a tube agglutination method, and those found to be *E coli* 0157 were grown in a modified brain heart infusion broth and tested for verotoxin production. Samples were also examined for free faecal verotoxin; if this was found in the absence of *E coli* 0157 then other toxigenic serogroups were sought by culture of the faecal sample on ordinary MacConkey agar (Oxoid CM7b) and by checking all *E coli* isolates for verotoxin production as above. All samples were also examined for the presence of other recognised enteric pathogens. All methods have been detailed in previous reports.[2 3]

We found VTEC in 31 (78%) of 40 patients with haemorrhagic colitis, but in only two (0·9%) of 229 control patients ($p < 0·001$). With the exception of one verotoxin producing *E coli* 0128, all VTEC isolated were sorbitol negative *E coli* 0157. Other recognised enteric pathogens were not isolated from any patients with haemorrhagic colitis; they were recovered from 52 (23%) of 229 control patients.

Thus our findings differ sharply from those of Larson and Welch.[1] Our findings are, however, similar to those of workers who studied sporadic cases of haemorrhagic colitis in England and Wales[4] and Canada.[5] We therefore disagree with the view expressed by Larson and Welch, and indeed regard VTEC, particularly serogroup 0157, as the maor aetiological agent in sporadic cases of haemorrhagic colitis.

PA CHAPMAN,
DJ WRIGHT,
P NORMAN,
Public Health Laboratory,
Northern General Hospital,
Herries Road,
Sheffield S5 7AU

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3 Chapman PA, Mitchelmore DL. A two-year survey of the incidence of heat-labile enterotoxin-producing Escherichia coli and other enteric pathogens in travellers returning to the Sheffield area. *Epidemiol Infect* 1988;**101**:239–47.
4 Smith HR, Rowe B, Gross RJ, Fry NK, Scotland SM. Haemorrhagic colitis and vero cytotoxin-producing Escherichia coli in England and Wales. *Lancet* 1987;**i**:1062–5.
5 Pai CH, Gordon R, Sims HV, *et al.* Sporadic cases of haemorrhagic colitis associated with Escherichia coli 0157:H7. *Ann Intern Med* 1984;**101**:738–42.

Dr Larson comments:

If acute diarrhoea in adult patients caused by *Salmonella, Shigella, Campylobacter* and *Clostridium* species are regarded as separate clinical entities, haemorrhagic colitis not due to these organisms is the single most common clinical presentation of acute diarrhoea to our infectious disease unit.[1] It was patients with this clinical presentation whom we subsequently studied for evidence of infection with verotoxin producing *Escherichia coli* and failed to find it. If these cases are, in fact, caused by infection with *E coli* 0157, this organism would be the single most prevalent gastrointestinal pathogen in the community. But I continue to be sceptical about this conclusion.

Drs Chapman, Wright, and Norman do not describe the patient population which was the source of the faecal samples they tested. Thus it is not possible to estimate a prevalence for *E coli* 0157 infection from their data (nor from data in their references 6 and 7) to compare it with our own. The percentage of cases of haemorrhagic colitis they found to be due to *E coli* 0157 is very high, however, significantly higher even than that found by Smith *et al*, or Pai *et al*. This suggests that they describe not sporadic but epidemic haemorrhagic colitis.

Reference

1 Jewkes J, Larson HE, Price AB, Sanderson PJ, Davies HA. Aetiology of acute diarrhoea in adults. *Gut* 1981;**22**:388–92.

Staphylococcus lugdunensis endocarditis

We were interested to read the description of a new type of staphylococcal endocarditis by Smyth *et al*.[1] No species identification was done for the staphylococcal strain which has most of the characteristics of *Staphylococcus lugdunensis*, a new coagulase negative staphylococcal species.[2] This species produces smooth, glossy colonies, initially cream-coloured, but becoming pale yellow to golden after five days. Using the API Staph gallery (AIP-System, Montalieu-Vercieu, France), *S lugdunensis* is incorrectly recognised as *S hominis* biotype 3. Such strains are identified as *S lugdunensis* if they have an ornithine decarboxylase and a fibrinogen affinity factor. They also have a thermostable DNAase activity, like *S aureus* but not like *S epidermidis*.

S lugdunensis, however, is probably more responsible for infections such as infective endocarditis. Three cases of infective endocarditis due to *S lugdunensis* occurred in France in 1977, 1982, and 1983.[3] The three strains, primarily recognised as coagulase negative staphylococci close to *S hominis*, but with atypical characters, were correctly identified in 1988.[2] Unlike the usual hospital *S epidermidis* isolates, the strains were susceptible to all the antibiotics tested (benzylpenicillin, meticillin, aminoglycosides, chloramphenicol, tetracycline, macrolides, fusidic acid, vancomycin . . .).

If *S lugdunensis* seems, like *S epidermidis* and *S saprophyticus*, to be a coagulase negative staphylococcus isolated from human infections, its correct identification is a necessity and can be done easily. Coagulase and fibrinogen affinity factor (clumping factor) must both be detected, and the positivity of the second test suggests only that the isolate is *S lugdunensis*; its definite identification is achieved by the other biochemical tests, including ornithine decarboxylase detection.[1 2]

J ETIENNE,
Y BRUN,
J FLEURETTE
Centre National de Référence
des Staphylocoques,
Laboratoire de Bactériologie,
Faculté de Médecine Alexis Carrel,
Rue Guillaume Paradin,
69372 Lyon Cedex 8. France

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1 Smyth EG, Wright ED, Marples RR. New type of staphylococcal endocarditis. *J Clin Pathol* 1988;**41**:809–14.
2 Freney J, Brun Y, Bes M, *et al.* Staphylococcus lugdunensis sp. nov. and Staphylococcus schleiferi sp. nov., two species from human clinical specimens. *Int J Syst Bacteriol* 1988;**38**:168–72.
3 Etienne J, Pangon B, Leport C, *et al.* Staphylococcus lugdunensis endocarditis. *Lancet* 1989;**i**:390.

