Comparison of Archival Plasma and Formalin-Fixed Paraffin-Embedded Tissue for Genotyping in Hepatocellular Carcinoma

Malin I.L. Sjöholm,[1,2,4] Gunilla Hoffmann,[3] Stefan Lindgren,[3] Joakim Dillner,[2,4] and Joyce Carlson[1,4]

Departments of [1]Clinical Chemistry, [2]Medical Microbiology, and [3]Gastroenterology; and [4]the Swedish National Biobanking Program, Lund University, Malmö University Hospital, Malmö, Sweden

Abstract

Biobanks containing formalin-fixed paraffin-embedded tissue, as well as frozen serum or plasma, are important resources for molecular epidemiologic studies. However, few studies have compared the reliability of formalin-fixed tissue samples and archival plasma samples for genotyping. We determined the genotype of four proposed genetic risk factors for hepatocellular carcinoma [hereditary hemochromatosis (HFE 63 and 282), α_1-antitrypsin deficiency (AAT 342) and cystic fibrosis (CFTR 508)] on formalin-fixed tissue samples, stored for up to 25 years, from 318 patients diagnosed with hepatocellular carcinoma and on plasma or serum samples from 31 of these patients. The genotypes were analyzed by RFLP or allele-specific amplification as well as by TaqMan assays. In addition, genotyping was attempted after whole genome amplification by multiple displacement amplification (MDA). Genotyping was successful in 94% of the tissue samples and successful and identical to the tissue samples from the same subjects in 98% of the plasma/serum samples. DNA from plasma samples could be amplified >5,000-fold by MDA and genotyping after MDA gave identical results to the genotyping of the same subjects before whole genome amplification. MDA amplification of the tissue samples was not successful. In summary, archival plasma was found to be an adequate source of efficiently amplifiable DNA. MDA on plasma samples allows analysis of multiple genotypes in epidemiologic studies. (Cancer Epidemiol Biomarkers Prev 2005;14(1):251–5)

Introduction

The use of the huge biobanks containing biological material that have been collected for routine healthcare purposes has enabled efficient use of informative study designs in molecular epidemiology, notably case-control studies nested in cohorts of samples collected before disease occurrence. Although this approach has been highly successful in a large number of epidemiologic studies (1-3), genetic epidemiology has not exploited such study designs to the same extent because the largest health care biobanks contain sample types considered suboptimal for genotyping purposes. For example, a survey at the University Hospital in Malmö found that >8,000,000 tissue or cytology samples were stored in the diagnostic pathology department and >800,000 serum or plasma samples were stored in the diagnostic microbiology department (see http://www.biobanks.se/).

Even short-term formalin treatment of tissue has been shown to reduce DNA solubility and induce DNA degradation. Denaturation is initiated at the AT-rich regions of double-stranded DNA by interchain hydrogen bond breakage and unstacking of bases (4). DNA from formalin-fixed tissues exhibits a high frequency of non-reproducible sequence alterations compared with frozen tissue. This could be due to cross-linking of cytosine nucleotides by the formalin. The Taq polymerase in a PCR reaction is thereby unable to recognize the cytosine and incorporates an adenine instead of a guanine creating C to T or G to A mutations (4).

Reports on the content of genomic DNA in serum or plasma in healthy subjects have shown a wide range from nil to 4 μg/mL (1 ng DNA corresponds to ~270 copies of the human genome; refs. 5-10). Sample handling techniques may affect the DNA content, e.g., when harvesting plasma from EDTA blood samples, some of the buffy coat cells may be included (8). Also, prolonged storage of blood samples before harvesting serum may increase the DNA content due to lysis of nucleated cells after clot formation (5).

One possible approach to overcome the problem of low and variable amounts of DNA in serum and plasma samples is the use of whole genome amplification techniques such as multiple displacement amplification (MDA). The MDA technique was originally developed for amplification of circular templates (11) but has recently been modified for amplification of linear templates (12). This isothermal procedure uses bacteriophage ϕ29 DNA polymerase to catalyze strand-displacement amplification of the template with random hexamers as primers. ϕ29 DNA polymerase contains a proofreading activity with a very low error rate (13).

