patient was already undergoing treatment with broad-spectrum antibiotics at the time the sample was obtained. The results presented here show that significant organisms are more likely to be isolated from enrichment culture of tissue, bone and pus.

The recovery of *Peptostreptococcus* species from peritoneal fluid emphasises the importance of subculturing enrichment broths in air (plus 8% carbon dioxide) and anaerobically.

Little scientific evidence was found in the literature for the utility of enrichment culture for tissue and fluid samples in clinical microbiology as assessed by a large comparative trial of different enrichment media. Although the data presented here involved the use of only one enrichment medium, the choice is based on tradition, with no attempt made to compare various enrichment broths. Any organisms isolated from this medium were considered relevant only if the clinical condition of the patient suggested the organism's role in causation of disease.

This retrospective audit demonstrates the value of enrichment culture for samples of bone, tissue and pus, but not for various other fluids received in the UCH laboratory. The results suggest that a large trial of different enrichment media on such fluid samples is needed on patients before therapy begins and on those already undergoing treatment.

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Extraction of DNA from paraffin sections with proteinase K and DNAzol

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Currently, the use of phenol/chloroform is the method of choice for DNA isolation from paraffin sections. However, in addition to its toxicity, the phenol/chloroform process is time-consuming and DNA can be lost. In the present study an alternative method is described which uses proteinase K and DNAzol for DNA extraction from paraffin-embedded tissue that is simple, quick and efficient.

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Three 5 µm sections were cut from formalin-fixed, paraffin wax-embedded blocks of oesophageal cancer tissue and placed in Eppendorf tubes. Xylene (1 mL) was added, mixed, incubated at room temperature for 15 min then centrifuged for 2 mins at 12,000 ×*g*. The supernatant was discarded. The dewaxing step was repeated with 1 mL xylene and then 1 mL 100% ethanol was added and incubated for 15 min, then centrifuged to remove the ethanol. This step was repeated (x2) to remove the xylene. The tubes were then placed in a 60°C oven for 30 min to dry the pellet.

30 µL lyses buffer (10 mmol/L Tris-HCl [pH 8.0], 1 mmol/L EDTA, 1% SDS) and 20 µL proteinase K (20 mg/mL) were added to the tissue pellet and incubated at 55°C in a water bath overnight. DNAzol (1 mL) was added, mixed and allowed to stand at room temperature for 10 min, then centrifuged to remove the undigested fragments. The supernatant was transferred to a new tube.

Genomic DNA was precipitated by the addition of 0.5 mL 100% ethanol and 8 µL glycogen (20 g/mL). The homogenate was stored at room temperature for 10 min. The DNA was collected by centrifugation at 12,000 ×*g* for 10 min. The DNA precipitate was washed with 70% ethanol. Finally, the DNA was dissolved with 8 mmol/L NaOH.

The dewaxed tissue sections were digested as above. The protein was removed with phenol/chloroform/isopropanol extraction. The upper aqueous supernatant was transferred carefully into a fresh tube. A one-tenth volume of 3 mol/L NaAC (pH 5.2) and two volumes of 100% ethanol were added and inverted, then stored at –20°C overnight.

The DNA was precipitated by centrifugation at 12,000 xg for 10 min. The DNA pellet was rinsed and resuspended as described above. A DNA sample (5 µL) was electrophoresed in 1% agarose (80 V, 45 min) then photographed with the EagleEye digital gel documentation system. A DNA sample (2 µL) was mixed with 6 mL ethidium bromide (EB, 0.8 µg/mL), dotted on the plastic membrane, photographed and analysed with Labworks software.

DNA (2 µL) was used as the template for the polymerase chain reaction (PCR). GAPDH primers (GAPDHg sense: 5′ TACAAGCGTTTTCTCCCTAAA 3′, GAPDHg anti: 5′CCCAATACGACCAAATCTAA 3′) of genomic sequence were designed according to the sequence (Accession number: NC_000012, Region: 6513945.6517797), and synthesised by Bosia Co (Shanghai, China). PCR was performed as described using r*Taq* (Takara, Japan). The PCR program began with initial denaturation at 94°C for 5 min, followed by 40 cycles (94°C for 30 sec, 51°C for 30 sec and 72°C for 35 sec) of amplification, with a final extension at 72°C for 10 min. Products were visualised and photographed with Genesnap (Gene Co, USA) after electrophoresis.

DNA extraction from a wide range of biological organisms has been used worldwide to assist in the diagnosis of diseases and in forensic identification, for example. Despite the many methods available by which DNA can be utilised, there are only are a few protocols for collecting DNA from paraffin tissues. Although the traditional proteinase K/phenol/chloroform extraction method is still in use, there are some disadvantages as the process is time-consuming

and there is DNA loss.

Recently, commercial DNAzol reagent has been reported to be a rapid, efficient method for DNA extraction from various sources;[1–4] however, its suitability for use with paraffin wax-embedded tissues is unknown. In the present study, proteinase K and DNAzol reagent were used to produce DNA from such samples. In addition, the inclusion of an additional step to disaggregate the sections prior to proteinase K digestion may improve DNA yield further. It is not suggested that tissue be rehydrated after dewaxing as the fragments could float, even after centrifuging, due to their lower density in water.

