cancer location and features corresponded to those seen in the frozen sections (fig 6).

Discussion

Our case presentation shows that an entire prostate can be successfully and rapidly examined from the histological point of view with macrocryosections.

The main advantages with the approach followed in this case are that the quality of the tissue is preserved and that the examination can be completed in a reasonably short time (table 1). The time from receiving the specimen to rendering the diagnosis to the clinician is approximately 1 h.

The requirements are those usually needed in routine frozen-section consultation—with

cryostats in good condition, including a low temperature in the chamber and a blade with a razor-like edge. The assistance of a skilled technician, like the one we had with us in the tissue-processing period, is necessary.

The major disadvantage is that a certain amount of tissue is lost when the first sections are discarded until complete ones are obtained. We did not calculate the amount of tissue discarded. However, our impression was that it was greater than that discarded when routinely processed material is cut with a microtome.

The limitation of the present description is that our experience is limited to a single case, even though this was successfully examined.

There was a previous short report on the feasibility of macrocryosectioning of the prostate.⁶ In that study, the authors reported their experience in 13 radical prostatectomies with clinically detected cancer. Only a single entire prostate slice per case was processed. They used a technique similar to ours. Comparison with permanent sections was not made.

Our report has some similarities with a recently published study, in which the frozen-section technique of the prostate was used in the setting of multiorgan donor cancer screening.⁷ In that study, however, only 50% of each specimen was analysed and macrosectioning was not performed.

Few papers have been published on the use of frozen-section examination of radical prostatectomy specimens with clinically detected cancer. In such cases, the intraoperative approach is used for the management of intraoperatively detected palpable tumour lesions during nerve-sparing scheduled radical prostatectomy and surgical margins, whereas the pathological examination of the main bulk of the prostate is performed on permanent sections.^{8–11}

In conclusion, macrocryosectioning of the prostate is feasible. However, more experience is needed with additional cases.

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Plasma DNA restoration for PCR applications

Plasma DNA is a valuable source of a wide range of molecular indicators that relate to the general condition and stage of cancer patients. However, continual patient monitoring and/or long-term studies require the use of archived plasma samples, frequently yielding a poor quality DNA upon storage. A restoration treatment was developed for plasma DNA that includes a pre-PCR incubation with *Taq* polymerase, dNTPs and 1× PCR buffer that improves the efficiency of subsequent PCRs. Pre-PCR treatment of isolated DNA resulted in superior PCR efficiency and sensitivity of detection both on a fresh plasma DNA isolate and on the plasma DNA isolates from 12 long-term archived plasma samples from lung cancer patients. This procedure allowed the recovery of a group of plasma samples considered otherwise useless. It is concluded that the quality of archived plasma DNA can be remarkably improved by subjecting the samples to a pre-PCR restoration treatment.

Human blood plasma from both healthy and sick individuals contains variable quantities of nucleic acids. The majority of the mechanisms proposed for its release from cells are related to cell death by necrosis or apoptosis, though active release has also been suggested.^{1,2} Patients suffering from different diseases where there is a clear increase in cell death have comparatively higher plasma DNA levels than healthy individuals.^{3,4} This phenomenon

Figure 4 Slice of prostate after de-freezing and fixation in formalin.

Figure 5 H&E-stained macrosection obtained after tissue fixation. (see also figs 2 and 4 for comparison).

Figure 6 Adenocarcinoma as seen in the permanent section (see fig 3 for comparison).

has been consistently observed in patients diagnosed with different cancers, whose plasma DNAs exhibit many genetic and epigenetic tumour DNA alterations, such as Ras and p53 mutations⁵⁻⁸ or microsatellite instability.⁹⁻¹⁰ This trait make plasma DNA a valuable diagnostic source for the assessment of different genetic alterations in tumours and is probably correlated with the general condition of the patient and the treatment response.^{4,7,11}

Large clinical studies must often be performed on archived plasma samples that have been stored for many years. It has been reported that DNAs present in long-term archived plasma samples show a progressive decrease in quality.¹² In some cases, plasma samples can become completely useless for the detection of low copy number sequences by PCR. We applied the restoration strategy outlined by Bonin *et al.*¹³ on one fresh plasma DNA isolate from a breast cancer patient and found an important increase in amplification efficiency, observed as a higher concentration of product and band intensity when visualized in agarose electrophoresis than in the non-restored sample. Similar results were obtained when the protocol was applied to plasma DNA isolates from a group of 12 long-term archived samples from lung cancer patients. After >1 year storage, none of these samples was suitable for genetic studies since no good quality DNA could be isolated from them. Application of the pre-PCR treatment described here noticeably improved PCR efficiency compared with non-restored isolates.

