



# Assessment of central venous catheter-associated infections using semi-quantitative or quantitative culture methods

Storti, A.<sup>1</sup>; Manzato, A.J.<sup>2</sup>; Pizzolitto, A.C.<sup>1</sup>; Pizzolitto, E.L.<sup>1\*</sup>

<sup>1</sup>Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, UNESP, Araraquara, SP, Brasil.

<sup>2</sup>Departamento de Ciências de Computação e Estatística, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, UNESP, São Jose do Rio Preto, SP, Brasil.

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## ABSTRACT

**Semiquantitative (Maki) and quantitative (Brun-Buisson) culture techniques were employed in the diagnosis of catheter-related bloodstream infections (CRBSI) in patients who have a short-term central venous catheter (inserted for 30 days). The diagnosis of CRBSI was based on the results of semiquantitative and quantitative culture of material from the removed catheters. Catheter tips (118) from 100 patients were evaluated by both methods. Semiquantitative analysis revealed 34 catheters (28.8%) colonized by  $\geq 15$  colony-forming units (cfu), while quantitative cultures (34 catheters, 28.8%) showed the growth of  $\geq 10^3$  cfu/mL. Bacteremia was confirmed in four patients by isolating microorganisms of identical species from both catheters and blood samples. Using the semiquantitative culture technique on short-term central venous catheter tips, we have shown that with a cut-off level of  $\geq 15$  cfu, the technique had 100.0% sensitivity, specificity of 68.4%, 25.0% positive predictive value (PPV) and 100.0% negative predictive value (NPV), efficiency of 71.4% and a prevalence of 9.5%. The quantitative method, with a cut-off limit of  $\geq 10^3$  cfu/mL, gave identical values: the sensitivity was 100.0%, specificity 68.4%, positive predictive value (PPV) 25.0%, negative predictive value (NPV) 100.0%, efficiency 71.4% and prevalence 9.5%. We concluded that the semiquantitative and quantitative culture methods, evaluated in parallel, for the first time in Brazil, have similar sensitivity and specificity.**

*Keywords:* central venous catheter; semi-quantitative culture; quantitative culture; catheter-related bacteremia.

## INTRODUCTION

Central venous catheters are frequently used for the assessment and monitoring of critically ill patients (Kamal et al., 1991). Catheters may be inserted for the administration

of fluids, blood products, medication, nutritional solutions, and for hemodynamic monitoring (Polderman & Girbes, 2002). Central venous catheters pose a greater risk of device-related infections than any other types of medical device (Maki, 1994) and are major causes of morbidity and mortality (Elliot, 1988). They are also the main source of bacteremia and septicemia in hospitalized patients (Elliot, 1993). It has been proved that bacteria have the capacity to adhere to and multiply on the surfaces of catheters (Marrie et al., 1983). Microorganisms attach to surfaces and develop biofilms (Donlan, 2002), which have been shown to be present on central venous catheters on either the outside or the inner lumen (Raad et al., 1993; Donlan & Costerton, 2002). The presence of biofilms is critical; usually such infections can be cured only by removing the catheter (Horvath & Collignon, 2003).

The diagnosis of catheter-related bloodstream infection (CRBSI) requires a positive culture of blood from a peripheral vein and clear evidence that the catheter is the source. The culture of 15 or more colonies forming units (cfu) of a pathogen from a catheter tip is diagnostic of CRBSI (Horvath & Collignon, 2003).

Biofilms on central venous catheters have been detected routinely by a semiquantitative procedure, also termed the roll-plate technique or the "Maki roll", first described in 1977 (Hodge & Puntis, 2002), in which the distal tip of the catheter is removed aseptically and rolled over the surface of a nonselective solid medium, which is thus inoculated with those microorganisms adhering to the outer surface. Catheter colonization is indicated by  $\geq 15$  cfu (Hodge & Puntis, 2002). Estimation of the biofilm population on the catheter tip depends on the number of organisms recovered by contact with the agar surface.