When DNAzol was added, the homogenate needed to be centrifuged in case a little incompletely digested debris remained. In the DNA precipitation procedure, 8 µL glycogen DNA carrier was supplemented in the lysed sample in 0.5 mL DNAzol in order to increase DNA recovery.

Two samples were tested with the proteinase K and DNAzol method and compared with the results obtained with the proteinase K/phenol/chloroform protocol. The DNA yield with DNAzol was greater than that with phenol/chloroform (Fig. 1) and the results show a yield of approximately 150 ng and 80 ng for samples 1 and 2, respectively, analysed with Labworks software.

Figure 2 shows that DNA extracted with DNAzol includes a large DNA fragment in addition to the smaller fragments, whereas DNA extracted using phenol/chloroform mainly comprises small-sized fragments. Thus, DNA yield with DNAzol may be more suitable as a template for PCR than is DNA produced with the phenol/chloroform method (Fig. 3). The PCR products are clearly visualised under ultraviolet (UV) light.

The proteinase K/DNAzol protocol described is a rapid, efficient approach to the extraction of DNA, with satisfactory production from paraffin-wax embedded tissue. This method may prove more suitable for isolating DNA from precious, small-sized biological materials.

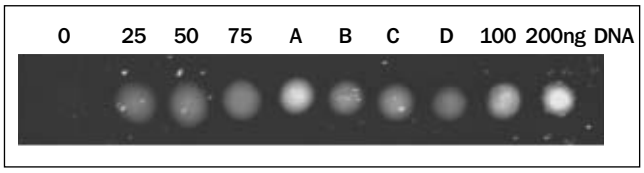

Fig. 1. DNA dot blot detection. Dots A and B show the DNA extracted from samples 1 and 2 with DNAzol; dot C and D show the DNA extracted by the phenol/chloroform method.

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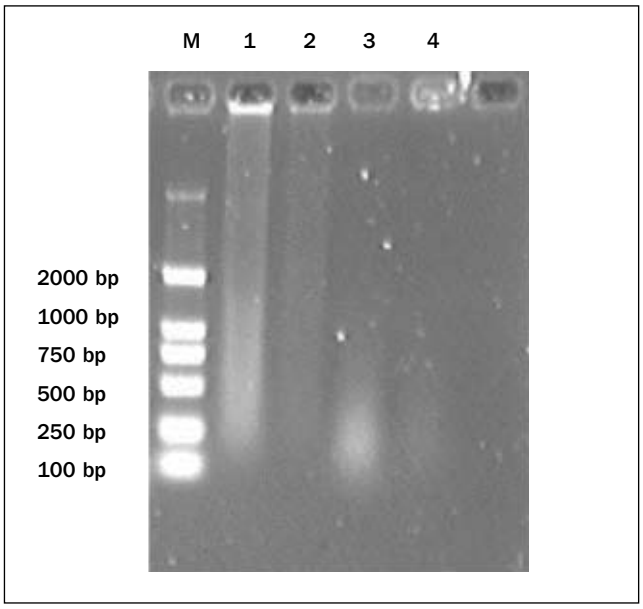

Fig. 2. Electrophoresis of DNA extracted from paraffin wax embedded tissue. M, Marker DL2000; lanes 1 and 2 show samples 1 and 2 extracted using the proteinase K/DNAzol method; lanes 3 and 4 show the same samples extracted using the phenol/chloroform method.

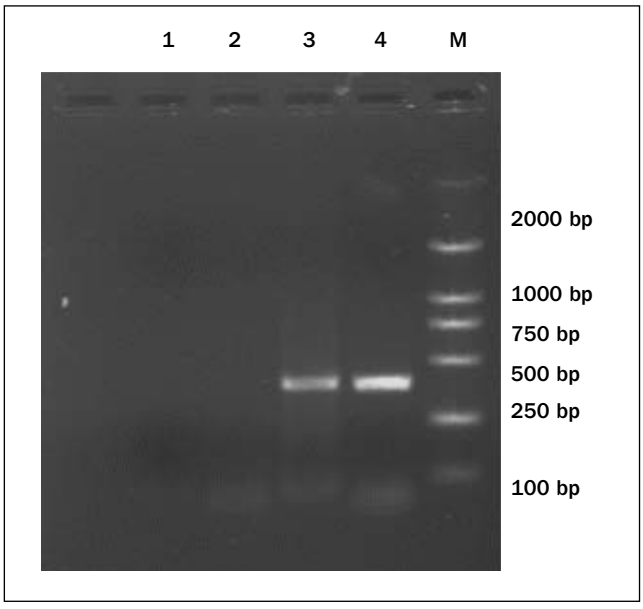

Fig. 3. PCR products of DNA amplified with GAPDH primer. M, Marker DL2000; Lanes 1 and 2 show results of samples 1 and 2 extracted with the phenol/chloroform method; lanes 3 and 4 show results with the DNAzol protocol.