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Extraction of genomic DNA from *Pseudomonas aeruginosa*: a comparison of three methods

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In diagnostic clinical microbiology there is growing interest in the adoption of molecular techniques to enhance and supplement conventional techniques. The majority of these molecular methodologies involve the use of extracted genomic DNA from bacterial cells for either diagnostic purposes and/or epidemiological subtyping. Regardless of the downstream application, all methods share a common procedure in the extraction of DNA from cells.

Previously our group has reported on the difficulties associated with the reliable extraction of bacterial DNA from blood cultures.[1] Here, we describe a simple observation that may be beneficial to laboratory personnel involved in the extraction of genomic DNA from bacterial pure cultures.

Pseudomonas aeruginosa isolates (n=60) were obtained by conventional selective culture from the sputa of adult cystic fibrosis (CF) patients attending the Northern Ireland Regional CF Centre. Three DNA extraction protocols were compared by several criteria as detailed in Table 1. For all methods, a loop (10 µL) of overnight culture of *P. aeruginosa* from Columbia blood agar (Oxoid CM0331; Oxoid , Bassingstoke, England), supplemented with 5% (v/v) defibrinated horse blood, was extracted.

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Method A (boiling): Bacteria were placed in molecular grade water (200 µL; BioWhittaker, Maryland, USA) and boiled for 10 min, then centrifuged at 12,000 x**g** for 5 min. The supernatant was stored at 4˚C.

Method B (QIAamp blood kit; Qiagen, Crawley, UK): Bacteria were placed in phosphate-buffered saline (PBS; 200 µL) and proteinase K (25 µL) was added along with 'binding buffer' (200 mL) as supplied. This was vortex-mixed immediately for 15 sec, or until the pellet dissolved. The mixture was incubated at 70°C for 10 min. DNA was extracted and eluted from spin columns, following the manufacturer's instructions, and then stored at 4°C.

Method C (High-purity PCR template preparation kit; Boehringer Mannheim, Lewes, UK): Bacteria were suspended in 200 µL of 10 mmol/L Tris-EDTA buffer (pH 8.0). Lysozyme (30 µL; pH 7.4) was added and the samples were incubated at 37°C for 45 min. Binding buffer (200 µL) and proteinase K (40 µL) were added and incubated at 72°C for 15 min. DNA was extracted and eluted from spin columns, following the manufacturer's instructions, and stored at 4°C.

Extracted DNA (15 µL) was examined by 2% (w/v) agarose gel electrophoresis (80V, 45min) and ethidium bromide (5 µg/100 mL) stained gels were visualised under ultraviolet illumination with a gel image analysis system (UVP Products, England). All images were archived as digital (*.bmp) graphic files. The ability to amplify extracted DNA was determined by a specific polymerase chain reaction (PCR) assay that targeted the outer membrane protein of *P. aeruginosa*, as previously described.[2]

The quality and quantity of extracted DNA obtained using the three methods are described in Table 1. All DNA extracts were able to generate a PCR amplicon of the correct molecular size.

The ability to extract genomic DNA from bacterial cells grown in culture is the cornerstone of any downstream molecular manipulation. Table 1 details the outcomes against several criteria for the three extraction methods employed; however, Wilson provides a comprehensive review of PCR inhibition and its causes.[3]

Overall, we would discourage use of DNA extraction from bacterial culture by boiling. We have experienced difficulty in the repeatability of downstream PCR reactions, particularly when boiling is used to extract DNA from pigmented isolates grown on agar culture and from cells that produce thermostable nucleases, including methicillin-resistant *Staphylococcus aureus* (MRSA),[4] and when such extracts are re-amplified after storage. Therefore, we would encourage the use of commercially available DNA extraction kits, which allow relatively rapid extraction of high-quality genomic DNA that is stable at 4˚C and can be used after

Table 1. Comparison of three methods for the extraction of genomic DNA from *Pseudomonas aeruginosa*

DNA extraction method	Speed	Quantity of DNA	Degree of shearing	Cost	Viability of DNA on storage at 4˚C
Boiling	Fast	Low	High	Low	Short (<7 days)
QIAamp blood kit (QIAGEN)	Moderate	High	Low	High	Long
High-purity PCR template preparation Kit (Boehringer Mannheim).	Moderate	High	Low	High	Long

extended storage. Boiling extraction represents a false-economy and leads invariably to frustration as laboratory staff have to repeat the extraction, usually by employing a commercial kit. □

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Hyperparathyroidism – pitfalls in management

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Parathyroid cancer is uncommon and easy to miss, and the limited studies available suggest the need for family screening and radical surgery. Medical options include chemotherapy, immunotherapy and bone protection with bisphosphonates, calcium and vitamin D or hormone therapy. The following case history illustrates some of the problems encountered.