We wished to compare the reliability of genetic studies done on biobanks comprised of formalin-fixed paraffin-embedded autopsy tissue with banks of frozen EDTA plasma or serum samples from the same donors, both stored for 10 to 30 years. Genetic traits proposed to associate with hepatocellular carcinoma (HCC), i.e., the single nucleotide polymorphisms (SNP) causing α_1-antitrypsin deficiency (AAT E342K; ref. 14) and hereditary hemochromatosis (HFE C282Y and HFE H63D; ref. 15) as well as the triplet deletion causing cystic fibrosis (CFTR ΔF508; ref. 16) were analyzed.

Received 6/17/04; revised 7/30/04; accepted 8/18/04.

Grant support: Grants from the MAS Cancer Fund, the Swedish Cancer Society, the Swedish Society of Medicine and the Lundström Foundation. The Swedish National Biobanking Program is funded by the Knut and Alice Wallenberg Foundation via SWEGENE and the Wallenberg Consortium North.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Joyce Carlson, Clinical Chemistry, University Hospital MAS, Entrance 71, 205-02 Malmö, Sweden. Phone: 46-4033-1493; Fax: 46-4033-6296.
E-mail: Joyce.Carlson@klkemi.mas.lu.se

Copyright © 2005 American Association for Cancer Research.

Downloaded from cebp.aacrjournals.org on April 19, 2021. © 2005 American Association for Cancer Research.

Materials and Methods

This study was approved by the Institutional Review Board of Lund University.

Patient Samples. From 1974 to 1996, 384 patients resident in Malmö were reported to the Swedish Cancer Registry with the diagnosis of primary hepatocellular carcinoma. This corresponds to an incidence of 6.7/100,000/year. Autopsy had been done in 318 of these cases (82.8%) and liver tissues were still available for all of these 318 patients. All autopsies were done within 3 days of death. Tissue was fixed in 10% neutral formalin for ~1 week and tissue blocks (of both normal and tumor tissue) of about 1 × 1 × 1/2 cm were then dehydrated and embedded in paraffin. The blocks were stored at room temperature. Samples consisting of 10 sections, 5 μm in thickness, of formalin-fixed paraffin-embedded normal liver tissue from the HCC cases as well as from one recently deceased anonymous autopsy case were obtained, in 1.5 mL polyethylene tubes, from the Pathology Department at Malmö University Hospital.

Serum and plasma samples from all patients investigated for liver disease at the Department of Internal Medicine of the University Hospital in Malmö since 1978 have been stored at −20°C. The number of freeze-thaw cycles from the time of sampling is not known. Thirty-two of these 384 HCC patients (31 of the 318 autopsied cases) had serum and/or plasma samples in this collection (17).

Tissue DNA Extraction with QIAquick Method. DNA was extracted using the QIAquick Gel Extraction Kit and protocol with the following modifications: 180 μL digestion buffer [50 mmol Tris-HCl (pH 8.3), 1 mmol EDTA, 0.5% Tween 20 (Merck-Schuchardt, Stockholm, Sweden) was added to the tissue sections. They were then incubated at 80°C for 20 minutes and interrupted once for vortexing after 10 minutes. The samples were cooled to 56°C and 20 μL protease solution was added, followed by incubation at 56°C for at least 3 hours. Buffer QG (600 μL) and isopropanol (200 μL) were then added to the lysate and the protocol was followed from step 6. The DNA was eluted in 50 μL buffer EB.

Tissue DNA Extraction with Proteinase K Digestion/QIAamp Method. DNA was extracted as described by Forslund et al. (18). After heat inactivation of proteinase K, samples were centrifuged at 15,800 × *g* for 15 minutes and the supernatant was collected. The DNA was purified using the QIAamp 96 spin blood kit according to the manufacturer's instructions and nucleic acids were eluted in 100 μL sterile water.

