

# Analysis of an alternative technique for exit site culture in peritoneal dialysis catheters using pediatric blood culture bottles

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## ABSTRACT

**Introduction:** The identification of the causative microorganism of infection at the peritoneal catheter exit site and the subsequent antibiogram will allow the establishment of appropriate antibiotic treatment aimed at avoiding complications.

**Objective:** To describe a new method used in sample collection for exit site culture, microbiological, epidemiological, and clinical results in patients on peritoneal dialysis.

**Material and Method:** We conducted a descriptive, retrospective, and cross-sectional study, in the Peritoneal Dialysis Unit of Complejo Asistencial Universitario de León (Spain), over a period of 18 years. Adult patients with peritoneal catheters, with equivocal/infected exit sites, and sample collection for culture in pediatric blood culture bottles were included. Sociodemographic variables, catheter dwell time, microorganisms, episodes, peritonitis, and technique cost were collected.

**Results:** A total of 331 patients were studied, with a mean catheter dwell time of  $37.44 \pm 107.06$ . Culture was collected from 171 patients, with 385 positive culture samples. A total of 63% were men, with a mean age of  $59.66 \pm 16.35$  years. A total of 465 microorganisms were identified, with 63 mixed cultures. Gram-positive bacteria, 365; Gram-negative, 86; Yeasts, 14. Peritonitis associated with the microorganism isolated at the exit site 41 resulted in 22 catheter removals.

**Conclusions:** This method has proven to be effective in identifying microorganisms. The most frequent: *S. epidermidis* and *Corynebacterium* spp, highlighting *C. amycolatum* with a multi-resistance profile and a tendency to create biofilm. Although this method is more expensive than the swab, it improves efficacy and performance while promoting the recovery of slow-growing microorganisms, avoiding sample handling and contamination, as well as false negatives in patients on antibiotic therapy.

**Keywords:** chronic kidney disease; peritoneal dialysis; blood culture; microorganisms; peritonitis.

## RESUMEN

**Análisis de una técnica alternativa para el cultivo del orificio de salida en catéteres de diálisis peritoneal utilizando frascos de hemocultivo pediátrico**

**Introducción:** La identificación del microorganismo causal de infección en orificio de salida catéter peritoneal y posterior antibiograma, permitirá instaurar tratamiento antibiótico adecuado dirigido para evitar complicaciones.

**Objetivo:** Describir nuevo método empleado en la recogida de muestras para cultivo del orificio de salida, resultados microbiológicos, epidemiológicos y clínicos en pacientes en diálisis peritoneal.

**Material y Método:** Estudio descriptivo, retrospectivo y transversal, en la Unidad de Diálisis Peritoneal del Complejo Asistencial Universitario de León, en un periodo de 18 años. Se incluyeron pacientes adultos portadores de catéter peritoneal, con orificio de salida equivoco/infectado y recogida de muestra para cultivo, en botella de hemocultivo pediátrico. Se recogieron variables sociodemográficas, tiempo permanencia catéter, microorganismos, episodios, peritonitis y coste de técnica.

**Resultados:** Se estudiaron 331 pacientes, tiempo medio de catéter 37,44±107,06. Se recogió cultivo a 171 pacientes, 385 muestras cultivo positivo. 63% varones, edad media 59,66±16,35 años. Identificados 465 microorganismos, 63 cultivos mixtos. Bacterias Grampositivas 365, Gramnegativas 86, Levaduras 14. Peritonitis asociadas al microorganismo aislado en orificio 41, ocasionando 22 retiradas de catéter.

**Conclusiones:** Este método ha demostrado ser efectivo, en identificación de microorganismos. Los más frecuentes: *S. Epidérmidis* y *Corynebacterium SPP*, destacando *C. Amycolatum* con perfil de multiresistencia y tendencia a crear biofilm. Aunque este método es mas caro que el hisopo, mejora el rendimiento y eficacia, favorece la recuperación de microorganismos de crecimiento lento, evita manipulación muestras y contaminación, evita falsos negativos en pacientes en tratamiento antibiótico.

