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Early Detection of Colonic Anastomotic Leakage in a Swine Model Using Continuous pH Monitoring

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Abstract

Background: Anastomotic leaks (AL) are a deadly postoperative complication. This study evaluated the efficacy of *Stream*™ Platform, a medical device designed to provide early prediction of AL using changes in peritoneal fluid pH and electrical conductivity (EC). **Methods**: *Stream*™ Platform was attached to the JP drain of 17 Yorkshire pigs, continuously measuring pH and EC or peritoneal effluent in a model of colorectal AL. Anastomotic leaks were surgically induced and confirmed through re-laparotomy. **Results:** Significant differences in pH were observed between experimental and sham animals two hours following leak induction via continuous measurements ($P = 0.02857$), at the 24-hour time point in reservoir fluid ($P = 0.008$), and during final peritoneal fluid collection at re-laparotomy ($P = 0.024$). **Conclusion:** This study illustrates the potential of continuous pH monitoring for early detection of anastomotic leaks, using a non-invasive technology—*Stream*™ Platform—in a model that closely resembles human physiology.

Keywords

Anastomotic Leak, *Stream*™ Platform, pH, Electrical Conductivity, Early Prediction

1. Introduction

The incidence of colorectal anastomotic leaks (AL) ranges from 0.5% - 30%, with mortality rates ranging from 27% to 67% [1]-[8]. ALs can lead to secondary complications such as wound infection and sepsis which may prolong hospitalization,

and may require readmissions and reoperations [1] [2] [9]. Unfortunately, predicting AL before patients progress to more serious, advanced stages remains a challenge.

Computed tomography (CT) is a widely used imaging modality for detecting AL in the postoperative period. Benefits of this technology include its non-invasiveness, ease-of-use, and relatively rapid ability to make leak diagnoses. CT scans involve identifying signs of leak, such as fluid collections and inflammatory changes in tissues. For patients with AL, CT with rectal contrast enema (RCE-CT) has grown in popularity as a key imaging modality for diagnosing AL. Building on standard CT imaging by introducing water-soluble contrast material via a rectal catheter, RCE-CT allows for direct visualization of extravasation at the anastomotic site and increases the sensitivity of traditional CT scans.

Despite the value that technologies such as CT bring, several studies have highlighted limits in this approach, including low sensitivity of CT scans in detecting AL, ranging from 0.59 - 0.73 [10]-[12]. As an example, work from Kornmann *et al.*, explored the impact of false-negative results from CT scans—which can provide a false sense of security to clinicians, whose patients are actually experiencing a leak [12]. This study included 524 patients undergoing colonic surgery, with an overall leak rate of 10.9% (n = 57), and 12 patients dying from a leak (mortality rate 21.1%). Results from CT scans had an overall sensitivity of 0.59 (95% CI 0.43 - 0.73), specificity of 0.88 (95% CI 0.75 - 0.95), positive predictive value (PPV) of 0.82 (95% CI 0.64 - 0.92), negative predictive value (NPV) of 0.70 (95% CI 0.57 - 0.81), and accuracy of 74% [12]. Critically, delayed reintervention for AL due to CT false negatives resulted in death for 62.5% of these cases (n = 5). Thus, while CT scans are routinely used in the clinical setting to diagnose leaks, they are not without challenges. Clinical assessment itself, although important, has sensitivity and specificity of 50% and 89%, respectively, and a significant time delay between leak formation and onset of clinical symptoms [13] [14].

Endoscopic assessment can also play a role in evaluating anastomotic integrity, both intraoperatively and postoperatively. Although valuable in standard-of-care practices, diagnostic limitations remain. For instance, the accuracy of endoscopic evaluation is heavily dependent on a surgeon's expertise, and false negatives are possible if subtle mucosal changes are missed. A technical publication by Barzola *et al.* noted that combining perioperative flexible endoscopy with intraoperative ICG fluorescence angiography to improve the identification of poorly perfused tissue and reduce AL rates is a key area for further research [15].

Biomarkers such as pH and EC offer a potential new avenue for improving the timeliness and reliability of leak prediction. Dehiscence at the anastomosis is associated with elevated inflammatory responses, such as serum C-reactive protein, procalcitonin, and white blood cell counts [13] [16]. The role of ischemia has also been extensively discussed in literature, with pH being a primary biomarker of interest [13] [16]-[19]. Between tissue hypoxia, increased cytokine release, and impaired fibroblast function, these factors collectively contribute to local acidosis

and by extension, lower pH levels. A prospective analysis of the pelvic drainage fluid from 753 patients demonstrated that a cut-off pH value of 6.978 on postoperative day (POD) 3 had a diagnostic sensitivity of 98.7% and a specificity of 94.7% for AL detection [17]. In a similar study of 173 patients, peritoneal drain fluid pH was found to be an independent predictor of anastomotic leakage with the maximum sensitivity (93.75%) and specificity (97%) obtained using cut-off pH < 7.53 on POD-1 and pH < 7.21 on POD-3 [19].

