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ORIGINAL ARTICLE

# Polymerase chain reaction for *Enterococcus faecalis* in drain fluid: the first screening test for symptomatic colorectal anastomotic leakage. The Appeal-study: Analysis of Parameters Predictive for Evident Anastomotic Leakage

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**Abstract**

*Purpose* With current diagnostic methods, the majority of patients with symptomatic colorectal anastomotic leakage (CAL) is identified approximately 1 week after operation. The aim of this study is to determine whether real-time polymerase chain reaction (RT-PCR) for detection of *Escherichia coli* and *Enterococcus faecalis* on drain fluid can serve as a screening test for CAL in the early postoperative phase.

*Methods* All patients included in this multicenter prospective observational study underwent left-sided colorectal resection for both malignant and benign diseases with construction of an anastomosis. In all patients, an intra-abdominal drain was placed during operation. During the first five postoperative days, drain fluid was processed for RT-PCR. The quantitative results of the RT-PCR on days 2 to 5 were compared to the results of day 1 in order to detect concentration changes.

*Results* In total, 243 patients, with both benign and malignant diseases, were included of whom 19 (7.8 %) developed symptomatic CAL. An increase in *E. coli* concentration was found in significantly more patients with CAL on day 4 and 5 [$p = 0.0004$; diagnostic odds ratio (DOR) 7.9]. For *E. faecalis*, this result was found for days 2, 3, and 4 ($p < 0.003$) with highest DOR on day 3 (31.6). Sensitivity and negative predictive values were 92.9 and 98.7 %, respectively, virtually ruling out CAL in case of negative test results on the third postoperative day.

*Conclusion* Quantitative PCR for *E. faecalis* performed on drain fluid may be an objective, affordable and fast screening tool for symptomatic colorectal anastomotic leakage.

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**Keywords** Colorectal surgery · Anastomotic leakage · Real-time polymerase chain reaction · *E. faecalis* · Screening

## Introduction

Despite the vast body of evidence concerning colorectal anastomotic leakage (CAL), it remains a poorly understood complication of colorectal surgery. The reported incidence of CAL is estimated between 2.4 and 19 % [1–3] and mortality rates due to sepsis and multiple organ failure are around 15 % in patients who develop CAL [4].

With current screening and diagnostic methods, the interval between construction of the colorectal anastomosis and diagnosis of leakage varies between 6 and 13 days [5–7]. Several studies have suggested that delay of diagnosis of CAL is associated with higher mortality rates and that only early management improves clinical outcome [8–10]. Therefore, new screening methods allowing detection of CAL in the early postoperative phase are needed in addition to current methods.

Morbidity caused by CAL is due to the bacterial load leaking through an anastomotic defect. Gram-negative *Escherichia coli* and gram-positive *Enterococcus faecalis* belong to the most common species of the colon [11, 12]. When present in wound fluid obtained from the anastomotic site, it means there is contamination from the bowel. Increased concentrations of these bacteria are most likely reflecting an anastomotic defect. Therefore, these bacteria might be suited to screen for CAL [13, 14].

The golden standard for detection of bacterial contamination is culture. However, bacteria present in drain fluid in a collection bag outside the patient may not always be viable, which could render false negative results. In addition, it takes about 48 h of incubation before bacteria can be identified [14], which is an unacceptable delay. Real-time polymerase chain reaction (RT-PCR) is an alternative, molecular-based technique that can be used to identify bacterial species. It is faster, more sensitive and less susceptible to contamination than culture and might therefore be a valuable screening tool for CAL. In addition, since virtually every clinical laboratory already has a RT-PCR machine, it is a cheap technique.

The aim of this study is to study whether RT-PCR determination of *E. coli* and *E. faecalis* can serve as a screening test for CAL in the early postoperative phase after (left-sided) colorectal surgery.

## Methods

Patients included in the APPEAL study received left-sided colorectal resection with construction of an anastomosis and were given an intra-abdominal drain. Seven medical centers in the Netherlands and Belgium participated in this study. The study, registered in the Dutch Trial Register (http://www.trialregister.nl, study number NTR 1258), was approved by the medical ethical committee of all the participating centers, in accordance with the ethical standards of the Helsinki Declaration of 1975 and all patients gave informed consent. Participating centers included patients consecutively between January 2007 and December 2009.

