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1 **Title:** Comparison of time-to-positivity between two blood culture systems: a detailed
2 analysis down to the genus-level

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13

14 **Abstract**

15 A recently developed, automated blood culture system and medium improve the time-
16 to-positivity (TTP) for bacteremia. However, there have thus far been no genus-level
17 analyses using this novel system. We evaluated and compared the changes in blood
18 culture TTP between two systems: BacT/Alert 3D with a blood culture medium
19 containing activated charcoal versus the more recent BacT/Alert Virtuo with a blood
20 culture medium containing polymeric beads. This before-and-after study included blood
21 cultures collected between July 2010 and April 2014 (3D, activated charcoal) and
22 between July 2015 and April 2018 (Virtuo, polymeric beads). A total of 554,732 blood
23 cultures were included, 267,935 (48.30%) during the first period and 286,797 (51.70%)
24 during the second period. Overall, 55,611 (10.02%) tested positive for at least one
25 microorganism. The incubation of the blood culture medium in the Virtuo system was
26 associated with reduced TTP for the most prevalent bacteria, those representing
27 91.72% (n=51,006) of all the positive blood cultures. The median TTP was reduced by
28 0.99 hours for *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Pseudomonadales*, and
29 most of the genera within the order *Enterobacterales* (except the family
30 *Morganellaceae*). However, strictly anaerobic bacteria belonging to the genus
31 *Bacteroides*, representing 0.85% (n=474) of all positive blood cultures, were detected
32 4.53 hours later using the Virtuo system. Virtuo was associated with a shorter TTP for
33 most bacteria, but this improvement was heterogeneous to the genus level.

34 **Keywords:** Bacteriological Techniques; Bacteremia; Bloodstream Infection;
35 Bacteroides Infection; Time-to-positivity

36 **Introduction**

37 Bloodstream infections (BSI) are frequent and severe systemic infections associated
38 with a 30-day mortality rate of 13–21% [1]. Empiric antimicrobial therapy is usually
39 prescribed but is not always appropriate and should be re-evaluated depending on
40 bacteriological analyses. Thus, fast and accurate pathogen diagnostics lead to better
41 care of patients with BSI [2].

42 Recent blood culture automated systems have been developed to optimize BSI
43 pathogen detection by improving the broth composition of the blood culture bottles and
44 the monitoring system. The BacT/Alert Virtuo (bioMérieux, Marcy l'Etoile, France;
45 referred to as Virtuo hereafter) automated system presents improved temperature
46 stability and high-fidelity optics compared to the older version BacT/Alert 3D
47 (bioMérieux, Marcy l'Etoile, France; referred to as 3D hereafter) [3]. The Virtuo is
48 intended to be used with a blood culture medium containing antibiotic-binding polymeric
49 beads instead of the former blood culture medium containing activated charcoal [4]. The
50 broth composition of blood cultures with activated charcoal is based on soybean-casein
51 digest with brain heart infusions solids. whereas for blood cultures with polymeric beads
52 the broth composition is based on peptones and yeast extracts without brain heart
53 infusions solids [5]. Polymeric beads provide a cleaner background for Gram-staining
54 and less interference for MALDI-TOF mass spectrometry or molecular assays,
55 compared to activated charcoal.

56 Time-to-positivity (TTP) of blood cultures is an important parameter to optimize because
57 lower TTP leads to faster pathogen identification, guiding the antimicrobial therapy.

58 Utilization of the Virtuo with blood culture media containing polymeric beads reduced
59 the time-to-positivity (TTP) of blood cultures for *Enterobacterales* and *Enterococcus*
60 spp., and increased bacterial recovery [3, 4, 6]. However, a detailed genus-level
61 analysis was not feasible in this type of prospective studies because the number of
62 blood culture bottles required would have been prohibitively large. Indeed, only about
63 10% of the blood culture samples are positive, and most of the bacteria recovered
64 belong to the same genera (*Staphylococcus*, *Escherichia*, *Enterococcus*, or
65 *Streptococcus*) [7]. Thus, the impact of switching to a newer automated system and
66 blood culture medium on the detection of less prevalent bacteria remains unknown.

