HPV mRNA test in triage of women with minor cervical lesions; experiences from the University Hospital of North Norway

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Abstract

In the Norwegian Cervical Cancer Screening Programme tests for detection of human papillomavirus (HPV) are used to triage women with minor cytological cervical lesions. The material in this study comprises samples from 1 798 women in the period 2006-08. The HPV test was performed according to the guidelines of the Norwegian Cancer Registry. The HPV mRNA test (PreTect HPV-Proofer) detects and types 5 high-risk genotypes (16, 18, 31, 33 and 45). The HPV mRNA results were compared to cytology and then biopsy up to December 2009. Women with minor cytological cervical lesions and negative HPV test were followed with a new PAP smear after 12 months. A total of 327 women (18 %) were HPV mRNA positive. Of the 1 798 women with minor cytological lesions, 232 women (13 %) had moderate dysplasia, severe dysplasia or cancer and 144 women (8 %) had severe dysplasia or cancer in biopsy. 57 % of the women with a positive HPV mRNA test had moderate dysplasia, severe dysplasia or cancer. 37 % had severe dysplasia or cancer. The sensitivity of the HPV mRNA test to detect moderate dysplasia, severe dysplasia or cancer was 81 %. The specificity for moderate dysplasia, severe dysplasia or cancer was 91 %. The negative predictive value (NPV) of the HPV mRNA test for moderate dysplasia, severe dysplasia or cancer was 97 %. Of 11 women with cervical cancer, 10 were positive for HPV type 16 or 18.

Compared to existing literature the HPV mRNA test seems more suitable than HPV DNA tests to triage women with minor cytological cervical lesions due to its higher specificity.

Key words: HPV, mRNA, dysplasia, cytology, cervical cancer, screening.
Cervical cancer is the second most common cancer affecting women worldwide. About 300 women in Norway get cervical cancer each year and 80 of these die from the disease. In 1995 a Norwegian National Cervical Cancer screening programme was established. As a part of the programme, women aged 25 – 69 years are invited to cytological screening every three years, totalling 400 000 smears a year. Every year 25 000 women are retested due to minor cytological lesions. Approximately 10 000 women are referred to colposcopy, and 3 000 treated by conisation because of high grade changes confirmed by biopsy (Cancer Registry of Norway; http://www.kreftrregisteret.no).

It has been estimated that without the screening programme, one might expect 600 cases of cervical cancer in Norway each year (Cancer Registry of Norway; http://www.kreftrregisteret.no). The Norwegian guidelines recommend treatment with conisation of the cervix after histologically confirmed high grade dysplasia. 3 000 women are conised every year to avoid 300 cases of cancer, which suggests considerable overtreatment. Currently no methods exist for distinguishing high grade dysplasia with high risk of progression to invasive cancer from high grade dysplasia that is going to regress spontaneously without treatment. The assay PreTect HPV-Proofer (NorChip AS, Norway) detects the E6/E7 oncogene expression which is necessary for malignant transformation and may therefore be a possible candidate to identify underlying cervical cancer precursors with high probability of progression to cancer among women with minor cytological abnormalities.

Women with minor cytological cervical lesions have an increased risk of having, or developing, high grade dysplasia compared to women with normal cytology. However, most minor cytological lesions regress spontaneously and therefore careful triage is crucial. In this short communication the routine diagnostic practice at the University Hospital of North Norway is evaluated including the use of the HPV E6/E7 mRNA test PreTect HPV-Proofer in triage of women with minor cytological cervical lesions for the detection of cervical dysplasia and cancer.
About 23,000 cervical smears are analysed annually at the Department of Clinical Pathology at the University Hospital of North Norway. Between 2006 and 2008, smears from 48,781 women aged 25-69 years were analysed. A total of 2,314 women (4.7%) were diagnosed with minor cervical lesions. For these women, repeat cytology and HPV mRNA test after 6 months is recommended. The compliance was high; as liquid based control cytology was received 1,798 women were tested with the HPV mRNA test (78% of the 2,314 women). Cells were extracted from the ThinPrep (Cytyc Corporation, Marlborough, MA, USA) for cytological examination.

