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# The Standardization Process of cfDNA Research: A New Molecular Marker

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## 1. Abstract

With the further development of research, cfDNA, as a new molecular marker, plays an increasingly important role in disease prediction, tumor prevention and treatment. However, there are still many obstacles before achieving a wide range of clinical application, the main one is the lack of standards for cfDNA, which leads to the lack of comparability of published research reports, the lack of repeatability of results, and the lack of reliability to achieve clinical application. This review will focus on the research progress related to cfDNA standardization in recent years, involving the sample source, sample preservation, extraction and preparation, detection, qualitative and quantitative analysis of cfDNA. Summarize various methods to explore the advantages and disadvantages, further consider the current results, and instruct the formulation of cfDNA standards, in order to directly measure the development of cfDNA technology in future research reports, and promote the clinical application of theoretical knowledge at the same time.

**2. Keywords:** cfDNA; Standardization; Extraction kit; Detection; Quantitative analysis.

## 3. Introduction

Cell-freeDNA (cfDNA) is a DNA fragment released into serum and plasma, which is usually thought to exist in plasma, but can be also included in other body fluids, such as bile, feces and urine [1]. It can be utilized to characterize different types of DNA, in circulating blood, including circulating tumor DNA (ctDNA) and fetal free DNA (cffDNA). CfDNA is released by all tissues of the body, including normal functional tissues, as well as by tissue sources with different or abnormal functions, such as intestinal flora or cancer. Nucleic acid sources include cells, bacteria and viruses, as well as nucleic acid-protein complexes [2-3].

Elevated cfDNA concentration was reported for the first time in patients with autoimmune diseases and leukemia. In the ensuing decades, many studies [4] have shown that patients with cancer and some inflammation usually have higher levels of cfDNA than healthy subjects. Therefore, the use of cfDNA detection for human health monitoring [5-7] has functional significance. Fetal chromosome aneuploidy and non-invasive prenatal detection based on cfDNA [8] is the first successful application of cfDNA technology. CfDNA detection is particularly suitable for tumor prevention and treatment [9]. Many adult tumor patients have to go through 20-30 years of lesions before they finally form cancer [10-11]. In cancer diagnosis, changes in cfDNA can be detected months in advance. The high fatality rate of cancer is due mainly to late medical intervention caused by failure to detect it early [12]. CfDNA has been shown to better represent the complete genetic characteristics of tumors than tumor biopsies. Therefore, cfDNA can be used to screen for early detection, diagnosis, and treatment of healthy and asymptomatic groups, as well as as an alternative marker to evaluate therapeutic efficacy and prevent and control disease recurrence [13-16]. The role of cfDNA as a molecular marker in clinical applications such as tumors has been reported in detail [17-19].

Numerous studies have reported that the use of cfDNA base length differences in fetal prenatal non-invasive detection, tumor, and transplant treatment monitoring. CfDNA naturally fragments at the average length of 167bp through the potential mechanism of nucleosome-mediated protection of cyclic nucleases [20-21]. Consistent findings include: cfDNA is much smaller than genomic DNA, more than 70% of plasma cfDNA are less than 300bp, and the average size is 170bp [22-24]. In order to prevent the interference of complex factors in vivo, Bronkhorst et al [25]. established an in vitro model and explored the properties of cfDNA by culturing cells in vitro and extracting cfDNA. The same as the experiment of Yu et al [26], cfDNA accounted for a prominent proportion of bands at 166bp. Different sizes of cfDNA fragments released into the body may be due to the different sizes of cfDNA released into the circu-

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latory system through different pathways. In most cases of plasma or serum DNA electrophoresis, a ladder pattern similar to that of apoptosis cells can be seen. There are various speculations about the pathway of cfDNA release from the body. In general, the size of these fragments is related to the multiple of nucleosome DNA extension, ranging from 150bp to 1000bp [25]. The size distribution of DNA fragments in different samples is also unlike, and the DNA fragments from necrotic cells are usually larger than 10000bp [27]. These findings indicate that most of cfDNA is derived from apoptosis in disease and healthy individuals [28-30]. Conjectures that have been denied include: first, the tumor cells and the cells at the circulatory junction dissolve and enter the bloodstream [31], because follow-up studies have confirmed that the concentration of cfDNA in the patient's blood exceeds the load of the tumor cells; second, tumor micro metastasis and the destruction of circulating cancer cells [32]. In addition, there are three suspicious sources: apoptosis, necrosis, and active cell release [33].

At present, relevant research reports on cfDNA generally have the phenomenon of incomplete information, and many publications do not fully explain the details such as the collection and preparation of cfDNA. We should mention in detail the source of the sample and whether it is fresh, how to preserve the sample that has not been treated immediately, and whether the sample has cell and DNA enzyme pollution or sample degradation during qualitative and quantitative cfDNA [9]. In addition, there is little mention of the repeatability of the results. Parallel experiments are lack of effective comparability, and the quality of the results cannot be widely evaluated. The purpose of this discussion is to summarize recent research status of cfDNA so as to provide a thinking direction for further promoting the research of cfDNA standardization.