67 We performed a large before-and-after study to examine the impact of switching to a
68 modern automated system and blood culture medium. The principal aim was to
69 evaluate the changes in TTP values, detailed to the genus level, between two systems:
70 3D with a blood culture medium containing activated charcoal versus the more recent
71 Virtuo with a blood culture medium containing polymeric beads.

72 **Methods**

73 This before-and-after study was conducted at Lille University Hospital, a 3,200-bed
74 hospital in France. In the first period from July 2010 to April 2014, BacT/Alert FA
75 (aerobic), FN (anaerobic), or PF (pediatric) blood cultures (containing activated
76 charcoal) were incubated on a 3D automated blood culture system. In the second period
77 from July 2015 to April 2018, BacT/Alert FA Plus (aerobic), FN Plus (anaerobic), or PF
78 Plus (pediatrics) blood cultures (containing polymeric beads) were incubated on a
79 Virtuo. In both periods, bottles were incubated for five days or until they signaled

80 positive for growth. Blood cultures from May 2014 to June 2015 were excluded from the
81 analysis because they were collected within a transition period during which another
82 blood culture medium (containing polymeric beads) was used on a 3D.

83 Blood cultures were transferred from the clinical wards to the laboratory using a
84 pneumatic tube system. Positive blood cultures were processed according to standard
85 laboratory protocols. Bacteria were identified using MALDI-TOF mass spectrometry
86 (Bruker Daltonics, Wissembourg, France). If identification at the species level was not
87 possible using MALDI-TOF, 16S rDNA sequencing was performed, as described
88 elsewhere [8].

89 Data collected included the type of blood culture, time of specimen collection, time of
90 introduction into the automated blood culture system, and time of positivity or negativity.
91 Time-to-positivity was defined as the duration from when the sample was first incubated
92 on the automated blood culture system to signaled positive for growth by the system.
93 The following patient demographic data were automatically collected during the data
94 extraction: age, sex, ward, and date of entry into the hospital. No names or birthdates
95 were included to preserve the privacy of patients, following the recommendations from
96 the French National Commission on Informatics and Liberty.

97 Polymicrobial blood cultures were not excluded. If a blood culture was positive with
98 multiple species from the same genus (e.g., two different *Staphylococcus* isolates), it
99 was counted only once for this genus. The denominator was the total number of blood
100 culture bottles included and not the number of different bacteria identified.

101 Statistical analyses were performed using R software version 3.6.3 [9]. The statistical
102 unit was blood culture bottles, which were considered individually for the analysis, even
103 if they were sampled as a set. Bacterial names were automatically classified by the
104 AMR R package [10], using the microbial taxonomy from the Catalogue of Life
105 database. Failures or uncertainties in the classification were manually reviewed.
106 Qualitative variables were compared with Fisher's exact test and continuous variables
107 with the Mann–Whitney U test. Survival distributions, as defined by the delay until blood
108 cultures signaled positive for growth, were plotted using the survminer R package.

109 Results

110 A total of 554,732 blood cultures were included. Of these, 267,935 (48.30%) were in the
111 charcoal-containing blood culture medium incubated on a 3D, and 286,797 (51.70%)
112 were in the polymeric bead-containing blood culture medium incubated on a Virtuo. In
113 total, 55,611 blood cultures (10.02%) were positive for at least one microorganism,
114 including 1,514 (0.27%) with yeasts. Polymicrobial growth (blood culture positive with
115 multiple microorganisms) was detected in 3,613 (0.65%) blood cultures.

