clear that the microbiology laboratory and its staff support a broad range of clinical services and initiatives within hospital trusts, despite financial constraints. This service frequently remains covert, and only comes to light when results exceed set limits, and action is required (thankfully infrequently).

Despite the variation in practice noted in this survey, it is important that the contribution made by the laboratory is not underestimated, especially in terms of infection control within the hospital setting, and the results presented here demonstrate, once again, the flexibility and commitment of laboratory staff. □

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# Infective discitis due to *Staphylococcus lugdunensis* – a case of missed opportunity


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Bone or joint infection due to *Staphylococcus lugdunensis* is rare, there being only a small number of case reports in the literature.[1-9] In six cases, infection was either a local complication of recent surgery[2-4] or associated with an underlying prosthetic joint.[7,8] However, in other reports, particularly relating to vertebral osteomyelitis[5,8,9] and an epidural abscess,[6] there were no apparent risk factors. These cases demonstrate that bone and joint infection due to *S. lugdunensis* may be associated with severe clinical manifestations and therapeutic difficulties. Furthermore, the bacteriological diagnosis may prove elusive due to the potential for *S. lugdunensis* to be misidentified.

To illustrate this point, an unusual case of infective discitis due to *S. lugdunensis* is described in a patient who similarly had no apparent risk factors. In this patient, the diagnosis of *S. lugdunensis* bacteraemia was missed, and the subsequent diagnosis of *S. lugdunensis* infective discitis delayed by six months, due to the lack of a laboratory screening strategy to distinguish *S. lugdunensis* from other coagulase-negative staphylococci (CNS) isolated from blood culture.

In addition, recommendations for laboratory procedures to avoid such misdiagnoses are discussed.


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A 73-year-old woman with severe osteoarthritis underwent corrective foot surgery in July 2000. Apart from penicillin allergy, she was otherwise well. Four weeks later she presented with diffuse low back pain, constipation and painful retention of urine. She was afebrile and general examination was normal. Her erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were both elevated at 138 mm/hr and 231 μg/mL, respectively.

She was treated for five days with intravenous cefuroxime for a presumed urinary tract infection. At the time of admission, however, urine culture was negative but a penicillin-sensitive CNS (clumping factor- and protein A-negative; Pro-Lab Diagnostics, Canada) was isolated from two separate blood culture sets. The CNS isolates had similar antibiograms but were not speciated because they were thought to reflect skin contamination.

A plain X-ray at presentation demonstrated degenerative changes at multiple levels within the lumbar spine, with loss of disc height at L4/L5; however, the vertebral endplates were well preserved. Subsequently, a magnetic resonance imaging (MRI) scan of the lumbosacral spine showed high signal within the L4/L5 intervertebral disc, which was of reduced height.

Disc degeneration most commonly leads to loss of hydration and therefore low signal on the T2 weighted images. The appearance of high signal within this disc raised the suspicion of discitis. Minimal enhancement after gadolinium was demonstrated, however, and therefore the appearances were thought clinically to be most likely due to degenerative changes.

The patient was re-admitted six months later with a five-day history of severe low back pain. ESR and CRP remained elevated at 100 mm/hr and 97 ng/mL, respectively. A repeat MRI examination of the lumbosacral spine showed extensive marrow signal abnormality within the L4 and L5 vertebral bodies, with extensive enhancement within the L4 and L5 vertebral bodies and within the peripheral margin of the L4 / L5 disc and adjacent endplates.

A lumbar spine X-ray obtained prior to computed tomography (CT)-guided biopsy now demonstrated ill-defined lytic destruction of the vertebral endplates around the L4/L5 disc, with features in keeping with infective discitis and osteomyelitis. CT-guided bone biopsies of the L4/L5 intervertebral disc space and superior endplate of L5 both yielded penicillin-sensitive CNS (clumping factor- and protein A-negative). Isolates were identified as *S. lugdunensis* by API 20 Staph (bioMérieux, SA, France), and were confirmed by the Division of Hospital Infection, Public Health Laboratory Service, London.

Antibiotic profiles of the *S. lugdunensis* isolates and the previous penicillin-sensitive CNS grown from blood cultures were identical. All strains were sensitive to penicillin, erythromycin, clindamycin, gentamicin, fusidic acid, tetracycline, rifampicin, ciprofloxacin, chloramphenicol and vancomycin.