Additional researchers have also evaluated the use of EC for predicting AL. For example, an early study from DeArmond *et al.* used a rat model whereby animals underwent celiotomy and creation of a 5-mm gastronomy [20]. An NaCl electrolyte solution was introduced via gavage, with the authors finding that leakage from the gastrotomy induced by as little as 1cc of the electrolyte solution was detectable as a significant drop in electrical resistance compared to negative controls [20]. Other work, for instance, by Ben-David *et al.* has used both mice and pig models to further support the utility of EC in leak detection [21]. The relationship between increased EC and leaks may be attributed to tissue edema, hyperemia, and cellular infiltration that occurs from the earliest phases of AL, as well as simultaneous increase in conductivity due to an influx of charge-carrying ions around the anastomotic site.

Anastomotic leaks extend length of hospital stay, increase reoperation and readmission rates, and increase ICU admission and length of stay (LOS). For instance, the secondary complications stemming from AL increase risk of reoperation by more than 10-fold [22]. One study with a sample of over 600 patients reported reoperation rates of 91.7% in those with leaks, compared to 5.4% in those without [8]. Reoperation rates of 50% - 60% have been reported in additional research articles [23].

Resources required to treat anastomotic leaks result in significant economic impact to the healthcare system. A large study with over 23,000 patients found the economic impact of AL in the UK to be approximately £1.1 million to £3.5 million [24]. Moreover, additional research has found that costs for patients who develop leaks are nearly three times higher than those without, with estimated cost differences equivalent to $28.6 million USD per 1000 patients [25].

The investigational device described herein, FluidAI's *Origin*™, a component of *Stream*™ Platform, uses nanosensing technology for bedside monitoring of pH and EC as early harbingers of anastomotic leak. This platform was designed to connect in-line, outside of the abdominal cavity, with currently used intra-abdominal drains. The objective of this experimental study was to assess the utility of pH and EC, measured using *Stream*™ Platform, in distinguishing between leak and non-leak cases, in a swine model of AL.

2. Materials and Methods

This study followed the guidelines of the Animal Welfare Act of 1966 and was approved by the Keenan Research Centre in the Li Ka Shing Knowledge Institute

of St. Michael's Hospital Animal Care Committee. All animals arrived at the vivarium facility seven days prior to the initial laparotomy for acclimatization and were individually housed in double-sized stainless-steel cages equipped with video surveillance for constant monitoring by vivarium staff. Daily assessment of the animals included activity levels, appetite, signs of pain/discomfort, and emesis. Additionally, daily vital sign assessment included heart rate (HR), respiratory rate (RR), and temperature. Normal ranges for swine are respectively: 60 - 100 beats/minute, 8 - 18 breaths/minute, and 38˚C - 40˚C [26]. All animals received a standard diet and fasted for 12 hours prior to surgery to prevent aspiration; water was provided ad libitum.

2.1. Colon Anastomosis and Device Attachment

Seventeen Yorkshire pigs of both sexes with an average weight of 29.9 kg (SD 3.5 kg) and average age of 3.8 months (SD 0.6 months) were included in the study. Animals received an intramuscular injection of ketamine (20 mg/kg) (Ketalean, Bimeda-MTC Animal Health Inc., Distributed by Vetoquinol N.A. Inc., Lavaltrie, QC), xylazine (2 mg/kg) (Rompun, Elanco Canada Limited, Mississauga, ON), and atropine sulphate (1 mg/25kg) (Atropine, Teligent Canada, Mississauga, ON). Subsequently, the animals were intubated and mechanically ventilated with tidal volume of 10 ml/kg (Ohmeda 7000, Division of Canadian Oxygen Limited, Rexdale, ON). General anesthesia was maintained with 2% - 3% isoflurane for the duration of the procedure. An intravenous (IV) catheter was inserted in an ear vein using a 20 g IV catheter and 0.9% saline was administered. Anesthesia levels and cardiopulmonary function were monitored using jaw tone, pulse oximetry, and ECG.

