How to Make High Quality Finished Microscope Slides:

Start with the Quality of the Slides Themselves

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HOW TO MAKE HIGH QUALITY FINISHED MICROSCOPE SLIDES: Start with the Quality of the Slides Themselves

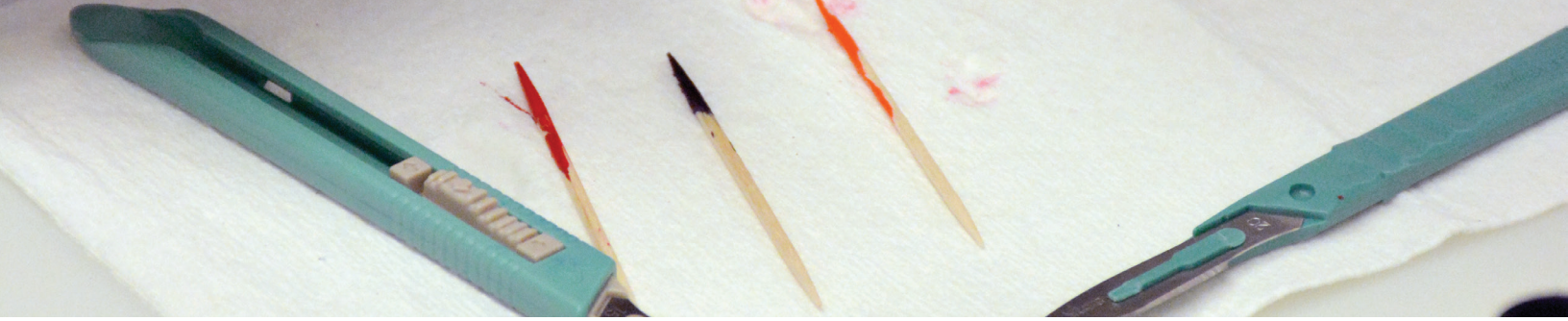
In the histopathology laboratory, laboratory personnel work as a team to produce finished microscope slides of the highest quality possible. The final slides must show the best quality of staining, starting with correct processing and embedding of the specimen and including the best microtomy possible.

But what about the microscope slides themselves? Efforts to produce the best quality final microscope slide for the pathologist are all for naught if the sections fall off the slides during staining, get jammed in a coverslipper or contain artefacts which may preclude an accurate diagnosis. In addition, many tiny specimens are received that, if lost during section fall off, could result in a "lost specimen". Clearly, the histopathology laboratory must start with microscope slides of the highest quality to begin with.

Microscope slides are usually made of optical quality glass, such as soda lime glass or borosilicate glass. With regard to research and special procedures, plastic slides may be utilized, which are composed of organic polymers which may include other materials as well. Additionally, fused quartz slides may be used when ultraviolet transparency is important, such as procedures involving fluorescence staining and microscopy. However, in the majority of histopathology laboratories, glass microscope slides are used.

When choosing microscope slides for your laboratory, initial consideration should be given to the size and shape of the slide. As we know, most laboratories use automated equipment requiring standardized slides. The majority of this equipment uses a slide that measures 1 mm thick by 25 mm wide by 75 mm in length (approximately 1/8 inch x 1 inch x 3 inches). Shape refers to the corners of the slide: they can be square at a 90 degree angle, or "clipped corners" with a 45 degree angle. The latter are important when using slide printing equipment. Finally, the edges of the slide may have an unfinished sharp edge, or have ground edges to make them smooth. Ground edges are safer for the histologist to handle and have fewer tendencies to "jam" in slide printers, stainers and coverslippers. When slides get "jammed" in a stainer or coverslipper, that means down time, which results in decreased efficiency and increased turnaround time in your laboratory. That is a result you can do without.

Microscope slides are also available with various "label ends". Plain ends are simply that: smooth glass which requires some type of glass etching for labeling. Frosted end slides include a rough, frosted label end on one or both sides of the slide. The frosted area allows information to be written on by using pencil or a special laboratory marker. Please be aware: an office "Sharpie ™" type marker is not recommended, as the subsequent exposure to solvents used during staining may dissolve and wash off the writing. Be sure to use microscope slide label-specific markers for writing.



The same marker can be used on microscope slides which contain a “colored” label end on the slide. This surface is smooth, easy to write/print on and provides good adhesion for slide labels. There are many different colors available and many labs use the different colors to designate workflow priority, stain type (i.e. special stains, immunohistochemistry (IHC) stains, etc.) and/or client designation. The slides may also be used on certain automated slide printers. These printers contain specific inks designed to withstand the subsequent stains and solvents used during various staining procedures. It is important to select a microscope slide with a label end that is compatible with your laboratory’s printer, such that there is excellent reproduction of any bar codes which will then ensure a 100% scan rate during use.