The diagnoses of the cervical smears and the biopsies were taken from the diagnostic database for cytological and histological samples (SymPathy) at the Department of Clinical Pathology, University Hospital of North Norway. The HPV mRNA testing was performed according to the national guidelines for HPV testing (Figure 1). In the department of clinical pathology all biopsies with high-grade dysplasia and cancer are evaluated by experienced pathologists. Biopsies with uncertain cellular changes are immunostained with p16.

The sensitivity of the HPV mRNA test is defined as the proportion of high grade dysplasia detected by HPV mRNA or repeat cytology that is positive for HPV mRNA. In the calculations of specificity and negative predictive values (NPV) it is assumed that cytonegative and HPV mRNA negative samples without detected dysplasia during the follow up period do not contain disease.

Of the 1,798 women who had a HPV-test done, 327 (18%) were HPV mRNA positive. A total of 232 women (13%) had moderate dysplasia or worse and 144 women (8%) had severe dysplasia or cancer in biopsy. The absolute number of true-positives (TP) for moderate dysplasia or worse was 188. The TP was 121 for severe dysplasia or cancer. 57% (188/327) of the women with a positive HPV mRNA test had moderate dysplasia or worse. 37% (121/327) had severe dysplasia or cancer. The sensitivity of the HPV mRNA test to detect moderate dysplasia or worse was 81% (188/232). The sensitivity for severe dysplasia or cancer was 84% (121/144). The specificity for moderate dysplasia or worse was 91% (1427/1566). The specificity for severe dysplasia or cancer
was 88 % (1448/1654). The negative predictive value (NPV) of the HPV mRNA test was 97 % (1427/1471) for moderate dysplasia or worse. NPV for severe dysplasia or cancer was 98 % (1448/1471).

For women with equivocal cervical lesions (atypical squamous cells of undetermined significance) the positive predictive value (PPV) for moderate dysplasia or worse was 57 % (83/145). The PPV for severe dysplasia or cancer was 41 % (60/145). The specificity was 95 % (1088/1150) and 93 % (1092/1177), respectively. For women with low-grade cytological lesions (low grade squamous intraepitelial lesion) the PPV for moderate dysplasia or worse was 58 % (105/182). The PPV for severe dysplasia or cancer was 34 % (61/182). The specificity of low grade cytological lesions was 82 % (339/416) and 75 % (356/477), respectively (Tables 1 and 2).

Of the 327 women with a positive HPV mRNA test, 204 women (62 %) were infected with HPV type 16 or 18. Of the 187 HPV positive women with moderate dysplasia or worse, 129 (69 %) had HPV 16 or 18. Of the 121 HPV positive women with severe dysplasia or cancer, 94 (78 %) had HPV type 16 or 18. Despite low-grade changes in PAP-smear six months earlier, 11 women actually had cancer in biopsy. 10 of these (91 %) had HPV type 16 or 18 (figure 2). One woman with a lymphoepithelial carcinoma of the cervix was HPV mRNA negative.

In general, the use of HPV DNA tests generates more positive results than the HPV mRNA test. This is because DNA tests detect the mere presence of the virus and will therefore also detect harmless transient infections which are handled by the immune system (Cattani et al., 2009b). The HPV DNA test Hybrid Capture 2 detects 13 HPV types and the HPV mRNA test PreTect HPV Proofer detects five HPV types. In Europe most of the HPV positive cervical cancer cases are types 16, 18, 31, 33 and 45 (Smith et al., 2007), and these HPV types are included in the HPV mRNA test. The specificity and the positive predictive value (PPV) of HPV DNA tests for moderate dysplasia or worse are low, especially when young, sexually active women are tested. The real cause of cervical cancer is not the HPV-virus infection per se, but continuous over-expression of
the viral oncogenes E6 and E7 from oncogenic HPV-types (zur Hausen, 2002). There may be several reasons why E6 and E7 are over-expressed, but one of the main reasons is integration of the virus into the human genome (loss of E2 gene results in loss of regulation of transcripts). The loss of the E2 gene only occurs in a small fraction of the high number of women with HPV infection (Collins et al., 2009). This implies that a test that detects the over-expression of E6 and E7 mRNA is more specific than a test that detects the general presence of viral DNA.