The anterior abdominal skin was prepared with a povidone-iodine solution (Teva Canada Ltd., ON, Canada) and draped in sterile fashion. A 10-cm midline laparotomy was performed and a 10-cc syringe (Terummo, Vaughan, ON, Canada) was used to aspirate an initial sample of free fluid from the peritoneal cavity for baseline pH and EC measurements. Afterwards, a mobile segment of the right colon was identified and exposed through the laparotomy incision. The total circumference of that segment of colon was measured and recorded. Subsequently, an electrocautery (AARON 1250, Bovie Medical, FL, USA) was utilized to perform an enterotomy encompassing 80% of the circumference of the colon. A hand-sewn, single-layer, continuous suture anastomosis was performed using non-absorbable 2-0 prolene sutures (Ethicon, Somerville, NJ, USA) leaving two 10 cm long ends of the suture untied at the beginning and at the end of the anastomosis. A needle was used to pierce two holes, 2 cm apart through a sterile plastic square (4 × 4 cm) to pass both ends of the anastomotic suture. The 4 × 4 plastic was interposed between the parietal peritoneum and the anastomosis to prevent adhesion of the anastomotic site to the lateral abdominal wall. The ends of the suture were externalized through the right abdominal wall, passed through a small latex tube (Bard Catheters, Covington, GA, USA), and tied to anchor and maintain the suture taut. Subsequently, a Jackson-Pratt® (JP) silicone catheter (Cardinal Health,

Ohio, USA) was positioned inside the abdomen near the anastomosis. The JP catheter was also exteriorized on the right side of the abdominal wall.

A retro-rectus sheath nerve block was performed with bupivacaine hydrochloride (5 mg/ml) (Sterimax Inc., Oakville, ON, Canada) prior to closing the laparotomy. The linea alba was closed with continuous number 1 polyglactin 910 single-layer suture (Vicryl Johnson & Johnson Intl., Somerville NJ, USA). The skin was sutured separately.

Two matching investigational devices (*Origin*™ Waterloo, Ontario, Canada) were used for each animal. One *Origin*™ device, coined JP-*Origin*, was attached in-line to the Jackson-Pratt drain tubing before the site of the 400 ml bulb reservoir. The JP-*Origin* was utilized for continuous assessment of the peritoneal effluent pH and temperature throughout the experiment, and EC for the first 24-H; temperature was assessed for the correction of pH and EC of the drainage fluid flowing through the drain.

The inlet port of the JP-*Origin* device was connected to the tubing coming from the animal's abdominal cavity and the outlet port of that device was connected to the tubing that led to the 400ml bulb reservoir. Subsequently, the JP-*Origin* device was placed in a protective 3D-printed plastic enclosure and stitched in four points to the animal's back wrapped in bandaging tape (3M Vetrap TM St-Paul, MN, USA) (**Figure 1**). Data captured from the JP-*Origin* device was transferred electronically to a tablet-based application for the duration of the study.

The other *Origin*™ device, coined "Lab-*Origin,*" was used in a laboratory setting to assess the pH, EC, and temperature of the fluid obtained from the 400 ml bulb reservoir; temperature was assessed for correction of pH and EC. This "Lab-*Origin*" device served to validate the accuracy of the continuous JP-*Origin* measurements using a benchtop workflow. Reservoir fluid from both experimental and sham groups were analyzed with the Lab-*Origin* device at 24-H intervals, and at the time of the second laparotomy. An additional reservoir fluid analysis was performed in the experimental group prior to suture pull-out.

A transdermal fentanyl patch (75 mcg/hr) (Sandoz Canada, Boucherville, QC, Canada) was applied to the animal's skin for postoperative pain management. The fentanyl patch was secured using a bandage (3M Vetrap TM St-Paul, MN, USA) to minimize tampering. The animals were returned to their cages upon completion of the procedures until the re-laparotomy.

2.2. Study Groups, Anastomotic Dehiscence, and Re-Laparotomy

The animals were randomized into two groups by vivarium staff: experimental (n = 11) and sham (n = 6). In experimental group animals, the externalized suture line was cut and subsequently pulled out to cause dehiscence of the colonic anastomosis on average, three hours after laparotomy closure. In the sham group, the externalized suture line was left intact. A defined postoperative reference point ("time zero", POH-0) was established at the moment the initial laparotomy was closed. As described above, in experimental animals, the externalized anastomotic

suture was cut at POH-3 to induce leak. This delay was intentional to mirror the typical clinical progression in which anastomotic dehiscence typically develops hours after surgery, rather than intraoperatively. All continuous and interval measurements were analyzed relative to this POH-based timeline.

