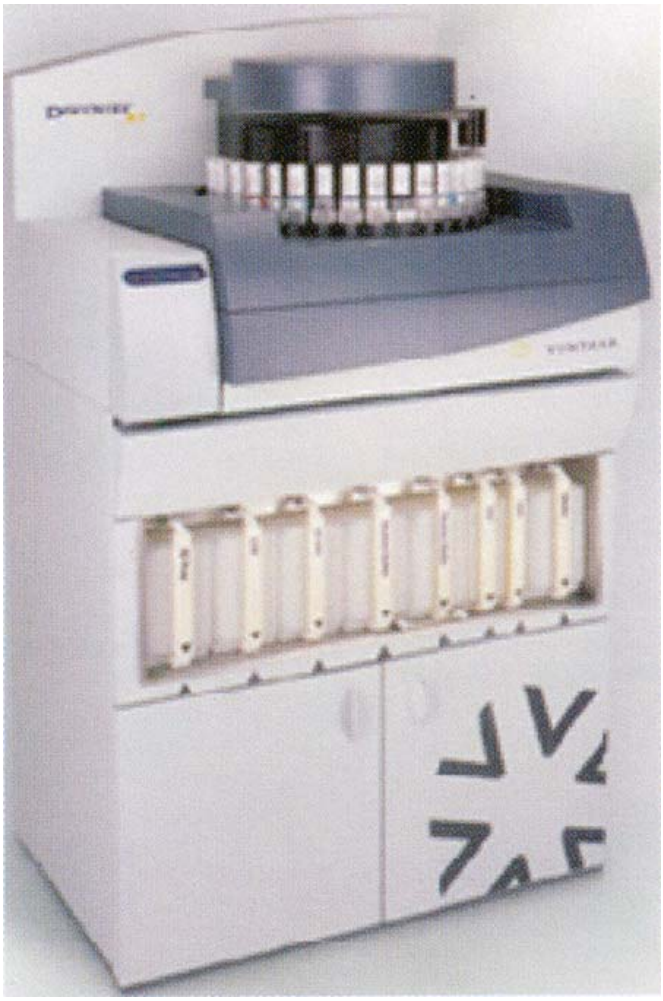
BASIC OPERATING PRINCIPLES AND FEATURES OF THE VENTANA AUTOMATED STAINER.



Discovery XT

Operated by QLMP

Queen's UNIVERSITY

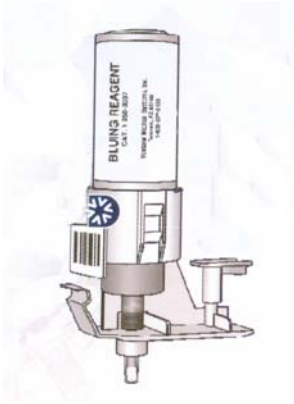
Anybody who has performed IHC, IF, ISH, or FISH staining manually knows that the process involves multiple applications of rinses, washes, and incubations. This is time consuming, and when processing multiple slides that will be graded and compared, care must be taken to ensure that incubation times, and all other protocol parameters are consistent, in order for the researcher to be sure that experimental variation is real, and not procedural.

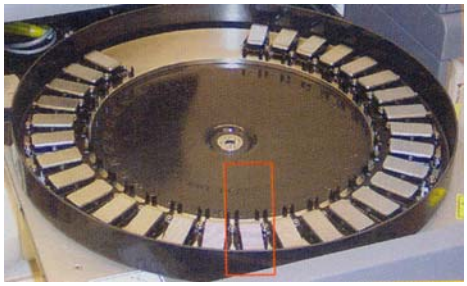
These are the difficulties that automation of the process can lessen or eliminate.

Use of the automated stainer represents a significant savings in time, and ensures a level of consistency from run to run that the best technician could not guarantee.

On top of the stainer dispensers are placed under a rotating plunger. Following software instructions, at the right time the plunger delivers a 100 µl bolus of reagent to each slide inside the carousel drawer, through a small opening. Bulk rinsing fluids, kept in containers under the carousel, are applied through spray nozzles located just above the slide.

