one generation to the next during reproduction process. DNA was discovered by Swiss biochemist, Frederich Miescher in the late 1800s. However, the realization of the DNA importance became significant when double helix stranded DNA was noticed by studying X-ray diffraction patterns and building models in 1953 by James Watson, Francis Crick, Maurie Wilkins and Rosalind Franklin (Langridge et al., 1957; Meselson and Stahl, 1958). The first molecular structure of DNA was proposed by James Watson and Francis Crick, and altered by Maurie Wilkins and Rosalind Franklin with remains its main features. DNA is present in the nucleus of every cell in the body. The structure composed of two polynucleotide chains bound helically. It made up of nucleotides like a building blocks structure in three parts: a phosphate group, a sugar and four types of nitrogen bases, which includes adenine (A), thymine (T), guanine

(G), and cytosine (C). Typically, nucleotides linked to the phosphate and sugar groups of chains alternatively to form a DNA strand while the nitrogen bases determine biological information (e.g. eyes color) of the individual in the DNA strand. It also acts as instructor to produces complex molecules of proteins called messenger ribonucleic acid (mRNA) to do most of the works in our bodies (Langridge et al., 1957). DNA has unique ability to duplicate itself during cell division. During cell division, the double stranded helix splits turns into two single strands as template to synthesis its compliment or copies of two double-stranded DNA as shown in Figure 1.

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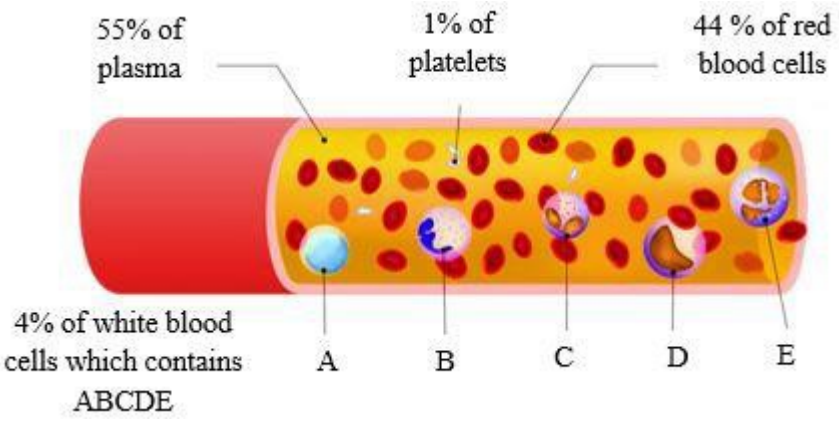

Figure 1. Mechanism of DNA replication based on Watson-Crick Model. Molecules of every generation contain one of the original parents (dark) molecules and paired with one new molecule (white)

Each DNA sequence makes a protein known as gene and it is important for growth and development of an individual. The application of DNA analysis in clinical research and medicine has been using widely to identify several genetic related diseases, developing diseases and for pharmacogenomics studies. Typically, DNA can be extracted from blood, salivary fluid, tissue cells and urine. Each sample requires different methods to extract high quality of DNA. DNA analysis also known as genetic analysis has expended enormously to provide rapid, accurate and cost-effective diagnostic information with minimal amount of sample requirements. The main purpose of DNA testing is to identify and confirm diseases related to genes for further treatments. Meanwhile, it also can provide predictive implication of certain clinical status. The genetic makeup of every person is slightly different, and every biological or immune response is eventually guided by the genes possessed by the individual. Thus, genetic analysis will prevent development of disease by early detection where treatment can be applied successfully. Adverse drug reaction also can be reduced by improving further treatment options.

1.1. Raw Sources of DNA

Profoundly, a microliter of biological sample amount is sufficient for DNA analysis in microfluidic platform to monitor diseases and infections. Biological samples are

mixture of heterogeneous cell populations; thus, it requires multiple steps to extract uncontaminated DNA. Moreover, each sample involves different procedures, reagents, handling steps and conditions. Generally, sources for DNA extraction are samples consist of complex matrices such as blood, saliva, tissue section and urine. However, the most suitable DNA sources especially for microfluidic research are blood and saliva as DNA extraction method using these samples is relatively functioning and the presence of exact DNA cells are quite high.

