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## Diagnostic accuracy of serum (1,3)-beta-d-glucan for neonatal invasive candidiasis: systematic review and meta-analysis

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# 1           **Diagnostic accuracy of serum (1,3)-Beta-D-Glucan for neonatal invasive** 2           **candidiasis: Systematic review and meta-analysis**

3  
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29  
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**Figures:** 3

32 **ABSTRACT (300/300)**

33 **Background:** Neonatal invasive candidiasis (NIC) is a leading cause of infection-related morbidity and  
34 mortality in preterm neonates. Several studies have shown that (1,3)-Beta-D-Glucan (BDG) was accurate  
35 in detecting invasive fungal infection in adults, but studies in neonates are scarce.

36 **Objectives:** To obtain summary estimates of the accuracy of BDG detection in serum for the diagnosis of  
37 NIC.

38 **Data sources:** We searched Medline, Embase, Clinicaltrials.gov, and Google Scholar (inception to July  
39 2019). We checked the reference lists of included studies, clinical guidelines, and review articles.

40 **Study eligibility criteria:** We included studies that assessed the accuracy of BDG against a reference  
41 standard that defined groups of patients with ordinal levels of NIC probability (e.g., proven, probable,  
42 possible) and included fungal blood culture.

43 **Participants:** Neonates suspected of having NIC.

44 **Interventions:** BDG measurement in serum (Fungitell® assay).

45 **Methods:** We assessed risk of bias and applicability using QUADAS-2. We used bivariate meta-analysis to  
46 produce summary estimates of diagnostic accuracy at prespecified positivity thresholds of 80 and 120  
47 pg/ml. This study was registered with PROSPERO (CRD42018089545).

48 **Results:** We included eight studies (465 participants). Of these, two were judged at low overall risk of  
49 bias. There was substantial variability across studies in the reference standards used. At a positivity  
50 threshold of 80 pg/ml, summary estimates of sensitivity and specificity of BDG were 89% (95% CI: 80% -  
51 94%) and 60% (53% - 66%), respectively; summary sensitivity for detecting proven cases of NIC was 99%  
52 (93% - 100%). At a positivity threshold of 120 pg/ml, summary estimates of sensitivity and specificity  
53 were 81% (71% - 88%) and 80% (67% - 88%), respectively.

54 **Conclusions:** Because of high sensitivity, BDG seems promising to rule-out NIC. It might be too early to  
55 recommend its use because of the scarcity of reliable clinical data, heterogeneity in case definitions, and  
56 unstable accuracy estimates.

57 **Abbreviations**

58 BDG: (1,3)-Beta-D-Glucan

59 CRP: C-reactive protein

60 NIC: Neonatal invasive candidiasis

61 IQR: Interquartile range

62 EORTC/MSG: European Organization for Research and Treatment of Cancer/Mycoses Study Group

63 95CI: 95% confidence interval

64 BW: Birth weight

65 ROC: Receiver operating characteristic

66 WG: Weeks of gestation

67

68 **Keywords:** Neonatal invasive candidiasis; (1,3)-beta-D-glucan; diagnostic tests; sensitivity; specificity;

69 neonates; systematic review; meta-analysis.

70

71 **Running title:** (1,3)-Beta-D-Glucan to detect neonatal invasive candidiasis

72

73 **Previous presentation:** Preliminary results of this work were presented in part at the 36<sup>th</sup> Annual

74 Meeting of the European Society for Paediatric Infectious Diseases (ESPID), Malmö, Sweden, May 2018

75 (E-poster ESP18-0223).

## 76 INTRODUCTION

77 Neonatal invasive candidiasis (NIC) is one of the leading causes of sepsis in neonatal intensive care units.  
78 *Candida* species are the third most common infectious agents isolated in late-onset neonatal sepsis (1),  
79 with *C. albicans* and *C. parapsilosis* accounting for 80 to 90% of neonatal fungal infections (2). In the USA,  
80 the incidence of NIC varies between 3 and 10 % for very low birth weight neonates (VLBW; 1000 – 1500  
81 g) and between 6 and 20% for extremely low birth weight neonates (ELBW; <1000 g) (1, 3). NIC is a  
82 severe illness, with a case-fatality rate up to 30% (4), and carries an additional risk of subsequent  
83 neurodevelopmental impairment due to *Candida* spp. meningoencephalitis (4). The significant decrease  
84 of NIC incidence since the end of the 2000s is partly explained by the identification and control of its  
85 modifiable risk factors (5), such as administration of broad-spectrum antibiotics, presence of a central  
86 venous catheter, exclusive parenteral nutrition, and mechanical ventilation (6).

87 The diagnosis of NIC remains difficult. Only 40% of neonates suspected of NIC have fever, and other  
88 clinical manifestations of neonatal sepsis such as tachycardia, poor perfusion, respiratory distress,  
89 feeding difficulties, and jaundice, are insufficiently specific (7). Laboratory findings such as  
90 thrombocytopenia, leukocytosis, and elevated C-reactive protein (CRP) levels are not specific for  
91 candidiasis (7-9). NIC can only be proven by culturing *Candida* spp. from a normally sterile site, e.g.,  
92 blood or cerebrospinal fluid samples (10), but the sensitivity of fungal blood culture may vary from 21 to  
93 71%, as suggested by autopsy-proven invasive candidiasis studies in adults (11). The sensitivity of blood  
94 culture is even lower in neonates because the blood sample obtained for culture is often less than 1 ml  
95 (12). Moreover, the delay in obtaining a positive culture result might be considered too long, usually 2 to  
96 5 days, to enable early diagnosis and treatment (13). Delays in instituting appropriate antifungal therapy  
97 may lead to an increase in morbidity and mortality (14). There is no single gold standard for determining  
98 the presence or absence of NIC. Therefore, most clinicians and researchers rely on risk stratification

99 systems such as European Organization for Research and Treatment of Cancer/Mycoses Study Group  
100 (EORTC/MSG) criteria, which grade the diagnosis of invasive fungal infection with three levels of  
101 probability (proven, probable, and possible).

