LABORATORY TECHNIQUES

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Examination of faeces for bacterial pathogens

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Introduction

The examination of a specimen of faeces is potentially one of the most complex and time consuming investigations undertaken in the microbiology laboratory. For this reason the processing of such specimens can be carried out most efficiently if the laboratory has a set routine to follow, which should preferably be written down and accessible to all members of staff. The purpose of this broadsheet is to present such a set of procedures which could be adopted in the general microbiology laboratory, together with a discussion of appropriate technical methods. It should be noted that methods for the isolation of *Clostridium difficile* are not included as this has been the subject of a previous broadsheet.¹

All laboratories should be able to examine specimens routinely for salmonellae, shigellae, and campylobacters. Whether examination will also be performed for other organisms such as vibrios, *Escherichia coli*, yersiniae, and others depends very much on the clinical details received with the specimen, particularly with regard to the age of the patient, recent foreign travel, or the possibility of "food poisoning".

The specimen is collected into a screw-cap container using a small wooden or plastic spoon. For solid specimens a single spoonful is satisfactory; liquid specimens should not fill the container to more than one third to avoid spillage when the container is opened.

Examination

For macroscopic examination, note the consistency of the specimen and the presence of any blood or mucous exudate.

Microscopic examination of bacterial infections is unlikely to yield useful information. If required, an examination for pus cells and red cells can be made. A small portion of a liquid specimen, or any bloodstained or mucous exudate on a solid specimen, is mixed with a drop of methylene blue on a glass slide and examined under the $\times 10$ and $\times 40$ objectives.

Culture

This is the mainstay of the examination of a fecal specimen for bacterial pathogens and

methods are presented below for each type of organism in turn.

CULTURE FOR SALMONELLAE AND SHIGELLAE

At least two and possibly three different media should be inoculated. In the United Kingdom the most frequently used media are desoxycholate-citrate agar (DCA) and xylose-lysine-desoxycholate agar (XLD), and other possible media include Hektoen enteric agar and Salmonella-Shigella agar.² All these media rely on selection of salmonellae and shigellae by the inclusion of bile salts, and on the exclusion of potential pathogens by production of acid from carbohydrates; an indicator system for the production of hydrogen sulphide is also included. By contrast, bismuth sulphite agar contains brilliant green as the selective agent and uses the reduction of sulphite to sulphide in the presence of glucose as an indicator. This medium is highly selective for salmonellae, especially *S typhi*, and is often used by laboratories with a special interest in this organism. It has the additional advantage that salmonellae belonging to the *Arizona* group (late lactose fermenters) will not be overlooked as might be the case on media which rely on the absence of lactose fermentation by salmonellae. In addition to two or three solid media, a selective enrichment medium should be included. Selenite broth is most frequently used in the United Kingdom.

Salmonellae produce colourless colonies on DCA, often with a black centre indicating H_2S production. *Proteus* spp may produce the same appearance, as can late lactose fermenting citrobacters. Shigellae produce small, translucent colonies without H_2S production, while lactose fermenters produce small, deep pink colonies.

XLD contains three fermentable carbohydrates (lactose, xylose, and sucrose) and the colour changes produced are more complex. Colonial appearance depends on the balance between the lowering of pH by sugar fermentation and the increase in pH resulting from lysine decarboxylation. Organisms which ferment two or more of the carbohydrates produce acid in excess, and whether or not lysine is

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decarboxylated, bright yellow colonies are produced; these include *E coli*, *Klebsiella* spp, and some *Proteus* spp. Organisms which do not decarboxylate lysine nor ferment any of the carbohydrates, such as the shigellae and some *Proteae*, will produce colourless colonies. Finally, those which decarboxylate lysine and ferment none or only one of the sugars will yield red or red-purple colonies. This is the typical reaction of *Salmonella* spp. H_2S -producing organisms may yield colonies with black centres.

Shigellae and most other coliforms do not grow on bismuth sulphite agar. *S typhi* produces black colonies with a characteristic metallic sheen and a deep brown or black zone surrounding the colony. Other salmonellae produce a variable appearance, with grey, grey-black, or green colonies. There may be a metallic sheen and the medium may be discoloured, although this is frequently absent.

