Is enrichment culture necessary for clinical samples?

K. I. MILES, M. W. D. WREN and S. BENSON
Clinical Microbiology, University College Hospital, London, UK

Inoculation of enrichment broth in addition to direct plating of tissue and fluid specimens has been recommended by some authors but not others.[1,2] It is assumed that broth inoculation improves the recovery of clinically relevant isolates as it provides favourable growth conditions for organisms in low numbers, or where the sample may already contain antibiotics as a result of current treatment.

Clinical microbiology at University College Hospital (UCH) uses Robertson's cooked meat medium for enrichment culture (Oxoid, Basingstoke, UK). We can find only limited scientific evidence[1,2] to determine whether or not enrichment culture is useful in clinical microbiology, although it is used extensively for food and environmental microbiology.[3]

The data presented in this paper were the result of an internal audit carried out to determine which samples, if any, might benefit from enrichment culture. Benefit was determined by the isolation of any organism that was deemed clinically relevant by assessment of the patient's clinical condition at the time of isolation. This was investigated by one of the laboratory medical staff.

All body fluids (excluding cerebrospinal fluid) and tissues received in the department are cultured routinely on a variety of media, which includes Robertson's cooked meat medium for enrichment. In-house protocols for joint revision samples are based on published work.[4]

The deposit from centrifugation of approximately 15 mL of the fluid samples (or the grindings from tissue samples) were cultured on a range of solid agar media, as determined by the laboratory standard operating procedures, using one drop from a standard sterile Pastette plated with a bacteriological loop. The remainder of the deposit was transferred into a bottle of cooked meat medium and a direct Gram stain performed.

If the direct agar cultures failed to grow any bacteria after two days' incubation, the cooked meat medium was subcultured on suitable media incubated aerobically (plus 8% carbon dioxide) and anaerobically. All cultures were performed in a class I cabinet to avoid exposure to possible aerosols.

Criteria determining the need to subculture the broth included moderate to large numbers of white blood cells (WBCs) seen on a direct Gram-stained smear of the original sample (>5 WBCs/high power field), tissue sent for culture or when the patient was diagnosed as human immunodeficiency virus (HIV)-positive or suffering from leukaemia or lymphoma.

Table 1 shows an annual summary of different organisms isolated from cooked meat broth used as an enrichment medium and demonstrates the number of organisms isolated from enrichment culture over the period.

A total of 28 (1.8%) organisms were recovered from enrichment of 1474 samples that were negative by direct agar culture. Eleven out of 500 tissue samples were positive using enrichment broth. Four isolates of coagulase-negative staphylococci were isolated from 37 bone samples.

A total of 11 organisms were isolated from 874 fluids, including various aspirates, pleural fluid and ascitic fluid. A further two organisms (*Staphylococcus aureus* and *Streptococcus milleri*) were isolated from pus samples after enrichment culture and considered to be clinically relevant. One peritoneal fluid yielded a growth of a *Peptostreptococcus* species.

Enrichment is used to increase low numbers of organisms that are not recovered on direct agar culture to detectable levels. This is used successfully in the food industry, for environmental microbiology and for increasing target numbers for use in a subsequent polymerase chain reaction.

Although enrichment has been a key feature in many clinical microbiology laboratories, it has been used traditionally and without sound scientific evidence. The present work has shown the benefit of enrichment culture for certain types of specimen (tissue, bone and pus) and that blanket use of enrichment may be unnecessary and wasteful of time and resources. However, a large-scale comparison of various enrichment media should be undertaken using clinical samples taken before therapy begins and probably on those where treatment has already started.

From 1474 samples, only 28 (1.8%) yielded clinically significant isolates from enrichment culture in the presence of a negative direct agar culture. Perhaps surprising is the low numbers of fluid and pus samples that yielded relevant organisms. This is most likely to be due to the fact that the

Table 1. *Enrichment results using Robertson's cooked meat medium.*

Specimen	Direct culture	Enrichment		Organisms
	Neg	Neg	Pos	
Tissues	500	489	11	CoNS x8 *Bacillus* sp. *Enterococcus* sp. Diphtheroids
Pus	54	52	2	*Staphylococcus aureus* *Streptococcus milleri*
Pleural fluid	387	383	4	CoNS x2 Diphtheroids Gemella
Peritoneal fluid	215	214	1	*Peptostreptococcus* sp.
Aspirates (various)	159	157	2	*Pseudomonas* sp. CoNS
Ascitic fluid	91	88	3	CoNS α-haemolytic streptococci *Acinetobacter* sp.
Skin biopsy	6	6	–	–
Synovial fluid	22	21	1	CoNS
Bile	3	3	–	–
Bone	37	33	4	CoNS x4
Total	1474	1446	28	

CoNS: coagulase-negative staphylococcus

Correspondence to: Dr. M. W. D. Wren
Email: mike.wren@uclh.nhs.uk

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.

References

1 Pollock AV, Evans M, Parida S. The prediction of abdominal surgical wound infection: the value of an enrichment broth for initial culture of operative parietal swabs. *J Hosp Infect* 1986; **8:** 242–7.

2 Morris AJ, Wilson SJ, Marx CE, Wilson ML, Mirrett S, Barth Reller L. Clinical impact of bacteria and fungi recovered only from broth cultures. *J Clin Microbiol* 1995; **33:** 161–5.

3 Bohaychuk VM, Gensler GE, King RK, Wu JT, McMullen LM. Evaluation of detection methods for screening meat and poultry products for the presence of foodborne pathogens. *J Food Prot* 2005; **68:** 2637–47.

4 Atkins BL, Bowler CJW. The diagnosis of large joint sepsis. *J Hosp Infect* 1998; **40:** 263–74.

Extraction of DNA from paraffin sections with proteinase K and DNAzol

B. ZHANG*†, X. HUO*, X. XU*, Z. QI*, H. YANG*, L. PENG*, B. QIU* and L. ZHENG*

**Confocal Laboratory, Shantou University Medical College, Shantou; and †Department of Biochemistry, Southern Medical University, Guangzhou 510515, P. R. China*

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.

Correspondence to: Xia Huo
Confocal Laboratory, Shantou University Medical College, Shantou 515041, P. R. China. Email: xhuo@stu.edu.cn

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