Presumptive diagnosis of brucellosis from contaminated blood cultures in an area of endemicity

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Brucellosis is a typical zoonosis that is endemic in large areas of the Middle East,[1] and, despite improved medical care, it remains a major cause of morbidity in the area. In Saudi Arabia, *Brucella* sp. is one of the most common isolates from blood culture samples, and 75 examples were isolated from a total of 1903 positive blood culture specimens (4%) at the King Fahad National Guard Hospital during 2000.

Institution-specific blood culture contamination rates vary from 1% to more than 6%,[2] and in this institution the contamination rate for the total number of blood cultures collected during 2000 was 3.8%. Owing to the high isolation rate and the fastidious nature of this organism, it is possible that some of the contaminated blood cultures may mask the presence of *Brucella* sp. when these specimens are subcultured on routine bacteriological culture media. A previous study has showed that a direct urease test on positive blood cultures that are suggestive of *Brucella* sp. is useful in the presumptive diagnosis of brucellosis.[3]

The aim of this study is to explore the utility of the direct urease test to detect the presence of *Brucella* sp. in blood culture vials that also contain coagulase-negative staphylococci, a common contaminant.

Simulated blood cultures were prepared using a 0.5 Macfarland standard suspension of *Staphylococcus epidermidis* (ATCC 12228) and similar suspensions of 50 separate patient isolates of *Brucella* sp. isolated during 2000. Each suspension was further diluted to give a final concentration of 10–100 colony-forming units (CFU)/mL and this was used as the working dilution. Each of 50 BACTEC aerobic blood culture vials were inoculated with 5 mL of the working dilution of both coagulase-negative staphylococci and one of the brucella suspensions. Three control vials were also prepared: one containing an isolate of *Brucella* sp., one containing a test strain of coagulase-negative staphylococci and one with no organisms added. Each blood culture vial was then loaded onto the BACTEC 9240 automated blood culture machine.

All the test vials automatically flagged as positive after 18–48 h incubation. The control vial containing the coagulase-negative staphylococci alone flagged as positive after 43 h and the control vial containing *Brucella* sp. alone flagged as positive after 83 h. The uninoculated blood culture vial remained negative at seven days.

All specimens were Gram-stained and subcultured onto a sheep blood agar plate and a urea slant, both of which were incubated at 37°C in an aerobic atmosphere. The original blood culture vials were retained and placed in a 37°C incubator.

Gram-stained preparations from all the culture vials showed Gram-positive cocci in clusters, indicative of *Staphylococcus* sp. The urea slants were checked after 4, 6, 8 and 24 h. After 4 h, 44 (88%) of the 50 urea slants were positive. After 6 h, a further three urea slants were positive, giving a total of 47 (94%) and one more slant was positive at 24 h, giving a grand total of 48 (96%). The control vial inoculated with *Brucella* sp. gave a positive urease test after 4 h. The control vial inoculated with *S. epidermidis* and the uninoculated control vial both remained urease-negative at 24 h.

The retained blood culture vials were subcultured onto fresh urea slants after four, five and seven days' incubation. A total of 49 (98%) out of the 50 urea slants inoculated after four days' incubation of the original vial gave a positive reaction within 4 h. Control vials gave the same reaction as previously described. This profile did not change when repeated after five and seven days' incubation.

Coagulase-negative staphylococci is the most frequently isolated blood culture contaminant.[4–6] The problem of pseudobacteraemia and the clinical and financial consequences of unnecessary treatment are well documented.[7] In Saudi Arabia, where brucellosis is endemic, there is the added possibility that brucella bacteraemia may be masked by the presence of contaminating organisms in blood cultures.

The present study showed that a simple biochemical test used directly on blood cultures that flag positive and show what may prove to be contaminating organisms on primary Gram-stain (Gram-positive cocci in clusters) can detect underlying, coexistent *Brucella* sp. This direct test appeared to be more useful if used on retained blood culture vials, once the result of the culture is known, and would ensure that test is only applied to those organisms highlighted as probable contaminants. In the present study, 98% *Brucella* sp. masked on subculture by coagulase-negative staphylococci gave a positive urease test after 4 h on blood culture vials retained for four days.

Although it is possible that a urease-positive organism may contaminate the blood culture vial, it is unlikely that one would do so that produces such potent urease activity as to give a positive reaction within 4 h. Thus, it is concluded that a direct urease test on blood cultures containing coagulase-negative staphylococci that may mask the presence of co-existing *Brucella* sp. may prove a useful tool in the presumptive diagnosis of brucellosis from contaminated blood cultures in an area of endemicity. □

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Severe acquired chylomicronaemia syndrome – a challenge to the routine laboratory

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Lipaemia is a common interfering (spectral or chemical) factor that can complicate biochemical analysis.[1,2] In patients with chylomicronaemia syndrome, gross elevations of plasma triglyceride-rich lipoproteins, particularly chylomicrons and/or very-low-density lipoproteins are found.[3,4] This causes sample turbidity that can interfere with some analytical methodologies involving spectrophotometric measurements. Hence, special attention must be paid to the removal of such interfering lipaemia prior to analysis.

