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TECHNICAL NOTE

# Improved reproducibility in preparing precision-cut liver tissue slices

**Martina Zimmermann · Johanna Lampe · Sebastian Lange · Irina Smirnow · Alfred Königsrainer · Claus Hann-von-Weyhern · Falko Fend · Michael Gregor · Michael Bitzer · Ulrich M. Lauer**

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**Abstract** Precision-cut liver tissue slices (PCLS) have been used for decades to study pharmacological metabolism as well as toxicology and efficacy of novel substances on primary material under standardized conditions. Slicing of primary liver tissue has been done using different slicing machines. Since there has been great variability in the results, we sought to compare the reproducibility of tissue slices generated using the newly developed Leica VT1200 S vibrating blade microtome with Vibrocheck (LV) and the Krumdieck tissue slicer (KD) which has been the standard apparatus for this application so far. Liver samples from five different species (human, pig, cattle, rat, mouse) were cut and the reproducibility of slice thickness was analyzed by cross sectioning the PCLS. The quality of the sliced tissue was determined via measurement of the ATP content. As a result, we found an improved accuracy and reproducibility of rat, mouse and human tissue slices using the new Leica vibrating blade microtome.

**Keywords** Primary culture · Precision-cut liver slices (PCLS) · Leica VT1200 S vibrating blade microtome · Krumdieck tissue slicer

M. Zimmermann · J. Lampe · S. Lange · I. Smirnow · M. Gregor · M. Bitzer · U. M. Lauer (✉)
Department of Gastroenterology and Hepatology, University Hospital Tuebingen, Otfried-Mueller-Str. 10, 72076 Tuebingen, Germany
e-mail: ulrich.lauer@uni-tuebingen.de

A. Königsrainer
Department of General, Visceral and Transplant Surgery, University Hospital Tuebingen, Tuebingen, Germany

C. Hann-von-Weyhern · F. Fend
Department of Pathology, University Hospital Tuebingen, Tuebingen, Germany

## Introduction

In vitro studies of cancer therapeutics in human cancerous and non-cancerous tissue specimen exhibiting natural tissue structures are not only a powerful method to elucidate many aspects of tissue/drug interactions which cannot be investigated in cell cultures representing single cancer/normal cell types only. In addition, comparative testing of patient-derived (i) primary cancer tissue slices vs. (ii) slices obtained from surrounding normal tissue paves the way for a patient-individualized preselection of cancer therapeutics to be used for tumor patients following an initial surgical removal of large tumor masses.

Primary and secondary tumors of the liver are among the leading causes of death and therefore are in the focus of many studies testing investigational new compounds. For this purpose, a system is required (i) which not only contains liver tumors, but also adjacent non-tumorous liver tissue (to test for selectivity of drugs), (ii) which also exhibits an intact

intercellular matrix (enabling testing of the capability of drugs to cross this barrier), and (iii) in which therapeutics that depend on specific mutations, e.g. in intracellular signaling pathways, can be tested functionally on a patient-individual basis. For pharmacological and toxicological tasks like this and other new therapy approaches, precision-cut (liver) slices have been used for decades (Brendel et al. 1987; Fisher et al. 1995; Kirby et al. 2004; Parrish et al. 1995; Rots et al. 2006; Stoff-Khalili et al. 2006; van de Bovenkamp et al. 2006; Zimmermann et al. 2009).

To prepare such slices, special automates, so-called tissue slicers, are used to cut the tissue into slices of defined size. During this procedure the samples should be treated as gentle as possible to preserve maximum viability.

The methods to produce and maintain precision-cut liver slices have been reviewed in detail (Gandolfi et al. 1996; Lerche-Langrand and Toutain 2000, Olinga et al. 1998). So far, slicing of liver samples has been possible only with a small selection of instruments (Lerche-Langrand and Toutain 2000). Recently, the Leica VT1200 S microtome (LV) has been newly developed. As a new slicing feature it includes a so-called Vibrocheck function, which minimizes vertical vibrating of the blade to reduce shearing of the tissue. Therefore, it was of interest to compare the new LV apparatus with the Krumdieck tissue slicer (KD) which has so far been used as the standard machine for slicing of liver samples (Krumdieck et al. 1980; Price et al. 1998).

In our study, we not only used liver tumor pieces, in which tissue integrity is often found to be diminished as a result of heavy treatment procedures (such as chemotherapies), but also naïve liver tissues with reproducible quality from five different species. As a result, we found that generation of high-quality precision-cut slices can be achieved with both instruments. However, several differences were observed, which are described and discussed in detail.