The contents of each DNA source vary the screening procedures thus affect the quality of the yield for further process. A dime-sized spot of *blood* is one of the superb sources of human DNA. Blood composed of 55% of plasma which composed of water, ions, plasma, and proteins along with 45% of cellular components including erythrocytes, leucocytes and platelets (Figure 2). Erythrocytes, also known as red blood cells (RBCs), are cells that does not have nucleus, thin in structure, biconcave disk shaped with 6-8µm in diameter. The red colour of blood is from haemoglobin that present in RBCs and the haemoglobin group contains iron atoms used to bind oxygen molecules. Thus, RBCs are primarily responsible for gaseous exchange and transport throughout body. Leucocytes also known as white blood cells (WBCs) are colourless and contain nucleus. It is worth mentioning that genomic DNA can be extracted from nucleus of WBCs. They are made by stem cells in the bone marrow with larger in size than RBCs, typically 8-20µm in diameter but irregular in shape. The core purpose of WBCs is to fight infections, pathogens and diseases. Platelets are another part of cellular elements, with no nucleus which usually 2-3µm in diameter and important in blood clotting mechanism. There is approximately one white blood cell for every 1000 red blood cells in a healthy person (Calder et al., 1939). Blood source naturally contains 4.5 to 11 × 10^5 white blood cells thus yielding 10-18 µg/ml of high-quality genomic DNA without contamination with foreign DNA (Ng et al., 2006; Sun and Reichenberger, 2014). Hence, the mean amplifiable human DNA percentage in blood samples is approximately 87.6% (Hu et al., 2012).

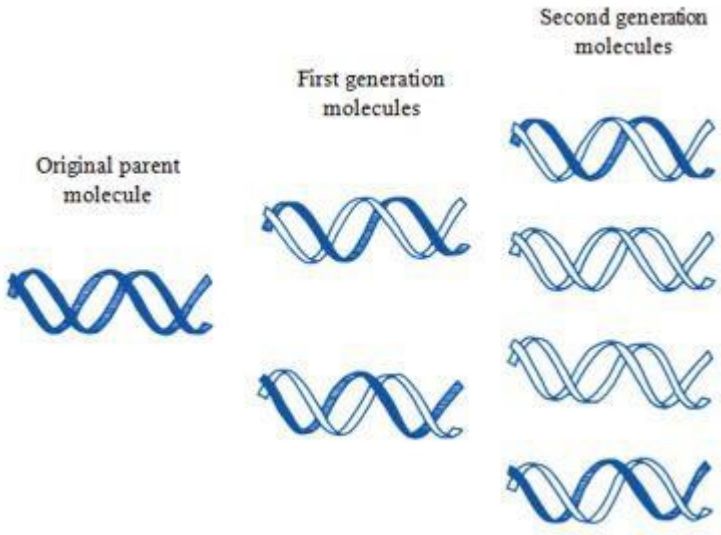

Figure 2. Proportion of plasma and cellular components in blood. A: lymphocyte, B: basophil, C: eosinophil, D: monocyte, E: neutrophil

There are abundance of studies and commercial kits available for efficient extraction of DNA in smaller scale compared to conventional method. Conventional method requires large equipment such as incubator, centrifuge and others. Typically 10 ml BD Vacutainer® Tubes (BD Biosciences, San Jose, CA) has been used to collect blood sample from patients for fast extraction method and then followed by DNA isolation with commercially available solutions such as the Gentra Puregene Blood Kit (Qiagen, Valencia, CA), DNA Isolation Kit for Mammalian Blood (Roche, Nutley, NJ) or NucleoSpin Blood XL (Clontech, Mountain View, CA) (Sun and Reichenberger, 2014). On top of this, inexpensive high-salt extraction methods or traditional phenol-chloroform isoamyl alcohol extraction also has been widely studied in laboratory especially for genomic research or testing (Quinque et al., 2006; Loparez et al., 1991). Although yield of DNA extracted from blood is quite high typically 10 to 15 g/mL, however invasive procedure and specific training in phlebotomy are needed to collect blood sample as most blood samples were obtained by venepuncture by trained staff.