A 77-year-old lady with a background of hypertension (controlled on methyl-dopa and bendrofluazide) was referred as an emergency with severe hypercalcaemia. Physical examination revealed significant dehydration. Initial investigations showed normal renal function but a corrected serum calcium of 3.96 mmol/L.[1] Emergency management consisted of intravenous rehydration and a standard infusion of disodium pamidronate (90 mg). Bendrofluazide treatment was stopped.

Serial serum calcium measurements are shown in figure 1. The intact serum parathyroid hormone level was markedly elevated at 533 pg/mL (reference range 10-50 pg/mL), 24-hour urinary calcium was elevated at 8.2 mmol/L (2.5-7.5 mmol/L), and a diagnosis of primary hyperparathyroidism was established.

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Despite initial response to rehydration, calcium levels remained high and began to rise further one month after the initial pamidronate infusion (Figure 1). Bone scan was normal and X-rays of the patient's hands showed degenerative changes only. A parathyroid subtraction scan showed increased uptake at the right upper pole of the thyroid, which was measured on ultrasonography as 2.0 x 1.7 x 1.8 cm, and a smaller area of uptake at the left lower pole. Following preoperative vocal cord assessment, which was normal, a parathyroidectomy was carried out, during which both masses were excised.

Histopathological examination of the right upper mass revealed a parathyroid nodule (2.5 cm diameter) with a follicular growth pattern. The nodule was surrounded by a thickened capsule and strands of fibrous connective tissue that extended into the parathyroid tissue, dividing it into bands. The capsule showed extensive haemorrhage and haemosiderin deposition, and the parathyroid parenchyma contained focal areas of calcification. Although there was minimal pleiomorphism and no frank vascular invasion, infiltration of the capsule by parathyroid tissue was seen. These features were suggestive, but not diagnostic, of parathyroid carcinoma. The nodule removed from the left lower pole proved to be a normal parathyroid gland.

Corrected calcium was 2.6 mmol/L on the first post-operative day, and this level fell gradually over consecutive days to 2.16 mmol/L and then stabilised. A subsequent dual emission X-ray absorbiometry (DEXA) scan showed decreased right hip bone mineral density (BMD) compared to the mean of the young adult female population (T score: 2.4 SD) and that of the age/gender matched control (Z score: 0.7). Lumbar spine values were normal. The patient was discharged to her home, symptom-free. On follow-up, three months after initial presentation, she remained well, with a calcium level of 2.36 mmol/L and PTH of 44 pg/mL. She continues to be monitored to check for evidence of recurrence.

Parathyroid carcinoma is rare and an uncommon cause of hypercalcaemia. It usually presents in the fourth decade of life. Clinical pointers include severe hypercalcaemia (>3.5 mmol/L), a palpable mass and unilateral vocal chord paresis,[2] but not all malignant parathyroid tumours are functional. Histopathological diagnosis in isolation can be difficult but markers include a solid growth pattern, extensive fibrosis, necrosis, nuclear atypia and mitotic figures. Sandelin *et al* reported a series of parathyroid carcinomas in which an initial benign diagnosis was give in 50% of cases.[3] The presence of DNA aneuploidy has been shown to correlated with adverse histological features and a worse prognosis.[4,5] The retinoblastoma tumour suppressor gene is found in some malignant tumours and may be of diagnostic and prognostic value.[6]

Diagnosis of parathyroid carcinoma has implications both for the individual and for family members, as there may be an associated hereditary syndrome (MEN1, MEN2A, hyperparathyroidism-jaw tumour syndrome or familial isolated hyperparathyroidism) in approximately 10% of cases.[7,8]

Experience in the management of parathyroid malignancy is limited as it is an uncommon condition, but it is a very slow-growing tumour and radical surgery can be curative, even in what appears to be invasive disease. Such surgery needs to be extensive and include resection of the ipsilateral