102 New biomarkers would be helpful for decision-making in neonates with sepsis. PCR based-assays for  
103 *Candida* have shown promising results, but clinical studies in neonates are scarce (15, 16). Serum (1,3)-  
104 Beta-D-Glucan (BDG), a cell wall component of most pathogenic fungi, has been introduced in the  
105 revised version of EORTC/MSG diagnostic criteria for invasive candidiasis in 2008 (10). Presence of BDG  
106 in the serum (Fungitell® > 80 ng/ml) allows the clinician to classify the patient as having “probable”  
107 instead of “possible” systemic fungal infection. Results of serum BDG levels can be obtained in less than  
108 24 hours, and the amount of blood needed for the measurement of BDG is acceptable for neonates (<  
109 100 µL).

110 In a recent meta-analysis in adults (17), BDG showed a summary sensitivity and specificity for detecting  
111 invasive fungal infection of 80% (95CI: 77% - 82%) and 82% (81% - 83%), respectively. BDG seems able to  
112 rule out invasive fungal infection in children and adults (18-20), but there is limited data for its use in  
113 neonates, and a diagnostic accuracy systematic review and meta-analysis is lacking. The accuracy of BDG  
114 deserves being investigated in neonates because of specific risk factors and epidemiology of *Candida*  
115 infections, and higher rates of dissemination and end-organ damage in this population (21). This study  
116 aimed to determine the diagnostic accuracy of BDG in NIC.

117

## 118 **METHODS**

119 This systematic review was reported following the *Preferred Reporting Items for a Systematic Review and*  
120 *Meta-analysis of Diagnostic Test Accuracy Studies* statement (**Appendix 1**) (22). This study was registered

121 with PROSPERO (CRD42018089545).

122

### 123 **Eligibility criteria**

124 We included published and unpublished studies which compared BDG (Fungitell® assay, Associates of  
125 Cape Cod, Inc., Falmouth, MA) with any reference standard that defined groups of patients with ordinal  
126 certainty of NIC (e.g., EORTC/MSG criteria) and included fungal blood culture. We included prospective  
127 and retrospective cross-sectional studies, randomised controlled trials, and case-control studies. We  
128 excluded studies in which another diagnostic kit than Fungitell® (e.g., GKT®) was evaluated, and case  
129 series with less than 10 participants. We included only studies reported in English, French, and Spanish.

130

### 131 **Literature search strategy**

132 The search strategy was developed in consultation with an information specialist (RS). We searched  
133 Medline via Pubmed using the search strategy described in **Appendix 2**. The search was adapted to  
134 search Embase via Ovid. We did not use methodological filters to identify diagnostic studies because  
135 such filters may result in the omission of relevant studies (23), but used the Cochrane Neonatal group's  
136 standard search strategy for the neonatal population (available at neonatal.cochrane.org). We hand-  
137 searched reference lists of included studies, of guidelines for the management of candidiasis, and of  
138 previous reviews about BDG (17, 19, 24-27). We also searched for eligible articles using the "similar  
139 articles" function in PubMed (20 first related articles of each included article). We used Google Scholar to  
140 search for newer reports citing included articles. Conference abstracts and proceedings listed in Embase  
141 were included in our screening. Furthermore, one author (JFC) additionally searched ClinicalTrials.gov  
142 and browsed through all available issues of the Fungitell® Bulletin

143 ([www.acciusa.com/clinical/fungitell/Fungitell\\_Bullitens.html](http://www.acciusa.com/clinical/fungitell/Fungitell_Bullitens.html)), a quarterly newsletter from the test  
144 manufacturer (March 2010 until March 2019).

145

#### 146 **Study selection**

147 First, two review authors (AO, JFC) independently excluded studies that were not related to NIC or BDG  
148 on the basis of the titles and abstracts identified by the search strategy. Then, two review authors (AO,  
149 JFC) retrieved the full text of relevant articles and independently evaluated them for inclusion using  
150 predetermined inclusion criteria.

151

#### 152 **Data extraction and management**

153 For each included study, the following set of data was extracted using a pro forma: first author, year of  
154 publication or presentation, inclusion criteria, study setting, study design, reference standard used,  
155 number of participants, participant characteristics, threshold values for BDG investigated in the study  
156 report, data needed to assess methodological quality, and numbers to construct 2 x 2 tables (number of  
157 true positives, true negatives, false positives, and false negatives) at predefined positivity thresholds of  
158 80 pg/ml (as recommended by the manufacturer) and 120 pg/ml. When study authors did not provide  
159 such numbers, we either calculated these numbers based on the reported estimates of sensitivity and  
160 specificity, or attempted to contact the authors to obtain additional information. According to the clinical  
161 and biological data available for each study, we reclassified participants according to the following three  
162 risk categories: proven NIC, probable NIC, and possible or excluded NIC. Data extraction was performed  
163 by one author (AO) and checked by a second (JT). One review author (JFC) acted as arbiter in case of  
164 discrepancies.



