Food-borne Pathogens

MONOGRAPH
NUMBER 7
VIBRIOS

OXOID

SETTING STANDARDS

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VIBRIOS

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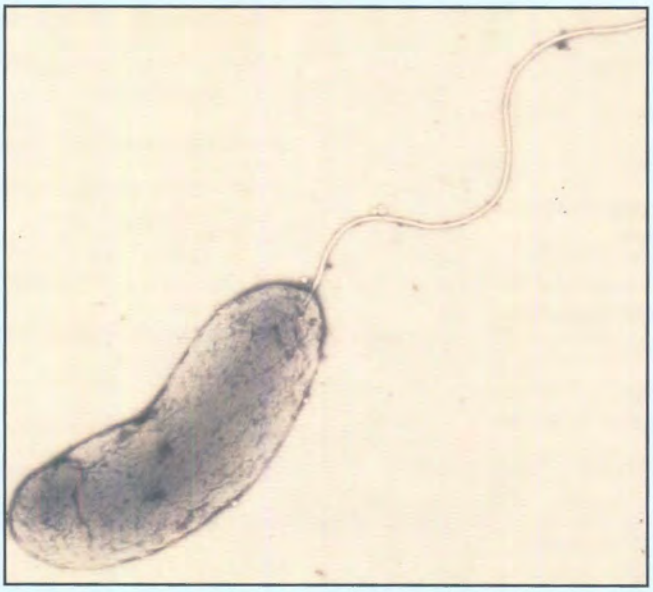
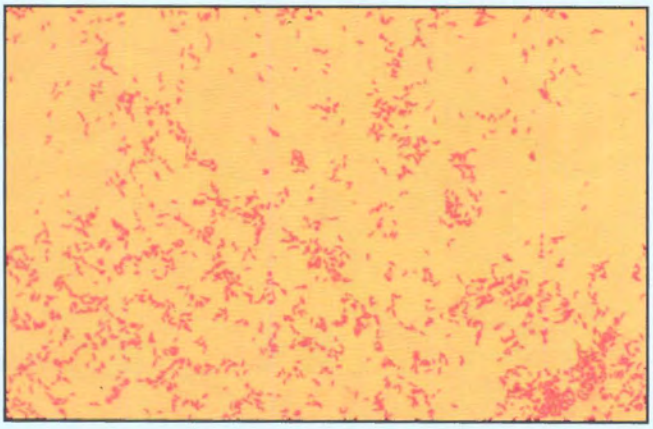
Contents

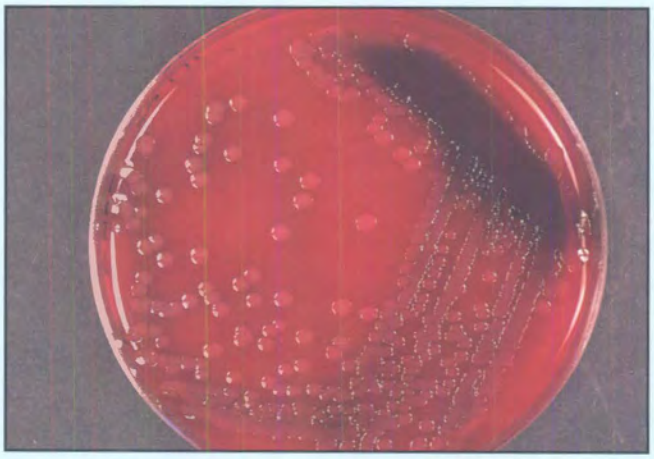


A *Vibrio* cell showing the characteristic curved appearance.



Gram-stain appearance of *Vibrio cholerae.*

Photograph by Dr Matthew Dryden, Microbiology Department, Royal Hampshire County Hospital, Winchester.



Typical appearance of *V. cholerae* colonies on blood agar.

Vibrios

Introduction

The genus *Vibrio* consists of at least thirty-five species. Vibrios are motile Gram-negative bacteria which microscopically appear curved in the shape of a comma. They will grow under both aerobic and anaerobic conditions. Vibrios are oxidase-positive and, as some species will grow on media used for isolating enteropathogenic Gram-negative bacilli, the oxidase test is a fundamental one for the initial distinction of vibrios from *Enterobacteriaceae*.

Most species of *Vibrio* are natural inhabitants of marine and estuarine environments. They are at their most numerous in the summer months when water temperatures are at their highest. Detection in the environment during winter is difficult and it seems likely that the organisms survive winter temperatures in sediment and in association with plankton. *V. parahaemolyticus* at least, appears to survive in this way. Seasonal decline in quantities of zooplankton due to changing weather conditions may be a trigger for entry into the viable but non-culturable (VNC) state of dormancy commonly shown by vibrios. There is evidence that large-scale weather disturbances like El Niño which affect sea-surface temperatures influence the incidence of cholera and other infectious diseases[1]. Colwell[2] has hypothesised that disease outbreaks are related to plankton blooms that occur when sea-surface temperatures rise and *Vibrio* numbers increase proportionally with the much greater numbers of copepods available in the plankton population. The significance of the viable but non-culturable state in public health has been reviewed by Oliver[3].

Almost all pathogenic species of *Vibrio* will grow in culture media only if the sodium chloride content is increased above the usual level of 0.5%. These species are termed "Halophilic" and include *V. parahaemolyticus* but not *V. cholerae*. The optimal level of sodium chloride for the different species varies, probably reflecting the salinity of their natural habitat. Concentrations of 1 to 3% are generally satisfactory but some species will tolerate levels much higher than this. However, halophism of enteropathogenic vibrios is not so extreme that they will not grow well on normal laboratory media containing additional salt and should not be confused with the extreme halophism demonstrated by bacteria for which very high levels of sodium chloride are obligatory, e.g. *Halobacter* spp.

All species of *Vibrio* are tolerant of alkaline conditions and this characteristic is commonly exploited in formulating selective culture media.

Vibrio spp. may infect a number of human body sites including wounds, ears and eyes as well as the gastrointestinal tract. Some species are invasive via the gastric mucosa and septicaemia is a risk for vulnerable subjects. The classical enteric disease cholera is the best-known *Vibrio* infection. Cholera is now a rarity in the developed world as a result of improved water treatment since the recognition in the 19th century by William Budd in Bristol and John Snow in London (in the better-known Broad Street pump incident), of the importance of contaminated water in disease transmission. Water-borne cholera continues to be a serious problem in parts of the world where sanitary conditions remain poor but it is now recognised that food also can be a cause of cholera in otherwise satisfactory conditions, apparently as a result of contamination of foods with unclean water and through unhygienic food handling.

The majority of *Vibrio* spp. are environmental organisms and have not been implicated in human disease. Only a few have been found responsible for food-borne infection and of these, three, *V. parahaemolyticus, V. vulnificus* and *V. cholerae* occur most frequently. However, as many of the species appear identical on the usual culture media it seems likely that the rarer species may sometimes be misidentified.

Vibrio enteric infections almost always arise from seafood and infection with *V. parahaemolyticus* is probably the most common.

Epidemics of food-borne cholera have occurred, including cases on the West Coast of the United States of America following importation of seafood. Infections caused by a variety of species including *V. mimicus*, which closely resembles *V. cholerae*, have been reported from US Gulf Coast states, resulting from uncooked or poorly-cooked seafood. The halophiles, *V. damsela* and *V. hollisae* are two species that have been isolated from diarrhoeal patients but *V. damsela* (*Photobacterium damsela*) is more commonly associated with infected wounds. *V. furnissii* is another species that more recently has been implicated in food-borne diarrhoea. It seems probable that the list of non-cholera vibrios associated with food-borne enteritis will lengthen.

Infection arising from foods in which *V. vulnificus* is present is unusual in showing life-threatening septicaemia as the major manifestation, particularly amongst those who have pre-existing liver disease. This species is responsible for more than 90% of seafood-related deaths in the USA. Most cases occur after eating raw oysters. *V. vulnificus* also causes wound infections that may be fatal. These can occur in susceptible persons who injure themselves when handling molluscs and during recreational activities in the sea.

The major species of *Vibrio* associated with food-borne illness will be described briefly in the following pages. Culture media used in their detection will be described in detail, together with a brief review of methods for identification and a short discussion about toxins and other virulence factors possessed by the different species.

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The occurrence of *Vibrio* species in foods

Vibrios are natural inhabitants of sea-water and brackish estuarine water in both tropical and temperate climates and therefore their presence in seafoods can be expected.

Human infections arise from exposure to water and after consumption of raw or contaminated cooked food. Subjects who are immunocompromised or have pre-existing disease are at greater risk. AIDS patients are particularly prone to food-borne *Vibrio* infections[1] and the pathogenicity of *Vibrio vulnificus* is strongly associated with existing liver disease. Risk assessment is complicated by the lack of correlation of the usual bacterial indicators of pollution and the presence of pathogenic vibrios.This can cause particular problems in controlling water quality in fish farming and for interpretation of the significance for human health of the vibrios present. The occurrence of *Vibrio* spp. in aquaculture has been reviewed by Dalsgaard[2].

Studies of the incidence of vibrios in a variety of seafoods have shown the presence of pathogenic species in approximately one-third of samples[3] but some species/food combinations may show rates much higher than this[4]. High numbers of *V. parahaemolyticus* and *V. vulnificus* are often present in prawns, oysters and clams. *V. vulnificus* and oysters are strongly associated and cholera and other *Vibrio* infections are a serious risk for eaters of raw oysters. Seafoods that have been cooked may not always be safe; Lowry *et al.*[4] showed viable *V. parahaemolyticus* and *V. vulnificus* present in 50% and 25% respectively of cooked oysters, presumably because of post-processing contamination. Preservation of seafoods by refrigeration may not eliminate pathogens. *V. cholerae* has been shown to survive for periods of several months in contaminated cooked prawns and crabmeat under storage at –20°C[5].

Amongst edible crustaceans, shrimps and prawns have long been known to harbour pathogenic vibrios.