In triage of minor cytological cervical lesions, in comparison with follow-up cytology, HPV DNA testing was more sensitive and equally specific for triage of equivocal lesions and for predicting recurrence of cervical intraepithelial neoplasia in women treated for high-grade dysplasia (Arbyn et al., 2005). Due to the high rate of HPV positivity, this is not the case for patients with cytological low grade lesions, as the DNA test Hybrid Capture 2 showed a substantially and significantly lower specificity than the repeat Pap smear, as demonstrated in a meta-analysis by Arbyn et al (Arbyn et al., 2004;Arbyn et al., 2005). When triaging women with low grade lesions, a reflex Hybrid Capture 2 test did show a significantly higher sensitivity, but at the same time a significantly lower specificity compared to repeat cytology (Arbyn et al., 2006).

In studies where cervical samples have been tested with both the HPV mRNA test and HPV DNA tests, the test positivity rate of the HPV mRNA test is one third of the HPV DNA tests. The sensitivity for moderate dysplasia or worse is lower, but the specificity is higher (Cattani et al., 2009a;Keegan et al., 2009;Lie et al., 2005;Szarewski et al., 2008). It is well-known that many cervical lesions with moderate or severe dysplasia will regress spontaneously. Only 31% of colposcopically visible lesions with severe dysplasia will progress to invasive cancer within 30 years (McCredie et al., 2008). Although the HPV mRNA test has a lower sensitivity in detecting moderate or severe dysplasia, it is probable that it still identifies the lesions that are destined to progress to cancer. Furthermore, several studies have shown that the sensitivity of the HPV mRNA
test for cervical cancer is the same as for the HPV DNA tests (Basu et al., 2009; Hovland et al., 2010; Kraus et al., 2006; Lie et al., 2005).

The HPV test is used to triage women with minor cytological cervical lesions. In triage it is important to have a test with high specificity in order to identify who should be referred to colposcopy and biopsy from those that only need control cytology 12 months later. The sensitivity is of minor importance because a positive diagnosis has already been made using cytology. A positive HPV test will result in biopsy when the control cytology has minor lesions. In cases where a HPV test with a low specificity is used, a high number of women will be referred to colposcopy and biopsy unnecessarily. Many unnecessary conisations are performed because many lesions with moderate or severe dysplasia will regress spontaneously without treatment. Moreover, unnecessary conisations should be avoided given the risk of obstetrical complications associated with such treatments (Arbyn et al., 2008).

One of the most used HPV tests in Norway is the Digene Hybrid Capture® HPV DNA Test (Qiagen, Gaithersburg, MD, USA). This test detects whether or not any of the 13 high risk HPV viruses are present but a positive test does not give any information concerning which type of virus is causing the positive result. The HPV DNA test is shown to have a high clinical sensitivity and a high negative predictive value (NPV), but the specificity for high grade dysplasia is low (Solomon et al., 2001). For instance, using high grade lesions as outcome, the average specificity of the HPV DNA test Hybrid Capture in triage of equivocal cervical lesions is 62.5% (95% CI: 57.8-67.3%) and only 28.6% (95% CI: 22.2-35.0) in triage of low grade cervical lesions (Arbyn et al., 2006). While HPV DNA tests correlate poorly with the grade of dysplasia (Trope et al., 2009), the experiences from the University Hospital of North Norway show that the HPV mRNA test correlates well with histologically confirmed grade of dysplasia. This indicates that HPV E6/E7 mRNA is a good biomarker for severe dysplasia and cancer. As moderate dysplasia regresses spontaneously more often than severe dysplasia, it is probable that use of a HPV mRNA test in
Triage reduces overtreatment of young women compared to the use of a HPV DNA test. However, if moderate or severe dysplasia is confirmed by biopsy, ethical considerations make it difficult to delay treatment to evaluate the rate of regression even though the women have a negative HPV mRNA test.