A number of investigators have used the semiquantitative culture of the catheter tip to quantitate biofilms and determine the relationship between bloodstream infection and biofilm formation (Corona et al., 1990; Aufwerber et al., 1991; Anaissie et al., 1995; Maki et al., 1997). The semiquantitative method plays an important role

\*Autor correspondente: Elisabeth Loshchagin Pizzolitto - Coordenadoria de Análises Clínicas e Hemoterapia - Unidade Auxiliar de Estrutura Complexa do Núcleo de Atendimento à Comunidade (CACH-NAC) - Faculdade de Ciências Farmacêuticas - Universidade Estadual Paulista, UNESP - Rua Expedicionários do Brasil, 1621 - CEP: 14801-360 - Araraquara - SP, Brasil - Telefone: (16) 3301-6107 - E-mail: pizzolel@cfar.unesp.br

in the diagnosis of CRBSI. However, since this method only recovers microorganisms attached to the outer surface of the catheter, bacteria growing on the luminal surface of the catheter will not be detected (Hodge & Puntis, 2002), and the method has a positive predictive value of 16-31% because most catheter tip cultures are negative (Maki et al., 1977).

For this reason, other diagnostic approaches have been investigated. In several studies it has been shown that central venous catheter-related infections may also be confirmed after removal of the catheter by the Brun-Buisson et al. (1987) technique, in which the catheter tips are subjected to quantitative culture. This is a sensitive and specific method to detect biofilm and diagnose catheter-related infections. Several methods (semiquantitative and quantitative) have been applied to catheter tips after removal of the device (Maki et al., 1977; Cleri et al., 1980; Linares et al., 1985; Brun-Buisson et al. 1987; Cercenado et al., 1990; Raad et al., 1992; Raad et al., 1994; Bouza et al., 2004; Catton et al, 2005). Culture of the catheter tip by semiquantitative or quantitative methods is the standard microbiological test for the diagnosis of catheter colonization and CRBSI (Rijnders et al., 2002; Bouza et al., 2004).

In Brazil, our group has assessed catheter tip colonization by semiquantitative (Aoki et al., 2004; Storti et al., 2005) or quantitative culture methods (Aoki et al., 2005). The objective of the present study was to examine the effectiveness and practicality of the semiquantitative and the quantitative techniques of central venous catheter tip culture, when employed in parallel in the diagnosis of catheter-related bloodstream infections in patients with short-term central venous catheters.

## **MATERIAL AND METHODS**

### **Sampling procedures**

This study was carried out on 100 catheterized Intensive Care Unit (ICU) patients in hospitals in the State of Sao Paulo (Brazil), from January 2004 to January 2005. One hundred and eighteen catheters were removed and blood samples were taken from peripheral veins before removal of the device.

The patients were divided into III groups. From group I, comprising 30 live patients, 36 catheter tips and 36 blood samples were taken and studied. Group II consisted of 5 patients who subsequently died, and 12 catheter tip and 6 blood samples were examined from patients in this group. Group III comprised 65 deceased patients and 70 catheter tips were taken from this group and studied. All catheters removed were studied by culture.

The data on catheter tips and patients were recorded in accordance with pre-established protocols, as follows: age, sex, date of ICU admission, type of disease on arrival, location of insertion, cause of catheter removal, length of time in ICU, duration of catheter insertion, reason for catheter insertion, suspicion of infection at the site of insertion and

fever after removal. The hospital ethics committee vetted and agreed to this study. Table 1 shows the clinical and demographic characteristics of the patients and the catheter tips.

Samples were collected as follows. A 10-mL sample of blood was collected from a peripheral vein from groups I and II at the time of catheter removal. The intravascular catheters were aseptically removed by the ICU medical team. The tip (distal 5.0 to 6.0 cm of the catheter) was cut off with sterile scissors and sent to the laboratory in a dry sterile Falcon tube (15.0 mL), where it was subsequently sectioned into two 2.0 cm fragments. The two segments were cultured, one by means of the Maki et al. (1977) technique and other by means of the Brun-Buisson et al. (1987) method.

### **Microbiological studies**

#### *Blood cultures*

Qualitative blood cultures were performed using the Oxoid Signal Blood culture system (OXOID®, Hampshire, England).

#### *Semiquantitative technique*

Catheter tips were cultured by the semiquantitative method proposed by Maki et al. (1977). A 2.0 cm catheter segment was placed on a plate of agar containing sheep blood and rolled back and forth at least four times. The culture was incubated at 37°C and examined daily for five days, and resulting colonies were counted. This technique permitted differentiation between colonized (infected) catheters ( 15 cfu per catheter tip) and contaminated catheters ( 15 cfu per catheter tip). The semiquantitative technique distinguishes infection ( 15 cfu) from contamination and is specific in diagnosis of catheter-related infections.