Recently, a case of poorly controlled diabetes complicated by severe hypertriglyceridaemia (maximum plasma triglyceride [TG] concentration: 130 mmol/L) was encountered. Samples received for lipid profile and liver/renal function tests (L/RFT) were grossly milky.

The aim of this short study is to discover which tests in the routine L/RFT profile are affected, what mechanism is involved and what appropriate measures should be used in the clinical laboratory for adequate lipaemia clearance at various hypertriglyceridaemia levels.

Pooled plasma with a clear appearance and normal L/RFT was used (neat TG: 1.2 mmol/L). Intralipid (20% emulsion; KabiVitrum, Stockholm, Sweden) was spiked into the pooled plasma at four TG levels (23, 49, 58 and 95 mmol/L). The samples were then analysed by both wet and dry chemistry analysers (Hitachi 747-100 [Roche/Boehringher Mannheim, Germany]) and Ortho Vitros-950 (Johnson & Johnson, Ortho Clinical Diagnostics, USA) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), glucose, lactate dehydrogenase (LDH),) phosphate and urea. Only tests quantitated at an ultraviolet wavelength were selected.

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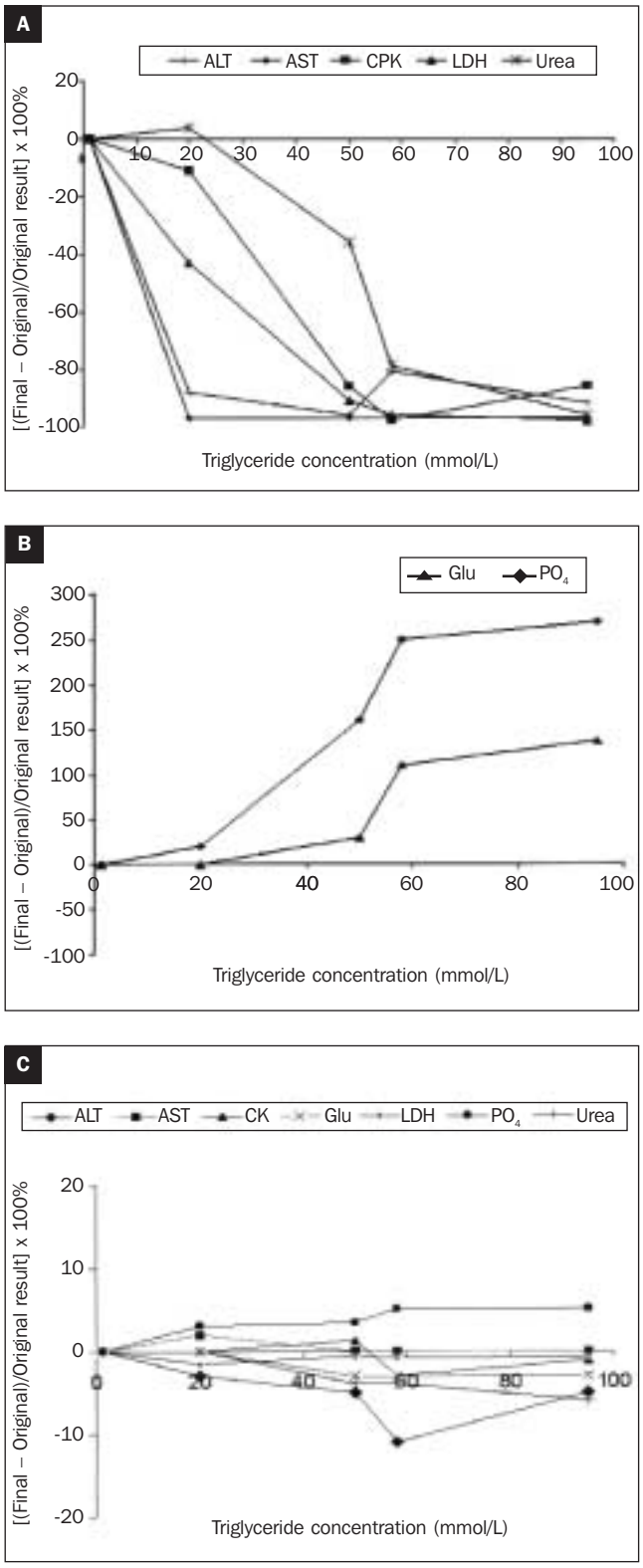

Fig. 1. Triglyceride interferographs. A and B) Hitachi 747-100; C) Vitros-950.

Subsequently, a further set of TG concentrations (26, 48, 78 and 126 mmol/L) was prepared and each of these was subjected to three lipaemia clearing procedures (high-speed