Clinical Performance of the Full Genotyping Agena MassARRAY HPV Assay Using SurePath Screening Samples within the VALGENT4 Framework

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The clinical performance evaluation of the novel MassARRAY human papillomavirus (MA-HPV) assay was performed using Danish SurePath cervical cancer screening samples under the fourth validation of HPV Genotyping Tests (VALGENT) framework. The MA-HPV assay is a mass array-based assay that individually detects 14 oncogenic HPV genotypes and five nononcogenic types. The MA-HPV assay was validated using the VALGENT4 panel, which constitutes 997 consecutive samples from a screening population in addition to 297 disease-enriched samples with abnormal cytologic findings. The clinical accuracy of the MA-HPV assay for sensitivity and specificity was assessed relative to that of the general primer 5+/6+ PCR enzyme immunoassay (GP-EIA), by a noninferiority test. The type-specific concordance of the MA-HPV assay with the GP5+/6+ PCR with Lumimx (GP-LMNX) genotyping was assessed as well. The relative sensitivity of the MA-HPV assay for cervical intraepithelial neoplasia $\geq 2$ or $\geq 3$ was 1.02 (95% CI, 0.98–1.05) and 1.01 (95% CI, 0.99–1.04), respectively. The sensitivity of the MA-HPV was noninferior to that of the GP-EIA ($P = 0.0001$), whereas the specificity of the MA-HPV was inferior (0.89; 95% CI, 0.85–0.91; $P > 0.99$). The genotype concordance between the MA-HPV and GP-LMNX was good for most oncogenic types. The MA-HPV assay is a clinical sensitive assay with a lower clinical specificity compared with the GP-EIA. The assay in its current form seems more suited to play a role in settings where specificity is of lesser importance but where high sensitivity is paramount. (J Mol Diagn 2022, 1–9; https://doi.org/10.1016/j.jmoldx.2021.12.009)

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over time, genotyping will allow clinicians to assess HPV persistence as a risk-defining element in the evaluation of HPV-positive women. HPV tests can be categorized into 4 assay design types: i) consensus assays, which report only positive or negative outcomes that measure the presence of the 13 or 14 oncogenic HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 with or without HPV66); ii) consensus assays with limited (partial) genotypingreporting for HPV16 and HPV18; iii) HPV assays with extendedgenotyping, typically HPV16 and HPV18 combined with more but not individual reporting of all the oncogenic genotypes; and iv) full genotyping assays with individual reporting of the 14 oncogenic HPV genotypes. The number of commercially available HPV assays has increased notably in the last years, with >200 assays now on the market. At the same time, HPV assay designs for cervical cancer screening has evolved from category 1 designs described above to entail increasingly more individual genotype reporting. However, only a few of these assays have been validated according to the international guidelines.

Besides cervical cancer screening, HPV assays are also used in a variety of related clinical modalities. These modalities include test-of-cure (ToC) after conization, epidemiologic monitoring of HPV vaccination effects, and HPV test on a number of samples, such as vaginal self-samples and urine, and specimen types from various medical specialties, including dermatology, venerology, head-and-neck, and infectious medicine. Common to the non-cervical cancer screening applications of the HPV test is the need for agile HPV assays that can run on a variety of sample types.

The Validation of HPV Genotyping Tests (VALGENT) framework is an international cooperation that was designed for clinical validation and comparison of HPV assays with genotyping capabilities for use in primary HPV screening. The VALGENT panels by design constitute a cohort of consecutive cervical screening samples (screening population) and disease-enriched samples with abnormal cytologic test results (enriched population). Until now, four VALGENT panels have been collected from well-established laboratories around Europe with screening samples from the Belgian (VALGENT1), Scottish (VALGENT2), Slovenian (VALGENT3), and Danish (VALGENT4) cervical cancer screening programs.

