Comparison of Commercially Available Urinary Cell-Free DNA Isolation Kits

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ABSTRACT

Liquid biopsies are a relatively new way of detecting various oncogenic biomarkers from body fluids. Their huge potential stems in their minimal invasiveness and simple collection. These properties are expected to help increase the number of individuals participating in screening programs and ultimately enable early intervention should it be needed. A completely non-invasive approach for biomarker testing is urine-based liquid biopsy. In order to properly evaluate the health status of each individual based on the content found, there is need for efficient extraction kits providing us with reliable information that can be used for further analysis. Currently, there is no standardized protocol for the extraction of urinary cell-free DNA and a comprehensive overview of available kits is lacking. A literature search up to May 30, 2022 was conducted to summarize the efficiencies of commercially available urinary cell-free DNA isolation kits and to discuss their advantages and disadvantages. Out of the kits mentioned in this article Norgen exhibits highest recovery rate of short cell-free DNA from urinary sample but lacks sufficient PCR inhibition resistance. On the other hand kits such as QIAamp and MagMAX displayed relatively high PCR inhibition resistance but at the same time were not able to detect short DNA fragments. Since using suboptimal methods may jeopardize the results, it is crucial to prudently choose a method that best suits one's research scenario.

INTRODUCTION

Cancer is a leading cause of death with an ever-increasing number of patients diagnosed. In 2020 it was responsible for almost 10 million deaths accounting for approximately 17% of all the deaths recorded (www.who.int). One would think that finding a cure for such a widely spread disease is something that our current day technologies can solve. This, however, is rather difficult since cancer is just an umbrella term for a broad spectrum of diseases with similar hallmarks. Some of the hallmarks according to Hanahan (2022) are sustained proliferation, growth suppressors evasion, replicative immortality, or cell death resistance. All of these and many more contribute to tumor growth and the spreading of the cancerous cells throughout the body. Tumor growth is the primary cause of lethality. By spreading and expanding in space the tumor eventually starts obstructing other healthy organs and tissues resulting in their deficient function. Additionally, as with all the living cells, tumor cells also require nutrients for their growth and thereby form competition to healthy tissues.

As already mentioned, curing cancer remains a difficulty and therefore it is important to find other ways to combat it. One of these is prevention. Cancer is a result of a multi-step process leading to the accumulation of mutations in the genome (www.who.int). These mutations could arise spontaneously or they can be induced by external carcinogens. Some of the external carcinogens are viruses, parasites, UV and ionizing radiation but also tobacco smoke and alcohol (www.who.int). By adjusting one's lifestyle and decreasing the amount and time the organism is exposed to these harmful agents, the risk of carcinogenesis can be lowered (www.who.int).

Although prevention is an important way to reduce the chances of cancer development, it does not always guarantee the absolute absence of tumor formation. That is why the second most important way of fighting cancer is early detection. Early detection comprises two components - early diagnosis and screening (www.who.int). These two go hand in hand and together allow for early intervention. Time is a key element when it comes to cancer treatment. The earlier the tumor is identified the higher the chances that it can be cured, excised, or eliminated. In order to identify patients suffering from the initial stages of cancer, although not yet symptomatic, it is important to test them in screening programs even before they experience any complications (www.cancer.org). Following the screening program, tissues or cells may be collected for diagnostic testing and further analyzed for cancer identification. This is done through tissue resection or biopsy. Biopsy by definition itself represents an invasive surgical intervention isolating part of the tissue and separating it from the organism for further examination (www.cancer.org). It usually requires medical assistance and is often associated with discomfort.

A relatively recent alternative to regular biopsy is a so-called liquid biopsy. Liquid biopsy is based on detecting various tumor biomarkers in body fluids such as saliva, blood, urine, and cerebrospinal fluid (Schiffman et al., 2015). Tumor biomarkers found in these fluid samples include circulating tumor cells, cell-free DNA (cfDNA), and exosomes (Kustanovich et al., 2019). Circulating tumor cells represent carcinogenic cells

that got separated from the original tumor and are present in the bloodstream (Zhang et al., 2021). Cell-free DNA are sequences of the DNA molecules present freely in the body fluids or bound to nucleosomes and lastly, exosomes represent protein- or RNA-containing vesicles capable of changing the function and behavior of neighboring cells (Zhang & Yu, 2019). Out of these three, it is especially cfDNA that is considered to be a potentially useful biomarker for cancer. By analyzing DNA fragments in the circulation, one can examine the sequences for abnormal genetic and epigenetic differences and their possible patterns for specific types of cancer (Kustanovich et al., 2019).

