**Bacterial translocation in horses with colic and the potential association with surgical site infection: a pilot study.**

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**SUMMARY**

**Background:** Surgical site infection (SSI) is a leading cause of morbidity in horses undergoing emergency exploratory laparotomy for the treatment of acute colic. The exact mechanism by which SSI develops in these cases is unclear. This prospective observational study investigated whether bacterial translocation occurs in horses with acute colic and if there is an association between bacterial translocation and development of SSI.

**Methods:** Peripheral venous blood (PVB) and peritoneal fluid (PF) samples were collected on admission and PF samples were collected at the end of surgery from horses presenting for investigation of acute colic. Any discharge from the laparotomy incision in horses that developed SSI was also collected. All samples were submitted for bacterial culture.

**Results:** In total, 7.7% of PVB samples (3/39), 11.8% (4/34) of admission PF samples and 8.7% (2/23) of the PF samples at surgery were culture positive. The prevalence of SSI was 10%. No association was identified between a positive PVB or PF culture and development of a SSI or survival to hospital discharge. **Conclusion:** Bacterial translocation can occur in some horses with acute colic. However, we were unable to identify any association between bacterial translocation and the development of SSIs following emergency exploratory laparotomy.

**INTRODUCTION**

Surgical site infection (SSI) is one of the most frequent morbidities that can develop following emergency exploratory laparotomy for the treatment of equine colic(1). SSIs result in increased hospitalisation and economic costs and are a major risk factor for subsequent incisional hernia formation, which may limit future athletic function(1,2). The prevalence of SSI following emergency exploratory laparotomy varies from 7.4 to 40%(3–9), while the prevalence of SSI following equine fracture repair is around 27.6%(10) and is as low as 0-1.5% following minimally invasive surgery such as laparoscopy and arthroscopy(11,12). Similar to equine surgery, gastrointestinal surgery in people also has a higher rate of SSI(13) compared to minimally invasive procedures(14) and orthopaedic surgery(15). In people, the risk of SSI after colon surgery can be up to 20%(13) whereas for fracture fixation it is around 2-5%(15).

A multitude of risk factors have been identified to increase the likelihood of SSI in horses following laparotomy for treatment of colic, and these have also differed between studies. Risk factors identified include repeat laparotomy, greater bodyweight, increased packed cell volume on admission, small intestinal resection, post-operative colic, application of a stent bandage, performing an enterotomy, administration of intraperitoneal heparin and antimicrobials, development of endotoxaemia, incisional closure method, intra-operative arterial oxygenation, duration of general anaesthesia and season in which surgery was performed(1,3,4,16–18). There is significant variation between studies as to the risk factors identified. Furthermore, factors such as use of a stent bandage and closure of the subcutaneous tissues have been shown to increase risk of SSI in some studies(16,19) but to be protective in others(8,20,21). The wide range of risk factors identified and differences between studies would suggest that SSI is multifactorial in nature and the exact route by which bacteria colonise the incision is unclear.

One theory is that SSI develops following direct bacterial contamination of the laparotomy incision at the time of surgery. However, positive intra-operative midline culture has not been associated with subsequent development of SSI(22) and where bacteria are cultured at the time of surgery, different isolates have been cultured when subsequent SSI has developed(23). Studies in people and dogs have also identified that a positive intra-operative bacterial culture is not a predictor of SSI(24–27). An alternative theory is that bacterial translocation occurs, where bacteria move across the intestinal wall to extraintestinal sites including the bloodstream(28). In people, bacterial translocation has been demonstrated following intestinal manipulation, intestinal obstruction, open fractures and burns(29) and has been reported to occur in 14% of human patients undergoing laparotomy(30). To our knowledge, no human studies have investigated bacteraemia and subsequent surgical site infection as most studies focus on bacteraemia as a result of SSI(31,32). Bacteraemia has also been reported in cats and dogs and positive blood cultures have been reported in 49% of critically ill cats and dogs(33). Bacterial translocation has been documented to occur in horses undergoing laparotomy with a reported prevalence of 13.8%(34). However, this study did not sample all horses prior to administration of antimicrobial agents and concurrent sampling of blood and peritoneal fluid was not performed.