The full genotyping MassARRAY HPV (MA-HPV) assay from Agena Bioscience (Hamburg, Germany) was evaluated using a general primer (GP) 5+/6+ PCR enzyme immunoassay (GP5+/6+ EIA) as comparator, supported by the GP5+/6+ PCR with Luminex (GP-LMNX) genotyping for individual genotypes. The assay was validated with 1294 SurePath screening samples from women ≥30 years of age who participated in the Danish cervical cancer screening program, which constituted the fourth VALGENT panel (VALGENT4). The MA-HPV is a newly designed HPV assay based on matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS), which has not previously been validated for HPV cervical cancer screening.

Material and Methods

Sample collection and data retrieval for VALGENT4 were approved by the Danish Data Inspection Agency. A European Union General Data Protection Regulation—compliant data handler agreement was established between Copenhagen University Hospital, Copenhagen, Denmark (the principal site), and Sciensano, Brussels, Belgium, for the data analysis. All collected samples were cross-referenced and found eligible via the Danish register (Vævsanvendelsesregistret), relating to the collection, storage, and use of human biological material in health research projects.

Sample Collection and Histologic Follow-up

The sample collection procedure has been described in detail previously. In short, the VALGENT4 panel consists of SurePath screening samples collected from women participating in the Danish cervical cancer screening program at the Department of Pathology, Hvidovre Hospital, Denmark (parent laboratory). The VALGENT panel is standardized and comprises two study populations: 997 consecutive routine screening samples (screening population), comprising 946 samples negative for intraepithelial lesions or malignancy (NILM), 6 atypical squamous cells of undetermined significance, 21 low-grade squamous intraepithelial lesions, and 24 high-grade squamous intraepithelial lesions, atypical glandular cells (AGCs), atypical squamous cells (cannot exclude high-grade squamous intraepithelial lesions), or adenocarcinoma in situ (mean age, 42.4 years; range, 30 to 59 years) (Table 1). The disease-enriched component included 297 cytologically abnormal samples (100 atypical squamous cells of undetermined significance, 100 low-grade squamous intraepithelial lesions, and 97 high-grade squamous intraepithelial lesions,) (enriched population), with a mean age of 40.4 years (range, 30 to 59 years) (Table 1). All cytologic and histologic procedures were performed at the parent laboratory as described previously. Subsequent histologic screening history on women included in the study was retrieved from the Danish PatoBank by linkage to the central personized registry. Histologic follow-up was assessed 32 months (range, 32 to 35 months) after baseline testing and revealed 122 women with cervical intraepithelial neoplasia ≥2 confirmed at histologic follow-up, with most derived from the enriched population (N = 109). A total of 897 women had two consecutive cytologic NILM samples at baseline and 12 to 24 months earlier, and these samples were used for the specificity calculations.
Clinical Validation of MassARRAY HPV Assay

Table 1 Characteristics of the VALGENT4 Study Population with MA-HPV and GP-EIA Testing

| Characteristic | Screening population | Enriched population |
|---------------|----------------------|---------------------|
|               | Entire population, N | Total, N            | Total, N |   |
| All           | 1294                 | 997 (245 (24.6))    | 297      | 267 (89.9) |
| Age, years    |                      |                     | 143 (14.3) | 253 (85.2) |
| 30–39         | 530                  | 382 (117 (20.2))    | 148      | 134 (90.5) |
| 40–49         | 519                  | 408 (51 (12.5))     | 111      | 100 (100.1) |
| 50–59         | 245                  | 207 (31 (15.0))     | 38       | 33 (86.8) |
| Cytologic analysis |                  |                     |          |           |
| Normal        | 946                  | 946 (205 (21.7))    | 105      | 101 (96.2) |
| ASCUS         | 106                  | 6 (6) (66.7)        | 100      | 98 (98) |
| LSIL          | 121                  | 21 (18 (85.7))      | 100      | 86 (86) |
| HSIL          | 106                  | 13 (12 (92.3))      | 93       | 84 (90.3) |
| AGC, ASC-H, or AIS | 15                   | 11 (8 (72.7))       | 6        | 4 (100) |
| Histologic follow-up |                  |                     | 4        | 4 (100) |
| No follow-up  | 945                  | 894 (190 (21.3))    | 102      | 100 (91.7) |
| CIN0          | 154                  | 71 (25 (15.5))      | 83       | 72 (86.7) |
| CIN1          | 73                   | 19 (17 (89.5))      | 54       | 52 (96.3) |
| CIN2          | 39                   | 5 (5 (100))         | 34       | 31 (91.2) |
| CIN3          | 75                   | 7 (7 (100))         | 68       | 64 (94.1) |
| Cancer        | 8                    | 1 (1 (100))         | 7        | 7 (100) |
| CIN ≥2        | 122                  | 13 (13 (100))       | 109      | 102 (93.6) |

