Histological staining is a series of technique processes undertaken in the preparation of sample tissues by staining using histological stains to aid in the microscope study. The process of histological staining takes five key stages which involve; fixation, processing, embedding, sectioning and staining. Great changes have been done on techniques used for histological staining through chemical, molecular biology assays and immunological techniques collectively and have facilitated greatly in the study of organs and tissues (Alturkistani *et al.,*2015).

Staining

Staining is used to highlight important features of the tissue as well as to enhance the tissue contrast. Hematoxylin is a basic dye that is commonly used in this process and stains the nuclei giving it a bluish color while eosin (another stain dye used in histology) stains the cell's nucleus giving it a pinkish stain. However, there are other several staining technicques used for particular cells and components (Black, 2012). Staining is a commonly used medical process in the medical diagnosis of tumors in which a dye color is applied on the posterior and anterior border of the sample tissues to locate the diseased or tumorous cells or other pathological cells (Musumeci, 2014). In biological studies staining is used to mark cells and to flag nucleic acids, proteins or the gel electrophoresis to aid in the microscopic examination (Jackson & Blythe, 2013). In some cases, various multiple staining methods are us

ed such as differential staining, double staining or the multiple staining (Iyiola & Avwioro, 2011)

Histology has evolved considerably since its beginnings in the 17th century, with advances in both specimen processing and analysis. Consequently, histology departments now face increasingly larger workloads. To adapt, they have integrated automated systems, which save time and allow histology professionals to work on other skill-based tasks while maintaining enough flexibility to process and stain according to the needs of the medical or research lab. Here, we’ll explore how automation has been integrated into histology to speed up the workflow of both medical technicians and researchers.

Slide staining and coverslipping

Slide staining is where automation necessitates the most flexibility, as it must be adapted for both basic histochemical as well as immunohistochemical analysis. There are two types of systems available: open and closed systems. Open staining systems provide the most flexibility and are often used for research purposes, where protocols may need to be customized depending on the tissue or biomarker. Closed systems provide less flexibility and are more suited for clinical labs, which prioritize reproducibility using established protocols.1 These systems may also come equipped with automated coverslippers that will dry your slides and immediately affix a coverslip to them.

Some of the features that can vary for automated slide stainers include slide and reagent management. For slide management, slides are loaded horizontally onto trays or racks and covered with reagent, which is spread around using either air or capillary action.1 However, the number of slides that can be processed at once varies, with some of the higher-end models capable of processing up to 330 slides per hour. Many machines, like the HistoCore SPECTRA ST from Leica, allow you to run high-throughput staining protocols for common stains like hematoxylin and eosin (H&E), while others, like the Ventana HE 600 system, allow you to stain individual slides, which is ideal for research labs with many users relying on differing staining protocols. With regards to reagent management, most systems use a matrix architecture, where both the slides and reagents are lined up in rows and columns and the robotic arm moves around dispensing reagents onto the slides. Reagent management can also be rotary, where the slides and reagents are organized around 2 rotating circular trays, and the rotation brings the slides into contact with the reagent-dispensing containers.1IHC and H&E slide stainers stain prepared slides of processed and cut human, animal or plant tissue to enhance the contrast within a specimen for study under a microscope. Automated slide stainers consist of the following major components::

A control and monitoring system – the display where all operations are controlled.

A solution reservoir, which includes slide heating stations, staining stations, wash stations and drying stations.

A robotic arm, which is a three-axis transport mechanism for slide baskets.

A fume control system, which uses activated-carbon filters to remove harmful vapors from inside the instrument.

The operator loads the slides into the solution reservoir where they are heated; stained once or twice, depending on need; and rinsed and dried – which prepares specimens to be coverslipped. Automated models can carry out multiple staining protocols simultaneously.

Common automated slide stainer problems:

Staining quality is poor. This will cause the tissue to be unreadable and produce the potential for a misdiagnosis.

Fluids leak from the solution reservoir. This will cause a spill that requires cleanup.

Flooding of the slide stainer. This will cause a spill that requires cleanup.

Solution reservoir doesn’t drain. This will cause a spill that requires cleanup.

Solutions overflow from the reservoir. This will cause a spill that requires cleanup.

Excessive noise is produced. This will cause a nuisance.

No movement in the solution reservoir. This will cause poor staining.