## Experimental procedures

### Materials and methods

Primary liver material from five different species (human, pig, cattle, rat, mouse) or primary human liver tumor samples (colorectal liver metastasis) were put into ice-cold Custodiol transplantation media (Dr. Franz Koehler GmbH, Alsbach-Haehnlein, Germany) directly after surgical resection. Human material was collected with informed patient consent by the Department of General, Visceral and Transplant Surgery, University Hospital Tuebingen, according to the guidelines of the local ethics committee.

Tissue specimens were cut into slices of 200 μm as fast as possible using the Leica vibrating blade microtome and the Krumdieck Tissue Slicer. Viability post slicing was measured by ATP quantification. Slice thickness was measured after paraffin embedding and cross sectioning of the tissue slices.

### Slicing

*Leica vibrating blade microtome VT1200 S (Leica, Wetzlar, Germany):* first, tissue samples were cut into $1.5 \times 1.0 \times 0.5$ $cm^3$ sized cubes and fixed with Roti Coll 1 (Roth, Karlsruhe, Germany) onto specimen plates; sectioning was performed after Vibrocheck (0;0) using stainless steel razor blades (Personna Medical, Stainton, VA, USA) under buffered conditions with ice-cold Krebs-Henseleit buffer (KHB) containing 25 mM glucose (Merck, Darmstadt, Germany), 25 mM $NaHCO_3$ (Roth, Karlsruhe, Germany) and 10 mM HEPES (Roth) at the following adjustable settings: knife angle: 15°; sectioning speed: 0.4–1 mm/s; oscillation amplitude: 3 mm; step size: 200 μm; retract: 10 μm; continuous stroke. To obtain equally sized sections, 8 mm diameter cutouts were generated using a stainless steel 8 mm diameter punch (Alabama Research and Development, Munford, TN, USA).

*Krumdieck tissue slicer (Alabama Research and Development, Munford, USA):* first, tissue cores of 8 mm diameter were prepared using a stainless steel 8 mm diameter punch (Alabama Research and Development). These tissue cores were cut using stainless steel razor blades (Personna Medical, Stainton, VA, USA) under buffered conditions with ice-cold KHB at a cycle speed of 40/min. The resulting slices with 8 mm in diameter and an approximated thickness of 200–300 μm were collected in ice-cold KHB.

### Slice culture

After slicing, samples were cultivated in 10 cm diameter Petri dishes using oxygenated William's E

medium (Lonza, Braine-l'Alleud, Belgium) supplemented with 25 mM glucose and 50 μg/mL gentamycin (= WEGG medium) in a highly oxygenated atmosphere (80% oxygen, 5% $CO_2$) at 37 °C for up to 3 days post slicing. 1 hour post slicing the medium was replaced with 20 ml fresh WEGG medium.

### Thickness determination

Ten randomly chosen slices (for each individual sample and slicing machine) were put into 4% formaldehyde solution (Fischar GmbH, Saarbruecken, Germany) for at least 1.5 h. After dehydration with alcohol concentrations rising from 70 to 99%, the slices were embedded in paraffin in an upright position and 5 μm cross sections were generated using a standard microtome (Leica). The cross sections were deparaffinized and stained with Hematoxylin (Roth) and Eosin (Roth) (H & E). For every species, five cross sections were photographed and measured using analysis 3.1 software (Soft Imaging System GmbH, Muenster, Germany) at 30 points of measurement on average along the microtome slice.

### Viability

The viability of the tissue slices was measured via ATP determination using the Celltiter Glo Kit (Promega, Mannheim, Germany). For this purpose, we collected three slices per time point (1.5 h, 1 d, 2 d, 3 d) post slicing for each individual sample and stored them at −80 °C. 500 μL of Dulbecco's Modified Eagle Medium (DMEM, Biochrom AG, Berlin, Germany) were added to the frozen slices followed by dissociation using a high intensity cup horn sonifier (Branson, Danbury, CT, USA) for 30 s at maximum power. Then, the ATP content in the lysate was measured.

## Results and discussion

### General parameters of slice production

An ideal apparatus for production of precision-cut liver slices would be one which is easy to handle and with which reproducibly sized slices can be prepared quickly to preserve maximum viability of samples.

Many different apparatuses have been tested for this pupose and so far, the Krumdieck tissue slicer (KD) and the Brendel-Vitron tissue slicer have been considered superior to other tissue slicers (Price et al. 1998).

