

Increase a Routine Pathology Lab's Productivity with the use of High-Throughput MOZAIC™ Immunostainer

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INTRODUCTION

Clinical laboratory services, particularly anatomic pathology labs, are increasingly relying on automation. Effective initiation of automation in laboratory services has in the past provided better patient management and future advances will certainly continue this trend.

The increasing number of immunohistochemical (IHC) tests and the continuing shortage of qualified histotechnologists are common challenges for pathology laboratories. Therefore, it is critical that large runs of IHC tests can be reliably and reproducibly automated and turnaround to maximize the lab's productivity.

The type of automated IHC stainers on the market today vary in several aspects, including the technology for the processing of slides, the slide capacity per run and in the flexibility of reagents ("open" versus "closed" systems).

One of the first technologies employed for the automated staining of slides was the use of the *capillary gap* principle. In this technology, tissue specimens on two microscope slides, or one microscope slide and a cover plate, are placed together to form a gap of defined width between them. Typically, this gap is 50 microns wide and will draw and hold a consistent volume, e.g. 100 μ l, thereby contributing significantly to the reproducibility in staining. In systems employing a cover plate in place of one slide, reagents are pipetted through a small funnel located on the top of the plate. The capillary gap retains the defined amount of fluid by surface tension while the excess overflows and is automatically discarded. Washing is performed by the flow of buffer through the gaps. Sufficient fluid is retained at all times to prevent any drying of the sections.

MOZAIC™ comes with many user-friendly features, including choice of IHC detection systems, automated CISH™ protocols, and a simple, convenient, easy user interface. MOZAIC™ can improve a pathology lab's productivity, as each run of 96 slides, using up to 48 different primary antibodies, can be completed in less than 2 1/2 hours.

The purpose of our study is to verify the performance, throughput, capacity, turnaround time, and staining quality of MOZAIC™.

MATERIALS AND METHODS

Specimens: 100 formalin-fixed, paraffin-embedded (FFPE) human cancer tissues from Zymed's tissue bank were used in the study. 4-5 mm sections from each tissue were used.

Antibodies: 80 Zymed 2nd Gen prediluted IHC antibodies, routinely used in pathology labs, were used in the study.

IHC Detection kits: Zymed Histostain®-LAB-SA, which is labeled biotin-avidin (streptavidin), detection kits (DAB and AEC); Zymed SuperPicture™ Polymer detection kits (DAB and AEC) were used.

CISH™ DNA probes: SP●T-Light® HER2 DNA probe and SP●T-Light® Chromosome17 Centromeric DNA probe.

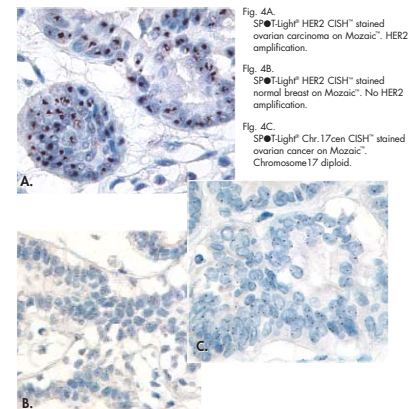
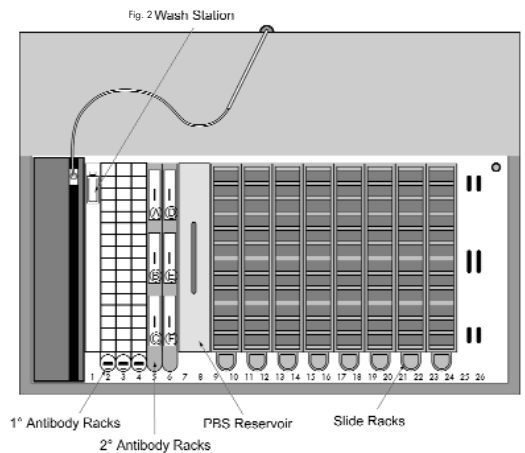
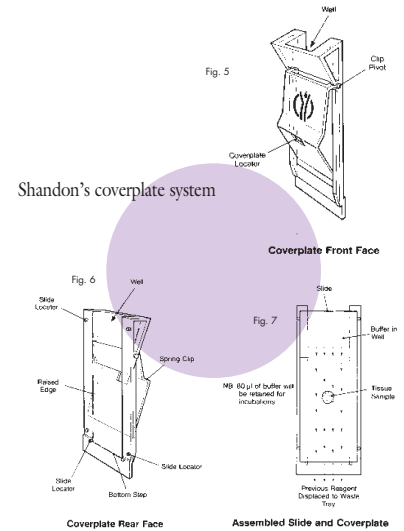
CISH™ Detection kits: CISH™ Polymer detection kit and CISH™ Centromere detection kit.