As with all the diagnostic tools, liquid biopsy has its advantages and disadvantages. Considering the heterogeneity of tumors, a common biopsy might not show the whole representative collection of accumulated mutations (Poulet et al., 2019). Liquid biopsy, on the other hand, provides us with a mixture of broad biomarkers from the tumor present in the organism. Next, it allows for detecting cancerous DNA from tumors for which regular biopsy would not be safe or possible at all (Poulet et al., 2019). Furthermore, as already mentioned, one of the substantial advantages of liquid biopsies is the non-invasiveness. Thanks to that and its relatively easy collection, in the case of urine, for instance, liquid biopsies have the potential to uncover many possible tumors and therefore enable early intervention (Poulet et al., 2019).

Some of the things that have to be kept in mind, though, are the level of dilution and the high fragmentation of cfDNA in urine. The combination of renal filtration, stronger DNase I activity, large collection volumes, and contamination by genomic DNA (>500 bp) (Augustus et al., 2020) from healthy cells of the urinary tract leads to a relatively low concentration of cfDNA molecules. This can substantially hamper the sensitivity of the kits to properly detect and identify DNA fragments in the sample. Most of the cfDNA detection kits were optimized for plasma cfDNA isolation which peaks around 116 to 161 bp (Augustus et al., 2020) whereas urinary cfDNA ranges from 40-250 bp. It is, therefore, necessary for the kit to be able to detect small cfDNA fragments from a highly diluted sample.

Out of the four liquid biopsy samples mentioned above, urine and saliva are the easiest to obtain and do not require any medical assistance. By making the analysis as efficient as possible both healthy and diseased patients will be able to obtain valuable information about their health status in a reasonable time and act accordingly (Poulet et al., 2019). In order to find the most effective procedure for urinary cfDNA extraction, this article will take a look at and compare different methods and kits for urinary cfDNA isolation, namely Norgen Urine Cell-Free Circulating DNA Purification Kit Midi, Qiagen QIAamp Circulating Nucleic Acid Kit, Thermo Fisher Scientific MagMAX Cell-Free DNA Isolation Kit, PerkinElmer NEXTprep-Mag Urine cfDNA Isolation Kit (NOVA-3826-02), and Zymo Research Quick-DNA Urine Kit (Table 1).

METHODS

Search strategy

In order to find relevant facts regarding the above-mentioned kits, information has been collected from various sources on May 30, 2022. Comparison analyses were found using search engines Google Scholar and PubMed by searching for key words ‘comparison of urinary cell-free DNA isolation’, ‘urinary cell-free DNA’, ‘cell-free DNA isolation’, ‘urinary DNA extraction’. Studies were selected based on their title, abstract, and full text, including articles in the English language only. Due to the scarcity and a limited number of available literature, none of the studies found were excluded based on their sample sizes or methods to assess isolation efficiency. For technical parameters and protocols, the official website of each kit has been used.

Data extraction

Data from the selected studies were extracted from the full text and summarized in a comprehensive overview including the following information: first author, year of publication, name of the cfDNA isolation kit used, input volume, minimal effective size detected, processing time, price, technology.

RESULTS

Search results

A total of 3 articles discussing the efficiency of cfDNA isolation kits were found and retrieved from Google Scholar and PubMed. No articles were excluded.

Studies included urine of both healthy volunteers and cancer patients to assess the efficiency of different cfDNA extraction kits. The number of included urine samples ranged from 5 to 10. In total 5 kits were examined, including Norgen, QIAamp, MagMAX, Zymo and PerkinElmer. All studies analyzed the total DNA concentration, which was assessed by real-time quantitative PCR (Oreskovic et al., 2019) or electrophoresis system Agilent (Lee et al., 2020; Streleckiene et al., 2018).

Norgen

A column-based cfDNA isolation kit often used in research is the Urine Cell-Free Circulating DNA Purification Midi Kit from Norgen Biotek. The official website presents that this kit is capable of analyzing urine sample volumes ranging from

2-10 ml with the ability to detect DNA sequences as short as 50 bp. According to a comparison analysis by Oreskovic et al. (2019), however, the Norgen kit was able to detect even shorter 25-nt DNA fragments. Moreover, the recovery rate for these short fragments was reported to be 72% which was actually higher than the recovery rate for the 40- to 150-nt fragments which was 30-40%. Additionally, this kit yielded cfDNA with high purity and low genomic DNA contamination (Lee et al., 2020). Some of the disadvantages of this kit include proneness to PCR inhibition and weak detection of low concentrations of DNA limited by small urine input (Oreskovic et al., 2019). Officially, 40-45 minutes are sufficient for complete purification with the total processing time being 90-120 minutes (Streleckiene et al., 2018; Oreskovic et al., 2019; Lee et al., 2020). The kit contains 20 preps, and even though the website does not display the price, the kit should cost somewhere between 410$ and 460$ (calculated from the reported price per sample from articles by Streleckiene et al. (2018) and Lee et al. (2020), respectively).