Plasma DNA Extraction. DNA was extracted using the QIAamp MinElute Virus Spin kit according to the manufacturer's protocol with the following modifications: to each 1 mL sample of thoroughly mixed plasma or serum, we added 30 μg tRNA (Sigma, Stockholm, Sweden) in a 1.5 mL polyethylene tube. Each sample was mixed and divided into five aliquots of 200 μL. Each aliquot was incubated for 1 hour after the addition of lysis buffer. The protease was then immediately inactivated by boiling for 5 minutes at 96°C. After lysis, all five aliquots from each sample were filtered through the same column and nucleic acids were eluted twice in 60 μL 10 mmol Tris-HCl (pH 8.5). The samples were then vacuum-dried in a Speed Vac centrifuge and dissolved in 10 μL sterile water. In some cases, <1 mL plasma or a dried residue was present in the biobank sample tubes. These samples were reconstituted to 1 mL with sterile water or 0.9% sodium chloride. Genotyping results, but no quantitative data, are reported for the dried samples. Qiagen kits were purchased from VWR International, Sweden.

Internal Controls. A plasma pool was created from 1-day-old clinical EDTA-plasma samples after routine hematologic analyses. This pool contained 21 ng DNA/mL and was used to test extraction protocols. DNA extracts of known concentration, isolated from EDTA whole blood with the QIAamp mini-kit, and known to have each potential genotype were used as positive controls in genotype analyses after removal of donor identities.

Determination of DNA Concentration. The concentration of DNA in the liver tissue eluates was determined using the PicoGreen dsDNA Quantification Kit (Molecular Probes, Gothenburg, Sweden) according to manufacturer's instructions on a FLOUstar Optima plate reader (BMG Labtech, GmbH, Offenburg Germany). Due to the minimal amount of DNA in the plasma samples, the DNA quantity in these samples was determined by real-time PCR using 1 μL (10%) of the concentrated DNA eluate and the reagents designed for the CFTR ΔF508 TaqMan assay (see below).

Structural Integrity of Extracted DNA. The size of extracted DNA was evaluated by electrophoresis through a 0.8% agarose gel (SeaKem, Cambrex BioScience Rockland, Inc., Rockland, Maine) in Tris-borate EDTA buffer (0.09 mol/L Tris, 0.09 mol/L boric acid, and 1 mmol/L EDTA) stained with 0.04% ethidium bromide (Applichem, GmbH, Malmö, Sweden) and compared with the size of *Hin*dIII-digested lambda phage (Marker II, Roche Molecular Biochemicals, Stockholm, Sweden) mixed with a 50 bp marker (GeneRuler 50 bp DNA ladder, Fermentas, St. Leon-Rot, Germany).

Whole Genome Amplification by MDA. A titration series of DNA, ranging from 0.02 to 2 ng, from plasma and tissue samples heterozygous for at least one genotype, was subjected to MDA using the GenomiPhi Amplification Kit (Amersham Pharmacia Biosciences, Uppsala, Sweden) according to the manufacturer's protocol. The quantity of human DNA after amplification was determined by real-time PCR using the reagents designed for the CFTR ΔF508 TaqMan assay.

Gel Electrophoretic Fragment Size Assays. Standard PCR amplifications using 10 ng of HCC tissue DNA as template and 1.5 mM $MgCl_2$, were done for HFE C282Y, HFE H63D, AAT E342K, and CFTR ΔF508 mutations and genotypes were analyzed by RFLP or allele-specific amplification. Primer sequences and amplicon lengths are listed in Table 1. PCR amplification was initiated with denaturation at 95°C for 9 minutes, followed by cycles with annealing for 30 seconds at 65°C for the HFE analyses, at 58°C for the AAT and at 60°C for the CFTR analysis and synthesis at 72°C for 45 seconds. HFE H63D and HFE C282Y PCR products were cleaved with *Bcl*I and *Rsa*I (Fermentas), respectively. These fragments and the allele-specific AAT and CFTR PCR products were analyzed on a 4% agarose gel (SeaKem, Cambrex BioScience Rockland) in Tris-borate EDTA buffer-stained with 0.025% ethidium bromide (Applichem). Genotypes were determined by comparing the fragment sizes of each sample to those of corresponding controls and a molecular weight marker (Marker XIII, Roche Molecular Biochemicals).

