

## ORIGINAL ARTICLE

# Processing of radical prostatectomy specimens for correlation of data from histopathological, molecular biological, and radiological studies: a new whole organ technique

S G Jhavar, C Fisher, A Jackson, S A Reinsberg, N Dennis, A Falconer, D Dearnaley, S E Edwards, S M Edwards, M O Leach, C Cummings, T Christmas, A Thompson, C Woodhouse, S Sandhu, C S Cooper, R A Eeles

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**Aims:** To develop a method of processing non-formalin fixed prostate specimens removed at radical prostatectomy to obtain fresh tissue for research and for correlating diagnostic and molecular results with preoperative imaging.

**Methods/Results:** The method involves a prostate slicing apparatus comprising a tissue slicer with a series of juxtaposed planar stainless steel blades linked to a support, and a cradle adapted to grip the tissue sample and receive the blades. The fresh prostate gland is held in the cradle and the blades are moved through the cradle slits to produce multiple 4 mm slices of the gland in a plane perpendicular to its posterior surface. One of the resulting slices is preserved in RNAlater™. The areas comprising tumour and normal glands within this preserved slice can be identified by matching it to the haematoxylin and eosin stained sections of the adjacent slices that are formalin fixed and paraffin wax embedded. Intact RNA can be extracted from the identified tumour and normal glands within the RNAlater preserved slice. Preoperative imaging studies are acquired with the angulation of axial images chosen to be similar to the slicing axis, such that stained sections from the formalin fixed, paraffin wax embedded slices match their counterparts on imaging.

**Conclusions:** A novel method of sampling fresh prostate removed at radical prostatectomy that allows tissue samples to be used both for diagnosis and molecular analysis is described. This method also allows the integration of preoperative imaging data with histopathological and molecular data obtained from the prostate tissue slices.

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Prostate cancer is the most common cancer diagnosed in men in the UK.<sup>1</sup> A major current problem is that it is not possible to predict the behaviour of early prostate cancer, which is now frequently diagnosed as a result of prostate specific antigen testing. Some cases may remain dormant for many years without progressing, whereas others will progress rapidly to metastases. Tests to predict the likelihood of recurrence after treatment and to predict prognosis are required to help individual treatments. In addition, we need to identify patients who should be treated more aggressively and separate them from those who can be managed by active surveillance,<sup>2</sup> thus sparing these last patients the adverse consequences of unnecessary treatment. Modern techniques involved in molecular pathology in combination with research tools such as microarrays can help identify new molecular markers that will help us to tackle these issues.<sup>3</sup> Many of these techniques require fresh tissue, which has been appropriately stored to preserve the molecular content (DNA, RNA, and proteins).

Radical prostatectomy specimens can be an invaluable source of fresh tissue in prostate cancer research. However, the standard procedure is to process the entire radical prostatectomy specimen using formalin fixation. This is necessary for adequately determining (1) margin status and tumour volume and (2) histological tumour grade, particularly for small volume or multifocal cancers.<sup>4</sup> However, this practice makes it impossible to analyse molecular data for research purposes, because this requires fresh non-formalin fixed material.

“The semisolid consistency of the fresh prostate gland makes it difficult to slice it evenly”

Sakr *et al* have reviewed various issues in the handling of radical prostatectomy specimens.<sup>5</sup> Diagnostic sampling of a whole fresh prostate gland poses great challenges for the following reasons: (1) the semisolid consistency of the fresh prostate gland makes it difficult to slice it evenly; and (2) as soon as a scalpel nicks the capsule of the fresh prostate to slice it, the tissue within the gland emerges through this cut because of the high pressure of any hypertrophic nodules. This deforms the gland and disturbs its orientation. Consequently, the time taken to store the fresh tissue appropriately may increase and affect the maintenance of the molecular profile within it as a result of RNA and protein degradation.<sup>6,7</sup>

Novel radiological imaging techniques, such as dynamic magnetic resonance imaging<sup>8</sup> and magnetic resonance spectroscopic imaging,<sup>9</sup> show promise in terms of improved intraprostatic tumour localisation. These, in combination with advanced radiotherapy techniques such as intensity modulated radiotherapy, may allow effective targeting of additional radiation dose focused on the tumour, resulting in an improved therapeutic ratio of prostate cancer irradiation.<sup>10</sup> Evaluation of these imaging techniques necessitates spatially accurate comparisons to be made between preoperative

**Abbreviations:** H&E, haematoxylin and eosin

imaging studies and postoperative histopathological findings and molecular analyses.