165

166 **Assessment of methodological quality**

167 Methodological quality assessment involved the use of the *Quality Assessment of Diagnostic Accuracy*  
168 *Studies-2* (QUADAS-2) tool (28). For each study, we scored risk of bias and concerns regarding  
169 applicability according to the four domains of QUADAS-2 (i.e., patient selection, index test, reference  
170 standard, and flow and timing).

171

172 **Statistical analysis and data synthesis**

173 Based on data from the 2 x 2 tables, we displayed estimates of sensitivity and specificity on forest plots  
174 and in the receiver-operating characteristic (ROC) space to represent the variability in diagnostic test  
175 accuracy within and between studies. We used Stata/SE version 13 (StataCorp, College Station, TX) to fit  
176 the hierarchical bivariate model (29), which allows for calculating summary estimates of sensitivity and  
177 specificity and the associated 95% confidence intervals, at prespecified positivity thresholds of 80 pg/ml  
178 and 120 pg/ml.

179 In our main analysis, participants with proven and probable NIC were considered as having NIC  
180 (reference standard positives), while participants with possible or excluded NIC were considered as not  
181 having NIC (reference standard negatives). In an additional analysis, we estimated the sensitivity of BDG  
182 to detect proven NIC cases, at a prespecified threshold of 80 pg/ml. We also carried out a sensitivity  
183 analysis by including only studies judged at low risk of bias.

184

185 **RESULTS**

186 **Results of the search**

187 The database literature search was performed on July 25, 2019 (last update). A chart displaying the flow  
188 of studies through the review appears in **Figure 1**. Searching in Medline and Embase identified 1697  
189 unique records. We excluded a total of 1674 reports on the basis of their title, abstract or both. After  
190 evaluating the full text of 23 studies, we excluded 18 (**Appendix 3**). Additional searches allowed us to  
191 include three other studies (30-32). Overall, we included eight studies in our systematic review and  
192 meta-analysis (16, 30-36).

193

194 **Characteristics of included studies**

195 **Table 1** summarises the main characteristics of the included studies. Four originated from France, one  
196 from Italy, one from Spain, one from South Africa, and one from Egypt. Five of them were retrospective  
197 (and among them, two were case–control studies), one was a single centre prospective study, and two  
198 were multicenter prospective cohort studies. A total of 465 participants were included across studies  
199 (range 13 to 155), with a median gestational age of 30 weeks of gestation (WG) (range 27 to 31.7) and a  
200 median birth weight of 1027.5 g (range 991.1 to 1811.4). Inclusion criteria varied (see detailed study  
201 characteristics in **Appendix 4**). Each author used case definitions derived from EORTC/MSG criteria (10),  
202 but there was substantial heterogeneity in risk stratification systems from a study to another; only the  
203 definition of proven cases was homogeneous (i.e., *Candida*-positive culture from a normally sterile site;  
204 see **Table 2**). Authors used various rules combining host, clinical and mycological criteria to define NIC  
205 status. Some of them used additional biological criteria such as CRP level, leukocytosis, and platelet  
206 count (33, 35). NIC risk factors and colonisation by *Candida* spp. were often taken into consideration

207 when defining probable NIC (31-35). The number of proven NIC cases ranged from 4 to 15 per study, for  
208 a total of 60.

209

### 210 **Methodological quality of included studies**

211 **Table 3** summarises quality assessment of the studies included in the review (see detailed quality  
212 assessment in **Appendix 4**). Two of the studies were judged as being of low overall risk of bias ( $\geq 3$   
213 QUADAS-2 items) (31, 34). Five studies avoided clinical selection of participants and therefore included a  
214 representative spectrum of patients. Interpretation of the results of the reference standard was made  
215 with blinding of the result of the BDG in 3 of 8 studies. There was low concern about applicability for  
216 patient selection, the index test, and the reference standard, except for the two case-control studies  
217 (30, 36).

218

### 219 **Summary estimates of accuracy**

220 The meta-analysis included eight studies encompassing 465 participants in total. At an 80 pg/ml  
221 positivity threshold, sensitivity varied from 75% to 100% and specificity from 47% to 100% (**Figure 2A**);  
222 bivariate meta-analysis showed a summary sensitivity of 89% (80% - 94%) and a summary specificity of  
223 60% (54% - 66%) (**Figure 3A**). At a 120 pg/ml positivity threshold, sensitivity varied from 65% to 100%  
224 and specificity from 66% to 100% (**Figure 2B**); the summary estimates of sensitivity and specificity of BDG  
225 were 81% (71% - 88%) and 80% (67% - 88%), respectively (**Figure 3B**).

226

### 227 **Additional analyses**

228 When restricting the meta-analysis to patients with proven NIC, and using a positivity threshold of 80  
229 pg/ml, BDG had a summary sensitivity of 99% (93% - 100%); only 2 out of 60 cases of proven NIC would  
230 have been missed by BDG (**Appendix 5**).

231 We initially planned a sensitivity analysis restricted to studies judged at low risk of bias in at least three  
232 QUADAS-2 domains, but only two studies fulfilled this quality criterion. We investigated the impact of  
233 study quality by restricting the meta-analysis to non-case-control studies. Compared with the overall  
234 results (summary sensitivity and specificity of 89% and 60%, respectively), at a threshold of 80 pg/ml,  
235 sensitivity was lower and specificity was stable when considering exclusively the six non-case-control  
236 studies (summary sensitivity and specificity of 85% and 59%, respectively).