The objectives of this prospective pilot study were therefore to investigate whether bacterial translocation occurs in horses with acute colic by determining the prevalence of positive bacterial cultures from blood and peritoneal fluid in a UK hospital population and to determine if there was an association with subsequent SSI. Our hypothesis was that bacterial translocation would occur and that in individual horses the same bacterial species would be isolated from either blood and/or peritoneal fluid and any SSI.

**MATERIALS AND METHODS**

**Study design and case selection**

This observational study was performed at the University of Liverpool Equine Hospital. Adult horses (>1 year of age) admitted to the hospital for the investigation of acute colic over a 12-month period (January 2018 - February 2019) were included in the study following informed owner consent. Peripheral blood samples (10ml) were collected from the jugular vein using a 19 gauge needle and 10ml syringe on admission as part of routine haematological investigations using strict aseptic technique, and prior to administration of any antimicrobials. Peritoneal fluid samples (0.5ml) were also collected on admission via transcutaneous centesis through the ventral midline using a 21 gauge 2 inch needle directly into a plain uncoated tube (BD Vacutainer, plain tube with silica) as part of routine investigations and (for cases undergoing exploratory laparotomy) peritoneal fluid samples were also taken following surgical manipulation of the abdomen, just prior to closure of the *linea alba* using a sterile syringe to aspirate peritoneal fluid that could be located and immediately transferred using aseptic technique into a plain uncoated tube (BD Vacutainer, plain tube with silica). Prior to collection of peripheral blood and peritoneal fluid on admission, a strict protocol for sterile preparation of the skin was followed using 4% chlorhexidine gluconate scrub for 3 minutes followed by cleaning of the site with 2% chlorhexidine gluconate and 70% isopropyl alcohol mixture solution (20:80). Blood samples were collected into Oxoid signala blood culture bottles and were either submitted directly to the laboratory for processing or were placed into an incubator and submitted for processing within 12 hours. Peritoneal fluid samples were collected into a plain uncoated tube (BD Vacutainer, plain tube with silica) and were either submitted immediately for processing or were placed into a refrigerator and submitted for processing within 12 hours of collection. SSI was defined as any purulent or serous discharge from the laparotomy incision of >24 hours duration(22). If a SSI developed post-operatively, a sample of the discharge was collected aseptically on a charcoal medium transport swabc and was submitted to the laboratory for culture and susceptibility testing. Bacterial culture was performed by direct plating onto 5% sheep blood agar. Plates were incubated aerobically and anaerobically for 2–7 days at 37.0°C. Results were reported as pure or mixed growth with all isolates identified at species level using API kitsd and GNID and GPID Sensititre Identification platese.

All horses undergoing surgery received procaine penicillin (14-22mg/kg intramuscularly q12h) and gentamicin (6.6mg/kg intravenously q24h) immediately prior to induction of general anaesthesia and after collection of the admission blood and peritoneal fluid samples. Antimicrobials were continued for 3-5 days post-operatively depending on clinician preference. Following recovery from general anaesthesia, abdominal bandages were placed consisting of a sterile absorptive contact layerf on the incision secured with an elastic cohesive dressingg wrapped around the abdomen and an elastic adhesive dressingg at the cranial edge of the bandage. Abdominal bandages were changed every 48 hours until horses were discharged from the hospital and the incision was monitored carefully for signs of a SSI.

As a control group, blood samples were also obtained from 10 clinically healthy horses. These horses were either undergoing elective surgery without any systemic disease in which bacterial translocation might occur, involving placement of an intravenous catheter and subsequent perioperative antimicrobials (n=4 elective orthopaedic surgery, n=3 laser sarcoid removal) or had routine intravenous catheter placement for nuclear scintigraphy as part of a lameness investigations without subsequent antimicrobial administration and anaesthesia (n=3).

**Data analysis**

Simple descriptive statistics were carried out using Microsoft Excel. The outcomes evaluated were 1: the frequency of positive cultures for blood and peritoneal samples; 2: the types of bacterial species and the antibiotic susceptibility profile and 3: the prevalence of both SSI and positive blood and/or peritoneal fluid culture. A Chi-squared test was used to test for significant differences between outcomes. A p value of ≤0.05 was deemed to be significant.