V. parahaemolyticus may be found in crabs[6] and a report[7] has recently identified crayfish as a source of pathogens.

Those for whom frog legs are a delicacy are at risk from *V. cholerae*[8]. Septicaemia caused by *V. hollisae* after eating catfish has been reported[9]. However, despite the presence of vibrios in water used in fish farming, the risk of infection from farmed fish appears not to be great.

Outbreaks of cholera have occurred following the eating of raw vegetables[10], lettuce[11] and cooked rice which has been contaminated by water[12]. Contaminated bottled mineral water was responsible for cholera cases in Portugal[13] although this source appears unusual; a study of eight different bottled waters sold in the UK detected the presence of various organisms but not *Vibrio* spp.[14].

Foods contaminated by infected handlers have caused cholera[5]. Illness, including cholera[15], has been caused by in-flight meals on aircraft, probably due to breakdown of basic hygiene measures in the catering establishments – long-term carriage of *V. cholerae* is extremely rare but short-term asymptomatic carriage is common.

Conflicting views are held about the ability of vibrios to multiply in foods but, for *V. cholerae* at least, growth has been demonstrated in a variety of foods that includes seafoods, fish, rice, pulses, meat and eggs[16]. Whether growth occurs is highly dependent on the pH of the food; vibrios do not tolerate acid conditions well but foods with pH values above 7.0, e.g. boiled egg and prawns, tend to promote rapid, vigorous growth. However, *Vibrio* spp. do not compete well in mixed microbial populations and this can act as a natural control on numbers in foods and lower the risk of disease.

Although vibrios are sensitive to cold, foods appear to exert a protective effect and survival over long periods in refrigerated seafoods is known to occur. Additionally, psychotrophic strains of *V. parahaemolyticus* and *V. mimicus* have been isolated[17].

Vibrios are sensitive to heat and a temperature of 60°C maintained for several minutes is generally effective, although conditions needed to kill the organisms may vary somewhat.

Irradiation is effective at doses that are not lethal for oysters and greatly reduces the risk from eating this shellfish raw.

V. parahaemolyticus is susceptible to bacterial inhibitors present in herbs and spices. Sorbic acid is very inhibitory for this species.

The susceptibility of vibrios to low pH ensures that there is a minimal risk of infection from acidic foods. However, much has yet to be learnt about the susceptibility of vibrios to food preservation methods. At the present time, thorough heating of shellfish is probably the only reliable protective measure.

Depuration, the bacterial cleansing that occurs when living bivalve shellfish are placed in clean water that they pump through their tissues, is of limited value in removing vibrios naturally associated with molluscs although very effective for removing *Enterobacteriaceae* that adhere far less tightly to the animal.

Pathogenic vibrios must receive minimum exposure to cold during laboratory investigation to prevent inactivation and consequent failure to detect them by culture. Subjection of *Vibrio* cells to osmotic shock in suspending fluids and culture media may also result in failure to grow. Differences in salt content of identification media can have a profound effect on test results and may lead to misidentification. The success or otherwise of detection may also be influenced by the diluent used for preparing samples. Superiority of 0.1% peptone water over phosphate-buffered saline when enumerating *V. vulnificus* has been reported[18].

Vibrios are natural inhabitants of sea-water and brackish estuarine water and therefore their presence in seafoods can be expected.

Photograph: David E. Post

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Table 1 – Pathogenicity of *Vibrio* species for humans

Vibrio species that cause gastroenteritis	Some other infectious conditions caused by the same species		
	Wound infection	**Ear infections**	**Septicaemia**
Vibrio cholerae 01	Reported		
Vibrio cholerae non 01	Reported	Reported	Reported
V. mimicus	Reported	Reported	Reported
V. parahaemolyticus	Reported	Reported	
V. fluvialis			
V. hollisae			Reported
V. vulnificus (rare)	Reported		Reported
V. furnissii (rare)			
V. alginolyticus (rare)	Reported	Reported Eye infection reported	Reported
V. damsela	Reported		

Note: Some species of *Vibrios* that have not yet been reported to cause enteritis in humans infect other body sites. These infections may have arisen from handling contaminated raw seafood.

Source

Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C. and Winn, W.C. Jnr. *Color Atlas and Textbook of Diagnostic Microbiology* 5th ed. Chapter 6. 339–345. Lippincott, Philadelphia, P.A.

The Major Pathogenic Vibrios

The number of recognisable species of *Vibrio* seems set to rise with increasing knowledge of the place the genus occupies in the environment and the development of increasingly sophisticated techniques applied to classification.

At least 12 species are currently known to infect humans but, of these, only a few are isolated with some frequency from diseased persons. Infections occur after contact with contaminated water or consumption of fish and other seafoods.

This short review is limited to those species most likely to be detected when investigating foods and food poisoning.

Vibrio cholerae

This organism is the type species of the genus. Unlike most of the infective species found in foods it is not halophilic.

V. cholerae is the cause of cholera which is most frequently contracted from drinking sewage-contaminated water. Strains that are isolated are routinely divided into those that belong to serogroup 01 and those that do not. With the exception of serogroup 0139, the non-01 serogroups are far less pathogenic but may occasionally cause sporadic infections.

The potential of *V. cholerae* 01 to cause epidemic and pandemic spread is rare amongst food-borne pathogens. Until the emergence of serogroup 0139 as the cause of the epidemic of 1993, only strains of serogroup 01 that produce cholera toxin (CT) were thought responsible for the extremely severe diarrhoea that characterises cholera. Non-toxigenic 01 strains and other serogroups were considered not to be pathogenic or only minor pathogens.

Epidemic 0139 strains possess virulence factors found in 01 strains including cholera toxin typical of the El Tor biotype. Other characteristics are shared with a variety of non-01 serogroups.

The 01 El Tor biotype was first isolated in 1900 at a quarantine station of that name from pilgrims visiting Mecca. For 60 years it was considered non-pathogenic because it lacks haemolytic activity. Early isolates were haemolytic for goat and sheep red cells and presumably haemolytic capability was subsequently lost. Before the recognition that non-haemolytic strains can be pathogenic, haemolysis was thought to be a key marker of pathogenesis. However, most recent cholera epidemics have been due to *Vibrio* El Tor. Non-El Tor *V. cholerae* 01 has almost disappeared although occasional isolations suggest it might re-emerge. The *V. cholerae* 01 serotype can be further divided into subtypes named Ogawa, Inaba and a third subtype Hikojima which agglutinates with both Ogawa and Inaba antisera[1]. Variability occurs in the subtypes; Inaba colonies may be isolated from Ogawa strains and the reverse also occurs[1,2].

The disease caused by serogroup 0139 is indistinguishable from that due to serogroup 01 and must be treated in the same way by rapid replacement of fluid and electrolytes. The extremely severe diarrhoea is due to an enterotoxin and cell invasion by the vibrio does not appear to play a part as it does with *Shigella* and some *E. coli* infections. *V. cholerae* is very susceptible to acidic conditions and it is necessary for large numbers of cells to be ingested to enable sufficient to survive the gastric acidity of normal subjects and pass into the intestinal tract to initiate infection. Persons with weakly acidic stomachs because of antacid therapy or surgery may develop cholera following ingestion of few cells. The higher pH of the stomach when food is present will also reduce the infective dose.

Following passage across the gastric barrier, *V. cholerae* cells adhere to the intestinal wall and secrete toxin which interferes

Haemolytic colonies of *V. cholerae* on horse-blood agar.

with cell function resulting in rapid loss of body fluid into the intestine and to the exterior, resulting in dehydration. Replacement of fluid and the electrolytes responsible for fluid retention is essential for successful treatment of cholera. Antibiotic therapy has little place because of the rapidity with which drugs are flushed from the body, although administration of cotrimoxazole and tetracycline will generally be successful in reducing the duration of diarrhoea.

Occasional cases of diarrhoeal disease are caused by strains belonging to neither 01 nor 0139 serogroups but as they do not produce CT it appears that a different enterotoxin or toxins and other virulence factors are responsible.

Rarely, *V. cholerae* may cause systemic infection in susceptible persons. A recent report[3] of an HIV-positive restaurant kitchen worker who contracted fatal *V. cholerae* serogroup 02 sepsis underlines the importance of minimising occupational exposure to shellfish and the water they are taken from.

V. parahaemolyticus

This halophilic species is one of the most frequent causes of food poisoning where seafoods are a large part of the diet. Cases are at their most numerous in the summer months, presumably because of sensitivity of the organism to winter cold. *V. parahaemolyticus* is commonly associated with prawns and crabs but may be found in a variety of seafood animals including white fish and molluscs.

Infection causes diarrhoea accompanied by nausea and stomach cramps 12 to 24 hours after eating contaminated food. Illness lasts for 2 to 5 days and fatalities are rare. The infective dose is fairly high and is generally reached prior to consumption, in foods in which multiplication has occurred because of inadequate refrigeration. *V. parahaemolyticus* may invade the gut wall and spread to cause systemic infection. Extra-intestinal infections may occur in wounds, eyes and ears.

The pathogenic mechanisms leading to enteritis are not entirely understood although invasion of the gastric mucosa probably plays a significant part. Various toxins are produced and although pathogenic strains generally produce a heat-stable haemolysin (TDH) detectable by the Kanagawa test[2,4] (see also page 37), gastroenteritis caused by non-haemolytic strains has been reported. Hydrolysis of urea has been correlated with TDH production, but the evidence for usefulness of this as a pathogenicity marker is inconclusive.

Rehydration therapy is usually the only treatment necessary.

Somatic O and capsular polysaccharide antigens can be

identified in order to serotype strains[5] but there appears not to be correlation between serotype and virulence.

V. parahaemolyticus is exceptional amongst the vibrios for the rapidity of its growth. Generation times of 8 to 9 minutes in culture at 37° have been recorded and in seafoods times of 12 to 18 minutes have been observed. Work to determine the infective dose indicates that mishandling of foods at temperatures that allow growth to occur is a prerequisite for the number of cells to be reached needed to cause disease[6].