Robotic arm jams. This could cause the slide to break. The operator would have to repeat all steps to produce a slide, which becomes a real problem if there isn’t enough tissue to produce another specimen.

Display errors. This will cause the operator to have to solve the problem behind the error, or it will shut down the slide stainer, depending on the error message.

Control panel display is blank. This will cause the instrument not to operate properly.

No power to the slide stainer: This will prevent the instrument from operating.

Excessive odor. This is likely from a chemical, creates a nuisance and could indicate a deeper problem.

Fan doesn’t operate. This will likely lead to you smelling chemicals from the slide stainer and create a nuisance.

Automated slide stainer troubleshooting:

In the case of error codes on the tissue processor control panel display, please refer to the product user manual. Different manufacturers use different codes and have different troubleshooting steps. In the case of a loss of power, remember to check circuit breakers and fuses.

When a Tech One technician is needed to make repairs, he will diagnose the problem and make the appropriate repairs. The typical service call is one to three hours.

Automated slide stainer maintenance tips:

Clean all components daily to prevent dirt from building up. Dirt build up can cause the stainer to jam and slides to break.

Make sure to replace the slide stainer’s charcoal filter every three months.

When unsure, call a Tech One technician for assistance.

Automated slide stainer.Context: The increasing demand for immunohistochemistry for clinical diagnostics, in combination with an ongoing shortage of staff in the histology laboratory, has brought about a need for automation in immunohistochemistry. The current automated staining platforms vary significantly in their design and capabilities.

Objective: To review how technology has been applied to automating the process of immunohistochemical staining.

Data sources: Literature review, vendor interviews, and personal practice experience.

Conclusions: Each of the commercially available, automated immunohistochemistry platforms has strategic design differences that produce advantages and disadvantages. Understanding those differences can help match the demands of testing volumes, turnaround time, standardization, and labor savings to the appropriate automated instrumentation.

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The transition from manual to semi- and fully automated processes for immunohistochemistry has contributed significantly toward achieving higher efficiency and superior quality of testing results.

By Taiying Chen, MD, PhD; Ronald Raab, PhD; Priya Ratnam, PhD; Sheena Das, MS; Rueyming Loor, PhD; and Kris Kalra, PhD

Immunohistochemistry (IHC), special stains and in situ hybridization (ISH) are indispensable tools for surgical pathology diagnostics. Due to the artistic nature of these assays, well-trained and highly skilled technicians are needed to perform the manual assays. The problems involved with manual performance are low-volume output, high incidence of false results and the inconsistency of staining results from person to person and laboratory to laboratory. Manual assays are time consuming and associated with high labor and material costs. There has been a great need to improve staining quality, slide output, speed, reliability and standardization.

Automated Staining Techniques in Histopathology Laboratories

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To address all of these needs, automated immunostainers were introduced into pathology laboratories in the early 1990s. The transition from manual to semi- and fully automated processes for IHC has contributed significantly toward achieving higher efficiency and superior quality of testing results. Improvements in efficiency and quality have, in turn, increased the value of these test results in patient management.

Automated Immunostaining System

An automated immunostaining system consists of a slide carrier (incubating/staining chamber), a reagent delivery system, a computer controller to initiate and control individual staining steps and a user interface with a suitable operating system. Dependent upon the type of autoimmunostainer, there are different methods for applying reagents to tissue sections. Early models used a capillary-gap immunolabeling method. In this type of immunostainer, a technician places two vertically positioned slides facing each other in a slide holder. The reagents and buffer move upward by capillary action in the space between the two slides. Later models simply used gravity flows to add reagents and rinse buffer on vertically positioned slides.

Another type of immunostainer uses a horizontal platform for automated staining. There are currently two different ways for applying reagents in a horizontal platform system. The system, developed by Ventana Medical System (Tucson, AZ) uses a mixture of aqueous reagents and aliphatic oil. The mixtures are dispensed by testpacks onto slides placed in a heated incubation chamber. Reactions are carried out at 40ºC.

Another horizontal platform system, developed by BioGenex Laboratories (San Ramon, CA), uses an incubation chamber in which reactions are carried out at ambient temperature. In this system, a technician places the slide rack in the incubation/staining chamber where the reagents are delivered via disposable tips and all steps are performed at ambient temperature. The horizontal platform immunostainer simulates the manual operation performed by a technician.

