PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Primary cervical cancer screening with an HPV mRNA test – a prospective cohort study
AUTHORS	Sørbye, Sveinung Wergeland; Fismen, Silje; Gutteberg, Tore; Mortensen, Elin; Skjeldestad, Finn Egil

VERSION 1 - REVIEW

REVIEWER	Kimon Chatzistamatiou Hippokratio Hospital of Thessaloniki, Greece I have had travel expenses and congress registration fees covered by Roche diagnostics.
REVIEW RETURNED	18-Apr-2016

GENERAL COMMENTS	Dear author, The presented study is a prospective population-based study which aims to retrospectively analyse the effectiveness of HPV mRNA testing. The study has a limitation, which is acknowledged in the manuscript, and refers to the fact that the study population was tested with HPV mRNA initially but the follow up was performed according to screening guidelines for Norway, meaning that initially positive women for HPV mRNA were not subjected to colposcopy if tested normal for cytology. They were subjected to colposcopy only depending on the cytology report (until 2005) or cytology plus HPV triage from 2005 to 2009. This is a source of bias against the HPV mRNA test, therefore the cumulatve CIN2+ incidence could be underestimated. However this does not have an impact on the presented analysis since no comparisons are being made between screening modalities. My questions/comments are the following: 1) Could the authors calculate diagnostic accuracy indices (sensitivity, specificity, PPV and NPV) for the HPV mRNA testing? If yes it could be useful to present this data (crude and adjusted for verification bias) because this could make their point on the better trade-off presented by the HPV mRNA test more clear. Moreover, a comparison concerning sensitivity of the HPV mRNA test with the one presented for the HPV DNA test in the literature is vital since sensitivity is crucial for primary screening. 2) Which HPV test is used for cytology triage since 2005 in Norway? It should be mentioned in the methods section. (This is the reason for the "NO" on question 4 above). 3) Why did they chose the age of 33 as a cut-off to distinguish younger to older women? 4) In the discussion section (paragraph 2) it is mentioned that HPV DNA primary screening could lead to huge administrative costs due to the high HPV DNA positivity in younger ages. This is true but the authors do not mention at all the various triage strategies for HPV

	positive women implemented to overcome this obstacle. I think that this should be included in the discussion section. (This is the reason for the "NO" on question 8 and 12 above; References should be included commenting on cervical cancer screening strategies focusing on triage of HPV positive women to colposcopy). 5) A comment should be made concerning the fact that the mRNA HPV test presented identifies only 5 hr HPV types which are all a target of the nonavallent vaccine which has already been available in some European countries. Therefore, if the vaccine is widely accepted this test might not be useful in the future for cervical cancer prevention strategies combining HPV test on vaccinated cohorts. In addition I have the following minor corrections to suggest: 1) Page 7, line 26: The authors state that the cumulative proportion of CIN3+ was 0.95% and 2.32% respectively, for women aged 25–33 years, and 0.55% and 0.87% respectively, for women aged 34–69 years, shown in figure 1. However, the y-axis of figure 1 presents the cumulative incidence of CIN3+ not showing the unit which measures it. Therefore they should clearly specify this unit on the y-axis and this applies to figure 2 too. 2) Page 7, line 49: The authors describe the differences between various HPV types and say that this is presented in table 3 and figure 2. However, figure 2 does not provide that information. It should be deleted and quote only table 3. 3) Page 9, line 36: The authors say that in a US cohort of.....cytology/HPV.....Do they mean cytology/HPV negative? It should be clarified since there is something clearly missing.

