

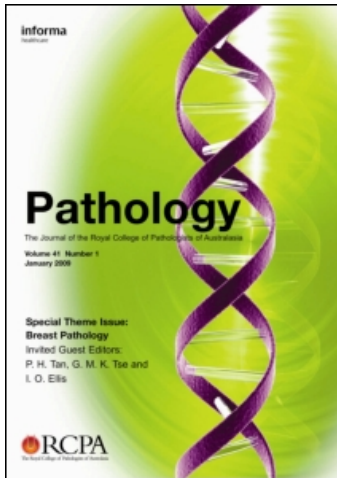
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MOLECULAR PATHOLOGY

Molecular confirmation of pathological specimen integrity in Australasia

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Summary

Aim: Investigations into 14 suspected pathology sample identification errors and mix-ups were performed, as a service for several public hospital and private laboratories, from 2005 to 2007.

Methods: Analyses were performed with the forensic ABI Identifiler kit of 16 microsatellites (15 autosomal and amelogenin) on DNA from paraffin-embedded tissues or blood specimens and compared to independently verified (single or multiple) patient samples.

Results: Of 23 unique patient specimens referred for sample integrity confirmation because of pathologist, clinician or patient concern, six (26.1%) were demonstrated to be discordant, indicating that specimen identification errors or mix-ups had occurred.

Conclusions: Due to their great sensitivity and high discrimination power, forensic identity multiplex systems using either microsatellites or single nucleotide polymorphisms (SNPs) can resolve concerns about pathology specimen identity and integrity.

Key words: Microsatellite, human identification, sample integrity.

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INTRODUCTION

Patient identification errors have been estimated to occur in 0.0055% of released pathology test results in a study by the College of American Pathologists.¹ These errors can affect all types of pathology specimens with potentially serious results. The ability to accurately and rapidly confirm tissue sample identity, in cases with suspect specimens, can greatly assist pathologists. Without the correct matching of individuals to appropriate specimens, further complex efforts undertaken to reduce diagnostic errors² or requirements for second opinions upon samples are worthless.

Specimen integrity error may occur at many stages in the analysis process. In the pre-laboratory setting, this may be due to a sample being labelled incorrectly in a doctor's office or operating theatre. Inappropriate use of pre-labelled specimen containers are a major source of this error.³ Extraneous tissue from a different patient may be incorporated into the paraffin sample or histological slides as 'floaters'. The rate of extraneous tissue in surgical or cytological cases has been estimated as 0.6% in a prospective study, which subsequently rose to 2.9% when viewed

retrospectively.³ Additional errors may occur during the testing process, or after laboratory analysis due to clerical errors. A meta-analysis of laboratory errors found the majority (68–87%) occurred at the pre- or post-analytic stages, not during the analytical process itself.⁴

Nevertheless under optimum work flow processes, the possibility of sample mix-up cannot be excluded and it has even been recently suggested that all patients undergoing radical therapy have a 'DNA time-out', whereby DNA identity tests are used to confirm integrity of patient samples.⁵ One published study has found that the incidence of no residual malignancy or 'vanishing cancer' on prostatectomy ranges from 0.07–0.5%⁶ and specimen integrity errors need clearly to be excluded.

Personal profiling facilitated by genotyping common polymorphic microsatellites (short tandem repeats) within the human genome in comparison with a reference sample can be used to determine the likelihood that samples do or do not come from the correct individual. This method has been utilised in specimen integrity queries by many laboratories.^{7,8}

Over the last 3 years this laboratory has performed 14 investigations into suspected histopathology or haematology specimen integrity error. This has been achieved by, in the majority of cases, the Identifiler kit (Applied Biosystems, USA) of 16 microsatellites (15 autosomal plus the sex-determining amelogenin marker). This is the same level of genotyping required for legally compliant paternity and forensic investigations. We describe our experience with nine cases involving concerns over specimen identity in prostate biopsy specimens; one case, six blood specimens; one case, eye tissue biopsies; one case, a skin biopsy query; and two cases of possible extraneous contaminating tissue on a breast and bone histology slide.

MATERIALS AND METHODS

Genomic DNA was extracted from the paraffin-embedded histology specimens initially by the use of the chelating resin Chelex 100 (BioRad, USA)⁹ and quantified prior to PCR amplification. Additionally, for difficult amplifications, a fast spin-column silica-gel membrane procedure was utilised (QIAamp DNA Mini Kit; Qiagen, Australia) in order to retrieve an effective amount of DNA.^{10,11} With difficult-to-extract DNA, the final DNA volume is kept low in order to maximise the concentration.⁸

A histopathologist identified appropriate normal or possible contaminating tissue on patient case slides for analysis. Specimens were photographed before and after removal of either representative or possible contaminating tissue used for DNA extraction.

