Tissue-Tek AutoSection® and Accu-Edge® Blades

The effect of consistency in section thickness

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Summary

The quality of a tissue section will affect all downstream activities within the laboratory as well as the ease of which a patient sample can be diagnosed. High quality sectioning will reduce the time and costs associated with diagnosis and the elimination of rework due to poor sectioning. This time and cost saving can have a positive impact on the laboratories budgets and ability to cope with workloads. This paper examines the effect of different disposable microtome blades used on the Tissue-Tek AutoSection® and their effect on the thickness of the tissue sections produced. It brings together the theory behind tissue sectioning and the testing that took place to achieve the ultimate quality of tissue section.

Method

This study was conducted in co-operation with 2 external companies. The tissue sections were produced by a laboratory that runs an international quality assurance scheme within histology. This laboratory is used to routinely cutting tissue sections from a variety of tissues for sending out to laboratories involved in their scheme. The section thickness was then measured by an independent company, Mitutoyo, equipped and experienced in measuring this sort of thicknesses.

This testing was performed using the 4689 – Accu-Edge® Microtome Blade and the 7089 – Paraform® Microtome Blade, manufactured by Feather in Japan. Both blades were used to cut tissue sections on the same Tissue-Tek AutoSection Microtome.

The 4689 microtome blade is one of the most commonly used Microtome blades for routine sectioning in Europe. On launching the AutoSection Microtome Sakura recommended the 7089 Paraform Microtome Blade for use on this instrument.

A total of 50 sections per blade type were taken using the AutoSection Microtome. The AutoSection was set to obtain sections of a 4µm thickness. These sections were then mounted on to glass slides in the usual way.

Mitutoyo then took 2 readings from each tissue section. This was performed by scraping away part of the tissue. This allowed the establish of a baseline - the surface of the glass slide. Using an Ultra QuickVision CNC Vision Measuring System, white light interferometry (WLI) measurements and analysis were completed to determine the thickness of each section. This produced 100 data points per blade type, which was enough to provide statistically significant results.

Insight on the best blade to produce consistent thickness of sections

This paper shows the results of those tests and provides insight on the best blade to produce sections of consistent thickness, describing the potential impact this may have on histological diagnosis.

The Accu-Edge 7089 – Paraform Blade is the best blade to use in conjunction with the Tissue-Tek AutoSection

Results

The results show that a laboratory that wants to attain the sections with the most consistent thickness should use the 7089 – Paraform Microtome Blade in conjunction with the Tissue-Tek AutoSection Microtome.

The benefit of obtaining routine sections of consistent thickness to the laboratory is that a reduction in the cost of rework could be achieved. This is due to the section having a uniformity of staining dependent upon the tissue structure rather than the thickness of the tissue within the section. The reject rate of slides at the QA check can then be reduced. Also the pathologists can potentially diagnose the section more quickly as they do not have to think about the effect of a change in tissue thickness on staining intensity.

Reduction of reject rate at QA so a reduction of rework costs

Figure 1 shows the distribution curves of the 2 different blades (4689 and 7089) when used in conjunction with Tissue-Tek AutoSection. In this way the degree of variation from the mean can be seen. It clearly shows that the 7089 has the least amount of variation in thickness of tissue sectioned when compared to those sectioned with the 4689 blade in this study.

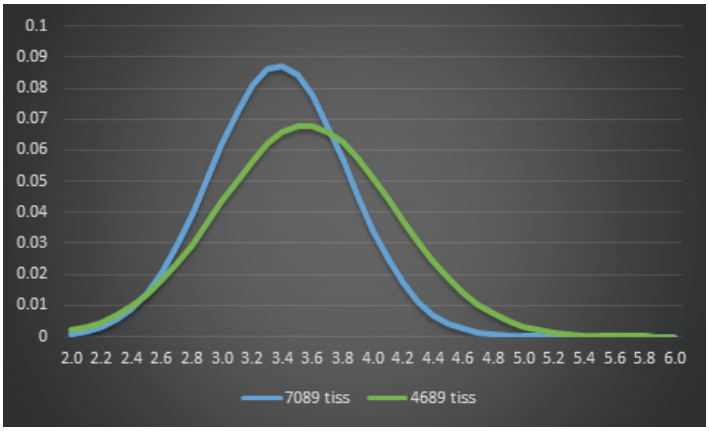


Figure 1 Variance of section thickness.

Results overview

	7089 blade tissue section	**4689 blade** tissue section
average	3.38	3.56
St div	0.457	0.587

To establish if this degree of variation between the 2 blades statistical testing was performed on these results using Levene methodology. The p value in this statistical analysis scored 0.011634. we can therefore conclude that the variation between the sections cut with the 7089 and 4689 blades are statistically significant. The results show that the degree of variation between the thickness of tissue sections is least in the 7089 Paraform Blade.

Discussion

Why is the consistency between sections so important to histological testing?

There has been very little research into the effect of thickness in variation in tissue on diagnosis within histology. On the whole it is understood that when a section is taken at 4µm that this is the thickness of the section that is achieved. There are occasions attributed to a microtome incorrectly set up / possessing a fault or a particular difficult tissue that will produce a result otherwise and are known as microtomy artifacts.

These artifacts such as thick / thin alternating sections, or chatters within a section are identified at QA stage of the laboratory and the source identified and corrected.

These microtomy issues due to the set-up of the microtome are eliminated by the AutoSection due to its fully automated use, with the ability to autoalign removing some of the manual set up nature of microtomy. Therefore achieving a quality section every time.