Alternatively, numerous studies have investigated different sources of DNA such as saliva, dried blood spots, plucked hairs and cheek swabs. Among all, *salivary fluid* is another most focused non-invasive substitute source of DNA (Quinque et al., 2006). It is composed of 99% mixture of water with electrolytes including sodium, potassium, calcium chloride, magnesium bicarbonate, phosphate and 1% of proteins with leukocytes cells (Figure 3). A healthy person produces 1 to 1.5 L of saliva per day. Each fluid in the body has its specific function. In the same vein, saliva lubricates, protects the oral tissues from irritations and dehydration. Saliva also protects the mouth from pathogens by controlling environmental conditions for instance, neutralizes the acids produces by acidogenic microorganisms and prevents enamel demineralization (De Almeida et al., 2008). Epithelial cells or leukocytes cells are typically found in saliva which contains DNA. There are approximately 4.3×10^5 cells including either epithelial cells or leukocytes per millilitre in saliva (Dawes, 2003; Looi et al., 2012).

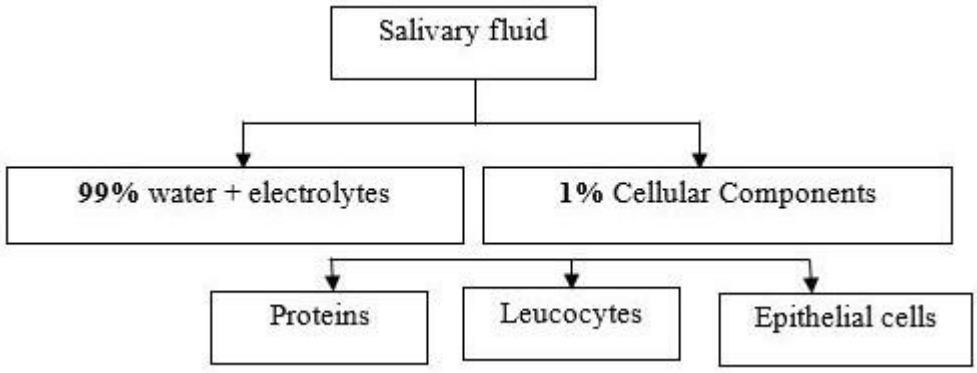

Figure 3. Salivary fluid components

The number of leukocytes with DNA is likely to fluctuate significantly depending on health condition of the donor. Moreover, the quality or yield of DNA from salivary fluid may be lower compared to blood sample as it may contains foreign DNA (bacteria cells and potentially food remnants). There is few precaution steps taken into account to collect fresh samples to generate higher yield of DNA for instance, the donor should not eat, drink, smoke or chew gum for at least 30 minutes before donating saliva and the donor should rinse their mouth with water for 15 seconds to remove any remaining food particles. Unlike blood sample, saliva can be obtained easily in non-invasive method without requiring professional training. It also reduces the pathogen transmission and infection that is common for blood driven circumstances. In addition, saliva also contains numerous ubiquitous species of bacteria that are not infectious and can be used as control organisms for disease diagnostic. In terms of sample collection and storage, there are few considerations need to consider including cost and duration of storage. Based on several studies, quality of DNA sample attested high even though after 30 days of storage at room temperature (Quinque et al., 2006) and almost 50 days at 30°C (Ng et al., 2006). Whereas, collected blood sample in vacutainer tubes must be refrigerated and the extraction must be carried out within a week of collection for better result. The yields of full-length DNA that are stored more than 2 weeks and shipped at ambient temperature from tropical countries tend to decrease by 80%. However, blood sample can be stabilized with reagent during shipment that require another additional handling step to dilute blood 4:1 (blood: stabilizer) before storage. Unlike blood, an appropriate preservative found readily in saliva allows it to be stable at room temperature for longer storage period (Sun and Reichenberger, 2014).