**RESULTS**

A total of 39 horses undergoing investigation for acute colic were recruited onto the study. The average age was 14 years old (range 3-27 years) and a variety of breeds were represented including Cob (n=6), Warmblood (n=6), Irish sports horse (n=6), Thoroughbred-cross (n=5), Welsh (n=4), Thoroughbred (n=3), Shire (n=2), Irish draft (n=2), Connemara (n=2), Shetland (n=1), Spanish (n=1) and Friesian (n=1) breeds. For the colic group, 38 peripheral blood samples (admission), 34 peritoneal fluid samples (admission)and 22 peritoneal fluid samples (end of surgery) were collected. For the control group, the average age was 11 years old (range 6-18 years) and the breed distribution was as follows: warmblood (n=5), Cob (n=2), Spanish (n=1), Cleveland Bay (n=1) and Irish Draft (n=1). A summary of the clinical and clinicopathological data collected on admission for the colic cases is displayed in Table 1.

|  |  |
| --- | --- |
| Variable (on admission) | Mean (± standard deviation) |
| Heart rate (per minute) | 61 (± 18.4) |
| Respiratory rate (per minute) | 25 (± 18.6) |
| Temperature (⁰C) | 37 (± 0.7) |
| Packed cell volume (%) | 40 (± 8.1) |
| Total protein (g/L) | 68 (± 14.5) |
| Peripheral lactate (mmol/L) | 2 (± 2.8) |
| Peritoneal fluid total protein (g/L) | 22 (± 18.5) |
| Peritoneal fluid lactate (mmol/L) | 5 (± 4.8) |

*Table 1: Summary of clinical and clinicopathological data on admission for the 38 colic cases recruited onto the study.*

Thirty-three of the colic group (84.6%) required emergency exploratory laparotomy and 6 were managed medically. Twenty-five (64.1%) horses had lesions affecting the small intestine whereas 14 (35.9%) had lesions affecting the large colon. Of the cases managed surgically, an enterotomy or anastomosis was performed in 28 cases. In 14 cases a pelvic flexure enterotomy was performed, in 10 cases a small intestinal resection or enterotomy and in 4 cases a small intestinal biopsy was performed.

Small intestinal strangulation was the most common lesion type representing 38.4% (n=15) of the colic cases. The distribution of primary lesions was: small intestinal strangulation due to a pedunculated lipoma (n=10), large colon displacement (n=10), other small intestinal strangulation (n=6), epiploic foramen entrapment (resulting in small intestinal strangulation) (n=4), equine dysautonomia (n=2), anterior enteritis (n=3), large colon impaction (n=1), large colon volvulus (n=1), peritonitis (n=1 ), idiopathic focal eosinophilic enteritis (n=1). The prevalence of SSI during hospitalisation was 10.2% (n=4/39). This included 3 horses with no positive cultures and one horse with a positive peritoneal fluid culture. There was no significant difference in development of a SSI between horses with a positive culture and those without (p=0.81). The median length of hospitalisation was 6 days (range: 0-16 days). Overall survival to hospital discharge for all horses recruited onto the study was 59.0% (23/39) and for those horses who stood following exploratory laparotomy, this was 64% (21/33).

The frequency of positive cultures in the colic group was very low as shown in Table 2. Overall, 7.7% (3/38) of peripheral venous blood samples were culture positive as were 11.8% (4/34) peritoneal fluid samples on admission and 8.7% (2/23) of peritoneal fluid samples taken immediately prior to closure of the abdomen at surgery. There were no positive blood cultures in the control group. A wide range of bacterial species were cultured as shown in Table 3.