AGC, atypical glandular cells; AIS, adenocarcinoma in situ; ASC-H, atypical squamous cells (cannot exclude HSIL); ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; GP-EIA, general primer 5+6+ PCR enzyme immunoassay; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; MA-HPV, MassARRAY human papillomavirus; NILM, negative for intraepithelial lesions or malignancies; VALGENT4, Validation of HPV Genotyping Tests 4.

DNA Extraction

As previously described, DNA extraction in VALGENT4 was performed at the parent laboratory using the MagNA Pure96 system from Roche Diagnostics (MagNA Pure 96 DNA and Viral NA Small Volume Kit). The mean number of days from sample reception date to DNA extraction was 27 days (range, 11 to 71 days).

MassARRAY HPV Assay Testing

The MA-HPV assay (Agena Bioscience) is a newly designed full genotyping assay based on PCR and MassARRAY using MALDI-TOF MS) technology. The HPV panel allows for individual detection of 19 HPV genotypes (6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, and 73). In this analysis, the focus was on the 14 oncogenic HPV genotypes. The assay is performed during a 2-day period with 94 samples per run. On day 1, an initial multiple amplification PCR is set up with 2 μL of biobanked DNA, followed by a shrimp alkaline phosphatase reaction (which removes the excess nucleotides). After the shrimp alkaline phosphatase reaction, an iPLEX pro single base extension PCR (Agena Bioscience) is set up. A mix of oligonucleotide extension primers designed to anneal to the amplified DNA fragments are added together with extension enzyme and mass-modified dideoxynucleotide terminators. On day 2, the extension products are desalted with clean resin before being loaded into the MassARRAY Dx Nanodispenser RS1000 (Agena Bioscience), transferring the analyte to a spectroCHIP, which is then analyzed on the MassARRAY Dx analyzer in concordance with the manufacturer’s specifications. The MA-HPV assay has an internal glyceraldehyde-3-phosphate dehydrogenase control for sample sufficiency and assay performance. One sample was found twice to be invalid for MA-HPV and was excluded from the analysis, leaving 1294 samples for the final analysis. The mean number of days from DNA extraction to MA-HPV testing was 75 days (range, 2 to 162 days).

Comparator Assay Testing

An DNA aliquot was shipped to DDL Diagnostics Laboratory (Rijswijk, the Netherlands), where all GP PCR testing was performed. The clinical validated high-risk GP-EIA for pooled detection of 14 HPV oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) was used as a comparator for the clinical validation of the MA-HPV assay. For genotyping comparison analysis, a LMNX-based readout for individual genotyping of the 14 oncogenic HPV types was used. GP-LMNX testing on biobanked DNA aliquots was completed 685 days after sample reception. The GP-EIA was subsequently performed and completed on the GP5+/6+ amplicons 1008 days after samples reception.
Table 2  HPV Genotypes for MA-HPV and GP-LMNX Stratified by Age in the Screening Population