V. vulnificus

V. vulnificus is halophilic and shares many biochemical characteristics with *V. parahaemolyticus*. It differs in fermenting salicin and cellobiose. α-nitrophenyl-β-D-galactopyranoside is hydrolysed (ONPG test) and lactose also is fermented. This last feature led to strains being known as "lactose-fermenting vibrios" before classification as a species.

V. vulnificus is a rare cause of gastroenteritis but, very importantly, is invasive and causes septicaemia. The species can infect wounds and the fatality rate for these cases is high. Abnormally high levels of plasma iron appear necessary for multiplication in humans.

V. vulnificus infections typically occur as single cases amongst groups of people who have eaten the same food, usually raw oysters. The organism is common in oysters and may be harboured in the intestinal tract of asymptomatic persons who eat uncooked seafood. Unhealthy subjects are at great risk and it is essential that any seafood they eat should be cooked.

The number of infections correlates closely with water temperature, most occurring during the warmest months of the year in geographical areas where temperatures are high. However, infections also occur in temperate climates[7].

Seasonality in infection incidence and inability to isolate *V. vulnificus* when water temperature is low is probably attributable to the cells entering the viable but non-culturable state (VNC).

Infection in wounds received when handling oysters in the shell is an ever-present risk and as a precaution handlers should wear protective gloves.

Food microbiologists should not find it difficult to isolate *V. vulnificus* but the only guide to the virulence of isolates that is available to most laboratories is the appearance of the colonies. Not all strains are virulent; those that are produce colonies having an opaque appearance. The colonies of avirulent strains are translucent. Virulent strains may produce both opaque and translucent colonies in the same culture due to mutation occurring in some cells[8].

Strains of *V. vulnificus* divide into biotypes 1 and 2. Biotype 2 is a natural pathogen of eels but has been reported to be an opportunistic pathogen in humans[9]. Eel farmers in particular are at risk when infections occur amongst eel stocks.

V. mimicus

This species, previously thought to be a sucrose-negative variant of non-01 *V. cholerae*, is usually isolated from persons who have developed diarrhoea following consumption of raw seafood. It may also cause ear infection in bathers and water-sport participants.

Raw oysters are commonly associated with enteric infection. Raw fish, crayfish, shrimp and crab have also been identified as vehicles for the species. Persons who are normally healthy readily become infected. Illness is characterised by diarrhoea, abdominal pain and nausea lasting from 2 to 10 days followed by complete recovery.

V. fluvialis

Strains of this species were known as "Group F. vibrios" before assignment of the species name[10]. Biochemically it is similar to *Aeromonas hydrophila* but is halophilic.

V. fluvialis may readily be isolated from brackish water and fish, crab, shrimp and shellfish.

Infection is similar to cholera; diarrhoea is profuse and leads to severe dehydration in the absence of fluid replacement. Typically, symptoms last from less than 24 hours to about 3 days.

V. furnissii

The two species *V. furnissii* and *V. fluvialis* are very alike but *V. furnissii* may be identified biochemically by its production of gas from glucose.

V. furnissii is also closely related to *A. hydrophila* but is easily distinguished by its ability to grow in 6% sodium chloride.

V. furnissii is present in river and estuarine waters and can be isolated from molluscs and crustaceae. It occasionally causes gastroenteritis. Salads containing shrimp and crab have been implicated.

V. hollisae

V. hollisae, a halophilic species previously known as CDC Enteric Group 42, may occasionally be isolated from persons who have developed diarrhoea following consumption of raw seafood.

The organism may invade and cause septicaemia in patients who have existing liver abnormalities. This species grows poorly or not at all on TCBS agar but may be isolated on MacConkey agar and XLD agar although the latter appears unreliable.

V. alginolyticus

V. alginolyticus was originally classified as biotype 2 of *V. parahaemolyticus*. It is found in sea-water. Infection may be enteric but much more frequently the organism causes superficial infections of wounds, and ear and eye infections. Bacteraemia may rarely occur.

V. alginolyticus is halophilic and may be isolated on the usual enteric culture media if supplemented with sodium choloride.

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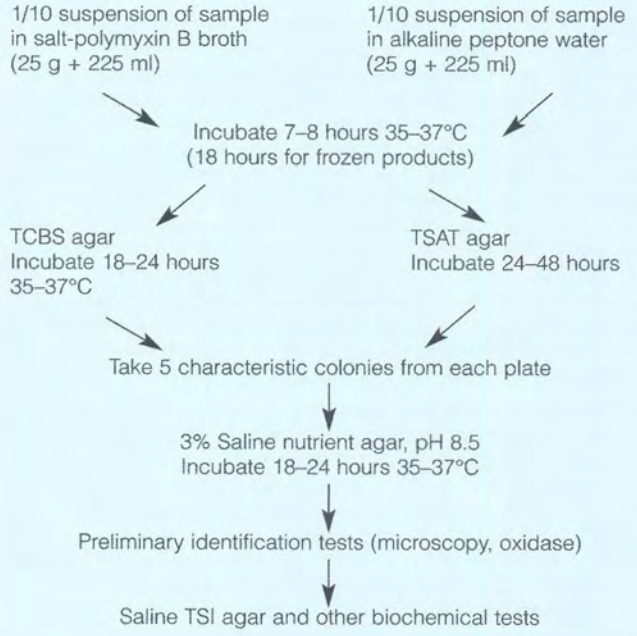
Table – Official Bodies that specify Detection Procedures and the Culture Media to be used

Official Body	Species	Enrichment	Plating	Identification
Australian/New Zealand Standard AS/NZS 1766.2.9.1997	*V. parahaemolyticus*	Alkaline Peptone water	TCBS Agar	Tryptone water Broth; Buffered glucose Agar; Nutrient Agar; TSI Agar; Bromocresol purple-cellobiose Broth All the above to contain 30 g/L sodium chloride
Canada. Health Protection Branch MFHPB – 15 1997	*V. parahaemolyticus* detection	Bismuth sulphite Broth	TCBS Agar	Various biochemical test media. Wagatsuma Agar
Canada. Health Protection Branch MFLP – 57 1995	*V. parahaemolyticus* enumeration	Salt-polymyxin Broth Glucose-salt-Teepol Broth	TCBS Agar TCBS Agar	Various biochemical test media supplemented with sodium chloride. Wagatsuma Agar
USA Food and Drug Administration. FDA. *Bacteriological Analytical Manual* 8th ed. 1995 and revision 1998	Pathogenic *Vibrio* spp.	Alkaline Peptone water	TCBS Agar modified CPC Agar	Various biochemical test media, most supplemented with 2–3% sodium chloride. Wagatsuma Agar
British Standard/ISO standard BS 5763 Part 14: 1991 ISO 8914: 1990	*V. parahaemolyticus*	(a) Alkaline Peptone water (b) Saline-glucose-Sodium dodecyl sulphate Broth	TCBS Agar TSAT Agar	Salt nutrient Agar; Salt TSI Agar; Salt-Lysine-decarboxylase Medium; Salt-Tryptone Medium
Nordic Committee on Food Analysis	Pathogenic *Vibrio* spp.	Alkaline Peptone water	TCBS Agar	Salt O/F Medium; Tryptone Broth; Blood Agar
Norme Française (AFNOR) NF ISO 8914 1991	*V. parahaemolyticus*	(a) Alkaline Peptone water (b) Salt-polymyxin B Broth (c) Saline-glucose-Sodium dodecyl sulphate Broth	TCB Sugar TSAT Agar	Salt-Tryptone Medium; Salt-nutrient Agar; Salt-meat-yeast Agar; Salt-Lysine-decarboxylase Medium; Salt-Tryptone Medium
Italian Istituto Superiore di Sanita Rapporti istison 96/35	*V. parahaemolyticus*	(a) Alkaline Peptone water (b) Salt-polymyxin B Broth (c) Saline-glucose Sodium dodecyl sulphate Broth	TCBS Agar TSAT Agar	Salt-nutrient Agar; Salt-TSI Agar; Salt-Lysine-decarboxylase Medium; Salt-Tryptone Medium
Italian Istituto Superiore di Sanita Rapporti istison 96/35	*V. cholerae*	(a) Alkaline Peptone water (b) Saline-glucose-Sodium dodecyl sulphate Broth	TCBS Agar Salt gelatin phosphate Agar	Kligler Agar; Tryptone soya Agar; Glucose Agar
Canada. Health Protection Branch MFLP – 72 1995	*V. cholerae* 01 and non-01 (0139)	Alkaline Peptone water	TCBS Agar	Various biochemical test media
USA. AOAC Official Method 988 – 20 1990	*V. cholerae*	Alkaline Peptone water	TCBS Agar	TI NI Agar; Kligler Agar; Tryptone Broth; O/F Medium; Purple carbohydrate Broth; Moeller decarboxylase Medium
Canada. Health Protection Branch MFLP – 73 1995	*V. vulnificus*	Alkaline Peptone water	TCBS Agar Modified CPC Agar	Various biochemical test media

Note: Many of the methodologies are complex and some require many different media to carry out the identification procedures specified. For reasons of brevity these have not all been listed in the above table. The publications of the various official bodies should be consulted for full details of methods and the materials used.

