



## Diagnostic Study

## Evaluation of different methods to improve culture-negative peritoneal dialysis-related peritonitis: A single-center study

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## ABSTRACT

**Background:** ISPD recommends culture-negative peritonitis in each peritoneal dialysis (PD) center should less than 15%. The hospital in Thailand, however faced a significantly high rate of culture negative peritonitis, even using blood culture bottles technique. This study evaluates the performance of three different culture methods in detecting organisms in PD related peritonitis.

**Methods:** A prospective cohort diagnostic study was performed in PD patients diagnosed with PD related peritonitis in Surin Hospital from October 2018 to June 2020. The Diagnosis of peritonitis was followed ISPD guidelines. PDF sample from each patient was processed by three different blood culture bottle-based techniques, including i) 50 ml PDF centrifugation, and ii) 10 ml PDF centrifugation before inoculated into blood culture bottles, and iii) inoculation into blood culture bottles without centrifugation. The sensitivities and isolated organisms were compared among the individual methods.

**Results:** Of 126 PD patients with clinical peritonitis, PD related peritonitis was diagnosed in 87 patients with 105 PDF analysis. PDF culture showed gram-positive organisms 34%, gram-negative organisms 41%, fungal 5.71%, and culture-negative result in 22.86%. The direct blood culture method was positive in 59.05%, while centrifugation before inoculated into blood culture bottles, has a higher percentage of positive results, 60.95% and 64.76% from 10 ml to 50 ml PDF volume; respectively. The sensitivity was 84% and 76.5% for 50 ml PDF centrifugation and blood culture without centrifugation.

**Conclusion:** Large volume PDF centrifugation before inoculating into blood culture bottles may improve the positive culture rate in PD related peritonitis.

## 1. Background

Peritonitis is the common complication in peritoneal dialysis (PD), which impact patient survival [1–4]. Moreover, PD related peritonitis is the major cause of technical failure in PD and lead to transfer to hemodialysis [5,6]. Early diagnosis and prompt antibiotic initiation are vital clues for treatment success. Collection of PDF for bacterial culture is routinely performed in all patients with suspected CAPD peritonitis. As such, an accurate culture technique is of paramount importance to provide a guideline for an appropriate antibiotic selection. ISPD guideline recommend that culture-negative peritonitis should not represent more than 15% of episodes [5]. The administration of antibiotics before collecting peritoneal dialysis fluid (PDF) is one of the common causes of negative cultures [7,8]. Another important cause of negative culture is

the culture techniques [8]. However, traditional techniques often return negative results, with culture-negative peritonitis being diagnosed in up to 58.1% of cases. (8) For instance, the use of a hemoculture bottle was shown to provide a higher diagnostic yield than using a culture dish [9]. A higher yield could also be achieved by pretreating a PDF sample (e.g., centrifugation) before transferring into a hemoculture bottle [10,11].

Several methods to improve the testing efficiency of PDF culture in suspected peritonitis have been developed. The dialysis fluid can be directly collected into the hemoculture bottle following the guidelines. To further improve the diagnostic yield, the pre-treatment of PDF via centrifugation may be used. In this technique, the sediment collected through centrifugation of 50 mL of PDF is transferred into a hemoculture tube for subsequent determination of microorganisms. This additional step, together with an adoption of updated treatment guidelines, was

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found to reduce the rate of culture-negative peritonitis to 22% [12]. However, the implementation of this centrifugation technique in community hospitals is the major challenge due to limited laboratory facilities. One of the variations to overcome this limitation is to lower PDF volume (e.g. ~10–15 mL) [5].

To our knowledge, there is no previous study that systematically compares the difference in the diagnostic yield among different culture methods for PDF analysis. Therefore, this study aims to fill in this gap in order to find a suitable method that displays a diagnostic yield similar to the standard method and at the same time saves cost and resources, which will be highly beneficial in low-resource facilities.

## 2. Material and methods

### 2.1. Patients and study design

This prospective diagnostic cohort study aimed to compare the diagnostic yields of three culture techniques. Patients with an age of 15 years and above who were suspected of having CAPD peritonitis at Surin hospital were enrolled between November 2018 and May 2020. The diagnosis of PD-associated peritonitis needs at least two of the following features: (1) clinical features of peritonitis, i.e., abdominal pain or cloudy dialysis effluent; (2) dialysis effluent white cell count  $>100/\mu\text{l}$  (after a dwell time of at least 2 h), with  $>50\%$  neutrophils; and (3) positive dialysis effluent culture [5].

The fluid from the dialysis bag was centrifuged at 3,000 rpm for 10 min (KUBOTA 5420, China) and the sediment of 5 mL was injected into hemoculture bottle and incubated at  $37^\circ\text{C}$  (Virtuo BacT/Alert, France). PDF in the hemoculture bottle was removed and plated onto a blood agar plate, a MacConkey agar plate and chocolate agar plate. The plates were then incubated at  $37^\circ\text{C}$  for 24 h under  $5\% \text{CO}_2$  atmosphere. Microorganisms were identified, and susceptibilities were determined by the standard methods. Three culture methods were done with details as follows.

