PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Opportunistic pathology-based screening for diabetes
AUTHORS	Nolan, Christopher; Simpson, Aaron; Krowka, Renata; Kerrigan, Jennifer; Southcott, Emma; Wilson, J; Potter, Julia; Hickman, Peter

VERSION 1 - REVIEW

REVIEWER	Dr Zhong X Lu Chemical Pathologist Melbourne Pathology Australia Competing interests: None.
REVIEW RETURNED	15-Jul-2013

GENERAL COMMENTS	This study has examined the usefulness of opportunistic HbA1c testing for diabetes screening using samples already in the pathology laboratory for other tests in three different groups: community-based, emergency department and inpatients. The manuscript is well written and clearly presented. It provides some evidence for how the scared resources in hospitals could be allocated for maximising detection rate of undiagnosed diabetes in clinical practice.

REVIEWER	Ezekiel Uba Nwose (PhD, CSci, FIBMS, MAIMS) Coordinator, Medical Laboratory Science Charles Darwin University, Darwin AUSTRALIA There is no competing interest
REVIEW RETURNED	15-Jul-2013

THE STUDY	There is no "supplementary document" associated with the manuscript
GENERAL COMMENTS	I would suggest that authors consider to include a statement whether (or how many %) participants identified as undiagnosed diabetes are undergoing diabetes management indicated by regular blood sugar and HbA1c checks tests. Otherwise, a statement of limitation may be good. The manuscript is almost flawless. It dwells on the subject of diabetes screening that I am interested in. I consider the strength of this study to be explanation of real-life diagnostic scenario whereby default confounding factors are considered; compared with other studies. Further demonstration of a role of pathology that has yet to be fully appropriated. A pointer to the healthcare management.

REVIEWER	Prof Garry John Consultant Clinical Biochemist Norfolk and Norwich University Hospital Norwich NR4 7UY UK No competing interests
REVIEW RETURNED	19-Jul-2013

THE STUDY	The authors should state that the analyser was calibrated to the IFCC refefence method (if it was) as that would be required to set targets. The unit mmol/mol represents the SI Unit rather than an IFCC recommended unit.
RESULTS & CONCLUSIONS	I am unclear about the take home message. The study is well designed and performed. The study shows differences in HbA1c between the groups investigated; the study confirms what may have been suspected. But if the study objective is that HbA1c provides a good tool for detecting diabetes in the groups studied, then the lower detection rate needs to be discussed. The authors state that it is a lot lower than seen in other studies (eg AUSDIAB), but this needs to be futher discussion; are patients being missed?
GENERAL COMMENTS	This is a good study, but if the objective is the potential for HbA1c to detect previously unknown diabetes there needs to be more discussion around its predictive value.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Dr Zhong X Lu
Specific:
Introduction:
Page 6, 1st paragraph: NHS in UK, in addition to WHO and ADA, has also endorsed using HbA1c for diagnosis of diabetes. This may worthy mentioning in the introduction.
Response: This is now included and referenced Page 6, 2nd line.

Methods:
Page 6, line 31 & page 7 line 29: ACT Pathology first appears in page 6. Description in the brackets needs to be moved from page 7 to page 6. ACT needs to spell out when it first appears.
Response: The information in brackets “(Canberra, ACT, Australia) has been moved as suggested to page 6. ACT Pathology is the registered trade name- it is not “Australian Capital Territory Pathology”- so we have decided not to spell out ACT here.

More information is required for the description of the unselected participants. The study screened 22,396 samples and finished with 4,050 samples (20.1%) for HbA1c testing. Although exclusion criteria were mentioned, it was unclear from how many people the 22,396 samples were collected nor the breakdown of the unselected participants. This information is required to allow for the assessment possible selection bias.
Response: The 22,396 FBC requests were consecutive during the collection windows. The LabWizard program (Pacific Knowledge Systems, Surry Hills, NSW, Australia) was used daily to filter the requests and remove those from the study that fitted the exclusion criteria such as pregnancy, under 18 years of age, post surgery, or any of the other exclusions mentioned in the paper. The extracted list of samples produced by LabWizard were the ones stored for HbA1c analysis. The LabWizard program, due to the volume of electronic history surveyed, could only produce a list of

samples that fitted the criteria for the study as an output. Unfortunately it did not provide a list of sample/patients that were excluded and why they were removed. For that reason we are unable to provide a more detailed breakdown of those meeting exclusion criteria, for example, how many excluded samples were from individuals under 18 years of age.

Page 8, line 31: Samples were stored at -80°C. The study was conducted between Apr 2010 and Jan 2011. It was unclear how long the samples were stored for before analysis. The stability of frozen samples needs to be mentioned if it was stored for a long duration before analysis.
Response: Samples were not stored for more than 6 months prior to testing. This statement has been added to the text- Page 8, end of second paragraph. Stability of HbA1c store at -80C has been reported out to 10 years (Rolandsson O, Marklund SL, Norberg M, Agren A, Hagg E. Hemoglobin A1c can be analyzed in blood kept frozen at −80°C and is not commonly affected by hemolysis in the general population. Metabolism 2004; 53: 1496-99).