In one study the HPV mRNA test was as sensitive for high grade dysplasia but more specific than HPV DNA testing with PCR (Molden et al., 2005). In a Norwegian study by Vintermyr et al. using samples from 435 women with minor cervical lesions the test positivity rate of the HPV DNA test Hybrid Capture 2 was 53% (232/435); the sensitivity for high grade lesions was 94% (47/50); the specificity 52% (200/385); and the PPV was 20% (47/232) (Vintermyr et al., 2008). In the material from the University Hospital of North Norway 18% had a positive HPV mRNA test; the sensitivity for high grade lesions was 81%; specificity 91%; and the PPV was 57%.

The experience from the University Hospital of North Norway is that the HPV mRNA test has a high specificity and a high positive predictive value. This makes it useful in triage of women with equivocal and low grade cytological lesions. Together with the cervical cytology, this HPV mRNA test detects more high-grade dysplasia than cytology alone without increasing the number of biopsies. Compared to existing literature the HPV mRNA test seems more suitable than HPV DNA tests to triage women with minor cytological cervical lesions due to its higher specificity.

**Conflicts of interest**

None

**Table 1**
Women with equivocal cervical lesions (atypical squamous cells of undetermined significance), HPV mRNA test results and subsequent high grade histological diagnoses

Table 2
Women with low grade cytological lesions (low grade squamous intraepithelial lesion), HPV mRNA test results and subsequent high grade histological diagnoses

Figure 1
Flow chart showing the follow up HPV mRNA test to triage women with equivocal (atypical squamous cells of undetermined significance) and low grade cytological lesions (low grade squamous intraepithelial lesion). The figure shows the number of women in each group. The numbers in brackets represent women with subsequent high grade histological diagnoses. Samples from 1 798 women were tested with the HPV mRNA test (78 % of the 2 314 women). The blue boxes denoted “No cytology” represent women lost in follow-up. 419 women were referred to colposcopy (the red box), 215 of these had high grade lesions (51 %).

Figure 2
Frequency of different HPV types detected by the HPV mRNA test and rate of subsequent high-grade histological diagnoses. Of 327 women with a positive HPV mRNA result, 232 had moderate dysplasia or worse, 144 had severe dysplasia or worse and 10 women had cancer. Of 162 women with HPV type 16, 112 had moderate dysplasia or worse, 85 had severe dysplasia or worse and 8 women had cancer. In total, 11 women had cancer, of which 10 were positive for HPV type 16/18. One tested negative for all the five HPV types.
References


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Response to comments from the reviewers

Reviewer #1:

General comments:
Cervical cancer screening is still based on cytology, with recent introduction of detection of HPV DNA along with cytology. The change now being proposed is in the detection of the mRNA of HPV oncogenic proteins (E6 and E7) as a marker of clinically significant cervical disease because detecting DNA alone yields too many negative biopsies (low specificity).

Specific comments:
The authors reported comprehensive evaluation of the utility of HPV E6 and E7 mRNA testing. Their histologic-virologic investigation, testing a large number of women (1798) for HPV mRNA, in the triage of ASCUS and LSIL, convincingly demonstrate that the detection of E6 and E7 viral transcripts is a specific tool for prediction of high grade lesions, correlating significantly with histological confirmed grade of dysplasia.

These findings have important clinical implications. HPV mRNA testing may be more efficient and effective for triage and follow-up of females with abnormal cytology, reducing the number of patients referred to colposcopy and biopsy.