**Palabras clave:** enfermedad renal crónica; diálisis peritoneal; hemocultivo; microorganismos; peritonitis.

## INTRODUCTION

Exit-site infection (ESI) of the peritoneal catheter is a major complication in peritoneal dialysis, being a risk factor for peritonitis, catheter removal, and technique failure<sup>1</sup>.

The prevention and treatment of ESIs have been a serious concern since the early days of the technique. The International Society for Peritoneal Dialysis (ISPD) first published guidelines in 1983 with recommendations for the prevention and treatment of ESIs associated with the peritoneal catheter and has issued subsequent updates<sup>2</sup>. The recently published recommendations have reduced the infection rate <0.40 episodes/year<sup>1</sup>.

In 1997, Twardowski et al. established a histomorphological classification to standardize criteria regarding the condition of the exit-site (ES), defining seven categories: perfect, good, equivocal, acute infection, chronic infection, external cuff infection, and traumatized<sup>3</sup>.

According to ISPD recommendations, exit-site infection is defined as the presence of purulent discharge, with or without erythema of the skin at the catheter-epidermis interface<sup>4</sup>.

The clinical guidelines of the Spanish Society of Nephrology for the prevention and treatment of peritoneal infection in peritoneal dialysis recommends, with an evidence level of 1C, the monitoring of the peritoneal catheter skin exit-site, establishing early treatment of catheter-associated infections to prevent their progression to peritoneal infection<sup>5</sup>.

Therefore, identifying the causative microorganism and performing subsequent antibiotic susceptibility testing is vital to initiate targeted antibiotic treatment and prevent complications. When no organism is identified after culture of purulent discharge from an ES swab, the diagnosis is negative-culture ESI<sup>1</sup>.

Sample collection is typically performed using a sterile swab. To improve culture yield and reduce the number of negative cultures, an alternative technique has been used that involves inoculating the sample into aerobic/anaerobic blood culture bottles (pediatric bottle).

Our aim was to describe a new method used for collecting exit-site culture samples, presenting the microbiological, epidemiological, and clinical results in peritoneal dialysis patients.

## MATERIAL AND METHOD

**Study design and period:** We conducted a descriptive, retrospective, cross-sectional study at the Peritoneal Dialysis Unit of Complejo Asistencial Universitario de León over an 18-year period, from March 2006 through March 2024.

**Sample:** Inclusion criteria were: adult patients with advanced chronic kidney disease (CKD) carrying a peritoneal catheter, presenting an equivocal/infected ES according to Twardowski's classification, and sample collection performed using aerobic/anaerobic pediatric blood culture bottles. Pediatric patients were excluded.

**Variables:** Sociodemographic and clinical variables were collected: age, sex, catheter dwell time, microorganisms, number of episodes, ESIs associated with peritonitis, and estimated technique cost. ESI was considered the cause of peritonitis when the same organism was isolated from both the ES culture and peritoneal fluid.

**Data collection:** Conducted in the second half of 2024. Data were collected retrospectively, with cut-off points set from March 25, 2006 through March 24, 2024, covering an 18-year study period.

**Sample collection method:** The ES culture collection protocol is as follows:

- Clean the pericatheter area with sterile gauze and 0.9% saline.
- Draw 0.1 mL of saline with a sterile syringe and needle (1 ml syringe with dead-space-free cone).
- Lift the catheter to expose the ES tract.

- Irrigate the ES tract with saline.
- Aspirate the saline, attempting to recover as much as possible.
- Attach a new needle to the syringe and inoculate the sample into a pediatric aerobic/anaerobic blood culture bottle (BACTEC FX, Becton Dickinson). For optimal use, perform multiple rinses with the culture medium.