237

## 238 **DISCUSSION**

### 239 **Main findings**

240 NIC is a serious illness for which diagnosis is particularly difficult. In this meta-analysis, including eight  
241 studies encompassing 465 patients, summary sensitivity and specificity of BDG were 89% (80% - 94%)  
242 and 60% (54% - 66%) at an 80 pg/ml threshold, and 81% (71% - 88%) and 80% (67% - 88%) at a 120 pg/ml  
243 threshold. There were substantial variations in inclusion criteria and risk stratification systems across  
244 studies. In an additional analysis, we found that BDG was able to detect 99% of proven NIC cases when  
245 using an 80 pg/ml positivity threshold. In non-case-control studies, the sensitivity of BDG was lower, and  
246 specificity was stable (85% and 59%, respectively).

247

### 248 **Comparison with previous findings**

249 The accuracy of BDG for detecting invasive fungal infection was studied in pediatric patients with  
250 oncologic and hematologic malignancies (18). Using an 80 pg/ml threshold, the sensitivity and specificity  
251 of BDG were 42% and 71%, respectively. Thus, BDG seems less sensitive in this type of patients than in  
252 neonates. Guitard *et al.* explained this lower sensitivity by the frequent use of empiric and pre-emptive  
253 echinocandins prior to BDG measurement. The authors hypothesised that echinocandins might cause  
254 false-negative BDG results by inhibiting the activity of enzyme BDG-synthase (18).

255 In adults, two recent systematic reviews with meta-analysis evaluated the diagnostic accuracy of BDG for  
256 detecting invasive fungal infection. At an 80 pg/ml threshold, sensitivity and specificity were 77% and  
257 85% (19), respectively, and 78% and 81% (24). Again, BDG seems more sensitive in neonates, at the  
258 expense of specificity. False-positive BDG results could be explained by cellulose-containing membranes  
259 used in hemodialysis, surgical glucan-containing gauzes, administration of human blood products such as  
260 albumin and intravenous immunoglobulins, treatment with certain antibiotics such as piperacillin-  
261 tazobactam, and glucan-containing test tubes used the laboratory (11, 37). Of note, no false positivity  
262 related to the preterm neonatal status *per se* has been reported.

263 Three studies that evaluated BDG in neonates were excluded from this review because they didn't use  
264 the Fungitell® assay but a different commercial kit (i.e., GKT® and Dynamiker Fungus® assays)(38-40).  
265 Reported positivity thresholds for the GKT® assay were lower than for Fungitell® (14 pg/ml for Liu *et al.*  
266 and 10 pg/ml for Zhao *et al.*). Sensitivity and specificity of the GKT® assay were 75% and 91%,  
267 respectively, in Liu's study, and 68% and 75%, respectively, in Zhao's. The reported threshold for the  
268 Dynamiker fungus® assay was 99 pg/ml, which seems in the same range than that of Fungitell®; its  
269 sensitivity and specificity were 64% and 95 %, respectively (40).

270

271 **Strengths and limitations**

272 In this review, two reviewers independently performed study selection, but data extraction and quality  
273 assessment was performed by one reviewer and checked by a second. Studies included in this review are  
274 not exempt from methodological weaknesses, and results should be considered with caution. Also, the  
275 number of studies (n = 8) and participants (n = 465) are rather low. This led to imprecision in meta-  
276 analysis summary estimates and impeded further analysis of potential sources of heterogeneity between  
277 studies, for example by use of meta-regression. Also, because of the lack of reported data, we were only  
278 able to analyse the accuracy of BDG at two prespecified thresholds (i.e., 80 pg/ml and 120 pg/ml).

279

280 **Implications**

281 At an 80 pg/ml threshold, BDG has a high sensitivity for detecting proven NIC (99%). When focusing on  
282 “proven and probable NIC”, a less stringent outcome, sensitivity dropped to 89% in our meta-analysis,  
283 which means that one out of ten patients with “proven and probable NIC” would be missed in a strategy  
284 relying only on BDG. Although BDG has a high sensitivity for proven NIC, it has only moderate accuracy  
285 for “proven and probable NIC” combined. As a consequence, BDG should not be used as a stand-alone  
286 test to rule out NIC and eventually stop empirical antifungal treatment. We probably should incorporate  
287 BDG in a clinical decision rule combining clinical, biological and radiological information, as suggested by  
288 Benjamin *et al.* (4). BDG could also be incorporated in a clinical scoring system such as the “Candida  
289 Score” developed by Leon *et al.* to assess the risk of invasive candida infection in non-neutropenic adults  
290 (41). We are not aware of studies assessing the clinical usefulness of BDG-based compared to such risk-  
291 based approaches.

292 There was high variability in reference standards and case definitions across studies. The presence of  
293 such variability highlights that the EORTC/MSG criteria seem challenging to apply in neonates. We  
294 suggest using a simplified rule-based risk stratification system relying on the presence of signs and  
295 symptoms of neonatal sepsis, risk factors for invasive candidiasis, evidence of *Candida* colonisation, and  
296 *Candida* being cultured from a normally sterile site (**Table 4**). We believe a more consensual risk  
297 stratification system would increase homogeneity in case definition and help interpreting results from  
298 future studies.

299 More prospective multicenter cohort studies with blinding of test readers are needed. Such studies  
300 should not focus on BDG alone, but should also investigate the value of other biomarkers such as platelet  
301 and leukocyte counts, CRP, mannan and anti-mannan antibodies, and *Candida* PCR, for example. This  
302 would allow direct comparisons of test performance. It could also be interesting to measure BDG  
303 systematically and repeatedly in the serum and cerebrospinal fluid of newborns with NIC because BDG  
304 monitoring might allow reducing the length of antifungal therapy.