| Assay and HPV type | Age (years), n (%) | 30–39 (n = 382 MA-HPV and 380 GP-LMNX) | 40–49 (n = 408 MA-HPV and 405 GP-LMNX) | 50–59 (n = 207 MA-HPV and 205 GP-LMNX) | Total, n (%) (N = 997 MA-HPV and 990 GP-LMNX) |
|--------------------|--------------------|----------------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| MA-HPV             |                    |                                        |                                        |                                        |                                        |
| HR-HPV             | 117 (30.6)         | 72 (17.6)                              | 56 (27.1)                              | 245 (24.6)                             |                                        |
| HPV16              | 14 (3.7)           | 9 (2.2)                                | 3 (1.4)                                | 26 (2.6)                               |                                        |
| HPV18              | 14 (3.7)           | 9 (2.2)                                | 5 (2.4)                                | 28 (2.8)                               |                                        |
| HPV31              | 17 (4.5)           | 13 (3.2)                               | 6 (2.9)                                | 36 (3.6)                               |                                        |
| HPV33              | 10 (2.6)           | 7 (1.7)                                | 7 (3.4)                                | 24 (2.4)                               |                                        |
| HPV35              | 6 (1.6)            | 5 (1.2)                                | 2 (1.0)                                | 13 (1.3)                               |                                        |
| HPV39              | 10 (2.6)           | 5 (1.2)                                | 3 (1.4)                                | 18 (1.8)                               |                                        |
| HPV45              | 16 (4.2)           | 9 (2.2)                                | 9 (4.3)                                | 34 (3.4)                               |                                        |
| HPV51              | 16 (4.2)           | 6 (1.5)                                | 5 (2.4)                                | 27 (2.7)                               |                                        |
| HPV52              | 15 (3.9)           | 3 (0.7)                                | 4 (1.9)                                | 22 (2.2)                               |                                        |
| HPV56              | 7 (1.8)            | 4 (1.0)                                | 2 (1.0)                                | 13 (1.3)                               |                                        |
| HPV58              | 7 (1.8)            | 7 (1.7)                                | 2 (1.0)                                | 16 (1.6)                               |                                        |
| HPV59              | 20 (5.2)           | 11 (2.7)                               | 11 (5.3)                               | 42 (4.2)                               |                                        |
| HPV66              | 14 (3.7)           | 11 (2.7)                               | 8 (3.9)                                | 33 (3.3)                               |                                        |
| HPV68              | 13 (3.4)           | 8 (2.0)                                | 6 (2.9)                                | 27 (2.7)                               |                                        |
| GP-LMNX            |                    |                                        |                                        |                                        |                                        |
| HR-HPV             | 77 (20.3)          | 51 (12.6)                              | 31 (15.1)                              | 159 (16.1)                             |                                        |
| HPV16              | 14 (3.7)           | 10 (2.5)                               | 7 (3.4)                                | 31 (3.1)                               |                                        |
| HPV18              | 14 (3.7)           | 10 (2.5)                               | 4 (2.5)                                | 28 (2.8)                               |                                        |
| HPV31              | 9 (2.4)            | 6 (1.5)                                | 1 (0.5)                                | 16 (1.6)                               |                                        |
| HPV33              | 10 (2.6)           | 5 (1.2)                                | 5 (2.4)                                | 20 (2.0)                               |                                        |
| HPV35              | 4 (1.1)            | 4 (1.0)                                | 1 (0.5)                                | 9 (0.9)                                |                                        |
| HPV39              | 3 (0.8)            | 1 (0.2)                                | 1 (0.5)                                | 5 (0.5)                                |                                        |
| HPV45              | 8 (2.1)            | 5 (1.2)                                | 2 (1.0)                                | 18 (1.8)                               |                                        |
| HPV51              | 5 (1.3)            | 5 (1.2)                                | 3 (1.5)                                | 13 (1.3)                               |                                        |
| HPV52              | 8 (2.1)            | 1 (0.2)                                | 2 (1.0)                                | 11 (1.1)                               |                                        |
| HPV56              | 7 (1.8)            | 4 (1.0)                                | 2 (1.1)                                | 13 (1.3)                               |                                        |
| HPV58              | 5 (1.3)            | 3 (0.7)                                | 1 (0.5)                                | 9 (0.9)                                |                                        |
| HPV59              | 4 (1.1)            | 0 (0)                                  | 1 (0.5)                                | 5 (0.5)                                |                                        |
| HPV66              | 7 (1.8)            | 6 (1.5)                                | 5 (2.4)                                | 18 (1.8)                               |                                        |
| HPV68              | 4 (1.1)            | 2 (0.5)                                | 1 (0.7)                                | 7 (0.7)                                |                                        |

GP-EIA, general primer 5+/6+ PCR enzyme immunoassay; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; MA-HPV, MassARRAY human papillomavirus.