BACTEC FX is a non-invasive blood culture system that continuously monitors, agitates, and incubates the bottles. When microorganisms are present, they metabolize nutrients and release carbon dioxide (CO<sub>2</sub>). A dye sensor at the bottom of the vial reacts to CO<sub>2</sub>. Photodetectors at each station measure fluorescence levels, which correspond to CO<sub>2</sub> production. The system interprets these readings according to pre-set positivity parameters<sup>6</sup>.

**Data analysis:** Microbiological data were obtained from the clinical microbiology lab software (Servolab, Siemens SA), and epidemiological data from the Versia<sup>®</sup> software program, with analysis using JASP statistical software. Measures of central tendency and dispersion were calculated for quantitative variables (expressed as means and standard deviations), and absolute frequencies and percentages for qualitative variables.

**Ethical considerations:** No personal data capable of identifying patients directly or indirectly were included, in full compliance with ethical and universal principles, international data protection standards, and current Spanish legislation. The study adhered to the Declaration of Helsinki and took into account applicable ethical and legal guidelines for biomedical research, as per Regulation (EU) 2018/1725 of the European Parliament and Council (October 23, 2018), and Organic Law 3/2018 (December 5), on Personal Data Protection and Digital Rights.

## RESULTS

We studied 331 patients with a mean catheter duration of 37.44±107.06 months. Cultures were collected from 171 patients, 6 of whom had not yet started peritoneal dialysis. A total of 63% were men (n=108) with a mean age of 59.66±16.35 years. A total of 471 samples were collected; 385 tested positive and 86, negative. Of the negative samples, 79 were classified as equivocal ES, and 7 as having clinical signs of ESI. The rate of negative cultures in acute infection ES was 1.78%. In 83 patients, >1 positive culture sample was collected (minimum: 2 - maximum: 16). A total of 465 microorganisms were identified. Sixty-three samples were mixed cultures: 57 had two microorganisms, 4 had 3, and 2 had 4.

Among the microorganisms, 365 were Gram-positive bacteria, with the most common being *Staphylococcus epidermidis* (n=113), *Corynebacterium spp.* (n=97), of which 74.2% were *C. amycolatum*, and *Staphylococcus aureus* (n=63).

There were 86 Gram-negative bacteria, primarily *Pseudomonas aeruginosa* (n=50) and *Escherichia coli* (n=7), and 14 yeasts, with *Candida parapsilosis* (n=12) being the most frequent. Full analysis is shown in **table 1**.

We identified a total of 41 peritoneal infections associated with the organism isolated from the exit-site: 48.8% were caused by *P. aeruginosa* and 39% by *S. aureus*. Full results are shown in **table 2**. These peritoneal infections resulted in 22 catheter removals, mostly due to *P. aeruginosa* and *S. aureus*.

Cost analysis comparing the 2 sample collection methods showed the per-sample cost for the blood culture bottle method was €29.49 vs €25.93 for swab collection. Cost breakdown is shown in **table 3**.