## 4. Biological Sample Collection and Preparation

Extraction of cfDNA involves in vivo and in vitro, and the samples used in vivo extraction are from a wide range of sources, including plasma, serum, and whole blood. Through quantitative RT-PCR measurement, the level of cfDNA in serum can be 10 times higher than that in plasma [34]. The test tube with the function of cell lysis should not be used in the process of sample collection, and the blood used to prepare plasma should be collected with the test tube treated with anticoagulant. Diverse kinds of blood collection tubes have their own advantages and disadvantages [35]. Wong et al. carried out molecular analysis of circulating free fetal DNA (cffDNA) in maternal plasma, and systematically studied the operation and processing conditions of time, temperature, strength and test tube batches [36]. It was concluded that the advantage of BCT tube was better than that of EDTA tube. The transportation process before sample extraction will bring uncontrollable changes, including stirring amount and pressure difference, settling time, centrifugal force, and the centrifuge used to settle cells will affect the late extraction of cfDNA [37]. In plasma samples or isolated cfDNA, 30% of DNA is degraded each year [38]. For samples that cannot be processed immediately and have to be preserved preserved for a short time, they can be placed in a BCT tube and choose a low temperature environment [39]. Care should be taken so that the sample is not exposed to extreme temperatures during transportation [40].

## 5. Sample Pre-Processing

If the collected samples cannot be extracted immediately, it is also necessary to control the time interval and temperature before treatment [41-42] to prevent the ultimate impact on the determination of cfDNA. The consequences of Risberg et al [39]. confirmed that there was no statistically significant difference in the number of mutant molecules expressed in copies per milliliter of plasma in the sample processing interval experiment. In view of the low yield of DNA in acellular plasma separated by Qiagen column, Xue et al found that the plasma was heat-treated in the presence of TritonX-100 and purified by phenol/chloroform (THP regimen) [42]. Bronkhorst et al confirmed that quick freezing before extraction did not increase the extraction amount of cfDNA, and the high yield could be completely attributed to the addition of protease K [25]. Fong et al used seven different methods to extract plasma DNA, from a small number of samples using three manual methods and four column-based procedures. A manual method using sodium iodide gave the best results [42]. In the process of processing and extracting cfDNA, it should be noted that the operational methods and reagent consumables used have no selective bias for the collection of cfDNA, and will not omit small fragments and will not degrade genomic DNA and cause cfDNA pollution.

## 6. CfDNA Extraction Kit

At present, there are many kinds of kit for cfDNA extraction, and a series of DNA extraction kit produced by QIAGEN Company is the most common in existing research experiments. Many researchers use Qiagen column to separate and purify [43] acellular plasma DNA, but it has been proved that this system has some disadvantages, such as low yield and partial loss of DNA fragments smaller than 150bp [44]. With the continuous deepening of the research, the requirement of precision is higher and higher, and the cfDNA extraction kit produced by QIAGEN is also becoming more and more abundant. In the early study, Sozzi et al [45]. used QIAamp DNA Mini kit to separate plasma at low speed twice at $4^0$C, purified by Qiagen column for 5 times, extracted cfDNA, and constructed ROC curve combined with DNA Dipstick TM Kit kit to realize cfDNA quantification. After that, Xue et al compared the QIAamp Blood DNA Midi Kit and Triton heat phenol (THP) [42] schemes, using bcl-2 as the reference plasmid, through Pico Green analysis, the results showed that the cfDNA yield of the THP scheme was higher than that of the kit method, and the addition of protease K during the operation of the kit would appropriately increase the cfDNA yield of the kit. The data published by Fong [46] in the same year showed that it was also extracted by QIAamp DNA Blood kit

and through fluorescent Quant-iT dsDNA Hs experiment, combined with the results of cfDNA content and fragment size detection, it was concluded that the kit method/phenol-chloroform method and sodium iodide method had higher extraction rate, and the result of sodium iodide method was the best.

Fleischhacker et al [47]. carried out quantitative analysis of cfDNA with three DNA separation kits in two different laboratories using three reference materials of a human endogenous virus (ERV), the GAPDH gene, and the é-globin gene, and compared the effects of QIAamp DNA Hemal Midi Kit kit/Nucleo Spin plasma F kit (MN) and Roche automatic system (Magna Puer LC DNA separation kit-mass) (MP) extraction). The results showed that the yield of plasma DNA extracted by automatic Magna Pure system was the highest, while the concentration of plasma DNA extracted by Qiagen column was relatively subtle.

The recent research data [48] compared with six QIAamp® circulating nucleic acid kit (QIA), PME free-circulating DNA Extraction Kit (PME), the Maxwell RSC ccfDNA Plasma Kit (RSC), the EpiQuick Circulating Cell-Free DNA Isolation Kit (EQ), and two consecutive versions of the NEXT prep-Mag cfDNA Isolation Kit ($NpM_{V1/2}$) kits provide complete reference data for the current experimental research, and the results show that the separation efficiency of QIA kit and RSC kit for KRAS mutated ctDNA and non-mutated cfDNA is similar, while the yield of PME and $NpM_{V2}$ kit is significantly lower.