Currently, most modern automated immunostainers are able to handle 40 to 50 slides and allow 20 to 40 staining protocols in the same run. Computer software not only offers the option of multiple protocols but also the traceability of each staining protocol. The bar code reading capability of some automated immunostainers allows accurate execution of the antibody staining protocol programmed for each slide. A bar code reader eliminates human and technical errors and dramatically reduces the time for setting up each run.

The hands-on labor requirement for IHC staining has been reduced dramatically by fully automated systems. Bar coded systems also minimize the amount of computer entry that is required by the technician. In addition to automating the entire set-up and staining process, preliminary steps, such as deparaffinization, can now be performed online by some systems.1 Based on the dramatic reduction in the hands-on time dedicated to IHC testing and the speed and capacity of automated systems, labs are able to devote time to other lab requirements and to process significantly greater numbers of tests per unit time. Automation has led to a standardized process for IHC testing by minimizing human errors, providing surgical pathologists with reliable and consistent staining results.

Fully Automated Staining Systems

The important features of an automated horizontal platform immunostainer are its capability of performing multitask operations, multireagent dispensing and the ability to perform other procedures, such as in situ hybridization and special stains, in addition to IHC. Technological breakthroughs are allowing histopathology laboratories to receive the benefits of automation across multiple applications, resulting in greater laboratory efficiency, optimized quality and complete workstation consolidation.

Special stains are classical, histochemical staining methods that are used for differentiating cell components and tissue types. The basic principle is to make an otherwise colorless substance or structure visible under the microscope. The manual special stains methods are time consuming, require complex reagent preparation and sometimes require the technician to deal with hazardous chemicals. Automation of special stains changes all of that.

Plateletspecial stains system,1 slides and reagents are bar coded, and set-up takes only minutes. With this type of system, there is no reagent wastage since only microliter amounts of reagent are used to stain slides. Another advantage of automated special stains method is that all reagents are prepared by the manufacturer and pre-tested to assure optimal performance. When one takes into account the technician’s time, elimination of preparation of hazardous materials and minimizing the time to prepare reagents, automation for special stains procedures becomes very cost effective.

Molecular level information is becoming increasingly important in the diagnosis, prognosis and therapy decisions relating to cancer, infectious diseases and other disorders. As a result, ISH is gaining prominence as an important test and research procedure. Compared to IHC, manual ISH techniques are more complicated and require extra hands-on time, more reagents and many time-consuming steps. Two of the most important steps in an ISH procedure are thermal or heat denaturing and the need for stringency washes. Several systems are now available for automation of ISH techniques, although developments in this area are fairly recent.

The first automated system named GENII (Ventana Medical System) was introduced only a few years ago. In one of the current systems,1 the thermal-denaturing step has been replaced by a room-temperature denaturization that produces results similar or better than the traditional manual thermal-denaturing ISH techniques. This automated room-temperature ISH system provides an assay for the detection of DNA or mRNA in tissue using fluorescein-labeled probes and an amplified anti-fluorescein immunodetection technology. As the diagnostic importance of ISH assay results continues to grow, it is anticipated that many more automated systems will be seen in the marketplace.

Additional features desirable in a fully automated staining system include: online deparrafinization using environmentally safe solutions; bar coded protocols and user-modifiable protocols in which the user has the flexibility of editing incubation times, the number of incubations or adding additional steps in the protocol as needed; ability to modify the volume of reagent applied to the slide based on the size of the tissue section, thus minimizing reagent wastage; and a reagent volume and expiration tracking feature to eliminate run interruptions and improve management of reagent inventory.

Future Automation Developments

Antigen retrieval (BioGenex), a widely accepted protocol for unmasking tissue and cellular antigens in formalin-fixed, paraffin-embedded tissues, has become an integrated part of IHC procedures. Staining of many important tumor markers, prognostic markers and drug selection markers require pre-treating tissue sections by microwave heating in an antigen retrieval solution. Since antigen retrieval can be a critical step in IHC staining, its automation and standardization is essential for eliminating staining variation and interpretation discrepancies among surgical pathology laboratories.Transforming a tissue specimen from fixed material

to stained sections is a multiple step process which

began as separate manual tasks. Indeed, histology in the last century has been the slowest of the

laboratory medicine departments to innovate and

keep pace with the speed required for a modern

dynamic hospital. Whereas the availability of highthroughput analyzers has made same-day results

the expected norm in the blood sciences, histology,

with its labor-intensive preparations and processes

usually sees result turnaround time (TAT) for biopsies and surgical samples being counted in days or

weeks rather than hours or minutes.

