MICROTOMES AND MICROTOME KNIVES – A REVIEW AND PROPOSED CLASSIFICATION

F. Mohammed, T.F. Arishiya, S. Mohamed. Microtomes and Microtome Knives – A Review and Proposed Classification. Annal Dent Univ Malaya 2012; 19(2): 43–50.

Review Article

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ABSTRACT

Microtome is a mechanical instrument used to cut biological specimens into very thin segments for microscopic examination. Biological specimens can be presented in many ways. But more often, these specimens are embedded in paraffin wax blocks and the commonest way of sectioning these specimens can be achieved by the microtome. The earliest form of the microtome enabled free hand sectioning of fresh or fixed material using a sharp razor. Modern microtomes are precision instruments designed to cut uniformly thin sections of a variety of materials for detailed microscopic examination. Central to the production of good sections is the microtome knife. Microtomy virtually begins and ends with a sharp, blemish-free cutting edge. The introduction of disposable blades has made easier the production of good quality, thin sections, but they are often unsatisfactory for sectioning harder tissues, especially bone. A sharp knife edge free from imperfections is essential for the production of good sections. Since many types of microtomes are commercially available in the market, choosing the right microtome is essential for producing the best result as required. A classification is proposed that unifies and organizes the various microtomes based on the mode of operation.

Key words: microtome, microtomy, knives, sharpening, biological specimens

INTRODUCTION

Microtome is a mechanical instrument used to cut biological specimens into very thin segments for microscopic examination (1). Micro = *Small,* Tome = *Cut*. Sectioning paraffin wax embedded tissue blocks is the commonest way of achieving this, but the tissue can also be presented in other ways for microtomy. The basic instrument used in microtomy is the microtome into which a cutting tool is clamped.

Cummings (1770), a manufacturing company, designed manual sectioning instruments made from wood, which were exclusively used in botany for cutting plants (2). Chevalier (1839) first introduced the term ***"microtome"*** in the scientific terminology. Rudolf Thoma, a Heidelberg pathologist in concert with Rudolf Jung, a precision engineer, designed the first microtome in series production called *"Thoma microtome"* enabling the beginning of a new era in histology (3).

Modern microtomes are precision instruments designed to cut uniformly thin sections of a variety of materials for detailed microscopic examination (4). With practice, sections which are quite thin and translucent could be produced (5). For light microscopy, where magnifications can reach up to 1,800x, the thickness of a section can vary between 1 and 10 microns (thin sections). For electron microscopy, where magnifications of about 10,000,000x is possible, the thickness of a section is usually of the order of 10 nanomicrons (ultra-thin sections).

Parts of a microtome

Microtomes consist of three main parts:

- Base (microtome body)
- Knife attachment and knife
- Material or tissue holder

With most microtomes a section is cut by advancing the material holder towards the knife whilst the knife is held rigidly in place. The cutting action which can be either in a vertical or horizontal plane is coupled with the advance mechanism, so that the material holder is moved after each cut. The distance moved is pre-selected using a scale setting on the microtome body and usually extends between 0.5 and 50 microns on microtomes cutting thin sections and from less than

60 nm to over 500 nm on machines cutting ultra-thin sections (6).

Classification of microtomes *(Proposed)*

There are different types of microtomes available, depending on the manufacturer's specifications and based on the application required for sectioning of the samples. But the previous literature doesn't cite any classification of microtomes, thereby making it difficult to select an appropriate microtome. So an attempt is made to classify these commercially available microtomes according to their mode of operation.