Schematic Diagrams of Typical Procedures for Detection of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*

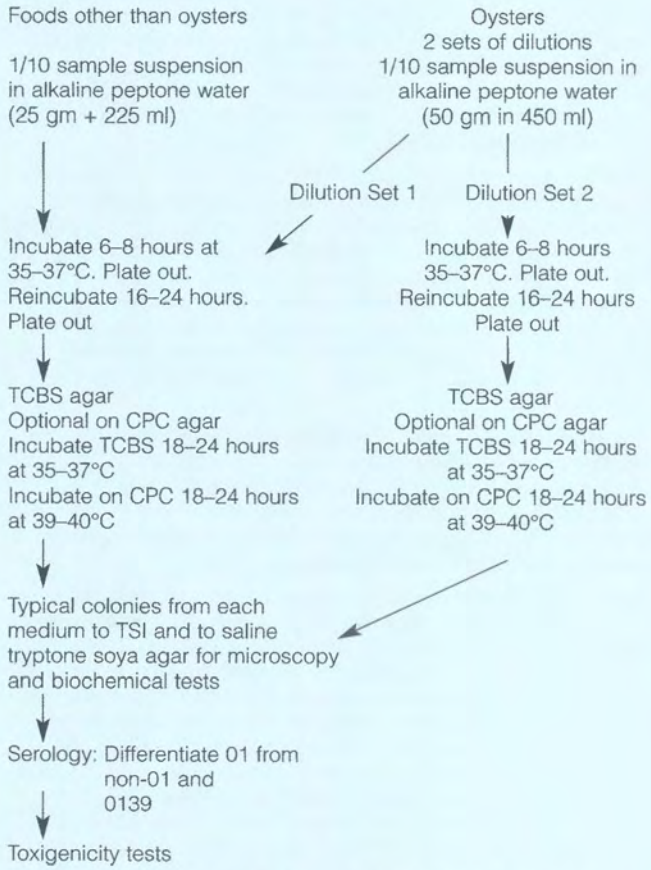
Figure 1 – Simplified schematic diagram of a typical procedure for detection of *Vibrio parahaemolyticus**



*Based on BS5763 part 14: 1991/ISO 8914: 1990

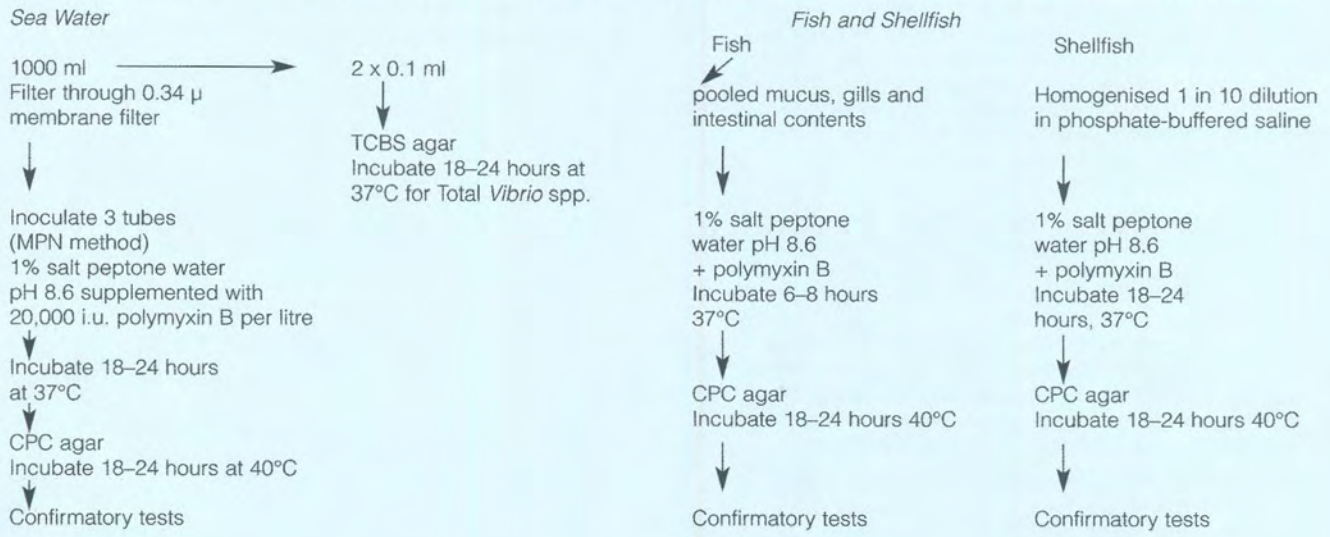
The Standard should be consulted for the detailed procedure.

Figure 2 – Simplified schematic diagram of a procedure for detection of *Vibrio cholerae**



*Based on FDA BAM 1995 method. The *Bacteriological Analytical Manual* should be consulted for the complete procedure.

Figure 3 – Schematic diagram of procedures for detection of *Vibrio vulnificus* in marine environments, fish and shellfish



Reference

For full details of the procedures see: Hoi, L., Larsen, J.L., Dalsgaard, I. and Dalsgaard, A. (1998) *Appl. Env. Microbiol.* **64,** 7–13. A method is also given for isolation from sediments.

Liquid media for enrichment culture of *Vibrio* species

Vibrios are not nutritionally demanding; Robert Koch was able to use simple meat infusion for his investigations into the cause of epidemic cholera. Dunham[1] used a solution of peptone and sodium chloride for detecting *V. cholerae* and he and Harvey[2] recognised that the relative tolerance of vibrios towards alkaline conditions could be employed to select *V. cholerae* in culture. To this day alkaline peptone water remains the selective enrichment medium of choice for growth of most species of vibrios.

Alkaline conditions alone are not always sufficiently selective and a number of modifications have been made to the basic medium over the years but, with few exceptions, they have not become commonly used. Increase in the salt content of standard peptone water is the simplest modification but, of the more complex formulae, Monsur's[3] tryptone-tellurite-taurocholate-peptone (TTP) medium has probably become most widely used. A plating medium (see page 24) made by the addition of agar to modified TTP medium is also strongly recommended[4].

Pal *et al.*[5] found that the addition of potassium tellurite to alkaline peptone water improved the inhibition of *Enterobacteriaceae* and *Pseudomonas* spp., although enterococci were still able to grow. However, both this medium and Monsur's TTP medium were superior to plain alkaline peptone water for growth of *V. cholerae* El Tor.

Many other modifications to alkaline peptone water were made in the 19th and early 20th centuries because of the prevalence of cholera amongst persons living in insanitary conditions. All were directed towards isolation from sick patients. Most modifications involved the addition of bile, potassium tellurite and inhibitory dyes. A number of workers used dyes in their selective media including Dishon[6] who combined three of them with sucrose in acid fuchsin-gentian violet-brilliant green broth. Modifications to Wilson and Blair's bismuth sulphite medium for *Salmonella typhi* were made by Read[7] and subsequently Wilson with Reilly[8]. More recently a medium based on nutrient broth with added salt and incorporating Eosin y was found by Hofer and Silva[9] to be the most effective of seven media tested when used with TCBS agar as the plating medium.

Vibrio parahaemolyticus is an important pathogen belonging to a group of halophilic vibrios, species that require a high concentration of salt to thrive. Satisfactory enrichment culture of halophiles in alkaline peptone water requires a raised salt content[10]. Many other media have been described for enrichment culture of this species including glucose-salt-Teepol broth[11], salt-colistin broth[12], salt-polymyxin broth[13], salt-meat broth[14], and Horie's arabinose-ethyl violet broth[15] reported by Beuchat[16] to be superior for recovering unstressed and heat- and cold-stressed cells of *V. parahaemolyticus.*

A period of resuscitation in tryptone soya broth containing 1% sodium chloride has been reported to improve detection rates still further[17]. An ICMSF methods study[18] which compared salt-polymyxin broth and glucose-salt-Teepol broth for enumerating *V. parahaemolyticus* in naturally contaminated samples using a Most Probable Number (MPN) technique showed neither medium to be superior. However, use of both media prevented false-negative results. Supplementation of glucose-salt-Teepol broth with magnesium ions has been reported to improve the recovery of chill-stressed *Vibrio* cells from oysters[19].

An enrichment medium, starch-gelatin-polymyxin B broth, has been formulated specifically for *V. vulnificus*[20] but alkaline peptone water may also be used for this species[21].

Spira[22] incorporated gelatin as the sole energy and nitrogen source in phosphate-buffered saline to stimulate the growth of environmental vibrios that produce gelatinase, but found the medium to be of limited use for detection of *V. cholerae* in situations where other gelatinase-positive organisms are found.

Many earlier formulae exist for enrichment media and examples will now be mentioned for historical interest. Amongst the media are some that contained ingredients designed to enable presumptive identification of isolates to be made. An example is the medium of Signorelli[23] which depended on the differential staining of cholera organisms by the dye dahlia added to peptone water. *Vibrio* cells were stained and the medium itself became decolourised. A few early media were solutions of salts that provided nitrogen, and carbohydrates including glycerol. Increased concentrations of sodium chloride were not a feature of these media, presumably because *V. cholerae* is not a halophile, but the selective property of alkaline media was generally recognised. One formula contained phenolphthalein and growth of *V. cholerae* in the medium caused it to decolourise.

Asparagine and aspartic acid were considered by some workers to have an important role in culture. Many organic sources of nitrogen including blood, egg, albumen and serum were used, as well as the inorganic salts preferred by some. Two of the more unusual nutrient bases were mussel and oyster infusions[24] (presumably because the author was working with shellfish) and melon juice neutralised with sodium carbonate[25]. Cucumber juice was tried as an alternative to melon but found to be inferior! In the year of publication of his melon juice formula Ottolenghi also described a more conventional enrichment medium that contained nitrate and bile to confer selectivity.

Other unusual medium ingredients that have been used include Nutrose and caffeine.

"Lemco" meat extract, manufactured by Liebig Extract of Meat Company was included in a 1906 formula[26] for a peptone-starch medium intended for use in a test for starch fermentation. The use of "Lemco" (now Oxoid Lab-Lemco L29) had been described by Buchner[27] as early as 1885 in a sucrose-peptone medium solidified using gelatin.

Reference to many early formulations for detection of *V. cholerae* is made in a comprehensive survey of culture media published in 1930[28].

Alkaline peptone water, some modifications to it and some other enrichment media are mentioned in the following pages.