Historically the lengthy TATs of the histopathology laboratory have been unavoidable due to the

technical requirements and the multiple manual

stages involved from tissue handling through to

the preparation of slides. However, alongside the

demands of modern medicine many laboratories

have introduced new semi and fully automated

processes and tracking systems designed to enable

rapid, accurate and safe histopathology reporting.

The modern manipulations to deliver these tissue

handling steps are covered in the various chapters of

this book, but increasingly this is an automated and

standardized reality. Histology automation is perceived as a relatively recent movement, but examples

began to be seen as far back as 1945 (Titford, 2006).

This chapter will deal with various components of

laboratory activity and the automation which currently exists. Whilst there are many companies and

systems to facilitate these automated solutions to

histology laboratory practice, only some illustrative examples are given and the discussion cannot

be all-encompassing. Indeed, one must look at this

evolving technology arena regularly in order to to

keep up to date with the companies which serve this

aspect of laboratory practice and their equipment.

The drivers for change

Drivers for the automation of histology processing

are various, but principally hinge on two elements:

financial budgets (generally constrained) and the

need for rapid sample analysis (patient and clinician

led). The increase and availability of preventative

medicine such as screening protocols, and the development of specific testing for personalized medicine

within an aging population, have all contributed to

an increase in histology workload. Globally, laboratories are expected to be more efficient than ever.

The trend being seen in terms of economies of scale

and diagnostic national and international guidelines

has pushed towards a minimum number of samples

for laboratory efficiency. There are fewer, larger

laboratories processing thousands of specimens per

month.

The changing nature of laboratory accreditation

is another driver to the introduction of automation.

The required standards for an accredited laboratory

have been expanded to include the validation and

verification of all processes, as well as standardizing

the equipment and reagents used. The innate production of this type of process normalization and

audit information is one of the strengths of an automated procedure.

Technology now exists which, combined with

adaptations to work practices, allows results to be

available within 24 hours of a biopsy being taken for

small and straightforward samples. This should lead

to an improved clinical response and patient outcome without an increase in cost. The goal of introducing a more automated process into histology is

to enable a leaner, more efficient process which benefits staff, patients and the service user/s.

Finally there are subsidiary drivers to be considered. For example, looking at processing, one can

appreciate that tissue samples needing to move from

fixative to paraffin wax requires transfer between

multiple solvents and impregnation periods at each

stage; this normally takes several hours. Automation

of this stage reduces the requirement for manual

intervention and allows the process to occur faster

or overnight, assisting TATs. Other beneficial outcomes to these systems include the increased safety

of the user as the process occurs in an enclosed environment with minimal reagent handling. This has

significant health and safety benefits.

Barcode technology and automated

sample tracking

A laboratory’s responsibility for a specimen (and

the laboratory’s TAT) begins as soon as the tissue

has been removed from the patient. It is from here

automation and tracking can begin. Systems such

as the Menerini Tissue SAFE are available. These

record the time the specimen was taken, when formaldehyde was added and the temperature at which

the specimen was transported to the laboratory,

therefore maintaining standardization. This allows

comprehensive verifiable data on the pre-analysis

handling of the specimen (Menerini, 2016).

Sample tracking continues once the specimen is

received and passes through all the laboratory processes, ultimately producing a diagnostic report and

recording both the disposal of excess material, and

specimen storage. The production of an audit trail

and the associated chain of custody can be a labor

intensive process both to complete and interrogate.

Indeed, this mundane task of the completion of an

audit trail can account for a significant portion of the

departmental workload, the majority of which is the

responsibility of the laboratory staff.

In many laboratories the creation of this audit

trail has been constructed piecemeal over a period

of time, with each step often recorded in a different format and the records often stored in separate

physical locations within the laboratory. As a result,

laboratories have a plethora of different systems in

use including (but not limited to) colored slides and

beads, lists of specimens, log books, worksheets and

initials here, there and everywhere! This approach

arises as a result of the different requirements for the

histology material as the specimen moves through

the histology process. It is not easy to capture all the

required information in a centralized location and

there has been a lack of computer software designed

specifically for this task.