Devonshire et al [49]. compared three specific cfDNA extraction methods: QIAamp ®Circulating nucleic acid (CNA) kit, NucleoSpin ®Plasma XS (NS) kit and FitAmp™ plasma/serum DNA isolation (FA) kit with the commonly used QIAamp DNA blood mini (DBM) kit, analyzed seven reference genes by GeNorm method and combined with digital PCR results, it was concluded that the extraction efficiency of each kit was in the order of CNA kit > DBM kit > NS kit > FA kit, and CNA and NS kits could better reflect the smaller DNA fragments in the extract than DBM kits.

Based on discrete quantitative analysis methods, the extraction efficiency of each kit cannot be compared uniformly. Widely accepted conclusions include: the extraction and purification process of QIAGEN kits will cause the loss of small fragments of DNA below 150bp, so the quantitative results are generally low; most kits contain PCR inhibitors, so PCR quantitative analysis will lead to low results; the sample stored for a long time will produce cell dissolution and contamination of cfDNA, resulting in high results. In addition, sizes of cfDNA fragments extracted from different kit are different, so the quantitative analysis of total cfDNA yield is not conducive to the standardization of cfDNA.

## 7. CfDNA Detection and Quantitative Method

Combined with long-term research, it is found that there are numerous quantitative methods for cfDNA, including ultraviolet spectrum and mass calculation realized by fluorescent magnetic particles [50]. However, the low concentration of cfDNA extracted often leads to the arithmetical error of this method. The comparison of the size of visible fragments under nucleic acid gel staining method [51] also does not possess the sensitivity to detect a small amount of cfDNA. In view of the fragile nature of cfDNA, differences in sample treatment and treatment, and differences in manual operation that may be caused by laboratory equipment, the determination of cfDNA fragment size is hindered [1]. In addition, sequence comparison based on sequencing technology [52-53], especially the emergence of second-generation sequencing technology has improved the low proportion of mutation signals in the wild-type DNA background to a certain extent [54], and the accuracy has been further improved [55]. Tumor cells release DNA into serum or plasma through a variety of mechanisms, so that cancer-related genetic changes can be detected, including point mutations [56], copy number changes, chromosome rearrangements and epigenetic aberrations. Yang [57] et al have developed a powerful and universal acellular DNA (CfDNA) allele counting system based on next generation sequencing, called cfDNA barcode for non-invasive prenatal diagnosis of single gene diseases. Adalsteinsson reported the ichorCNA software, which quantifies the number of tumors in cfDNA from genome-wide sequencing data covered by 0.1x without knowing the tumor mutation in advance [58]. However, sequencing technology for the detection of cfDNA gene fusion and point mutation results still has variability [59].

In addition, real-time quantitative PCR and rtPCR, and droplet digital PCR-facilitated concentration and copy number analysis are also covered. The primary problem of using PCR and quantifying through the standard curve is that the universal target gene has not been determined. At present, there are various kinds of reference genes available, including GAPDH, β-globin and ERV gene [47,60]. Among them, β-globin is significantly different from that obtained by GAPDH and ERV. Comparing the DNA yield obtained by different methods and at two locations: multivariate tests showed that the DNA yield was strongly dependent on the isolation procedure and the target genes used, and less reliant on the laboratory location. No interaction was noted among the three variables. In addition, for quantitative reference genes, you can use them alone or in combination with multiple reference genes. As a result, the extraction rates of each experimental platform cannot be compared uniformly. Droplet digital PCR (DdPCR) [61-63] is more sensitive than standard quantitative PCR or NGS, and its workflow is simpler than other digital PCR (such as BEAMING). The problems are caused by detection based on a single mutation site are worth thinking deeply, because large copy number variation can lead to false positive phenomenon [64]. A method called Cerebro [65] for analyzing the next generation of cancer sequence data can identify highly reliable somatic mutations while minimizing false positives.

Information of mutation sites detected by ddPCR is known. For unselected mutations, most studies use pre-screening methods, such as denatured high-performance liquid chromatography, time-temperature gradient electrophoresis and single strand conformation polymorphism (SSCP) [54]. In the past, countless methods for screening cancer-derived cfDNA have focused on the targeted detection of somatic single nucleotide mutations in cancer genes with repeated mutations [66-67]. However, somatic copy number change (SCNAs) may be more suitable for a wider range of applications, as the vast majority of metastatic cancers contain somatic SCNAs at the arm level [68]. Previous benchmark tests have shown that somatic changes can be reliably detected in tumor samples containing at least 5-10% tumor content using the prevailing depth of WES (~ 150x coverage) [69].

