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](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

<table>
<thead>
<tr>
<th>TITLE (PROVISIONAL)</th>
<th>Predicting late-onset sepsis by routine neonatal screening for colonization by gram-negative bacteria in neonates at intensive care units: protocol for a systematic review</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUTHORS</td>
<td>Harder, Thomas; Seidel, Juliane; Eckmanns, Tim; Weiss, Bettina; Haller, Sebastian</td>
</tr>
</tbody>
</table>

### VERSION 1 - REVIEW

| REVIEWER                        | Prof. Bernhard Resch  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medical University of Graz. Austria</td>
</tr>
<tr>
<td>REVIEW RETURNED</td>
<td>25-Nov-2016</td>
</tr>
</tbody>
</table>

| GENERAL COMMENTS | Review  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manuscript ID bmjopen-2016-014986</td>
</tr>
<tr>
<td></td>
<td>The authors present a protocol for a systematic review investigating the value of routine screening for colonization by gram-negative bacteria in neonates for sepsis prediction.</td>
</tr>
</tbody>
</table>
|                  | Major concerns  
|                  | Methodology is clearly described and interesting to read, even if the topic of a systematic review of the literature is well-known. |
|                  | In the abstract the authors mention to include infants up to an age of 12 months. This is confusing as far as the aim of the planned study is screening for colonization by gram-negative bacteria in neonates for sepsis prediction. And this is limited to a corrected for prematurity age of 28 days. Please clarify. |
|                  | Otherwise no major concerns arised.  
|                  | Minor concerns  
|                  | Page 7, line 10: Confounding: Confounding will occur if interventions are delivered to study participants… Please correct the sentence. |

| REVIEWER                        | Irija Lutsar  
|---------------------------------|----------------------------------------------------------|
|                                 | University of Tartu  
|                                 | Estonia                                                    |
| REVIEW RETURNED                 | 02-Jan-2017                                              |

| GENERAL COMMENTS | The manuscript describes a protocol to evaluate the value of screening for mucosal colonisation by Gram negative microorganisms in neonates. If my understanding is correct the authors by doing systematic review aim to demonstrate whether mucosal screening is meaningful or not in predicting neonatal sepsis. I do agree with the authors that several studies have been conducted but no systematic reviews have been performed. Although I do believe that such review would be useful I still have some comments to authors. |


1. The aim is to look at the relationship between colonization by Gram negative microorganisms and development of neonatal sepsis. Gram-negative microorganisms, however, are part of normal microflora and are common colonisers of the mucosal surfaces. In other words Gram negative colonisation is not necessarily a negative phenomenon. Furthermore, using the term of Gram negative is a very high level term. For example the baby could be colonised with E.coli but get an infection with Klebsiella spp., both are Gram negative organisms. Having just Gram negative colonisation and Gram negative sepsis does not mean that these two are related or that one predicts the other. The authors should do their analysis on species level at least.

2. Furthermore the authors are suggested to restrict their analysis or at least do sub-analysis on species that are clinically relevant or difficult to treat. For example, colonisation by non-fermentative microorganisms, or colonisation by antibiotic resistant species (ESBL, MDR microorganisms etc.). I believe that there is sufficient amount of evidence that screening just for any Gram negative microorganism has very low specificity and sensitivity for several reasons as mentioned above.

3. The authors are suggested to distinguish between prospective studies designed specifically to evaluate the value of colonisation cultures and retrospective studies.

4. The authors use the cut-off value for prematurity of <37 weeks. This may be too high cut-off as majority of infections occur in profoundly premature babies. I would suggest of using a cut-off of 32 weeks and/or 1500g as well.

5. The authors are suggested to have clear definition for type of ward as they may have include studies from very different units.

6. The authors propose the age bracket <1 years. This, however, could be mixed population. If looking at specific organisms (see point 2) this is likely not an issue if, however, looking at the Gram negative colonisation this may be a problem as feeding habits and level of maturity are very different.