A minor suggested change is that the authors may consider reducing the number of tables (table 1 and 2 could be incorporated comparing results for CIN2+ and CIN3+ in the same table)

*Our response: CIN2+ and CIN3+ are now in the same table. However, reviewer #2 recommended dividing the material into women with cytological ASC-US versus women with LSIL, respectively. For this reason there are still two tables.*

Reviewer #2:

**GENERAL COMMENTS**

Women with ASCUS/LSIL have an increased risk for having or developing high grade CIN compared to women with normal cytology. However, most minor lesions regress spontaneously and therefore careful triage is crucial. This study demonstrates good specificity of the HPV Proofer containing probes for the 5 main HPV types in triage of women with minor cytological lesions (ASCUS/LSIL). Triage with HPV DNA testing using the HC2 assay has significantly higher sensitivity and similar specificity compared to repeat cytology. However in LSIL triage, HC2 is highly sensitive but has very low specificity. For this reason it would be appropriate that the authors provide separate data split by triage group: ASCUS and LSIL.

*Our response: The data is now split by triage group ASC-US (table 1) and LSIL (table 2), as recommended by reviewer #2.*

It is recommended also to present the performance of triage by repeat cytology.
Our response: Unfortunately, we cannot compare triage by repeat cytology with triage by HPV-testing in our material. According to the guidelines from the Norwegian Cervical Cancer Screening Programme, colposcopy and biopsy are not recommended after repeat cytology (ASC-US / LSIL x 2) without a positive HPV-test. However, colposcopy and biopsy are recommended if the woman has minor cytological abnormalities (ASC-US / LSIL x 3) over a period of 18 months even though the HPV-test is negative.

Certain improvements in describing accuracy parameters are needed (see below).

Our response: Accuracy parameters have been improved according to Reviewer 2’s recommendations (see below)

References to studies addressing primary screening are irrelevant. The authors should refer to studies where HPV tests in a triage setting are used.

Our response: We agree and have included the following paragraph (page 6, ln 5-14)

“In triage of minor cytological cervical lesions, in comparison with follow-up cytology, HPV DNA testing was more sensitive and equally specific for triage of equivocal (ASC-US) lesions and for predicting recurrence of cervical intraepithelial neoplasia (CIN) in women treated for high-grade dysplasia (CIN2+). Due to the high rate of HPV positivity, this is not the case for patients with cytological low grade dysplasia (LSIL), as the DNA test Hybrid Capture 2 showed a substantially and significantly lower specificity than the repeat Pap smear, as demonstrated in a meta-analysis by Arbyn et al. When triaging women with low-grade squamous intraepithelial lesions (LSIL), a reflex Hybrid Capture 2 test did show a significantly higher sensitivity, but at the same time a significantly lower specificity compared to a repeat Pap smear.”

Pages could be numbered to facilitate reviewing.

Our response: The pages have been numbered.

INTRODUCTION

Page 5,3rd §: the accuracy estimates of cytology in primary screening (Nanda, Koliopoulous, Gage) are irrelevant for this study, where triage of ASCUS/LSIL is addressed. The authors should refer to previously published meta-analyses on triage of these lesions: (see Arbyn JNCI 2004; Gynecol Oncol 2005 and Vaccine 2006).

Our response: We agree and have included the following paragraph (page 6, ln 5-14)

“In triage of minor cytological cervical lesions, in comparison with follow-up cytology, HPV DNA testing was more sensitive and equally specific for triage of equivocal (ASC-US) lesions
and for predicting recurrence of cervical intraepithelial neoplasia (CIN) in women treated for high-grade dysplasia (CIN2+). Due to the high rate of HPV positivity, this is not the case for patients with cytological low grade dysplasia (LSIL), as the DNA test Hybrid Capture 2 showed a substantially and significantly lower specificity than the repeat Pap smear, as demonstrated in a meta-analysis by Arbyn et al. When triaging women with low-grade squamous intraepithelial lesions (LSIL), a reflex Hybrid Capture 2 test did show a significantly higher sensitivity, but at the same time a significantly lower specificity compared to a repeat Pap smear.”