After overnight incubation the selenite broth is subcultured to a solid medium such as DCA or XLD. Examination of the plates will usually show one or more organisms which, based on the reactions described above, may be a *Salmonella* or *Shigella*. Although it is possible to attempt immediate identification of the organism by slide agglutination with polyvalent antisera, this is not recommended as erroneous results may be obtained. To identify each isolate to species level would be a waste of time and resources, so a simple screening procedure should be used to enable most non-pathogens to be discarded. Those that remain can then be identified fully. Several screening methods are available, including a short series of biochemical tests (such as ONPG and urease activity plus fermentation of sucrose and salicin), tubed composite media such as Kligler's iron agar or Kohn's two-tube medium, or commercial kits such as the API Z kit (API Systems, France). Whichever method is used, a blood or nutrient agar plate must also be inoculated to serve as a purity check and to provide material for agglutination tests.

Kligler's iron agar and triple sugar iron (TSI) agar are two composite media, either of which may be used to differentiate members of the Enterobacteriaceae according to sugar fermentation and hydrogen sulphide production. Both are poured as agar slopes and should be inoculated from pure cultures or with single colonies picked off selective media by stabbing the butt with a straight wire and streaking the surface of the slope. Kligler's medium contains glucose, an excess of lactose, ferric citrate for detecting hydrogen sulphide production and a pH indicator phenol red. TSI has a very similar composition but contains sucrose in addition to glucose and lactose.

Fermentation of glucose produces acid which initially turns the medium yellow. This change reverts under aerobic conditions so that organisms which ferment only glucose produce an acid (yellow) butt and an alkaline (red) slope. The excess of lactose (and sucrose in TSI) present in the media allows lactose or sucrose fermenting organisms, or both, to produce sufficient acid to turn both the butt and slope yellow. Any gas produced from glucose can be seen to disrupt the medium, and hydrogen sulphide production turns the medium black due to the production of ferric sulphide. Typical reactions in these media are shown in table 1. If Kligler's or TSI media are to be used then a test for urease production or the presence of phenylalanine deaminase should also be performed to distinguish the *Proteae* from salmonellae and shigellae.

Kohn's composite media consists of two tubes. Tube 1 is poured as an agar slope and contains urea, glucose, an excess of mannitol and the pH indicator phenol red. It is inoculated in the same way as Kligler's iron agar or TSI. Organisms which ferment glucose alone turn the butt yellow; those which also ferment mannitol produce a yellow butt and slope; and organisms which hydrolyse urea turn the butt and slope a bright pink colour. Tube 2 is allowed to set vertically and contains sucrose, salicin, sodium thiosulphate as a sulphur

Table 1 Typical reactions in Kligler's and TSI agar

Organisms	Butt	Slope	H_2S production
<i>E coli</i> , <i>Klebsiella</i> spp, <i>Enterobacter</i> spp, some <i>Citrobacter</i> strains, <i>Aeromonas</i>	Acid	Acid	—
Some <i>Citrobacter</i> strains, some <i>Proteae</i>	Acid	Acid	+
<i>Shigella</i> , <i>Plesiomonas</i> , some <i>Proteae</i> , some strains of <i>Aeromonas</i> , <i>Citrobacter</i> and <i>Salmonella</i>	Acid	Alkaline	—
<i>Salmonella</i> , <i>Proteae</i> , some <i>Citrobacter</i> strains	Acid	Alkaline	+
Non-fermenting bacteria such as <i>Pseudomonas</i> spp	Alkaline	Alkaline	—

Table 2 Typical reactions in Kohn's two tube media

Organism	Fermentation of:		Urease production	Fermentation of sucrose/salicin	Production of:	
	Glucose	Mannitol			H_2S	Indole
<i>Salmonella typhi</i>	A	A	—	—	+	—
<i>Salmonella</i> spp	AG	A	—	—	+/-	—
<i>Shigella dysenteriae</i>	A	—	—	—	—	+/-
<i>Shigella flexneri</i> and <i>boydii</i>	A	A	—	—	—	+/-
<i>Shigella sonnei</i>	A	A	—	—	—	—
<i>Proteae</i>	P	P	+	AG or —	+/-	+/-

Fermentation tests:

AG acid and gas

A acid only

— no reaction

P apparent negative reaction as bright pink colour from urease activity masks effect of fermentation

Other tests:

+ positive

— negative

+/- variable reaction

Table 3 Initial colour changes in cupule A of API Z system

Colour	Interpretation
Yellow	Salmonella, Shigella, and <i>Y enterocolitica</i> excluded
Green	
Blue-green	
Brown-orange	
Blue	Investigate organism further as Salmonella, Shigella, or <i>Y enterocolitica</i> suspected
Colourless	
Pale beige	

source, and a pH indicator bromothymol blue. After inoculation indicator papers for the production of indole and hydrogen sulphide are suspended over the medium. Organisms which ferment sucrose or salicin, or both, change the medium from blue-green to yellow. Certain typical reactions in Kohn's media are shown in table 2. As neither tube contains lactose a test for ONPG activity should also be performed.