TaqMan Assays. TaqMan assays were done as recommended by the manufacturer on a 7900HT (Applied Biosystems, Foster City, CA, USA) using TaqMan MGB "assay by design" reagents, no AmpErase UNG (P/N 4354018) Master Mix and 50 PCR cycles. Reaction mixtures (25 μL) contained 1 to 10 ng tissue DNA (1 ng in most cases) as template and 0.125 μL assay mix. Primers and probes are listed in Table 2. An annealing temperature of 58°C was used for the AAT E342K assay.

Real-time PCR for DNA quantification monitored fluorescence of the wild-type CFTR ΔF508 allele during PCR followed by end-point measurements to verify genotypes. In the presence of the rare CFTR ΔF508 allele, real-time PCR was repeated, monitoring fluorescence of the wild-type factor II 20210 G allele followed by end-point measurements.

Downloaded from cebp.aacrjournals.org on April 19, 2021. © 2005 American Association for Cancer Research.

Table 1. Primer sequences for the gel electrophoresis assays

Genotype	Primer sequences	Amplicon length (bp)
HFE H63D	Forward 5′-GAC CTT GGT CTT TCC TTG TTT GAA GC-3′	90
	Reverse 5′-GGG CTC CAC ACG GCG AC-3′	
HFE C282Y	Forward 5′CCA GGG CTG GAT AAC CTT GGC T-3′	101
	Reverse 5′-CCC AGA TCA CAA TGA GGG GCT G-3′	
AAT E342K	Forward 5′-GCC TGG GAT CAG CCT TAC AAC GT-3′	127
	Reverse 5′-CAT GGG TAT GGC CTC TAA AAA CAT GG-3′	
	PiZ ASO + 5′-CAT AAG GCT GTG CTG ACC ATC AAC AA-3′	PiZ, 75
	PiM ASO − 5′-CCA GCA GCT TCA GTC CCT TCC TC-3′	PiM, 99
CFTR ΔF508	Forward 5′-TTC TGT TCT CAG TTT TCC TGG AT-3′	120
	Reverse 5′-TCT TAC CTC TTC TAG TTG GCA TG-3′	
	Inner F 5′-GCA CCA TTA AAG AAA ATA TCA TCT T-3′	89
	Inner R 5′-ACC AAT GAT ATC TTC TTT AAT GGT G-3′	57

Our laboratory routinely uses three positive (wt/wt, wt/mut, and mut/mut) internal controls and a nontemplate control in each batch of genotype assays. It performs stringent quality control based on statistical analysis of fluorescent signal intensity for TaqMan assays. We subscribe to available external quality assurance programs and are accredited by the Swedish Board for Accreditation and Conformity Assessment to perform diagnostic DNA analyses.

Results

Formalin-Fixed, Paraffin-Embedded Tissue. Ten sections of 5 μm formalin-fixed paraffin-embedded liver tissue from an anonymous autopsy case were extracted using the QIAquick and the proteinase K/QIAamp method. Comparison of DNA amounts, measured by PicoGreen fluorescence assay, and amplifiable DNA amounts measured by real-time PCR found that ~40% of the DNA was functional, i.e., of sufficient quality to be detectable by real-time PCR. The linear range of the quantification was between 0.1 and 3.2 ng of DNA.

Ten sections of 5 μm formalin-fixed paraffin-embedded liver tissue from 318 HCC patients were extracted using the modified QIAquick protocol. The amount of DNA obtained ranged from 0.01 to 1.6 μg/10 × 5 μm with a mean of ± 0.02 (SEM) μg/10 × 5 μm, as determined by PicoGreen fluorescence assay. The genotypes were determined by fragment size on gel electrophoresis and verified by TaqMan assays. Samples for which genotype determination was unsuccessful were re-extracted using the proteinase K method. The amount of DNA obtained using this method ranged from 0.9 μg/10 × 5 μm to 18.4 μg/10 × 5 μm with a mean of 6.6 ± 1 (SEM) μg/10 × 5 μm. Real-time PCR analysis of these samples was largely unsuccessful and the DNA was therefore purified using QIAamp 96 spin blood kits, reducing the amount of DNA obtained to 0.4 ± 0.1 (SEM) μg/10 × 5 μm. Despite the low amounts obtained, real-time PCR of purified samples was successful for 4/16 samples that had been unsuccessful before purification.

