White paper

Comparative analysis of H&E stain quality between the VENTANA HE 600 system and a traditional linear stainer
Introduction

Most tissue-based diagnostic decisions in anatomic pathology (AP) are based solely on hematoxylin and eosin (H&E) tests, making H&E one of the most important stains in tissue diagnostics. However, despite individual slide staining technologies made available in recent years, many AP labs continue using traditional linear “dip and dunk” stained for H&E testing, which may pose risks to stain quality. *

With dip and dunk staining, reagents can carry over between containers and tissue can transfer as patient slides move from one reagent bath to the next. Open baths expose reagents to the lab's environmental conditions: oxidation can deplete the amount of hematoxylin dye available, and alcohol evaporation throughout the day can cause further deterioration of H&E stain quality. For these reasons, controlling the reagent baths – and subsequently, stain quality – can be a major challenge for labs using dip and dunk stained, particularly when there are fluctuations in slide volume. Depletion, dilution, evaporation and oxidation may all negatively affect H&E stain quality.

Unlike dip and dunk, the VENTANA HE 600 system optimizes H&E stain quality with individual slide staining. As many as 200 slides can be stained per hour, but the system detects and handles each slide as an individual specimen, automatically applying fresh, ready-to-use reagents to every slide. No alcohol is used, which eliminates the risk of declining stain quality due to evaporation. With the VENTANA HE 600 system, fresh reagents, unexposed to the elements, result in continuously crisp, high-quality H&E stains.

To document the variability in staining quality, Ventana Medical Systems, Inc., a member of the Roche Group, conducted a multi-slide study comparing the VENTANA HE 600 system to a traditional linear dip and dunk stainer, the Leica Autostainer XL. The comparison of slides from both systems in the study indicates that the VENTANA HE 600 system improves H&E stain quality significantly by eliminating reagent carry over, dilution and dye depletion.

Materials and Methods

Participant and study protocol

The participant in this study was Sandra Mueller, M.D., Ph.D., pathologist and medical director for PETROGLYPH Pathology Services in Rio Rancho, New Mexico, U.S.A. Dr. Mueller evaluated stained slides for six tissue samples: Lymphoma 1, Lymphoma 2, Melanoma, Signet cell carcinoma of the stomach (Gastric Ca), Infiltrating lobular breast (Lobular Ca), and Liver. The same block was used for each tissue sample.

Dr. Mueller observed two slides per instrument for each tissue sample (totaling 480 slides, or 240 per instrument, throughout the course of the study). After closely observing each set of slides, Dr. Mueller chose which system she preferred based on five stain quality criteria: consistency, color balance, overall quality, cross contamination, and quality of coverslipping.

* Decrease in stain quality is characterized by shifts in intensity of eosin and hematoxylin.
Stain quality scoring
Dr. Mueller used a four-point scale (4=excellent, 3=good, 2=fair, 1=poor) to assess stain quality for the purpose of diagnosis. Scoring was limited to the area under the coverslip. To keep the focus purely on H&E stain quality, in her observations Dr. Mueller ignored any extraneous tissue and stain effects that resulted from poor microtomy. She also disregarded residual keratin shed from the histotechnician.

Staining protocols and materials
The same protocol was used for each tissue sample. Dip and dunk staining was based on the participant's routine validated protocol; individual slide staining protocol on the VENTANA HE 600 system was based on Dr. Mueller's preference from a review of available stain protocols prior to the start of the study. Materials included:

Ventana staining platform and reagents:
1. VENTANA HE 600 Wash
2. VENTANA HE 600 Hematoxylin
3. VENTANA HE 600 Bluing
4. VENTANA HE 600 Eosin
5. VENTANA HE 600 Organic Solution
6. VENTANA HE 600 Differentiating Solution
7. VENTANA HE 600 Transfer Fluid
8. VENTANA HE 600 Cleaning Solution

Dip and dunk staining platform and reagents:
1. Leica Autostainer XL
2. Drying oven
3. Hematoxylin
4. Differentiator
5. Eosin
6. Bluing Agent
7. Alcohol
8. Xylene
9. Cytoseal
10. Water
11. Filters for hematoxylin
12. Leica CV5030 glass coverslipper
Results

H&E stain quality rated “excellent” in nearly 60% more slides with the VENTANA HE 600 system

A side-by-side comparison of Dr. Mueller’s quality scores revealed a significant difference in stain quality by instrumentation across all tissue samples. Of the slides stained by the VENTANA HE 600 system, 85.0% received an excellent rating; none (0.0%) were rated poor. By contrast, 26.2% of dip and dunk slides were rated excellent and 6.7% were rated poor. Figure 1 illustrates the ratings and overall average scores for all slides evaluated in the study, including Dr. Mueller’s commentary on how she defined each of the four ratings.