Other methods aimed at improving the sensitivity of detection have been reported, including competitive binding radioimmunoassay [4] sensitivity (range 25-1000ng/ml) and direct radiolabeling of purified DNA [70] (sensitivity as high as 1.6ng/ml), DNA embedding method [71], Microbeads, latex, amplification and magnetism (light beam) [72] and methylation detection method [73-77] which can be used for tumor localization at the same time and their combination with hmC-CATCH [78] can greatly improve the sensitivity of cfDNA detection. In addition, there are short oligonucleotide mass spectrometry [79] (SOMA) and bead amplification magnetism [80], and immunoassays for histone and double-stranded or single-stranded DNA [34].

With the maturity of cfDNA technology in clinical application, third-party verification of test parameters, such as sensitivity, reliability, use of internal and external controls, and regulatory guidance from the FDA and laboratory regulators must be considered [1].

## 8. Current Problems

First, the standard of cfDNA is the unity of units. At present, the reported units include the copy number of mass concentration ng/mL, the total DNA of cfDNA/ (%) [27] and sequence information. The second is the uniformity of fragment size, the difference of cfDNA fragment size can be used in clinical application [81]. It is assumed that the distribution pattern of ctDNA fragment size of 145bp can reflect the cfDNA, released during cell proliferation, while the fragment at 167bp may reflect the cfDNA released by apoptosis or blood cell maturation/transformation. Size of cfDNA fragments not only depends on their own characteristics. Various methods used in the extraction process, such as simple gel electrophoresis, Sanger sequencing, electron microscope and atomic force microscope, will produce different estimates of the average size of cfDNA. The lower and upper limits of fragment size range from more than ten base pairs to tens of thousands of bases. In addition to the influence in the extraction process, the initial stage of extraction involves the source of the sample, and there are also differences in the content of cfDNA in plasma, whole blood and the primary site of the tumor [82]. The root cause of the difference may lie in the singular detection period, and the high content of cfDNA in the primary site may also guide the exploration of the mechanism of cfDNA release.

Secondly, the transport and storage conditions of samples will also affect the level of cfDNA. For samples that cannot be addressed in time, to avoid sample contamination or degradation, the use of BCT tube preservation in low temperature environment has been widely recognized. In the process of extraction, according to the source of the experimental sample, combined with the product description, the fitting kit should be adopted according to the size of the target fragment.

There are many different methods for the detection and quantification of tumor DNA, but the most common method for analyzing tumor DNA is to detect the mutation of KRAS [83] genes, which account for only about half of the patients. With the improvement of methodology and the addition of more tumor-specific gene changes, the detection rates of mutations based on EGFR [84], BRAF [85], ESR1 [86] and $PIK_3CA$ [87] are different, so it is difficult to compare directly with other studies.

No consensus has been reached on the best methods of purification, quantification, and mutation detection of DNA, as well as the same scope of application, verification, and nomenclature. At present, there is no mention of measuring the purity of A260/A280 by measuring cfDNA. The reason may be that the content of cfDNA obtained in the laboratory is too low and the measured concentration is not accurate, so the determination of cfDNA purity needs to develop a new technical means or promote a new concept to describe its purity.

The accurate detection of cfDNA should not only achieve accurate quantification, but also achieve the location of primary tumors in vivo. The existence of tumor heterogeneity leads to great obstacles in the screening of primary tumors of unknown sources. The existing detection techniques still have many problems, such as unstable results, poor accuracy, high detection cost and so on. Technical methods suitable for clinical detection of cfDNA have not been unified. In addition, due to the heterogeneity between research methods and calculation endpoints, statistical comparison is not applied.

## 9. Conclusion and Perspectives

According to the current experimental data, we have sufficient theoretical and factual basis to prove that cfDNA can be utilized as an effective marker in clinical diagnosis, especially in disease prevention and control. Therefore, it is necessary for us to establish a standard, including a sample pretreatment, cfDNA extraction and detection, qualitative and quantitative. Altered tumor cells have different mutations, and different stages of the development of the

same tumor cells also have different mutations [34, 88]. It is not known whether the mutation frequencies of separate mutation sites interfere with each other in the detection process.

At present, only two cfDNA-based detection methods approved by FDA are: Cobas EGFR mutation test for EGFR mutation in cfDNA of lung cancer patients v2 [89] and Epi proColon analysis for methylation of SEPT9 promoter in cfDNA of colorectal cancer screening patients [90]. The more perfect standard setting is the further deepening of the research and development of cfDNA, which is conducive to the design and reporting of related experiments with greater intrinsic value. Only when the methods of each laboratory are comparable can we promote the gradual maturity of the technology, achieve accurate quantification more quickly to avoid false positive or false negative results [25], ensure that the experimental results are stable and repeatable, and finally realize the value of clinical detection application in an all-round way.