### Inclusion and exclusion criteria

Subsequent patients undergoing left hemicolectomy, sigmoid resection, high anterior resection [HAR; with partial mesorectal excision (PME)], low anterior resection [with total mesorectal excision (TME)], and subtotal colectomy with ileorectal anastomosis were included. Oncologic resections as well as resections for inflammatory disease were included.

Emergency operations were excluded due to the high probability of coexisting tissue damage and logistical difficulties. Reversals of colostomy were also excluded since the primary disease was already treated. Furthermore, patients under 18 years of age, patients who refused to participate, and patients who did not receive a drain were excluded.

### Surgical procedure

The surgical procedure was left to the surgeon's discretion. All patients received preoperative antibiotic prophylaxis and an intra-abdominal drain. Guidelines concerning bowel preparation differed for each center and were respected. Patients were operated by laparotomy or laparoscopy, and the anastomosis was stapled or hand sutured. A diverting stoma was constructed according to the surgeon's preference. To obtain drain fluid, a drain was placed at the anastomotic site and was left in place during the first five postoperative days. The drains were all passive and closed drainage systems. The exact type of drain used was left to the surgeon's discretion.

### Drain fluid

Drain fluid reservoirs were emptied two times a day with 12 h intervals, respecting rules of sterility. The evening collection was disposed of. The morning collection was centrifuged for 10 min at 2,800×*g* and 4 °C. The supernatant was brought into different cryotubes that were frozen at −80 °C to allow PCR analysis in batch.

### Real-time polymerase chain reaction

RT-PCR analysis of the drain fluids was performed in batch for efficiency purposes. The applied technique was described earlier [13].

*DNA isolation*

After thawing, each sample was centrifuged at room temperature for 5 min at 100 g. Supernatant was diluted 10 times in a total volume of 250 μl and centrifuged at room temperature for 5 min at 8,000 g. The resulting pellet was resuspended in 180 μl buffer containing 20 mM Tris, 2 mM EDTA, 1 % Tween 80, and lysozyme (50 mg/ml) and incubated for 30 min at 37 °C on a shaking device at 600 rpm (Sanyo Orbital Shaker, München, Germany). DNA extraction was performed using a Macherey–Nagel NucleoSpin® Tissue Kit (Clontech Laboratories, Inc., Mountain View, CA, USA). First, 25 μl of protease was added to the sample, followed by incubation at 56 °C for 2 h at 700 rpm in a shaking device (Thermomixer Compact, Eppendorf, Hamburg, Germany). Protocol proceeded according to the manufacturer's instructions. Finally, template DNA was eluted in nuclease-free water in a total volume of 100 μl.

*Semi-quantitative real-time polymerase chain reaction*

All PCR reactions were performed in a total volume of 25 μl. The PCR mix for detection of *E. coli* consisted of 12.5 μl 2× DyNAmo™ HS SYBR® Green mix (Finnzymes Oy, Espoo, Finland), 0.25 μl forward primer (50 pmol/μl), 0.25 μl reverse primer (50 pmol/μl), 0.25 μl 100 nM fluorescein calibration dye (Bio-Rad, Hercules, CA, USA), 5 μl template DNA, and 6.75 μl water. In order to detect *E. faecalis*, a PCR mix containing 12.5 μl 2× DyNAmo™ HS SYBR® Green mix (Finnzymes Oy), 0.45 μl forward primer (50 pmol/μl), 0.15 μl reverse primer (50 pmol/μl), 0.25 μl 100 nM fluorescein calibration dye (Bio-Rad), 5 μl template DNA, and 6.65 μl water was used. As an extraction process control (internal control), phocine herpes virus (PhHV) was performed with a PCR mix consisting of 12.5 μl 2× DyNAmo™ HS SYBR® Green mix (Finnzymes Oy), 0.2 μl forward primer (50 pmol/μl), 0.25 μl reverse primer (50 pmol/μl), 0.25 μl 100 nM fluorescein calibration dye (Bio-Rad), 5 μl template DNA, and 6.75 μl water. The following primers were used for real-time quantitative PCR: *E. coli* uidA gene forward primer 5′-GGC TTC TGT CAA CGC TGT TT-3′, *E. coli* uidA gene reverse primer 5′-CCC ATG GAA GAG AAA TGG AA-3′, *E. faecalis* 23S rRNA gene forward primer 5′-AGA AAT TCC AAA CGA ACT TG-3′, *E. faecalis* 23S rRNA gene reverse primer 5′-CAG TGC TCT ACC TCC ATC ATT-3′, PhHV forward primer 5′-GGG CGA ATC ACA GAT TGA ATC-3′, and PhHV reverse primer 5′-GCG GTT CCA AAC GTA CCA A-3′. The Bio-Rad IQ5 ICycler (Bio-Rad, Veenendaal, The Netherlands) was used as real-time PCR platform, and the PCR conditions for *E. coli*, *E. faecalis*, and PhHV were as follows: a single predenaturation step of 15 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 59 °C. Finally, the sample temperature was gradually increased to 95 °C in order to generate dissociation curves. These curves were used to assess the specificity of the PCR product. The dissociation temperature was 76.0 °C for the *E. coli*-specific PCR product and 77.0 °C for the *E. faecalis*-specific product. The PCR efficiency was calculated using the slope of the standard curve (efficiency=10−1/slope−1).

