WHITEPAPER

Troubleshooting: Coverslipping

BIOCARE MEDICAL

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So, you've done it. You've successfully created a strong, crisp, cleanly stained slide. All that's left to do now is apply a coverslip and look at it under a microscope. However, as you may have learned, nothing in immunohistochemistry (IHC) is ever so simple. Your choice of mounting media and coverslip will have a direct effect on your ability to visualize your slide, regardless of staining.

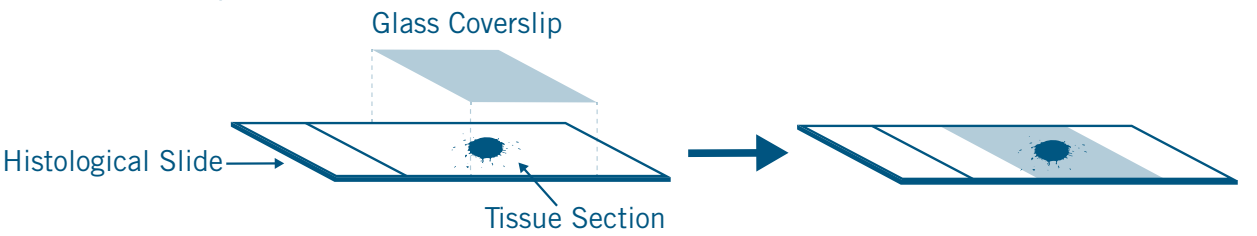
Mounting and coverslipping serve dual purposes in microscopy. First, they protect the tissue section from damage. The coverslip should be large enough to cover the tissue section entirely, and the appropriate amount of mounting medium should be applied at the correct dilution. If the medium is too scarce or too dilute, it will retract from the edges of the coverslip as it dries, leaving parts of the section exposed to the air, as seen in Figure 1.[1]

Second, mounting and coverslipping improve visual clarity by controlling the light refraction around the tissue.[3] In other words, the mounting medium and coverslip will ideally bend light in a way that makes the stained section clearly visible beneath them. The extent to which a substance bends light is measured by a value known as its refractive index (RI). If the RIs of the coverslip material and mounting media are too different, the mismatch in their refraction angles will create optical aberrations, reducing clarity.[3] This is also the reason why air bubbles (RI 1.0) or water droplets (RI 1.3) can be so visually disruptive under a glass coverslip (RI 1.5). [3] As such, technicians should check the RIs of their coverslip and mounting materials and prevent air or water from being trapped beneath the coverslip.

Additionally, the mounting media should preserve staining quality. Care must be taken in your choice of mounting media to ensure that stains are not exposed to substances in the medium that could damage them. For example, some chromogens can lose their color when exposed to organic solvents and so must be mounted with an aqueous mounting medium.[2] For example, Biocare's EcoMount is a polymer-based mounting medium for IHC slides that does not contain organic solvents such as xylene. Other mounting media prevent fading in fluorescent stains, such as Biocare's Fluoro Care Anti-Fade Mountant, which is specially designed to preserve fluorescent specimens, extending their shelf life. With the wide variety of mounting media available on the market, technicians should always check the specifications of their staining reagents and mounting media to ensure they are compatible.

To learn more about how Biocare Medical's mounting media can benefit your lab, please visit us at biocare.net or call 1-800-799-9499.

Glass Slide Coverslip



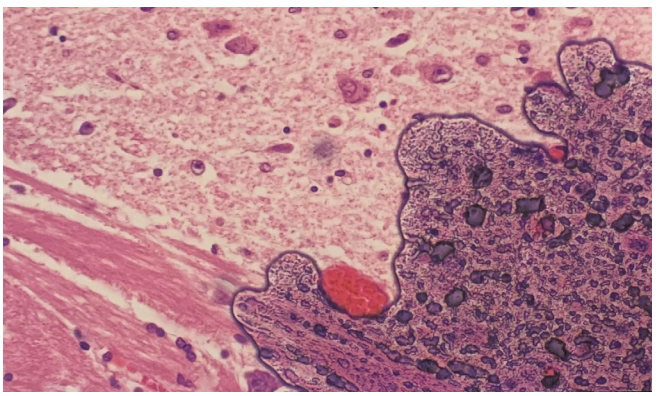


Figure 1 Retraction artefact can be seen in this slide image
From Histotechnology: A Self-Instructional Text (p. 131), by Carson, F. L., & Cappellano, C. H., 2009, United States: American Society for Clinical Pathology Press. Copyright 2009

1. Carson, F. L., & Cappellano, C. H. (2009). Histotechnology: A Self-Instructional Text (3rd ed.). ASCP.
2. Kalyuzhny, A. E. (2016). Immunohistochemistry. Techniques in Life Science and Biomedicine for the Non-Expert.
3. Ravikumar S, Surekha R, Thavarajah R. Mounting media: An overview. J NTR Univ Health Sci 2014;3, Suppl S1:1-8