Evaluating Adjunctive p16 IHC on Cervical Biopsies According to LAST Recommendations –
Results from the CERTAIN (Cervical Tissue Adjunctive Analysis) Study

Thomas C. Wright Jr¹, Mark H Stoler², James Ranger-Moore³, Monesh Kapadia³, and Ruediger Ridder³

¹Columbia University, ²University of Virginia, ³Ventana Medical Systems / Roche
Disclosures

• Drs. Wright and Stoler served as pathologists for the study and are consultants and speakers for Ventana Medical Systems / Roche

• Other authors are employees of Ventana Medical Systems / Roche

• Study was funded by Ventana Medical Systems / Roche
• It is well recognized that interpretation of cervical biopsies is subjective and has a substantial inter- and intraobserver variability.

• This can lead to both the under-reporting and over-reporting of significant cervical disease.

• p16 is a cyclin-dependent kinase inhibitor that is in widespread use as a biomarker for HPV-associated CIN.
Objectives

- To evaluate the performance of p16 immunohistochemistry as an adjunctive diagnostic aid to H&E-stained slides for identification of CIN2+ status

- To assess the performance of published criteria for selecting which biopsies should be stained with p16
CERTAIN Study Design

Establishing “Expert” diagnosis

• 1,100 H&E-stained cervical biopsies were reviewed by 2 GYN pathologists using 3-tiered CIN terminology

• If diagnoses agree, “Expert H&E” diagnosis

• If not, 3rd pathologist reviewed and “Expert H&E” diagnosis achieved if agreement of 2 of 3

• Repeated using H&E stained AND p16 (CINtec® Histology) stained slides for “Expert p16” diagnosis
Biopsies were divided into 4 reading sets of 275 biopsies each.

Each reading set was reviewed by 17 or 18 community pathologists (CPs; total of 70 CPs)

**Read 1:** H&E stained slides only (CP H&E)

4-week washout period

**Read 2:** H&E with p16 stained slides (CP p16)

Total of 19,250 reads per method
# Impact of p16 on Performance

## Community vs “Expert H&E” diagnosis

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Read 1: H&amp;E</th>
<th>Read 2: H&amp;E + p16</th>
<th>Difference</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>89.9%</td>
<td>92.0%</td>
<td>2.1%</td>
<td>0.0006</td>
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<tr>
<td>Sensitivity</td>
<td>85.7%</td>
<td>92.0%</td>
<td>6.3%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Specificity</td>
<td>91.8%</td>
<td>93.0%</td>
<td>1.2%</td>
<td>0.1334</td>
</tr>
</tbody>
</table>

**Accuracy** = Overall percent agreement  
**Sensitivity** = Positive percent agreement, *eg. for “positive” cases*  
**Specificity** = Negative percent agreement, *eg. for “negative” cases*  
*All results based on generalized linear mixed models*
## Impact of p16 on Performance

*Community vs “Expert p16” diagnosis*

<table>
<thead>
<tr>
<th>Endpoint (for CIN2+)</th>
<th>Read 1: H&amp;E</th>
<th>Read 2: H&amp;E + p16</th>
<th>Difference</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td>89.7%</td>
<td>93.3%</td>
<td>4.6%</td>
<td>$&lt; 0.0001$</td>
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<tr>
<td><strong>Sensitivity</strong></td>
<td>74.7%</td>
<td>86.2%</td>
<td>11.5%</td>
<td>$&lt; 0.0001$</td>
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<tr>
<td><strong>Specificity</strong></td>
<td>93.5%</td>
<td>96.1%</td>
<td>2.6%</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

**Accuracy** = Overall percent agreement  
**Sensitivity** = Positive percent agreement, *eg. for “positive” cases*  
**Specificity** = Negative percent agreement, *eg. for “negative” cases*

All results based on generalized linear mixed models.
Impact of p16 on Performance
Community vs “Expert H&E” diagnosis
Impact of p16 on Performance
Community vs “Expert p16” diagnosis
Results By Individual Pathologist

Reference “Expert p16” diagnosis

Differences in Sensitivity and Specificity for H&E + p16 vs H&E

Sensitivity

Specificity

Lower

Higher
Use of LAST Criteria for p16 Staining
Use for all biopsies versus selected biopsies

• CAP / ASCCP have made recommendations (*LAST criteria*) on when cervical biopsies should be stained with p16

• During **Read 1** (H&E only), community pathologists were asked whether according to LAST they would request p16 immunostaining

• *Wide variation in requests for p16 staining.* Overall, p16 requested in 42.3% of cases.
# Impact of p16 on Performance

**p16 used on LAST vs non-LAST biopsies**

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<td><strong>Yes</strong></td>
<td>Accuracy</td>
<td>77.6%</td>
<td>87.9%</td>
<td>10.4%</td>
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<td>Sensitivity</td>
<td>73.4%</td>
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<tr>
<td></td>
<td>Specificity</td>
<td>79.6%</td>
<td>89.3%</td>
<td>9.7%</td>
<td>&lt; 0.0001</td>
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<tr>
<td><strong>No</strong></td>
<td>Accuracy</td>
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<td>97.0%</td>
<td>0.5%</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
<td>73.1%</td>
<td>84.1%</td>
<td>11.0%</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>99.2%</td>
<td>98.5%</td>
<td>-0.8%</td>
<td>&lt; 0.0001</td>
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Conclusions

- Adjunctive use of p16 with H&E stained slides improved accuracy and sensitivity of community pathologists compared to both reference standards.

- Positive effects doubled when using more accurate reference standard ("Expert p16").

- p16 staining used according to LAST criteria improved accuracy, sensitivity, and specificity.

- Equally large improvement in sensitivity when performed on non-LAST cases - but small loss in specificity (0.8%).