The API Z kit is a rapid method of screening for *Salmonella*, *Shigella*, and *Yersinia enterocolitica* by enzyme detection. The documentation accompanying the kit should be consulted for full details of how to use, read, and interpret the results of the test. The system comprises two cupules (A and B) which are inoculated from a single colony of a non-lactose fermenting organism. Cupule A contains substrates for an enzyme specific for *Salmonella* and for four enzymes not possessed by *Salmonella*, *Shigella*, or *Y enterocolitica*. Various colour changes may occur, on the basis of which the organism may either be discarded as non-pathogenic or investigated further as a possible *Salmonella*, *Shigella*, or *Y enterocolitica*. Cupule B maintains the viability of the organism should further investigation be necessary. Typical results with this system are shown in table 3.

The API Z is read after two hours, and a urease slope can be initially examined after four hours. Other tests will require overnight incubation. Organisms which are not excluded by these tests should be identified by standard laboratory methods,^{3,4} and slide agglutinations performed using the growth on the blood or nutrient agar plate. The results of slide agglutinations with salmonellae should be checked by tube agglutination tests. This is often not done with shigellae provided the slide agglutinations and biochemical identification are clear-cut.

METHODS FOR SLIDE AND TUBE AGGLUTINATIONS

To perform slide agglutinations a small amount of the culture to be tested is emulsified in a drop of physiological saline on a glass slide. A uniformly smooth suspension is essential. A loopful of antiserum is added to the suspension and mixed with it by gently rocking the slide backwards and forwards. Examination is aided by viewing the reaction against a black tile, and a positive result consists of visible clumping of the suspension within 60 seconds. Agglutinations should initially be performed with polyvalent O and H (specific and non-specific phase) antisera. If a positive result is obtained

Table 4 *Salmonella* serotyping using Rapid Diagnostic Sera

Agglutination with RDS			H antigen
1	2	3	
+	+	+	E complex
+	+	—	b
+	—	+	d
—	+	+	k
+	—	—	r
—	+	—	L complex
—	—	+	G complex

RDS 1 contains antisera to: b; d; r; E complex.

RDS 2 contains antisera to: b; k; E complex; L complex.

RDS 3 contains antisera to: d; k; E complex; G complex.

the test should be repeated with monovalent sera. Time may be saved when performing H agglutinations by using the three Rapid Diagnostic Sera which contain a mixture of antisera to H antigens. The pattern of results obtained allows the serotype to be determined (table 4). Note that these sera do not contain antiserum to H "i" antigen, which must be tested separately.

If an isolate of a salmonella is found to be in the non-specific phase it may be changed to the specific phase with a Craigie tube. This is a tube containing 10 ml of semisolid (0.5%) agar mixed with 0.2 ml of polyvalent non-specific phase H antiserum, and a small inner tube whose upper end projects above the agar surface. The tube is inoculated with the non-specific phase culture inside the inner tube at the top. Organisms in the non-specific phase are immobilised by the antiserum, while those in the specific phase migrate down the inner tube and up to the surface of the agar, from where a subculture can be made. This method, without antiserum, can also be used to select a motile variant from a poorly motile strain.

Slide agglutination results should be confirmed by performing tube agglutinations. Antigen for salmonella O and shigella agglutinations is prepared by suspending the growth from an agar plate culture in saline. For salmonellae, the culture is killed by heating the suspension to 100°C for 10 minutes to remove flagellae, while for shigellae a few drops of formalin should be added to the suspension. For salmonella H agglutinations, antigen is prepared by adding a few drops of formalin to an overnight broth culture of the organism. In all cases the suspension should be adjusted with saline to about 2×10^8 bacteria/ml (it may be compared with an opacity standard).

The antiserum to be tested is first diluted 1 in 10, then a series of six doubling dilutions of antiserum in saline is prepared in small test tubes (such as 1 cm $\times$ 7.5 cm round-bottom tubes). To each tube an equal volume of antigen is added, giving a final range of dilutions from 1 in 20 to 1 in 640. A control tube should also be set up containing saline and antigen without antiserum.