Statistical Analysis

A sample was considered MA-HPV positive if >1 of the 14 oncogenic types was detected, and a sample was considered MA-HPV negative for all other outcomes, including detection of low-risk HPV genotypes; the same applied for GP-LMNX. The level of genotype agreement between MA-HPV and GP-LMNX was determined using κ statistics. The following definitions were used: poor, 0.00 to 0.20; fair, 0.21 to 0.40; moderate, 0.41 to 0.60; good, 0.61 to 0.80; and excellent, 0.81 to 1.00; excellent. 34

The accuracy of the MA-HPV assay for detection of CIN ≥2 and CIN ≥3 was assessed and compared with the GP-EIA. Noninferiority of the MA-HPV assay compared with the GP-EIA was assessed according to the international validation guidelines, using the 0.90 and 0.98 benchmarks for relative sensitivity and specificity, respectively. 35,36 Relative accuracy measures (MA-HPV vs GP5+/6+ PCR) with McNemar 95% CIs were computed.

Testing results for MA-HPV, GP-EIA, and GP-LMNX were sent to the Unit of Cancer Epidemiology, Sciensano, for statistical analysis, which was performed using STATA software version 14 (StataCorp, College Station, TX).

Results

HPV Characteristics of the VALGENT4 Panel

The prevalence of hrHPV for MA-HPV was 24.6% in the screening population and 89.9% in the enriched population (Table 1). In comparison, the HPV prevalence for GP-EIA was 14.3% and 85.2% in the screening and enriched populations, respectively. When considering the screening population only, the prevalence was higher in women 30 to 39 years of age (30.6%) and 50 to 59 years of age (27.1%) compared with women 40 to 49 years of age (17.6%). This high prevalence was observed in none of the age groups except for women over 50 years of age (78.9 in women 60 to 69 years of age).
Table 3  HPV Genotyping Prevalence for MA-HPV Assay and GP-EIA Stratified by Cytology Diagnoses

| Cytopathology finding, n (%) | Assay and type (n = 946 MA-HPV and 939 GP-LMNX) | Total, n (%) |
|-----------------------------|-----------------------------------------------|--------------|
| Normal (n = 106 MA-HPV)     | HR-HPV (21.2%)                              |               |
| ASCUS (n = 106 MA-HPV)      | HPV16 (20.1%)                               |               |
| LSIL (n = 121 MA-HPV)       | HPV18 (23.4%)                               |               |
| HSIL* (n = 123 MA-HPV)      | HPV31 (29.3%)                               |               |
| NNILM (n = 121 GP-LMNX)     | HPV33 (17.8%)                               |               |
| GP-LMNX (n = 121 GP-LMNX)   | HPV35 (12.1%)                               |               |
|                              | HPV39 (15.1%)                               |               |
|                              | HPV45 (26.7%)                               |               |
|                              | HPV51 (21.2%)                               |               |
|                              | HPV52 (18.9%)                               |               |
|                              | HPV56 (7.0%)                                |               |
|                              | HPV58 (14.5%)                               |               |
|                              | HPV59 (16.3%)                               |               |
|                              | HPV66 (25.2%)                               |               |
|                              | HPV68 (19.2%)                               |               |
|                              | HPV4+ (12%)                                 |               |
|                              | HPV4- (12%)                                 |               |
|                              | HPV26 (25)                                  |               |
|                              | HPV28 (23)                                  |               |
|                              | HPV30 (11.2)                                |               |
|                              | HPV32 (14.1)                                |               |
|                              | HPV34 (8.9)                                 |               |
|                              | HPV36 (4.0)                                 |               |
|                              | HPV38 (15.6)                                |               |
|                              | HPV40 (9.0)                                 |               |
|                              | HPV42 (7.5)                                 |               |
|                              | HPV44 (10.9)                                |               |
|                              | HPV46 (9.0)                                 |               |
|                              | HPV48 (7.0)                                 |               |
|                              | HPV50 (5.9)                                 |               |
|                              | HPV52 (0.3)                                 |               |
|                              | HPV54 (13.4)                                |               |
|                              | HPV56 (7.0)                                 |               |
|                              | HPV58 (5.0)                                 |               |
|                              | HPV60 (5.0)                                 |               |
|                              | HPV62 (7.0)                                 |               |
|                              | HPV64 (5.0)                                 |               |
|                              | HPV66 (5.0)                                 |               |
|                              | HPV68 (5.0)                                 |               |

