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Time-to-positivity, type of culture media and oxidase test performed on positive blood culture vials to predict *Pseudomonas aeruginosa* in patients with Gram-negative bacilli bacteraemia

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ABSTRACT

Introduction. The aim of this study was to determine the usefulness of oxidase test and time-to-positivity (TTP) in aerobic and anaerobic blood culture vials to detect the presence of *Pseudomonas aeruginosa* in patients with Gram-negative bacilli (GNB) bacteraemia.

Material and methods. TTP was recorded for each aerobic and anaerobic blood culture vial of monomicrobial bacteraemia due to GNB. Oxidase test was performed in a pellet of the centrifuged content of the positive blood culture. An algorithm was developed in order to perform the oxidase test efficiently taking into account TTP and type of vial.

Results. A total of 341 episodes of GNB bacteraemia were analysed. Sensitivity, specificity, positive predictive value and negative predictive value of the oxidase test performed on positive vials with GNB to predict *P. aeruginosa* were 95%, 99%, 91%, and 99%, respectively. When growth was first or exclusively detected in anaerobic vials, *P. aeruginosa* was never identified hence the performance of the oxidase test could be avoided. When growth was only or first detected in aerobic vials, a TTP≥8h predicted *P. aeruginosa* in 37% or cases (63 of 169), therefore oxidase test is highly recommended.

Conclusions. Oxidase test performed onto positive blood culture vials previously selected by TTP and type of vials is an easy and inexpensive way to predict *P. aeruginosa*. In most cases, this can lead to optimization of treatment in less than 24 hours.

Keywords: Time to blood culture positivity, culture media, bloodstream infection, *Pseudomonas aeruginosa*, oxidase test.

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Tiempo de positividad, tipo de medio de cultivo y prueba de oxidasa realizada en viales de hemocultivo positivos para predecir *Pseudomonas aeruginosa* en pacientes con bacteriemia por bacilos gramnegativos

RESUMEN

Introducción. El objetivo del estudio fue determinar la utilidad de la prueba de oxidasa y del tiempo de positividad del hemocultivo (TPH) para detectar la presencia de *Pseudomonas aeruginosa* en pacientes con bacteriemia por bacilos gramnegativos (BGN).

Material y métodos. Se registró el TPH de cada vial aerobio y anaerobio en todos los episodios de bacteriemia monomicrobiana por BGN. La prueba de oxidasa se realizó sobre el contenido centrifugado del hemocultivo positivo. Se diseñó un algoritmo para optimizar la realización de la prueba de oxidasa según el TPH y el tipo de vial.

Resultados. Se analizaron 341 episodios de bacteriemia por BGN. La sensibilidad, especificidad, valor predictivo positivo y valor predictivo negativo de la prueba de oxidasa para predecir *P. aeruginosa* fueron del 95%, 99%, 91% y 99%, respectivamente. Cuando el crecimiento se detectó primero o exclusivamente en viales anaerobios, nunca se identificó *P. aeruginosa* pudiendo evitar la realización de la prueba de oxidasa. Cuando el crecimiento se detectó antes o exclusivamente en viales aerobios un TPH ≥8h predijo la presencia de *P. aeruginosa* en el 37% de los casos (63 de 169), por lo que es recomendable la realización de la prueba de oxidasa.

Conclusiones. La prueba de oxidasa realizada a viales de hemocultivos positivos previamente seleccionados por el TPH y el tipo de medio es una forma fácil y económica de predecir *P. aeruginosa*. En la mayoría de los casos, esto puede contribuir a la optimización del tratamiento antibiótico en menos de 24h.

Palabras clave: Tiempo de positividad del hemocultivo, medio de cultivo, bacteriemia, *Pseudomonas aeruginosa*, prueba de oxidasa.

INTRODUCTION

Pseudomonas aeruginosa is the most prevalent Gram-negative oxidase-positive bacilli involved in hospital acquired infections, it requires specific management due to drug resistance and in bacteremic patients has a higher mortality when compared with other Gram-negative bacilli (GNB)[1]. Prognosis can still be improved in patients with severe sepsis due to GNB who receive inappropriate empirical treatment if therapy is switched to an appropriate one within 48 h of having their cultures drawn[2]. Therefore, the immediate identification of the microorganism in a positive blood culture vial may be of vital importance[3].