## References


1. Zandvakili I, Lazaridis KN. Cell-free DNA testing: future applications in gastroenterology and hepatology. Therap Adv Gastroenterol. 2019; 11: 1-13.
2. Gahan PB, Stroun M. The virtosome—a novel cytosolic informative entity & intercellular messenger. Cell Biochem Funct. 2010; 28: 529-38.
3. Pinki C, Debasis P, Nibedita M, Poulomee K, Amit P, Chakrabarti MK. Thermostable direct hemolysin downregulates human colon carcinoma cell proliferation with the involvement of E-cadherin, and β-catenin/Tcf-4 signaling. Plos One. 2011; 6: e20098.
4. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res. 1977; 37: 646-50.
5. Hayashi Y, Fujita K, Matsuzaki K, Matsushita M, Kawamura N, Koh Y, et al. Diagnostic potential of TERT promoter and FGFR3 mutations in urinary cell-free DNA in upper tract urothelial carcinoma. Cancer Sci. 2019; 110: 1771-9.
6. Parikh AR, Corcoran RB. Monitoring resistance through liquid biopsy. Ann Oncol. 2018; 29: 8-11.
7. Hidestrand M, Tomita-Mitchell A, Hidestrand PM, Oliphant A, Goetsch M, Stamm K, et al. Highly sensitive noninvasive cardiac transplant rejection monitoring using targeted quantification of donor-specific cell-free deoxyribonucleic acid. J Am Coll Cardiol. 2014; 63: 1224-6.
8. Schmid M, White K, Stokowski R, Miller D, Bogard PE, Valmeekam V, et al. Accuracy and reproducibility of fetal-fraction measurement using relative quantitation at polymorphic loci with microarray. Ultrasound in Obstetrics & Gynecology. 2018; 51: 813-7.
9. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. Cancer Discov. 2014; 4: 650-61.
10. Jones Sn, Chen W-d, Parmigiani G, Diehl F, Beerenwinkel N, Antal T, et al. Comparative lesion sequencing provides insights into tumor evolution. PNAS. 2008; 105: 4283-8.
11. Yachida S, White CM, Naito Y, Zhong Y, Brosnan JA, Macgregor-Das AM, et al. Clinical significance of the genetic landscape of pancreatic cancer and implications for identification of potential long-term survivors. Clin Cancer Res. 2012; 18: 6339-47.
12. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018; 68: 394-424.
13. Spindler KG, Boysen AK, Pallisgard N, Johansen JS, Tabernero J, Sqrensen MM, et al. Cell-Free DNA in Metastatic Colorectal Cancer: A Systematic Review and Meta-Analysis. Oncologist. 2017; 22: 1049-55.
14. Hendriks RJ, Dijkstra S, Smit FP, Vandersmissen J, Voorde HVD, Mulders PFA, et al. Epigenetic markers in circulating cell-free DNA as prognostic markers for survival of castration-resistant prostate cancer patients. Prostate. 2018; 78: 336-42.
15. Mehra N, Dolling D, Sumanasuriya S, Christova R, Pope L, Carreira S, et al. Plasma Cell-free DNA Concentration and Outcomes from Taxane Therapy in Metastatic Castration-resistant Prostate Cancer from Two Phase III Trials (FIRSTANA and PROSELICA). Eur Urol. 2018; 74: 283-91.
16. Christie EL, Fereday S, Doig K, Pattnaik S, Dawson SJ, Bowtell DDL. Reversion of BRCA1/2 Germline Mutations Detected in Circulating Tumor DNA From Patients With High-Grade Serous Ovarian Cancer. J Clin Oncol. 2017; 35: 1274-80.
17. Bronkhorst AJ, Ungerer V, Holdenrieder S. The emerging role of cell-free DNA as a molecular marker for cancer management. Biomol Detect Quantif. 2019; 17: 1-23.
18. Jiang P, Chan KCA, Lo YMD. Liver-derived cell-free nucleic acids in plasma: Biology and applications in liquid biopsies. J Hepatol. 2019; 71: 409-21.
19. Quigley D, Alumkal JJ, Wyatt AW, Kothari V, Foye A, Lloyd P, et al. Analysis of Circulating Cell-Free DNA Identifies Multiclonal Heterogeneity of BRCA2 Reversion Mutations Associated with Resistance to PARP Inhibitors. Cancer Discov. 2017; 7: 999-1005.
20. Thierry AR, El Messaoudi S, Gahan PB, Anker P, Stroun M. Origins, structures, and functions of circulating DNA in oncology. Cancer Metastasis Rev. 2016; 35: 347-76.
21. Jung K, Fleischhacker M, Rabien A. Cell-free DNA in the blood as a solid tumor biomarker--a critical appraisal of the literature. Clin Chim Acta. 2010; 411: 1611-24.
22. Elshimali YI KH, Sarkissyan M, Wu Y, Vadgama JV. The clinical utilization of circulating cell free DNA (CCFDNA) in blood of cancer

patients. Int J Mol Sci. 2013; 14: 18925-58.