Typically, further analysis of DNA only requires 1µg of DNA. Based on numerous studies, saliva sample passed quality control call rate threshold and further analysis was a success. Bahlo et al. (2010) Abraham et al. (2012) and Hu et al. (2012) stated in their work that the call rate and genotype concordance for saliva were up to 96% to 97 %. Several studies have emphasized the usage of salivary fluid to extract DNA for further medical applications (Rylander-Rudqvist et al., 2006; Hansen et al., 2007). Thus far, those studies reported that the total DNA yield were lower from saliva (around 5g/mL) than from blood sampling. Garcia-Closas et al. (2001) compared the DNA yield from various sources and found that only 50% of the DNA is of human origin from saliva as experimented by hybridization to the D17Z1 probe. In a valuation of real time PCR for the human prothrombin gene, 68% is found to be human DNA by Rylander-Rudqvist et al. (2006) and 77% by Nishita et al. (2009). The average number of bacteria, fungi or food remnants in the oral cavity that enclosed in total amount of DNA from saliva was estimated to be 1.7×10^7/mL (Dawes, 2003). Even though DNA from saliva has various advantages including lower cost, lower infectious risk and non-invasive DNA extraction, but the contamination of bacteria cells in saliva reduces DNA yield. The typical commercial kit for DNA isolation from saliva sample is by using Oragene DNA collection kits (DNA Genotek, Kanata, Ontario, Canada). According to few recent studies, salivary fluid collection by commercially available kits is comparatively non-invasive technique for genomic studies with sufficient amount DNA as well as desired quality. It is significant to extract higher purity DNA for further process without any relative errors. As mentioned by Abraham et al. (2012) and Bahlo et al. (2010), the

extracted DNA from saliva can be used for genotyping and simple PCR reaction for clinical research.

1.2. DNA Testing for Healthcare Analysis

DNA testing can be termed depending on the situation as genetic testing, carrier testing, pharmacogenomics testing, diagnostic testing, new-born screening, prenatal testing, pre-implantation testing, predictive and pre-symptomatic testing, and forensic testing. This DNA testing is entirely based on person's DNA to provide health information.

Genetic and carrier testing is used to identify gene mutation that causes genetic disorder or inherited disease. It offers information about cause of disease in family and clarifies suspected inherited disease by identifying specific family member who are at risk of developing inherited disease. Accordingly, this test also provides appropriate planning on diagnosis as an early preventive treatment. For instance, a member of family suffers from Alzheimer disease, heart disease, diabetes and cancer, the risk of developing this disease to the next generation of the family is higher than general population due to gene mutation in DNA. By an appropriate DNA testing, the inherited disease can be identified for early prevention or treatment. Similarly, predictive or pre-symptomatic testing carried out to detect gene mutation that could lead to particular diseases. It is based on early symptoms of the patient's condition. However, the test also can predict the development of genetic disorder such as hereditary hemochromatosis even before any early symptoms. Besides another explanation for DNA testing is diagnostic testing which carried out to identify and confirm specific genetic for disease when particular conditions are suspected based on sign and symptoms. In most cases, diagnostic testing is carried after several stages of diseases infested (Cirini and Ho, 2013). Most of chronic diseases and infections are possibly detected through DNA testing of individual. Among Alzheimer, asthma, cancer, diabetes, skin related disease, rheumatoid arthritis and others non-communicable disease, drug hypersensitivity reactions are one of major clinical concern. Thus, pharmacogenomics testing would be very helpful before prescription of any medicine to patient as it is combined study of the science of drug and functions of gene accordingly. It provides information about suitable dosage of any drug or medicine for every individual based on their DNA testing particularly to evade from dangerous side effects or infections.

There is a large volume of published studies describing about the drug hypersensitivity reactions which affected numerous hospitalized patients who have been exposed to certain drugs are considered a life-threatening concern as severe adverse cutaneous drug reaction (cADR). ADR is increasingly recognized as a serious, worldwide public health concern. Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are most significant potent cutaneous eruptions (Hussein and Craven, 2005; Baldo and Pham, 2013). They share ordinary clinical characteristics and together account for the majority of drug-induced skin eruptions. SJS and TEN are potentially fatal, severe, rare, adverse cutaneous drug reactions involving both the skin and mucous membranes. The issue of SJS has received considerable critical attention as it related to thick epidermal necrosis with lesser

indifference of the cutaneous surface and involvement of mucous membrane. Besides, TEN is an acute dermatologic disease which described by widespread erythematous macules and targeted lesions associated thick epidermal necrosis with more than 30% of cutaneous surface (Lim et al., 2008). A common anticonvulsant drug (CBZ) used to treat seizures, bipolar disorder and other chronic neuropathic pain; allopurinol which is a usual prescription for gout and hyperuricemia has been highly associated with severe hypersensitivity reactions of SJS/TEN. A recent study in Malaysia stated numerous SJS and TEN cases have been reported since past decades. For instance, based on retrospective study of hospitalized cases from 1987 at Hospital Universiti Science Malaysia (HUSM), situated in the north-eastern state of Malaysia, 54.5% of SJS cases and 10.3% of TEN cases have been reported with the mortality rate of 4.5% and 33.5% respectively (Kamaliah et al., 1998). Other than this, drugs for instance, statins for regulation of cholesterol, metformin for regulation of blood sugar level, perindopril for treatment of stable coronary artery disease, warfarin for anticoagulants and others also could lead to other side effects. However, the medication (the dosage of drugs consumption) and their side effect are highly dependent on our genes and body's immune system.