|  |  |  |  |
| --- | --- | --- | --- |
| Case  | Blood sample (admission) | Peritoneal fluid (admission) | Peritoneal fluid (surgery end) |
| 1 | x | - | X |
| 2 | x | x | X |
| 3 | x | x | - |
| 4 | x | ✓ | X |
| 5 | x | x | X |
| 6 | ✓ | x | ✓ |
| 7 | x | x | X |
| 8 | x | x | X |
| 9 | x | - | - |
| 10 | x | x | X |
| 11 | x | x | ✓ |
| 12 | x | x | - |
| 13 | x | x | - |
| 14 | x | x | X |
| 15 | x | x | X |
| 16 | x | x | X |
| 17 | x | x | X |
| 18 | x | x | - |
| 19 | x | x | - |
| 20 | x | x | X |
| 21 | x | x | - |
| 22 | x | ✓ | - |
| 23 | x | x | - |
| 24 | x | x | X |
| 25 | x | ✓ | X |
| 26 | x | x | - |
| 27 | x | x | - |
| 28 | ✓ | x | - |
| 39 | x | - | - |
| 30 | x | x | - |
| 31 | ✓ | x | X |
| 32 | x | - | - |
| 33 | x | ✓ | X |
| 34 | x | x | X |
| 35 | x | x | X |
| 36 | x | x | X |
| 37 | x | - | X |
| 38 | x | x | X |
| 39 | x | x | - |
| Control 1 | x | - | - |
| Control 2 | x | - | - |
| Control 3 | x | - | - |
| Control 4 | x | - | - |
| Control 5 | x | - | - |
| Control 6 | x | - | - |
| Control 7 | x | - | - |
| Control 8 | x | - | - |
| Control 9 | x | - | - |
| Control 10 | x | - | - |
| Total positive samples (%) | **3 (7.7)** | **4 (11.8)** | **2 (8.7)** |

*Table 2: Summary of the samples collected and frequency of positive microbiological culture from 39 horses with acute colic and 10 non-colic control horses. X indicates no bacterial growth, ✓ indicates bacterial growth, - indicates that no sample was taken.*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Case | Sample type | Bacterial species | Penicillin sensitivity | Gentamicin sensitivity  | Diagnosis | Did SSI develop at the laparotomy incision? |
| 4 | PFadmission | *Burkholderia cepacia* (light growth ) | Resistant | Resistant | Epiploic foramen entrapment | No |
| 6 | BloodPFsurgery | *Bacillus* spp *Clostridium* spp | SuceptibleSusceptible | Suceptiblen/a | Large colon displacement | No (euthanised 5 days post-surgery due to persistent reflux) |
| 12 | PFsurgery | *Escherichia coli* | IR | Suceptible | Anterior enteritis | No |
| 22 | PFadmission | *Staphylococcus saprophyticus* | Suceptible | Suceptible | Large colon displacement | No |
| 25 | PFadmission | *Escherichia coli (1) , Enterococcus (2), Actinobacillus (3)*  | 1: IR 2: Susceptible 3: n/a | 1: Suceptible2: n/a3: resistant  | Epiploic foramen entrapment | No |
| 28 | Blood | *Staphylococcus epidermis and Staphylococcus warneri* | IR (both isolates) | Suceptible (both isolates) | Epiploic foramen entrapment | No (euthanised 2 days post-surgery due to acute renal failure and intestinal hyperammonaemia resulting in severe neurological deficits) |
| 31 | Blood | *Staphylococcus warneri*  | IR | Resistant | Strangulating pedunculated lipoma | No (euthanised 2 days post-surgery due to persistent endotoxaemia and reflux) |
| 33 | PFadmission | *Actinobacillus suis* | n/a | Suceptible | Strangulating pedunculated lipoma | Yes (*Klebsiella pneumoniae* MDR, ESBL positive) |

*Table 3: Sample type, microbiological isolate and lesion type in horses with a positive microbiological culture (PF: peritoneal fluid MDR: multi-drug resistant, ESBL: extended spectrum β-lactamase, IR: intrinsic resistance*(35)*).*

Of the horses with a positive culture, five (62%) survived to hospital discharge. There was no significant difference in survival between horses with a positive culture and those without (p = 1.00). Only one horse in the study that had a positive peri-operative culture (*Actinobacillus suis* from peritoneal fluid on admission) developed a subsequent SSI. The bacteria cultured from the SSI (*Klebsiella pneumoniae*) which was identified as an extended spectrum β-lactamase (ESBL) producing multidrug resistant (MDR) organism, was different to that cultured from the peritoneal fluid on admission (*Actinobacillus suis*).

**DISCUSSION**

This pilot study has demonstrated that bacterial translocation occurs in horses investigated for colic with a variety of different intestinal lesions in a UK hospital population, but at a low frequency. The findings from the current study do not support our hypothesis that bacterial translocation is a key factor in the development of SSI in horses following exploratory laparotomy. Only one horse in which bacterial translocation into blood or peritoneal fluid was confirmed at hospital admission developed a SSI; other horses with positive cultures including those that survived to hospital discharge did not develop a subsequent SSI. These results would suggest that although bacterial translocation into blood or peritoneal fluid does occur, it is not a major or single mechanism/contributor in the development of SSI following emergency laparotomy in the population of horses investigated.