Figure 1: Comparison of H&E stain quality scores between the VENTANA HE 600 system and a traditional dip and dunk stainer (n=240 slides per instrument). Highest possible average score is 4.0.
The highest discrepancies in stain quality between the two instruments occurred in breast and liver tissue. Nearly two-thirds (62.5%) of the dip and dunk stains rated as poor were Lobular Ca (breast tissue). In all instances of poor dip and dunk stain quality for breast tissue, the same-day counterparts from the VENTANA HE 600 system received excellent quality scores—a stark dichotomy relating to breast cancer testing. Likewise, liver tissue comprised the remaining 37.5% of the poor quality ratings received on dip and dunk stains, while all same-day liver equivalents from the VENTANA HE 600 system received a rating of either excellent or good.

Stain quality consistency improved with the VENTANA HE 600 system

In a summary of her impressions related to stain quality and consistency for each tissue sample on each staining platform, Dr. Mueller noted: “On stains from the VENTANA HE 600 system, eosin was more differentiating, meaning it displayed more shades, and hematoxylin was crisper.” Furthermore, she says this superior differentiation made low power magnification more effective in terms of achieving a diagnosis, which is particularly important when looking at tissue samples like Lobular Ca and others that often “hide” tumors on low magnification if the differentiation is poor.

“While both systems show some inconsistency, mostly with regard to eosin staining, variation was more prevalent on dip and dunk stains,” Dr. Mueller continued. She estimated 11–12% variation on dip and dunk, compared to 2–4% variation on the VENTANA HE 600 system. Dr. Mueller’s full summaries by tissue sample and staining method are provided in Table 1 below.

Table 1: A summary of Dr. Mueller’s overall impressions on stain quality and consistency by tissue sample and staining method.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>VENTANA HE 600 system</th>
<th>Traditional dip and dunk stainer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma 1</td>
<td>This is very pretty tissue! Consistent. Great detail. Nice H&amp;E balance.</td>
<td>Tissue has an edge effect (gradient effect) with improper staining. Eosin is less differentiating than it should be and has a basophilic hue compromising the degree of contrast in the tissue.</td>
</tr>
<tr>
<td>Lymphoma 2</td>
<td>Eosin varies somewhat from pink to orange to brownish. A few slides had diminished eosin. Hematoxylin is very crisp, which highlights all the nuclear features. Would be nice if eosin was a little more stable. Overall gorgeous stain on tissue.</td>
<td>Many slides score low because eosin is lacking; lack of contrast gives a flat, featureless appearance to tumor/ tissue. Also, hematoxylin itself lacks crispness. Some slides have the appearance of having been poorly fixed.</td>
</tr>
<tr>
<td>Melanoma</td>
<td>VERY consistent staining. Very crisp detail.</td>
<td>Eosin is inconsistent with many slides. Very heavy (the hematoxylin has a pink hue in many nuclei). I scored it at 3 (good) – would have scored 2 (fair) but realize that many pathologists like pink. Other slides are more in balance – those I scored at 4 (excellent).</td>
</tr>
<tr>
<td>Gastric Ca</td>
<td>This is a great stain. Beautiful contrast! Very consistent (rare slide with slightly paler eosin).</td>
<td>This is a good stain, but not a great stain. Consistent. Not great contrast.</td>
</tr>
<tr>
<td>Lobular Ca</td>
<td>Overall very nice. Slight variation in eosin (pink to orange to brownish); kept going back and forth between slides to compare.</td>
<td>Slides that score 1 (poor) and 2 (fair) have numerous nuclei that are black (reminded me of tissue fixed in mercury-based fixatives where the mercury precipitates out in a bizarre fashion).</td>
</tr>
<tr>
<td>Liver</td>
<td>Very slight variation in shade and intensity of eosin. Rare edge effect but limited to 1-2 cells.</td>
<td>This is an awful set to review. All slides have a gradient (edge) effect. Some have absence of staining. A few have streaks of eosin only on the tissue. Almost all of the slides have residual hematoxylin on the glass.</td>
</tr>
</tbody>
</table>
As shown below, every stain (100%) from the VENTANA HE 600 system for Gastric Ca received an excellent quality rating, compared to none (0.0%) of the stains produced on a traditional dip and dunk stainer for the same tissue sample. Similarly, 100% of the stains from the VENTANA HE 600 system for Melanoma were rated excellent, versus 60.0% with dip and dunk. Percentages for each rating by tissue sample and by staining system are shown in Table 2.