All animals underwent a re-laparotomy based on the presence of specific criteria for each group. Re-laparotomy was performed if two or more of the following findings were detected:

Experimental group criteria for re-laparotomy:

1) Clinical signs and behavioral changes: Heart rate (HR) > 120 beats/min, body temperature (>38˚C), respiratory rate (>18 breaths/min), lethargy, food avoidance. Vital signs were assessed by vivarium staff.

2) Laboratory findings: White blood cell count (WBC) > 21 × 10^9/L, hypokalemia (<3.4 mm/mol)

Sham group criteria for re-laparotomy:

1) Presence of less than 5 ml of peritoneal drainage fluid in the reservoir in 24-hours.

2) Stable pH and conductivity values in the peritoneal drainage fluid for at least three hours after laparotomy closure.

Re-laparotomies were performed through the same incision of the initial laparotomy under general anesthesia as previously described. Upon opening the peritoneum, a final sample of free fluid was aspirated from the peritoneal cavity for pH and conductivity analysis using a 10-cc syringe (Terummo, Vaughan, ON, Canada). Subsequently, the peritoneal cavity was examined thoroughly for signs of diffuse peritonitis, abscesses, and phlegmon. The colonic anastomosis was assessed for anastomotic leak. Subsequently, animals were euthanized with intravenous injection of pentobarbital sodium (0.2 mL/kg).

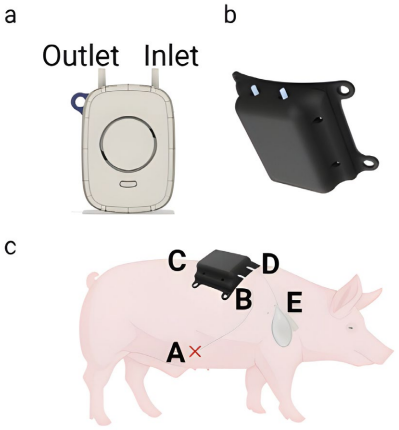


Figure 1. (a) Front visual of the investigational device; (b) Protective enclosure for the device, to minimize tampering by the animals; (c) Attachment of the device to the animals.

Within **Figure 1(c)**, (A) represents the site of exteriorization of catheter on right ventral abdominal, (B) indicates attachment of catheter to the device inlet, (C) represents the protective enclosure sutured dorsally on the study animal, (D) repre-

sents the attachment of the device outlet to the 20 cm tubing connected to the evacuator, and (E) represents the Jackson-Pratt ® 400 ml silicone bulb reservoir.

2.3. *Origin*™ Device Calibration

The JP-*Origin* and the Lab-*Origin* devices were calibrated to ensure adequate readings. Three-point calibrations were performed on both devices at the time of the initial surgical procedure using three standard pH buffers (pH 4.00, 7.00, and 10.00) (Reagecon Diagnostics Ltd, Shannon, Co. Clare, Ireland) and three standard EC solutions (5.00, 12.88, and 80.00 mS/cm) (HANNA Instruments, Quebec, Canada). Additional 3-point calibrations were performed on the Lab-*Origin* devices and 1-point calibrations performed on the JP-*Origin* devices (pH 7.00 and EC 12.88 mS/cm standards) every 24-H. Final 3-point calibrations were performed on both devices at the time of the re-laparotomy (Figure 2).

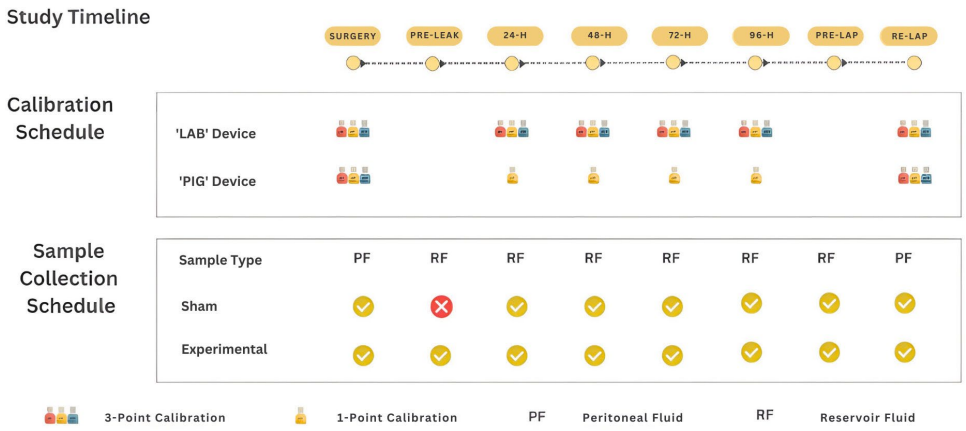


Figure 2. Daily calibration and sample collection schedule. Note that no **reservoir** fluid was collected from sham animals "pre-leak", as no leak was induced in this group.