REVIEWER	Kate Cuschieri Scottish HPV Reference Laboratory, Royal Infirmary of Edinburgh NHS Lothian Scotland UK
REVIEW RETURNED	25-Apr-2016

GENERAL COMMENTS	This is an interesting study and to my knowledge the largest to address the potential utility and performance of a 5 type mRNA assay in a screening context. Given that the modality of primary screening is shifting from cytology to HPV testing - the information presented is timely. The Norweigan context with its excellent linkage capabilities and history of application of this assay is an appropriate place to deliver this work. I do have some issues with the article which are listed below General issues The abstract is too definitive - I do not think that the data do support the use of the mRNA test for primary screening. While the cumulative rate of CIN3+ is presented, the other key measures of whether an assay is suitable for primary screening (including sensitivity) are passed over. Thus I do not agree with the conclusion that "HPV mRNA testing may be used as a primary screening both for women 25-33 and 34-69" In addition - one could argue that the responsibility of any screening

	test is to (within reason) be sensitive. Appropriate triages can then stratify the relevant infections from the noise. While we are not in possession of a perfect triage one could argue that this is not the "fault" of HPV DNA tests and changing the modality to reduce the sensitivity of a screening test may be shortsighted. Specific comments Page 4 - Line 6: I thought according to WHO data cervical cancer is now the fourth most comment female cancer. Page 4 - Line 10: "young female population" is vague Page 4: Line 40-44. Not all cervical cancers have integrated viral forms - between 15-20% will have episomal forms only. Integration is not a requisite for transformation Page 4 Line 58: In line with above comment, I am not sure I agree with the logic that a more specific test is appropriate for screening purposes. The data indicate that 3/5 cancers were missed using this approach... Page 5: Line 10 - not sure what is meant by "selected gynaecologists" Page 5: Line 30: What exactly do the gyane clinicians report that the pathology lab do not? Need to get a handle on how representative and comprehensive the data sources are. Page 6: Line 28: Who were the women with the positive test? women associated with the study only? Presumably the mRNA test results had to be disclosed - a fuller explanation would be helpful here. Page 7: Lines 43-48 are hard to understand - consider rephrasing. Page 8: Line 4/5: Sentence beginning "Case numbers" - does not make sense to me Page 9: Line 22 - I don't agree with the "huge administrative challenge" IF (as indicated above) there is a useful triage. Page 10: Line 10 - The discussion on types in Danish cancers will not necessarily transfer to a global perspective - consider a broader reference? Page 10: Line 35 - The discussion on triage is off topic Page 11: Lines 13-31 are repetitious with the introduction and are not needed.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1) Could the authors calculate diagnostic accuracy indices (sensitivity, specificity, PPV and NPV) for the HPV mRNA testing? If yes it could be useful to present this data (crude and adjusted for verification bias) because this could make their point on the better trade-off presented by the HPV mRNA test more clear. Moreover, a comparison concerning sensitivity of the HPV mRNA test with the one presented for the HPV DNA test in the literature is vital since sensitivity is crucial for primary screening.

Our response: It is possible, but not common to calculate the baseline sensitivity and specificity of a screening test with six years of follow-up. We have not included data for sensitivity/specificity as we find It, along with other publications on the issue, inappropriate.

2) Which HPV test is used for cytology triage since 2005 in Norway? It should be mentioned in the methods section.

Our response: In the period 2005-2009, the HPV-tests used in Norway were Hybrid Capture II, PreTect HPV-Proofer and Amplicor. This information is added in the M&M section (lines 150-1).

3) Why did they choose the age of 33 as a cut-off to distinguish younger to older women?

Our response: In Norway, there is an ongoing pilot study for HPV DNA test (Cobas 4800) in primary screening in women 34-69 years, while women 25-33 years still are screened with cytology. The same is the case in Italy, while Sweden and Nederland are planning to start HPV DNA primary screening in women 30-60 years. Our age categorization reflects the current screening situation in Norway.

4) In the discussion section (paragraph 2) it is mentioned that HPV DNA primary screening could lead to huge administrative costs due to the high HPV DNA positivity in younger ages. This is true but the authors do not mention at all the various triage strategies for HPV positive women implemented to overcome this obstacle. I think that this should be included in the discussion section. (This is the reason for the "NO" on question 8 and 12 above; References should be included commenting on cervical cancer screening strategies focusing on triage of HPV positive women to colposcopy).