Kovac´s oxidase test has been used for decades to identify GNB that produce the enzyme cytochrome oxidase. The procedure consists of rubbing a colony of the organism on a filter paper saturated with oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) which in 10 seconds becomes dark blue if the production of the enzyme is present[4]. On the other hand, GNB time to positivity (TTP), as provided by automatic blood culture processing machines, has been reported to be useful to disclose the kind of microorganism involved[5–7], the presence of resistance[8], endovascular origen[5,9–11] and prognosis of bacteraemia[5,6,12–17]. Furthermore, Defrance et al., described an algorithm to differentiate Enterobacteriaceae, strict anaerobes and *P. aeruginosa* in positive blood cultures integrating information of type of vials and TTP[7].

The aim of the present study was to assess the diagnostic accuracy of the oxidase test performed on GNB positive blood cultures and, in order to avoid laboratory overload, to propose an algorithm of application of the test depending on TTP and type of vial where growth is first detected in patients with GNB monomicrobial bacteraemia.

MATERIAL AND METHODS

From January to December 2011 the Microbiology Laboratory recorded TTP and type of vial and performed the oxidase test in all blood cultures vials positive for GNB obtained at a 700-bed university hospital in Barcelona, Spain. Blood cultures were processed by the BACTEC 9240 system (Becton-Dickinson, MD, USA). Vials with or without resins were used (BACTEC Plus Aerobic/F and BACTEC Plus Anaerobic/F or BACTEC Standard 10 Aerobic/F and BACTEC Lytic /10 Anaerobic/F). Polymicrobial blood cultures were excluded. TTP was defined as the length of time from the beginning of culture incubation to the detection of bacterial growth by the automatic system. For the purpose of the study, the shortest time registered in any positive vial was considered. If a patient had persistent bacteraemia, only the first positive sets of blood cultures were taken into account. To perform the oxidase test 10 mL of blood-broth mixture from the first positive vial of each patient were centrifuged at 1500 rpm for 5 minutes. Then 2 mL of the supernatant were centrifuged at 13000 rpm for 1 minute and the oxidase test was performed on a pellet using Oxidase Reagent test (Biomérieux, France). In order to rule out non-specific reactions, the oxidase test was also performed in forty negative blood culture vials. The GNB were subcultured onto MacConkey and blood agar plates and identified by the Phoenix System (Becton-Dickinson MD, USA) and matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen, Germany).

Medians and interquartile ranges (IQ) were calculated

Table 1 **Results of the oxidase test performed onto positive blood cultures[a].**

Specie	Number of isolates	Number of positive oxidase tests
Oxidase-positive species		
Pseudomonas aeruginosa	66	63
Pseudomonas nitroreducens	2	2
Brevundimonas spp.	1	1
Achromobacter spp.	5	3
Aeromonas sobria	2	0
Vibrio alginolyticus	1	1
Oxidase-negative species		
Escherichia coli	154	0
Klebsiella pneumoniae	40	0
Klebsiella oxytoca	10	0
Enterobacter cloacae	12	0
Enterobacter aerogenes	1	0
Pantoea aglomerans	2	0
Proteus mirabilis	9	0
Serratia marcescens	9	0
Serratia liquefaciens	1	0
Citrobacter freundii	6	0
Citrobacter koseri	2	0
Morganella morganii	3	0
Salmonella typhimurium	2	0
Stenotrophomonas maltophilia	5	0
Acinetobacter spp.	5	0
Bacteroides fragilis	2	0
Fusobacterium nucleatum	1	0

[a]**Performance characteristics of the oxidase test for oxidase producers GNB: Se 90%, E 100%, PPV 100%, NPV 97%. Performance characteristics of the oxidase test for *P. aeruginosa*: Se 95% E 99%, PPV 91 %, NPV 99%.**

for TTP and pairwise comparisons between GNB groups (glucose-fermenters, non-glucose-fermenters and strict anaerobes) were performed with non-parametric Mann-Whitney test. Proportions were compared by the chi-square or Fisher's exact test. Sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) were calculated for the oxidase test to predict oxidase-positive species as well as *P. aeruginosa*. Statistical analysis was done using the SPSS statistical package for Windows (version 20.0; Chicago, IL, USA).

RESULTS

A total of 341 episodes of Gram-negative monomicrobial bacteraemia were analysed.

Results of the oxidase test performed onto positive blood cultures are shown in table 1. All oxidase-negative species as well as all the negative blood cultures used as controls were negative in the direct oxidase test. Among oxidase producers, 3 of 66 (4.5%) *P. aeruginosa*, 2 of 5 (40%) *Achromobacter* spp. and 2 of 2 *Aeromonas sobria* yielded a negative oxidase test, so the performance characteristics were: 90% Se, 100% Sp, 100% PPV and 97% NPV. On the other hand, the performance characteristics of the test to predict *P. aeruginosa* from a blood culture positive for GNB were: 95% Se, 99% E, 91% VPP and 99% VPN.