Dispensers can be purchased from Ventana with antibodies or reagents ready to go, or they can be user filled in the lab for tailor-made applications, such as specialized blocking solutions, enzyme digestions, commercial antibodies, etc.



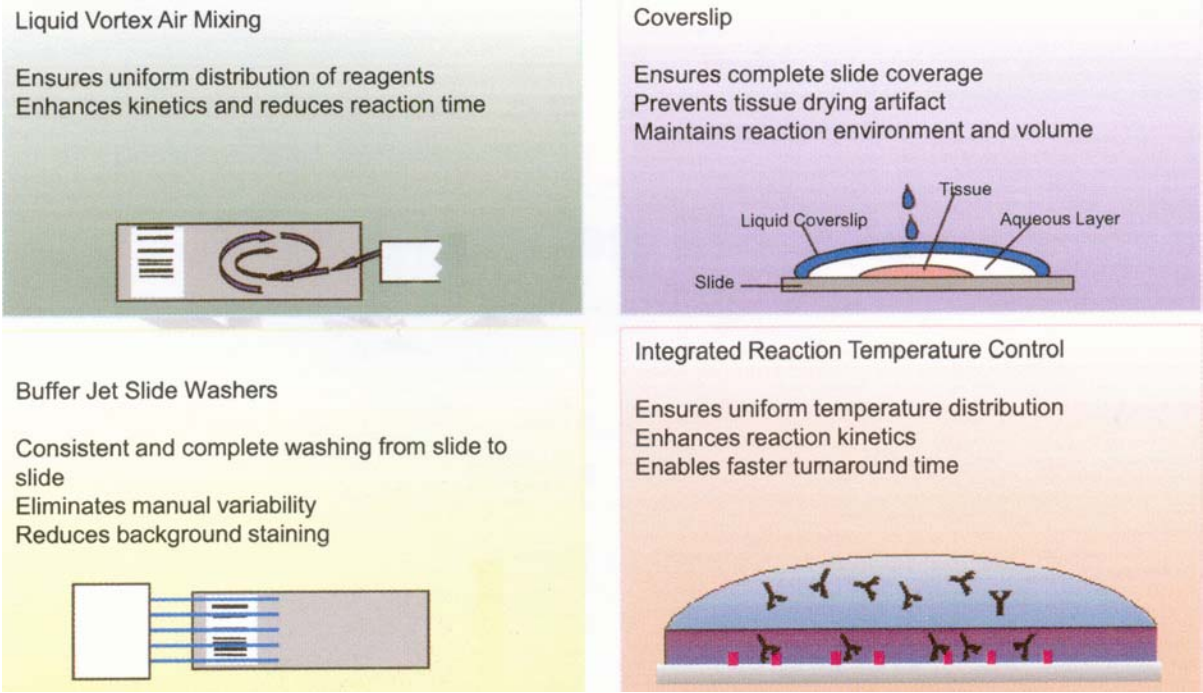


At the core of the machine is a 30 pad carousel that can accommodate 30 slides, and deliver a different protocol to each slide, in one run. Each pad heats and cools individually, according to the protocol it is following. There are 2 basic types of runs: Auto, whereby the machine applies the primary antibody through a dispenser, and titration, where the machine allows access to apply the primary or secondary antibody manually.

The stainer deparafinizes with heat and detergents, and applies heat through the thermo-pad. The reagent bubble on the slide is swirled c/w and cc/w via air jets positioned over the slide.

An important principle to keep in mind with the Ventana stainer is that because the reaction is so dynamic, manual protocols do not translate into Ventana protocols. Many antibodies have manual protocols that use a low pH citrate antigen retrieval, but 99% of antibodies on the Ventana use higher pH EDTA antigen retrieval. Optimization of antibodies usually involve 2 to 3 runs of maybe 2 to 3 slides each, with appropriate controls. Most IHC staining in the QLMP lab is done with DAB with streptavidin horse radish peroxidase, but alternate chromogens are available. If using heat-sensitive alkaline phosphatase systems, the heating cycle can be turned off.