REVIEWER
Matthew Bizzarro
Yale University School of Medicine
United States of America

REVIEW RETURNED
07-Jan-2017

GENERAL COMMENTS
The authors present a proposed protocol for a systematic review aimed at assessing the value of routine screening for Gram-negative rod colonization as a predictor for late-onset sepsis. The methodology, particularly with respect to systematic reviews of prognostic tests (and how they differ from diagnostic tests), is well described.

Comments/inquiries:
1. The major comment that I have with respect to this proposal is whether or not the topic is actually a relevant one in 2017. While some NICUs still routinely screen for antimicrobial resistant organisms (e.g. MRSA, VRE), it is typically done to facilitate cohorting and isolation and not necessarily as a method for predicting late-onset sepsis. Furthermore, given that we know that most infants in NICU are eventually colonized with gram-negative rods, routine screening for gram-negative rod colonization is no longer standard practice in most institutions (at least not in the United States). This is supported by the fact that the references
provided by the authors cite studies from 1978-1992, with the most recent (Dobson et al) titled, "Reduced use of surface cultures for suspected neonatal sepsis and surveillance." I would therefore ask whether or not the authors truly feel that this is a relevant subject worth analyzing.

2. The authors list that their main objective is "to assess the usefulness and value of routine screening for colonization by gram-negative bacteria performed in the NICUs as predictive measures for sepsis." The title of the manuscript suggests that the authors only wish to focus on late-onset sepsis. I would therefore suggest changing the wording of the objective and the rest of the manuscript to reflect this more specific terminology (i.e. change sepsis to late-onset sepsis). I also assume that the authors wish to review whether or not gram-negative rod colonization is a predictor for gram-negative rod sepsis (i.e. that the same organism detected on surveillance culture is the one responsible for the late-onset sepsis episode) and not sepsis from any and all organisms, correct? If so, I would also make that distinction.

3. I might suggest specifying in the section "Participants" that "Studies that include infants up to an age of 12 months who are still in a neonatal intensive care unit will be considered..."

4. The authors might consider including other potential confounders for late-onset sepsis (if available) in their "Patient/population characteristics" such as presence of comorbidities (like NEC), central line use, and need for surgery.

5. In the "Subgroup analysis" the authors may wish to select additional gestational age and birth weight categories such as: <26 weeks, 26-29 weeks, 30-32 weeks... and <750 grams, 751-1000 grams, 1000-2500 grams, >2500 grams. I know much of this will be dependent on individual study inclusion criteria, but defining a preterm cohort as <37 weeks includes a very broad and heterogeneous population, particularly as it pertains to risk for late-onset infection.

6. I think the authors should consider removing Table 1 as I think there is adequate comparison of prognostic and diagnostic testing and analysis in the manuscript and in Figure 1.

7. Do the authors wish to include the term "Gram-negative" in their search strategy (Table 2)?

8. In Table 3, there are several references to the "reference standard". What is the "reference standard" as it pertains to this review?

9. The only aspect of the PRISMA checklist that I believe has not been outlined in the process for data management. The authors may wish to include this.

10. There are several grammatical errors in the "Introduction" that should be addressed.
Reviewer: 1
Comment 1: Methodology is clearly described and interesting to read, even if the topic of a systematic review of the literature is well-known. In the abstract the authors mention to include infants up to an age of 12 months. This is confusing as far as the aim of the planned study is screening for colonization by gram-negative bacteria in neonates for sepsis prediction. And this is limited to a corrected for prematurity age of 28 days. Please clarify. Otherwise no major concerns arised.
Answer 1: Thank you for this comment. Following the suggestions by Reviewer 3, we did not change this item (see Comment 3 by Reviewer 3).

Comment 2: Minor concerns: Page 7, line 10: Confounding: Confounding will occur if, , interventions are delivered to study participants… Please correct the sentence.
Answer 2: We corrected the sentence.