Microtomes can be classified as:

– Manual microtomes
– Semi- automatic microtomes
– Automatic microtomes

Manual microtomes:

– Rocking microtome
– Rotary microtome
– Sledge microtome
– Freezing microtome
– Vibrating microtome
– Ultra microtome
– Cryostat
– Sliding microtome
– Saw microtome
– Hand microtome

Automatic microtomes:

– Laser microtome
– Computer microtome
– Ultra-thin computer microtome

Rocking Microtome

This instrument is one of the oldest in design, relatively cheap, and is exclusively designed for sectioning paraffin blocks (Figure 1). The name of the microtome derives from the rocking action of the cross-arm and is simple to use. This microtome comprise of three moving parts, which is extremely reliable and requires minimum maintenance. The tissue moves through an arc as it advances towards the knife (the slightly biconcave Heiffor knife) which is held rigidly causing the sections to be cut in a curved plane (7).

However, the disadvantages are its tendency to move during cutting because of the lightness of the frame and very thin sections are difficult to obtain. The rocking microtome has largely been replaced by the more precise rotary microtome although it is re-appearing in portable cryostats.

Rotary Microtome

This microtome derives its name from the rotary action of the hand-wheel which actuates the cutting movements. Machines of this sort are general purpose microtomes for cutting semi-thin to thin sections for light microscopy (Figure 2). The operation is based upon the rotary action of a hand wheel activating the advancement of a block towards a rigidly held knife. The block moves up and down in a vertical plane in relation to the knife and therefore cuts flat sections. It has the advantage of being heavier and therefore more stable than the rocking type, and is ideal for cutting serial sections (8). Larger blocks of tissue may be cut on this machine, and the cutting angle of the knife (tilt of knife) is adjustable. Since a heavier and larger knife is used with this type of microtome, there is less likelihood of vibration when cutting.

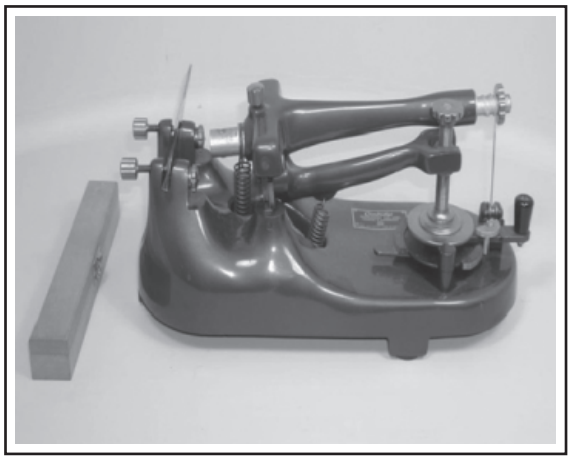

Figure 1. Rocking microtome.

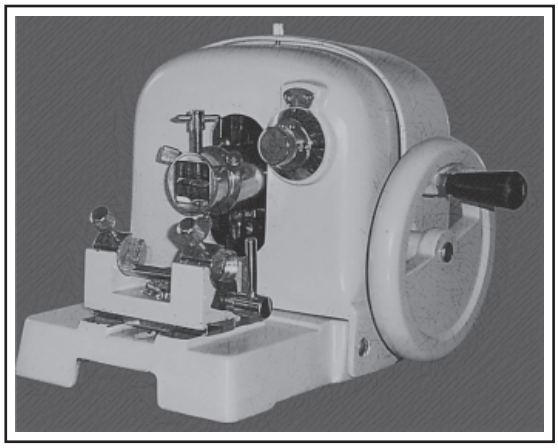

Figure 2. Rotary Microtome.

Section thickness settings range from 0.5μm to 60μm on most machines. Sections of paraffin wax embedded tissues are normally cut within the range 3 to 5μm, while resin sections are attained between 0.5 to 1μm (9).

Sledge microtome

This microtome was originally designed for cutting sections of very large blocks of tissue (e.g. whole brain). The sledge microtome has become a popular machine for routine use since the World War

II (Figure 3). The block-holder is mounted on a steel carriage which slides backwards and forwards on guides against a fixed horizontal knife. This microtome is heavy and consequently very stable and not subjected to vibration. A large knife is used (24 cm in length) and the knife is usually wedge-shaped which reduces the possibility of vibration and requires less honing. The knife-holding clamps are adjustable and allow the tilt and the angle (slant) of the knife to the block to be adjusted with ease (10). Its action is much slower when compared to rocking or rotary microtome which is its major disadvantage.