23. Green C HJ, Talbot E, Mwaba P, Reither K, Zumla AI. Rapid diagnosis of tuberculosis through the detection of mycobacterial DNA in urine by nucleic acid amplification methods. Lancet Infect Dis. 2009; 9: 505-11.
24. Li Y ZB, Rusterholz C, Kang A, Holzgreve W, Hahn S. Size separation of circulatory DNA in maternal plasma permits ready detection of fetal DNA polymorphisms. Clin Chem. 2004; 50: 1002-11.
25. Bronkhorst AJ, Wentzel JF, Aucamp J, van Dyk E, du Plessis L, Pretorius PJ. Characterization of the cell-free DNA released by cultured cancer cells. Biochim Biophys Acta. 2016; 1863: 157-65.
26. Yu SC, Chan KC, Zheng YW, Jiang P, Liao GJW, Sun H, et al. Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. Proc Natl Acad Sci U S A. 2014;111: 8583-8.
27. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res. 2001; 61: 1659-65.
28. Holdenrieder S, Stieber P. Apoptotic markers in cancer. Clin Biochem. 2004; 37: 605-17.
29. Nagata S, Nagase H, Kawane K, Mukae N, Fukuyama H. Degradation of chromosomal DNA during apoptosis. Cell Death Differ. 2003; 10: 108-16.
30. Nagata S. DNA degradation in development and programmed cell death. Immunology 2005; 23: 853-75.
31. Sorenson G. Communication at the XXVth Anniversary Meeting of the International Society for Oncodevelopmental Biology and Medicine. Montreux, Switzerlland, September 1997.
32. Anker P, Lefort F, Vasioukhin V, Lyautey J, Lederrey C, Chen XQ, et al. K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. Gastroenterology. 1997; 112: 1114-20.
33. Stroun M, Lyautey J, Lederrey C, Olson-Sand A, Anker P. About the possible origin and mechanism of circulating DNA: apoptosis and active DNA release. Clin ChimActa. 2001; 313: 139-42.
34. Gormally E, Caboux E, Vineis P, Hainaut P. Circulating free DNA in plasma or serum as biomarker of carcinogenesis: practical aspects and biological significance. Mutat Res. 2007; 635: 105-17.
35. Deans ZC, Butler R, Cheetham M, Dequeker EMC, Fairley JA, Fenizia F, et al. IQN path ASBL report from the first European cfDNA consensus meeting: expert opinion on the minimal requirements for clinical ctDNA testing. Virchows Archiv. 2019; 474: 681-9.
36. Wong D, Moturi S, Angkachat.hai V, Mueller R, DeSantis g, Boom DVD, et al. Optimizing blood collection, transport and storage conditions for cell free DNA increases access to prenatal testing. Clin Biochem. 2013; 46: 1099-1104.
37. Chiu RW PL, Lau TK, Leung TN, Wong EM, Lo YM. Effects of blood-processing protocols on fetal and total DNA quantification in maternal plasma. Clin Chem. 2001; 47:1607-13.
38. Sozzi G, Roz L, Conte D, Mariani L, Andriani F, Verderio P. Effects of prolonged storage of whole plasma or isolated plasma DNA on the results of circulating DNA quantification assays. J Natl Cancer Inst. 2005; 97: 1848-50.
39. Risberg B, Tsui DWY, Biggs H, Almagro ARV, Dawson SJ, Hodgkin C, et al. Effects of Collection and Processing Procedures on Plasma Circulating Cell-Free DNA from Cancer Patients. J Mol Diagn. 2018; 20: 883-92.
40. Hidestrand M, Stokowski R, Song K, Oliphant A, Deavers J, Goetsch M, et al. Influence of temperature during transportation on cell-free DNA analysis. Fetal Diagn Ther. 2012; 31: 122-8.
41. Angert RM LE, Lo YM, Chan LY, Delli-Bovi LC, Bianchi DW. Fetal cell-free plasma DNA concentrations in maternal blood are stable 24 hours after collection: analysis of first- and third-trimester samples. Clin Chem. 2003; 49: 195-8.
42. Xue X TM, Holen I, Zhu YM, Woll PJ. Optimizing the yield and utility of circulating cell-free DNA from plasma and serum. Clin Chim Acta. 2009; 404: 100-4.
43. Vermeulen C, Geeven G, Wit Ed, Verstegen MJAM, Jansen RPM, Kranenburg MV, et al. Sensitive Monogenic Noninvasive Prenatal Diagnosis by Targeted Haplotyping. The American Journal of Human Genetics. 2017; 101: 326-39.
44. Schmidt B WS, Witt C. Improved Method for Isolating Cell Free DNA. Clin Chem. 2005; 51: 1561-3.
45. Sozzi G, Conte D, Mariani L, Vullo SL, Roz L, Lombardo C, et al. Analysis of Circulating Tumor DNA in Plasma at Diagnosis and during Follow-Up of Lung Cancer Patients. CANCER RESEARCH. 2001; 61: 4675-8.
46. Fong SL ZJ, Lim CK, Eu KW, Liu Y. Comparison of 7 methods for extracting cell-free DNA from serum samples of colorectal cancer patients. 2009; 55: 587-98.
47. Fleischhacker M, Schmidt B, Weickmann S, Fersching DMI, Leszinski GS, Siegele B, et al. Methods for isolation of cell-free plasma DNA strongly affect DNA yield. Clin Chim Acta. 2011; 412: 2085-8.
48. Sorber L, Zwaenepoel K, Deschoolmeester V, Roeyen G, Lardon F, Rolfo C, et al. A Comparison of Cell-Free DNA Isolation Kits: Isolation and Quantification of Cell-Free DNA in Plasma. J Mol Diagn. 2017; 19: 162-8.
49. Devonshire AS, Whale AS, Gutteridge A, Jones G, Cowen S, Huggett CA JF. Towards standardisation of cell-free DNA measurement in plasma: controls for extraction efficiency, fragment size bias and quantification. Anal Bioanal Chem. 2014; 406: 6499-512.
50. Dressman D YH, Traverso G, Kinzler KW, Vogelstein B. Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. Proc Natl Acad Sci USA. 2003; 100: 8817-22.