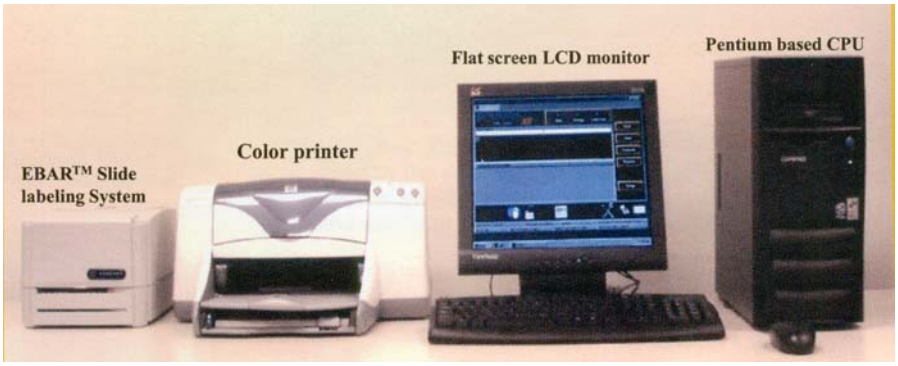
Under certain circumstances, protocols using SA-HRP can be combined with SA-AP detection, yielding double stained tissue.



Reaction dynamics involve 2 directional swirling, heating and cooling, and the application of a thin layer of light oil, called liquid cover slip. This allows the program to pause mid-run, and hold until the technician is ready to apply something to the slide, or remove it from the machine. The oil prevents evaporation, allowing overnight and interrupted runs.

Programming on the Discovery XT is designed with the researcher in mind, and allows variation for procedures such as Immunofluorescence, In Situ Hybridization, Laser Capture, FISH, and SISH. Double and triple stains are possible with different detection kits, and with the use of fluorescent labeled secondaries, multiple stain IF slides are achievable. Researchers and students often need to supply step-by-step procedural descriptions for reports and publications, and these reports can be provided by the Discovery XT software.

The secondary antibody application is with a Ventana “universal” cocktail that includes Goat Anti Mouse IgG, Goat Anti Rabbit IgG, and Goat Anti Mouse IgM. This cocktail will recognize a broad range of Primary Antibody types. Under certain conditions mouse tissue can be stained as well.

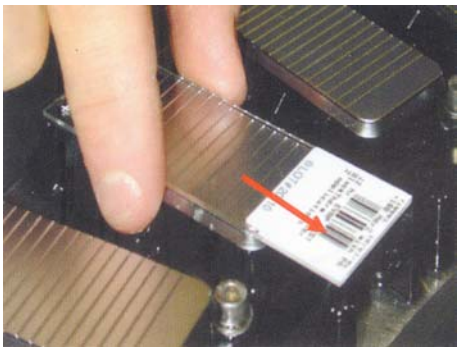


These four components, plus the stainer, comprise the system.