305

## 306 **Conclusions**

307 BDG seems a promising biomarker in the management of newborns with suspected NIC. Notably, BDG  
308 might be helpful in clinical practice to exclude NIC diagnosis. However, it seems too early to recommend  
309 its use because of the scarcity of reliable clinical data, heterogeneity in case definitions, and unstable  
310 accuracy estimates. Given the current evidence, BDG cannot be used as a stand-alone test to decide  
311 whether or not the treatment threshold for NIC is reached. Larger high-quality clinical studies in  
312 neonates with sepsis are warranted.

313 **TRANSPARENCY DECLARATION**

314 **Protocol:** The protocol of this study was registered and published on the PROSPERO website (number  
315 CRD42018089545).

316 **Conflicts of interest:** None.

317 **Funding:** There was no external funding for this study.

318 **Acknowledgements:** We thank Drs Cliquennois, Cornu, Mackay, and Goudjil for having shared additional  
319 unpublished data.

320 **Access to data:** JFC had full access to the data; he is the guarantor for the data.

321 **Authors' contribution:** AO and JFC performed study selection. AO and JT performed data extraction. JFC  
322 analysed the data. JFC and AO wrote the first draft of the manuscript. RS designed the literature search  
323 strategy. JFC and JT coordinated the development of the study, wrote the first draft of the protocol,  
324 designed the study, and finalised the manuscript. All authors revised the manuscript for important  
325 intellectual content and approved the final version submitted for publication.



## TABLES

**Table 1. Characteristics of included studies**

Study	Country	Inclusion criteria	Study design	N	Median gestational age (weeks)*	Median birth weight (g)*	Risk stratification system (n)**	Fungal species when identified
Cliquennois 2018	France	Neonates suspected of NIC	Prospective single center cohort	61	27.6	1035	Proven NIC (4) Probable NIC (4) Possible NIC (53)	7 <i>C. albicans</i> 1 <i>C. Parapsilosis</i> 1 unspecified yeast
Cohen 2017	France	Neonates suspected of NIC, with available BDG serum measurements	Retrospective single centre cohort	13	27.3	1020	Proven NIC (5) Probable NIC (4) Possible NIC (4)	4 <i>C. albicans</i> 1 <i>C. glabrata</i>
Cornu 2018	France	Neonates suspected of late-onset infection, with available BDG serum measurements	Retrospective single centre cohort	38	30	1200	Proven group (4) Probable group (13) Control group (21)	16 <i>C. albicans</i> 1 <i>C. parapsilosis</i>
Goudjil 2012	France	Neonates suspected of NIC, with available BDG serum measurements	Retrospective single centre cohort	61	28.5	1000	Infected group (18, including 6 proven NIC) Non-infected group (43)	14 <i>C. albicans</i> 3 <i>C. parapsilosis</i> 1 <i>C. lusitanae</i>
Mackay 2011	South Africa	Neonates suspected of late-onset infection, with high risk of NIC	Prospective multicenter cohort	72	31	1340	Definite fungemia (10) Probable fungemia (9) Possible fungemia (22) No fungemia (31)	4 <i>C. albicans</i> 4 <i>C. parapsilosis</i> 1 <i>Saccharomyces cerevisiae</i>
Montagna 2011	Italy	Preterm neonates with NIC, and non-infected preterm neonates	Retrospective single centre case-control	20	Not reported	1145	Proven <i>Candida</i> bloodstream infections (10) Negative controls (10)	6 <i>C. albicans</i> 3 <i>C. parapsilosis</i> 1 <i>C. glabrata</i>
Ramos 2017	Spain	Neonates suspected of late-onset sepsis or meningitis in NICU	Prospective multicenter cohort	155	27	882	IC confirmed (6) IC probable (2) Controls (147)	8 <i>C. albicans</i> 1 <i>Candida spp</i>
Tabl 2012	Egypt	Neonates with NIC, neonates with bacteremia, and healthy controls***	Retrospective single center case-control	45	Candidemia: 30.4 Bacteremia: 30.5 Control: 31.7	Candidemia: 1018.7 Bacteremia: 991.1 Control: 1811.4	Candidemia (15) Bacteremia (15) Control (15)	Not reported

Abbreviations: WG, weeks of gestation; BDG, Beta-D-Glucan; NIC, neonatal invasive candidiasis; NICU, neonatal intensive care unit. \*Gestational age and birth weight reported as median except for Ramos and Tabl (mean). \*\* According to case definitions used in the original articles. \*\*\*In meta-analysis, neonates with bacteremia and controls were grouped as NIC-negatives.