Our unit has a requirement to obtain fresh tissue for molecular pathology research and to assess the accuracy of new imaging modalities. As a result, we have developed a novel method of sampling a fresh prostate gland removed at radical prostatectomy that allows the integration of pre-operative imaging data with data obtained from histopathology and high quality molecular data from the prostate slices, in addition to maintaining tissue integrity for histopathology.

## METHODS AND RESULTS

### Apparatus

The processing of a fresh prostate gland removed at radical prostatectomy described here involves a tissue slicing apparatus made up of (1) a tissue slicer (fig 1A), comprising a series of juxtaposed planar stainless steel blades linked to a support (UK Patent Application Number 0318125.2) and (2) a cradle (Lakeland Ltd, Windermere, Cumbria, UK) adapted to grip the tissue sample and receive the blades (0-11-3-1-18 mm Quick-Point™ blades; Stanley Works, New Britain, Connecticut, USA) such that, in use, a tissue sample is held in the cradle (fig 1B) and the blades can be moved through the slits to slice the tissue sample (fig 1C). The local ethics committee approved our study and written informed consent was given by all patients taking part.

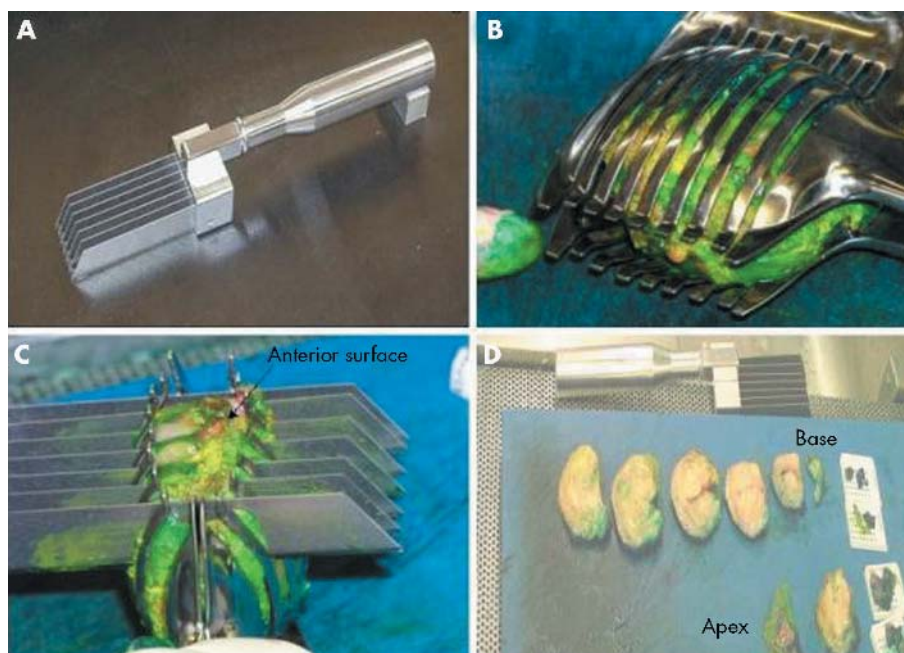
### Slicing method

The fresh prostate, removed from a patient undergoing radical prostatectomy, is collected from the operating theatre in a labelled plastic bag placed on dry ice. It is then promptly (within five minutes) transported to the histopathology cutup room, where it is further processed as follows. The prostate gland is weighed and inked in the traditional manner (within the next five minutes). After inking the gland, the right and left seminal vesicles are transected, and each is placed in separate labelled small cassettes. Shavings are then taken from the basal and apical urethral margins, and these are placed in separate labelled small cassettes. The