Alternatively, there are quite a few numbers of DNA testing available mainly for preventive steps. Pre-implantation testing is a specialized technique to reduce the risk of having child with certain genetic disorder. The genetic changes are detected in embryos by in-vitro fertilization method. A small number of cells are taken from embryos and tested for genetic disorder before implanted in the uterus to initiate pregnancy. On the other hand, prenatal testing also available and often offered during pregnancy if there is a developing risk that the infant will have a genetic disorder. It helps to identify birth defect or inherited disorder of an infant before birth. Whereas, new-born screening is used just after birth to discover genetic disorder that can be treated at early stage. Apart from that, DNA is used for forensic testing to recognize plus solve cases related crime suspect or catastrophe victim for legal purposes (Cirino and Ho, 2005).

2. Personalized Medicine Based on DNA Analysis

The practice of empiric medicine or treatment prescribed is usually tested on broad general populations revolves around in terms of "standards of care for average person". Regrettably, due to genetic variances it only works for few patients and the rest of the patients under inappropriate medications for them. According to Schork (2015), in the United States, the highest grossing drug helps 1 in 25 people who consume them and even routinely used drugs such as statin to lower cholesterol help a small number of patients as 1 in 50. Besides, if the prescribed treatment does not work for the patient after several phases, the doctor proceeds to the next medication which is entirely based on clinical experimental. The lack of genetic information that causes major diseases will have an impact on ineffective and imprecise treatments. Thus, personalized medicine was introduced by physician to consider individual genetic variability to respond towards a particular treatment.

Personalized medicine is still in its infancy and new approaches are reported in recent times. This effort to revolutionize health care enables to deliver right

treatment for the right person at right time for disease diagnosis. It is also variously known as precision medicine, stratified medicine, individualized medicine or genomic medicine relies on each patient's biomarkers that help clinicians to provide customized medication, more accurate diagnoses, safer drug prescriptions and lead to effective treatments. As well, in January 2015, United States former President Barack Obama announced Precision Medicine Initiative to establish database of patient's genetic information for more accurate treatment. Previously, genetic analysis was studied by collecting countless amount of data on side effect, responsiveness and amount of dosage consumption for every patient before and after treatment. These testing interventions were considered as time consuming process as it has to be done before and after according to every prescribed medicine. Although some researchers have designed formalized approach, still it is unmanageable to collect information for all the people. Hence, electronic probe and devices would be supportive to gather all measurable parameters for precision medicine. The collection of sufficient data may benefit for appropriate usage of drug and lead to positive response towards treatment. In view of that, exploration in variety of heath monitoring device to guide clinicians to prescribe precise medicine has increased tremendously.

Personalized medicine is relying on each patient's unique genetic makeup and it can definitely overcome limitations of empiric medicine. Recently, one of the greatest interests to perform DNA testing and developing biodevice is to develop personalized medicine especially as diseases preventive step. The escalating personalized medicine allows health care providers to test individual DNA onset, thus an appropriate recommendation or preventive actions to reduce developing disease are highly possible. Plus, tendencies to develop or susceptibility to certain genetic disease and side effect from exposure of inappropriate number of pharmaceutical drugs also can be identified. Based on genetic studies, potential response of medications and their side effect (adverse drug reaction) can be evaluated and more effective drug can be prescribed. The analysis also enables early stage detection for diseases such as Alzheimer, cancer, diabetes and heart attack for more successful treatment by reducing failure rate of pharmaceutical clinical trials. Those disease risks are in conjunction with the predispositions of individual genome at birth and also lifestyle. Hereafter, suitable tailored treatment which will work better for that particular patient can be provided magnificently. Besides, healthy people are encouraged to perform DNA analysis to maintain healthy lifestyle.