116 The characteristics of the analyzed blood cultures are provided in Table 1. Samples
117 were collected mainly from patients hospitalized in the medical (35.17%), intensive or
118 critical care (33.88%), surgical (11.08%), emergency (8.73%), and pediatric (5.03%)
119 wards. There were small but statistically significant differences in the following
120 demographic data between the two populations: patients from the Virtuo group were
121 older (58 vs. 60 years), more frequently female (39.03% vs. 40.00%), and more
122 frequently from emergency wards (6.65% vs. 10.66%). The blood culture TTP was
123 lower for the Virtuo (16.53 hours on 3D vs. 15.40 hours on Virtuo, $P < 0.0001$). In
124 subgroup analysis, the blood culture TTP was reduced for aerobic blood bottles (15.98
125 hours on 3D vs. 14.75 hours on Virtuo, $P < 0.0001$), for anaerobic blood bottles (17.07
126 hours vs. 16.65 hours, $P < 0.0001$), and for pediatric blood bottles (17.88 hours vs.
127 15.28 hours, $P < 0.0001$).

128 For Gram-positive bacteria, we compared the most frequent genera, identified in at least
129 100 blood cultures (Table 2). *Staphylococcus* represented the most prevalent genus,
130 identified in 53.05% of the positive blood cultures, with a lower TTP for the Virtuo.

131 *Enterococcus* and *Streptococcus* were present in 6.69% and 5.45% of the positive
132 blood cultures, respectively, also with lower TTP in the second period. The TTP was
133 higher in the Virtuo for *Micrococcus*, which are usually considered contaminating
134 bacteria with little clinical relevance. Table S1 shows the subgroup analysis for Gram-
135 positive bacteria, depending on the type of blood culture bottles.

136 For Gram-negative bacteria, we compared the most frequent genera, identified in at
137 least 100 blood cultures (Table 3). *Enterobacterales* represented the most frequent
138 order, identified in 28.11% of the positive blood cultures. Of these, the TTP for the
139 Virtuo was lower for genera belonging to the family *Enterobacteriaceae* (*Escherichia*,
140 *Klebsiella*, *Enterobacter*, *Citrobacter*) or *Yersiniaceae* (*Serratia*). However, there were
141 no differences in the detection of two genera belonging to the family *Morganellaceae*
142 (*Proteus* and *Morganella*) despite high numbers of positive blood cultures ($n=556$ and
143 $n=267$, respectively). *Pseudomonas* was identified in 3.20% of the positive blood
144 cultures, and the TTP was lower for the Virtuo. *Bacteroides* were the most frequently
145 strictly anaerobic bacteria identified (0.85% of the positive blood cultures) and had a
146 higher TTP for the Virtuo (26.62 hours on 3D vs. 31.15 on Virtuo, $P<0.0001$). The TTP
147 values of blood cultures positive with *Bacteroides* were plotted as cumulative events in
148 Figure 1. Table S2 shows the subgroup analysis for Gram-negative bacteria, depending
149 on the type of blood culture bottles.

150 For yeasts, the TTP tended to be lower in the Virtuo, but this was not statistically
151 significant (31.39 vs. 33.50 hours, $P=0.056$).

152 **Discussion**

153 In this before-and-after study on 554,732 blood cultures, incubation with the system
154 combining the Virtuo and polymeric beads was associated with a reduced TTP for the
155 most prevalent bacteria. The median TTP was reduced by 0.99 hours for
156 *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Pseudomonadales*, and most of the
157 genera within the order *Enterobacterales* (except the family *Morganellaceae*),
158 representing 91.72% ($n=51,006$) of all the positive blood cultures.

159 These results are consistent with previous studies. Altun *et al.* showed that the TTP was
160 significantly reduced in the Virtuo compared with the 3D using simulated blood cultures
161 spiked with 115 bacterial or fungal isolates, for all the bacteria tested (*Enterobacterales*,
162 *Acinetobacter*, *Staphylococcus*, *Enterococcus*, *Streptococcus*) [3]. The TTP was
163 reduced in the Virtuo for both blood culture media, containing either polymeric beads
164 (FA Plus or FN Plus) or activated charcoal (FA or FN). Another study using simulated
165 blood cultures spiked with 330 bacterial or fungal isolates showed a reduction of the
166 TTP in the Virtuo for most microorganisms, except *Stenotrophomonas maltophilia* and
167 some *Candida* species [11]. Additionally, a multicenter study on 5,709 blood culture sets
168 compared the 3D to the Virtuo using either a blood culture medium containing polymeric
169 beads (FA Plus or FN Plus) or a medium containing no beads and no charcoal (SA or
170 SN) [6]. In these conditions, the TTP was reduced by almost 2 hours for the blood
171 cultures incubated on the Virtuo compared to the 3D. The TTP was significantly
172 decreased only for *Enterobacterales* and *Enterococcus*. For *Streptococcus* and
173 coagulase-negative *Staphylococcus*, there was a trend of the Virtuo reducing the TTP,
174 but this effect was not statistically significant ($P=0.08$ and $P=0.10$, respectively). A
175 recent study compared the TTP of the 3D to Virtuo on 1,904 blood culture sets