Genotyping was regarded as successful if identical results were obtained twice, generally by fragment size and TaqMan assays (Table 3A). This occurred in 295 samples for the HFE H63D, 302 samples for the HFE C282Y, 268 samples for the AAT E342K, and 282 samples for the CFTR assay of QIAquick extracted DNA. After proteinase K/QIAamp re-extraction, an additional 46 genotypes were successfully determined by TaqMan assays (Table 3A). Genotyping was successful in 94% (1193/1272) of tissues obtained between 1974 and 1995. Tissues obtained between 1974 and 1978 were the most difficult to genotype, especially for AAT E342K, for which the genotyping was successful in only 67% of the samples.

DNA extracted by both the QIAquick method and the proteinase K/QIAamp method was largely degraded and shorter than 1 kb (Fig. 1, *lanes 1-3*). Numerous attempts at MDA amplification of tissue DNA isolated by either method failed consistently.

Archival Plasma or Serum. Plasma was available from 30 of the 318 HCC patients, and serum from 1 additional patient. DNA from these samples was extracted with the QIAgen MinElute kit. The amount of DNA obtained ranged from 0.2 to 72 ng/mL (mean 14.4 ± 3.5 (SEM) ng/mL). The amount of DNA obtained from the serum sample was 7.4 ng/mL. The genotypes were determined by TaqMan assays using 1 μL or 100 genome copies as template. Genotyping was successful in 30/31 samples for HFE H63D and HFE C282Y and in 31/31 samples for AAT E342K and CFTR ΔF508 (Table 3B). DNA from all plasma and serum samples gave identical genotyping results as obtained from tissue DNA from the same subject.

Table 2. Primer and probe sequences for the TaqMan assays

Genotype	Primer sequences	Reporter sequences*	Amplicon length (bp)
HFE H63D	F 5′-GAT GAC CAG CTG TTC GTG TTG-3′	5′-VIC-CGA CTC TCA T**G**A TCA TA-MGB-3′	93
	R 5′-CCA CAT CTG GCT TGA AAT TCT ACT G-3′	5′-FAM-CGA CTC TCA T**C**A TCA TA-MGB-3′	
HFE C282Y	F 5′-GGC TGG ATA ACC TTG GCT GTA C-3′	5′- VIC-CCT GG**C** ACG TAT AT-MGB-3′	75
	R 5′-TCC AGG CCT GGG TGC TG-3′	5′-FAM-A CCT GG**T** ACG TAT AT C-MGB-3′	
AAT E342K	F 5′-GCC TGG GAT CAG CCT TAC AAC GT-3′	5′- VIC-AAC ATC GAC **G**AG AAA-MGB-3′	
	R 5′-CAT GGG TAT GGC CTC TAA AAA CAT GG-3′	5′-FAM-CC ATC GAC **A**AG AAA-MGB-3′	127
CFTR ΔF508	F 5′-GAT TAT GCC TGG CAC CAT TAA AG-3′	5′-VIC-A ACA CCA **AAG** ATG ATA TT-MGB-3′	79
	R 5′-TGC TTT GAT GAC GCT TCT GTA TCT A-3′	5′-FAM-CA CCA ATG ATA TTT T-MGB-3′	76

NOTE: F, forward primer; R, reverse primer.
*Genetic variants are in boldface.

Downloaded from cebp.aacrjournals.org on April 19, 2021. © 2005 American Association for Cancer Research.

Table 3. Genotyping results

Genotype	Successful and identical			Unsuccessful	Irreproducible
	wt/wt	wt/mut	mut/mut		
(A) Genotype results for tissue samples					
HFE H63D	245 (6)	56 (4)	4 (0)	(0)	13 (8)
HFE C282Y	286 (10)	22 (0)	4 (0)	(0)	6 (6)
AAT E342K	254 (10)	25 (4)	3 (0)	(4)	32 (15)
CFTR ΔF508	285 (12)	9 (0)	0 (0)	(9)	15 (13)
(B) Genotype results for plasma and serum samples*					
HFE H63D	25	5	0	1	0
HFE C282Y	24	5	1	1	0
AAT E342K	29	2	0	0	0
CFTR ΔF508	30	1	0	0	0

NOTE: Proteinase K/QIAamp extracted samples in parentheses.
*Compared to genotype determination of tissue samples from the same subjects.