Following the biopsy procedure, blood culture was repeated and this grew *S. epidermidis* (penicillin- and fusidic acid-resistant), which was considered to be a contaminant. The patient was treated with clindamycin for a total of four weeks and made a good clinical recovery. There was no clinical evidence of infective endocarditis and an echocardiogram showed no abnormality. Two months later her CRP had returned to normal.

*S. lugdunensis* is a CNS named after the Latin name for Lyon, the French city where it was first described in 1988. It is characterised by a failure to produce free tube coagulase with rabbit and bovine plasma, but is clumping (fibrinogen affinity) factor- and ornithine decarboxylase (ODC)-positive.[10] Isolates are typically penicillin-sensitive.[1-9, 11,12]

*S. lugdunensis* has been associated most commonly with skin and soft-tissue infection, including abscess formation.[11] It is also an important cause of infective endocarditis, often attacking native valves, and follows an aggressive course.[12-14] The clinical picture, therefore, can be similar to *S. aureus* infection, particularly as there may not be an obvious portal of entry. A single case of *S. lugdunensis* meningitis following ventriculostomy has also been reported recently.[15] *S. lugdunensis* has also been linked to brain abscess, peritonitis and vascular prosthetic infections.[1]

In the case reported here, *S. lugdunensis* bacteraemia probably occurred following foot surgery. The subsequent infective discitis with vertebral osteomyelitis is typical of haematogenous spread. The penicillin-sensitive CNS originally isolated from blood culture showed an identical extended antibiogram to the *S. lugdunensis* strains cultured from intervertebral disc and bone-marrow biopsies. Unfortunately, they were not available for further examination when the patient was re-admitted. If further identification tests had been undertaken when the blood culture CNS were first isolated, it is likely that the patient would have avoided the prolonged delay in diagnosis.

Few laboratories have screening strategies in place to distinguish *S. lugdunensis* simply from other blood culture CNS because infection is so rare. In a study of 258 CNS in blood cultures, *S. lugdunensis* accounted for only 1.1% of true bacteraemic isolates and 1.6% of contaminants. The most common species were *S. epidermidis, S. hominis* and *S. haemolyticus*.[16]

In an earlier study of 978 tube coagulase-negative staphylococcal isolates from blood culture, no strains of *S. lugdunensis* were found.[13] Similarly, a 20-year review of 89 staphylococcal isolates from patients with bacterial endocarditis identified *S. lugdunensis* in only four cases.[17]

Nevertheless, in view of the clinical importance of *S. lugdunensis* infection, a variety of screening strategies have been proposed, including tests for pyrrolidomylarylamidase (PYR), ODC, alkaline phosphatase and maltose/mannose fermentation.[11,18,19] For clumping factor-positive CNS, only PYR and ODC are required to identify *S. lugdunensis*. If clumping factor-negative, then an additional fermentation test is required for confirmation.

Although these tests are available as commercial media, they may prove expensive because CNS are the most frequently isolated bacteria from blood culture sets. Therefore, their suitability in busy diagnostic laboratories is questionable. However, the recently introduced 'ID staphylococcus ring' (Mast Diagnostics, UK), which incorporates tests for novobiocin and desferrioxamine sensitivity with p-nitro phenol phosphate, is a promising alternative. Although requiring further evaluation, it is designed to distinguish simply the main CNS pathogens (*S. epidermidis, S. saprophyticus, S. haemolyticus* and *S. lugdunensis*), and therefore may provide an alternative screening system.

Clumping factor-positive, tube coagulase-negative staphylococci clearly warrant further follow-up because *S. lugdunensis* and *S. scheiferi* are the most likely species. However, as demonstrated in the case study presented here, clumping factor alone cannot be relied upon because the test is not 100% positive (87% from human plasma, 73% from rabbit plasma) for *S. lugdunensis* strains.[18]

Furthermore, the ability of commercial reagents widely used in diagnostic laboratories to detect staphylococcal clumping factor have also not been specifically evaluated against *S. lugdunensis* strains. Similarly, ODC is not a unique characteristic for *S. lugdunensis* as mis-identifications due to weakly positive results in some cases of *S. haemolyticus* isolates have been reported.[18]

Most case reports of *S. lugdunensis* infection have been due to β-lactamase-negative, penicillin-sensitive strains. This is a relatively unusual characteristic for CNS. In our own laboratory, only 5% of CNS from blood culture exhibit penicillin sensitivity. Hence, the routine speciation of penicillin-sensitive CNS may prove to be a simple screening strategy to distinguish *S. lugdunenis* from other CNS isolated from blood culture.