#### *Quantitative technique*

Catheter tips were also cultured by the quantitative method, as described by Brun-Buisson et al. (1987). Sterile water (1mL) was dripped into a 2.0 cm segment that was then placed in a 15.0 mL Falcon tube, to introduce a flow of liquid through the internal lumen of the segment. The tube was vortexed for one minute, and a 0.1 mL fraction of the suspension was removed and spread evenly over the entire surface of a 90-mm diameter 5% sheep blood agar plate. The culture was incubated at 37°C and examined daily for five days in order to observe any bacterial growth. In the quantitative technique, bacterial growth of 103 cfu indicates catheter tip colonization.

#### *Identification of microorganisms*

The microorganisms were identified by standard methods (Murray et al., 2003). Staphylococci were determined with the ID API 20 Staph system (bioMérieux®,

Table 1 - Clinical and demographic characteristics of patients and catheter tips

Patient Characteristics	Group I		Group II		Group III		Total	%
	N	%	N	%	N	%		
Number in each group	30	30.0	5	5.0	65	65.0	100	100.0
Age	(57.8±3.0)		(67.7±3.9)		(67.6±1.9)			
Sex								
Male	18	60.0	4	80.0	34	52.3	56	56.0
Female	12	40.0	1	20.0	31	47.7	44	44.0
Length of time in ICU (days)	(19.9±2.0)		(24.1±2.3)		(6.6±0.8)			
Catheter tips	36	30.5	12*	10.2	70	59.3	118	100.0
Number of catheterizations								
Three	2	6.5	2	40.0	0	0	4	4.0
Two	2	6.5	3	60.0	5	7.7	10	10.0
One	26	87.0	0	0	60	92.3	86	86.0
Site of catheter insertion								
Subclavian vein	29	80.5	12	100.0	65	92.8	106	89.9
Internal jugular vein	5	13.9	0	0	2	2.9	7	5.9
External jugular vein	2	5.6	0	0	2	2.9	4	3.4
Right femoral vein	0	0	0	0	1	1.4	1	0.8
Duration of catheterization (days)	(16.1±1.2)		(16.6±2.1)		(4.2±0.5)			
Motive for catheter removal								
Local inflammation	25	69.4	5	41.7	0	0	30	25.4
Suspension of venous access	4	11.1	1	8.3	0	0	5	4.2
Pain	2	5.6	0	0	0	0	2	1.7
Bad function	5	13.9	0	0	0	0	5	4.2
Death	0	0	6	50.0	70	100.0	76	64.5
Bacteremia								
Positive	3	8.3	1	16.7	0	0	4	9.5
Negative	33	91.7	5	83.3	0	0	38	90.5

Age: (median ± standard error); length of time in ICU: (median±standard error); ICU: intensive care unit; \*(6 alive and 6 died.)

Marcy l'Etoile, France) and Gram-negative bacteria with the BBL CRYSTAL™ Identification Systems Enteric/Nonfermenter ID Kit (BD Biosciences, Becton Dickinson and Company, Sparks, MD United States).

#### Antibiotic susceptibility assay (Disc diffusion)

Disc diffusion tests were performed with Ciprofloxacin (5 µg), Clindamycin (2 µg), Chloramphenicol (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Levofloxacin (5 µg), Oxacillin (1 µg), Penicillin (10 U), Rifampicin (30 µg), Tetracycline (15 µg), Teicoplanin (30 µg), Trimethoprim/sulfamethaxole (1.25/23.75 µg) and Vancomycin (30 µg), the using Kirby-Bauer method (NCCLS, 2003).

**Definitions** (O'Grady et al., 2002; Fraenkel et al., 2000)

Colonized catheter: Colonization was revealed by

the growth of ≥15 bacterial colonies in the semiquantitative culture prepared from the catheter tip (Maki et al., 1977) or ≥10<sup>3</sup> cfu/mL detected in the quantitative culture of the tip (Brun-Buisson et al., 1987).

Catheter-related bacteremia: Blood infection was defined by isolation of the same organism (identical genus, species and antibiotic type) from a semiquantitative or quantitative culture of the catheter tip and from blood taken from the peripheral vein of the patient, together with clinical symptoms of bloodstream infection and no other apparent sources of infection (Raad & Bodey, 1992).

#### Statistical analysis

The Fletcher et al. (2003) method was used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), efficiency and

prevalence. Semiquantitative and quantitative tests were compared by means of the coefficient of linear correlation ( $r$ ) (Zar, 1999), the Kappa correlation, and McNemar tests (Soares & Siqueira, 2002).