Dr Smyth *et al* comment:

We agree that the organism we described as causing endocarditis can probably be identified as *S lugdunensis*, a new species first described by Etienne *et al* in 1988. The description was not available to us at the time we studied the strain from this patient, but we were cognisant that ornithine decarboxylase positive staphylococci resembling *S*

hominis could be pathogenic. Because of space limitations, this is only implicit in the biotyping scheme presented in Warsaw (Marples 1981) where a taxonomic collection, mostly for non-clinical sources, was studied. The isolates were ornithine decarboxylase positive and two were from the blood. At that time, we had not recognised any anomalies in clumping factor tests, though we have become aware of these in this and other species of coagulase negative staphylococci.

Recognition that tests for clumping may not be specific for *S aureus* when rapid bench kits are used is important, as it may affect treatment and management. In our case, the initial misidentification as an anomalous *S aureus* was not of clinical importance because appropriate treatment was given. There continues to be a need to identify organisms causing endocarditis.

KC WATSON
EJC KERR
Central Microbiological Laboratories, Western General Hospital, Crewe Road, Edinburgh EH4 2XU

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2 Watson KC, Kerr EJC. Studies on antistreptolysin O activity generated in serum by micro organisms. *J Med Microbiol* 1976;**57**:105–13.
3 Watson KC, Kerr EJC. Partial characterisation of an inhibitor of streptolysin O produced by bacterial growth in serum. *J Med Microbiol* 1975;**8**:465–76.

Comparison of latex and haemolysin tests for determination of anti-streptolysin (ASO) antibodies

Curtis *et al* compared latex and standard haemolysis inhibition tests for determining ASO antibodies.[1] May we offer one obvious explanation for the discrepancies in results referred to in their table 3? They make no distinction between antistreptolysin antibody (ASO) and the entity that we have designated as antistreptolysin factor (ASF).[2] The latter is produced by the activity of cholesterol esterase on low density lipoproteins and is contained in peptide fragments where cholesterol is spatially orientated in such a manner that it is capable of binding to streptolysin O.[3] For years we have routinely distinguished between ASO and ASF by measuring activity before and after precipitation of liproproteins with dextran sulphate. Only the latter fraction contains true antibody activity and titres are almost always lower than they are before precipitation. Raised ASF titres may be found in some patients with chronic staphylococcal infection, in rheumatoid arthritis, and in glomerulonephritis. Even in patients with Lancefield group A infections, the titre measured by haemolysis inhibition is usually due to ASF with true ASO only about two thirds that of ASF. Occasional contaminated sera give high ASF concentrations due to production of cholesterol esterase by *Staphylococcus aureus* or *Pseudomonas aeruginosa*. Both produce high ASF concentrations when grown in serum broth mixtures.

Marking planes of surgical excision on specimens with mixture of India ink and acetone

In a recent paper by Paterson and Davies,[1] the use of artists' pigments suspended in acetone to mark planes of surgical excision on breast specimens was advocated. The pigment preparations were reported to be better than India ink in that spreading did not occur and drying was much quicker. These authors did not mention the use of India ink in acetone or other volatile liquids in their methods nor in their discussions. In our laboratory the pathologists routinely use India ink (Pelikan AG, West Germany) to mark planes of surgical excision. The possibility of improving the performance of India ink by mixing it with a suitable volatile reagent immediately came across our minds. We were unaware of anyone who had explored this possibility.

We mixed equal parts of acetone with India ink (Pelikan AG, West Germany) and compared the application of this mixture with undiluted India ink on two breast specimens. When the India ink and acetone mixture was applied with a brush, no spreading of the marker beyond the area painted was noted. Drying was quick and completed in less than five minutes. The mixture was easy to prepare and was free from potential health hazards associated with the artists' pigments.[1] If the mixture was allowed to dry, however, a hardened gel would result that could be very difficult to break up. The undiluted India ink was slow to dry and tended to spread beyond the area painted. The intensity of labelling using the two preparations was indistinguishable when viewed under the microscope.

We tried to use absolute ethanol to replace acetone in the mixture. Spreading of the marker was noted, and we concluded that ethanol was not a good substitute for acetone for this purpose.

KW CHAN
I LUI
WB CHUNG
Department of Pathology, University of Hong Kong, Hong Kong

Reference

1 Paterson DA, Davies JD. Marking planes of surgical excision on breast biopsy specimens: use of artists' pigments suspended in acetone. *J Clin Pathol* 1988;**41**:1013–16.

Dr Davies *et al* comment:

We were intrigued to see the above letter from Drs Chan *et al* about the use of India ink mixed with acetone. A brief trial in this laboratory confirms the apparent benefit of their novel method, although the very fluidity of the mixture—admittedly an asset in rapidly coating a biopsy specimen—would make differential marking difficult. Indeed, it must be pointed out that by itself India ink, even admixed with acetone, precludes microscopic identification of more than one plane of resection. Coloured pigments are necessary for this purpose. Use of the many older, if more muted artists' pigments[1] would avoid the potential toxic hazards of cadmium, cobalt, and other bright modern colours. Furthermore, the use of India ink diluted in acetone does not prevent the unfortunate tendency for the marker to penetrate between lobules of fat. The main advantage of acetone as an ancillary mountant for India ink is that it shortens the drying time. Like Chan *et al*, we, too, found in pilot studies that ethanol was a poor substitute for acetone.

JD DAVIES,
J ARMSTRONG,
DA PATERSON
Department of Pathology, Bristol Royal Infirmary, and Edinburgh University Medical School

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