Materials and methods

Twelve archived plasma samples collected from July 2004 to July 2005 from 12 lung cancer patients were included in this study. All of the samples were processed as described below and the plasma was stored at -20°C . However, after 1 year of storage, no further DNA study could be performed since no good quality DNA could be obtained and PCR analysis no longer yielded positive amplification. For comparison, the pre-PCR treatment proposed here was applied both on DNA isolated from these samples and on a plasma DNA isolate from a breast cancer patient not subjected to long-term storage. All blood samples were collected by venipuncture, using EDTA as an anticoagulant, and centrifuged at 3000 *g* for 20 min at room temperature. The top plasma layer was transferred to a microfuge tube and further centrifuged at 8000 *g* for 3 min to remove cellular debris.

DNA extraction

DNA was extracted from 200 μl of archived or fresh plasma following the Blood and Body Fluid Spin protocol of the QIAamp[®] DNA Mini Kit (QIAGEN; Germany). A 1 μl aliquot of mussel glycogen (Sigma, catalogue number G1767) was added at the precipitation step, along with the AL solution from the Kit, and incubated at -20°C overnight before adding the whole mix to the column. The DNA was eluted with 100 μl of buffer AE.

Pre-PCR restoration treatment

The DNA restoration method is based on the filling and restoration of single-strand breaks in DNA molecules, using the complementary strand as template.^{13,14} Restoration of plasma DNA from all archived samples was carried out by mixing 20 μl of unrestored DNA of varying concentration (the low quality of archived

samples does not allow for accurate quantification) with PCR buffer and dNTPs (Promega, USA). The final reagent concentrations were as follows: 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 3.0 mM MgCl_2 and 200 mM of each dNTP; and the final volume was 25 μl . After incubation at 55°C for 1 h, 1 U of *Taq* DNA polymerase (Promega) was added and further incubated at 72°C for 20 min.

HBB amplification

Two sets of primers for the human *HBB* gene coding for the beta chain of haemoglobin (β -globin) were used to test restored and non-restored plasma DNA samples for amplification. The following primer sequences were used: amplicon 1 (110 bp), PCO3 (forward) 5'-ACACAACGTGTCTCACTAGC-3' and PCO4 (reverse) 5'-CAACTTCATCCACCTTCACC-3'; and amplicon 2 (171 bp), GLO1 (forward) 5'-AGCAACCTCACAAACAGACACC-3' and GLO2 (reverse) 5'-CTACACATGCCAGTTTC-3'.

For comparison, equivalent quantities of "non-restored" and "restored" counterparts must be used. Since the DNA samples are slightly diluted at the pre-PCR treatment, the volume of template must be proportionally

increased. Therefore, whereas 10 μl of template DNA were added from each non-restored isolate, 12.5 μl of template were used from each restored counterpart for PCR. Other conditions for both non-restored and restored DNA samples were identical: 7.5 pmol of each primer, 50 mM KCl, 3.0 mM MgCl_2 , 10 mM Tris-HCl (pH 9.0), 200 mM of each dNTP and 1 U of *Taq* polymerase (Promega), in a final volume of 25 μl . Cycling was performed in a MyCycler[™] thermal cycler (BioRad, Laboratories, USA), using the following cycling conditions: 95°C for 3 min; and 95°C for 20 s, 58°C for 20 s, 72°C for 20 s, for 40 cycles.

Results and discussion

The proposed mechanisms resulting in increased free DNA levels in the plasma of cancer patients include the lysis of circulating tumour cells, increased necrosis and/or apoptosis, and spontaneous/active release,¹⁵ all producing extensively degraded DNA. Although these mechanisms cannot fully explain the variability in DNA levels among different cancer patients or cancer types, it was demonstrated here that a pre-PCR treatment as proposed here is suitable to repair the damage on DNA due to single-chain

Figure 1 Lanes 1 and 3: amplification of the human *HBB* gene using two primer sets on non-restored fresh plasma DNA (from a patient with breast cancer), one for a 110 bp segment and the other for a 171 bp segment. Lanes 2 and 4: corresponding amplifications on restored DNA. As seen, pre-PCR treatment notably increases the efficiency of amplification. The gel used was 1 $\times$ TBE, 2% agarose. Equivalent volumes of post-PCR solution (10 μl) were loaded for all lanes.

Figure 2 Amplification of the 171 bp *HBB* gene fragment on four archived lung cancer patient plasma DNA isolates. All samples were processed in parallel. Lanes 1-4 show amplification using restored DNA and lanes 5-8 show the same four samples unrestored. Lane 9 is the negative control. The gel type is the same as that in fig 1.

breakage. This strategy improved amplification efficiency, as was demonstrated for the detection of a single-copy gene.