Our response: Triage of HPV DNA positive women with cytology can reduce the burden of colposcopy. However, all women with a positive HPV DNA test in primary screening still need follow-up even though cytology is normal. If you accept to return HPV positive / cytology negative women back to screening, we would prefer to use a less sensitive, but more specific HPV-test as the primary screening test. There is limited documentation supporting HPV positive women with a negative triage test (like p16/Ki67 (CINtec PLUS)) may return back to screening at regular screening intervals. We have not added any information about this topic in the revised manuscript.

5) A comment should be made concerning the fact that the mRNA HPV test presented identifies only 5 hr HPV types that are all a target of the nonavalent vaccine that has already been available in some European countries. Therefore, if the vaccine is widely accepted this test might not be useful in the future for cervical cancer prevention strategies combining HPV test on vaccinated cohorts.

Our response: If the nonavalent HPV-vaccine is widely accepted, and the rate of cervical cancer is reduced by 80% there is no longer a need for screening for cervical cancer. In the meantime, the five most common HPV-types in cervical cancer are still the most dangerous types. That is why screening for HPV16, 18, 31, 33 and 45 makes sense until vaccination of the entire population have taken place. A discussion on screening in a post-vaccination scenario is not relevant for the present manuscript.

In addition, I have the following minor corrections to suggest:

1) Page 7, line 26: The authors state that the cumulative proportion of CIN3+ was 0.95% and 2.32% respectively, for women aged 25–33 years, and 0.55% and 0.87% respectively, for women aged 34–69 years, shown in figure 1. However, the y-axis of figure 1 presents the cumulative incidence of CIN3+ not showing the unit which measures it. Therefore, they should clearly specify this unit on the y-axis and this applies to figure 2 too.

Our response: Unit is added to the y-axis on both figure 1 and 2 in the revised ms.

2) Page 7, line 49: The authors describe the differences between various HPV types and say that this is presented in table 3 and figure 2. However, figure 2 does not provide that information. It should be deleted and quote only table 3.

Our response: The text is changed, figure 2 is deleted (line 198).

3) Page 9, line 36: The authors say that in a US cohort of.....cytology/HPV.....Do they mean cytology/HPV negative? It should be clarified since there is something clearly missing.

Our response: The text is refined (lines 244-5).

Reviewer: 2

This is an interesting study and to my knowledge the largest to address the potential utility and performance of a 5 type mRNA assay in a screening context. Given that the modality of primary screening is shifting from cytology to HPV testing - the information presented is timely.
The Norwegian context with its excellent linkage capabilities and history of application of this assay is an appropriate place to deliver this work.
I do have some issues with the article which are listed below

General issues

1) The abstract is too definitive - I do not think that the data do support the use of the mRNA test for primary screening. While the cumulative rate of CIN3+ is presented, the other key measures of whether an assay is suitable for primary screening (including sensitivity) are passed over. Thus, I do not agree with the conclusion that "HPV mRNA testing may be used as a primary screening both for women 25-33 and 34-69"

Our response: Primary HPV DNA testing is less useful in women 25-33 years because of high positivity rate and many transient infections. Our conclusion is based upon the finding that the 5-type HPV mRNA test had similar test performances in both age groups.

2) In addition - one could argue that the responsibility of any screening test is to (within reason) be sensitive. Appropriate triages can then stratify the relevant infections from the noise. While we are not in possession of a perfect triage one could argue that this is not the "fault" of HPV DNA tests and changing the modality to reduce the sensitivity of a screening test may be shortsighted.

Our response: A screening test, which is more sensitive and more specific than cytology is more useful in primary screening than cytology. HPV DNA testing is more sensitive, but less specific than cytology. Cytology in triage of HPV DNA positive women does not solve the problem. The less types that are screened for, the less overdiagnosing/repeated follow-up of women with HPV-types that have low oncogenic potential. HPV-types with a prevalence ratio less than 1 (types in cancer/types in CIN 2-3), are considered candidates for cancer, but have a minor, if any, contribution to the volume of cervical cancer. In our opinion restricting the screening to types -16, -18, -31, -33 and -45 will be the optimal types, preferably with an mRNA-test.