The microsatellite multiplex in the Identifiler kit consists of a five-dye fluorescent system enabling automated DNA fragment analysis. This was run on an ABI 3130 Genetic Analyser instrument (Applied Biosystems) and data were processed using the GeneMapper ID v3.2 (Applied BioSystems) software. According to the allele frequencies of each microsatellite, for the appropriate ethnicity for the patient, the probability of any individual possessing the exact genotypes for all loci was calculated and then expressed as a percentage likelihood.^{12,13} This is thus derived by multiplying the minimum allele frequencies, from the database, for each allele identified by genotyping, across all the tested loci and is expressed as a percentage by further multiplication by 100. Therefore, the chance of an individual possessing, or sharing, a genotyping identity at all loci is a direct product of the frequency of all the alleles in their appropriate sub-population at all loci.⁸

The ethnicity of subjects was Caucasian except for one subject in Case B and another in Case C, both of which were of Asian origin. Ayres *et al.* have found the use of the broad racial subcategories of Caucasian, Asian and Aboriginal for forensic analysis in Australia to be appropriate.¹² Australian forensic short tandem repeat (STR) allele data are derived from Ayres *et al.* which includes Caucasian frequencies for each Australian state, as well as Asian and Aboriginal statistics.¹² Additional United States data for comparison, including Caucasian, African-American, Hispanic and Native, are taken from Lins *et al.*,¹³ and the Applied Biosystems AmpFlSTR Identifiler PCR Amplification Kit User's

Manual.¹⁴ Genotype profiles of all molecular genetic laboratory staff have been determined, in order to allow tracing and identification of any sources of contamination.

RESULTS

The 14 cases required testing of 23 separate patient identities. Of these, six (26.1%) were found to be discordant (Table 1). Of the concordant samples, the average probability of a similar match was $9.77 \times 10^{-6}\%$ (range $7.55 \times 10^{-5}\%$ – $7.8 \times 10^{-12}\%$) (Table 1).

The vast majority of tissue cases arose from prostate specimens (Table 2). Of these nine cases, three patient specimen identity errors were demonstrated (Fig. 1).

Four cases included non-prostate tissue samples (Table 3). Case E involved a mix-up of skin samples collected from a husband and wife in the same doctor's surgery at the same time. Therefore sex identification by typing the amelogenin locus, which confirmed that the samples had indeed been labelled correctly, was performed. One case involving possible contamination in a specimen of bone from the greater trochanter (Case H) was unable to be examined, as the amount of contaminating tissue was too small for adequate DNA extraction.

One case involved the examination of haematological samples (Case B; Table 4). This involved a suspected case of misconduct by a phlebotomist who was established to be labelling multiple same patient samples with multiple different patient labels.

The origin of referral of the 14 cases was predominantly from a pathologist, although referring doctors and patients also initiated queries about specimen integrity.

DISCUSSION

Forensic identity microsatellite multiplex systems have great sensitivity and high discrimination ability.⁸ This technique has great utility for resolving cases where inappropriate labelling or mix-up of histological slides has been postulated to have occurred, particularly for 'irreplaceable' specimens.

In the 14 identity genotyping investigations performed by this laboratory during the period 2005–2007, which included 31 specimens for 23 individuals, a total of six mismatches were identified. Four of these mismatches were

TABLE 1 Case results for individuals

	Concordant	Discordant
Case A	1	0
Case B	4	2 (13 & 13 alleles)
Case C	1	2 (20 & 21 alleles)
Case D	1 (3 samples same individual)	0
Case E	2*	0
Case F	1 (2 samples)	0
Case G	1	0
Case H	No result	No result
Case I	1	0
Case J	1 (6 samples)	0
Case K	1	0
Case L	1	1 (20 alleles)
Case M	1	1 (17 alleles)
Case N	1 (2 samples)	0
Total	17 (73.9%)	6 (26.1%)

The suspected samples were compared with newly collected buccal swab samples or previously archived paraffin embedded samples. The number of conflicting alleles for the discordant subjects is shown in brackets.

*In Case E only the amelogenin locus was tested.