The amount of DNA obtained from the plasma samples was insufficient to visualize on gels (Fig. 1, *lane 4*). To investigate the functional integrity of the DNA and provide additional material for subsequent analyses, a titration series of DNA, ranging from 0.02 to 2 ng, from three plasma samples with at least one heterozygous genotype was amplified by MDA. All samples were successfully amplified, producing a majority of DNA fragments of 4 → 23 kb (Fig. 1, *lanes 5-7*). Real-time PCR of MDA products showed a 5,000- to 43,000-fold amplification when 0.2 ng plasma DNA was used as MDA template. All genotypes obtained from MDA products using >0.02 ng DNA as template were identical to the genotypes determined using DNA extracts from plasma or tissue directly. However, heterozygosity disappeared in two samples when only 0.02 ng (~ five genome copies) was used as MDA template. This effect was seen in several different dilutions of the MDA product and was therefore not due to insufficient DNA in the actual post-MDA genotype analyses.

Discussion

We found that DNA extracted from both archival tissue and plasma samples was sufficient for successful genotyping of nearly all samples tested. Furthermore, concordance of results from plasma and tissue DNA validates the genotypes obtained and precludes the presence of artifactual results due to sample handling procedures. A previous study reported 98% genotyping concordance between DNA extracted from freshly frozen tissue and serum (9). Identical genotyping results with DNA extracted from serum and whole blood have also been found (19). Our highly successful MDA amplification of the plasma samples shows the structural integrity of DNA in archival plasma, even when stored under suboptimal conditions for >20 years. The success of this method also provides the possibility of performing numerous rather than a very select few genotyping assays using the enormous plasma or serum biobanks that have previously been regarded as useless for this purpose.

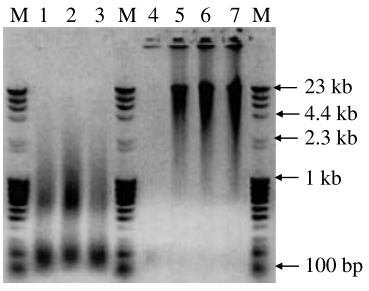

Figure 1. Gel analysis of tissue and plasma DNA size prior to, and after MDA. Approximately 100 ng of DNA isolated from three liver tissue samples (using the proteinase K digestion/QIAamp method); *lanes 1-3,* 50 ng DNA isolated from plasma; *lane 4,* 1 μL MDA product from three plasma samples; *lanes 4-7* were analyzed on a 0.8% agarose gel with a high molecular marker (*M*).

Although vast amounts of tissue and serum of potential interest for genetic studies are present in pathologic tissue archives and serum banks, the extraction and subsequent analysis of DNA from this type of starting material has presented a considerable challenge. The percentage of tissue samples that were successfully genotyped in this study can be compared with those of other studies; 97% for HFE H63D and HFE C282Y using an extraction buffer containing Nonidet P-40 (20), 52% for cytochrome *P*450 2D6 gene using Chelex-100 on samples stored for >10 years (21), up to 95% (22) or 88% for the β-globin gene (23), and 94% for BRCA1 mutations (24). The three latter publications used extraction methods similar to our proteinase K method.

In agreement with our observations, several of the abovementioned authors noted that the success of genotyping decreased with increasing storage time (21, 24) and amplicon length (24). For this reason, genotyping assays were designed to have the shortest possible amplicons. Because gel analysis patterns from the subsequent small fragments were difficult to interpret and because of the substantial decrease in hands-on time required, we turned to automated TaqMan assays. Prolonged delay between death and autopsy can be excluded as a cause of analytic failure. Only two of the irreproducible cases (from 1991 and 1994) had experienced delays of 5 and 4 days, respectively. The other cases had been autopsied within 2 days of death. The overall low success rate (84%) of the oldest samples, obtained at autopsy between 1974 and 1978, could either be due to differences in tissue processing (tissue dehydration techniques have varied over time) or to DNA alterations, presumably due to formalin, occurring slowly over time. It was therefore particularly important to analyze the few available plasma samples saved from these HCC cases.