Specific comments

Page 4 - Line 6: I thought according to WHO data cervical cancer is now the fourth most comment female cancer.

Our response: The text and the reference are updated (line 64).

Page 4 - Line 10: "young female population" is vague

Our response: The text is changed (line 67).

Page 4: Line 40-44. Not all cervical cancers have integrated viral forms - between 15-20% will have episomal forms only. Integration is not a requisite for transformation

Our response: The text is now updated (lines 91-2).
Page 4 Line 58: In line with above comment, I am not sure I agree with the logic that a more specific test is appropriate for screening purposes. The data indicate that 3/5 cancers were missed using this approach...

Our response: We have too short observation time to target cancer as outcome. We are expecting updated data within a few years, and hopefully we will have more data on cancers.

Page 5: Line 10 - not sure what is meant by "selected gynaecologists"

Our response: The text is changed to …..women 13-87 years of age visiting gynecologists…..(lines 102-4)

Page 5: Line 30: What exactly do the game clinicians report that the pathology lab do not? Need to get a handle on how representative and comprehensive the data sources are.

Our response: The gynecologists report type of treatment in CIN and cancer. They also report FIGO stadium. This information is merged with the pathology reports. The Norwegian cancer registry receive all these reports through different surveillance systems. Validated data on other cancer forms show a 97-100% completeness of reporting.

Page 6: Line 28: Who were the women with the positive test? women associated with the study only? Presumably the mRNA test results had to be disclosed - a fuller explanation would be helpful here.

Our response: At baseline all women were screened with the mRNA-test. In follow-up different HPV-tests were used, depending upon laboratory reading the cytology slides. After 2005 HPV-testing was used in triage of ASC-US / LSIL. We have added the different HPV-test used. (lines 150-1)

Page 7: Lines 43-48 are hard to understand - consider rephrasing.

Our response: Text is rewritten (lines 191-201)

Page 8: Line 4/5: Sentence beginning "Case numbers" - does not make sense to me

Our response: Text is rewritten (lines 202-9).

Page 9: Line 22 - I don't agree with the "huge administrative challenge" IF (as indicated above) there is a useful triage.

Our response: Triage do not solve all the problems. HPV DNA positive women with a negative triage test still need follow-up. We provide more information on this issue in the revised ms (lines 224-9).

Page 10: Line 10 - The discussion on types in Danish cancers will not necessarily transfer to a global perspective - consider a broader reference?

Our response: In the manuscript there are references to studies regarding CIN3+ in Denmark, US, Germany, Nederland and a European meta-analysis. We also have references regarding HPV-types in cervical cancer in India and Norway.

Page 10: Line 35 - The discussion on triage is off topic

Our response: the text is deleted

Page 11: Lines 13-31 are repetitious with the introduction and are not needed.

Our response: text deleted.

We have to the best of our knowledge responded to the objections/suggestions for editing from the reviewers. We look forward to the editorial decision on our ms.

VERSION 2 – REVIEW

REVIEWER	Kimon Chatzistamatiou 2nd Department of Obstetrics and Gynecology, Hippokrateio Hospital of Thessaloniki, Aristotle University of Thessaloniki, Greece I have had travel expenses and congress registration fees covered by Roche diagnostics.
REVIEW RETURNED	08-Jul-2016

GENERAL COMMENTS	Although I do not agree totally with the views expressed in the submitted article or with the answers given to my comments, I think that the revised version of the manuscript presents the study in a more comprehensive and less biased way. Therefore I recommend acceptance so that your research could be available for the scientific community and initiate a debate on primary cervical cancer screening modalities other than HPV DNA testing, which in my opinion is the optimal one. Besides, as you acknowledge, there is a need for head to head comparison between HPV mRNA and DNA tests in order to clarify this issue.