**Table 1.** Isolated Microorganisms.

GRAM-POSITIVE BACTERIA	Count	Percentage
<i>Actinomyces neuii</i>	1	0.215%
<i>BGP</i>	1	0.215%
<i>Brevibacterium paucivorans</i>	1	0.215%
<i>Brevibacterium ravenspurgensense</i>	2	0.43%
<b><i>Corynebacterium amycolatum</i></b>	<b>72</b>	<b>15.484%</b>
<i>Corynebacterium aurimucosum</i>	8	1.720%
<i>Corynebacterium confusum</i>	1	0.215%
<i>Corynebacterium jeikeium</i>	1	0.215%
<i>Corynebacterium minutissimum</i>	4	0.860%
<i>Corynebacterium simulans</i>	1	0.215%
<i>Corynebacterium sp</i>	6	1.290%
<i>Corynebacterium striatum</i>	4	0.860%
<i>Dermabacter hominis</i>	6	1.290%
<i>Enterococcus faecalis</i>	7	1.505%
<i>Enterococcus faecium</i>	1	0.215%
<i>Coagulase-negative staphylococci</i>	4	0.860%
<i>Micrococcus luteus</i>	1	0.215%
<i>Staph. hominis-hominis</i>	8	1.720%
<b><i>Staphylococcus aureus</i></b>	<b>63</b>	<b>13.548%</b>
<i>Staphylococcus auricularis</i>	2	0.430%
<i>Staphylococcus capitis</i>	15	3.230%
<b><i>Staphylococcus epidermidis</i></b>	<b>113</b>	<b>24.301%</b>
<i>Staphylococcus haemolyticus</i>	9	1.935%
<i>Staphylococcus hominis</i>	6	1.290%
<i>Staphylococcus intermedius</i>	1	0.215%
<i>Staphylococcus lugdunensis</i>	6	1.290%
<i>Staphylococcus pasteurii</i>	1	0.215%
<i>Staphylococcus pettenkoferi</i>	1	0.215%
<i>Staphylococcus schleiferi</i>	1	0.215%
<i>Staphylococcus simulans</i>	4	0.860%
<i>Staphylococcus warneri</i>	9	1.935%
<i>Staphylococcus xylosus</i>	2	0.430%
<i>Streptococcus agalactiae</i>	3	0.645%

GRAM-NEGATIVE BACTERIA	Count	Percentage
<i>Acinetobacter lwoffii</i>	3	0.645%
<i>Enterobacter agglomerans</i>	1	0.215%
<i>Enterobacter asburiae</i>	1	0.215%
<i>Enterobacter cloacae</i>	10	2.151%
<i>Enterobacter ludwigii</i>	1	0.215%
<i>Escherichia coli</i>	7	1.505%
<i>Klebsiella ornithinolytica</i>	1	0.215%
<i>Klebsiella oxytoca</i>	1	0.215%
<i>Klebsiella pneumoniae</i>	1	0.215%
<i>Leclercia adecarboxylata</i>	1	0.215%
<i>Proteus mirabilis</i>	2	0.430%
<i>Proteus vulgaris</i>	1	0.215%
<b><i>Pseudomonas aeruginosa</i></b>	<b>51</b>	<b>10.968%</b>
<i>Serratia liquefaciens</i>	1	0.215%
<i>Serratia marcescens</i>	1	0.215%
<i>Stenotrophomonas (X.) maltophilia</i>	3	0.645%
YEASTS	Count	Percentage
<i>Candida glabrata</i>	1	0.215%
<i>Candida metapsilosis</i>	1	0.215%
<i>Candida parapsilosis</i>	12	2.581%

**Table 2.** Microorganisms causing Infections.

Microorganismos causantes de IP	N	%
<i>Candida parapsilosis</i>	1	2.44%
<i>Corynebacterium sp</i>	1	2.44%
<i>Escherichia coli</i>	1	2.44%
<b><i>Pseudomonas aeruginosa</i></b>	<b>20</b>	<b>48.8%</b>
<i>Serratia liquefaciens</i>	1	2.44%
<b><i>Staphylococcus aureus</i></b>	<b>16</b>	<b>39%</b>
<i>Staphylococcus lugdunensis</i>	1	2.44%

## DISCUSSION

The method of sample collection by flushing the exit-site peritoneal catheter (ESPC) and subsequent inoculation into a pediatric blood culture bottle (aerobic/anaerobic) proved effective in identifying microorganisms at the ESPC. A similar method was used by Twardowski et al. in 1996 in their validation and classification study of ES, sampling the ES tract for bacteria and cells<sup>7</sup>.