176 exclusively using a blood culture medium containing polymeric beads (FA Plus or FN
177 Plus) [12]. The TTP was significantly reduced for *Staphylococcus aureus* (-1.7 hours for
178 the Virtuo) and *Escherichia coli* (-0.6 hours). However, the overall reduction of the TTP
179 for clinically significant pathogens was only 18 minutes, less than the 2-hour reduction
180 reported previously [6]. Finally, another study compared two different periods, one with
181 the 3D, followed by another with the Virtuo, exclusively using a blood culture medium
182 containing polymeric beads (FA Plus, FN Plus, or PF Plus) [13]. The TTP was
183 significantly reduced for *Staphylococcus*, *Enterobacterales*, and *Streptococcus*, while no
184 difference was shown for *Enterococcus*.

185 In our study, the TTP was reduced in the Virtuo with polymeric beads for
186 *Enterobacterales*, but not for the *Morganellaceae* family. Data on bacteremia due to
187 *Morganellaceae* are scarce [14], even if *Morganellaceae* represented 1.50% of all
188 positive blood cultures in our study. The mechanism by which the *Morganellaceae* TTP
189 is not decreased with the more efficient automated system remains unknown. One
190 hypothesis might reside in activated charcoal inhibiting swarming from *Proteus* [15]. A
191 correlation between swarming and growth rate in liquid medium has been previously
192 suggested [16].

193 Our results also show that *Bacteroides* was associated with a higher TTP for the Virtuo
194 with polymeric beads compared with the 3D with activated charcoal. In 474 blood
195 culture positive with *Bacteroides*, the median TTP was 4.53 hours higher using the
196 Virtuo ($P < 0.0001$). *Bacteroides* is the most common genus detected in BSI due to
197 strictly anaerobic bacteria. This delayed detection could be clinically relevant. Failure to
198 add appropriate antimicrobials was previously associated with higher mortality (55% vs.

199 17%) in the case of anaerobic bacteremia (mostly due to *Bacteroides*) [17]. Empirical
200 antimicrobial therapy includes anti-anaerobic coverage for only half of the patients with
201 anaerobic bacteremia [17]. The reasons for this delayed detection remain hypothetical. As
202 the Virtuo improved temperature stability and high-fidelity optics compared to the 3D,
203 the difference in TTP for *Bacteroides* might be linked to the use of a blood culture
204 medium containing polymeric beads. Indeed, De Keukeleire *et al.* compared the
205 performance of two blood culture media containing either polymeric beads (FN plus) or
206 activated charcoal (FN) on the detection of anaerobes with the 3D, using blood cultures
207 experimentally spiked with anaerobic bacteria [18]. The TTP was significantly increased
208 for resin-based compared with charcoal-based blood culture bottles for *Bacteroides* spp.
209 (49 hours vs. 27 hours) and most of the other anaerobes. Rapid diagnosis of
210 bacteremia due to *Bacteroides* would allow for the rapid adaptation of antimicrobial
211 therapy, which may lead to lower mortality.

212 The principal limitation of our study is the before-and-after design. First, the type of
213 patients included in our hospital may have changed during the study period.
214 Demographic characteristics of the patients were significantly different: in the first
215 period, patients were younger (58 vs. 60 years), more frequently male (60.97% vs.
216 60.00%), and more frequently hospitalized in intensive or critical care (34.59% vs.
217 33.22%). All these differences were highly significant ($P < 0.0001$). However, due to a
218 large number of blood cultures included (554,732), even small differences in values
219 could result in statistical significance. Second, additional parameters affecting the TTP
220 of blood cultures include the volume of blood sampled, transportation time, and
221 concomitant antimicrobial therapy, which have not been monitored in our study [19].