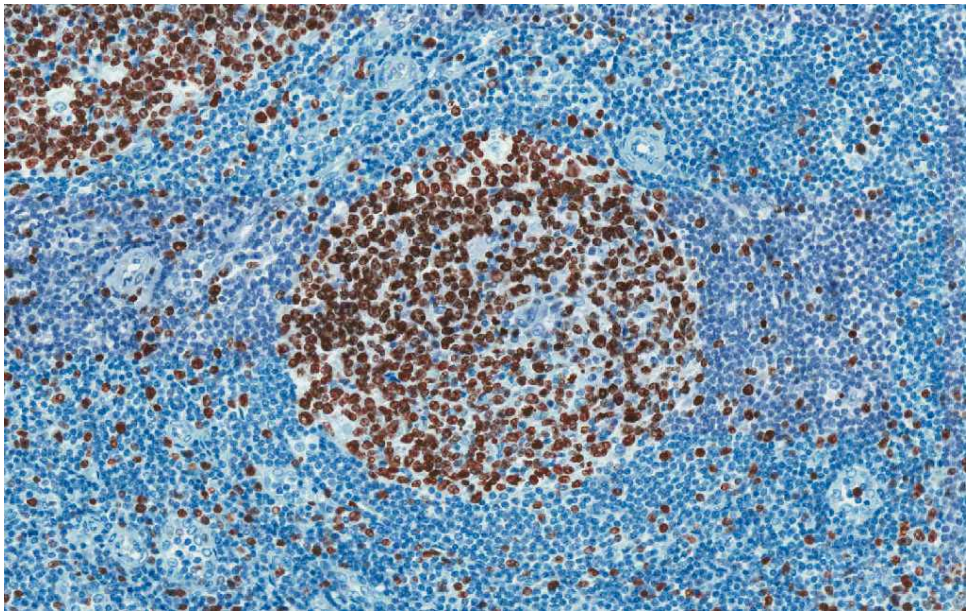
All this manipulation by the software requires that the slide, dispensers, and protocols all be recognizable to the program.

For this purpose the program uses the bar code. The technician creates a protocol that directs the stainer to manipulate a slide, using reagents in dispensers, which have their own bar codes. The technician creates and applies a bar code label to the slide. When the stainer starts a run, it "sees" the slide, goes to the stored protocol to gain instruction, then "sees" which dispensers have been placed on the machine. If the reagents on the machine are correct for the job, the stainer proceeds with the protocol.

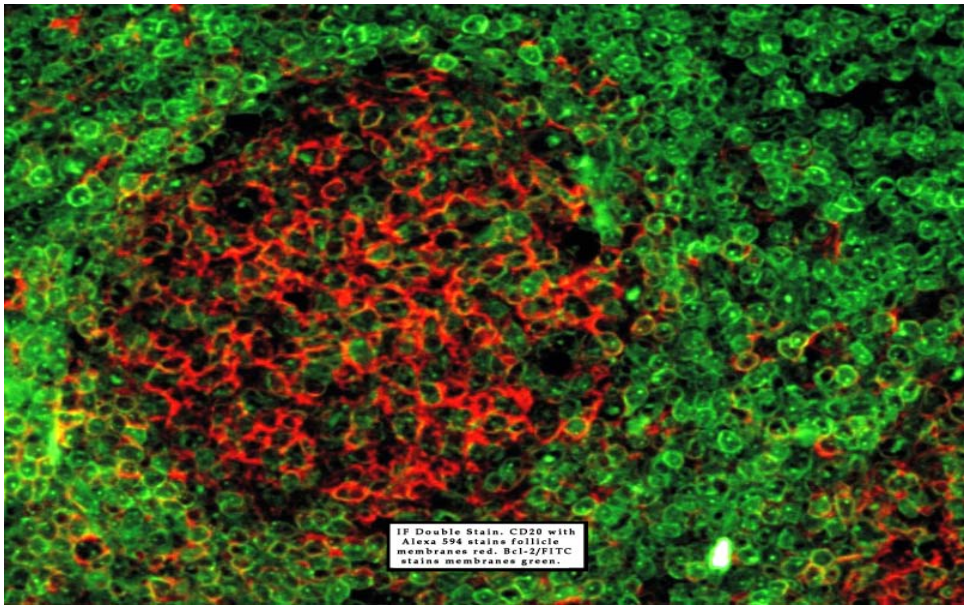
Standard Super Frost charged slides are recommended, and when the section is mounted on the slide, proper adhesion is achieved with an o/n incubation at 60°C. The stainer takes the slide from deparafinization to counterstain. At the end of the run the technician removes the slide, dehydrates to toluene or xylene, and cover slips.



Examples of DAB staining and Immunofluorescence



Tonsil follicle, staining for proliferating B cells with Ki67



Tonsil follicle, double stain IF with Bcl-2 and CD20

Compiled by Lee Boudreau, QLMP
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