51. Wang BG, Huang HY, Chen YC, Bristow RE. Increased plasma DNA integrity in cancer patients. Cancer Res. 2003; 63: 3966-8.

52. Cristiano S, Leal A, Phallen J, Fiksel J, Adleff V, Brumn DC, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature. 2019; 570: 385-9.

53. Cheng ML, Solit DB. Opportunities and Challenges in Genomic Sequencing for Precision Cancer Care. Ann Intern Med. 2018; 168: 221-2.

54. Calvez FL, Ahman A, Tonisson N, Lambert J, Temam S, Brennan P, et al. Arrayed primer extension resequencing of mutations in the TP53 tumor suppressor gene: comparison with denaturing HPLC and direct sequencing. Clin Chem. 2005; 51: 1284-7.

55. Salk JJ, Schmitt MW, Loeb LA. Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations. Nat Rev Genet. 2018; 19: 269-85.

56. Grasselli J, Elez E, Caratu G, Matito J, Santos C, Macarulla T, et al. Concordance of blood- and tumor-based detection of RAS mutations to guide anti-EGFR therapy in metastatic colorectal cancer. Ann Oncol. 2017; 28: 1294-301.

57. Yang X, Zhou Q, Zhou W, Zhong M, Guo X, Wang Y, et al. A Cell-free DNA Barcode-Enabled Single-Molecule Test for Noninvasive Prenatal Diagnosis of Monogenic Disorders: Application to beta-Thalassemia. Adv Sci (Weinh). 2019; 6: 1802332.

58. Adalsteinsson VA, Ha G, Freeman SS, Choudhury AD, Stover DG, Parsons HA, et al. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. Nat Commun. 2017; 8: 1324.

59. Supplee JG, Milan MSD, Lim LP, Potts KT, Sholl LM, Oxnard GR, et al. Sensitivity of next-generation sequencing assays detecting oncogenic fusions in plasma cell-free DNA. Lung Cancer. 2019; 134: 96-9.

60. Chiu Chin Yuan WM, David Waters. A quantification of human cells using an ERV-3 real time PCR assay. Journal of Virological Methods. 2001; 91: 109-17.

61. Buder A, Setinek U, Hochmair MJ, Schwab S, Kirchbacher K, Keck A, et al. EGFR Mutations in Cell-free Plasma DNA from Patients with Advanced Lung Adenocarcinoma: Improved Detection by Droplet Digital PCR. Target Oncol. 2019; 14: 197-203.

62. Beck J, Hennecke S, Bornemann-Kolatzki K, Urnovitz HB, Neumann S, Strobel P, et al. Genome aberrations in canine mammary carcinomas and their detection in cell-free plasma DNA. PLoS One. 2013; 8: e75485.

63. Ballester LY, Glitza Oliva IC, Douse DY, Chen MM, Lan C, Haydu LE, et al. Evaluating Circulating Tumor DNA From the Cerebrospinal Fluid of Patients With Melanoma and Leptomeningeal Disease. J Neuropathol Exp Neurol. 2018; 77: 628-35.

64. Snyder MW, Simmons LE, Kitzman JO, Coe BP, Henson JM, Daza RM, et al. Copy-number variation and false positive prenatal aneuploidy screening results. N Engl J Med. 2015; 372: 1639-45.

65. Wood DE, White JR, Georgiadis A, Van Emburgh B, Parpart-Li S, Mitchell J, et al. A machine learning approach for somatic mutation discovery. Science Translational Medicine. 2018; 10.

66. Newman AM, Lovejoy AF, Klass DM, Kurtz DM, Chabon JJ, Scherer F, et al. Integrated digital error suppression for improved detection of circulating tumor DNA. Nature Biotechnology. 2016; 34: 547-55.

67. Lanman RB, Mortimer SA, Zill OA, Sebisanovic D, Lopez R, Blau S, et al. Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. PLoS One. 2015; 10: e0140712.

68. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010; 463: 899-905.

69. Cibulskis K, Lawrence MS, Carter SL, Sivachenko A, Jaffe D, Sougnez C, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol. 2013; 31: 213-9.

70. Fournie GJ, Gayral-Taminh M, Bouche JP, Conte JJ. Recovery of nanogram quantities of DNA from plasma and quantitative measurement using labeling by nick translation. Anal Biochem. 1986; 158: 250-6.