*Standard curves*

The semi-quantitative inoculum of indicator organisms potentially present in the peritoneal drain fluid at the time of anastomotic leakage has been determined by using a reference dilution series of *E. coli* and *E. faecalis* inocula. Reference series were produced by spiking 500 μl of culture-negative drain fluid with a 10-log serial dilution of both *E. coli* and *E. faecalis*. A standard curve was generated by comparing the real-time PCR results (threshold cycle or Ct value) to the inoculum sizes. The approximate inoculum size of the query patient sample was determined after interpolation of its Ct value within the standard curve. Patient samples were analyzed in duplicate.

Definitions

The endpoint of the APPEAL study was symptomatic colorectal anastomotic leakage. This was defined as a clinically manifest insufficiency of the anastomosis leading to a clinical state requiring intervention, confirmed by radiological studies, reoperation or fecal discharge from the drain. Radiologic confirmation of CAL was defined as extravasation of endoluminally administrated water-soluble contrast and/or significant perianastomotic air on computed tomography or X-ray. Radiological studies were not routinely performed only in case of clinical suspicion of CAL. Interventions to treat CAL consisted of therapeutic drainage (prolonged stay of drain), use of therapeutic antibiotics, or a surgical intervention, i.e., construction of a diverting stoma, disconnection of the anastomosis and construction of a new anastomosis or a colostomy, or suturing of the leakage site.

All postoperative fistulas communicating with the surgical anastomosis were classified as a leak. Postoperative abscesses were classified as anastomotic leakage if there was extravasation of enteric contrast on radiological studies, if there was significant perianastomotic air or if communication with the anastomosis was noted after radiologic drainage.

The bacterial load of drain fluid was expected to rise in case of CAL; therefore, an increase detected by RT-PCR was scored positive and a decrease was scored negative.

Postoperative mortality was defined as patients that died within 30 days of operation in hospital and after discharge.

Data collection

Patients were followed from their preoperative admission on the ward until the first postoperative follow-up at the outpatient clinic. Demographic data of the patients, operative details, postoperative events and follow-up data were obtained through a standardized case record form and entered into a database. In case of CAL, the postoperative day of diagnosis was noted along with the manifestation of CAL, the diagnostic tool for detection of the leak, and the treatment.

Statistics

Categorical data are presented as numbers with percentages, numerical data are presented as means ± standard deviation (normally distributed), or medians with interquartile ranges (not normally distributed). Univariate analysis was performed using a chi-square test or Fisher exact test in case of categorical data and a Mann–Whitney *U* test in case of numerical data.

As test performance indicators sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic odds ratio (DOR) were calculated. Calculations were performed using SPSS 20 (SPSS Inc., Chicago, IL, USA).

## Results

A total of 243 patients were included. The mean age was 64±12 years, 135 patients (56 %) were male and 108 (44 %) were female. Thirty-three patients (14 %) were treated for inflammatory diseases, 206 patients (84 %) were treated for malignancy, and four patients (2 %) had ischemic colitis. Fifty-six patients (23 %) underwent preoperative radiotherapy, and in 59 patients (25 %), a defunctioning stoma was constructed. A total of 92 (38 %) patients underwent a laparoscopic procedure, and 151 patients (62 %) were operated through laparotomy.

Nineteen patients (7.8 %) developed clinical CAL. In nine patients it became manifest as sepsis, in seven patients as peritonitis, two patients developed a presacral abscess, and one patient developed an intra-abdominal abscess. In eight patients, the diagnosis was made by CT scan, in seven patients by relaparotomy, and in four patients fecal discharge from the drain occurred. Median interval between operation and confirmation of CAL was 6 days (range 2–26 days). Two patients (0.8 %) developed an infection at the drain insertion site, both in the group without CAL. Average hospital stay of patients with CAL was significantly longer [28±22 days vs. 13±13 days ($p<0.0001$)]. In the group of patients with CAL, three died (16 %), whereas six patients (3 %) died in the group without CAL ($p=0.002$). Table 1 shows the baseline characteristics of the patients with and without CAL.