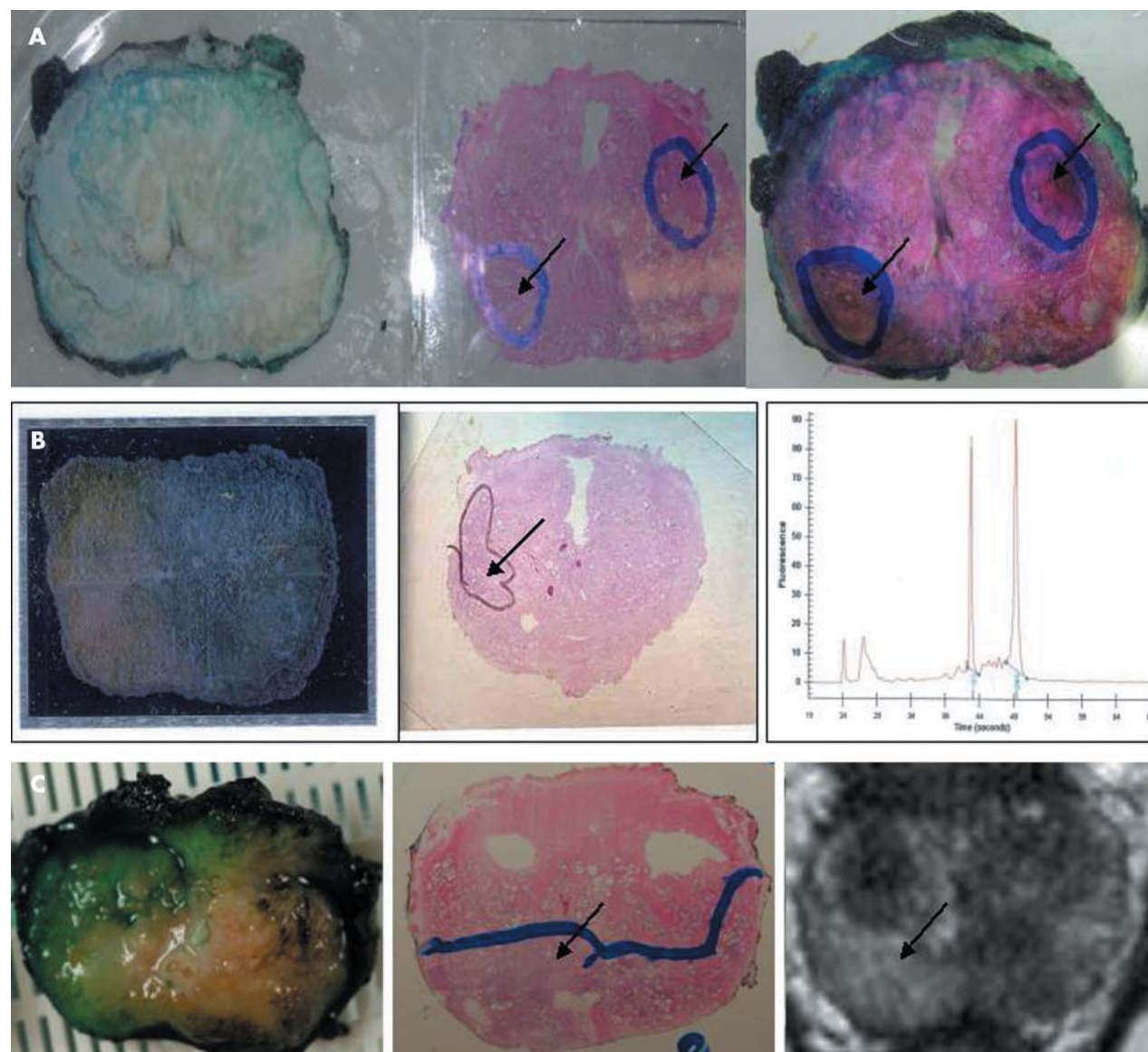
whole gland is then held in the cradle in such a way that it will be cut in a plane perpendicular to the posterior surface of the gland. The tissue slicer is then used to slice the whole gland, base to apex, to yield equally spaced cross sections of similar thickness (fig 1C). The resulting slices (fig 1D) are numbered from base to apex, and each is placed in separate labelled large cassettes. From the slices produced by this method, a single slice, which will not be formalin fixed and paraffin wax embedded, and can be used for further research, is selected and placed in a sterile petri dish filled with RNA storage solution (RNAlater™; Ambion Inc, Austin, Texas, USA) within 20 minutes after prostatectomy. The remaining slices are fixed in 10% (vol/vol) neutral buffered formalin and paraffin wax embedded in the traditional fashion, after which a whole mount section is cut from each of these for haematoxylin and eosin (H&E) staining. All the slices have to be consistently orientated in their individual cassettes—for example, apical surface facing down in all—so that the corresponding H&E sections from the respective cassettes represent similar surfaces for each slice. White gel foam pads (packaging material for knives; BDH Laboratories, Poole, Dorset, UK) are used to prevent curling up of the slices in their respective cassettes and petri dish. The slice in RNAlater is kept at 4°C initially and subsequently stored at -70°C after discarding the excess RNAlater solution. Slices (which are stored in RNAlater) are made available for further research only after a satisfactory histopathological diagnosis has been made. We have processed 12 radical prostatectomy specimens using this method and the average time from prostatectomy to preservation of research slices in RNAlater was 20 minutes.