Page 8, line 38: "Biorad Variant II Turbo Analyser (Bio-Rad Laboratories Pty., Ltd., Gladesville, NSW, Australia)" is wrong. The manufacturer, Bio-Rad, is in the United States, not in Australia.
Response: Thank you- This has been corrected (Bio-Rad Laboratories Pty., Ltd., Hercules, CA, USA)

Page 8, line 41: Was the inter-assay CV calculated based on the IFCC (mmol/mol) values or the NGSP (%) values for HbA1c? This needs to be specified.
Response: This is based on the NGSP (%) values- which is now stated in the text.

Page 9, line 17: Is "the variability in the measured HbA1c" a good term? It sounds like the authors are testing difference of HbA1c in analytical aspects. Is there a better term? Are we testing the determinants of HbA1c results?
Response: We have changed this sentence to "…to investigate age and gender determinants of the measured HbA1c in the three patient groups".

Page 9, lines 20 and 24: typo in HbA1c. The letter "c" should be a small letter.
Response: Corrected.

Results:
Page 10, line 31: Typo. 5.5±0.5% (37 ± 0.5% (37± 5 mmol/mol) for ED – should be: 5.5±0.5% (37± 5 mmol/mol)
Response: Corrected.

Page 10, line 53: Could the gender effect on HbA1c be due to difference in the mean glucose levels between men and women in the study, rather than the actual gender effect? Were the mean glucose results different between men and women in the study?
Response: This is a very interesting question. The lower HbA1c levels in females of 0.13% could be due to lower glucose levels in females or due to other factors such as differences in haemoglobin metabolism. We suspect that lower glucose levels in females are likely based on lower rates of diabetes in females diagnosed by OGTTs compared to males in epidemiological studies of diabetes in Australia (e.g. the AUSDIAB study). We have not added anything further to the text, as this issue is beyond the scope of this manuscript.

Page 11, line 8: The prevalence of previously undiagnosed diabetes was lowest at 0% in the CB group less than 40 years of age ---. Is the 0% right? Response: If this is right, the sentence may need to be rewritten for clarity.
This is correct and the sentence has been altered for clarity.

Discussion:
Page 13, line 27: "---random plasma glucose >5.5 mmol/L, --- rather those most likely to have diabetes." The work "most likely" needs to be modified. Selecting people with random plasma glucose of >5.5 mmol/L for HbA1c testing may increase the positive rate for diabetes but people with random plasma glucose >5.5 mmol/L are not most likely to have diabetes.
Response: Thank you- this has been corrected.

Figure legend:

Page 21 lines 17 and 20: Two comas (,) are not necessary. Line 24: 12/12-12 months – unclear what this means.
Response: 2 commas have been removed. The figure has been changed with year replacing 12/12. Thus 12/12 no longer needs to be defined in the legend.

Figure 1:
3rd raw of boxes: 12/12 – unclear what this means.
Response: This has been altered to improve clarity (12/12 replaced by year)

Reviewer: Ezekiel Uba Nwose (PhD, CSci, FIBMS, MAIMS)

I would suggest that authors consider to include a statement whether (or how many %) participants identified as undiagnosed diabetes are undergoing diabetes management indicated by regular blood sugar and HbA1c checks tests. Otherwise, a statement of limitation may be good.
Response: A sentence has been added (page 13- new 2nd paragraph) stating that the family doctors were notified of new diagnoses of diabetes.

Reviewer: Prof Garry John

The authors should state that the analyser was calibrated to the IFCC refefence method (if it was) as that would be required to set targets.
Response: We are able to confirm that the method is calibrated to the IFCC reference method.

The unit mmol/mol represents the SI Unit rather than an IFCC recommended unit.
Response: This sentence has been altered according to this comment- Page 9 first paragraph.

I am unclear about the take home message. The study is well designed and performed. The study shows differences in HbA1c between the groups investigated; the study confirms what may have been suspected.
Response: The sentence “This method of diabetes screening warrants further consideration” has been added at the end of the discussion as a take home message.

But if the study objective is that HbA1c provides a good tool for detecting diabetes in the groups studied, then the lower detection rate needs to be discussed. The authors state that it is a lot lower than seen in other studies (eg AUSDIAB), but this needs to be futher discussion; are patients being missed?
Response: We expect that the lower detection rate is due to (1) much greater efforts to determine if the diabetes had previously been diagnosed than in other similar studies, and (2) a high rate of prior screening of diabetes in subjects having blood tests in the community setting (i.e. family doctors are doing a good job). The fact that they are attending for a blood test means that they are attending a doctor- as opposed to persons who are not engaged in the health care system who are more likely to have previously undiagnosed diabetes. These points are already made in the discussion.

This is a good study, but if the objective is the potential for HbA1c to detect previously unknown diabetes there needs to be more discussion around its predictive value.
Response: A statement relating to a predictive value is now made in the new second paragraph of page 13.