The prevalence and TTP of the different species are shown in table 2. The median (IQR) TTP for all isolates was 10.7 (8.5-14.7) h. Median (IQR) TTP of glucose-fermenters [9.9 (8.1-12.3) h] was significantly shorter than that of non-glucose-fermenters and strict anaerobes [15.4 (12.4-19.4) h, $p<0.001$].

Growth detection according to the type of vials for each group is shown in table 3. All non-fermenters were detected earlier or exclusively in aerobic vials, but only 113 (44%) of glucose-fermenters met this criterion ($p<0.0001$).

When the GNB was detected exclusively in anaerobic vials, a TTP lower than 19 h ruled out the presence of strict anaerobes. When growth was only or first detected in aerobic vials, a TTP≥8h predicted *P. aeruginosa* in 37% of cases (63 of 169), while a TTP lower than 8 h had a predictive value of 11% (3/28).

Table 2 Prevalence and time to positivity of each specie and group of Gram-negative bacilli.

Microorganism	N (%)	Median in hours (IQR)
Glucose-fermenters	**254 (74)**	**9.9 (8.1-12.3)**
Escherichia coli	154	9.9 (8.3-12.2)
Klebsiella oxytoca	10	8.5 (7.2-11.3)
Klebsiella pneumoniae	40	9.6 (6.6-11.3)
Citrobacter freundii	6	10.2 (7.6-18.5)
Citrobacter koseri	2	11.9 (9.2-11.9)
Enterobacter aerogenes	1	6.1
Enterobacter cloacae	12	10.9 (9.5-14)
Morganella morganii	3	9 (6.1-9)
Proteus mirabilis	9	10.5 (8.1-13.2)
Pantoea aglomerans	2	4.9 (4.7-4-9)
Serratia liquefaciens	1	20
Serratia marscecens	9	14.2 (11.9-29)
Salmonella typhimurium	2	12 (11.2-12)
Aeromona sobria	2	6.2 (6-6.2)
Vibrio alginolyticus	1	6.3
Non-glucose-fermenters	**84 (25)**	**15.3 (12.3-18.9)**
Achromobacter xylosoxidans	5	23.6 (20.8-31.2)
Acinetobacter spp.	5	16 (12.9-20.3)
Brevundimonas spp.	1	58.1
Pseudomonas nitroreducens	2	14.7 (5.3-14.7)
Pseudomonas aeruginosa	66	14.7 (12.2-16.6)
Stenotrophomonas maltophilia	5	21.1 (10.8-21.1)
Strict anaerobes	**3 (1)**	**21 (19.1-21)**
Bacteroides fragilis	2	20 (19-20)
Fusobacterium nucleatum	1	29.6
Total	**341**	**10.7 (8.5-14.7)**

DISCUSSION

The main finding of the present study is that the oxidase test performed onto a positive blood culture has a 90% Se, 100% Sp, 100% PPV and 97% to predict an oxidase producer GNB. In order to obtain these results, two centrifugations, as described in methods section, were needed to separate the bacteria from the other components of the blood culture and avoid false positive cases that may occur when the test is performed on the unprocessed content of the vial. The whole procedure takes around ten minutes. The present study confirms what Sepúlveda et al. reported in 1990 about that a positive oxidase test plus ß-glucuronidase, ß-xylosidase and indole negative tests performed onto the filtrated content of positive blood culture vials were highly predictive of *P. aeruginosa*[18].

Another finding in this study was that TTP of glucose-fermenters is shorter than that of non-glucose-fermenters and strict anaerobes, which is consistent with previous reports[5–7]. Since the system used was the same in the three studies (BACTEC 9240), the absolute values of median TTP for each group were practically the same. Furthermore, in Defrance et al report[7], as in ours, all *P. aeruginosa* were detected earlier or only in aerobic flasks. However, when a higher number (n=693) of *P. aeruginosa* bacteraemia were analysed in our centre, one percent of these were detected only in anaerobic flasks and 7% were detected earlier in anaerobic than in aerobic vials (data not shown). A possible explanation may be that some air could have been accidentally introduced when filling the anaerobic blood culture vial changing the atmosphere to an aerobic one.