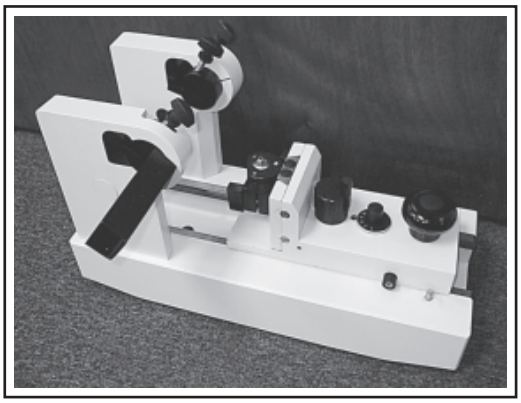

Figure 3. Sledge Microtome.

Sliding Microtome

In a sliding microtome, the knife is moved horizontally against a fixed block which progresses against it in an inclined plane (Figure 4). The sliding microtome can be used for paraffin-wax embedded sections although it was designed for cutting celloidin-embedded sections (11).

Figure 4. Sliding Microtome.

Freezing Microtome

Although other microtomes can be modified for cutting frozen sections, this type of microtome was known to be efficient, producing the best results and was used almost universally. The machine is clamped to the edge of a bench and is connected to a cylinder of CO_2 by means of a specially strengthened flexible metal tube (Figure 5). The cutting action of the freezing microtome differs from those described previously as in this case the knife is moved whilst the tissue block remains static. The block moves by a pre-set amount, in microns, at the end of each cut. However, consistent, high quality, thin sections are very difficult to obtain with this type of microtome (10).

This device enables tissue to be frozen without the necessity of solid carbon dioxide or liquid nitrogen. This method has acquired popularity before the cryostat became widely used for frozen sections. In this instrument, two dissimilar metals are placed in apposition with one another and when a direct electric current passes through them, heat is generated on one surface and lost from the other. This phenomenon is known as ***'Peltier' effect*** (12).

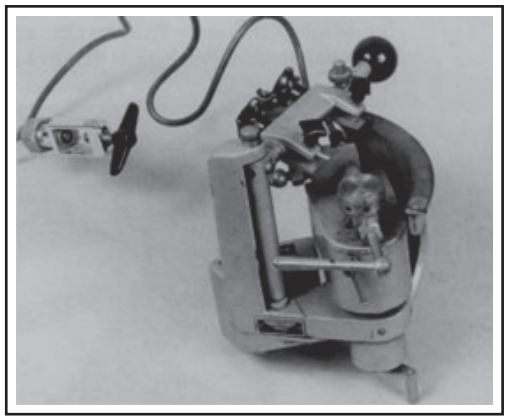

Figure 5. Freezing Microtome.

Vibrating Microtome

Originally thought to replace the hand microtome, the vibrating microtome was conceived as a microtome which could produce high quality sections of fresh, unfixed material from animal or botanical sources (Figure 6). This instrument has been designed to cut tissue which has not been fixed, processed or frozen and has the greatest application in enzyme histochemistry and ultrastructural histochemistry.

The name of the instrument was derived from the high speed vibration produced by a safety razor blade which provided the cutting power. The amplitude of vibration is adjusted by altering electrical voltage applied to the 'knife' (10). To prevent tearing, soft

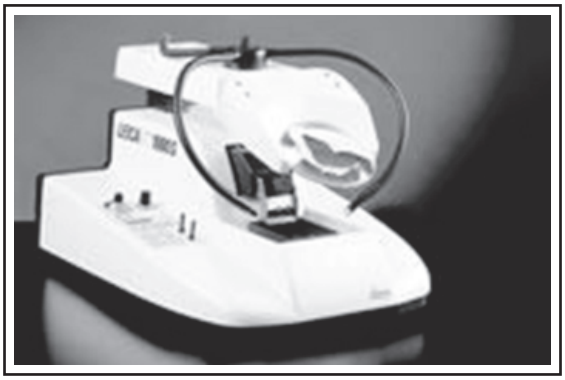

Figure 6. Vibrating Microtome.

material is cut whilst immersed in a fluid which also aids in dissipating heat produced at the vibrating edge of the razor during cutting.