Reviewer: 2
Comment 1: The aim is to look at the relationship between colonisation by Gram negative microorganisms and development of neonatal sepsis. Gram-negative microorganisms, however, are part of normal microflora and are common colonisers of the mucosal surfaces. In other words Gram negative colonisation is not necessarily a negative phenomenon. Furthermore, using the term of Gram negative is very high level term. For example the baby could be colonised with E.coli but get an infection with Klebsiella spp., both are Gram negative organisms. Having just Gram negative colonisation and Gram negative sepsis does not mean that these two are related or that one predicts the other. The authors should do their analysis on species level the least.
Answer 1: Thank you for this important comment. Of course, for all studies that provide respective data, the analysis will be made at the species level. In those cases where this is not possible, we will critically discuss the limitations (see page 13).

Comment 2: Furthermore the authors are suggested to restrict their analysis or at least do sub-analysis on species that are clinically relevant or difficult to treat. For example, colonisation by non-fermentative microorganisms, or colonisation by antibiotic resistant species (ESBL, MDR microorganisms etc.). I believe that there is sufficient amount of evidence that screening just for any Gram negative microorganism has very low specificity and sensitivity for several reasons as mentioned above.
Answer 2: As suggested, we will perform subgroup analyses restricted to those multidrug-resistant species or species that are difficult to treat. A respective sentence was added to the paper (page 12).

Comment 3: The authors are suggested to distinguish between prospective studies designed specifically to evaluate the value of colonization cultures and retrospective studies.
Answer 3: Thank you for this comment. In our systematic review, studies will be stratified according to study design (page 12).

Comment 4: The authors use the cut-off value for prematurity of <37 weeks. This may be too high cut-off as majority of infections occur in profoundly premature babies. I would suggest of using a cut-off of 32 weeks and/or 1500g as well.
Answer 4: To account for all possible definitions in the included studies, we will primarily include all studies and subsequently stratify their results according to different cut-offs for gestational age and birth weight (page 12).

Comment 5: The authors are suggested to have clear definition for type of ward as they may have include studies from very different units.
Answer 5: Thank you for this comment. We added “type of ward” to the list of variables for subgroup analyses (page 13).
Comment 6: The authors propose the age bracket <1 years. This, however, could be mixed population. If looking at specific organisms (see point 2) this is likely not an issue if, however, looking at the Gram negative colonisation this may be a problem as feeding habits and level of maturity are very different.

Answer 6: Please see our answer to comment 1 by reviewer 1.

Reviewer: 3

Comment 1: The major comment that I have with respect to this proposal is whether or not the topic is actually a relevant one in 2017. While some NICUs still routinely screen for antimicrobial resistant organisms (e.g. MRSA, VRE), it is typically done to facilitate cohorting and isolation and not necessarily as a method for predicting late-onset sepsis. Furthermore, given that we know that most infants in NICU are eventually colonized with gram-negative rods, routine screening for gram-negative rod colonization is no longer standard practice in most institutions (at least not in the United States). This is supported by the fact that the references provided by the authors cite studies from 1978-1992, with the most recent (Dobson et al) titled, "Reduced use of surface cultures for suspected neonatal sepsis and surveillance." I would therefore ask whether or not the authors truly feel that this is a relevant subject worth analyzing.

Answer 1: Thank you for this comment. While we are aware of the fact that routine screening for gram-negative colonization is not standard practice in many institutions, it is still recommended e.g. in Germany by the German Committee on Hospital Infections and Hygiene (KRINKO). Therefore, an important goal of our systematic review will be to assess the published evidence on this topic and report the results to the Committee. We added a respective explanation to the Introduction section of the paper (page 4).

Comment 2: The authors list that their main objective is "to assess the usefulness and value of routine screening for colonization by gram-negative bacteria performed in the NICUs as predictive measures for sepsis." The title of the manuscript suggests that the authors only wish to focus on late-onset sepsis. I would therefore suggest changing the wording of the objective and the rest of the manuscript to reflect this more specific terminology (i.e. change sepsis to late-onset sepsis). I also assume that the authors wish to review whether or not gram-negative rod colonization is a predictor for gram-negative rod sepsis (i.e. that the same organism detected on surveillance culture is the one responsible for the late-onset sepsis episode) and not sepsis from any and all organisms, correct? If so, I would also make that distinction.