### Mapping of prostate tissue for research

Identification of tumour and normal areas in the research slice is achieved by matching this slice with H&E sections taken from formalin fixed and paraffin wax embedded slices immediately above and below the research slice (fig 2A). The tissue can be cored using either a fresh scalpel or by using sterile hypodermic needles. The coring of tumour and normal areas from the research slice is performed with the slice still



**Figure 1** Slicing of radical prostatectomy specimens. (A) The slicing device with juxtaposed parallel blades linked to a support and a handle (UK Patent Application Number 0318125.2). (B) The inked fresh prostate gland held in the cradle obtained from Lakeland Ltd (Cumbria, UK). (C) Slicing of a prostate held in the cradle with the slicing device. (D) Slices from a fresh prostate obtained with the help of the slicing device and cradle.



**Figure 2** Integrated histopathology, molecular biology, and coordination with radiological imaging. (A) Left panel, research slice preserved in RNAlater™; middle panel, haematoxylin and eosin (H&E) histopathology section with arrows indicating areas of tumour; right panel, alignment of research slice and histopathology. (B) Left panel, research slice preserved in RNAlater and frozen at  $-70^{\circ}\text{C}$ ; middle panel H&E histopathology section with area of tumour marked with an arrow; right panel, RNA prepared from area of prostate cancer shown in the middle panel and subject to analysis on an Agilent Bioanalyser 2100 (Agilent Technologies, USA). (C) Left panel, fresh research slice; middle panel, H&E histopathology section with arrow indicating the location of the tumour; right panel, preoperative magnetic resonance image obtained from the same patient.

in its petri dish, over dry ice. The cored tissue corresponding to the tumour and normal areas can be stored at  $-70^{\circ}\text{C}$  or immediately processed further to extract nucleic acids such as DNA and RNA, or proteins. We have been able to identify tumour in the research slice in eight of the 12 samples collected.