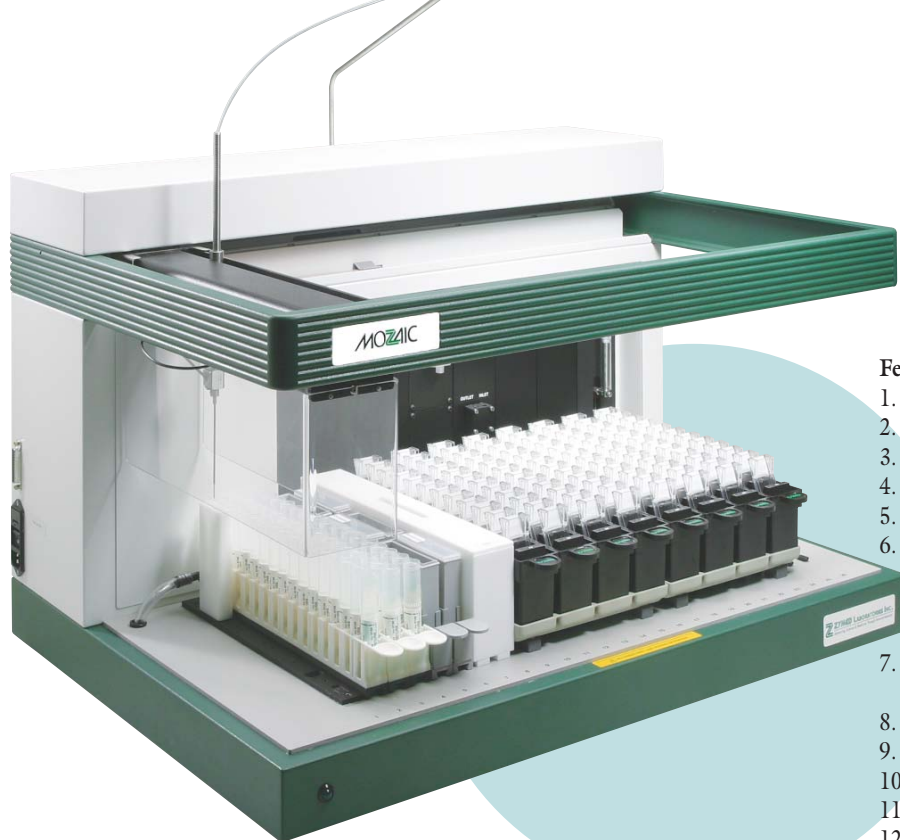
Automated Immunostainer: Zymed MOZAIC™ Automated Immunostainer (Figures 1 and 2)

Zymed's MOZAIC™ Automated Immunostainer is a vertical slide processing system that utilizes capillary gap technology to maintain an even reagent volume, which is necessary for reproducible and consistent staining results. MOZAIC™ is a desktop personal computer controlled staining system with easy-to-use and intuitive Windows® based software that allows for pre-programmed protocols as well as the flexibility to customize programs. Barcoded reagents are used to help ensure accuracy during and after the protocol; barcodes make sure that run reports are accurate. MOZAIC™ is compatible with most currently available reagents for staining paraffin-embedded tissue sections.

MOZAIC™ automates various sample manipulations, including successive reagent additions, washing, and incubation. However, an operator is required to manually perform sample preparation and load the slides and necessary reagents onto the MOZAIC™.

Shandon's coverplate system is used on MOZAIC™ (Figures 5-7). These coverplates retain approximately 80-100 μ l of reagent for incubation.