Sixty patients received a Penrose drain; all the other patients received a silicone tube drain. Drainage systems were all passive and closed. The production of drain fluid was not constant over time or between patients and varied greatly between 0 and 1,500 ml per day per patient (Table 2). The difference in production between patients with and without CAL was not significant.

The quantitative results are depicted in Table 3. An increase of *E. coli* or *E. faecalis* as detected by RT-PCR was scored positive, whereas no change or a decrease was scored negative. An increase in *E. coli* concentration was found in significantly more patients with CAL on days 4 and 5 ($p=0.0004$; DOR 7.9). For *E. faecalis*, this result was found for days 2, 3,

**Table 1** Univariate analysis of baseline characteristics of APPEAL-study patients

| Variable | No. CAL ($n=224$) | CAL ($n=19$) | $p$ value |
|---|---|---|---|
| Age | 64.3±12.0 | 65.3±13.9 | 0.765 |
| Gender | | | |
| Male | 125 (93 %) | 10 (7 %) | 0.789 |
| Female | 99 (92 %) | 9 (8 %) | |
| BMI | | | |
| <25 | 80 (91 %) | 9 (9 %) | 0.155 |
| 25–30 | 109 (98 %) | 5 (2 %) | |
| >30 | 35 (88 %) | 5 (12 %) | |
| ASA-score | | | |
| 1–2 | 173 (93 %) | 13 (7 %) | 0.532 |
| 3–4 | 50 (89 %) | 6 (11 %) | |
| Neoadjuvant radiotherapy | | | |
| Yes | 51 (91 %) | 5 (9 %) | 0.945 |
| No | 173 (93 %) | 14 (7 %) | |
| Type of resection | | | |
| TME/LAR | 76 (92 %) | 7 (8 %) | 0.727 |
| PME/HAR | 55 (95 %) | 3 (5 %) | |
| Left hemicolectomy | 21 (96 %) | 1 (4 %) | |
| Sigmoid resection | 68 (90 %) | 8 (10 %) | |
| Subtotal colectomy | 4 (100 %) | 0 (0 %) | |
| Height anastomosis | | | |
| >7 cm | 149 (94 %) | 10 (6 %) | 0.211 |
| <7 cm | 74 (89 %) | 9 (11 %) | |
| Construction anastomosis | | | |
| Stapled | 178 (93 %) | 14 (7 %) | 0.735 |
| Handsewn | 45 (90 %) | 5 (10 %) | |
| Configuration anastomosis | | | |
| End-to-end | 57 (86 %) | 9 (14 %) | 0.259 |
| End-to-side | 16 (94 %) | 1 (6 %) | |
| Side-to-end | 122 (94 %) | 8 (6 %) | |
| Side-to-side | 23 (96 %) | 1 (4 %) | |
| Protective ileostomy | | | |
| Yes | 55 (93 %) | 4 (7 %) | 0.933 |
| No | 167 (92 %) | 15 (8 %) | |

*CAL* Colorectal Anastomotic Leakage; *BMI* Body Mass Index; *ASA-score* American Society of Anesthesiologists score; *TME* Total Mesorectal Excision; *PME* Partial Mesorectal Excision; *HAR* High Anterior Resection; *LAR* Low Anterior Resection

**Table 2** Amount of drain fluid produced in ml per 24 h versus CAL

| 24 h Production | CAL | *N* | Mean | SD | *p* value |
|---|---|---|---|---|---|
| Day 1 | Yes | 13 | 121.0 | 155.2 | 0.084 |
| | No | 196 | 179.8 | 172.1 | |
| Day 2 | Yes | 14 | 79.3 | 85.0 | 0.551 |
| | No | 194 | 104.1 | 133.7 | |
| Day 3 | Yes | 11 | 94.9 | 110.5 | 0.769 |
| | No | 177 | 124.1 | 182.2 | |
| Day 4 | Yes | 11 | 129.7 | 159.3 | 0.737 |
| | No | 162 | 120.3 | 149.2 | |
| Day 5 | Yes | 10 | 91.1 | 76.7 | 0.875 |
| | No | 134 | 119.4 | 151.3 | |

*CAL* Colorectal Anastomotic Leakage; *N* number of patients; *SD* Standard Deviation

and 4 ($p<0.003$) with highest DOR on day 3 (31.6). Sensitivity and negative predictive values were 92.9 and 98.7 %, respectively. The results including sensitivity, specificity, PPV, NPV, and DOR for *E. coli* and *E. faecalis* are depicted in Tables 4 and 5, respectively.