Only recently, the Leica VT1200 S vibrating blade microtome (LV) has been developed which exhibits some new features: for example, the LV has a control panel on which all parameters being crucial for the slicing process are now adjustable in a precise manner. Depending on the stability of the material the sectioning speed can be adjusted on a mm/s level; if the tissue exhibits high stability it can be increased up to 1.5 mm/s, which shortens the overall duration of the slice production. Due to the relatively small size of the LV buffer tray, less than 500 mL Krebs-Henseleit buffer are needed to cover the tissue sample, minimizing buffer consumption.

In contrast, for the KD, slicing speed is the only precisely adjustable parameter. The only possibility to adjust the slice thickness when using the KD is to press the sample more or less strongly onto the blade; therefore, when using the KD, slice thickness depends mainly on the investigator's experience with this apparatus. As the microtome unit of the KD is relatively large, more than 1,000 mL buffer have to be used. An advantage of the KD is that preparation of 30 slices lasts between 15 and 30 min which is much faster than the slicing with the LV.

To provide a general overview, the main parameters are summarized in Table 1.

Additionally, there are several differences concerning the slicing procedure which one should keep clearly in mind. While the whole microtome unit of the Krumdieck slicer can be sterilized, only the buffer tray of the Leica apparatus can be autoclaved. But, and this is an important point when it comes to working with human samples of unknown pathogenicity, if one wants to replace the blade of the KD between preparation of two different samples, either a huge volume of buffer will have to be discarded or one will have to immerse their gloved hands in a potentially contaminated liquid. Additionally, the latter can cause cross contamination of different samples, as observed during our testing procedure with the porcine samples, leading to an artifact-based rise of the ATP curve (Fig. 3h).

**Table 1** Basic settings and parameters of slice preparation

| | Leica vibratome VT1200 S | Krumdieck tissue slicer |
|---|---|---|
| Settings | Sectioning speed: 0.4–1 mm/s | Cycle speed: 40–50/min |
| | Oscillation amplitude: 3 mm | Continuous mode |
| | Step size: 200 μm | |
| | Retract: 10 μm | |
| | Continuous stroke | |
| Preparation time per 30 slices | 45–60 min | 15–30 min |
| Blades needed per 30 slices | 1 | 1 |
| Krebs buffer | <500 mL | >1,000 mL |

## Measurement of slice thickness

To determine if slices produced with either machine display a reproducible and evenly distributed thickness, we determined this characteristic using cross sections of slices as described in materials and methods.

## Consistency of slice thickness depends on species and slicing machine

To visualize the distribution of expected thickness of 200 μm, all points measured per species samples were integrated in one histogram (Fig. 1). As a result, murine, rat, and human (tumorous/non-tumorous) tissue slices generated with the LV apparatus showed a good reproducibility with a consistent slice thickness of slightly less than 200 μm. This shrinking is most likely a result of dehydration during paraffin embedding.

In contrast, porcine and bovine slices displayed a much wider distribution of thickness (from less than 100 μm up to 700 μm). For slices cut with the KD, a much wider overall distribution in slice thickness was observed (Fig. 1), even for the species (human, rat) where slices generated by the LV showed a clear peak in thickness (Fig. 1b, e). Notably, very small samples like mouse liver cannot be sliced appropriately with the KD machine (the space in which samples are fixed is too big and they just slip away; in contrast even small samples can be fixed with glue onto the specimen plate of the LV).

Taken together, due to differences in the characteristics of the two apparatuses, more consistent results in terms of slice thickness were obtained with the LV slicer which also allows slicing of smaller sized tissue samples.

## Consistency of thickness in single slices

To investigate whether thickness is consistent throughout single slice specimen the average values and standard deviations for five slices per one single liver sample were calculated. The best results for slices prepared with the LV apparatus were obtained with murine tissues with a mean of about 200 μm each (Fig. 2a; only a single outlier (mouse 01) was observed). A similar pattern was observed for rat and human liver and human tumor samples (Fig. 2b, e, f).

Tissue slices obtained with the KD machine (Fig. 2, panels to the right) were on average thicker than corresponding samples produced with the LV apparatus (Fig. 2, panels to the left) most obvious in porcine slices (Fig. 2c vs. h), rat slices (animal 04 and 05, Fig. 2b vs. g) and human liver slices (Fig. 2e vs. j). In addition, slices cut with the KD exhibited higher variations throughout one distinct sample which can be seen especially in human liver slices (LV: Fig. 2e; KD: Fig. 2j). Porcine and bovine slices produced with either instrument showed similarly high variations in mean slice thickness (Fig. 2c, d, h, i).