Features of Zymed MOZAIC™ Automated Immunostainer

1. Open or closed system
2. Vertical processing system
3. 1 – 96 slide capacity
4. 1- 48 (12 ml/vial) antibody capacity
5. 7 reagent troughs (for detection, washing, counterstaining, etc.)
6. Detection systems:
 - Zymed Histostain® LAB-SA
 - Zymed SuperPicture™ Polymer
 - Or laboratory's own system
7. Dispense volume: 100 µl – 200 µl (closed system)
100 µl – 400 µl (open system)
8. Minimum reagent volume/slide: 100 – 200 µl
9. Reagents barcode: Yes
10. Number of protocols: 6 (closed system); unlimited (open system)
11. Liquid level sensor: Yes
12. Audible alarm: Yes
13. Temperature range: Ambient
14. Software: Windows-based
15. ISH and special staining: Immunostaining part of CISH

Ancillary: Zymed MOZAIC™ Wash Buffer.

MOZAIC™ staining protocols: Use MOZAIC™ provided staining protocols

Manual staining protocols: Follow the protocols provided in the Zymed IHC and CISH detection kits product inserts.

RESULTS

1. The specific staining for the antibodies tested was the same or stronger than the manual staining using Histostain® LAB-SA detection system (see Figure 3).
2. The specific staining for the antibodies tested was the same as the manual staining using SuperPicture™ Polymer detection system (see Figure 3).
3. The specific staining for CISH was the same or stronger than the manual staining using CISH™ Polymer detection system (see Figure 4).
4. Turnaround time per run

SuperPicture™ Polymer Detection Kit (DAB): 2 hours, 30 min

(Using 48 different Abs, 96 slides, including endogenous peroxidase blocking, non-overnight run)

SuperPicture™ Polymer Detection Kit (AEC): 2 hours, 40 min

(Using 48 different Abs, 96 slides, including endogenous peroxidase blocking, non-overnight run)

Histostain® LAB-SA Kit (DAB): 3 hours, 20 min

(Using 48 different Abs, 96 slides, including endogenous peroxidase blocking, non-overnight run)

Histostain® LAB-SA Kit (AEC): 3 hours, 30 min

(Using 48 different Abs, 96 slides, including endogenous peroxidase blocking, non-overnight run)

CISH™ Polymer Detection Kit: 2 hours, 30 min

CISH™ Centromere Detection Kit: 2 hours

Fig. 3

A. Rb anti-Chromogranin A stained pancreas on Mozaic™ using SuperPicture™ Polymer Kit.

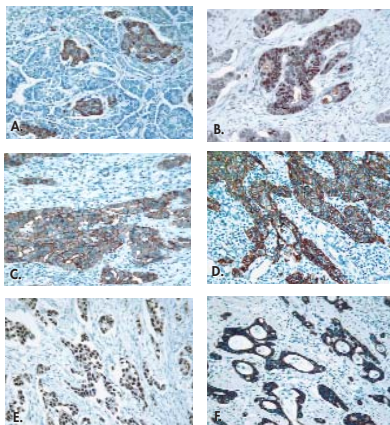
B. Ms anti-MSH6 (44) stained colon on Mozaic™ using SuperPicture™ Polymer Kit.

C. Ms anti-HER2 (TAB250) stained tissue on Mozaic™ using SuperPicture™ Polymer Kit.

D. Ms anti-EGFr (31G7) stained head & neck cancer on Mozaic™ using Histostain® LAB-SA kit.

E. Ms anti-ER (1D5) stained tissue on Mozaic™ using SuperPicture™ Polymer Kit.

F. Ms anti-pan Cytokeratin (PNAK-9) stained gastric carcinoma on Mozaic™ using Histostain® LAB-SA Kit.



5. Good staining reproducibility and staining consistency based on three 96-slides runs using the same antibodies, same tissues, same lot of reagents.

6. It should be noted that with capillary gap action, air bubbles may affect staining results. To maximize performance with MOZAIC™, we suggest immersing the slide and coverplate under water or buffer when applying the slide onto the coverplate.

DISCUSSION

Capillary gap is a proven technology that allows for the highest capacity yet small footprint immunostainer due to the vertical positioning of the slides (versus horizontal).

CONCLUSION

MOZAIC™ has high-throughput capability and is easy to operate. At <3 hours per run using Zymed's polymer system, it has a fast turnaround time. We observed that it gives good staining reproducibility and consistency.