Peace of Mind That You're Always Prepared:

An Educational Series on the Value of Routine PM Service

Part 6: Staining

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This article will focus on the automated hematoxylin and eosin (H&E) staining of paraffin sections on glass microscope slides. The principles discussed will apply to any special stains that have been set up to run on a like automated stainer as well. Routine preventative maintenance on such automated stainers is essential to obtaining consistent optimal staining in the final microscope slide.

There are many vendors and models of automated H&E stainers present in laboratories across the country and around the world. Most operate on the "X-Y-Z" axis operational principle. Cut slides are placed into slide racks which are compatible with the stainer platform. The slide racks are loaded onto/into the stainer and the proper staining protocol is chosen by the operator. The on-board computer control system will then direct the slide rack into and out of the reagents and stains designated by the staining protocol. The end point of the staining protocol is when the slides are held in a xylene/ xylene substitute reagent to await the coverslipping process. It is important to note that there are "closed system" stainers as well, in which reagents are applied in a "one use only" mode. These stainers are beyond the scope of this discussion.

It is important to remember that even though these stains are "automated", the operator still has the biggest influence on the final quality of the stain on an hour to hour, day to day basis. Stains must be filtered and changed out; reagents must be changed and rotated correctly – all within the parameters established by the laboratory director. The laboratory personnel who attend the slide stainers must be vigilant and precise in their actions. This includes daily maintenance tasks as well.

Beyond that observation, the equipment itself must undergo preventative maintenance by a qualified vendor representative at least annually. The mechanical tolerances within the equipment are very exacting. The "robot arm" must be precisely calibrated to consistently deliver the slide racks into and out of the various reagent vessels. If a slide rack "crashes", slides can break and this can have very dire consequences. This is especially true for laboratories which process extremely small specimens, in which the entire specimen may be on one slide.

In addition to mechanical tolerances, the on-board computer system must be maintained and checked as well. The timing clock must be calibrated in order to ensure that each staining step is performed for the specified duration. Stain procedure steps may be designated as "critical" or "non-critical". This designation is important and requires exact timing for "critical' steps within each stain procedure. Additionally, the timing of the "retraction" of slides from the reagent vessels is critical. It must be set to allow the last drops of reagent to fall into the vessel, prior to being moved to the next reagent. This will affect stain quality and reagent management.

Rinse water sources and water rinse stations must be maintained to prevent biomass contamination. This may cause the introduction of bacteria and/or fungi into the rinse water, which can show up on a final slide. Biomass contamination may also impede rinse water flow, and result in sub-optimal staining. Water connections should be checked for any signs of leakage. Vendor service technicians should inspect the rinse water system and replace any parts that show wear. The service technician will also check the “smart water rinse” control. This instructs the equipment to open and run water rinse stations only when they are needed – instead of running continuously and wasting water.

At the end of the staining run, the stainer may sound an alarm to alert the attending laboratory personnel to remove the slide rack from the stainer for subsequent coverslipping. Alternatively, some automated slide stainers are made to be coupled to a transfer station, which moves the slide rack from the stainer into an automated coverslipper. This equipment will be the subject of the next article.

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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.