In the current study, there was a low prevalence of positive bacterial culture from peripheral venous blood and from peritoneal fluid samples taken pre- and intra-operatively. These samples included samples taken on hospital admission, all of which were obtained prior to the administration of antimicrobial agents, unlike previous studies that have investigated bacterial translocation in horses investigated for colic(34,36). This study adds to the evidence that bacterial translocation from the gastrointestinal tract into the blood stream and/or peritoneal cavity and subsequent positive bacterial culture does occur but at a low frequency in horses with acute colic, consistent with the previous studies(34,36). The prevalence of positive blood culture in the current study (7.7%) was slightly lower than the 13.8% prevalence reported by Hurcombe *et al* (2012)(34). However, the latter study included culture results from mesenteric venous blood, mesenteric lymphatic tissue and intestinal luminal content as well as peripheral venous blood. In that study, only one peripheral venous blood sample was culture positive. Furthermore, the latter study only investigated horses with strangulating small intestinal lesions unlike the current study which included other types of colic. In small intestinal strangulation there is likely to be more severe intestinal wall compromise and disruption to the normal barriers to bacterial translocation than in horses with non-strangulating intestinal disorders; thus it is plausible that bacterial translocation across the gastrointestinal tract will be more likely to occur in those horses. Large colon strangulating lesions may plausibly result in bacterial translocation due to compromise to a large surface area of the gut but only one horse in this study had a large colon voluvulus. This horse had a negative blood culture preventing further investigation of this theory.

The prevalence of positive bacterial culture from peritoneal fluid in the current study was lower than a previous study(36), being positive in 11.8% of admission peritoneal fluid samples and 8.7% of peritoneal fluid samples taken at the end of surgery, prior to closure of the linea alba. The previous study reported positive bacterial cultures from 96% of peritoneal fluid samples taken during abdominal surgery in horses(36). That study sampled enterotomy and anastomosis sites, where some degree of bacterial contamination from intraluminal contents is likely but also included 11 positive samples from 13 horses not undergoing enterotomy or intestinal resection. The reason for the lower prevalence in this study is not clear and both groups would have received antimicrobials prior to surgery starting. Where a positive culture was obtained from peritoneal fluid, these horses did not develop overt peritonitis, suggesting that the type and duration of antimicrobial therapy was appropriate or that infection was cleared via natural defence mechanisms. Whilst penicillin and gentamicin are routine for prophylaxis for exploratory laparotomy, this is an area of variability about the need for antimicrobial use at all where the procedure is deemed to be clean(37). Both the timing and dosage of antimicrobials prior to exploratory laparotomy(37) and the duration of antimicrobial therapy after surgery(38) have been shown to have no association with development of SSI in equine colic cases.

We found no association between a positive culture and subsequent development of a SSI or altered survival to discharge in this pilot study. However, due to the small numbers, the study is under-powered to detect a significant difference between either group. Consistent with previous studies(23,34,36,39), the bacteria cultured from the pre- and intra-operative samples were not reflective of what was cultured from subsequent SSI. In addition, susceptibility testing performed on isolates from our study shows that five (42%) of the bacterial isolates were resistant to penicillin and a two (16%) of the isolates were resistant to both penicillin and gentamicin. However, the low prevalence of SSI in this study would support ongoing use of this regime. Previous work carried out at our hospital identified a similar pattern of resistance, with a high frequency of penicillin resistant bacteria in cultures from SSI(20). Ideally, all samples would have been collected prior to administration of antimicrobials as this may have affected the likelihood of a positive sample. However, it was not deemed ethical to withhold antimicrobials prior to surgery.

Determining whether a positive culture is reflective of bacterial translocation or sample contamination is often difficult. In human studies, up to 41% of positive blood cultures from septicaemic patients were considered to be due to contamination rather than true bacteraemia(40). The wide range of bacterial species cultured in this study was not consistent with the findings from previous studies in horses with colic. *E. coli* was reported to be the predominant organism cultured from blood samples in one study(34) whereas *E. coli, Streptococcus* spp. and *Enterococcus* spp.were the most common organisms cultured from peritoneal samples in another study(36). Other studies have shown that the most common bacterial isolates from equine skin following aseptic preparation are *Bacillus* spp. and coagulase negative *Staphylococcus* spp.(41,42), both of which were cultured from blood and peritoneal fluid samples in this study. *Staphylococcus* spp. have been reported to be present in the gastrointestinal tract of healthy horses(43,44). Therefore, determining contamination versus true bacteraemia is difficult. However, strict aseptic technique was followed in collection of the samples so the likelihood of contamination was considered to be low.