According to the survey conducted in United States and Germany by Kichko et al. (2016), the acceptance and awareness among public about personalized medicine are tremendous thus has been widely implemented with related genetic privacy protection. Moreover, in some developed countries, the desired infrastructure to provide personalized medicine is being enhanced with the help of molecular medicine and genetic laboratory and clinical laboratory specially to improve the care of patients in cost effective manner. Even though advances in technology accelerated discovery of reference genome in all variations, yet there is lack of product to provide rapid outcome for further suitable treatment in clinical setting where doctors able to use bed side to treat patient on-the spot. In short, with this flourishing technique, lifestyle of an individual can be transformed accordingly as

precautionary movements, improve health, prevent disease development, disease detection at an early stage where treatment will be successful and reduce adverse drug reaction by improving treatment. For that reason, an appropriate biodevice to deliver on-the-spot solution regarding genetic information and related medication would be beneficial for everyone.

3. Conventional Methods for DNA Analysis

The most typical methods used for genomic DNA extraction are by either organic solvent which known as phenol extraction or liquid or solid based extraction. Generally, all of the methods starting with cell lysis, proteins and contaminants removal by Proteinase k followed by DNA purification by isopropanol. DNA extraction by organic solvents also began with cell lysed by lysis buffer and mixed with phenol, chloroform and isoamyl alcohol. In high pH condition, the contaminants are separated in organic phase while DNA is recovered in aqueous phase by alcohol precipitation. The extracted DNA from this method may contain residual of used venomous compounds that inhibit enzyme reactions plus it involved cumbersome time-consuming technique. Thus, reproducible DNA yield would not be possible and sensitive for further downstream analysis such as polymerase chain reaction (PCR). In view of that, usage of hazardous waste guidelines is also vital for this technique. Another conventional way for DNA extraction is salting-out-method. High salt concentration of potassium acetate or ammonium acetate are used to precipitate proteins and contaminant and removed by centrifugation process followed by standard DNA recovery by alcohol precipitation. There are few drawbacks from this technique including inept of contaminant removal, requirement of repeated DNA recovery step and inconsistent DNA yield and purity. It is worth mentioning about the failure of PCR which mainly due to sensitivity and purity of sample input (Zhang et al., 2010). Hence, optimization of PCR condition for specificity before initiate the amplification is necessary for either bench top or microfluidic device.

Cesium chloride density gradient method through centrifugation process for DNA extraction is known as high quality DNA isolation method. The lysed cells are alcohol precipitated and mixed with cesium chloride and ethidium bromide for gradient centrifugation for several hours. Then, DNA refining is based on isopropanol extraction mainly to eliminate ethidium bromide. Subsequently, presence of pure DNA can be identified using diphenylamine (DPA) indicator by hydrolysis of DNA (heated in acid) and measured the absorbance intensity by spectrophotometer. Although the DNA yield from this method is high in purity, but it is time consuming, harmful and requires expensive ultracentrifuge. Liquid or solid based extraction based on interaction between negatively charged phosphates of nuclei acid and positively charged surface molecules of substrate used. Hence, under low salt and high pH condition DNA will bind to substrate and other impurities will be washed away by appropriate buffer solutions. Then, DNA is eluted and recovered by alcohol precipitation method. Mostly, the high throughput of DNA can be used for further downstream analysis. Comprehensively, liquid extraction technique separates DNA from proteins and impurities based on their affinity to different immiscible solvent phases. The extraction usually occurred in

micro centrifuge in aqueous organic phase to minimize interaction energies (Maaroufi et al., 2004; Roy, 2013).

3.1 Microfluidic Device for DNA Analysis

Microfluidic device in clinical setting refers to service or health care product provided by trained healthcare professionals at the time of care. For an example, microfluidic device enables physician to perform bedside testing for medical diagnostic and monitoring (Table 1); provide immediate and efficient solution to prevent further illness; and the miniature portable characteristic of microfluidic device makes analysis convenient to perform at time of care. Besides, the clinical analysis also can be done with raw sample in shorter time at the location of patient. It reduces the probability of sample contamination as the test being performed directly once sample is collected. Unlike the conventional method, there is high potential for sample contamination during sample transportation to laboratory due to uncertainty of sample when exposed to environment effects or temperature change.