Method 1: 50 mL of dialysis fluid was collected and centrifuged at 3,000 rpm. Then, 5–10 mL of the sediment was collected into a blood culture bottle and transferred into an agar plate.

Method 2: 10–15 mL of dialysis fluid was collected and centrifuged at 3,000 rpm. Then, 5 mL of the sediment was collected into a blood culture bottle and transferred into an agar plate.

Method 3: 10–15 mL of dialysis fluid was directly collected into a blood culture bottle without prior centrifugation and transferred to an agar plate (standard method).

Relevant clinical information was collected from the patients' medical records and referral letter before and after the culture of the fluid was performed. The study was approved by Human research Ethic Committee, Medical Staff Organization, Surin Hospital Ethic number 62/2561. The study was registered at [researchregistry.com](https://www.researchregistry.com) via unique identifying number (UIN) researchregistry 6389. This study has been reported in line with the STROCSS criteria [13].

### 2.2. Statistical analysis

Descriptive statistics including frequency, percentage, mean, standard deviation was used to summarize and present the data. McNemar test was used to compare the positivity yield of the culture methods. Diagnostic accuracy measures including sensitivity and specificity were calculated using composite culture results as the reference standard.

## 3. Results

Peritoneal dialysis fluid culture was done in a total of 126 patients. Among these, 87 patients were diagnosed with CAPD peritonitis. Culture was done 105 times in this group of patients (Fig. 1).

PDF culture was performed in a total of 126 patients. Eighty-seven patients were diagnosed with CAPD peritonitis (averaged age = 59.04 years; 45 females; 81 males). Culture was done 105 times in this group of patients. Among the 75 positive culture tests, 34 were gram positive and

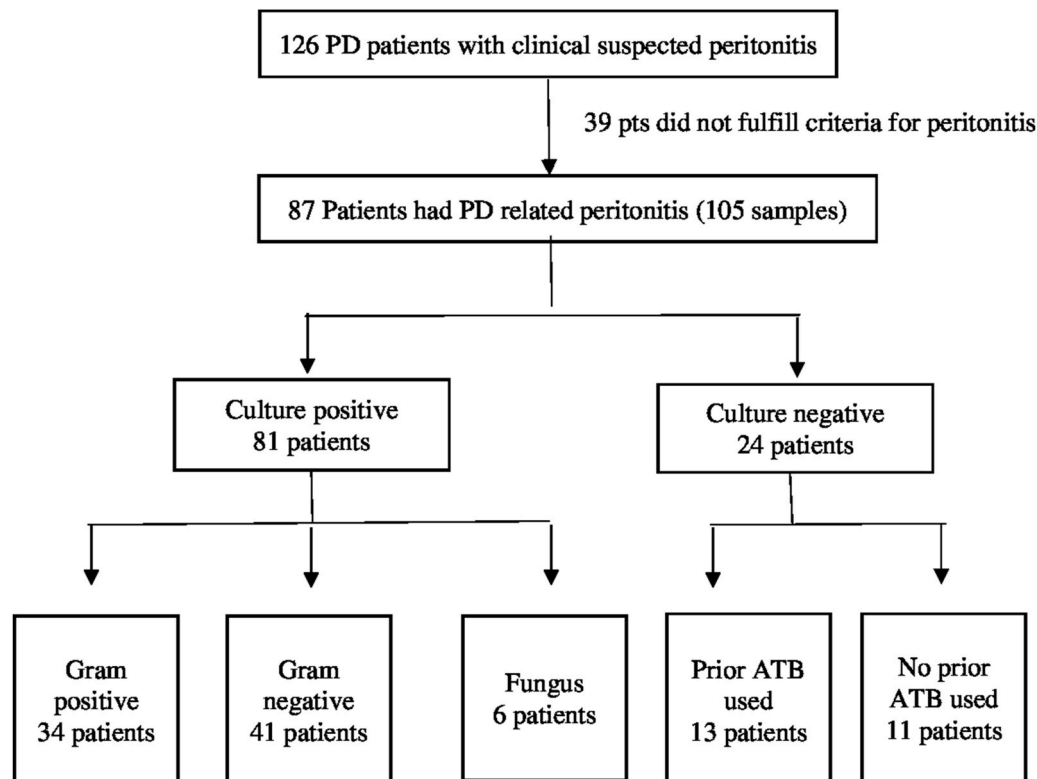


Fig. 1. Flow diagram of patient enrollment.

41 were gram negative. Three patients were infected with more than one pathogen. The three most common pathogens were *Staphylococcus*, *Klebsiella*, and *E. coli*. Six tests (5.71%) were positive for fungal infection. Table 1 summarizes the results of the microbial culture tests. On the other hand, the culture was tested negative in 24 patients (22.86%), where 13 patients received prior antibiotics and 11 did not. Thirty-eight patients received antibiotics within 30 days of diagnosis of peritonitis.