Prevalence of most of the individual oncogenic genotypes was highest in the younger age groups (Table 2). The prevalence of the individual genotypes was equal to or higher for MA-HPV for all 14 genotypes compared with GP-LMNX. MA-HPV had the highest prevalence for HPV59, followed by 45, 31, 66, 18 (Table 2). For GP-LMNX, HPV 16 had the highest prevalence, followed by HPV18, 33, 45, and 66. The HPV prevalence in NILM cytologic samples was highest for MA-HPV (21.2%) compared with GP-LMNX (13.0%), whereas in abnormal cytologic samples the prevalence was more at par (Table 3).

Clinical Accuracy of the MA-HPV Test

The accuracy for MA-HPV is presented in Table 4. MA-HPV detected 115 of 122 cases of CIN ≥2 (sensitivity, 94.3%; 95% CI, 88.5% to 97.7%). In comparison, the GP-EIA detected 113 of 122 cases of CIN ≥2 (sensitivity, 92.6%; 95% CI, 86.5% to 96.6%). The MA-HPV assay detected 79 of 83 cases of CIN ≥3 (sensitivity, 95.2%; 95% CI, 88.1% to 97.8%), and the GP-EIA detected 78 of 83 cases of CIN ≥3 (sensitivity, 94.0%; 95% CI, 86.5% to 98.0%). The relative sensitivity for CIN ≥2 and CIN ≥3 was 1.02 (95% CI, 0.98 to 1.05) and 1.01 (95% CI, 0.99 to 1.04), respectively. The sensitivity of the MA-HPV assay was noninferior to that of the GP-EIA for both CIN ≥2 and
Table 4  Sensitivity and Specificity of the MA-HPV Assay Using the GP-EIA as Comparator

| MA-HPV result | GP-EIA | Sensitivity, % (95% CI) | Relative sensitivity (95% CI) | P for noninferiority |
|---------------|--------|------------------------|-----------------------------|---------------------|
| CIN ≥3        |        |                        |                             |                     |
| Positive      | 112    | 94.3 (88.5–97.7)       | 1.02 (0.98–1.05)            | 0.0001*             |
| Negative      | 1      | 92.6 (86.5–96.6)       |                             |                     |
| Total         | 113    |                        |                             |                     |
| CIN ≥3        |        |                        |                             |                     |
| Positive      | 78     | 95.2 (88.1–98.7)       | 1.01 (0.99–1.04)            | 0.0009*             |
| Negative      | 0      | 94.0 (86.5–98)         |                             |                     |
| Total         | 78     |                        |                             |                     |
| Two times NILM|        |                        |                             |                     |
| Positive      | 86     | 9.2 (76.4–81.9)        | 0.89 (0.86–0.91)            | >0.99*              |
| Negative      | 11     | 89.2 (87.0–91.1)       |                             |                     |
| Total         | 97     |                        |                             |                     |

*P < 0.05 for noninferiority means that the accuracy of MA-HPV is not significantly lower than that of GP-EIA, using the benchmarks of 0.90 for relative sensitivity and 0.98 for relative specificity. Noninferiority is significant for sensitivity but not for specificity.

Relative sensitivity of MA-HPV to detect cervical precancer (CIN ≥2 and CIN ≥3) compared with GP-EIA.

Relative specificity of MA-HPV to exclude cervical precancer compared to GP-EIA (assessed for women with NILM at the prior screening round and the index screening).

CIN, cervical intraepithelial neoplasia; GP-EIA, general primer 5+/6+ PCR enzyme immunoassay; MA-HPV, MassARRAY human papillomavirus; NILM, negative for intraepithelial lesions or malignancies.