Page 5, last line: “.candidate”; complete: . candidate “to identify underlying cervical cancer precursors with high probability of progression among women with minor cytological abnormalities”.

Our response: We agree and have inserted this sentence according to the reviewers recommendations: (page 3, ln 17-19) “candidate to identify underlying cervical cancer precursors with high probability of progression among women with minor cytological abnormalities.”

Page 6, first §. This § essentially concerns primary screening. The authors should focus on the topic of their manuscript which is triage of ASC-US/LSIL. Only the Solomon reference is relevant. This paragraph should be dropped.

Our response: We agree and this paragraph has been omitted.

MATERIAL AND METHOD

Page 5, 3rd §. “Cells are extracted with the ThinPrep®. Change “with” into “from”

Our response: We agree and have changed the sentence accordingly (page 4, ln 9).

As mentioned before, the authors should provide separate data for ASC-US and LSIL.

Our response: We agree. The data are now presented separately (table 1 and 2).

The authors should put the assumption forward that cytonegative/RNA negative samples do not contain disease. Otherwise the specificity and NPV cannot be derived from these data since this would require verification of all mRNA neg samples.
Our response: We agree, and have added this sentence accordingly (page 4, ln 17-20) “In the calculations of specificity and negative predictive values (NPV) it is assumed that cytonegative and HPV mRNA negative samples without detected dysplasia during the follow up period do not contain disease.”

RESULTS
Page 7: Give the absolute number of true-positives (TP) for CIN2+ and CIN3+.

Our response: We agree and have included these data (page 4, ln 23-24) “The absolute number of true-positives (TP) for CIN2+ and CIN3+ is 188 and 121 respectively.”

It is recommended to provide systematically numerator and denominator for all computed accuracy measures in the paper.

Our response: We agree and we have included these numbers in all the presented data.

The PPV values are mentioned twice (0.57 and 57%; 0.37 and 37%). Be consistent in describing accuracy parameters: always as a percentage for instance (57%) but not in fraction (0.57).

Our response: We agree. All the PPV and NPV are now presented as percentages.

The authors should define sensitivity of mRNA as the proportion of CIN2/3+ detected by mRNA or repeat cytology that is positive for mRNA.

Our response: We agree. This sentence has been included accordingly (page 4, ln 16-17) “The sensitivity of the HPV mRNA test is defined as the proportion of high grade dysplasia (CIN2+) detected by HPV mRNA or repeat cytology that is positive for HPV mRNA.”

The title of fig 1 can be shortened, since the fig is self-explanatory.

Our response: We agree. It is now shortened.
Fig 2. It is recommended to mention the absolute values on top of each bar.

**Our response:** We agree. See updated figure 2.

Terminology tables 1 & 2:
Change detection rate by test positivity rate.
Change proportion CIN2/3+ by prevalence of CIN2/3+

**Our response:** We agree. See updated table 1 and 2.

**DISCUSSION**
Page 8: “Many unnecessary conizations are performed because many CIN2+ lesions will regress spontaneously without treatment”. Add the sentence: Moreover, unnecessary conisations should be avoided given the risk of obstetrical complications associated with such treatments (Arbyn BMJ 2008).

**Our response:** We agree. The sentence has now been included (page 7, ln 7-10)

Page 8, last but one sentence: “The HPV DNA test is shown to have a high clinical sensitivity and a high negative predictive value (NPV) in triage of minor cytology, but the specificity is low”. It would be useful to add: “For instance, using CIN2+ as outcome, the average specificity of HC2 in triage of ASCUS is 62.5% (95% CI: 57.8-67.3%) and only 28.6% (95% CI: 22.2-35.0) in triage of LSIL (Arbyn Vaccine 2006).”