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Table 2 – Selective agents of some enrichment media for *Vibrio* species

Medium	Intended use	Salt content %	pH	Selective agents	Reference
Alkaline peptone water	*Vibrio* spp.	0.5–1	8.6	High pH	1
Salt-alkaline peptone water	Halophilic *Vibrio* spp.	3	8.6	High salt High pH	2
Alkaline peptone water with electrolyte supplement	*Vibrio* spp.	1	8.6	High pH	3
Monsur tellurite-taurocholate peptone water	*Vibrio* spp.	1	9.2	High pH Potassium tellurite Sodium taurocholate	4
Alkaline tellurite peptone water	*Vibrio* spp.	Not stated but probably 1	9.2	High pH Potassium tellurite	5
Salt-free alkaline peptone water	*V. cholerae*	0	8.6	High pH Absence of salt	6
Alkaline taurocholate-peptone water	*V. cholerea*	1	8.6	Potassium tellurite High pH	6
Salt-polymyxin broth	*V. parahaemolyticus*	2	7.4	Polymyxin B	2
Salt-colistin broth	*V. parahaemolyticus*	2	7.4	Colistin	7
Glucose-salt-Teepol	*V. parahaemolyticus*	2	9.4	High pH	8a, b
Saline-glucose-sodium dodecyl sulphate medium	*V. parahaemolyticus*	3	8.6	Teepol* Sodium dodecyl sulphate methyl violet	9
Bismuth sulphite-salt broth	*V. parahaemolyticus*	2.5	9.1	High pH High salt Bismuth sulphite	10
Salt-meat broth	*V. parahaemolyticus*	5	7.2	High salt	11
Water blue-alizarin yellow broth	*V. parahaemolyticus*	3	6.9	High salt Teepol* Water blue Alizarin yellow	12
Starch-gelatin-polymyxin B broth	*V. vulnificus* *V. cholerae* El Tor	0.5	7.6	Polymyxin B	13

*Sodium dodecyl sulphate may be substituted for Teepol.

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Peptone Water

Peptone Water

Code: CM9

A basal medium for the production of alkaline peptone water for the selective enrichment of *Vibrio* species.

Formula

	grams/litre
Peptone	10.0
Sodium chloride	5.0
Water	1000 ml

pH before adjustment to alkaline peptone water 7.2 ± 0.2

Directions

Dissolve 15 gm in 1 litre of distilled water. To make alkaline peptone water adjust the pH to 8.6 or the pH value required if different. Add 1–2% sodium chloride for halophilic species. Mix well and distribute into the final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Peptone water adjusted to alkaline pH is used for the selective enrichment of *Vibrio* spp.[1] pH 8.4–8.6 has been found suitable for *V. cholerae*[2] but pH values ranging from 7.4 to 9.4 are stated in the literature; actual pH values specified are dependent on the medium, the species, type of food under investigation and the composition and quantity of accompanying flora that can be expected.[2] A pH of 8.6 and adjustment of sodium chloride content to 1% has been shown by Furniss and others[3] to be most suitable for a wide range of species.

Plain alkaline peptone water has been used since the 1880's following the observation by Dunham[4] of its efficacy for isolating *V. cholerae* from faeces. Modification of the formula may be necessary for other kinds of sample and for other species, usually to make it more selective by adding various chemicals, bile salts and either colistin or polymyxin B. Increase in sodium chloride concentration to make the medium suitable for halophilic species may be necessary. Supplementation with various electrolytes is stimulatory for *V. cholerae* and other pathogenic species[3]. The authors added their supplement to media as necessary but Roberts and her colleagues[5] routinely add it to all peptone water.

A sodium chloride-free solution of peptone may be advantageous when attempting to isolate *V. cholerae* from the environment by preventing the growth of halophilic species which also may be present[6]. If required, an alkaline 1% solution of bacteriological peptone L37 can be used instead of peptone water CM9.

The addition of nutrients such as blood, egg and serum to peptone water has been suggested by workers investigating *V. cholerae* but they do not appear to improve performance[7].

Important modifications to alkaline peptone water have been made by Monsur[8], Pal[9], and Spira[6] to prevent overgrowth of *V. cholerae* by unwanted *Vibrio* spp. and non-vibrio organisms and the antagonism that may be shown by the latter to vibrios. Monsur added bile salts and potassium tellurite to make taurocholate-tellurite-peptone water (TTP). Experience has shown this medium to be very useful, not only because of its improved selectivity and excellent growth of *V. cholerae*, but because it is easy to prepare and may also be used as a sample transport medium.

Pal[9] added potassium tellurite to alkaline peptone water to make peptone water-tellurite (PWT) medium, and Spira[6] used sodium taurocholate to make alkaline taurocholate peptone water (ABPW). Peptone water is specified by the American Food and Drug Administration (FDA) as the base for a number of media used for selective enrichment of *Vibrio* spp.[10]

Formulae of some modifications to alkaline peptone water are given in the following pages. The publications referred to should be consulted for further information.

Other uses for peptone water CM9

Peptone water which has not been adjusted to alkaline pH may be used as a simple growth medium. A pure culture grown in peptone water is a convenient inoculum for fermentation and other diagnostic tests.

Peptone water may be modified to make it suitable for carbohydrate fermentation tests by the addition of Andrade's indicator* and the required carbohydrates to make peptone water sugars.

**Andrade's indicator*

Andrade's indicator may be made by adding 1N sodium hydroxide to a 0.5% solution of acid fuchsin until the colour becomes just yellow.

Appropriate safety precautions must be taken to avoid inhalation of, and skin contact with, acid fuchsin.

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Simple Peptone Water Media for Enrichment of *Vibrio* species

Salt-Alkaline Peptone Water

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Sodium chloride	3	Sodium chloride L5
Water	1000 ml	
pH 8.6		

Description

The salt content in this modification of alkaline peptone water is increased to 3% to select halophilic *Vibrio* spp. including *V. parahaemolyticus* and *V. alginolyticus*. This concentration of salt is inhibitory to *Enterobacteriaceae*. If simultaneous examination for non-*Vibrio* enteric pathogens is a requirement, then a salt content no greater than 1% (as in unmodified alkaline peptone water) should be used but poorer recovery of halophilic vibrios must be expected.

Reference

Donovan, T.J. and van Netten, P. (1995) *Int. J. Food Microbiol.* **26,** 77–91.

Formulae of United States Food and Drug Administration Peptone Water Media for Enrichment of *Vibrio* species

Peptone water is specified by the U.S. F.D.A. as the basis of a number of media used for selective enrichment of *Vibrio* spp.

Only the formulae are given here; detailed descriptions of their preparation and use are outside the scope of this monograph but can be found in the Bacteriological Analytical Manual.

AKI Medium B.A.M., Formula M7

Formula

	grams/litre	Suggested Oxoid products
Peptone	15	Peptone bacteriological L37
Yeast extract	4	Yeast extract L29
Sodium chloride	5	Sodium chloride L5
Water	970 ml	

Sterilise by autoclaving. When cool, add 30 ml of filter-sterilised 10% sodium bicarbonate.

Final pH 7.4 ± 0.2

Alkaline peptone-salt broth, Formula M9

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Sodium chloride	30	Sodium chloride L5
Water	1000 ml	
pH 8.5 ± 0.2		

Alkaline Peptone Water, Formula M10

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Sodium chloride	10*	Sodium chloride L5
Water	1000 ml	
pH 8.5 ± 0.2		

*This medium is suitable for *V. cholerae*, *V. mimicus* and less demanding halophilic species.

Sodium chloride content should be increased to 2–3% to optimise the salt content for a wider range of halophiles including *V. parahaemolyticus* and *V. vulnificus*.

Reference

Elliot, E.L., Kaysner, C.A., Jackson, L. and Tamplin, M.L. (1998) Food and Drug Administration. *Bacteriological Analytical Manual*. Revision A (1998) of 8th ed. 1995. A.O.A.C. Int. Gaithersburg, M.D.

Some alternative enrichment media for *Vibrio* species

Alkaline Peptone water containing Electrolyte Supplement

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Sodium chloride	10	Sodium chloride L5
Magnesium chloride hexahydrate	4	
Potassium chloride	4	
Water	1000 ml	
pH 8.6		

Description

This medium[1] combines alkaline peptone water with the electrolyte supplement devised by Furniss, Lee and Donovan to provide the additional salts required by some species of *Vibrio*. It may be used for enrichment culture of all *Vibrio* spp. likely to be found in food. Excessive overgrowth by *V. alginolyticus* may occur and mask the presence of other vibrios. Overgrowth may be reduced by repeating culture using plain alkaline peptone water. Isolation of *V. cholerae* may be aided by subculturing after 6 hours incubation.

Reference

1 *Practical Food Microbiology*, Section 6.14 Method 1. Roberts, D., Hooper, W. and Greenwood, M. (1995) PHLS London.

Electrolyte Supplement for Peptone Water

Formula

	grams/litre	Suggested Oxoid product
Sodium chloride	100	Sodium chloride L5
Magnesium chloride: $6H_2O$	40	
Potassium chloride	40	
Water	to 1000 ml	

Distribute into 5 ml volumes and sterilise at 121°C for 15 minutes.

Add the supplement to an appropriate medium in proportions of 0.1 ml of supplement to 1.0 ml of medium.

Note

The total volume is made up to 1000 ml by addition of water after the salts have been dissolved in a smaller volume.

Reference

Furniss, A.L., Lee, J.V. and Donovan, T.J. (1978) *The Vibrios* page 13. Public Health Laboratory Service Monograph Series number 11, HMSO, London.

Alkaline Taurocholate-Peptone Water

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Sodium chloride	10	Sodium chloride L5
Sodium taurocholate	5	
Water	1000 ml	
pH 8.6		

Description

Alkaline-taurocholate-peptone water (ABP) was reported by Spira to be satisfactory for enrichment culture of *V. cholerae* in foods but had not been thoroughly evaluated for other vibrios. Inhibition of accompanying flora was more satisfactory than that shown by alkaline peptone water. Experimental work established that enrichment culture for 18 hours is optimal for *V. cholerae* if ABPW is the only enrichment medium used. Plating to solid medium at 6 hours incubation, which is recommended in some procedures for detection of *V. cholerae*, is not as satisfactory when using ABPW.