Table 3 **Performance of aerobic, anaerobic or both types of vials for the different groups of Gram-negative bacilli included in the study.**

Gram-negative bacilli	N	Growth detection in anaerobic vial only	Growth detection in aerobic vial only	Growth detection in anaerobic and aerobic vials	Growth detection only or earlier in aerobic vials
Glucose-fermenters	254	32 (12%)	35 (14%)	187 (74%)	113 (44%)
Non-glucose-fermenters	84	0	78 (93%)	6 (7%)	84 (100%)
P. aeruginosa	66	0	60 (91%)	6 (9%)	66 (100%)
Strict anaerobes	3	3 (100%)	0	0	0
TOTAL	341	35 (10%)	113 (33%)	193 (57%)	197 (58%)

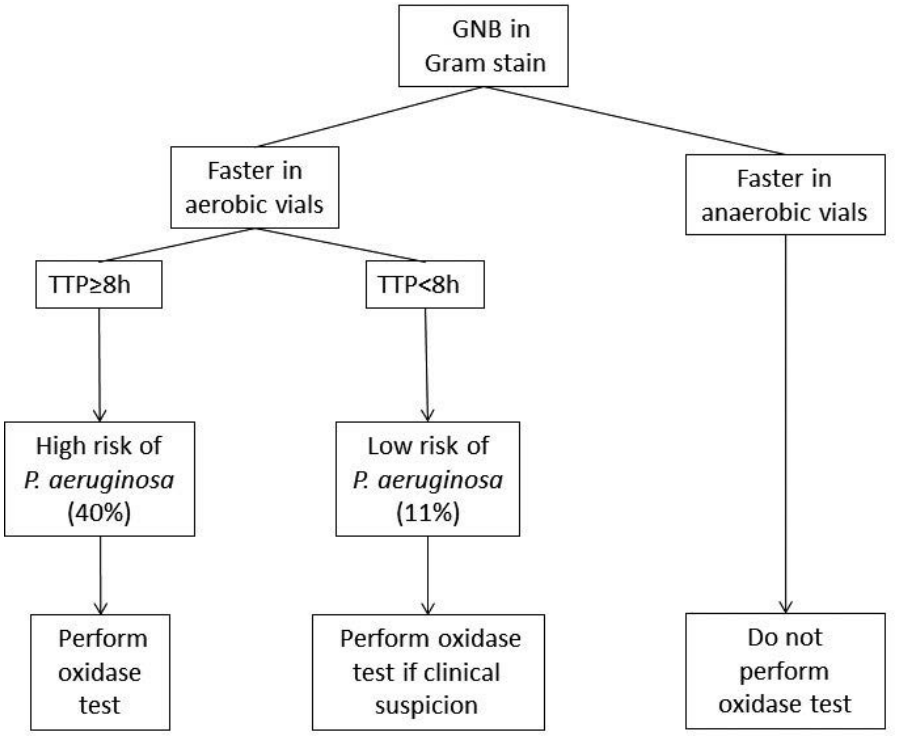

Figure 1 **Algorithm showing when to perform direct oxidase test depending on the type of flask on growth is first detected and time-to-positivity.**

The fact that *P. aeruginosa* may grow earlier or exclusively in anaerobic vials should be taken into account before considering de-escalation of an empirical treatment in severely ill or patients with high risk of *P. aeruginosa* infection.

In order to avoid unnecessary overloading of the laboratory, we recommend not performing the oxidase test when growth detection happens solely or earlier in anaerobic vials. In this manner 42% of tests will be spared. On the other hand, when growth is detected earlier or only in aerobic vials, we suggest to do always the test when the TTP is higher or equal to 8 h since the probability of *P. aeruginosa* is almost 40%, while in less than 8 h, where prevalence is low (11%), the test could be reserved for high-risk patients (figure 1). In any case, the test is fast and inexpensive and can be a useful tool to guide and promptly adjust the treatment for GNB bacteraemia in settings with limited resources where polymerase chain reaction or MALDI-TOF techniques may not be available[19].

The main limitation of this study is the relative low number of GNB bacteraemia included. A higher number of episodes should be analysed in order to determine more accurately the test performance for non-*P. aeruginosa* oxidase producers.

In conclusion, the oxidase test has an excellent sensitivity and specificity to identify *P. aeruginosa* when performed on positive blood culture vials. A positive test rules out the presence of a glucose-fermenter GNB while a negative test practically discards *P. aeruginosa*. In order to minimize laboratory overwork, TTP and type of flask may be useful to decide when to perform the oxidase test. However, the clinical impact of including this test in the lab routine should be evaluated in future studies

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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