The AAT E342K genotype was the most difficult to determine. The site of the AAT SNP is close to the telomere on chromosome14q32.1 (25) and corresponds to a CpG methylation. This and the AAT amplicon length (necessary for specific amplification among numerous homologous sequences) may partly explain the highly irreproducible results observed in repeated AAT SNP analyses of particularly the oldest tissues.

Most of the ''irreproducible'' samples produced at least two different genotypes on repeated assays. This phenomenon may

Downloaded from cebp.aacrjournals.org on April 19, 2021. © 2005 American Association for Cancer Research.

be explained by allelic drop-out because of single-allelic representation in the template or because of uneven allelic amplification in the PCR reaction. Thus, a selection bias against heterozygous genotypes may be present.

This study shows the need to adapt methods to the specific requirements and constraints of the available materials. We have, in the light of these experiences, developed specific strategies for the handling of tissue and plasma samples. DNA from tissue samples (where sufficient materials for repeat extraction are generally available) are extracted, purified, quantified by PicoGreen fluorescence assay for determination of the optimal template amount, and then genotyped. The sparse amounts of DNA present in archival serum and plasma samples, on the other hand, do not allow extensive analyses. The DNA from plasma samples is therefore extracted and quantified by real-time PCR. More than 0.2 ng of DNA is then subjected to whole genome amplification by MDA to ensure bi-allelic representation. MDA products are then quantified by real-time PCR to determine the optimal amounts of template before genotyping is done. The minimum amount of DNA we found to be necessary for use as MDA template without losing bi-allelic representation is similar to other studies where allelic drop-outs were seen when using <0.05 ng of DNA (26).

Quantification by PicoGreen fluorescence assay, as opposed to real-time PCR, does not discriminate between fragmented and functional DNA. In addition, the PicoGreen assay is subject to interference by some impurities and does not measure ssDNA. Real-time PCR is particularly preferred when quantifying MDA products as nonspecific amplification products may occur in negative controls (12) as well as in unsuccessful reactions and these may be detected in the PicoGreen assay.

In summary, archival plasma/serum seems to be a most useful starting material for genetic epidemiologic studies, particularly if whole genome amplification using the MDA technology is applied before genotyping.

Acknowledgments

We thank Christina Möller for retrieval of tissue samples, Christer Halldén for advice concerning the QIAquick isolation method, Eva Johansson, Ylva Johnson, and Frida Johansson for isolating DNA with the QIAquick method from tissue samples and for the fragment size genotyping analyses.