Ultra Microtome

The ultra-microtome is used to prepare ultra-thin sections for light and electron microscopy (Figure 7). Very small samples of tissue or industrial product are usually embedded in hard resin before cutting. It has been reported that sections can be cut as thin as 10 nanometers (13). Two forms of advance mechanism have been developed in this style of microtome.

The thermal mechanism relies upon heat induced expansion in a bifurcated metal strip. Whereas in the mechanical form a microprocessor coupled to a precise stepping motor controls the advance mechanism (14). The cutting stroke is motor driven to provide a regular, smooth motion for sections of even thickness and constant reproducibility. Knives are usually made from glass, diamond or sapphire. The block is brought to the knife edge under microscopical control and as each section is cut it is floated on to a water bath adjacent to the knife edge (10).

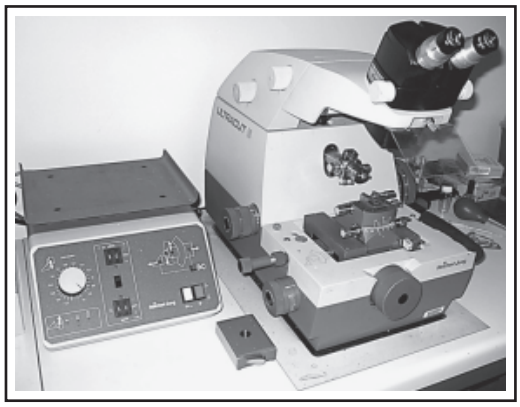

Figure 7. Ultra Microtome.

Saw Microtome

Saw microtomes cut sections from very hard material such as undecalcified bone, glass or ceramics (Figure 8). The samples, commonly embedded in resins, are moved extremely slowly against a diamond coated saw rotating at approximately 600 rpm. It is possible to produce sections of 20 μm or greater, provided the saw blade is in perfect condition (15). The saw microtome is not capable for producing very thin sections (9).

Figure 8. Saw Microtome.

Hand Microtome

The hand microtome is limited to sectioning intrinsically rigid botanical material and it is difficult to obtain thin sections from animal tissues (Figure 9) (10).

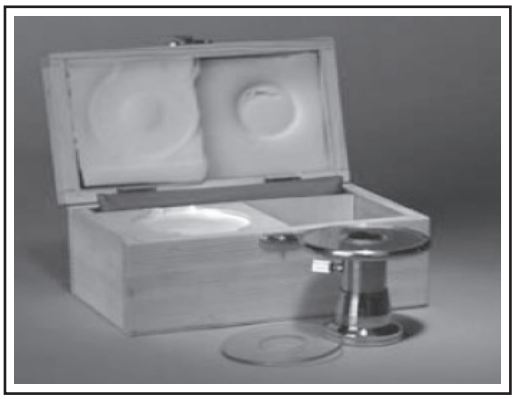

Figure 9. Hand Microtome.

Cryostat

The introduction of fluorescent antibody staining techniques by Coons, Creech and Jones in 1941 led to a need for thin sections (3-5 μm) of fresh frozen tissue free of ice crystal defects (16). To satisfy these criteria the tissue must be snap frozen at a very low temperature.

Linderstrom-Lang and Mogensen designed the first cryostat in 1938. Coons and his colleagues redesigned it in 1951 (Figure 10) (17).