## Both instruments produce slices with similar viability

Furthermore, to compare the viability of slices, ATP contents in three exemplary slices per harvesting time point per individual were determined. In all slices the ATP content declined over time (Fig. 3a–k) exhibiting no major difference between the two apparatuses. Only a single strong increase in ATP content over time was observed in porcine tissue cut with the KD (Fig. 3h), which can clearly be ascribed to a bacterial contamination observed during the incubation time of 3 days. Variations of the ATP content in human

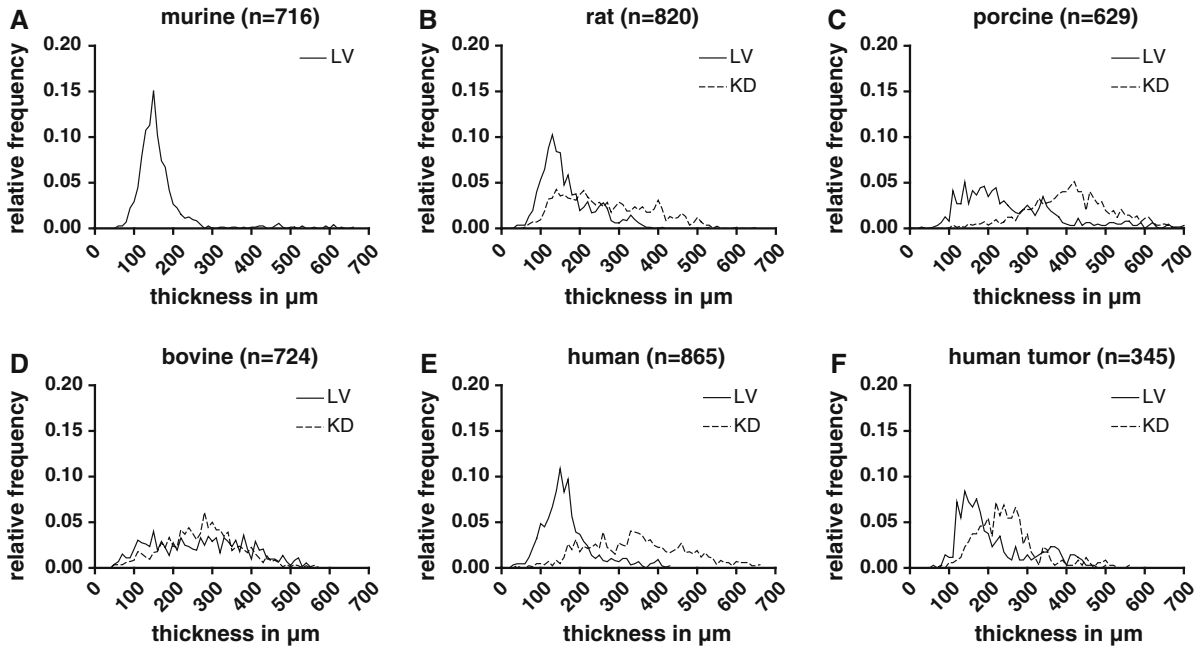


**Fig. 1** Consistency of slice thickness. Histograms display cross section thickness distribution of tissue slices generated from livers of different species. Slices were photographed and measured using analysis 3.1 software. **a** Murine liver tissues; **b** rat liver tissues; **c** porcine liver tissues; **d** bovine liver tissues; **e** human non-cancerous liver tissues; **f** human cancerous liver tissues. 30 points of measurement on average; histograms represent all points measured per species; results were sorted in 10 µm wide bins; *n*: number of measuring points. Very small samples like mouse liver can be sliced appropriately only with the LV apparatus, but not with the KD machine; therefore, only a single graph is depicted for murine slices)

tumor samples (Fig. 3f, k, human 4) may be due to the very inhomogeneous tumor tissue or dividing tumor cells.

When using the LV apparatus, we usually started with a slow sectioning speed (0.4 mm/s) in order to preserve tissue viability as good as possible; if the material turned out to be sufficiently stable we were able to increase speed up to 1.5 mm/s. For all samples cut with the LV apparatus we used the maximum oscillation amplitude of 3 mm to avoid any unwanted squeezing of the tissue sample. However, when using the KD slicer, one should be very careful with changing the sectioning speed because there is no function ensuring that slice thickness will be adjusted automatically.

