Quality review, validation and availability of HPV tests

Commercially available HPV tests in 2020

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Commercially available alpha-HPV molecular tests

- not a simple addition of newly developed tests to the old list of HPV tests
- the existence of all HPV tests double-checked with manufacturers at every update round

- data retrieved from:
  - Medline/Pubmed, Web of Science, Scopus, Bing, Google Scholar, Google without language or period restrictions – September 2019 and January 2020
  - abstracts from main HPV-related conferences (2015-2020)
  - internal files
  - the Chinese National Medical Products Administration (formerly the China Food and Drug Administration) was consulted to obtain a list of HPV tests approved by agency

- conservative estimate – very likely haven’t identified all HPV tests currently available
- omission of any particular commercially available HPV test was unintentional

Test (distinct, unique) vs test variant

particular HPV test was considered a variant if technologically identical or very similar to the original test but targeting different HPV type(s)

Human papillomavirus 16, 18 (DNA-Technology LLC, Moscow, Russia)

DISTINCT TEST

Human papillomavirus 18, 45, 39, 59

VARIANT

Human papillomavirus 16, 31, 33, 35, 58, 52, 67

VARIANT

Human papillomavirus 6, 11

VARIANT

Main groups of available commercial HPV molecular tests on the global market in 2020 (tests vs. variants)

<table>
<thead>
<tr>
<th>Tests</th>
<th>Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr-HPV DNA screening tests</td>
<td>40</td>
</tr>
<tr>
<td>hr-HPV DNA screening tests with concurrent or reflex partial genotyping for the main hr-HPV types</td>
<td>41</td>
</tr>
<tr>
<td>hr-HPV DNA full-genotyping tests</td>
<td>91</td>
</tr>
<tr>
<td>hr-HPV E6/E7 mRNA tests</td>
<td>9</td>
</tr>
<tr>
<td>in situ hybridization DNA in mRNA based HPV tests</td>
<td>33</td>
</tr>
<tr>
<td>hr-HPV DNA tests targeting miscellaneous HPV types</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>254</td>
</tr>
</tbody>
</table>

2020

254 distinct commercial HPV assays (and 425 variants) on the global market

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of Tests</th>
<th>Number of Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>79</td>
<td>51</td>
</tr>
<tr>
<td>2012</td>
<td>45</td>
<td>33</td>
</tr>
<tr>
<td>2015</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>2020</td>
<td>31</td>
<td>10</td>
</tr>
</tbody>
</table>

The number of identified HPV tests with at least one peer reviewed publication is 102/254 (40.3%).

**BUT**

- 46/254 (18.2%) of HPV tests with published performance evaluation (analytical and/or clinical)
- 56/254 (22.1%) of HPV tests cross-sectional descriptive studies only - no data for key test performance characteristics (sensitivity, specificity, reproducibility)
- "test A versus test B" approach with no reference standard
- ad hoc collections of heterogeneous clinical samples without follow-up
- various target population (including several non-genital)


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<table>
<thead>
<tr>
<th>No.</th>
<th>DNA chip</th>
<th>Blotter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>others</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Others</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

The clinical evaluation of the AccuPlex® HPV Genotyping Kit was performed in 5 clinical samples. AccuPlex® HPV Genotyping Kit is more sensitive than DNA chip assay.

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**BUFFALO BILL'S WILD WEST**

Accomplished Wild West Cowboys and Congress of Rough Riders of the World.
Main problems and limitations:
- manufacturers’ webpages in local languages only
- lack of transparent webpages
- indirect marketing
- exclusive orientation towards the Western market
- situation on emerging markets poorly understood
- publication bias

Sample extraction part?
the majority of HPV tests currently on the market are not complete diagnostic assays:
- sample extraction part not included in the kit
- recommended nucleic acid extraction methodology not even mentioned in manufacturer’s instructions
- only a minority of HPV tests on the market have internal controls
the extraction of DNA/RNA is an invaluable part of the HPV testing procedure!
manufacturers should validate sample extraction procedure for each of the recommended sample collection devices and clinical sample types
list of validated sample collection devices and specimen types should be provided in the manufacturer’s instructions

Issues to be resolved:
we need complete HPV diagnostic assays including sample extraction procedure!
manufacturers should put more effort into evaluating their HPV products
manufacturers should seek advice from established HPV researchers in the very early phase of development:
- how to properly design a novel test
- define intended use of future test (clinical, epidemiological, research)
- how to evaluate test performance that the HPV community will accept: evaluation/validation results
manufacturer-independent evaluations and publication of results in peer-reviewed journals are crucial
more comprehensive priced HPV tests needed!

COVID-19-related problems arising
- unprecedented health and economic impact of the COVID-19 pandemic
- extraordinary demand on a global scale for sampling devices, reagents, consumables, and diagnostic instruments needed for timely diagnosis of SARS-CoV-2 infection
- manufacturer’s shift toward new niche market with unprecedented market growth opportunity
- serious COVID-19-related supply chain problems (reagents and consumables)
- shifted interest of public, agencies and medical journals (“nothing is important but COVID-19”)
- temporary pause of cervical cancer screening programs
- “new normal”: unclear future; potential of second, third, fourth… waves of pandemic

Conclusions
- 254+ commercial HPV assays (and 425+ variants) on the global market
- 60% of HPV tests on the global market without a single peer-reviewed publication
- 81% of HPV tests on the market without published performance evaluation (analytical and/or clinical) in peer-reviewed journals
- great majority of performance evaluations not in line with standards agreed in the HPV community
- several clinically unvalidated HPV assays are used worldwide in daily practice - only a small subset of HPV tests on the market has validated clinical performance
- serious COVID-19-related supply chain problems arising

only clinically validated HPV tests should be used in cervical cancer screening