It consists of a microtome of any type but preferably rustproof, which is enclosed and operated within a deep freeze cabinet. The cabinet is fitted with a double glass window, and a door through which material may be passed in and out. The cabinet is equipped with a fluorescent light and a fan to ensure the circulation of cool air (15). The temperature may be regulated between-10ºC to -40ºC. Any cryostat can

Figure 10. Cryostat.

be used as an alternative to a freezing microtome for rapid sectioning. Some microtomes have been designed for this purpose by incorporating an internal Freon quick freeze stage. This stage holds four block holders which after the tissue is frozen on them, are transferred to the microtome. Because the sections adhere firmly to the warm slides, the staining and mounting may be carried out more rapidly. The microtome may be adjusted to cut sections from 2-16μm (11).

Laser Microtome

Laser microtome is used for precise, non-contact sectioning (Figure 11) and was designed to slice samples with high precision. It's equipped with state-of-the-art **femtosecond** laser technology. It enables non-contact cutting inside biological tissues and various materials without causing thermal damage (18). Depending on the material being processed, slice thicknesses of about 5 to 100 μm are feasible (18).

Non- contact processing, sub micrometer precision, cutting of the tissue in its native state, no thermal damage, fewer artifacts and less time consumption in tissue preparation are the added advantages of this laser microtome (19).

Computerized Microtome

It is equipped with the advanced rapid thermostatic switch, semiconductor freezing, cryo-scalped and cryoplate (Figure 12). The computerized microtome can carry out the rapid freezing section or routine paraffin section (dual-purpose). This microtome attains slice thickness in the range of 1-25μm with least slice adjusting graduation of 1μm and a maximal slice section of 32x32mm. The temperature of cryo scalpel and cryoplate range between 0ºC ~ -18ºC and -10ºC ~ -40ºC respectively (20).

Microtome Knives

Central to the production of good sections is the microtome knife. The first attempt to produce an adequate cutting surface was a sharpened razor blade; however these became blunt very quickly. In 1950 Latta and Hartmann discovered edges fine enough by using freshly cut glass (21).

Microtomy virtually begins and ends with a sharp, blemish-free cutting edge. The introduction of disposable blades has made easier the production of good quality, thin sections, but they are often unsatisfactory for sectioning harder tissues, especially bone. As these tissues constitute the greatest challenge to the microtomist, the necessity for maintaining a sharp knife has not been diminished. Microtome knives can be classified according to the material used for making the knife or based on the shape of the knife edge.

Figure 11. Laser Microtome.

Figure 12. Computerized Microtome.

Based on material of the knife:

- Steel knives
- Non-corrosive knives for cryostat
- Disposable blades
- Tungsten carbide knives
- Glass knives
- Diamond knives
- Sapphire knives

Based on shape of the knife edge *(Profile)*:

- Profile A: Strongly plano-concave/biconcave
- Profile B: Plano-concave
- Profile C: Wedge Shaped
- Profile D: Plane Shaped

Steel Knives

Steel microtome knives are manufactured from high quality carbon or tool grade steel which is heat treated to harden the edge (Figure 13) (22). The steel should be rust resistant, free from impurities and contain anti-corrosives.

The best knives are those that are fully hardened. Those which are only surface hardened lose the cutting edge very quickly once the hardened area is removed through repeated re-sharpening (10).

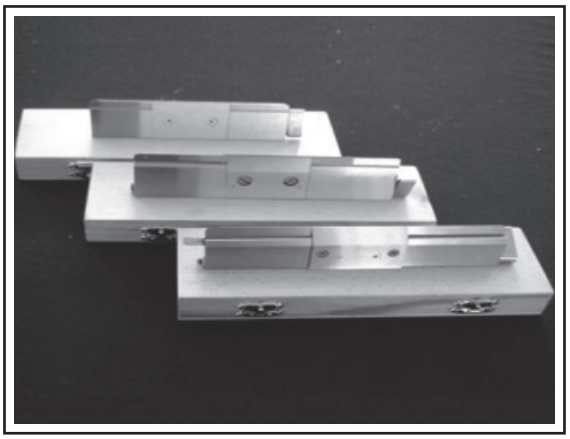

Figure 13. Steel Knives.