CIN ≥3 (P = 0.001 for noninferiority and P = 0.0009 for inferiority). The MA-HPV reported 710 of 896 two times NILM cases as negative (specificity, 79.2%; 95% CI, 76.4 to 81.9%), and the GP-EIA reported 799 of 896 two times NILM cases as negative (specificity, 89.2%; 95% CI, 87.0% to 91.1%). The specificity of MA-HPV was inferior to that of GP-EIA (relative specificity, 0.89; 95% CI, 0.86 to 0.91; P > 0.99 for noninferiority).

HPV Genotyping Concordance between MA-HPV and GP-LMNX

The overall HPV concordance in the entire VALGENT framework panel was 88.1% (κ = 0.74) (Supplemental Table S1). Differentiating the screening and enriched cohorts, the overall HPV agreement was slightly higher in women with disease (Supplemental Table S1). The agreement for the individual 14 HPV genotypes for the entire VALGENT panel was fair for HPV68 and HPV59 (κ = 0.33 and 0.38), moderate for HPV51 (κ = 0.58) and HPV66 (κ = 0.54), good for eight genotypes (κ = 0.68 to 0.80), and excellent for HPV16 (κ = 0.89) and HPV35 (κ = 0.81). When limiting the analysis to the screening population, the individual genotype concordance ranged from fair (κ = 0.21) to excellent (κ = 0.82); for the enriched population, the concordance was better for almost all genotypes, with fair (κ = 0.25) to excellent (κ = 0.92) agreement.

Discussion

The MA-HPV is a MALDI-TOF MS HPV assay with full genotyping. To the best of our knowledge, no study has previously been published on the MA-HPV using cervical cancer screening samples. In this study, the clinical validation of the MA-HPV assay is conducted within the VALGENT framework on SurePath-collected screening samples from women participating in the Danish organized cervical cancer screening program. Noninferiority for sensitivity of the MA-HPV compared with the GP5+/6+ comparator test was found for both CIN ≥2 (P = 0.0001 for noninferiority) and CIN ≥3 (P = 0.0009 for noninferiority) but not for specificity (P > 0.99 for noninferiority) (Table 4). The relative sensitivity was 1.02 (95% CI, 0.98 to 1.05) for CIN ≥2 and 1.01 (95% CI, 0.99 to 1.04) for CIN ≥3. The relative specificity was 0.89 (95% CI, 0.86 to 0.91) for NILM samples at baseline and 12 to 24 months earlier.

This finding is not surprising given that the MALDI-TOF assay runs without defined clinical cutoffs and that this technology platform allows for very sensitive analysis (ie, in the substantial detection of HPV59). However, with respect to cervical screening samples, the high sensitivity translates into substantially lower specificity.

The overall concordance between MA-HPV and GP-LMNX was good (κ = 0.74) for all 14 oncogenic HPV genotypes in the VALGENT4 panel (Supplemental Table S1). When the analysis between the screening and enriched population was differentiated as individual sample sets, the overall concordance was moderate (κ = 0.60) for the screening and good (κ = 0.63) for the enriched population set. When the individual specific genotype concordance is considered, a more diverse picture emerges, with κ values ranging from fair (κ = 0.21) to excellent (κ = 0.82) for the screening samples and from fair (κ = 0.25) to excellent (κ = 0.92) for the enriched population. The concordance between MA-HPV and GP-LMNX was higher...
for almost all genotypes in the enriched population (Supplemental Table S1). The higher concordance between HPV assays in women with disease has previously been observed in other studies.29,30,37 The observation is that HPV16 and HPV35 has the best concordance between the MA-HPV test and the comparator assay, whereas HPV68 has the poorest concordance. In addition, HPV68 is close to inconsequential in cervical cancer screening with respect to cancer risk despite being included on the International Agency for Research on Cancer high-risk list.2,38

The MA-HPV is a full genotyping HPV assay that individually reports on 19 HPV genotypes, and only a handful of full genotyping assays have been validated for clinical accuracy according to the international guidelines.20,23,24,26,39,40 Most full HPV genotyping assays require cutoff optimization to fulfill validation criteria for use in cervical screening.23,25,26,32,33,40 This requirement is typically a consequence of the assay design in which the manufacturers aim for high sensitivity of detection in a variety of sample types (ie, formalin-fixed, paraffin-embedded tissue samples, swab samples, and samples collected in liquid-based cytologic media). However, this focus on high sensitivity collides with the requirements of cervical cancer screening in which sensitivity and specificity must be balanced to reduce overdiagnosis.