## Discussion

Current screening methods for CAL consist of observation of clinical signs and symptoms and blood examination. These methods are not specific for CAL and may lead to various diagnostic procedures to exclude other less severe complications instead of ruling out CAL by means of highly specific imaging studies like CT scan and/or water-soluble contrast radiography [9]. These additional diagnostics could lead to a delay in diagnosis of CAL [10]. Therefore, there is a need for a screening method that is objective and specific for CAL.

This study shows that the number of patients with increased levels of *E. faecalis* between postoperative days 1 and 3 was significantly higher in case of CAL. This test has the highest DOR (31.6), reflecting the strong association between the test result and CAL. Considering high sensitivity (92.9 %) and NPV (98.7 %), a negative test result virtually rules out CAL at day 3 postoperatively. The false negative (1.3 %) rate is far lower than any other reported diagnostic test for CAL. However, since it does not equal zero, clinical observation remains important.

Specificity (70.9 %) and PPV (30.2 %) indicate a substantial number of false positive results. This is most likely due to subclinical anastomotic leakage, a long-known phenomenon with a reported incidence of 8 % [15, 16]. It could also be caused by intraoperative spill; however, the number of bacteria should have decreased at day 3. Regardless of the cause of the false positive results, positive test results should lead to additional imaging. Reported sensitivity and specificity of contrast radiography when performed in case of clinical suspicion are 68 and 94 %, respectively [17]. When performed routinely, reported sensitivity varies between 20 and 52 % and specificity is approximately 85 % [18, 19]. The reported sensitivity of CT scan in the early postoperative period varies between 15 and 52 % [17, 20, 21]. The reported negative predictive value is 73 %, and the false negative rates vary between 35 and 53 % [17, 21]. Even though sensitivity of CT scan is lower, it is preferable over contrast enema due to the additional information it provides.

As false positive RT-PCR results are most likely due to subclinical anastomotic leakage, CAL demonstrated on CT scan might also remain subclinical and specific treatment may not be absolutely necessary. However, subclinical leakage is also associated with reduced quality of life and impaired bowel function [16], perhaps rendering treatment beneficial. The latter remains speculative and requires more research.

The number of PCRs performed, as depicted in the tables, does not add up to the number of included patients. This is due to insufficient production of drain fluid in most cases as illustrated by Table 2. The great variability is a drawback of this study that cannot easily be solved. A peritoneal lavage could be a solution; however, this may interfere with the quantitative PCR analysis. In addition, a few samples are missing due to accidental drain removal by patient, early intervention for CAL, and accidental loss of drain fluid either at the ward or at the processing laboratory.

Prophylactic drainage (PD), as performed on patients included in the APPEAL study, originally aimed to evacuate wound fluid and blood collections from the surgical site to prevent infectious complications and to detect AL by fecal or purulent discharge [22–24]. To date, the use of prophylactic drainage remains controversial. Several level 1 studies have shown that PD does not have a beneficial or a detrimental effect on the incidence of AL and on the morbidity afterwards [25–27]. A prospective study

**Table 3** Results of the semi-quantitative real-time PCR. The values are presented in colony forming units per milliliter (CFU/ml)

| RT-PCR | CAL Y/N | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|---|
| *E. coli* (CFU/ml) | Y | 55 | 30 | 55 | 100,000 | 1,000,000 |
| | N | 0 | 0 | 0 | 0 | 0 |
| *E. faecalis* (CFU/ml) | Y | 300 | 7,500 | 75,000 | 75,000 | 100,000 |
| | N | 1,000 | 1,000 | 1,000 | 0 | 1,000 |

*RT-PCR* Real-Time Polymerase Chain Reaction; *CAL* Colorectal Anastomotic Leakage