A comprehensive computer system which records

key aspects of the process, such as who has booked

in, dissected, embedded, trimmed, cut and checked

a specimen can be a useful information management

to an improved clinical response and patient outcome without an increase in cost. The goal of introducing a more automated process into histology is

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intervention and allows the process to occur faster

or overnight, assisting TATs. Other beneficial outcomes to these systems include the increased safety

of the user as the process occurs in an enclosed environment with minimal reagent handling. This has

significant health and safety benefits.Manual staining is a time consuming process which

requires at least one trained individual to spend a

large proportion of the day juggling timers, stains and

rinse steps, often having to adapt the protocol due to

tissue type, humidity, dye variation or other variable.

If individuals are on a rota through this section, some

of the less common stains may not be performed by

an individual for months or more, making standardization and competency hard to maintain.

With the increasing need to categorically demonstrate the validity of results, the standardized, same

day result which is produced by a tinctorial autostainer can be a great asset to a laboratory. Many

of these automated tinctorial staining systems are

capable of being customized. Individual slide heaters and the ability to produce a custom protocol

enables consistency, whilst also catering to established pathologist preferences.

The overall TAT for individual stains is frequently

longer than the bench equivalent (with a few notable

exceptions), but the true saving is that of personnel,

who are only required to load, unload and maintain

the machine. As well as being less labor intensive,

these steps do not require the same experience level

or length of training as bench staining itself does,

reducing the training burden on the laboratory and

enabling staff to become competent in the section in

a reduced timeframe. As always, a back-up has to be

considered in case of technical failure.

There is also the potential for remote requesting:

many systems when used together with 3D barcodes and an LIMS system enable the pathologist

to request further work from remote workstations.

These systems can also resolve some commonly

found poor practice, such as the need for illegible

paper slips to request further work, stains being

missed, or re-labelling of slides by automatically

adding requests to both the pending label printing

software and pending staining protocols worksheet.

Once the barcode labelled slide is added to the

stainer it proceeds to confirm and log which stain is

required and if the required reagents are both available and in date, information which forms part of

the comprehensive audit trail.

Two widely used histochemical stainers are

the Dako Artisan Link System (Fig. 11.7) and the

Ventana Benchmark SS, both of which use proprietary reagents, individual heating plates and the ability to perform multiple stains in a single run (Roche

2013a; Dako, 2016).

In both cases the emphasis is placed upon the

quality and reproducibility of the staining. Reagent

management and validation is simplified with the

use of barcoded IVD marked reagents. The drawbacks of tinctorial stain automation at present

include the limited range of stains available, and

the comparatively high cost per slide of automated

staining when compared to the conventional manual approach (Table 11.3).

Immunohistochemistry

Immunohistochemistry (IHC) staining (see Chapter

19) revolves around sequential, well-defined steps,

time intervals and prescribed temperatures so the

whole process is ideal for automation. Indeed, in

most laboratories this now happens in one form or

another. Many different systems exist which can perform some, or all of the multistep process.

The instruments are a combination delivery system with or without heat, controlled by computer

software. Slides may be stationary or mobile and

either in a linear or rotary fashion, whilst reagents

are delivered via pipettes from reagent containers/

pre-packed cartridges. Some platforms also include

deparaffinization, multiple antigen retrieval options

and counterstain. All of these enable a wide range of

immunohistochemical stains to be produced.Automation of immunohistochemical techniques

has been a major step in improving quality efficiency and reproducibility of results. Most IHC

automated systems include flexibility for continuous flow and user defined steps. Some are capable

of performing all the steps within the process,

making them a true ‘walk-away’ system requiring far fewer man hours than previous manual or

partly automated systems. Workflow features such

as independent slide draws and reagent racks are

essential if a ‘first-in-first-out’ workflow system is

desired, and work can be continuously added and

sent out.

Integration of the platform’s software with the

LIMS system can allow a full patient tracking package and remote requesting options whilst also

removing the need for constant re-labelling of slides.

More advanced platforms are available with an

expanded range of functions, such as in situ hybridization and direct immunofluorescence.

Examples of IHC platforms on the market include

the DAKO Autostainer plus, Labvision Autostainer

720, Ventana Benchmark and Vision Biosystems

Bondmax.

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