**Table 2. Risk stratification systems used across studies**

Study	Risk stratification system
Cliquennois 2018	See Table 4
Cohen 2017	See Table 4
Cornu 2018	<ul style="list-style-type: none"> <li>• <b>Control group:</b> neonates who were neither colonised nor infected with any fungal pathogen;</li> <li>• <b>Probable group:</b> neonates who had probable invasive yeast infection (IYI) suspected from clinical and biological criteria; Probable IYI was defined as receipt of more than 5 days of antifungal therapy and presence of yeast in urine (urine bag) or digestive (stool and anal swab) colonisation with clinical or biological (white blood cell count, C-reactive protein [CRP])) symptoms of sepsis.</li> <li>• <b>Proven group:</b> neonates who had proven IYI. Proven IYI was defined as a positive culture for a yeast species from a normally sterile body site except urine obtained with a urine bag.</li> </ul>
Goudjil 2012	<ul style="list-style-type: none"> <li>• <b>Infected group:</b> patients with systemic infection, including a <i>Candida</i>-positive culture from a usually sterile body site: candidemia (only blood cultures positive for <i>Candida</i> spp.), disseminated candidiasis (blood, urine, CSF, peritoneal or pleural spaces, bone, joint or ocular sites positive for <i>Candida</i> spp.), renal candidiasis (urine cultures are positive for <i>Candida</i> spp. with ultrasound evidence of fungal lesions in the kidney);</li> <li>• <b>Non-infected group:</b> patients with symptoms of infection but without any positive fungal culture from sterile body sites.</li> </ul>
Mackay 2011	<ul style="list-style-type: none"> <li>• <b>No fungemia:</b> a high index of suspicion for fungemia including 3 or more risk factors; blood culture negative; white cell count (WCC), platelet count (&lt;100 000/mm<sup>3</sup>) and C-reactive protein (CRP) within normal limits; no evidence of colonisation with a fungal organism; or definite bacterial sepsis;</li> <li>• <b>Possible fungemia:</b> a high index of suspicion for fungemia including 3 or more risk factors; blood cultures negative; no evidence of colonisation; two or more of the following present: abnormal WCC, low platelet count or elevated CRP (&gt;10);</li> <li>• <b>Probable fungemia:</b> a high index of suspicion for fungemia including 3 or more risk factors and evidence of colonisation with a fungal organism in the form of positive stool or non-sterile urine sample (collected by urine bag) for yeasts; blood cultures negative; and one or more of the following are present: abnormal WCC, low platelet count (&lt;100 000/mm<sup>3</sup>) or elevated CRP (&gt;10);</li> <li>• <b>Definite fungemia:</b> positive culture for a fungal organism from a normally sterile site (including blood or tissue culture).</li> </ul>
Montagna 2011	<ul style="list-style-type: none"> <li>• <b>Proven <i>Candida</i> bloodstream infections</b></li> <li>• <b>Negative controls</b></li> </ul>
Ramos 2017	<ul style="list-style-type: none"> <li>• <b>Invasive candidiasis (IC) confirmed:</b> an episode was defined as IC confirmed if a culture of a sterile sample was found to be positive for <i>Candida</i> spp. or if a <i>Candida</i> species was detected during the autopsy of a deceased patient. Likewise, an episode was considered IC confirmed when endophthalmitis candidiasis was diagnosed by direct examination of the fundus of the eye or if fungus balls were observed by abdominal ultrasound.</li> <li>• <b>IC probable:</b> this designation was used for patients who tested negative for <i>Candida</i> spp. in a culture derived from blood, cerebrospinal fluid (CSF), or other sterile fluid samples but for whom (i) improvement was seen upon treatment with an antifungal(s) and/or (ii) the episode corresponded to a true IC (according to the clinician's criteria and based on the evolution) and/or (iii) the image from an echocardiogram was consistent with <i>Candida</i> endocarditis or a cerebral ultrasound showed micro-abscesses consistent with IC. In addition to fulfilling any of the criteria described above, one of the following conditions had to be met: colonisation by <i>Candida</i> spp. was detected by isolation of <i>Candida</i> spp. from stool, urine, bronchial aspirate, or gastric samples or a clinical diagnosis of <i>Candida</i> dermatitis was made; thrombocytopenia (platelet count of 100,000/mm<sup>3</sup>) was diagnosed; or, after an episode of sepsis, a complication such as meningitis, endocarditis, or pyelonephritis appeared as a result of the dissemination of <i>Candida</i> spp. This condition was determined by clinical judgment. Mucosal candidiasis in the absence of clinical signs of IC was not considered to represent IC.</li> </ul>
Tabl 2012	<ul style="list-style-type: none"> <li>• <b>Candidemia group:</b> culture-proven candidemia</li> <li>• <b>Bacteremia group:</b> culture-proven bacteremia</li> <li>• <b>Control group:</b> healthy preterm neonates</li> </ul>

**Table 3. Risk of bias and concerns regarding applicability of included studies**

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
<b>Cliquennois 2018</b>	Low	Low	Low	Low	Low	Low	Low
<b>Cohen 2017</b>	High	High	High	High	Low	Low	Low
<b>Cornu 2018</b>	Low	Low	High	Unclear	Low	Low	Low
<b>Goudjil 2012</b>	Low	Low	Low	Unclear	Low	Low	Low
<b>Mackay 2011</b>	Low	Unclear	Unclear	Unclear	Low	Low	Low
<b>Montagna 2011</b>	High	High	High	High	High	Low	High
<b>Ramos 2017</b>	Low	Low	Unclear	High	Low	Low	Low
<b>Tabl 2012</b>	High	High	Unclear	High	High	Low	High

**Table 4. Proposed simplified risk stratification system for neonatal invasive candidiasis**

<b>NIC</b>	<b>≥ One criterion for neonatal sepsis<sup>a</sup></b>	<b>≥ One risk factor for NIC<sup>b</sup></b>	<b>Evidence of <i>Candida</i> colonisation<sup>c</sup></b>	<b><i>Candida</i> positive culture from a normally sterile site<sup>d</sup></b>
<b>Proven</b>	Yes	+/-	+/-	Yes
<b>Probable</b>	Yes	Yes	Yes	No
<b>Possible</b>	Yes	Yes	No	No