We examined the effects of the restoration technique on a freshly isolated plasma DNA, obtained from a breast cancer patient (fig 1). The freshly isolated, non-restored DNA sample showed positive amplification for both the 110 and 171 bp β -globin amplicons (lanes 1 and 3). The corresponding PCRs where restored breast cancer plasma DNA was used as template displayed higher efficiency for both primer sets assayed, based on the band intensity (lanes 2 and 4). The restoration treatment does not increase the length of DNA chains but repairs those sites where some nucleotides have been lost.¹³

Given the increase in amplification efficiency obtained from the freshly isolated sample, we decided to try the restoration technique on archived plasma DNA samples. We selected 12 plasma samples from lung cancer patients stored at -20°C for 1 year or longer. These samples were selected based on the inability to amplify the β -globin gene on DNA isolated from them using standard PCR procedures. Using equivalent DNA input for each non-restored–restored pair, the two amplicons assayed could be obtained from the 12 restored samples, unlike the non-restored controls in which the result continued to be negative. Four of the 12 samples are shown in fig 2, where lanes 1–4 (fig 2A) show positive amplification with restored DNA, and lanes 5–8 (fig 2B) show no amplification on the corresponding non-restored samples.

A benefit of the restoration technique involves the quantity of template needed to obtain similar amplification efficiencies between restored and non-restored samples. Figure 1 showed that the amplification efficiency of newly isolated plasma DNA can benefit from the restoration technique, requiring less DNA input, thereby increasing the number of experiments on a given isolate. This is beneficial if the sample is difficult to obtain, very rare or no longer available. Restored DNA from the sample shown in fig 2A, lane 2 could not only be amplified, but could also be diluted $>1:10$ and still produce the amplicon of interest (data not shown).

In summary, the quality of plasma DNA isolates obtained from archived samples can be significantly improved by introducing a pre-PCR treatment. The technique is based on the filling and restoration of single-strand breaks in DNA molecules, using the complementary strand as template. The procedure allowed the amplification of two short segments from a single-copy gene on samples which could not be amplified by other strategies. However, since the procedure involves a “de novo” synthesis of DNA chains, it is not suitable for the evaluation of epigenetic features of DNA such as the methylation status of specific genes.

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Take-home messages

- A simple pre-treatment step can improve the integrity of archived plasma DNA.
- The pre-treatment technique is modified from a protocol used to restore the integrity of archived paraffin-embedded tissue DNA samples. Restoration is based on “de novo” synthesis, where single-chain breaks are repaired using the complementary strand as template.
- DNA restoration improves the detection of many genetic features, but it is not applicable for the detection of epigenetic markers such as CpG island methylation status.

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The effect of automated test rejection on repeat requesting

We present our experience of the effect of automated test rejection and computerised reminders on repeat requesting behaviour of hospital clinicians and general practitioners. In 2000, four tests (thyroid-stimulating hormone (TSH), ferritin, glycated haemoglobin and B12 + folate), which are frequently requested, were selected. Following discussion with key local clinicians, test-specific trigger intervals within which a repeat was unlikely to show clinical change were decided. A computerised scheme of automatic test rejection was introduced in 2000 and the effect on these four tests assessed by retrospective interrogation of the laboratory database in 2001. The data supported the contention that tests are being unnecessarily repeated. Re-audit after 4 years looked at the effect of these reminders on physician repeat requesting practice. Against a background increased workload of 37.4% for these tests over the 4-year period, the mean percentage of declined tests fell from 4.0% in 2001 to 2.8% in 2005. This suggests a positive learning effect on requesting behaviour from continuous computerised reminders about appropriate test repetition intervals.

Introduction

The appropriate use of laboratory tests is necessary for optimal patient care. Laboratory utilisation has steadily increased during the past several decades in many health care jurisdictions around the world.^{1,2} Inappropriate requesting can make up a large proportion of laboratories' workloads and cost the health care system significant amounts of money. There have been numerous attempts over the years to modify the diagnostic test-ordering behaviour of physicians, mostly because of perceived, or real, overutilisation.^{3–7} Repeat testing is one component of requesting which can be modified. A study in an immunology laboratory in Hong Kong concluded that unnecessary test repeats within 12 weeks of previous analyses constituted 16.8% of its total workload, with an estimated annual cost consequence of US\$ 132 151 (£66 344).⁸

In this report we present our experience of the effect of automated test rejection and computerised reminders on repeat requesting behaviour of hospital clinicians and general practitioners (GPs).

Methods

The study was conducted in the Clinical Chemistry Laboratory in the Royal Devon and Exeter Hospital in South West England with a workload of 306 000 patient requests annually in 2000–01 rising to 427 000 in 2004–05.

In 2000, four tests (thyroid-stimulating hormone (TSH), ferritin, glycated haemoglobin and B12 + folate), which are frequently requested, were identified as suitable. A literature review was performed and, following