It was interesting that the only horse that had a positive culture on admission and who subsequently developed a SSI cultured *K. pneumoniae* which was MDR and ESBL positive from the SSI. *Klebsiella* species are uncommonly reported as causative agents for SSI in equine colic cases. Isgren et al (2017) reported that *Klebsiella* spp. were cultured from 1.7% of SSI in horses undergoing exploratory laparotomy at the same hospital over a 3-year period (July 2010-July 2013). ESBL-producers are the leading cause of blood stream and nosocomial infections in people(45) and are becoming increasingly common in horses(46). ESBL-producing genes are located on large plasmids which often carry other resistance genes making them multidrug resistant. These plasmids can be horizontally transmitted via conjugation to other bacteria and in doing so will also transfer these MDR genes(47). Although gastrointestinal carriage of ESBL-producing *Enterobacteriaceae* are common in hospitalised horses(48) they are infrequently reported in SSIs(3). However, it is important to highlight that carriage is a pre-requisite for infection with one human study showing that *K. pneumoniae* GI tract colonization on admission was significantly associated with subsequent infection in intensive care patients(49).

The main limitations in this study were the small sample size and the use of solely conventional culture methods to diagnose bacterial translocation. This was in part due to the nature of the study (pilot study), the funding available and the low study power which precluded further statistical analysis, particularly given the low frequency of SSI. This study only assessed development of SSI during hospitalisation. Owner-reported occurrence of SSI can be unreliable and it was not feasible for this study to include regular follow-up visits by referring veterinarians once the horses had been discharged from the hospital. Blood culture has a poor sensitivity for detecting bacteraemia in horses(50) so the number of bacteraemic horses in this study may have been underestimated. Previous studies have revealed that polymerase chain reaction (PCR) detected significantly more microbial DNA within the bloodstream compared to blood culture in people(51,52) and dogs(53). PCR techniques have been shown to detect as low as one to 10 bacterial cells per sample(54) which means that while PCR is extremely sensitive for the detection of bacteraemia, false positive results are also likely, especially from sample contamination. Furthermore, PCR will also detect non-viable bacteria or components of bacteria that may not be clinically significant. It has previously been demonstrated that PCR-positive patients have a higher disease severity despite a negative blood culture suggesting that microbial DNA in the blood, whether viable or not, may be clinically relevant(51,52). Quantitative PCR (qPCR) provides an estimate of the number of bacteria per millilitre of sample and this technique is likely to prove useful in the future at determining threshold levels of bacterial counts that can be deemed clinically significant(55). PCR presents an exciting opportunity for improved detection of bacteraemia and further investigation of bacterial translocation using these techniques is warranted in horses. However, given the potential for false positive results using PCR, the data must be interpreted with caution and the usefulness of culture should not be overlooked.

This study has demonstrated a low prevalence of bacterial translocation in horses presenting for acute colic, including concurrent blood and peritoneal samples and samples obtained prior to the administration of antimicrobials. No association was found between bacterial translocation and subsequent SSI. Larger studies using more advanced molecular techniques, such as PCR and qPCR or whole genome sequencing are required to more accurately understand the mechanisms in which bacteria colonise the incision in horses that develop SSIs following exploratory laparotomy.

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**Authors’ declaration of interests:**

No competing interests have been declared.

**Ethical animal research**

Ethical approval was granted by the University of Liverpool Veterinary Research Ethics Committee (VREC614). Owners gave informed consent for their animals’ inclusion in the study.

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**Manufacturer’s addresses**

a Thermo Fisher, Basingstoke, United Kingdom

b Becton Dickinson, Oxford, U.K.

c Deltalab, Barcelona, Spain.

d Biomerieux, France.

e TREK Diagnostic Systems, West Sussex, UK.

f Smith & Nephew Medical Ltd, Hull, Yorkshire, UK.

g BSN Medical, Hull, Yorkshire, UK.

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