TABLE 2 Tissue cases: prostate

Case	Details	Outcome
C	3 patient samples possible switch	2 samples switched, 1 consistent
D	1 patient (3 biopsies) identity query	All 3 matched verified buccal mucosa genotype (7.8×10^{-12})*
F	1 patient (2 biopsies) identity query	Matched verified buccal mucosa genotype (6.6×10^{-8})*
G	1 patient (1 biopsy sample) identity query	Matched verified blood sample genotype (3.7×10^{-9})*
I	1 patient (1 biopsy sample) identity query	Matched verified blood sample genotype (2.1×10^{-9})*
J	Query mislabelling of 1 patient's 6 biopsies with another collected on same day	All 6 samples matched previous paraffin embedded sample genotype; confirmed labelling correct
K	1 patient (1 biopsy) sample identity query	Matched verified buccal swab sample (2.03×10^{-7})*
L	Query mismatch between 2 patients	Matched different patient paraffin sample and not 2 patient labelled samples; mismatch confirmed (1.85×10^{-6})†
M	Query mismatch between 2 patients	Matched different patient paraffin sample and not 2 patient labelled samples; mismatch confirmed (7.55×10^{-5})†

*By chance (%) probability of genotype matching of one biopsy sample with the genotype of patient (assuming Hardy-Weinberg equilibrium).

†By chance (%) probability of genotype matching of the two different patient samples (assuming Hardy-Weinberg equilibrium).¹²

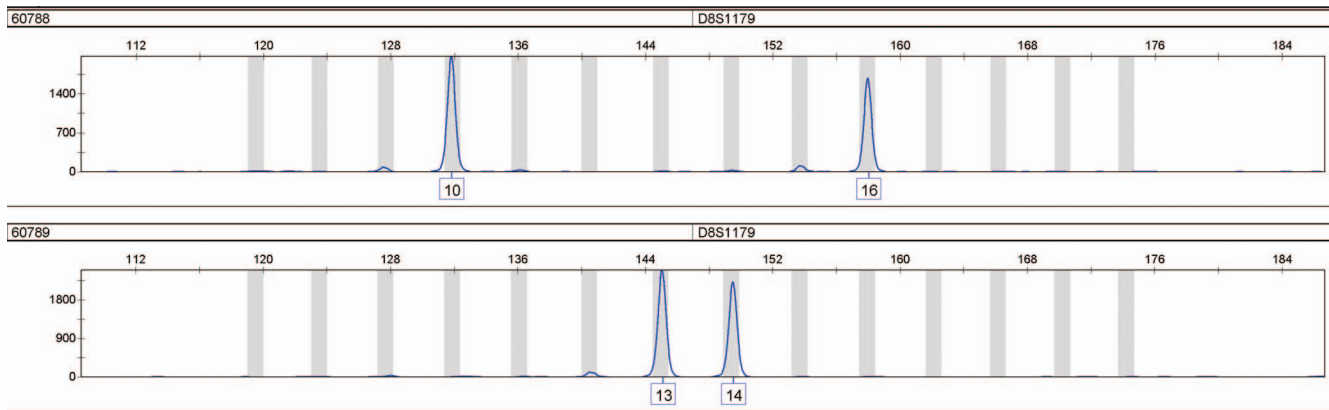


FIG. 1 Discordant genotypes for the tetranucleotide microsatellite *D8S1179* for two distinct individuals from Case C.

TABLE 3 Other tissue cases

Case	Tissue	Details	Outcome
A	Breast	Possible contamination	Genotypes consistent with rest of sample and contamination excluded
E	Skin	Query mix between husband and wife's sample collected at same time	Amelogenin typing confirmed sexes of samples consistent as labelled
H	Bone: greater trochanter of femur	Possible contamination	Poor DNA extraction as contaminant too small and testing could not be performed
N	Eyelid	2 biopsy samples; possible mix-up	Correctly labelled samples matched; those labelled with other patient did not (by chance= $5.4 \times 10^{-7}\%$)

TABLE 4 Haematological cases

Case	Details	Outcome
B	6 blood samples labelled with 6 unique patient identifiers; possible phlebotomist error	3 different patient samples found to be identical but 3 samples discordant

for prostate specimens and two were for haematological samples.

Limitations in contamination investigations are often due to the minute area of tissue available, which can be further reduced by cutting of further sections for additional stains.³ Extremely low level DNA may reduce interpretation at some loci as DNA amplification can be greatly reduced or fail, or allelic dropout (false homozygosity) may occur.⁸ If possible, isolation of normal tissue for DNA extraction investigation is required to avoid problems of microsatellite instability with additional alleles or loss of heterozygosity (LOH) in malignant tissue.

With the possibilities of low-cost large-scale SNP array genotyping and high throughput DNA sequencing within the near future, either DNA barcoding¹⁵ or parallel typing of polymorphic regions will enable identification and appropriate individualisation of all pathological specimens. Although this is currently plausible it may become standard practice for high value, high complexity pathology requests. At present, pathologists and clinicians should be aware of

genotyping solutions for the resolution of possible specimen identity errors.

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