<sup>a</sup> Fever, cardiovascular instability (tachycardia, bradycardia, poor perfusion), respiratory symptoms (respiratory distress, apnea, oxygen desaturation), neurological symptoms (lethargy, irritability, seizures), feeding difficulties (vomiting, abdominal distension), jaundice;

<sup>b</sup> Term < 28 WG, Neonatal weight < 1000 g, central venous catheter, exclusive parenteral nutrition, broad-spectrum antibiotics, mechanical ventilation with endotracheal intubation, post-natal corticosteroids, gastrointestinal tract disease (abdominal surgery, necrotising enterocolitis, spontaneous intestinal perforation);

<sup>c</sup> At least two positive sites among skin, stools or anal swab, mouth, respiratory tract, and urine without ultrasound findings suggestive of fungal lesions;

<sup>d</sup> Blood, cerebrospinal fluid, bone or joint, pleural or pericardial or peritoneal fluid, urine with ultrasound findings suggestive of fungal lesions, such as fungus balls, intraocular fluids and tissues.

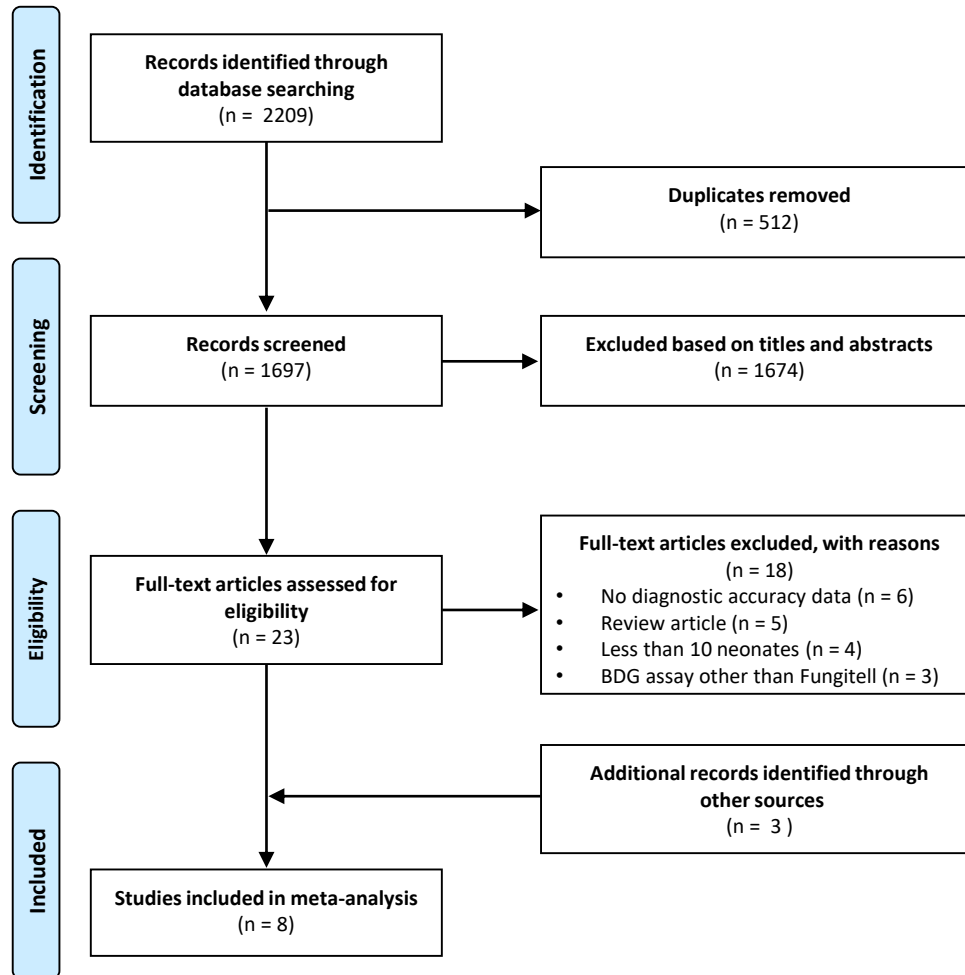
## FIGURE CAPTIONS

**Figure 1. Flow diagram of studies in the review.**

**Figure 2. Forest plots of (1,3)-Beta-D-Glucan sensitivity and specificity for detecting neonatal invasive candidiasis.** TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative.

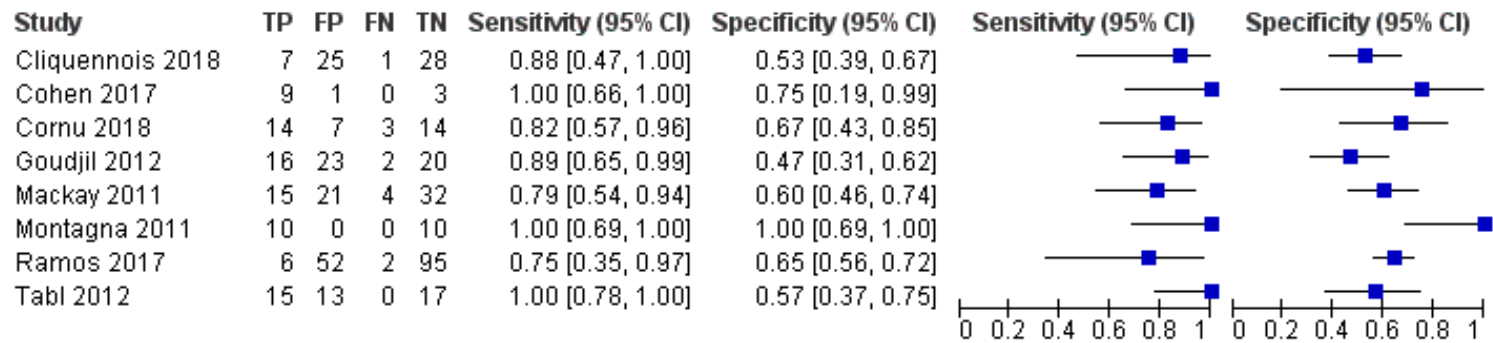
**Figure 3. Summary ROC plot of (1,3)-Beta-D-Glucan sensitivity and specificity for neonatal invasive candidiasis (n = 8).** Each study cohort is represented by an empty square. The filled circle is the pooled summary estimate for sensitivity and specificity. The dotted line represents the 95% confidence region for the summary operating point.