The tubes are incubated at 50°C in a water-bath for four hours. They should be viewed against a dark background with a hand lens, and agglutination is seen as clumping of the antigen with resultant clearing of the suspension as the bacilli settle to the bottom of the

tube. If no agglutination is present the tubes should be left at room temperature overnight and read again the next day. The result is taken as the highest dilution of the antiserum to produce visible agglutination, and to confirm the slide agglutinations the result must equal or exceed the titre stated on the antiserum package.

PROBLEMS IN THE SEROTYPING OF SALMONELLAE
Certain problems are sometimes encountered when serotyping strains of salmonellae. Negative H agglutinations may occur because the strain is non-motile (such as *S pullorum*) or poorly motile. In the latter case a motile variant may be selected using a Craigie tube without antiserum (see above). Negative O agglutinations may occur with rare serotypes for which antisera are not present in the polyvalent O antiserum, and in this case it will probably be necessary to refer the strain to a reference laboratory for serotyping. This problem may also arise if the strain possesses Vi antigen, which can mask the presence of an O antigen. Agglutinations with Vi antiserum should be performed and the Vi antigen can be removed by heating a suspension of the organism to 100°C for one hour. The O agglutinations may then be repeated.

2 Culture for campylobacters

Most media for campylobacters are rendered selective by the inclusion of antibiotics in the medium. A frequently used combination is that of vancomycin, polymyxin, and trimethoprim to which amphotericin B can be added to inhibit yeasts. The plate should be incubated at 42°C to increase selectivity and must be incubated in a microaerophilic environment with a gas mixture of 5% oxygen, 10% carbon dioxide, and 85% nitrogen.

After 48 hours' incubation campylobacters appear as large, flat grey colonies. An oxidase test and a Gram stain should be performed; an organism which grows on this medium at 42°C is oxidase positive, and microscopically a curved or S-shaped Gram negative rod can be presumptively identified as *Campylobacter jejuni/coli*. Further speciation is unnecessary in the routine laboratory.

3 Culture for *Yersinia enterocolitica*

Culture for *Y enterocolitica* still presents something of a problem for the routine laboratory, but it should be performed when clinical details are suggestive of mesenteric adenitis, terminal ileitis, or acute gastroenteritis, especially if it occurs in association with reactive arthritis or erythema nodosum. The best approach is probably to use a selective medium such as cefsulodin-irgasan-novobiocin (CIN) agar. This medium also contains mannitol and neutral red, and *Y enterocolitica* produces dark pink colonies as a result of mannitol fermentation. A similar appearance is produced by some citrobacters and *Serratia* spp, while *Pseudomonas* spp appear as non-mannitol fermenting colonies. Most other coliforms are inhibited. Isolates suggestive of yersiniae may be identified by standard methods.

4 Culture for vibrios

A satisfactory general purpose medium for the isolation of vibrios is thiosulphate-citrate-bile salt-sucrose (TCBS) agar, which is rendered selective by the inclusion of bile salts and a high pH (8.6). Sucrose fermenting organisms produce bright yellow colonies on this medium and H₂S producers yield colonies with black centres. Alkaline peptone water (APW, pH 8.6) is used as a semi-selective enrichment medium.

The specimen should be inoculated on to TCBS agar and into APW. Six hours later the APW should be subcultured on to TCBS and into another APW which is incubated overnight before subculture to a third TCBS plate (method of Furniss and Donovan).⁵ Most Gram negative bacilli are inhibited on TCBS; some *Proteaeae* will grow and may appear as either sucrose fermenters or non-sucrose fermenters. Many produce H₂S, and as none of the vibrios does, these organisms can be ignored.

After overnight incubation organisms growing on the TCBS plate should be stained by Gram stain. This allows enterococci, which frequently grow on this medium producing small yellow colonies, to be discarded. A heavy subculture of any Gram negative bacilli should be made on blood agar. Six to eight hours later there should be sufficient growth for an oxidase test, and if the organism is a sucrose fermenter it must be tested by slide agglutination to *V cholerae* O1 antiserum. An oxidase positive, Gram negative bacillus which ferments sucrose and agglutinates in the specific antiserum can be provisionally identified as *V cholerae* O1. Confirmation of identity and biotyping can be performed by standard laboratory methods.⁶ Organisms which do not agglutinate with the antiserum may be non-O1 *V cholerae*. These organisms may cause gastrointestinal infection but do not cause epidemic cholera.