222 Finally, different blood culture media and blood culture instruments were compared,
223 resulting in a mixed effect. However, the TTP decrease for the most prevalent bacteria
224 found is consistent with the literature [3, 6, 13] and externally validate our results.

225 Overall, our study confirms the Virtuo and the blood culture medium with polymeric
226 beads are associated with a reduced TTP for the most prevalent bacteria, compared to
227 the 3D system and the blood culture medium with activated charcoal. However, no
228 differences were observed for the family *Morganellaceae*, representing an exception
229 within the order *Enterobacterales*. Moreover, TTP values were even significantly
230 increased for *Bacteroides* using the most modern automated system.

231 **Declarations**

232 **Funding:** This work was supported by CHU Lille.

233 **Competing Interests:** The authors declare that they have no conflict of interest.

234 **Ethical Approval:** The study was approved by the local Institutional Review Board.

235 Patient consent was not requested for this study, since the study was based on
236 anonymized data collected retrospectively.

237 **Consent to Participate:** Formal consents were not required for this retrospective study
238 on anonymized data.

239 **Consent to Publish:** Not applicable.

240 **Availability of data and materials:** All data were anonymized. The dataset analyzed
241 during the current study is available from the corresponding author on reasonable
242 request.

243 **Authors Contributions:** RLG and RD conceived and planned the study. All authors
244 participated in data collection and microbiological analysis. RLG performed the data
245 analysis. RLG and RD wrote the first draft, all authors reviewed and edited the final
246 manuscript.

247

248 **Words:** 2289

249 **Figure Legends**

250 **Figure 1.** Cumulative probability of the time-to-positivity (TTP) for blood cultures
251 positive with *Bacteroides* sp.

252 BacT/Alert 3D = BacT/Alert 3D with the blood culture medium containing activated
253 charcoal; BacT/Alert Virtuo = BacT/Alert Virtuo with the blood culture medium containing
254 polymeric beads.

255 **References**

256

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311

312 **Table 1.** Characteristics of the included blood culture sample bottles

	BacT/Alert 3D Total (n = 267,935)	BacT/Alert Virtuo Total (n = 286,797)	P-value
Patient demographics characteristics			
Age (years)	58.0 (42.0–70.0)	60.0 (45.0–71.0)	< 0.0001
Sex			< 0.0001
Female	104,554 (39.03%)	114,705 (40.00%)	..
Male	163,355 (60.97%)	172,085 (60.00%)	..
Type of ward			< 0.0001
Medicine	94,750 (35.36%)	100,367 (35.00%)	..
Intensive/critical care	92,676 (34.59%)	95,273 (33.22%)	..
Surgery	30,592 (11.42%)	30,851 (10.76%)	..
Emergency	17,825 (6.65%)	30,583 (10.66%)	..
Pediatric	15,362 (5.73%)	12,530 (4.37%)	..
Geriatric	9,870 (3.69%)	9,511 (3.31%)	..
Rehabilitation	3,740 (1.40%)	4,983 (1.74%)	..
Obstetrics	2,958 (1.10%)	2,576 (0.90%)	..
Psychiatry	162 (0.06%)	123 (0.04%)	..
Blood culture type			< 0.0001
Aerobic	130,598 (48.74%)	141,543 (49.35%)	..
Anaerobic	118,757 (44.32%)	130,893 (45.64%)	..
Pediatric	18,580 (6.94%)	14,361 (5.00%)	..
Positive blood cultures			
Positive blood bottles	25,783 (9.62%)	29,828 (10.40%)	< 0.0001
Time to positivity (hours)	16.53 (11.88–24.63)	15.40 (10.95–22.72)	< 0.0001

313
314 Data are median (IQR) or n (%).