Comparing the three culture methods showed that using a blood culture bottle alone was positive in 62 of 105 tests (59.05%), using 10–15 mL of centrifuged dialysis fluid was positive in 64 of 105 tests (60.95%), and using 50 mL of centrifuged dialysis fluid yielded the highest positivity rate of 68 of 105 tests (64.76%). The sensitivity was highest in the 50 mL centrifugation method (84%) and lowest in the 10–15 mL centrifugation method (76.5%). However, the difference among the three methods was not statistically significant (i.e., p-value = 0.2008 for 50 mL centrifugation vs. 10–15 mL centrifugation; p-value = 0.3711 for 50 mL centrifugation vs. 10–15 mL centrifugation). The diagnostic yield and sensitivity of each culture method is shown in Table 2.

#### 4. Discussion

The present study showed a considerably low culture-negative rate of 22.86% in comparison to previous studies [14]. Among patients with positive-culture tests, there were 32% who received prior antibiotic treatment. Excluding these patients, the culture-negative rate decreased to only 10.47%. In the culture-positive group, both high and low white blood cell counts were detected in the PDF. This result is in contrast to the previous study by Males which reported that a culture was more likely to be positive when white blood cell count exceeded 500 cells [14]. This difference may be explained by our inclusion criteria, which adhered to the recommendations from the ISPD guideline, the antibiotic treatments prior to the PDF collection.

Our study showed that the culture method using 50 mL of centrifuged dialysis fluid led to the highest positivity yield, which is in agreement with previous studies [15,16]. It is of note that whether the additional centrifugation step is of advantage in improving the positivity rate of PDF analysis is still controversy [17]. Furthermore, we found that the additional centrifugation step resulted in a higher positivity rate in both 50 mL and 10–15 mL specimens, compared with the culture without centrifugation. However, this difference was not statistically significant. Considering that using 50 mL of centrifuged dialysis fluid was 8% higher in terms of positivity when compared to the culture without centrifugation, there is a trend that using a higher volume of dialysis fluid (even only 10–15 mL) and centrifugation would likely improve the positivity yield. Other factors that may affect culture positivity also needs to be taken into account.

**Table 1**

Results of peritoneal dialysis fluid culture among patients diagnosed with PD related peritonitis.

Organisms	Number of cultures	% of all cultures
No growth	24	22.86
Gram positive	34	32.39
Staphylococcus gr	16	15.24
Streptococcus gr	4	3.81
Enterococcus	4	3.81
Corynebacterium	3	2.86
Bacillus	7	6.67
Gram negative	41	39.05
E.Coli	11	10.48
Klebsellar pneumoniae	13	12.38
Pseudomonas	3	2.86
Acinetobacter	7	6.67
Enterobacter	5	4.76
Serratia	2	1.9
Fungus	6	5.71

**Table 2**

Positivity yield and sensitivity of three culture methods.

Method	% of culture positive	Sensitivity	95% confidence interval
Hemoculture bottle without centrifugation	59.05%	76.5%	65.8–85.2
Centrifugation 10 ml + Hemoculture bottle	60.95%	79%	68.5–87.3
Centrifugation 50 ml + Hemoculture bottle	64.76%	84%	74.1–91.2

Unfortunately, there were a few limitations in our study. Firstly, it is single-center nature with small patient numbers. Secondly, some patients received antibiotic before specimen collection, which may interfere sensitivity and specificity accuracy of each method. The future multicenter prospective cohort that included larger patient number may be need to evaluate the performance of each method.

#### 5. Conclusion

The centrifugation of the dialysis fluid before transferring to a hemoculture bottle may help improve the positivity rate in facilities where there is a high rate of culture-negative CAPD peritonitis and in community hospitals that are not equipped to centrifuge large volume of fluid.

#### Ethics approval and consent to participate

The study was approved by Human research Ethic Committee, Medical Staff Organization, Surin Hospital Ethic number 62/2561.

#### Research registration unique identifying number (UIN)

Name of the registry: Research Registry.

Unique Identifying number or registration ID: researchregistry6389.

Hyperlink to your specific registration: <https://www.researchregistry.com/browse-the-registry#home/registrationdetails/5fe225fbbd59cc001bc692cf/>

#### Availability of data and materials

Further clinical data are available from the corresponding author upon reasonable request.

#### Provenance and peer review

Not commissioned, externally peer-reviewed.

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None.

#### Authors' contributions

Dr. Tanratananon conceptualized, collected and analyzed data, drafted, reviewed and revised the manuscript. Deekae analyzed PDF culture. Dr. Sukit reviewed the manuscript. Dr. Srithongkul contributed to the concept, drafted and critically reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

### Declaration of competing interest

All authors have no relevant financial interests or conflicts of interest to report.

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