Reference

Spira, W.M. (1984) Tactics for detecting pathogenic vibrios in the environment. Chapter 17. In: *Pathogenic Vibrios in the Environment*. Colwell, R.R. (ed.) Wiley, New York.

Alkaline Tellurite-Peptone Water

Alkaline peptone water

Potassium tellurite to final concentration of 1/200,000.

pH 9.2

Description

This modification to alkaline peptone water was found by Pal *et al.* to be a more satisfactory enrichment medium than plain alkaline peptone water for detection of *V. cholerae* in faecal specimens.

The authors did not specify the exact composition of the alkaline peptone water to which they added potassium tellurite immediately before adding faecal specimens. The medium was not evaluated for use with food or environmental samples.

Reference

Pal, S.C., Murty, G.V.S., Pandit, C.G., *et al.* (1967) *Ind. J. Med. Res.* **55,** 318–324.

Alkaline Taurocholate-Tellurite-Peptone Water

Formula

	grams/litre	Suggested Oxoid products
Tryptone	10	Tryptone L42
Sodium chloride	10	Sodium chloride L5
Sodium taurocholate	5	
Sodium carbonate	1	
Water	1000 ml	
pH not stated*		

Potassium tellurite is added to a final concentration of 1/100,000 to 1/200,000 immediately before use.

Description

This medium was devised by Monsur[1] both for preservation of faecal samples during transport to the laboratory and for enrichment culture of *V. cholerae*. It was not evaluated for use with foods but may be used with environmental waters with suitable adjustment of the water content to account for dilution.

The final pH of the medium was not stated by the author. The content of potassium tellurite added at the pH value chosen by the user will need adjustment between the limits given above to ensure optimum performance by testing with suitable control strains. The author found the medium capable of detecting 1 organism per ml of sample.

Reference

1 Monsur, K.A. (1962) *Fed. Proc.* **21,** 394, item number 191.

*A subsequent publication stated the pH to be "about 9.2".[2]

Reference

2 Monsur, K.A. (1983) Bacteriological diagnosis of cholera under field conditions. *Bull. W.H.O.* **28,** 387–389.

Salt-Polymyxin Broth

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Sodium chloride	20	Sodium chloride L5
Polymyxin B	100,000 i.u.	
Water	1000 ml	
pH 8.6		

Description

This formula for salt-polymyxin broth (SPB) is specified by the Public Health Laboratory Service for enrichment culture of *V. parahaemolyticus*[1]. It is recommended that either alkaline peptone water containing electrolyte supplement or salt-glucose-sodium dodecyl sulphate is used as a second enrichment medium. SPB may be used in a Most Probable Number (MPN) procedure for enumeration of *V. parahaemolyticus*.

An alternative salt-polymyxin broth containing 1% peptone, 0.3% yeast extract, 2% sodium chloride and polymyxin B sulphate 0.25 µg/ml was formulated by Karunasager *et al.*[2] The reduction of Polymyxin B from 2.5 µg/ml contained in the medium of Sakazaki *et al.*[3] was found to increase significantly the isolation rate of *V. parahaemolyticus* from fish samples. A similar medium having a pH of 7.4 and containing 100,000 i.u. of polymyxin B per litre is specified in British Standard BS5763 part 14: 1991 (ISO 8914:1990).

Reference

1 Practical Food Microbiology: Roberts, D., Hooper, W. and Greenwood, M. (1995) Section 6.14 Methods 2 and 4. Public Health Laboratory Service, London.
2 Karunasager, I., Venugopal, M.N., Karanusagar, I. and Segar, K. (1986) *Appl. Env. Microbiol.* **52,** 583–585.
3 Sakazaki, R., Karashimada, T., Yodo, K. *et al.* (1979) *Arch. Lebensmittelhyg.* **30,** 103–106.

Salt-colistin Broth

Description

Colistin is polymyxin E and possesses a similar range of antibacterial activity to polymyxin B. Both colistin and polymyxin B have been used in formulae devised for selection of *Vibrio* spp.

One or other, (or, in one formula, both), antibiotics are generally incorporated in 1% salt peptone water.

Sakazaki[1] used colistin in a medium containing 2% sodium chloride for the detection of *V. parahaemolyticus*. Unusually, the pH is near neutral at pH 7.4 so that further stress to damaged cells can be minimised.

Care must be taken when preparing media containing colistin or polymyxin B to ensure the correct concentrations of antibiotic are used as the quantities required may be stated either as International Units (i.u.) or micrograms. The values may not be interchangeable and each author's instructions must be closely followed.

The salt content specified by other workers may differ.

Reference

1 Sakazaki, R. (1973) Control of contamination with *Vibrio parahaemolyticus* in seafoods and isolation and identification of the vibrio. Pages 375–383. In: Hobbs, B.C. and Christian, H.B. (eds.) *The Microbiological Safety of Foods*. Academic Press, London.

Salt Meat Broth

Formula

	grams/litre	Suggested Oxoid product
Cooked meat medium Oxoid CM81 prepared according to manufacturer's instructions	100	
Sodium chloride	50	Sodium chloride L5
pH 7.2 ± 0.2		

Description

Robertson's cooked meat medium with additional 5% of sodium chloride was used to investigate the occurrence of *V. parahaemolyticus* and *V. alginolyticus* in oysters and mussels. The neutral pH prevents further damage that can result from the highly alkaline conditions of some alternative media on cells which may already be damaged.

The authors concluded that, although they were successful in isolating the two species after subsequent plating on TCBS agar, their methodology and media should be used in parallel with others to maximise the isolation rate.

Reference

Kampelmacher, E.H., Mossel, D.A.A., van Noorle-Jansen, L.M. and Vincentie, H. (1970) *J. Hyg. Camb.* **68,** 189.

Starch-Gelatin-Polymyxin B Broth (SGPB)

Formula

	grams/litre	Suggested Oxoid product
Soluble starch	10	
Gelatin	10	Gelatin bacteriological L8
Sodium chloride	20	Sodium chloride L5
Polymyxin B	150,000 i.u.	
Water	1000 ml	
pH 7.6		

Description

SGPB broth was developed for use in conjunction with SDS-polymyxin B-sucrose agar in methodology to detect sulphatase activity of *V. vulnificus* and *V. cholerae* 01. For use with the latter, non-halophilic species, the sodium chloride concentration is reduced to 0.5%.

SGPB broth may be used routinely as an enrichment medium in investigations for the presence of *V. vulnificus*.

Reference

Kitaura, T., Doke, S., Azuma, I. *et al.* (1983) *FEMS Microbiol. Lett.* **17,** 205–209.

Glucose-Salt-Teepol* Broth (GSTB)

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Yeast extract	3	Yeast extract L21
Sodium chloride	20	Sodium chloride L5
Polymyxin B	0.25 mg	
Teepol*	10	
Water	1000 ml	
pH 9.4		

Description

This medium containing 0.25 µg/ml of polymyxin B is a modification by Malin and Beuchat[1] of the medium described by Twedt[2] for recovery of chill-stressed *V. parahaemolyticus*. Glucose-Salt-Teepol broths with varying concentrations of polymyxin B were evaluated by Karunasagar *et al.*[3] and 0.25 µg/ml found to be most satisfactory for detecting small populations of *V. parahaemolyticus* although higher concentrations may be required when preparing homogenates of fish tissue because of absorption of the antibiotic[4].

References

1 Malin, A.C.F. and Beuchat, L.R. (1980) *Appl. Env. Microbiol.* **39,** 179–185.
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4 Blanchfield, B., Stavric, S., Jean, A. and Pivnick, H. (1982) *J. Food Prot.* **45,** 744–746.

**Sodium dodecyl sulphate may be substituted for Teepol.*

Saline-Glucose-Sodium Dodecyl Sulphate-Peptone water (GST)

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Meat extract	3	Lab-Lemco L29
Sodium chloride	30	Sodium chloride L5
Glucose	5	
Methyl violet	0.002	
Sodium dodecyl sulphate	1.36	
Water	1000 ml	
pH 8.6		

Description

Sodium dodecyl sulphate may be specified instead of Teepol in media for selective enrichment of pathogenic *Vibrio* spp. This medium, containing 3% salt, also contains methyl violet to inhibit Gram-positive organisms. It is used in ISO standard methodology for detection of *V. parahaemolyticus.*

Reference

ISO Standard method 8914: 1990 (E).

Horie Broth

Formula

	grams/litre	Suggested Oxoid products
Peptone	5	Peptone bacteriological L37
Meat extract	3	Lab-Lemco L29
Sodium chloride	30	Sodium chloride L5
Arabinose	5	
Bromothymol blue	0.03	
Ethyl violet	0.001	
Water	1000 ml	
pH 9.0		

Description

This medium, which is designed to increase the range of nutrients available by partially replacing the peptone with meat extract, contains 3% of salt to optimise salinity for growth of halophilic *Vibrio* spp. Inhibitory activity at pH 9.0 is enhanced by the presence of ethyl violet. Arabinose and bromothymol blue are included to detect species able to ferment arabinose.

Horie broth has been found superior for detection of cold-damaged *V. parahaemolyticus.* Chilled cells of this species are greatly effected by the diluent and broth used in laboratory procedures and it is suggested that both glucose-salt-Teepol broth and Horie broth are used for enrichment culture.

Reference

Horie, S., Saheki, K., Kozima, T. *et al.* (1964) *Bull. Jpn. Soc. Fish* **30,** 786–791 (Japanese language) cited in: Ann Ma-Lin, C.F. and Beuchat, L.R. (1980) *Appl. Env. Microbiol.* **39,** 179–185.

Water Blue-Alizarin Yellow Broth (WBAY)

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Sodium chloride	30	Sodium chloride L5
Sucrose	10	
Water blue	0.02	
Alizarin yellow	0.02	
Teepol*	2.0	
Water	1000 ml	
pH 6.9		

Description

Beuchat[1] investigated the suitability of various enrichment broths and diluents for enumerating cold- and heat-stressed *V. parahaemoloyticus.* WBAY broth was found to be superior to GSTB broth for repair and division of cells. The author recommended that it should be used in parallel with GSTB broth to maximise the numbers detected.