Table 2. Gram-positive bacteria: time-to-positivity (median) and percentage of positivity

Microorganism group			Sample size (n)		Median time to positivity (hours)			Percentage of positivity (%)			
Phylum	Order	Genus	3D	Virtuo	3D	Virtuo	Difference	P-value	3D	Virtuo	P-value
<i>Firmicutes</i>			16,716	19,385							
	<i>Bacillales</i>		13,625	16,123							
		<i>Staphylococcus</i>	13,520	15,984	19.27	18.35	-0.92	<0.0001	5.05	5.57	<0.0001
		<i>Bacillus</i>	79	143	12.32	12.9	0.58	0.92	0.03	0.05	<0.001
	<i>Lactobacillales</i>		3,340	3,512							
		<i>Enterococcus</i>	1,789	1,931	13.05	12.50	-0.55	<0.001	0.67	0.67	0.81
		<i>Streptococcus</i>	1,506	1,526	13.30	12.46	-0.84	<0.0001	0.56	0.53	0.13
<i>Actinobacteria</i>			291	386							
	<i>Actinomycetales</i>		262	349							
		<i>Micrococcus</i>	101	145	37.02	43.68	6.66	<0.001	0.04	0.05	0.03
		<i>Corynebacterium</i>	86	73	47.05	49.58	2.53	0.59	0.03	0.03	0.17

Only genera identified in at least 100 blood cultures are shown. Statistically significant differences in time-to-positivity (TTP) are in bold. 3D = BacT/Alert 3D with the blood culture medium containing activated charcoal; Virtuo = BacT/Alert Virtuo with blood culture medium containing polymeric beads.

Table 3. Gram-negative bacteria: time-to-positivity (median) and percentage of positivity

Microorganism group			Sample size (n)		Median time to positivity (hours)				Percentage of positivity (%)		
Phylum	Order	Genus	3D	Virtuo	3D	Virtuo	Difference	P value	3D	Virtuo	P-value
<i>Proteobacteria</i>			8,248	10,001							
<i>Enterobacteriales</i>			6,970	8,660							
		<i>Escherichia</i>	3,197	3,791	11.08	10.30	-0.78	< 0.0001	1.19	1.32	< 0.0001
		<i>Klebsiella</i>	1,847	2,367	11.57	10.28	-1.29	< 0.0001	0.69	0.83	< 0.0001
		<i>Enterobacter</i>	1,170	1,581	12.07	10.77	-1.30	< 0.0001	0.44	0.55	< 0.0001
		<i>Serratia</i>	240	319	14.43	12.32	-2.11	< 0.0001	0.09	0.11	0.01
		<i>Proteus</i>	275	281	12.73	13.15	0.42	0.84	0.10	0.10	0.61
		<i>Citrobacter</i>	189	318	11.73	10.76	-0.97	< 0.001	0.07	0.11	< 0.0001
		<i>Morganella</i>	119	148	13.32	12.82	-0.50	0.37	0.04	0.05	0.25
<i>Pseudomonadales</i>			1,072	1,092							
		<i>Pseudomonas</i>	922	859	17.23	16.35	-0.88	0.03	0.34	0.30	0.004
		<i>Acinetobacter</i>	138	215	13.16	11.80	-1.36	0.04	0.05	0.08	< 0.001
<i>Xanthomonadales</i>			83	119							
		<i>Stenotrophomonas</i>	83	119	22.58	20.62	-1.96	0.23	0.03	0.04	0.05
<i>Bacteroidetes</i>			298	256							
<i>Bacteroidales</i>			277	226							
		<i>Bacteroides</i>	261	213	26.62	31.15	4.53	< 0.0001	0.10	0.07	0.004

Only genera identified in at least 100 blood cultures are shown. Statistically significant differences in time-to-positivity (TTP) are in bold. 3D = BacT/Alert 3D with the blood culture medium containing activated charcoal; Virtuo = BacT/Alert Virtuo with the blood culture medium containing polymeric beads.