Table. 1 Comparison of comprehensive sequence involving in general medical testing using microfluidic device and laboratory study

Sequence of Laboratory Analysis	Sequence of Microfluidic Analysis
1. Requirement of biological sample test is ordered	1. Requirement of biological sample test is ordered
2. Sample collection	2. Sample collection
3. Transference of sample to specific laboratory	3. Analysis of sample using microfluidic device
4. Sample is labeled and stored	4. Interpretation of result
5. Sample centrifugation process	5. Implementation of required treatment by physician
6. Analysis of sample using laboratory equipment	
7. The obtained result is recorded and submitted to higher staff	
8. Results is reviewed and reported to the related department	
9. Implementation of required treatment by physician	

Prior work for DNA extraction in microfluidic device using raw sample or finger-prick collection only needs sample input volumes of approximately 5 μL to 200 μL (Dimov, 2011; Shim and Browne, 2010; Oblath et al., 2013; Wu et al., 2014; Gauri et al., 2019). However, sample preparation can be complicated since it comprises complex matrices in microfluidic platform (Cao et al., 2014; Zhao et al., 2014; Chiou et al., 2013). Microfluidic device has great potential as personal diagnostic tool for patients as they able to perform simple clinical testing at home as self-

monitoring for their healthcare. The past decade has seen the rapid development of microfluidic device for ranges of healthcare considerations especially in microfluidic platform or chip. A single microfluidic chip able to perform series of analysis or experiments conducted in an entire lab. As the required sample and reagent volume is small, the microfluidic platform might be applicable for analysis of a single cell. Mostly, microfluidic device can be created as portable, transportable, pocket-sized instrument and also diminutive bench top instrument. It would be a great supporting device to deliver the right care at right time. However, there are some considerations in development of the microfluidic devices for medical essential. The poor quality of analysis and result interpretation will directly lead to inaccurate treatment options thus may affect patient's health. Although the raw specimen input to the device do not necessitate centrifugation process, yet the quality of specimen and analytical technique also plays important role to produce high yield result. Above and beyond, the micro total analytical system (μTAS) has been implemented for the microfluidic devices by using small amount of sample, reagent, cost effective and user friendly.

4. Conclusion and Future Perspective

Selecting a suitable method for DNA extraction is essential for further analysis and greatly influences the yield quality. Extraction of pure sample is the key factor for success of further downstream process especially in clinical analysis to diagnosis disease. In view of all that has been mentioned so far saliva has great potential as DNA source for all kind of patients from infants to elderly people. The long-term storage capability can facilitate easy collection of DNAs for developing a database of human genomic DNA from saliva. Extraction of analytes from most of biological sample is complicated thus, method using microfluidic device would be suitable for rapid analysis. The fluid mechanics of heterogeneous biochemical content to implement in microfluidic scale and high yield of sample preparation from appropriate source are remains as challenges in microfluidic device. Therefore, in order to develop great point of care microfluidic device with complete functions, selecting an appropriate source of sample is vital as it's greatly influences the quantity and quality of the yield. Such emerging approach has stemmed from technology revolution in medicine and health through instrumentation technologies and directed to remarkable development of microfluidic for clinical POC devices especially for DNA testing. This recent trend of smart innovation technologies has redefined the current empirical treatment or therapy where healthcare providers able to make precise decision for the right person for disease diagnosis based on individual genetic biomarkers.

The time has come for prediction model to be introduced within schools with the intention to help the teachers monitor their students' learning behaviour. School students need proper monitoring with their study as well because they are not only growing in numbers but also have diverse learning behaviour and show different attitude towards study. Past researcher urged for the students' behaviour and attitude to be learnt because these are important traits and it affects student academic performance (SAP) (Mccoy, 2016). Therefore, Kalboard360 an e-learning system enriched with X-API (Experience API) is suggested to be implemented within schools to capture factors impacting SAP such as learning behaviour of students.

Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest

The author declare that there is no conflict of interests regarding the publication of this manuscript.

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Legends to Figures

Figure 1. Mechanism of DNA replication based on Watson-Crick Model. Molecules of every generation contain one of the original parents (dark) molecules and paired with one new molecule (white)

Figure 2. Proportion of plasma and cellular components in blood. A: lymphocyte, B: basophil, C: eosinophil, D: monocyte, E: neutrophil

Figure 3. Salivary fluid components