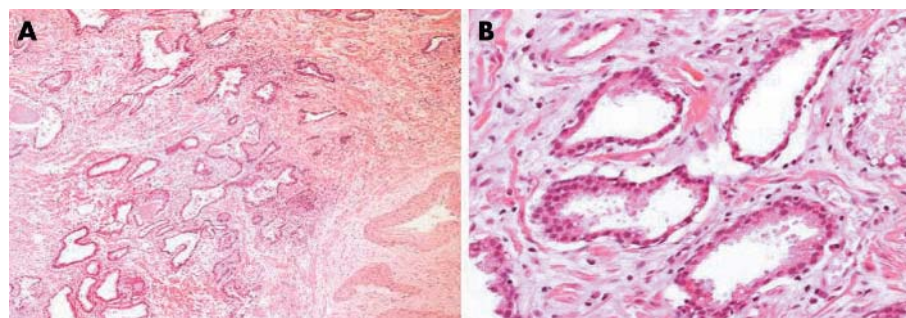
#### RNA extraction

RNA was extracted using the TRIzol (Invitrogen Ltd, Carlsbad, California, USA) protocol, according to the manufacturer's instructions, after immediate homogenisation (Ultra Turax; IKA Labor Technik, Staufen, Germany) in TRIzol reagent (Invitrogen Ltd). Figure 2B shows the integrity of the extracted RNA analysed by an Agilent Bioanalyser 2100 (Agilent Technologies, Palo Alto, California, USA). These results show that the RNA is highly intact, with excellent presentation of 28S and 18S ribosomal

RNAs. RNA extraction was successful in all six tissue cores obtained from slices stored for research.

#### Correlation of the slices with imaging

As described above, the ex vivo prostate is sectioned in a plane perpendicular to its posterior surface. In anticipation of this, preoperative imaging studies can be acquired with the angulation of axial images also chosen to be perpendicular to this surface, as seen on a sagittal "scout" view of magnetic resonance imaging. If required, the slice thickness on imaging can also be chosen to correspond to that used during the slicing procedure, to allow matching of the image with histopathology. Given that the stained histological section is taken from within a few microns of a known and consistent aspect of each slice, it then becomes relatively straightforward to match each stained slice with its counterpart on imaging (fig 2C).



**Figure 3** Histopathology from an RNAlater™ preserved research slice. The research slice preserved in RNAlater was transferred to formalin as described in the text. (A) Low power (original magnification,  $\times 4$ ) and (B) high power (original magnification,  $\times 20$ ) haematoxylin and eosin stained images are shown. This illustrates that the tissue stored for research is adequately preserved for diagnostic pathology purposes, should it be needed.

### Histopathological analysis of the research slice

If the research slice preserved in RNAlater is needed for histopathology, it is placed in 10% (vol/vol) neutral buffered formalin and embedded in paraffin wax. The quality of the H&E image obtained (fig 3) is similar to that obtained when the specimen is directly preserved in formalin.

### Tissue culture

Small pieces of the tissue can be used for primary cell culture if they are removed from an area of the slice that is distant from the margins before immersion in RNAlater. This does not compromise the requirements of the pathologist, who requires that intact margins should be preserved until the pathological examination is complete. To improve the sterility of the tissue, it can be briefly immersed in 70% (vol/vol) ethanol, followed by immediate thorough rinsing in the appropriate culture medium.<sup>11</sup>

### DISCUSSION

We have described a novel method for processing fresh radical prostatectomy specimens that facilitates the correlation of histopathology, molecular biology, and radiological studies. The method also allows tumour and normal tissue from the specimen to be made available for research without jeopardising diagnostic accuracy. Many histology laboratories prefer to slice formalin fixed prostate glands because it is easier to slice a firm fixed prostate than a fresh gland.<sup>12</sup> However, this prevents the procurement of fresh tissue samples and hence intact nucleic acids and proteins for research. Sectioning a fresh prostate gland from base to apex is a technically demanding procedure,<sup>5</sup> especially when dealing with an enlarged gland, which deforms as soon as the capsule is nicked because of the high pressure of the nodular hyperplasia within it. The procedure can become even more complicated when complete rather than partial sampling of the prostate gland is required. Our method of obtaining complete sections in one slicing action maintains the architectural orientation and produces fewer sections for the pathologist to evaluate. The cradle used in our method helps to prevent the prostate deforming and supports it during cutting, thus eliminating the need to fix it in formalin before slicing.

Bova *et al* have described a method of harvesting fresh prostate cancer tissue for research.<sup>12</sup> However, their technique makes it difficult to assess capsular penetration because it involves stripping of the capsule. In addition, tumour volume cannot be assessed accurately and radiological correlation is not possible. Given that the method of slicing described in our report results in complete tissue slices (without capsular loss), which are equally spaced and have been sliced in a chosen plane, the technique lends itself well to studies evaluating novel imaging techniques. When histopathological or molecular findings are correlated with the findings of preoperative imaging, such as functional magnetic resonance

imaging, then rigorous attention must be paid to matching corresponding images and pathology, a task facilitated greatly by the use of our technique.