**Our response:** We agree. The sentence has now been included (page 7, ln 16-18)

Page 9: It would be useful to add the ref of Molden T, Nygard JF, Kraus I, Karlsen F, Nygard M, Skare GB et al. Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-proofer and consensus PCR: A 2-year follow-up of women with ASCUS or LSIL pap smear. Int J Cancer 2005; 114: 973-6. In this paper it is shown that Pretect proofer is as sensitive for CIN2+ but more specific than HPV testing with PCR.

**Our response:** We agree. This reference has now been included (page 8, ln 3-4) “In a study by Molden et al., the HPV mRNA test was as sensitive for CIN2+ but more specific than HPV DNA testing with PCR”
Editor’s Comments:

- Do not use the first-person when rewriting (ie. “we” and “our”).

**Our response:** *the use of first-person has been deleted in the manuscript*

- There are grammatical errors which must be corrected.

**Our response:** *Grammatical errors have been corrected and the manuscript has been edited by a scientific English editing service*

- The paper must be rewritten in grammatical English with the help of a native English speaking-scientist or scientific English editing service:
  - [http://www.elsevier.com/wps/find/authors.authors/languagepolishing](http://www.elsevier.com/wps/find/authors.authors/languagepolishing)

- An example of the corrections necessary throughout the text will be e-mailed to you separately / is attached.

- Do not use the acronyms ASCUS, LSIL, CIN+, UNN. Do not use acronyms in the title of the manuscript.

**Our response:** *OK*

- Reference List:
  - Please check the style of the Journal
  - Do not use the word “and” in the list of authors

  **Our response:** *this has been corrected according to the style of the journal.*

- The title page must be retyped according to the style of the Journal.

  **Our response:** *this has been corrected according to the style of the journal.*
Figure(s)

- Minor cervical lesions: 2,314 (315)
  - No HPV mRNA test: 516 (83)
    - HPV mRNA test
      - New cytology: 1,798 (232)
        - HPV mRNA test
          - New cytology: 1,121 (9)
  - HPV mRNA pos.
    - Minor cervical lesions: 306 (20)
    - Normal cytology
      - HPV mRNA neg.
        - Minor cervical lesions: 174 (89)
          - High grade cervical lesions
            - HPV mRNA pos. or neg.
              - HPV mRNA pos.
                - Normal cytology: 45 (14)
              - Abnormal: 419 (215)
                - Colposcopy and biopsy
                  - HPV mRNA neg.
                    - Normal: 198 (2)
  - HPV mRNA neg.
    - Minor cervical lesions: 174 (89)
    - Normal cytology
      - HPV mRNA neg.
        - Normal cytology: 1,121 (9)
  - New cytology: 276 (20)
    - Abnormal: 78 (18)
      - HPV mRNA neg.
        - Normal: 198 (2)
  - HPV mRNA neg.
    - Minor cervical lesions: 306 (20)
    - Normal cytology
      - HPV mRNA neg.
        - Normal cytology: 1,121 (9)
  - Return to screening: 1,359 (11)
  - New HPV mRNA test
    - New cytology: 25 (8)
      - HPV mRNA neg.
        - Normal: 20 (6)
### Table 1
Women with equivocal cervical lesions (atypical squamous cells of undetermined significance), HPV mRNA test results and subsequent high grade histological diagnoses

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<th>HPV+</th>
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<td>1 248</td>
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Sensitivity 
Specificity 
Positive predictive value 
Negative predictive value 
Test positivity rate 
Prevalence of moderate dysplasia or worse

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<td>11</td>
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Sensitivity 
Specificity 
Positive predictive value 
Negative predictive value 
Test positivity rate 
Prevalence of severe dysplasia or worse

### Table 2
Women with low grade cervical lesions (low grade squamous intraepithelial lesion), HPV mRNA test results and subsequent high grade histological diagnoses

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<td>Total</td>
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Sensitivity 
Specificity 
Positive predictive value 
Negative predictive value 
Test positivity rate 
Prevalence of moderate dysplasia or worse

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Sensitivity 
Specificity 
Positive predictive value 
Negative predictive value 
Test positivity rate 
Prevalence of severe dysplasia or worse