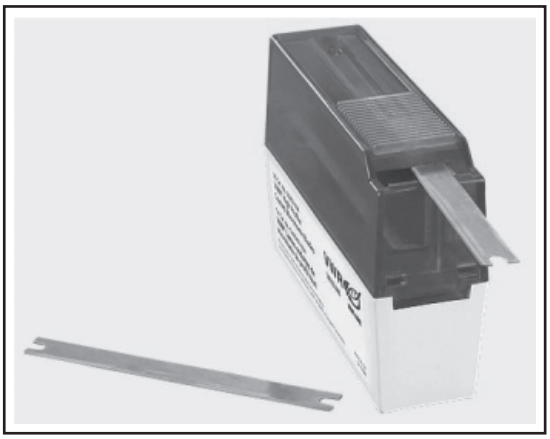

Figure 14. Disposable Knives.

Non-Corrosive Knives For Cryostats

These are manufactured from hardened, heat treated stainless steel free from all impurities. They contain 12 to 15% chromium (10).

Disposable Blades

These are essentially refined, thickened razor blades (Figure 14). When these are held in a specially adapted knife holder the blades consistently produce high quality sections. These have replaced conventional microtome knives in many instances. All disposable blades are manufactured from high quality stainless steel. The edge of disposable blades can be coated with platinum or chromium to enhance strength and prolong cutting life (23-24).

Teflon coated blades are particularly suitable for use in cryostats as these offer reduced cutting resistance and minimal friction. The smaller, thinner disposable blade also reaches cryostat chamber temperature more rapidly than a conventional knife, **minimizing the delay** during blade exchanges or temperature adjustments. Disposable blades need to be held rigid in a special holder to prevent vibration during the cutting stroke (10)

Tungsten Carbide

Knives manufactured from high quality tungsten carbide (Figure 15) are practically nonmagnetic and 100 times harder than hardened tool steel (25). The knives have excellent resistance to wear but are brittle because of their extreme hardness and should be handled carefully. But, it has been reported that up to 30,000 serial sections of undecalcified bone embedded in methacrylate can be obtained per sharpening (26).

Glass Knives

The cutting edge of glass knives ('Ralph knives' with edges of 25 or 38 mm) used for conventional sectioning is parallel to one surface of the glass while knives used for ultramicrotomy is positioned against / across the thickness of the glass (Figure 16). Different profiles of 'Ralph knife' for cutting sections from different embedding media can be produced very quickly. Glass knife holders are available so that 'Ralph knives' can be used with a rotary microtome. Glass knives are hard but brittle and therefore require care when handling. These knives deteriorate with storage due to changes in the 'flow' or 'strain' of the glass after fracture and from oxidation impurities remaining in the hardened glass after manufacture. Knives should thus be prepared immediately before use (27).

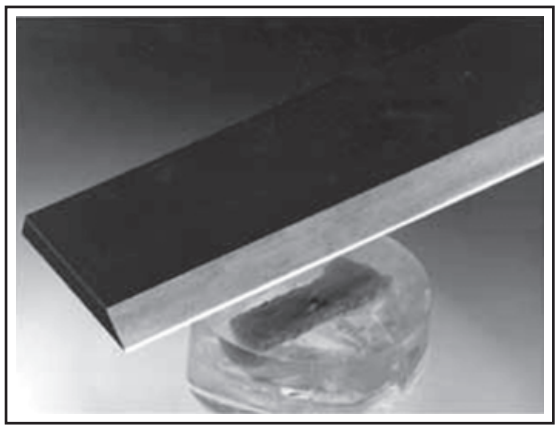

Figure 15. Tungsten Knives.

Figure 16. Glass Knives.

The ralph knives can be classified according to the profile of the cutting edge.