In this era of functional genomics, it has become essential to strike a balance between achieving good quality tissue for molecular analysis and maintaining histological integrity of the tissue. Most molecular research techniques require tissue samples in which the DNA, RNA, and/or protein content has been preserved, which necessitates their quick and appropriate storage. However, formalin fixation to preserve tissue integrity for histopathology and to simplify sampling of the tissue can take a considerable time, thereby jeopardising the molecular content of these tissues,<sup>6,7</sup> and thus hindering analysis at the genomic, transcriptomic, and proteomic levels. In the technique described here, the slices, in addition to not being formalin fixed and paraffin wax embedded, are produced in a single cutting action, so that the time required to cut the entire prostate into multiple slices is reduced, thus allowing quick storage of tissue and preservation of molecular content.

*“In this era of functional genomics, it has become essential to strike a balance between achieving good quality tissue for molecular analysis and maintaining histological integrity of the tissue”*

Other reports on sampling fresh prostate tissue, such as that described by Riddick *et al*,<sup>13</sup> involve storage of smaller quadrants of a slice of fresh prostate. The knowledge of areas suggestive of tumour in the technique described by Riddick *et al* is derived from inspection and information from digital rectal examination, transrectal ultrasound and biopsies, and other radiological imaging.<sup>13</sup> In addition, the tissue is banked by snap freezing. We have used the RNA preservative—RNAlater—which has been shown to provide improved preservation of both macromolecules and tissue integrity (for histological purposes, including immunohistochemistry) compared with snap freezing using liquid nitrogen.<sup>14</sup>

The slices that are initially stored in RNAlater and frozen at  $-70^{\circ}\text{C}$  can thereafter be retrieved at any time. This tissue can potentially be used for a wide range of molecular and molecular/cytogenetic techniques, including microarray studies.<sup>14–18</sup> One of the factors that affects the speed of tissue fixation is the rate of diffusion of fixative into the tissue,<sup>19</sup> and in practice it takes 48 hours to allow an entire prostate to fix at the rate of one hour/mm of tissue thickness.<sup>7</sup> Our method of producing slices that are 3–4 mm thick allows quicker fixation compared with an entire unsliced prostate, and has the potential to allow better preservation for immunohistochemistry.<sup>20</sup> The use of RNAlater also prevents the chemical damage of RNA, DNA, and proteins that accompanies formalin fixation.<sup>14</sup>

Hollenbeck *et al* found that partial sampling (50% submitted versus whole mount radical prostatectomy

### Take home messages

- We describe a novel technique of slicing a whole fresh prostate gland removed at surgery so that fresh tissue can be made available for wide areas of research without jeopardising the histological integrity for proper diagnosis
- There is an ethical concern that research needs could compromise diagnostics and our technique overcomes this
- This technique allows quicker, easier, and accurate slicing of the prostate and permits quicker formalin fixation
- This method allows integration of studies correlating preoperative imaging data with histopathological and molecular data obtained from the prostate slices
- Intact RNA can be extracted from areas of tumour and normal glands from the slices produced by this method

specimens) did not alter diagnostic accuracy.<sup>21</sup> It is our institute policy to sample the entire prostate gland, preferring whole mount sections rather than partial embedding of the samples, because it decreases the number of slides to be examined. Within our series of radical prostatectomies, multifocality of cancer was seen in four of the 14 samples. Adverse pathological features were seen in two of 12 samples (capsular invasion in one and seminal vesicle involvement in the other). In the device described, because we used disposable blades to avoid blunting, margins could be preserved in all cases.

In summary, we describe a novel technique of slicing a whole fresh prostate gland removed at surgery so that fresh tissue can be made available for wide areas of research without jeopardising the histological integrity for proper diagnosis. There is an ethical concern that research needs could compromise diagnostics and our technique overcomes this. The outcomes of such research from fresh tissue could be very wide ranging, from contributions to diagnosis and prognosis, to the targeted treatment of prostate cancer.

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