<table>
<thead>
<tr>
<th>Tissue Sample</th>
<th>Excellent Rating</th>
<th>Good Rating</th>
<th>Fair Rating</th>
<th>Poor Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma 1</td>
<td>77.5%</td>
<td>17.5%</td>
<td>5.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Lymphoma 2</td>
<td>75.0%</td>
<td>20.0%</td>
<td>5.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>100%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Gastric Ca</td>
<td>100%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Lobular Ca</td>
<td>97.5%</td>
<td>2.5%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Liver</td>
<td>60.0%</td>
<td>40.0%</td>
<td>75.0%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Table 2: H&E stain quality scores by tissue sample for the VENTANA HE 600 system versus a traditional dip and dunk stainer.

Key Conclusions

The results of this study demonstrate the extent to which H&E stain quality is enhanced by individual slide staining on the VENTANA HE 600 system. Compared to dip and dunk, stains from the VENTANA HE 600 system scored higher on quality across all six tissue samples, by a margin of 30-100% when examining excellent rating totals.

* 100% more Gastric Ca stains rated excellent *
* 77.5% more Lymphoma 1 stains rated excellent *
* 60% more Liver stains rated excellent *
* 45% more Lobular Ca stains rated excellent *
* 40% more Melanoma stains rated excellent *
* 30% more Lymphoma 2 stains rated excellent *

* On the VENTANA HE 600 system (compared to a traditional dip and dunk stainer)
Almost all stains from the VENTANA HE 600 system (98.3%) received the top two quality scores (excellent and good). Among them, nearly nine in every 10 stains were deemed excellent. By contrast, 83.8% of dip and dunk slides received the top two scores, and less than three in 10 of those were deemed excellent. More than one-sixth of dip and dunk slides (16.3%) received the two lowest ratings (fair and poor), compared to 1.7% from the VENTANA HE 600 system ranking as fair, and 0.0% ranking as poor.

Stain consistency was also enhanced on the VENTANA HE 600 system, as illustrated in Dr. Mueller’s observation summaries (see Table 1). Through individual slide staining that uses fresh reagents every time, the VENTANA HE 600 system prevents stain quality degradation that can be caused by reagent re-use, carryover and oxidation with traditional dip and dunk stainers. The results of this study suggest labs using the VENTANA HE 600 system can expect more consistent, higher quality H&E stains with sharper detail. The average scores shown below are based on Dr. Mueller’s quality ratings for all slides included in this study (highest possible average score is 4.0):

- **58.8%** more stains rated excellent on the VENTANA HE 600 system overall
- **10x** fewer slides rated fair on the VENTANA HE 600 system overall
- **3.8** average stain quality score on the VENTANA HE 600 system
- **3.0** average stain quality score with dip and dunk staining

At the completion of the study and based on her observations across all 480 slides, Dr. Mueller concluded: “From a staining standpoint, if given a choice, I would choose the VENTANA HE 600 system. Depletion, dilution, evaporation and oxidation – all commonplace with dip and dunk methods – are completely resolved with individual slide staining. Fewer defects means fewer distractions, decreased eye fatigue and improved diagnostic performance.”

Slide image above: Breast tissue sample at 4x magnification.

On the cover: Prostate tissue sample at 10x magnification.