2.4. Statistical Analysis

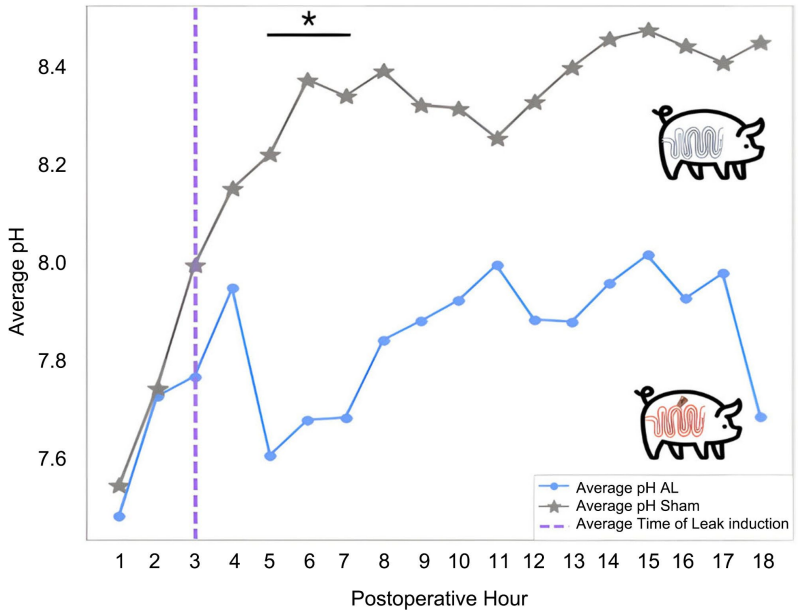
All statistical analysis was conducted in Python (version 3.9.16). Continuous variables are presented as means (SD), and normality of datasets was assessed using the Shapiro-Wilk Test. Parametric datasets were evaluated using Student's t-tests, while non-parametric data was evaluated through the Mann-Whitney U test. One-sided tests were used in cases where a priori assumptions existed based on existing literature. $P < 0.05$ was considered statistically significant. Bland-Altman analyses were used to compare pH measurements obtained from the benchtop pH probes, as well as blood gas analysis.

3. Results

3.1. pH and Electrical Conductivity of the Peritoneal Effluent and Re-Laparotomy Findings

The experimental group showed lower average pH values in the peritoneal effluent monitored continuously with the JP-*Origin* device in-line with Jackson-Pratt

drains compared to the sham group (7.54 (SD: 0.30) vs. 7.77 (SD: 0.50) respectively; $P < 0.001$) (Figure 3). Moreover, our findings showed that statistically significant differences between those groups commenced at postoperative hour (POH) 5, corresponding to two hours post suture pull out (average pH experimental group: 7.61 vs. average pH sham group: 8.22; ($P = 0.02857$)). Experimental group animals presented with clinical and laboratory signs of anastomotic leak and criteria for re-laparotomy at POH-28, on average. Initial and final/re-laparotomy vital signs are presented in the following Supplement Table. Continuous 24-H assessment of the average EC in the peritoneal effluent with the JP-*Origin* device also showed significantly higher EC overall in the experimental group vs. sham group, respectively (14.3 mS/cm (SD: 0.77) vs. 13.6 mS/cm (SD: 0.38) ($P < 0.0001$) (Figure 4).



*$P < 0.05$ (POH-5 to POH-7).

Figure 3. Continuous monitoring of peritoneal effluent pH, over the first 18 postoperative hours. Average pH values for experimental and sham animals including average time of leak induction (POH-3) are shown.

Assessment of the peritoneal cavity and colonic anastomosis during re-laparotomy showed that leaks were successfully created in 8/11 of the experimental group animals (seven with complete anastomotic breakdown and one with partial rupture). Fecal peritonitis and enteric spillage were observed macroscopically within the abdomen of the experimental pigs, confirming the presence of a leak. The remaining (3/11) experimental animals did not experience these findings due to a tamponade/containment of enteric contents by the abdominal wall and surrounding organs. There were no leaks in the sham group, with the suture remaining intact in all animals. One sham animal presented with local inflammation at the midline and serosal fluid in the surrounding peritoneum. This animal had partial dehiscence of the midline laparotomy suture.