In summary, we have shown that the new Leica slicing apparatus can be applied to generate precision-cut liver slices as an alternative to the standard apparatus (Krumdieck tissue slicer) used so far. Additionally, we were able to cut murine livers with the LV apparatus which was not possible with the KD machine. Concerning these results, the main advantage of the KD machine is the fact, that production of tissue slices is much faster than with the LV apparatus (factor 2–3). Thus, an investigator with lots of experience with the KD machine will be able to produce reproducible slices in a short time. Although slicing with the LV apparatus takes longer, handling is much easier, and thickness of the slices can be defined on a micrometer level; in fact, in this report we have found that production of liver slices with the LV apparatus is precise and reproducible and can be performed without any bacterial contamination.

We conclude that liver slices obtained with the LV are of high value for diverse applications, e.g. (i) *ex vivo* testing of novel drugs, giving additional information to cell culture experiments and leading to a minimized usage of laboratory animals and (ii) any patient-based analysis of drugs before application paving the way for individualized treatment regimes to be applied in tumor patients.

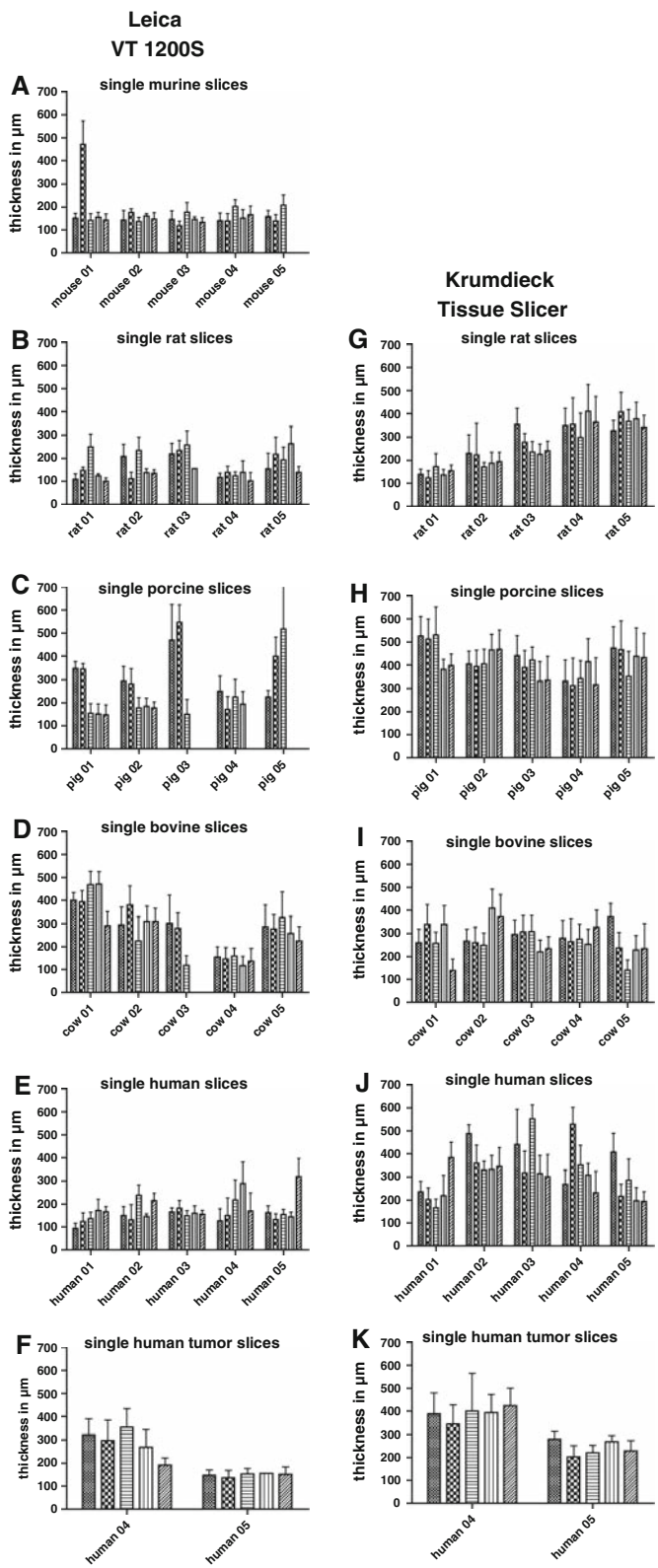


**Fig. 2** Reproducibility of equally sized slices. Average thickness of single slices (if possible five per individual liver sample); error bars indicate single standard deviation. **a–f** Liver slices obtained by the LV apparatus; **g–k** liver slices obtained by the KD machine. Very small samples like mouse liver could be sliced appropriately only with the LV apparatus, but not with the KD machine; therefore, only a single graph is depicted for murine slices