## RESULTS

Catheter tips ( $n=118$ ) were all tested by the semiquantitative and quantitative culture techniques. All the catheters assessed were central venous catheters made of Vialon and inserted in the subclavian, femoral, on internal jugular veins, the majority being located in the subclavian vein. The average duration of catheterization, in days, was  $16.1\pm 1.2$  (group I),  $16.2\pm 2.1$  (group II) and  $4.2\pm 0.5$  (group III).

The semi-quantitative and quantitative methods gave similar results. Out of 118 catheters analyzed, 34 (28.8%) showed growth of  $\geq 15$  cfu/plate in the former test and the same number showed  $\geq 10^3$  cfu/mL in the latter; thus, 84 (71.2%) had  $< 15$  cfu/plate or  $< 10^3$  cfu/mL. Distribution of the data obtained from the three groups of patients is shown in Table 2.

From the semi-quantitative plates prepared from the 34 colonized catheters, 55 microorganisms were isolated, 32 (58.2%) being classified as Gram-positive bacteria

(mainly *Staphylococcus aureus*), 19 (34.5%) as Gram-negative and 4 (7.3%) as *Candida species*.

By means of the quantitative catheter culture method, 52 microorganisms were isolated. Of these, 32 (61.6%) were classified as Gram-positive (mainly *Staphylococcus aureus*), 19 (36.5%) as Gram-negative and one (9.0%) as *Candida sp.*

The five microorganisms isolated most commonly from catheter tips were, in decreasing order: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*.

Results regarding the resistance patterns of the *Staphylococcus aureus* isolates from catheter tips and blood cultures are shown in Table 3.

The *Staphylococcus aureus* isolates were oxacillin-resistant, but vancomycin and teicoplanin were active against all *S. aureus* isolates from catheter tips and blood culture.

Of the 34 cultures identified by the semi-quantitative method, 14 (41.2%) were polymicrobial and 20 (58.8%) were monomicrobial. Of the 34 cultures identified by the quantitative method, 13 (38.2%) were polymicrobial and 21 (61.8%) were monomicrobial.

All catheter tips giving positive cultures by the quantitative method were also positive by the semi-quantitative method. A comparison of the two methods

Table 2 - Frequency of culture results using the semi-quantitative and quantitative methods.

Groups	Culture methods					
	semi-quantitative		quantitative		Total	%
	=15 cfu/plate or =10 <sup>3</sup> cfu/ml		<15 cfu/plate or <10 <sup>3</sup> cfu/ml			
n	%	n	%			
GI = alive	14	11.9	22	18.6	36	30.5
GII = alive→deceased	7*	5.9	5**	4.2	12	10.2
GIII = deceased	13	11.0	57	48.4	70	59.3
TOTAL	34	28.8	84	71.2	118	100.0

cfu=colony-forming units; \*(2 alive and 5 deceased); \*\*(4 alive and 1 deceased); G=groups

Table 3 - Resistance pattern of 37 *Staphylococcus aureus* isolates to commonly tested antimicrobial agents as determined by disc diffusion method.

Antibiotics	Abbreviation	Concentration	n and % of the strains		
			S	I	R
Gentamicin	GEN	10 µg	0	0	37(100.0)
Rifampicin	RIF	30 µg	5(13.5)	14(37.8)	18(48.7)
Chloramphenicol	CLO	30 µg	0	0	37(100.0)
Ciprofloxacin	CIP	5 µg	0	0	37(100.0)
Levofloxacin	LVX	5 µg	0	0	37(100.0)
Teicoplanin	TEC	30 µg	37(100.0)	0	0
Vancomycin	VAN	30 µg	37(100.0)	0	0
Clindamycin	CLI	2 µg	0	0	37(100.0)
Erythromycin	ERI	15 µg	0	0	37(100.0)
Oxacillin	OXA	1 µg	0	0	37(100.0)
Penicillin	PEN	10 UI	0	0	37(100.0)
Trimethoprim/sulfamethaxole	SUT	25 µg	0	0	37(100.0)
Tetracycline	TET	30 µg	0	0	37(100.0)

(S)=sensitive (I)=intermediate (R)=resistente

showed a Kappa coefficient of 89% and the McNemar concordance test showed  $\chi^2=0.107$ ,  $p=0.75$ .