The MA-HPV assay cannot be easily cut off optimized for use with cervical screening samples. This is due to the nature of MALDI-TOF technology which basically measures time of flight through a vacuum tube by laser evaporated DNA strands of defined length. Yet, the MA-HPV test system provides an agile platform with a reasonable throughput for advanced HPV genotype detection constituting a clear competitor to NGS as the MA-HPV does not require bioinformatics to obtain the final test results. Looking across the field of cervical screening, the ability to provide highly sensitive and detailed HPV genotype results could be utilized in a subset of screening derived samples. Specifically, most organized screening programs require an HPV analysis after treatment (ToC). Often these samples are analyzed using the same HPV assay as employed in primary HPV screening. However, the underlying question is different. In ToC, the aim is to detect residual HPV infection in the cervix post conization and, if possible, determine whether continued HPV positivity constitute same-genotype persistence or a genotype switch.2,41 Whereas a high-risk HPV test result is sufficient to predict recurrence with high sensitivity,8,41–43 its specificity is low and might be increased by identifying the same types before and after conization.8,44

In particular, full genotyping assays, such as the MA-HPV assay, are operated independently of sample type and sample collection media given that they analyze extracted DNA. In the case of ToC testing, HPV testing with any biopsy material and the ToC sample together compared with the original screening sample could help assess a woman’s risk of recurring disease. The ability to run on extracted DNA independent of sample types provides versatility because the DNA input material can be obtained from many different platforms and extraction kits. Moreover, the MA-HPV assay requires a comparatively small amount of DNA input material, making the assay useful for HPV analysis on samples with scarce material (ie, small biopsy specimens, whether fresh or formalin fixed).

The strength of the VALGENT4 study is that the samples were freshly collected SurePath samples from the Danish women in the primary screening target age range of 30 to 59 years who participated in organized cervical cancer screening with complete registration of follow-up. Another strength was that extracted DNA used for the MA-HPV, GP-EIA, and GP-LMNX testing was extracted at the parent laboratory and aliquoted from the same stock, thereby avoiding any bias introduced by different preanalytical DNA extraction methods.

A weakness of the study, also discussed in the published protocol,29 was the sample preparation protocol, which deviates from routine practice. However, because the MA-HPV and comparator assays use preanalytical extracted DNA rather than original sample medium, the effect of this is expected to be minimal. This expectation is also supported by the low number of invalid samples by MA-HPV, GP-EIA, and GP-LMNX in this study and other assays validated in the VALGENT4 study.

In conclusion, the MA-HPV assay is a clinically sensitive assay with a lower clinical specificity than the comparator assay. The lower specificity could be attributable to the detection of HPV infections at low viral loads, and a cutoff optimization could theoretically improve the low specificity in a screening setting. However, the assay in its current form seems more suited to play a role in evaluation of viral infection outcomes where specificity is of less importance but where sensitivity of detection is paramount. These applications include HPV genotype monitoring in epidemiologic studies of HPV vaccination outcomes, clinical TOC evaluation after conization, and clinical noncervical screening diagnostic samples for HPV testing.

### Author Contributions

M.A. coordinates the VALGENT frameworks; J.H.B. and M.A. designed the study; H.P., D.M.E., and W.Q. performed the laboratory work; J.H.B., D.M.E., L.X., and M.A. analyzed the data; J.H.B., H.P., and D.M.E. drafted the manuscript; all authors contributed to revisions of the manuscript, participated in the decision to submit, and had full access to all the data in the study.

### Supplemental Data

Supplemental material for this article can be found at http://doi.org/10.1016/j.jmoldx.2021.12.009.
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