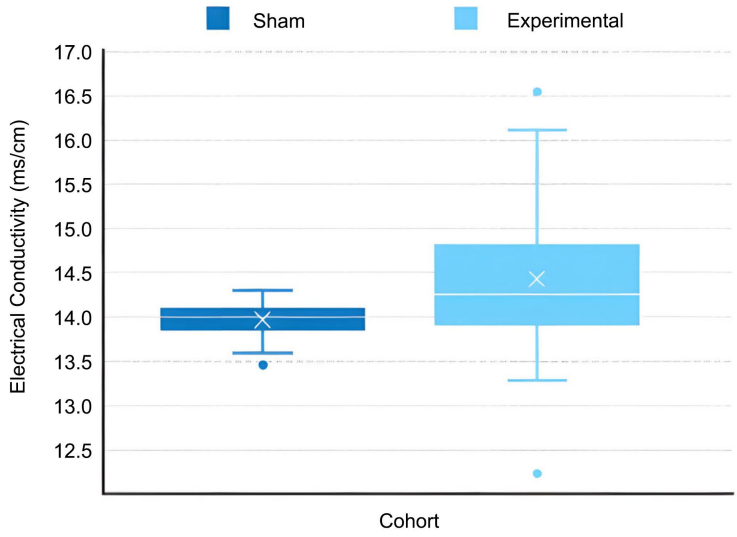


Figure 4. Average continuous EC (mS/cm) measurements of reservoir fluid made over the first 24 postoperative hours, using investigational devices directly connected to the animal (JP-*Origin*).

3.2. Free Peritoneal Fluid Assessments

The pH of the final free fluid sample taken at re-laparotomy was significantly lower in the experimental group than the sham group; respectively (7.76 (SD: 0.09) vs. 8.20 (SD: 0.12), $P = 0.024$). However, no statistically significant differences in the EC of the final free fluid samples were observed between those groups. As expected, pH and EC of the initial free fluid samples obtained at the initial laparotomy were similar between the two groups (Table 1).

Table 1. Analysis of peritoneal fluid pH and electrical conductivity during initial surgery and re-laparotomy.

	Initial Peritoneal Fluid		*P*-value	Final Peritoneal Fluid		*P*-value
	Experimental	Sham		Experimental	Sham	
Average pH (SD)	7.99 (0.23)	8.11 (0.20)	0.202	7.76 (0.09)	8.20 (0.12)	**0.024****
Average EC (mS/cm) (SD)	13.40 (0.56)	12.87 (0.69)	0.268	12.84 (1.17)	12.87 (1.24)	1.00

** $P < 0.05$.

3.3. Reservoir Fluid Analyses

The pH of the reservoir fluid assessed with the Lab-*Origin* device was significantly lower in the experimental group compared to the sham group sample at the end of the first 24-H; respectively (8.38 (SD: 0.42) vs. 8.93 (SD: 0.18), $P = 0.008$). However, those findings did not repeat in reservoir fluid obtained at 48-H and before re-laparotomy (Table 2). The differences in the EC of reservoir fluid assessed with the Lab-*Origin* device were not statistically significant between the experimental group compared to the sham group in any of the time points (Table 2).

Table 2. Analysis of peritoneal fluid pH and electrical conductivity during initial surgery and re-laparotomy.

Study Time Point	Experimental	Sham	*P*-value
Average pH (SD)			
24-H	8.38 (0.42)	8.93 (0.18)	**0.008****
48-H	8.27 (0.57)	8.79 (0.24)	0.143
Pre-relaparotomy	8.19 (0.22)	8.91 (0.10)	0.056
Average Conductivity (mS/cm) (SD)			
24-H	13.72 (1.80)	13.44 (0.46)	0.755
48-H	15.33 (4.02)	13.84 (0.90)	1.00
Pre-relaparotomy	14.02 (2.39)	11.59 (0.43)	0.111

** $P < 0.05$.

3.4. Receiver Operator Characteristic (ROC) Analysis—pH Sensitivity and Specificity

ROC analysis based on average pH values during the first 24-H period yielded an AUC of 0.9271, with sensitivity and specificity of 100% and 87.5% respectively, of detecting anastomotic leak based on pH (**Figure 5**).