Of the 42 catheters paired with blood cultures, in 16 (38.1%) there was a significant development of colony-forming units in both the semi-quantitative and quantitative methods (Table 4).

The data in Table 4 show that the two methods had the same results, with maximal correlation ( $r=1$ ), and that there was neither a significant number of colony-forming units isolated, nor did catheter-associated bacteremia exist, in 26 (61.9%) catheter tips examined by the semi-quantitative or quantitative methods. In 12 (28.5%) cases, (a significant number colony formation of colony-forming units) was found, which did not cause bacteremia. In the four catheter-associated bacteremia cases, the microorganisms isolated from the catheter tips were also isolated from the blood. Two cases showed *Staphylococcus aureus*, one case showed *Stenotrophomonas maltophilia* and remaining case, *Citrobater freundii*. The number of colony-forming units were uncountable by either the semi-quantitative or the quantitative methods. The CRBSI diagnosis for 4 (9.5%) patients with short-term catheter insertion was confirmed by both semi-quantitative and quantitative cultures of their removed catheters. Thus, for short-term central venous catheter insertion, a cut-off point of  $\geq 15$  cfu or  $\geq 10^3$  cfu/mL, together with peripheral blood cultures, has a sensitivity of 100.0%, a specificity of 68.4%, a positive predictive value (PPV) of 25.0%, a negative predictive value (NPV) of 100.0%, efficiency of 71.4% and prevalence of 9.5% for the diagnosis of CRBSI.

## DISCUSSION

Data presented here demonstrated that either the semi-quantitative or quantitative culture method for the assessment of central venous catheter-associated infections can be used as an indicator of bacteremia. These data on both technique tested concomitantly are described for the first time in Brazil.

The semi-quantitative culture of central venous catheter tips shows that catheter-associated bacteremia was only observed in catheters in which semi-quantitative cultures indicated the presence of over 15 cfu/plate. In a study using mostly peripheral venous catheters, Maki et al. (1977) showed that the accepted indicator for the risk of bacteremia

was the presence of at least 15 colonies/plate. Bouza et al. (2004) chose this positivity criterion (15 cfu), because the majority of patients with lower numbers (15 cfu) did not present symptoms of an infection, while all the bacteremia cases had counts above 15 cfu.

Positivity of at least one blood culture was used in the present study as a factor for the interpretation and to establish the diagnosis of catheter-associated infection (Raad & Bodey 1992). The criteria for establishing catheter-associated infection used here were based on studies carried out by Raad & Bodey (1992) and confirmed by Bouza et al. (2003), according to whom a certain microorganism has to be isolated from the catheter tip by the semi-quantitative or quantitative method and also from the peripheral blood. As pointed out by Stratton (1998), the isolated microorganism must be of the same genus and species, and have the same antibiotic sensitivity test profile. In the present study, the isolates of the three species (*S. aureus*, *S. maltophilia* and *C. freundii*) obtained from catheter tips and from blood each had the same profile of antibiotic sensitivity. However, this does not necessarily mean that these bacteria are from the same clone. For this to be verified, molecular genotyping would have to be done. Further studies are planned, in which molecular methods will be applied to these isolates.

According to data presented here the same microorganisms were isolated from the catheter tips by both culture methods and peripheral vein blood. The semiquantitative test showed 100% sensitivity and a specificity of 68.4%. These results differ from those of Collignon et al. (1986), who obtained values of 92% sensitivity and 83% specificity. However, Bouza et al. (2004) observed that the specificity of the semi-quantitative technique was 76%. The current results are also in good agreement with those obtained by Gutiérrez et al. (1992), who showed the method described by Maki et al. (1977) has 100% sensitivity and 76%-79% specificity for the diagnosis of catheter-associated bacteremia. Whereas, Cercenado et al. (1990), noted that the semi-quantitative method, used as a standard, is unable to show the degree of colonization inside the catheter, Fätkenheuer et al. (2003) suggested that the semi-quantitative culture method be considered the standard, even if it only captures bacteria that adhere to the external surface of the catheter. An increase of 15 or more colony-forming units on a plate is considered proof of catheter-associated infection.

Table 4 - Relationship between catheter-related bacteremia and the different methods of catheter culture.