71. Coulet F, Blons H, Cabelguenne A, Lecomte T, Lacourreye O, Brasnu D, et al. Detection of plasma tumor DNA in head and neck squamous cell carcinoma by microsatellite typing and p53 mutation analysis. Cancer Res. 2000; 60: 707-11.

72. Janku F AP, Tsimberidou AM, Fu S, Naing A, Falchook GS, Hong DS, et al. Actionable mutations in plasma cell-free DNA in patients with advanced cancers referred for experimental targeted therapies. Oncotarget. 2015; 6: 12809-21.

73. Shen SY, Singhania R, Fehringer G, Chakravarthy A, Roehrl MHA, Chadwick D, et al. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. Nature. 2018; 563: 579-83.

74. Zhang J, Han X, Gao C, Xing Y, Qi Z, Liu R, et al. 5-Hydroxymethylome in Circulating Cell-free DNA as A Potential Biomarker for Non-small-cell Lung Cancer. Genomics Proteomics Bioinformatics. 2018; 16: 187-99.

75. Shen SY, Burgener JM, Bratman SV, De Carvalho DD. Preparation of cfMeDIP-seq libraries for methylome profiling of plasma cell-free DNA. Nat Protoc. 2019; 14: 2749-80.

76. Song CX, Yin S, Ma L, Wheeler A, Chen Y, Zhang Y, et al. 5-Hydroxymethylcytosine signatures in cell-free DNA provide information about tumor types and stages. Cell Res. 2017; 27: 1231-42.

77. Li W, Zhang X, Lu X, You L, Song Y, Luo Z, et al. 5-Hydroxymethylcytosine signatures in circulating cell-free DNA as diagnostic biomarkers for human cancers. Cell Res. 2017; 27: 1243-57.

78. Zeng H, He B, Xia B, Bai D, Lu XY, Cai JB, et al. Bisulfite-Free, Nanoscale Analysis of 5-Hydroxymethylcytosine at Single Base Resolution. Journal of the American Chemical Society. 2018; 140: 13190-4.

79. Laken SJ, Jackson PE, Kinzler KW, Vogelstein B, Strickland PT, Groopman JD, et al. Genotyping by mass spectrometric analysis of short DNA fragments. NATURE BIOTECHNOLOGY. 1998; 16: 1352-6.

80. Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci USA. 2005; 102: 16368-73.

81. Mouliere F, Chandrananda D, Piskorz AM, Moore EK, Morris J, Ahlborn LB, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. Sci Transl Med. 2018; 10.

82. Miller AM, Shah RH, Pentsova EI, Pourmaleki M, Briggs S, Distefano N, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. Nature. 2019; 565: 654-8.

83. Janku F, Huang HJ, Fujii T, Shelton DN, Madwani K, Fu S, et al. Multiplex KRASG12/G13mutation testing of unamplified cell-free DNA from the plasma of patients with advanced cancers using droplet digital polymerase chain reaction. Annals of Oncology Advance Access. 2017; 28: 642-50.

84. Sacher AG, Paweletz C, Dahlberg SE, Alden RS, O'Connell A, Feeney N, et al. Prospective Validation of Rapid Plasma Genotyping for the Detection of EGFR and KRAS Mutations in Advanced Lung Cancer. JAMA Oncol. 2016; 2: 1014-22.

85. Hong DS, Morris VK, El Osta B, Sorokin AV, Janku F, Fu S, et al. Phase IB Study of Vemurafenib in Combination with Irinotecan and Cetuximab in Patients with Metastatic Colorectal Cancer with BRAFV600E Mutation. Cancer Discov. 2016; 6: 1352-65.

86. Schiavon G, Hrebien S, Garcia-Murillas I, Cutts RJ, Pearson A, Tarazona N, et al. Analysis of ESR1 mutation in circulating tumor DNA demonstrates evolution during therapy for metastatic breast cancer. Sci Transl Med. 2015; 7: 313ra182.

87. Arend RC, Londono AI, Montgomery AM, Smith HJ, Dobbin ZC, Katre AA, et al. Molecular Response to Neoadjuvant Chemotherapy in High-Grade Serous Ovarian Carcinoma. Mol Cancer Res. 2018; 16: 813-24.

88. Herrmann S, Zhan T, Betge J, Rauscher B, Belle S, Gutting T, et al. Detection of mutational patterns in cell-free DNA of colorectal cancer by custom amplicon sequencing. Mol Oncol. 2019; 13: 1669-83.

89. Odogwu L, Mathieu L, Goldberg KB, Blumenthal GM, Larkins E, Fiero MH, et al. FDA Benefit-Risk Assessment of Osimertinib for the Treatment of Metastatic Non-Small Cell Lung Cancer Harboring Epidermal Growth Factor Receptor T790M Mutation. Oncologist. 2018; 23: 353-9.

90. Warren JD, Xiong W, Bunker AM, Vaughn CP, Furtado LV, Roberts WL, et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Medicine. 2011; 9: 1-9.