- **High profile:** 1.0 to 1.5 cm cutting edge.
  - Suitable for cutting sections from soft embedding medium such as waxes.
- **Medium profile:** 0.5 to 1.0 cm high cutting edge.
  - Suitable for cutting sections from soft and hard plastics.
- **Low profile:** less than 0.5 cm high cutting edge.
  - Suitable for cutting sections from hard plastics.
- **Front profile:** The sloping edge artifact makes the knife unsuitable for use-this is caused by inadequately securing the glass strip at each end (10).

Diamond Knives

Diamond knives are manufactured from gem quality diamonds without flaws. Although the diamond knives are very expensive, the knives are extremely durable, due to its hardness. These knives are used primarily for cutting very thin sections (28-29).

Sapphire Knives

These knives are manufactured from one piece of solid sapphire artificially produced from an alumina monocrystal under computer controlled thermal conditions (24). Sapphire is harder than tungsten carbide or glass which ensures high durability of the cutting edge for all types of material. The only restriction when using a sapphire knife is the block size as the knife edge is limited to 11 mm. A special knife holder is required for this kind of knife (10).

The knife edge can be classified according to its shape / profile.

PROFILE – A: Strongly plano concave/biconcave

One surface of the plano concave knife is straight whilst the other is hollow ground (Figure 17). The bi-concave knife has two hollow ground surfaces. Both knives are extremely sharp and are used for cutting soft, celloidin embedded material or foam compounds (30). These knives are not suitable for relatively hard materials, which cause the edge to vibrate and produce the phenomenon known as chattering. To obtain the best result the knife should always be oblique to the object when cutting sections (10).

PROFILE – B: Plano concave

This knife is similar to a profile – A knife but has a thicker back. It is used for cutting sections from material which is too hard to cut with a profile – A knife but can also be used for softer materials embedded in paraffin wax. This knife should be positioned obliquely to the material being sectioned. Plano-concave knives are available with varying degrees of concavity (28).[28]

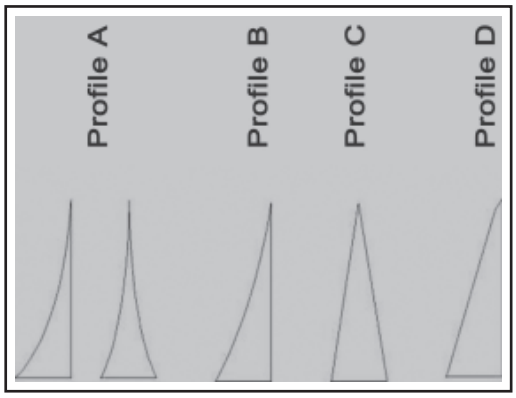

Figure 17. Profiles of the Knives.

PROFILE – C: Wedge Shaped

The wedge shaped knife has more rigidity than profile – A or B knives and can therefore be used for cutting harder materials. Because of the extra thick nature of the wedge at the tip, this type of knife cannot be ground as sharp as profile – A or B knives. It is commonly used for cutting sections from paraffin wax embedded material, frozen sections, cryostat sections and for small, synthetic resin embedded material. With this style of knife the cutting plane is transverse to the object (15).

PROFILE – D: Plane Shaped/Tool edge shaped

This knife will cut hard and tough material as it has greater stability than any of the other profile knives. As only one bevel provides the cutting edge this knife is the least sharp of all of the knife profiles. It is commonly used for cutting synthetic resin blocks, hard materials embedded in paraffin wax and large wax blocks. The cutting edge of all steel knives is produced by grinding a bevel on each side of the knife for profiles – A, B and C, or onto the angled surface of a profile – D knife. The bevel faces enclose a sharper angle than the main surfaces of the knife (15).

CONCLUSION

Many types of microtomes and knives are commercially available in the market and the selection of which microtome to be used will depend on the types of tissue sample and the requirement needed. Understanding the microtomes, its components and the cutting mechanism will be useful for selecting the suitable microtome required for cutting of the tissue samples and can minimize damage or distortion to the tissue sample.

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