If the organism is oxidase positive but does not ferment sucrose it may be *V parahaemolyticus*. It should be identified by standard methods.⁶

5 Culture for *Clostridium perfringens*

Nearly all of the healthy population carry this organism in faeces, usually in the region of 10⁴–10⁵ organisms per gram. The simple fact of isolation of the organism from faeces is therefore insufficient, and a quantitative estimation must be made. A spore count in addition to a count of vegetative cells should be performed because the enterotoxin of *C perfringens* is formed on sporulation of the organism in the large intestine. The following procedure includes vegetative cell and spore counts, plus a qualitative examination for heat resistant strains:

1 A thick (1/10) suspension of faeces is made in 1/4 strength Ringer's solution.

2 One ml of the suspension is inoculated into a tube of Robertson's cooked meat medium, which is then heated to 100°C for one hour and incubated overnight. Next day, it should be subcultured to neomycin blood agar. This provides a qualitative examination for heat resistant strains.

3 Total viable counts are performed on

neomycin blood agar using 10-fold dilutions of the suspension. This gives a vegetative cell count.

4 The remaining suspension is heated to 80°C for 10 minutes, then the viable counts are repeated. This heat shock will encourage spores to germinate, giving a spore count.

To confirm infection with this organism the counts of *C perfringens* in faeces (and food if available) should exceed 10^6 /g.⁷ Counts higher than this may be seen in otherwise healthy elderly people or in patients in hospital, so additional confirmation is provided if the strains from food and faeces are of identical serotypes.

6 Culture for *Staphylococcus aureus*

The specimen should be inoculated on to a selective indicator medium such as mannitol-salt agar. Most bacteria other than staphylococci will not grow on this medium due to the high concentration of sodium chloride (5%). Strains of *S aureus* produce pink colonies due to mannitol fermentation, and suspect colonies may then be identified by standard methods.

Many healthy people carry this organism in faeces, so the fact of isolation alone is not clinically important. Isolates should be phage-typed and tested for enterotoxin production; toxin producing strains of identical phage types isolated from food and faeces may be assumed to be the cause of the infection. It is the preformed and relatively thermostable enterotoxin of *S aureus* which is responsible for the aetiology of *S aureus* food poisoning. If food is heated before it is eaten it is possible that the organism may be killed but the toxin left intact, and so it should be remembered that failure to isolate enterotoxigenic strains of *S aureus* from faeces does not exclude a staphylococcal aetiology.

7 Culture for *Bacillus cereus*

This organism may be found in small numbers in a significant proportion of healthy people, and in cases of suspected *B cereus* food poisoning a quantitative culture method should be used.

A thick (1/10) suspension of faeces should be made in peptone water and 10-fold dilutions made of this suspension. Viable counts are performed by culturing 0.1 ml aliquots of each dilution of *B cereus* selective medium (Oxoid Ltd, Basingstoke, England). This medium is rendered selective by the inclusion of polymyxin B, and contains lecithin, mannitol, and a pH indicator. *B cereus* colonies are surrounded by an opaque zone due to the precipitation of hydrolysed lecithin and do not ferment mannitol. Gram positive bacilli growing on this medium with the colonial morphology described above can be presumptively identified as *B cereus*.

To confirm the organism as the cause of the disease the viable counts from faeces or food should be at least 10^5 /g.⁷ Isolates from faeces should be of the same serotype as that from the suspect food.

8 Culture for *Escherichia coli*

Several different types of *E coli* are capable of causing gastrointestinal tract infection—the enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohaemorrhagic (EHEC) strains. Examination of faeces for EPEC strains in children has been standard practice for many years, but techniques for detection of other types of enteropathic *E coli* have only recently become available.

EPEC strains: These strains are capable of causing outbreaks of infection in nurseries and hospital wards, and for this reason examination for them should be performed in all children under the age of 3 with suspected gastrointestinal infection. A non-selective medium such as MacConkey agar should be included in the set of culture plates, and after overnight incubation colonies of lactose fermenting organisms are tested by slide agglutination with polyvalent antisera for the EPEC serotypes. If agglutination is positive with the polyvalent sera it may then be repeated with monovalent antisera. Because cross-reactions are common any strain giving a positive slide agglutination test should be identified as *E coli* by standard methods, and the slide agglutinations must be confirmed by tube agglutination tests. The method is the same as for salmonellae or shigellae; antigen is prepared by heating a suspension of the organism to 100°C for one hour and adjusting to 2×10^8 organisms per ml with an opacity standard.