Methods		Catheter-associated bacteremia	
		yes	no
Semi-quantitative	$\geq 15$ cfu/plate	4	12
	$< 15$ cfu/plate	0	26
Quantitative	$\geq 10^3$ cfu/mL	4	12
	$< 10^3$ cfu/mL	0	26

cfu = colony forming units

The present studies demonstrate that the quantitative culture from the catheter tips is able to identify catheter-associated bacteremia. This method is considered a sensitive and reliable method (Blot et al., 1998). The comparison of the semi-quantitative and quantitative methods showed them to give similar results, as described by Brun-Buisson et al. (1987) and, in addition, the quantitative technique proved to be more sensitive, with highly positive predictive values (Blot et al., 1998). The present results showed that the quantitative technique was 100% sensitive in detecting catheter-related bacteremia. Siegman-Ingra et al. (1997) compared six methods, and proved that the most accurate one, with sensitivity and specificity above 90%, was a quantitative method, similar to that of Maki et al. (1977).

The present results show a similar correlation with both methods. The semi-quantitative and quantitative methods are highly sensitive, with 100% negative predictive value, meaning that both methods are appropriate for detecting the absence of catheter-associated bacteremia. In the present study, the positive predictive value was low (25.0%), similar to that reported by Sitges-Serra and Linares (1988), who found that the Maki method showed a low positive predictive value, varying from 10% to 75% depending on the duration of the catheterization. In the present study, the duration of catheterization was  $12.4 \pm 1.3$  days and the positive predictive values of this method were low (16-31%). The majority (71.2%) of the catheter tips showed negative results.

Regarding the specificity of the methods, the values obtained in the present study were lower (65.0%) than expected, compared with those of other authors: (Raad et al. (1992) 86%, Gutierrez et al. (1992) 83%, and Widmer et al. (1992) 96%). The results reported here showed no significant difference between the two techniques in sensitivity, specificity, PPV, NPV, efficiency, or prevalence. Nevertheless, the semi-quantitative method is the more recommended, as it is rapid and easy to perform, routinely, in the clinical microbiology laboratory, as pointed out by Fätkenheur et al. (2003).

Concluding, it can be reported that, in the semi-quantitative and quantitative methods employed in parallel tests here, all cultures from catheters with significant numbers of colony-forming units ( $\geq 15$ cfu/plate for semiquantitative culture or  $\geq 10^3$ cfu/mL for quantitative culture) have a 9.5% probability of indicating catheter-associated infections and high sensitivity. The confidence in both methods is similar, and both techniques can be recommended, although the semi-quantitative method is more widely used in clinical microbiology laboratories, because of its simplicity and low cost.

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#### RESUMO

*Avaliação do cateter venoso central associado à infecção por métodos de cultura semi-quantitativa e quantitativa*

**As técnicas de cultura semiquantitativa (Maki) e quantitativa (Brun-Buisson) foram usadas para o diagnóstico da infecção relacionada ao cateter em pacientes com cateter venoso central de curta duração (30 dias). O diagnóstico da infecção relacionada ao cateter foi baseado nos resultados das culturas semiquantitativa e quantitativa do cateter removido. As pontas de cateter (118) de 100 pacientes foram avaliadas usando ambos os métodos. A análise semi-quantitativa revelou que 34 cateteres (28,8%) tiveram crescimento  $\geq 15$  unidades formadoras de colônias (ufc) e, as culturas quantitativas (34 cateteres, 28,8%) crescimento  $\geq 10^3$  ufc/mL. A bacteriemia foi confirmada em quatro pacientes por isolamento de microrganismos de espécie idêntica, em ambos, cateteres e amostras de sangue. Usando a técnica semi-quantitativa de cultura em cateteres de curta duração, mostra-se que com ponto de corte  $\geq 15$  ufc, a técnica apresentou 100% de sensibilidade, especificidade 68,4%, valor preditivo positivo (VPP) 25,0% e valor preditivo negativo (VPN) 100%, eficiência 71,4% e prevalência 9,5%. Para o método quantitativo usando limite de *cut-off*  $\geq 10^3$  ufc/ml, a sensibilidade foi 100%, especificidade 68,4%, valor preditivo positivo (VPP) 25,0%, valor preditivo negativo (VPN) 100%, eficiência 71,4% e prevalência 9,5%. Conclui-se que as técnicas de cultura semi-quantitativa e quantitativa avaliadas concomitantemente neste estudo, pela primeira vez no Brasil, apresentam especificidade e sensibilidade similares.**

*Palavras-chave:* cateter venoso central; cultura semi-quantitativa; cultura quantitativa; bacteriemia relacionada ao cateter.

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