References

1. Hakama M, Aaran RK, Alfthan G, et al. Linkage of serum sample bank and cancer registry in epidemiological studies. Prog Clin Biol Res 1990;346:169–78.
2. Helicobacter and Cancer Collaborative Group. Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case control studies nested within prospective cohorts. Gut 2001;49:347–53.
3. Lehtinen M, Luukkaala T, Wallin KL, et al. Human papillomavirus infection, risk for subsequent development of cervical neoplasia and associated population attributable fraction. J Clin Virol 2001;22:117–24.
4. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. Am J Pathol 2002;161:1961–71.
5. Steinman CR. Free DNA in serum and plasma from normal adults. J Clin Invest 1975;56:512–5.
6. Wu TL, Zhang D, Chia JH, Tsao KH, Sun CF, Wu JT. Cell-free DNA: measurement in various carcinomas and establishment of normal reference range. Clin Chim Acta 2002;321:77–87.
7. Sozzi G, Conte D, Mariani L, et al. Analysis of circulating tumor DNA in plasma at diagnosis and during follow-up of lung cancer patients. Cancer Res 2001;61:4675–8.
8. Stemmer C, Beau-Faller M, Pencreac'h E, et al. Use of magnetic beads for plasma cell-free DNA extraction: toward automation of plasma DNA analysis for molecular diagnostics. Clin Chem 2003;49:1953–5.
9. Blomeke B, Bennett WP, Harris CC, Shields PG. Serum, plasma and paraffin-embedded tissues as sources of DNA for studying cancer susceptibility genes. Carcinogenesis 1997;18:1271–5.
10. 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.
11. Dean FB, Hosono S, Fang L, et al. Comprehensive human genome amplification using multiple displacement amplification. Proc Natl Acad Sci U S A 2002;99:5261–6.
12. Lage JM, Leamon JH, Pejovic T, et al. Whole genome analysis of genetic alterations in small DNA samples using hyperbranched strand displacement amplification and array-CGH. Genome Res 2003;13:294–307.
13. Esteban JA, Salas M, Blanco L. Fidelity of phi 29 DNA polymerase. Comparison between protein-primed initiation and DNA polymerization. J Biol Chem 1993;268:2719–26.
14. Jeppsson JO. Amino acid substitution Glu leads to Lys α_1-antitrypsin PiZ. FEBS Lett 1976;65:195–7.
15. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399–408.
16. Rommens JM, Iannuzzi MC, Kerem B, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989;245:1059–65.
17. Carlson J, Eriksson S. α_1-Antitrypsin and other acute phase reactants in liver disease. Acta Med Scand 1980;207:79–83.
18. Forslund O, Nordin P, Hansson BG. Mucosal human papillomavirus types in squamous cell carcinomas of the uterine cervix and subsequently on fingers. Br J Dermatol 2000;142:1148–53.
19. Andolfatto S, Namour F, Garnier AL, Chabot F, Gueant JL, Aimone-Gastin I. Genomic DNA extraction from small amounts of serum to be used for α_1-antitrypsin genotype analysis. Eur Respir J 2003;21:215–9.
20. Przygodzki RM, Goodman ZD, Rabin L, et al. Hemochromatosis (HFE) gene sequence analysis of formalin-fixed, paraffin-embedded liver biopsy specimens. Mol Diagn 2001;6:227–32.
21. Coombs NJ, Gough AC, Primrose JN. Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. Nucleic Acids Res 1999;27:e12.
22. Chan PK, Chan DP, To KF, Yu MY, Cheung JL, Cheng AF. Evaluation of extraction methods from paraffin wax embedded tissues for PCR amplification of human and viral DNA. J Clin Pathol 2001;54:401–3.
23. Cao W, Hashibe M, Rao JY, Morgenstern H, Zhang ZF. Comparison of methods for DNA extraction from paraffin-embedded tissues and buccal cells. Cancer Detect Prev 2003;27:397–404.
24. Bernstein JL, Thompson WD, Casey G, et al. Comparison of techniques for the successful detection of BRCA1 mutations in fixed paraffin-embedded tissue. Cancer Epidemiol Biomarkers Prev 2002;11:809–14.
25. Billingsley GD, Walter MA, Hammond GL, Cox DW. Physical mapping of four serpin genes: α_1-antitrypsin, α_1-antichymotrypsin, corticosteroid-binding globulin, and protein C inhibitor, within a 280-kb region on chromosome I4q32.1. Am J Hum Genet 1993;52:343–53.
26. Schneider PM, Balogh K, Naveran N, et al. Whole genome amplification—the solution for a common problem in forensic casework? Int Congress Ser 2004;1261:24–6.

Downloaded from cebp.aacrjournals.org on April 19, 2021. © 2005 American Association for Cancer Research.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

Comparison of Archival Plasma and Formalin-Fixed Paraffin-Embedded Tissue for Genotyping in Hepatocellular Carcinoma

Malin I.L. Sjöholm, Gunilla Hoffmann, Stefan Lindgren, et al.

Cancer Epidemiol Biomarkers Prev 2005;14:251-255.

Updated version	Access the most recent version of this article at: http://cebp.aacrjournals.org/content/14/1/251

Cited articles	This article cites 25 articles, 11 of which you can access for free at: http://cebp.aacrjournals.org/content/14/1/251.full#ref-list-1
Citing articles	This article has been cited by 4 HighWire-hosted articles. Access the articles at: http://cebp.aacrjournals.org/content/14/1/251.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cebp.aacrjournals.org/content/14/1/251. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.

Downloaded from cebp.aacrjournals.org on April 19, 2021. © 2005 American Association for Cancer Research.