**Figure 1**

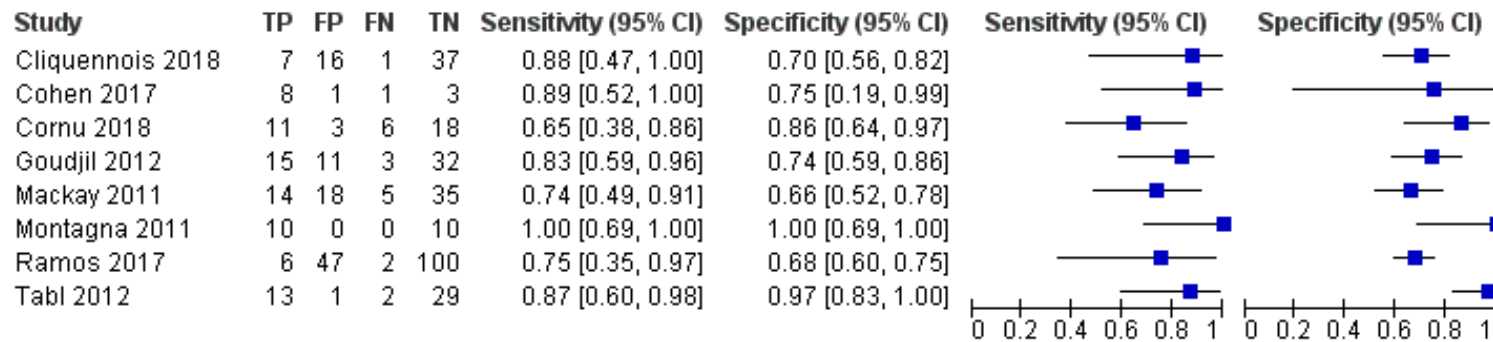


**Figure 2**

**2A. 80 pg/ml threshold**

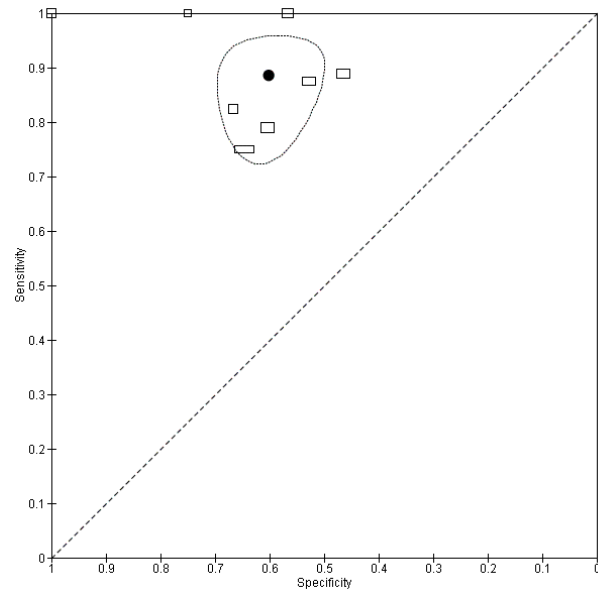


**2B. 120 pg/ml threshold**

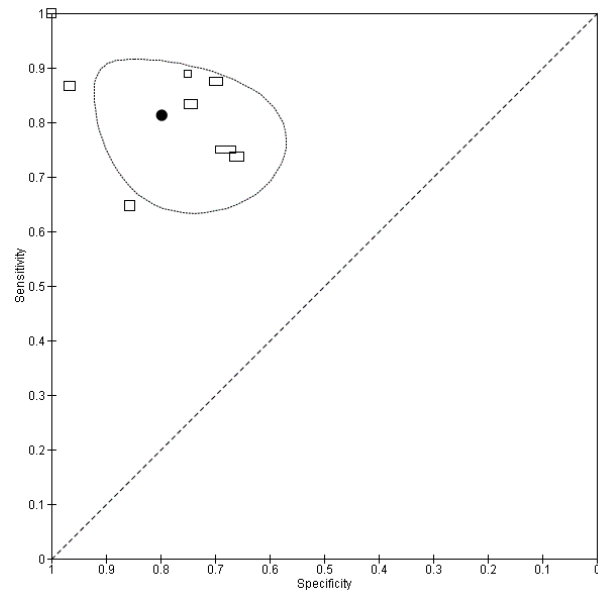


**Figure 3**

**3A. 80 pg/ml threshold**



**3B. 120 pg/ml threshold**





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