However as this study shows there is a variation between section thicknesses during routine microtomy that are not picked up by the user or during QA as the section is seen as ‘normal’.

These variations can be down to several microtomy elements such as the temperature of the tissue block, the experience of the user, the set-up of the microtome and the type of blade being used. As this study was controlled the variant was the type of blade used.

Reduction of the risk of misdiagnosis

The question that this poses is what impact does this variation have? Earlier it was described how a more consistent thickness in sections may have an impact on the cost of rework and the ease of diagnosis with routine qualitative H&E or tinctortial staining.

This is due to the increased consistency of staining. This in turn may reduce the risk of misdiagnosis and the stresses caused to the patient because of this, let alone the costs associated with repeating a biopsy or surgery or later treatment.

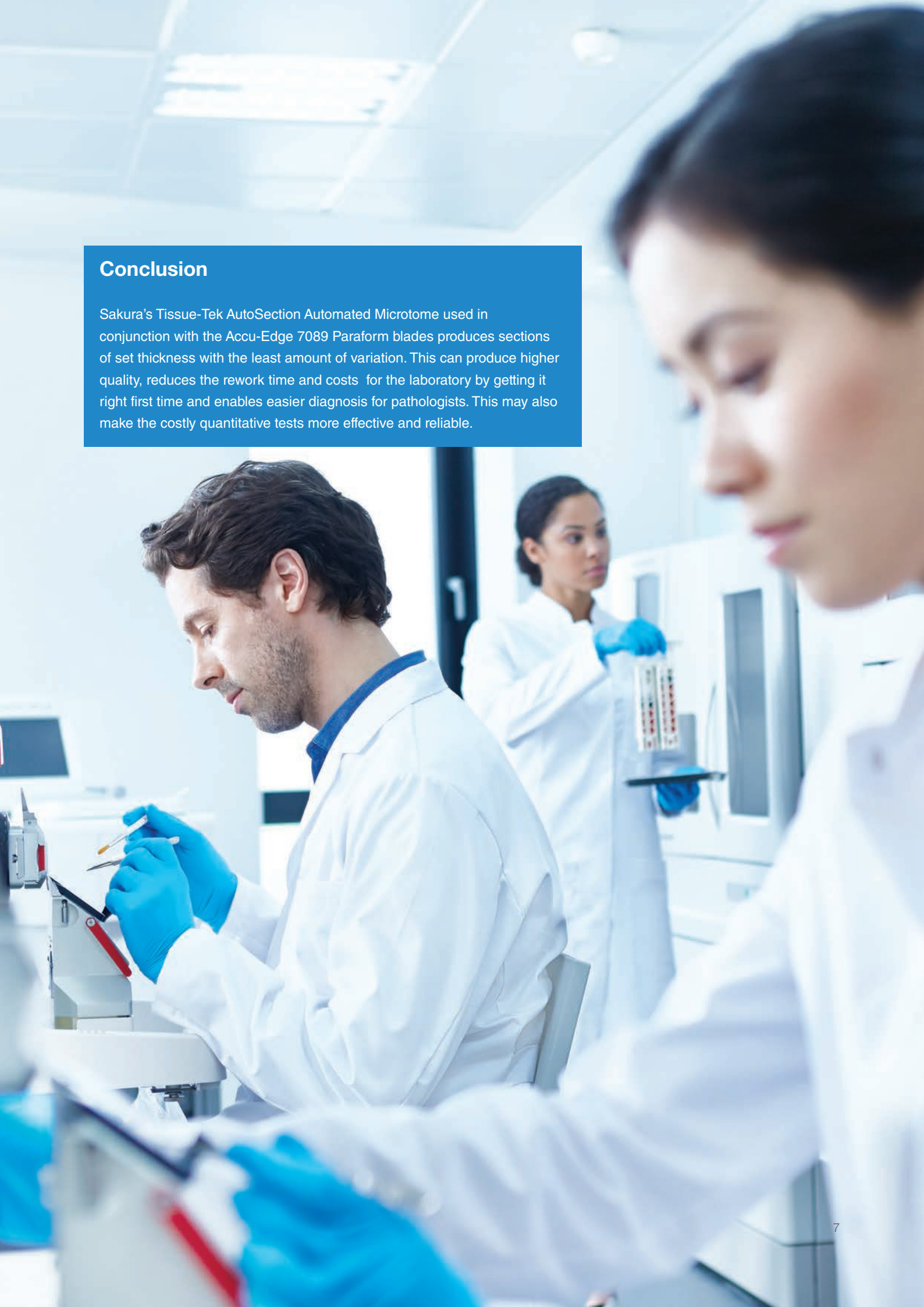
Increase the effectiveness and reliability of quantitative tests

More recently in histology however, more expensive and advanced quantitative testing is being performed. As this testing is based on counting the expression of certain epitopes it requires a greater degree of standardization. If therefore the same test is performed on a section of greater thickness than another section then the quantity of expression of an epitope could be higher on the thicker section. This might have impact on the treatment patient does or does not receive.

The AutoSection, when used in combination with the 7089 – Paraform Blade, producing sections with the least amount of variation in section thickness could therefore aid in standardizing this quantitative type of testing. This could make these costly tests more effective and reliable.

Conclusion

Sakura's Tissue-Tek AutoSection Automated Microtome used in conjunction with the Accu-Edge 7089 Paraform blades produces sections of set thickness with the least amount of variation. This can produce higher quality, reduces the rework time and costs for the laboratory by getting it right first time and enables easier diagnosis for pathologists. This may also make the costly quantitative tests more effective and reliable.



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