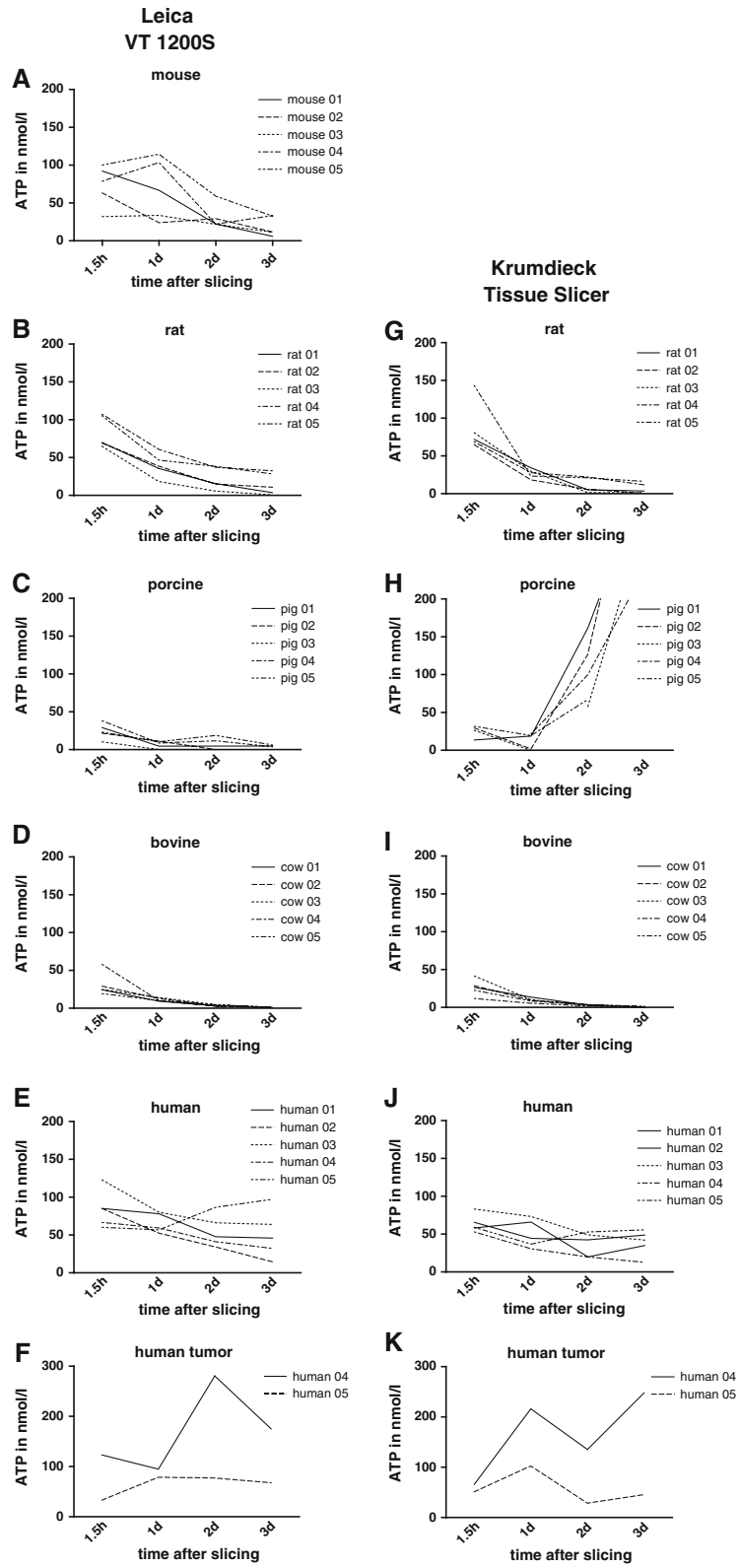


**Fig. 3** ATP determination. The decay of ATP content in the slices over time is shown. **a–f** Liver slices obtained by the LV apparatus; **g–k** liver slices obtained by the KD machine. At each harvest time point, three slices per liver specimen were lysed followed by measurement of the ATP concentration. A single strong increase in ATP content over time was observed in porcine tissue cut with the KD (*panel H*), which had to be ascribed to a bacterial contamination

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