**Table 4** Quantitative increase of *E. coli* as determined by RT-PCR. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic odds ratio (DOR) are shown. All values are accompanied by their 95 % confidence interval (CI)

| PCR | Interval | Increase | CAL[a] | | *p* value | Sens % (95 % CI) | Spec % (95 % CI) | PPV % (95 % CI) | NPV % (95 % CI) | DOR (95 % CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Yes | No | | | | | | |
| *E. coli* | Days 1→2 | Yes | 3 | 11 | 0.185 | – | – | – | – | – |
| | | No | 10 | 94 | | | | | | |
| | Days 1→3 | Yes | 6 | 23 | 0.102 | – | – | – | – | – |
| | | No | 9 | 91 | | | | | | |
| | Days 1→4 | Yes | 9 | 16 | 0.0004 | 69.2 (38.9–89.6) | 83.5 (74.3–89.9) | 36.0 (18.7–57.3) | 95.2 (87.7–98.5) | 7.9 (2.44–5.6) |
| | | No | 4 | 81 | | | | | | |
| | Days 1→5 | Yes | 10 | 21 | 0.0004 | 66.7 (41.7–84.8) | 79.8 (71.1–86.4) | 32.3 (17.3–51.5) | 94.3 (86.6–97.9) | 7.9 (2.4–25.6) |
| | | No | 5 | 83 | | | | | | |

[a] Numbers of PCRs are less than number of included patients due to the APPEAL study's priority to biochemical analysis, lack of production, accidental drain removal by patient, and early intervention for CAL

concerning pelvic anastomosis showed a higher leakage rate after routine irrigation–suction drainage in elective anterior resection [28]. A retrospective study showed drainage and the use of a defunctioning stoma to be beneficial in terms of reoperation rates as a result of anastomotic leakage after TME [29]. Despite this controversy surrounding the necessity to drain, prophylactic drainage remains common practice in many hospitals, particularly after rectal surgery [30]. In addition, the outcome measures used in these studies consist of leakage rates, hospital stay, radiological anastomotic leakage, infectious complications, and patient comfort. In this study, we have focused on and demonstrated the diagnostic capacity of the drain. Therefore, considering the low complication rate of PD, it should be placed routinely during surgery to allow collection of drain fluid for the first three postoperative days. In addition, it does not interfere with ERAS protocols since the results are known at day 3 and the drain can be removed [31].

Screening is defined by the World Health Organization as the systemic application of a test in an asymptomatic population in order to identify abnormalities that suggest presence of disease and refer these patients promptly for diagnosis and treatment [32]. The APPEAL study is the first study to define a promising screening tool for symptomatic CAL that is objective, fast, affordable and provides useful information concerning CAL as early as postoperative day 3.

## Conclusion

RT-PCR for *E. faecalis* performed on drain fluid may be a useful screening tool for symptomatic colorectal anastomotic leakage in the early postoperative phase. Negative test results virtually rule out the presence of CAL. Positive results should lead to highly specific imaging studies for diagnosis of CAL.

**Table 5** Quantitative increase of *E. faecalis* as determined by RT-PCR. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic odds ratio (DOR) are shown. All values are accompanied by their 95 % confidence interval (CI)

| PCR | Interval | Increase | CAL[a] | | *p* value | Sens % (95 % CI) | Spec % (95 % CI) | PPV % (95 % CI) | NPV % (95 % CI) | DOR (95 % CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Yes | No | | | | | | |
| *E. faecalis* | Days 1→2 | Yes | 10 | 26 | 0.001 | 71.4 (45.4–88.3) | 75.9 (67.1–83.0) | 27.8 (14.8–45.4) | 95.3 (87.9–98.5) | 7.9 (2.3–27.3) |
| | | No | 4 | 82 | | | | | | |
| | Days 1→3 | Yes | 13 | 30 | 0.00001 | 92.9 (68.5–98.7) | 70.9 (61.5–78.8) | 30.2 (17.7–46.3) | 98.7 (92.7–99.8) | 31.6 (4.0–252.7) |
| | | No | 1 | 73 | | | | | | |
| | Days 1→4 | Yes | 9 | 26 | 0.003 | 75.0 (46.8–91.1) | 72.6 (62.9–80.6) | 25.7 (12.5–43.2) | 95.8 (88.3–99.1) | 7.9 (2.0–31.8) |
| | | No | 3 | 69 | | | | | | |
| | Days 1→5 | Yes | 7 | 38 | 0.388 | – | – | – | – | – |
| | | No | 7 | 65 | | | | | | |

[a] Numbers of PCRs are less than number of included patients due to the APPEAL study's priority to biochemical analysis, lack of production, accidental drain removal by patient, and early intervention for CAL

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