QIAamp

QIAamp Circulating Nucleic Acid Kit is a column-based DNA/RNA isolation kit produced by QIAGEN capable of analyzing up to 5 ml of sample volumes. It has been identified as a highly efficient method for extraction of DNA outperforming 4 other isolation kits in a comparative analysis by Sorber et al. (2016). All the isolation kits used in this comparison analysis, including QIAamp, were however tested to isolate DNA from blood samples and plasma. When isolating DNA from a urine sample, QIAamp was able to extract fragments 150-nt long and that with only an 18% recovery rate (Oreskovic et al., 2019). For shorter fragments such as 80- or 40-nt long the recovery was very low, equal to 1% and 0.2%, respectively (Oreskovic et al., 2019). An advantage, however, is that QIAamp shows good resistance to PCR inhibition with no increase in the PCR quantification cycle measured up to 40% eluate. The processing time of QIAamp is reported to be 120 minutes for 12 samples that were analyzed according to Oreskovic et al. (2019) and 120 minutes per 24 samples according to the official website. The kit contains 50 preps and costs 1 237$.

MagMAX

Another tool for DNA extraction is the magnetic beads-based MagMAX Cell-Free DNA Isolation Kit. It is capable of analyzing sample volumes ranging from 0.5 ml to 10 ml. According to the official website, the efficiency of short DNA recovery is almost a hundred percent for DNA fragments of 100-700 bp. Comparison analysis by Oreskovic et al. (2019), however, obtained 66% recovery for 150-nt fragments and only 0.2% for 40-nt fragments from a urine sample. PCR inhibition testing showed that the kit is slightly sensitive to eluate, being able to withstand 20% eluate but displaying gross inhibition at 40% eluate (Oreskovic et al., 2019). The kit can be reportedly used to analyze 24 samples in 40 minutes and costs 630$ per 50 preps.

Zymo

Quick-DNA Urine Kit produced by Zymo Research offers another column-based tool for DNA extraction. It is able to process samples of volume as large as 40 ml and according to the official website can detect and effectively isolate DNA molecules ranging from 100 bp to 23 000 bp. Lee et al. (2020) report in their comparison analysis that the required time for a run is 2 hours while following the process in the official protocol on the website states approximately 60 minutes for the procedure. One kit contains 50 preps and costs 400$.

PerkinElmer

Lastly, a magnetic beads-based cfDNA isolation kit by Perkin Elmer - NextPrep-Mag Urine cfDNA Isolation Kit will be described. The kit is able to process 25 samples of the volume of 4 ml or less, or 5 samples of 20 ml. No information about the range of DNA lengths efficiently extracted from samples was given on the website or in the comparison by Streleckiene et al. (2018). Streleckiene et al. (2018) focused on capturing DNA fragments between 100 and 1000 bp in length and quantified DNA yield which was reported to be higher than with the Norgen isolation kit. The processing time for this particular kit was 45 minutes (Streleckiene et al. 2018). The kit costs 253$

NAME/MANUFACTURER	INPUT VOLUME (ml)	SIZE DETECTED (bp)	TIME (min)	PRICE ($)	PRICE/PREP ($)
Norgen	2-10	25	40-45	410-460	20.5
QIAamp	0-5	150	120	1237	24.74
MagMAX	0.5-10	80	40	630	12.4
Zymo	40	100	60	400	8
PerkinElmer	4-20	NA (100)	30	253	10.12

Table 1: **Comparison of 5 urinary cell-free DNA isolation kits.** The kits are compared according to the urinary input volume, minimal effective size detection, time required for processing, and price.

and is capable of analyzing 100 ml of urine in the form of either 25x4 ml samples or 5x20 ml samples.

DISCUSSION

In this article, 5 different urinary cfDNA isolation kits were compared according to their sample input volume, minimal effective size detection, the time required for processing, and price. Keeping in mind the relatively high fragmentation and low concentration of cfDNA in urine, an ideal isolation kit should be capable of recovering short DNA molecules from samples with highly diluted concentrations of DNA (Oreskovic et al. 2019).