However, in view of occasional reports of penicillin resistance and, more recently, oxacillin resistance linked to the *mecA* gene,[20] we suggest that all CNS repeatedly isolated from blood culture or predominant growths from deep tissue samples should be identified to species level. □

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# Effect of sample tube type and time to separation on *in vitro* levels of C-reactive protein

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Highly sensitive C-reactive protein (hs-CRP) is assuming increased importance in the evaluation of patients with coronary artery disease (CAD). It is a prognostic indicator in acute coronary syndromes[1-3] and a predictor of future coronary events in those with and without overt CAD.[4,5] As small changes in hs-CRP concentration potentially have considerable clinical impact, all aspects of the analytical process need to be evaluated.

Highly sensitive CRP has undergone numerous evaluations on pre-analytical and analytical variability. In healthy individuals, hs-CRP exhibits no diurnal variation,[6] and hs-CRP shows little variation over a 12-month period, with a similar stability of measurement to total cholesterol.[7] Analytical variability of hs-CRP assays has been assessed recently by Roberts and colleagues, who reported coefficients of variation (CV) <10% at 0.15 mg/L.[8]

It is widely recognised that sample collection tube type may affect analyte concentrations. In particular, 'gel' tube effects have been described for anticonvulsant drugs[9] and intact parathyroid hormone.[10] However, the effect of sample collection tube type on hs-CRP concentration has not been studied.

This study aims to assess the stability of serum and plasma hs-CRP in different collection tubes over a 24-hour period, using the DPC Immulite highly sensitive CRP assay.

Blood samples were collected from seven patients with chronic renal failure (on dialysis) into a 10 mL plain glass tube (tube A; Lip Z10/GN), a 2.7 mL EDTA tube (tube B [2.7 ml KE]; Sarstedt Monovet, Germany) and a 4.2 mL gel tube (tube C [4.2 ml,Z GEL]; Sarstedt Monovet).

Samples were transported to the laboratory on ice and remained unseparated at room temperature (17–23°C) until centrifuged. At 20 min (baseline), 1, 2, 4, 8 and 24 h, a 1 mL sample was taken from each tube and centrifuged. The resultant supernatant was frozen at –20°C until analysis, which was carried out in one batch using a DPC Immulite high sensitivity CRP assay (LKCR, Diagnostic Products, Los Angeles; intra-assay CV = 4.1%), immediately after thawing. Statistical analysis was by repeat measures ANOVA and, where significant, was followed by Tukey-Kramer post-test comparison.

Results of tube-type effect and time on hs-CRP values are shown in Table 1. Significant differences were observed in samples separated at 0.3 h (ANOVA $P$=0.0192), and hs-CRP values were significantly higher ($P$<0.0.5) in gel tubes than in plain and EDTA tubes. Otherwise, there were no significant between-tube differences in hs-CRP concentration.

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**Table 1.** Mean CRP concentration (mg/L) for each sample tube against time

| | Time | | | | | |
|---|---|---|---|---|---|---|
| | 0.3 h | 1 h | 2 h | 4 h | 8 h | 24 h |
| Tube A (Plain) | 17.0(17.3) | 16.6(16.8) | 17.2(17.9) | 17.4(17.7) | 17.7(18.1) | 17.8(17.6) |
| | A/C+ | | | | | |
| Tube B (EDTA) | 16.8(17.1) | 17.2(17.8) | 16.7(16.4) | 16.0(16.1) | 15.9(16.6) | 17.2(18.2) |
| | B/C+ | | | | | |
| Tube C (Gel) | 18.5(18.3) | 17.6(17.7) | 17.4(17.3) | 17.5(17.9) | 16.6(16.3)* | 16.7(16.9)* |

Results are mean (Standard Deviation)
Within sample tube C (Gel), compared to 0.3 h * = $P$<0.05
Between sample tubes. A/C + = $P$<0.05, B/C + =$P$<0.05