Reference

Beuchat, L.R. (1977) *Can. J. Microbiol.* **23,** 630–633.

**Sodium dodecyl sulphate may be substituted for Teepol.*

Bismuth Sulphite-Salt Broth

Formula

	grams/litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Sodium chloride	25	Sodium chloride L5
Potassium chloride	0.7	
Magnesium chloride $6H_2O$	5	
Water	950 ml	

pH 9.1 Adjust to this value by adding 10% aqueous sodium carbonate before autoclaving.

To cooled medium add:

Bismuth sulphite solution	100 ml
Ethyl alcohol 95%	1 ml

To make Bismuth sulphite solution:

Solution A

Sodium sulphite	20 gm
Boiling water	100 ml

Solution B

Ammonium bismuth citrate	0.1 g
Boiling water	100 ml

Mix Solution A with Solution B and boil for 1 minute.

Add: Mannitol	20 g
Boiling water	100 ml

Mix and dispense in 10 ml volumes.

Description

This medium is used in the Canadian Health Protection Branch method for isolating *V. parahaemolyticus* from sea fish and shellfish. It may also be used in examining human faeces for *V. parahaemolyticus.*

Reference

Government of Canada Health Protection Branch, Ottawa. HPB method MFHPB – 15 April 1997. Polyscience Publications, Quebec.

Agar Media for *Vibrio* species

Very many culture media have been formulated for use in investigating cholera, emphasising the extreme importance of this enteric infection in public health before the introduction of effective sanitation and water treatment.

The recognition of the importance of species other than *V. cholerae* in human disease has resulted in the development of media more appropriate to them and to detecting their presence in foods.

Some of the early formulae devised for culturing *V. cholerae* appear curious: Sobel[1] formulated an easily made medium that contained beer and lactose for use in isolating and identifying the organisms responsible for cholera, typhoid and dysentery. The dye Congo Red was included to enable *V. cholerae*, *Salmonella typhi* and *Shigella* species to be differentiated by the colour of the colonies they formed. Earlier, Richter[2] had used a medium containing Moselle wine. The wine served first to rehydrate dried shredded agar. It was then incorporated with the agar in a watery meat extract. In addition to its use in the diagnosis of cholera and typhoid the medium was used in the investigation of anthrax, another infection which was much more common in 19th Century Europe. Gelatin was included in the formula, although whether this was primarily present to demonstrate gelatinase activity of the organisms growing on it, or to supplement the gelling property of agar is not clear.

Deycke[3] certainly formulated his medium so that the gelatinase activity of *V. cholerae* could be used as a means of identifying the organism. He reported that colonies on his medium were similar in appearance to those grown by Robert Koch on a gelatin-containing medium. Deycke based his medium on peptone and made it favourable for *V. cholerae* by incorporating albumen treated with soda (presumably sodium carbonate) to give an alkaline pH.

Whole egg was used by other workers in a later peptone-based medium that included sodium carbonate to raise the pH.

Numerous other media have been described which utilise complex nitrogen sources, gelatin, salt and alkalinity in various combinations.

Potato starch was used, with litmus as a pH indicator, in a peptone-based alkaline medium which enabled the fermentation of starch by cholera vibrios to be demonstrated[5]. This medium was an improvement that followed at least one formula[6] for solid alkaline peptone media which did not possess a differential identifying system of any sort but depended on *V. cholerae* showing a typical colony appearance.

Meat extract (Lemco) manufactured by the Liebig Extract of Meat Company was used in a number of formulae in order to avoid the time-consuming preparation of fresh meat extract in the laboratory. The normal use of Lemco was in human nutrition. Teague and Travis[7] incorporated Lemco and Nutrose in a medium containing sucrose and a mixture of eosin and Bismark brown that served to detect fermentation of the sucrose. Goldberger[8] included Lemco in a medium containing whole egg and glucose. Following the extension of the use of Lemco into areas that were not originally intended, the product was modified to make it more suitable for use in bacteriological culture media and it is now extensively used for this purpose under the name Oxoid Lab-Lemco L29.

Few of the early formulations are now used although Dieudonné's alkaline blood medium[9] and Aronson's medium[10] are occasionally employed. The original formulae have undergone many modifications in attempts to improve performance and/or ease of preparation. Brief descriptions of the two media are given in the following pages.

Nearly all the early media were intended for isolating *V. cholerae* from clinical specimens but generally they lacked selectivity or reliable presumptive identification characteristics even for this purpose. Their performance is poor if used to detect low numbers of cells in the environment. Consequently, they have been replaced by better media, nearly all of which are also satisfactory for other pathogenic species of *Vibrio*. Although not all perform well when used in food investigations there are some that can be used with confidence for this purpose. Thiosulphate-citrate-bile-sucrose (TCBS) agar[11] is probably the most commonly used but is not always entirely satisfactory because in some circumstances it is insufficiently selective yet may inhibit some vibrios.

TCBS agar is a modification of a medium devised by Nakanishi[12] which was itself based on Salmonella-Shigella (SS) agar. SS agar was altered by increasing the pH and substituting ox bile for bile salts. Nakanishi used the medium as a replacement for Dieudonné's and Aronson's media which, although suitable for *V. cholerae*, were unsuitable for *V. parahaemolyticus*. Nakanishi's modification of SS agar does not inhibit enterococci. This limitation was overcome in TCBS agar by adding sodium cholate to the bile products already present. The starch and lactose present in Nakanishi's medium were removed, the content of sucrose increased and a more effective pH indicator system allowed sucrose-fermenting vibrios to be detected by the intense yellow colour of colonies. Morris and colleagues[13] working with El Tor *V. cholerae* strains, further enhanced the selectivity of TCBS agar for these organisms by increasing the salt content. Although the modified medium also enabled *V. parahaemolyticus* to grow, classical *V. cholerae* strains were suppressed.

Experience with TCBS agar has shown that performance may be variable because of differences in bile products. Oxidase tests conducted directly on colonies on the medium may give false results and serological tests may be difficult to interpret because colonies do not emulsify easily. Additionally, the medium may allow only poor growth or even no growth of some species of *Vibrio*. These disadvantages may make it necessary to use alternative media.

Monsur gelatin-taurocholate-tellurite (GTT) agar[14] allows oxidase and agglutination tests to be done directly from the medium without difficulty.

Chatterjee[15] found practical difficulties when using GTT agar and formulated a simple alternative to TCBS agar for culturing *V. cholerae*. Greater consistency of performance was achieved in his medium by substituting Teepol for bile. The omission of salt from the formula serves to inhibit halophilic vibrios.

The polymyxin antibiotics are not active against *Vibrio* spp. and have been used by some workers in selective media. Shimada[16] combined polymyxin B with potassium tellurite in polymyxin-mannose-tellurite (PMT) agar which enables *V. cholerae* 01 strains to be differentiated from non-01 strains. Colistin (polymyxin E) and polymyxin B possess similar activity but both are used in cellobiose-polymyxin B-collistin (CPC) agar[17] for selective isolation and identification of *V. vulnificus*. The medium was devised as a more selective alternative to *Vibrio vulnificus* (VV) agar[18].

A modified VV agar was formulated by Miceli with others[19] and given the name *Vibrio vulnificus* enumeration (VVE) agar. The medium is designed for use in a plate-count technique which replaces Most Probable Number (MPN) techniques for enumerating *V. vulnificus* in shellfish and water.

The sulphatase activity of *V. vulnificus* was exploited by Kitaura and colleagues[20] in a medium that differentiates *V. vulnificus* from similar non-sucrose-fermenting vibrios.

Sodium dodecyl sulphate is included as a selective agent.

Recognition of the severity and increasing numbers of *V. vulnificus* infections is driving development of methods and culture media directed specifically towards this species. Danish workers, as part of work not yet completed, have formulated a selective differential medium containing polymyxin and cellobiose for use in a membrane filtration procedure which is part of an intended standard method. (I. Dalsgaard, personal communication to Oxoid Limited.)

Kitaura's medium may also be used to isolate *V. cholerae* and *V. parahaemolyticus*. TCBS and Monsur's medium are reported to perform well for these species[21].
V. parahaemolyticus characteristically is very tolerant of many of the agents used in selective media and is able to thrive at pH values of 8.6 and above.

The robust and halophilic nature of *V. parahaemolyticus* is exploited in media specifically directed towards this species. Bile salts and 2.5% salt are both used in tryptone-soy-triphenyltetrazolium (TSAT) agar[22], a medium designed to differentiate *V. parahaemolyticus* and *V. alginolyticus* by using a combination of sucrose and triphenyltetrazolium that results in distinctively different coloured colonies.

Bromothymol blue-Teepol (BTBT) agar[23] utilises Teepol with 4% salt for selection of *V. parahaemolyticus*. The medium possesses the same system for differentiating species as TCBS agar but fewer species will grow on it.

Many of the media mentioned in this review are of interest now only from a historical perspective that demonstrates the progress made in methods. Even many of the newer formulae are now relatively old, perhaps because of the general usefulness of TCBS agar making further medium development less urgent until circumstances demand it. More recently Beazley and Palmer[24] described a medium (TCI agar) which takes a different approach to the detection of *Vibrio* species as a group by using sodium thiosulphate and potassium iodide as agents to recover vibrios from mixed populations. The major advantage claimed for the medium is the absence of bile salts and other heat-sensitive ingredients that might be adversely affected during preparation. The performance is similar to that of TCBS agar for growth of a range of vibrios and inhibition of normal faecal flora. However, also like TCBS agar, it will not grow all the species known to be pathogenic. An evaluation of TCI agar by independent workers[25] broadly confirmed the advantages claimed for it but drew attention to the quite different size of *Vibrio* colonies compared with that expected on other media. Because of the lack of carbohydrate in the formula, reliable results can be expected from oxidase tests conducted directly on cultures.