Once you have chosen the proper slide size, finish and label end type for your laboratory, it is extremely important to choose which coating, if any, your laboratory requires. Some laboratories are able to use uncoated slides. That is, the slides are manufactured, cleaned and then shipped. However, if your laboratory performs staining procedures such as special stains and IHC on routine specimens and decalcified bone and nails, you should consider using “coated” or “charged’ slides.

Back in the “good old days” many laboratories used gelatin in the tissue float bath during microtomy to help the sections “stick” to the slide. However, this method many times resulted in stains being retained by the gelatin which imparted a pink or blue background on the slide.

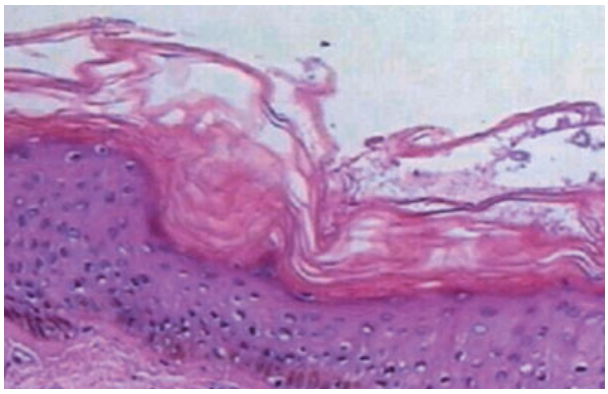
In the 1980’s microscope slide manufacturers began using poly-L-lysine to coat the microscope slide, such that a positive charge was imparted to the slide surface by this amino acid. It was found that this coating helped to keep sections on the slide during staining. However, these “charged” slides sometimes exhibit a “hydrophobic” artifact on the slide, in which focal areas of the section were not stained. Additionally, if slides were stored for lengthy times (i.e. months) the “charge” could dissipate, resulting in section fall off.

Manufacturers responded by updating their production procedures, and soon they were able to consistently manufacture coated slides which provided excellent, reproducible results. They also experimented with different “coatings”.

Lysine coated slides remain one of the top choices of slides for laboratories. In addition, manufacturers added a choice of silane coated slides. Aminoalkylsilane is used to provide a permanent positive charge to the slide surface. Both lysine and silane work on the principle of providing a constant electrostatic attraction of the glass to the tissue section and cells.

Utilizing the original procedures, manufacturers also provide gelatin coated slides for specialty use. Slides are coated with a solution of gelatin (0.5% to 2.0%) to provide a “sticky” slide surface. These are especially useful for decalcified bone and nail specimens. While there may

be some stain retention artifact on the slide, it does not interfere with the final stain of the section. Another specialty microscope slide is the "hydrophobic" slide. Wells are created by using a coating of Teflon ™ or epoxy ink for use in special procures such as direct immunofluorescence for tissue sections and cell cultures.



Selection of a proper coating for the microscope slides used in your laboratory is a necessary and essential step in the production of high quality slides. As mentioned at the beginning of this article, failure to use an appropriate slide may result in staining artefacts and tissue section fall off, which in turn could result in a lost specimen. Staining artefacts may require re-cutting and re-staining the affected slides. This means you are performing that task twice – to get one result. Your laboratory profit margin is now cut in half.

More ominous is the chance of a lost specimen. A good example is a tiny skin biopsy. Many times, that tiny specimen is the lesion – there is not more to re-sample. If this specimen is lost, a medical-legal issue may result, and no laboratory wants or needs that. Even if the patient can be re-biopsied, this is always done as a "no charge" which means your laboratory profit has now gone into the negative numbers.

In summary, it is not enough to automatically use the same slides the laboratory has been using since you started working there. If your laboratory is experiencing tissue fall off and staining artefacts due to poor quality microscope slides, it is time to change. Investigate by contacting your vendors to request samples of their different microscope slides, and run some test validations. You may be surprised that a better slide does not necessarily cost more. Even if it does, the increased cost is minimal, compared to the possible medical-legal result from one lost specimen.

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Clifford Chapman has over 40 years experience managing both private reference and teaching hospital pathology laboratories in the Boston area, including Massachusetts General Hospital, Pathology Services, Children's Hospital Boston, and StrataDx.

He also has over 25 years experience presenting lectures, workshops, teleconferences and webinars at the local, regional and national level for the Massachusetts Society for Histotechnology, Region I Histology and National Society for Histotechnology.

Clifford is a specialist in histological techniques, quality management, laboratory workflow and laboratory safety. He is an author and co-author of over thirty scientific publications, including his most recent book "Dermatopathology Laboratory Techniques". Clifford is currently the Technical Specialist at StrataDx and works as a consultant at Medi-Sci Consultants.