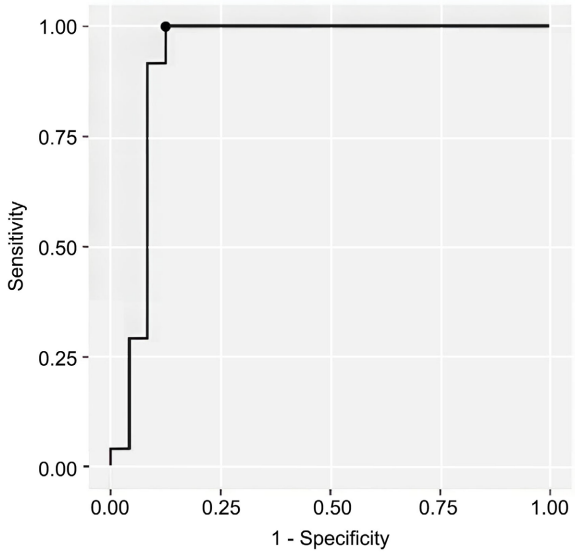


Figure 5. ROC curve for cut-off analysis of average pH values for first 24-H period.

3.5. Validation of the Lab-*Origin* Device Results Using Standard Benchtop Probes

Our findings showed strong positive correlations, high coefficients of determination, and satisfactory Bland-Altman analyses of the bias between pH results obtained with the Lab-*Origin* device and those assessed with standard benchtop probes (Mettler Toledo), as well as blood gas analyzers (Radiometer ABL800 Flex Blood Gas Analyzer). The results are summarized in **Table 3**.

Table 3. Results from linear regression and Bland-Altman analyses for comparisons of pH measurements obtained using Lab-*Origin* devices with measurements made using Mettler Toledo benchtop probes and blood gas analyzers (BGA).

	Lab-*Origin* device vs. Mettler Toledo pH	Lab-*Origin* device vs. BGA pH
Pearson product moment correlation coefficient	r = 0.943 ($P < 0.001$)	r = 0.926 ($P < 0.001$)
Coefficient of determination	$R^2 = 0.889$ ($P < 0.001$)	$R^2 = 0.857$ ($P < 0.001$)
Bland-Altman Analysis (Mean estimated bias ± standard deviation)	−0.116 ± 0.157 (95% CI: −0.081, −0.151)	0.226 ± 0.186 (95% CI: 0.270, 0.183)

4. Discussion

This study examined changes in peritoneal fluid pH and EC correlated with colonic anastomotic dehiscence in a swine model. Significant decreases in pH were detectable within two hours of leak induction, considerably sooner than clinical symptom onset. A pH value of 7.61 in the peritoneal effluent on POH-5 had a sensitivity of 100% for anastomotic leak. The pH of reservoir fluid was significantly lower in the experimental group than sham group at the end of the first 24-H ($P = 0.008$), however did not show statistical significance at 48-H ($P = 0.143$). These observations may be explained by sample size reductions that occurred over the course of the study, where some experimental animals were euthanized on POD-1, leaving fewer animals for collection of 48-H reservoir fluid. In the sham group, by the end of the study, some animals healed to the point that little reservoir and peritoneal fluid were left for testing, thus limiting statistical power. It is noteworthy that 3 out of the 11 experimental animals developed contained or walled-off leaks, in which extraluminal contents were tamponaded by surrounding tissues. Although these animals did not display the same gross peritonitis observed in complete dehiscence, they nonetheless showed biochemical disturbances in peritoneal drainage consistent with early leak physiology. This finding raises the possibility that continuous pH monitoring may support detection of sub-clinical or localized leaks.

The present model was also designed to minimize interference with current management of abdominal drains, providing smooth integration into clinical settings through use of technology used in-line with the JP tubing outside of the abdominal cavity. This feature also eliminated direct contact between the device and the anastomosis, precluding interference with the healing process at the anastomotic site. A high degree of alignment was found between measurements of pH obtained using *Stream*™ Platform and established techniques (Mettler Toledo benchtop probes and blood gas analyzers), substantiating the reliability of the readings obtained with the device.