The most frequently isolated microorganisms were *S. epidermidis* and *Corynebacterium spp.*, notably *C. amycolatum* with a multidrug-resistant profile. These organisms, commonly found on skin and mucosa<sup>8</sup>, tend to form biofilms on the catheter lumen, making them difficult to eradicate due to high antibiotic resistance<sup>9,10</sup>. Biofilm-forming bacteria are potential sources of recurrent peritonitis and are

**Table 3.** Estimated Price of Both Techniques.

Item	Precio estimado	
	Swab (€)	Pediatric Blood Culture Bottle * (€)
Saline 0.9%	0.25	0.25
Swab	0.19	-
Pediatric Blood Culture Bottle	-	3.50
Gloves	0.47	0.47
Insulin Syringe	-	0.13
Needles x 2	-	0.12
Mask	0.02	0.02
Blood Agar Plate	0.37	-
Mannitol Agar Plate	0.25	0.25
Chocolate Agar Plate	0.26	0.26
MacConkey Agar Plate	0.25	0.25
ATB ID**	23.87	23.87
<b>Total</b>	<b>25.93</b>	<b>29.49</b>

\* Pediatric blood culture bottle – aerobic/anaerobic.

\*\* ID ATB: automated identification and antibiotic susceptibility testing system.

linked to high catheter loss rates<sup>11,12</sup>. Other highly prevalent pathogens in our samples were *S. aureus* and *Pseudomonas spp.*, which are more virulent and frequently cause peritoneal infections<sup>4</sup>. In our series, these pathogens were responsible for the majority of peritoneal infections (87.8%), leading to high peritoneal catheter removal rates<sup>13,14</sup>. This result aligns with other studies demonstrating a strong association between ESIs and the development of peritonitis in PD patients, especially with these pathogens<sup>15</sup>.

Many studies support a strong association between ESIs and subsequent peritonitis<sup>16,19</sup>. Therefore, it is reasonable to assume that preventing ESIs and promptly treating infections at the ES and subcutaneous tunnel can reduce peritonitis rates. We found limited literature addressing negative cultures from ES samples. In 2005, Bernardini et al., in a comparative study of ES care with mupirocin vs gentamicin, reported negative culture rates of 0.06 vs 0.03 per year<sup>20</sup>. In 2012, Van Diepen et al. reported an 11.4% rate of negative cultures in ES with infection criteria<sup>21</sup>. In 2021, Sanchidrián et al. reported no negative cultures in ES with clinical signs of ESI<sup>8</sup>. In our study, the rate of negative cultures was low at only 1.78%, supported by a much larger sample size and collection period than previous studies.

According to Akoh JA, in his publication on infections related to peritoneal dialysis, any purulent discharge from the ES should be cultured and Gram-stained<sup>22</sup>. Monitoring and follow-up of the ES are essential for early detection of infection signs. Microbiological evaluation helps implement targeted antibiotic treatment early and avoids underdiagnosing infections by mistaking skin-resident pathogens for contaminants.

Having multiple effective sample collection methods improves microorganism identification, reduces negative cultures, and enables earlier intervention. Some studies suggest early, appropriate antibiotic administration improves outcomes by reducing catheter removal due to peritonitis and lowering mortality risk<sup>23,24</sup>.

Cost analysis showed that while blood culture bottles are slightly more expensive than swabs, they may improve diagnostic performance and efficiency. This method reduces sample handling (thus contamination risk), provides continuous microorganism growth monitoring with automated detection every 10 minutes, and enhances recovery of slow-growing organisms<sup>6</sup>. Additionally, pediatric culture bottles contain antibiotic-binding agents to prevent false negatives in patients on antibiotic treatment<sup>25</sup>.

**Limitations:** This study evaluated microorganism detection using a single technique. Prospective comparative studies between both techniques (blood culture and swab) would be beneficial to assess validity and provide usage recommendations.

**Practical considerations:** Collecting samples in pediatric blood culture bottles may serve as an alternative to swabs as an initial method or second-line option in cases of negative cultures.

In light of our results, we can conclude that this method of blood culture bottle sample collection is effective in identifying microorganisms at the peritoneal catheter ES, reduces the time to detect positive cultures, and may allow earlier targeted antibiotic treatment and prevention of complications.

Although this method is more expensive than swabbing, it may improve performance and diagnostic yield.

### Conflicts of interest

The authors declare no conflicts of interest related to the research, authorship, and/or publication of this manuscript.

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