Answer 2: We changed “sepsis” to “late-onset sepsis” throughout the text. Regarding the second part of the question (specific organism), please see our answer to comment 1 by Reviewer 2.

Comment 3: I might suggest specifying in the section “Participants” that “Studies that include infants up to an age of 12 months who are still in a neonatal intensive care unit will be considered...”

Answer 3: Thank you for this suggestion. We changed the respective paragraph accordingly (page 8).

Comment 4: The authors might consider including other potential confounders for late-onset sepsis (if available) in their “Patient/population characteristics” such as presence of comorbidities (like NEC), central line use, and need for surgery.

Answer 4: We added these potential confounders to the list (pages 10-11).

Comment 5: In the "Subgroup analysis" the authors may wish to select additional gestational age and birth weight categories such as: <26 weeks, 26-29 weeks, 30-32 weeks... and <750 grams, 751-1000 grams, 1000-1500 grams, 1500-2500 grams, >2500 grams. I know much of this will be dependent on individual study inclusion criteria, but defining a preterm cohort as <37 weeks includes a very broad and heterogeneous population, particularly as it pertains to risk for late-onset infection.

Answer 5: Thank you for this suggestion. We changed the respective paragraph accordingly (page
12).

Comment 6: I think the authors should consider removing Table 1 as I think there is adequate comparison of prognostic and diagnostic testing and analysis in the manuscript and in Figure 1.
Answer 6: We removed Table 1.

Comment 7: Do the authors wish to include the term "Gram-negative" in their search strategy (Table 2)?
Answer 7: Although it might appear plausible to include the term "gram-negative" in the search strategy, we decided to make the strategy as broad as possible to minimize the chance of missing publications that did not use this phrase.

Comment 8: In Table 3, there are several references to the "reference standard". What is the "reference standard" as it pertains to this review?
Answer 8: As explained in the Introduction section (page 5, lines 1-4), in prognostic accuracy studies the reference standard is substituted by a (clinical) outcome. We added a footnote to Table 2 to clarify this in the context of the risk of bias tool.

Comment 9: The only aspect of the PRISMA checklist that I believe has not been outlined in the process for data management. The authors may wish to include this.
Answer 9: Thank you for this comment. We addressed this issue in the protocol (page 10) and changed the PRISMA checklist accordingly.

Comment 10: There are several grammatical errors in the "Introduction" that should be addressed.
Answer 10: We corrected the grammatical errors.

---

**VERSION 2 – REVIEW**

| REVIEWER          | Prof. Dr. Bernhard Resch  
|                  | Medical University of Graz, Austria |
| REVIEW RETURNED | 05-Feb-2017 |

**GENERAL COMMENTS**  
Revision fulfills all concerns raised, thus, I endorse acceptance.

| REVIEWER          | Matthew Bizzarro  
|                  | Yale University School of Medicine  
|                  | New Haven, CT USA |
| REVIEW RETURNED | 07-Feb-2017 |

**GENERAL COMMENTS**  
The authors have clearly and sufficiently addressed all of my comments/questions from my initial review.
Predicting late-onset sepsis by routine neonatal screening for colonisation by gram-negative bacteria in neonates at intensive care units: a protocol for a systematic review

Thomas Harder, Juliane Seidel, Tim Eckmanns, Bettina Weiss and Sebastian Haller

BMJ Open 2017 7:
doi: 10.1136/bmjopen-2016-014986

Updated information and services can be found at:
http://bmjopen.bmj.com/content/7/3/e014986

These include:

References
This article cites 28 articles, 8 of which you can access for free at:
http://bmjopen.bmj.com/content/7/3/e014986#BIBL

Open Access
This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

Epidemiology (2139)
Infectious diseases (576)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/