Our findings corroborate previous reports demonstrating that peritoneal fluid pH could potentially be an early marker of AL. A recent systematic review of the literature by Walshaw *et al.* found robust evidence supporting the use of pH as a tool for early detection of AL following GI surgery [27]. Ten articles, including

two animal studies and eight human studies were included; five of these studies specifically examined the colorectal surgery context [17]-[19] [28] [29]. Notably, two studies found that as early as within the first 24 postoperative hours, significant differences in pH were observed between leak and non-leak subjects. Millan *et al.* conducted a prospective study of 90 patients with rectal or sigmoid cancer, where a primary anastomosis was conducted [18]. A catheter was placed directly at the anastomosis, to assess the intraluminal pH (pHi) via tonometry. Multivariate analysis showed that anastomotic pHi in the first 24 hours was an independent risk factor for AL ($P = 0.001$), with a pH cut-off of 7.28 yielding an AUC of 0.861, sensitivity 28.1%, and specificity 98.3% [18]. Corroborating these findings, Molinari *et al.* found significantly lower pH in drainage fluid on POD-1 and POD-3 ($P < 0.05$) [19]. pH cut-offs that maximized sensitivity and specificity included 7.53 on POD-1 (AUC = 0.80) and 7.21 on POD-3 (AUC = 0.86); sensitivity = 93.75% and specificity = 97% [19]. Critically, the timepoints at which pH provided a useful prediction of AL was significantly sooner than when subjects in these studies were diagnosed via standard-of-care (POD-6 to POD-12, average: POD-8) – pointing to the value of this biomarker in decreasing time to diagnosis and by extension, minimizing the devastating consequences of AL.

The pathophysiology behind pH and its connection with AL has been outlined in previous work, finding that various cells, cytokines, ions, and inflammatory substances present at the anastomotic site create an acidic microenvironment. Additionally, the production of substances like superoxide and nitric oxide is affected by pH levels [30] [31]. Studies have shown cellular dysfunction and delayed infection resolution when pH regulation of exudative cells is impaired [32]-[34]. Moreover, acute inflammation associated with leaks triggers neutrophil activation and prolongs their functional lifespan by activating specific molecular pathways through pH-dependent regulation of leukocyte activity in the inflammatory fluid [35].

Unlike pH, our model's EC findings did not show statistically significant differences in the assessment performed on both peritoneal and the reservoir fluids. Moreover, greater variability existed in conductivity readings in experimental animals compared to controls. Those findings are in keeping with previous studies that showed high variability in the use of electrical conductivity measurements for anastomotic leak detection [20] [25]. In a rat model of AL, the variability in measured EC was attributed to factors such as temperature inconsistencies, air pockets surrounding the electrodes, and omental positioning relative to the electrodes [20]. In our model, the device was positioned in-line with the tubing of the JP drain outside the abdominal cavity, and this could also have contributed to the variability and unreliable findings pertaining to EC assessed in the peritoneal and reservoir fluid. Ultimately, only a 64 kHz frequency was used for measuring EC, and a wider range of frequencies may be more effective in highlighting EC differences between experimental and control animals.

The present study has several limitations, including its small sample size and

use of an animal model. Limitations inherent to animal models precluded a clinical assessment that could be translated to humans, particularly related to the physical examination of the abdomen. Additionally, radiological methods (e.g. CT scans) were not used to investigate the anastomoses for dehiscence and leak, despite their frequent use in the clinical setting. Additional investigations, which would have further supported these results but were not able to be conducted, included an assessment of the systemic and local inflammatory response, and histopathological analyses of the anastomosis (which would have provided quantitative assessment of the inflammatory process). Further histopathological examination of the anastomotic tissue could have offered a more objective means of scoring inflammation, ischemia, necrosis, and early anastomotic breakdown, thus permitting a direct correlation between tissue-level pathology and the magnitude of pH alterations detected by the device. Including these data in future studies would help further substantiate the physiological basis of the biomarker changes observed. Of final note, comparisons between sham and experimental animals were restricted to a maximum of 48-H, given that by this time, all experimental animals had met criteria for re-laparotomy. This is considerably sooner than in the clinical setting. However, by this time, the study had achieved its objectives in detecting AL within experimental animals (hence why re-laparotomy and euthanasia was completed), and so this shorter window for comparison between groups is an unavoidable aspect of the study.

5. Conclusion

Our study demonstrated the use of a novel device for continuous pH and EC monitoring in a porcine model of colonic anastomotic leak. The device was applied inline with a Jackson-Pratt drain outside of the abdominal cavity, allowing for non-invasive monitoring of drainage fluid throughout the postoperative period, with significant differences in pH observed as early as two hours following leak induction. Conversely, EC measurements were more variable, and did not consistently differentiate leak from non-leak states, indicating that EC may be less reliable than pH for early leak prediction within the constraints of this experimental setup. Our findings highlight the utility of pH as a valuable biomarker in the early prediction of leaks, and support further investigation of *Stream*™ Platform in the clinical setting as a tool for bedside monitoring of AL.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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