ETEC strains: These strains of *E coli* are now believed to be a major cause of gastrointestinal infection in developing countries and in travellers from those countries. Although many laboratories do not examine faecal specimens routinely for these organisms, this position is rapidly changing and examination for ETEC is likely to become part of the routine processing of specimens from travellers in the near future. The diagnosis rests on the isolation of toxin producing strains of *E coli* from faeces. The specimen should be cultured on to MacConkey agar, and after incubation several colonies of lactose fermenting coliforms, including all colonial types, should be subcultured and tested for the ability to produce toxin.

Two types of toxin are produced—heat labile toxin (LT), and heat stable toxin (ST). Demonstration of the ability to produce ST is best accomplished with a gene probe, but this is not feasible at present in most laboratories. The demonstration of LT production, however, is relatively straightforward. Commercial kits are available, such as a reversed passive latex agglutination assay (Oxoid Ltd, Basingstoke, England) and a coagglutination assay (Pharmacia Diagnostics AB, Uppsala, Sweden). For small numbers of tests one of these kits may be used, but the kits are expensive; for larger numbers of specimens a tissue culture assay, as described by Chapman and Swift,⁸ is performed as follows.

The specimen is inoculated on to MacConkey agar, and after overnight incubation up to five colonies (including all colonial types) of lactose fermenting coliforms are subcultured

into tubes of brain-heart infusion broth supplemented with lincomycin to a concentration of 90 mg/l. Each organism should be inoculated into its own tube as the use of pooled cultures may suppress the production of LT. The tubes of broth are incubated for 20 hours, then polymyxin B is added to each tube to a concentration of 100 mg/l. The tubes are incubated for a further four hours.

The broth cultures from each specimen may now be pooled to make a single mixed culture. This will reduce the number of cell monolayers needed. The maintenance medium is removed from cell monolayers of Y1 mouse adrenal cells and replaced with the pooled broth culture. The cells are incubated for 30 minutes, the broth is then removed, and replaced with cell maintenance medium. The monolayers should be incubated for a further 24 hours.

After incubation the monolayer is examined for the characteristic rounding up of the cells. The effect should be confirmed as that of a heat labile toxin by retesting two portions of the pooled culture, one of which has been heated to 100°C for five minutes. The effect of the toxin should be neutralised by heat. Each of the original lactose fermenting isolates may now be identified and tested individually for the ability to produce LT.

EHEC strains: Recently, *E coli* has been associated with haemorrhagic colitis, often followed by renal failure (haemolytic uraemic syndrome), occurring particularly in children and sometimes in clusters of cases. Although several serotypes of *E coli* have been associated with this condition, serotype O157:H7 has been most commonly implicated. Most strains of this serotype do not ferment sorbitol, unlike most other strains of *E coli*, and a modified MacConkey medium containing 10/g/l of sorbitol instead of lactose may be used to detect possible strains of EHEC.⁹ Non-sorbitol fermenting strains of *E coli* may then be sent to a reference laboratory for serotyping and testing for toxin production.

9 Culture for *Aeromonas hydrophila*

A hydrophila may cause severe gastroenteritis.

It can be cultured on MacConkey, DCA, and other enteric agars, but different strains vary in their ability to ferment lactose. The organism may be more easily detected in faecal specimens by using a selective medium, sheep blood agar containing 15 mg of ampicillin/l. *A hydrophila* is an oxidase positive, Gram negative bacillus, on this medium and most strains appear as β haemolytic colonies which turn a dark green colour on prolonged incubation. Identification should be confirmed by standard methods which may include the use of Kligler's iron or TSI agar (table 1) or the API 20E system.

10 Culture for *Plesiomonas shigelloides*

P shigelloides, an occasional cause of diarrhoea, may be isolated from faeces cultured on MacConkey agar or DCA; most strains are non-lactose fermenters on these media. It is oxidase positive (when grown on media which do not contain fermentable carbohydrates) and is non-haemolytic on blood agar. Kligler's iron agar or TSI agar (table 1), the API 20E system or other conventional biochemical tests should be used to confirm identification.

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