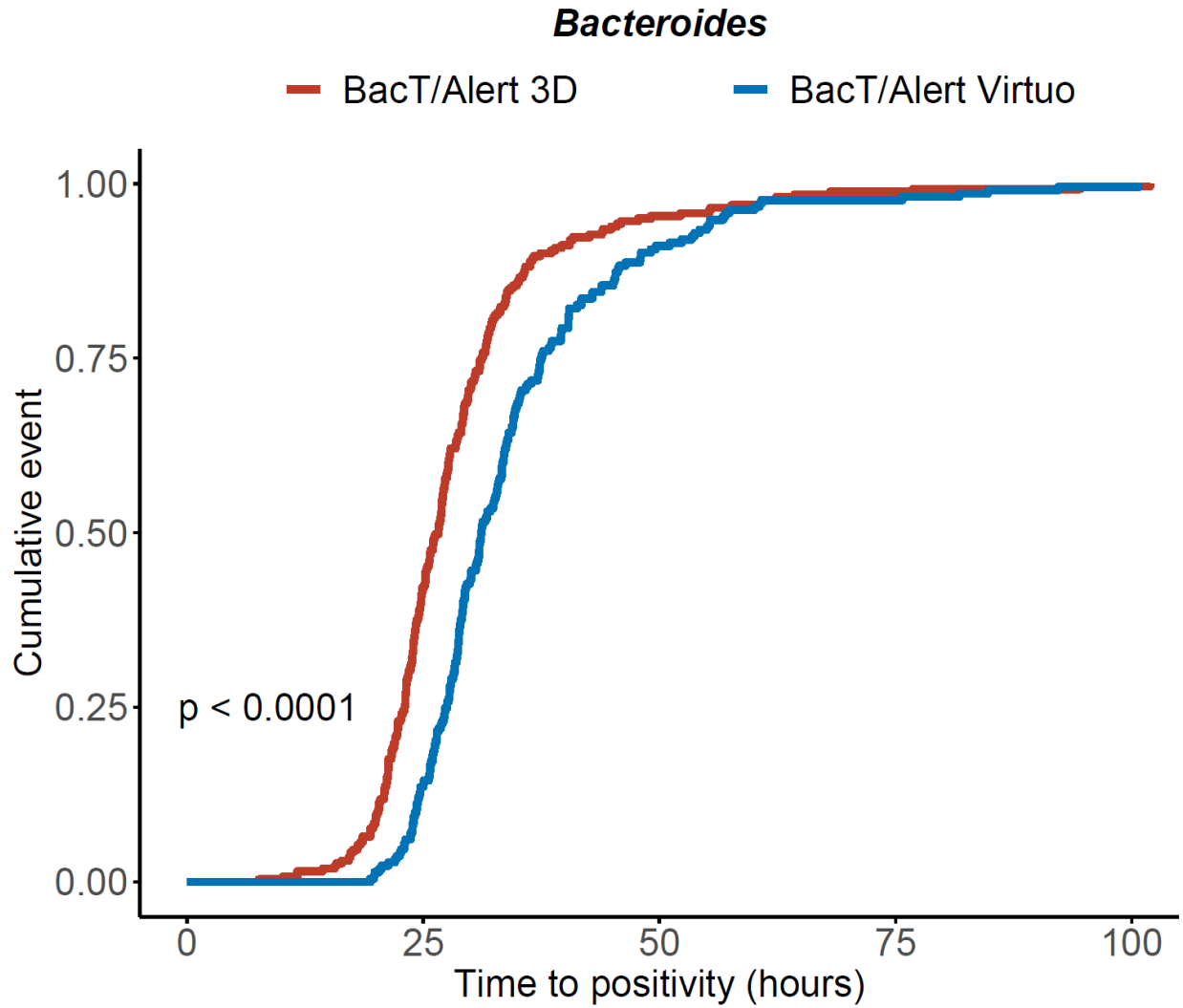


Figure 1. Cumulative probability of time to positivity for blood cultures positive with *Bacteroides* sp.

BacT/Alert 3D = BacT/Alert 3D with the blood culture medium containing activated charcoal; BacT/Alert Virtuo = BacT/Alert Virtuo with the blood culture medium containing polymeric beads.