Out of the 5 kits evaluated in this article, none of them displayed particularly outstanding results in both of the above-mentioned criteria at the same time. The most promising kit when it comes to isolating very short cfDNA fragments with high purity and low cellular genomic DNA contamination is the Norgen Cell-Free Circulating DNA isolation kit (Lee et al., 2020). This kit exhibits by far the most effective extraction of cfDNA molecules as short as 25-nt in length outperforming all the other isolation methods. The next one in line with effective short DNA molecules extraction is the MagMAX cfDNA Isolation kit capable of recovering fragments of 80-nt in length. Unfortunately, this might be already considered a suboptimal isolation kit when it comes to urinary cfDNA extraction since urinary cfDNA peaks at the length between 30- to 60-nt (Oreskovic et al., 2019). Therefore, this kit was reported as not recommended (Oreskovic et al., 2019). One of the findings that came as a surprise was the ineffective isolation of short fragments by QIAamp from urine samples. In a previous comparison analysis, QIAamp displayed one of the highest efficiencies of cfDNA extraction from plasma samples (Sorber et al., 2017). It was, therefore, viewed as a promising kit for urinary cfDNA extraction as well. This, however, was not confirmed, and apparently, the origin of liquid biopsy, being it a plasma sample or urine sample, affects both the recovery rate and minimal length of cfDNA detected. It has to be kept in mind that urine samples generally tend to have lower cfDNA concentration and contain shorter fragments due to renal filtration and DNA degradation in urine (Oreskovic et al., 2019). QIAamp is, therefore, not recommended for cfDNA extraction from urine samples (Oreskovic et al., 2019).

Even though Norgen seems to be a promising kit so far, there are also certain disadvantages. Oreskovic et al. (2019) reported this kit to be highly prone to PCR inhibition and therefore may ultimately lead to unreliable results due to no amplification of the genetic material in the sample. This, on the other hand, was not the case for the previously mentioned kits from QIAamp and MagMAX which both showed a higher level of PCR inhibition resistance. Furthermore, all three kits exhibited a poor recovery rate of cfDNA from low concentration samples.

One thing that could partly compensate for the low concentration of DNA in the urine is the input volume of the sample. This is without a doubt the highest in Quick-DNA Urine Kit by Zymo Research which enables the input volume to be as high as 40 ml. Zymo kit, however, on the other hand, was not able to detect short fragments of DNA and the effective extraction length started around 100 bp. Coming back to the initially very promising Norgen kit, a comparison analysis by Oreskovic et al. (2019) found that the combination of relatively low input volume of 2 ml and the proneness to PCR inhibition leads to poor sensitivity to cfDNA when it comes to low concentration samples.

The desirability of using PerkinElmer kit is difficult to evaluate since one of the most important criteria, the minimal effective size detection, has not been tested below 100 bp in the available literature and the official website also does not provide information regarding this parameter of the kit.

The selection of kits in this article contains both column-based and magnetic beads-based kits. Although the majority of kits is column-based, magnetic beads technology seems to be more efficient. Magnetic beads-based kits were shown to be capable of efficiently detecting fragments of plasma DNA shorter than 70-nt, which was not the case for column-based kits (Hudecova, 2022). Moreover, magnetic beads technology can be automated and therefore is not as hands-on demanding.

Comparing different extraction kits by collecting information from various articles and analytical comparisons poses a challenge since different authors used different metrics and criteria for evaluating the applicability and efficiency of the particular kits of their interest. It might be, therefore, useful to standardize the metrics and agree on parameters that best characterize different isolation kits. Thereby, it could be easier for the researchers to orient themselves among the analyses and to choose the method that suits best for their type of research. Additionally, from the available literature, it seems that there is no kit that would be able to efficiently detect small DNA molecules from low concentration samples of urine. This might cause a problem in the future and lead to unreliable results. One of the ways to solve this could be optimization of extraction protocols, rather than kits, such as the ones mentioned in the article by Oreskovic et al. (2019), for instance.

CONCLUSION

According to the available information each kit has its advantages and disadvantages. Out of the kits analyzed in this article, Norgen has the highest recovery rate of short cfDNA

fragments but shows high PCR inhibition. QIAamp and MagMAX are relatively resistant to inhibition but are not able to detect short DNA fragments. Zymo is the least expensive kit and can process large sample volumes. In general, it is recommended to use magnetic beads-based technologies as they can detect shorter DNA fragments than column-based and can be automated in most cases.

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