The media reviewed all have relatively complex formulae and where laboratory facilities and trained staff are minimal may not be entirely appropriate. In these circumstances, alkaline peptone agar specified by the American Food and Drug Administration[26] may be preferable. The basic formula is given on page 34. The sodium chloride content may be adjusted for growth of a wide range of species.

The following pages contain the formulae and more detailed descriptions of some of the media mentioned in this review.

Table 3 summarises the use, selective systems and diagnostic systems that are used in a number of media.

References

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Table 3 – Principal Selective Differential Agar Media for *Vibrio* species

Medium	Intended use	Salt content %	Selective agents	Diagnostic agents	Reference
Thiosulphate-citrate-bile salt agar (TCBS)	*Vibrio* spp. (not *V. hollisae*)	1	pH 8.6 Na thiosulphate Na taurocholate Ferric citrate	Sucrose	1
Gelatin-taurocholate-tellurite agar (GTT – Monsur)	*Vibrio* spp.	1	pH 8.5 Na taurocholate Pot. tellurite	Pot. tellurite	2
Tryptone-Soya-tri-phenyl tetrazolium agar (TSAT)	*Vibrio parahaemolyticus* *Vibrio* spp.	3	Bile salt	Sucrose Triphenyltetrazolium chloride (TTC)	3
Polymyxin-Mannose-tellurite (PMT) agar	*Vibrio* spp. Differentiation of *V. cholerae* 01 and non-01	1	pH 8.4 Polymyxin B Pot. tellurite Na dodecyl sulphate	Mannose	4
Cellobiose-polymyxin B-Colistin (CPC agar)	*V. vulnificus* *V. cholerae*	2	Polymyxin B Colistin Raised incubation temperature (40°C)	Cellobiose	5
Vibrio vulnificus (VV) agar	*V. vulnificus*	1	pH 8.6 Ox bile; Pot. tellurite Crystal violet	Salicin	6
V. vulnificus enumeration (VVE) agar	*V. vulnificus*	2	pH 8.5 Ox bile Bile salts; Pot. tellurite	Cellobiose Lactose; x-gal	7
Vibrio agar	*V. cholerae* *V. parahaemolyticus*	1	pH 8.5 Ox bile Na deoxycholate Na thiosulphate Na lauryl sulphate Na citrate	Sucrose	8
Sucrose-tellurite-Teepol (STT) agar	*V. cholerae*	Absent	pH 8.0 Teepol*; Pot. tellurite	Sucrose	9
Sodium dodecyl-sulphate-polymyxin-sucrose (SPS) agar	*V. vulnificus*	2	Na dodecyl sulphate	Sucrose sulphatase	10
Thiosulphate-chloride-iodide (TCI) agar	Pathogenic *Vibrio* spp. Not *V. vulnificus*	0.5	Potassium iodide Sodium thiosulphate	Appropriate carbohydrates and phenol red may be added to identify particular species	11
Alkaline peptone agar	*Vibrio* spp.	1–3	pH 8.5		12

*Sodium dodecyl sulphate may be substituted for Teepol.

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Cholera Medium TCBS (TCBS Agar)

Cholera Medium TCBS (TCBS agar)

Code: CM333

A selective isolation medium for pathogenic vibrios.

Formula

	grams/litre
Yeast extract	5.0
Bacteriological peptone	10.0
Sodium thiosulphate	10.0
Sodium citrate	10.0
Ox bile	8.0
Sucrose	20.0
Sodium chloride	10.0
Ferric citrate	1.0
Bromothymol blue	0.04
Thymol blue	0.04
Agar	14.0
Water	1000 ml

pH 8.6 ± 0.2

Directions

Suspend 88 grams in 1 litre of distilled water. Boil to dissolve the medium completely. DO NOT AUTOCLAVE.

Pour plates without further heating and dry before use.

Description

Kobayashi, Enomoto, Sakazaki and Kuwahara[1] developed TCBS medium from the selective isolation agar of Nakanishi[2].

The Oxoid TCBS medium conforms to the formulation of Kobayashi *et al.*, except that it contains specially processed ox bile, free from the defects noted by Nakanishi and Kobayashi.

The complexity of the composition of this medium means that uniformity of growth is a difficult standard to maintain. Several investigations have shown variation between batches of TCBS medium made by different companies[3,4,5,6].

Quality control by the manufacturers of TCBS medium is especially important because satisfactory inhibition of normal gut flora and lack of inhibition of certain *Vibrio* species is very critical. West *et al.*[7] showed that Oxoid TCBS Medium came closest to their criteria for a satisfactory product.

WHO has established a minimum acceptable guideline for the recovery of *Vibrio* species on TCBS medium[8].

The Oxoid medium is suitable for the growth of *Vibrio cholerae*, *V. parahaemolyticus*, and most other vibrios[9].

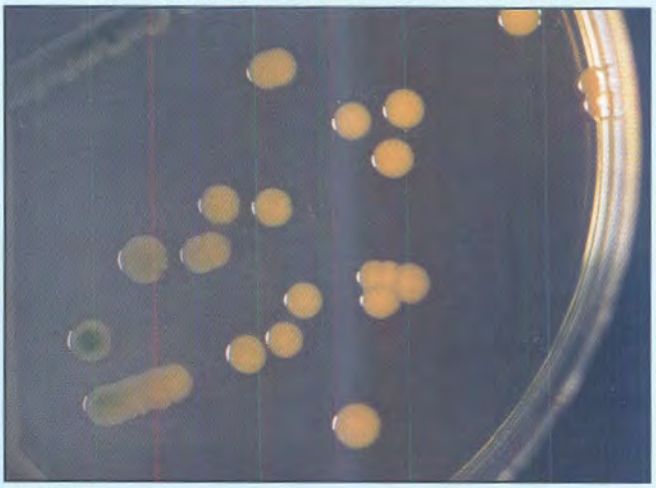
Most of the *Enterobacteriaceae* encountered in faeces are totally suppressed for at least 24 hours. Slight growth of *Proteus* spp. and *Strept. faecalis* may occur but the colonies are easily distinguished from the colonies of *Vibrio* spp.

Morris[10] further increased the selectivity of TCBS agar by increasing the sodium chloride content to 2.5%. This simple modification enhanced inhibition of *Proteus* spp., *Klebsiella* spp. and enterococci without any adverse effect on growth of *V. cholerae* biotype El Tor.

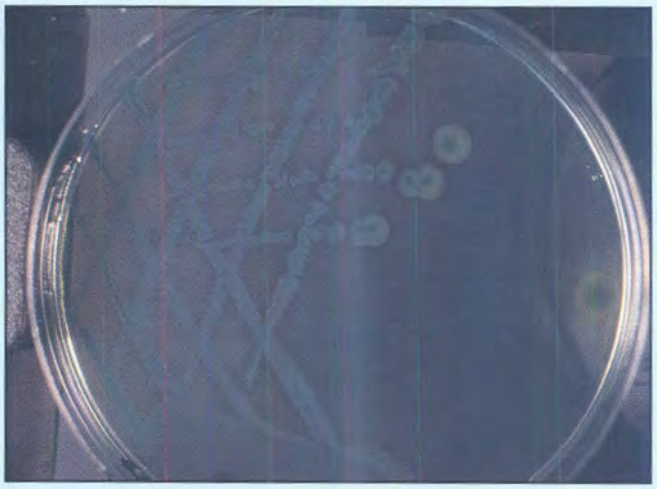
The modified TCBS medium must be tested using appropriate cultures to ensure that other pathogenic vibrios are not inhibited.

Oxoid TCBS cholera medium is complete and requires no additives or aseptic additions of blood. It therefore shows a considerable advantage over lauryl sulphate-tellurite agar which requires further additions after sterilisation. Apart from this convenience factor, it also possesses superior growth characteristics for *Vibrio* species, compared with media containing tellurite. Whilst inhibiting non-vibrios, it promotes rapid growth of pathogenic vibrios after overnight incubation at 35°C. For the isolation of other vibrios from environmental samples, incubation at lower temperatures, commonly 20–30°C, is needed.

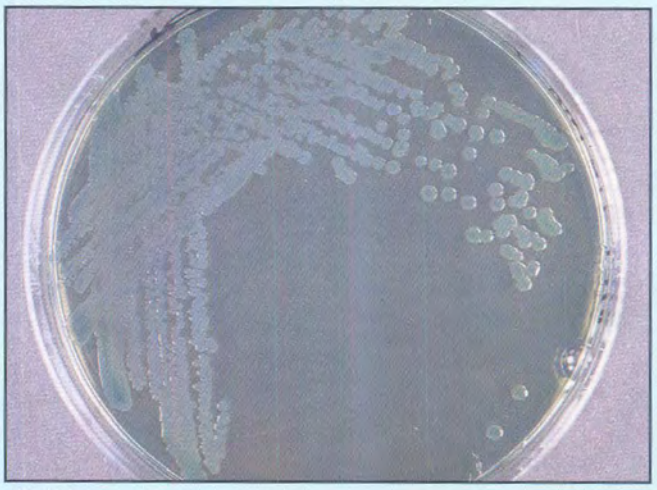
Appearance of some species of *Vibrio* on TCBS agar



V. cholerae



V. parahaemolyticus



V. vulnificus

Colonial appearance of organisms on TCBS Medium

24 hours incubation at 35°C.

Organisms	Colonies
V. cholerae and El Tor 01, 0139, non-01/0139 biovar	Yellow, flat 2–3 mm diameter
V. parahaemolyticus	Blue-green 3–5 mm diameter
V. alginolyticus	Yellow 3–5 mm diameter
V. metschnikovii[11]	Yellow 3–4 mm diameter
V. fluvialis[12]	Yellow 2–3 mm diameter
V. vulnificus[13]	Blue-green 2–3 mm diameter
V. mimicus[14]	Blue-green 2–3 mm diameter
Enterococcus spp.	Yellow 1 mm diameter
Proteus spp.	Yellow-green 1 mm diameter
Pseudomonas spp.	Blue-green 1 mm diameter

Some strains of *Aeromonas hydrophila* grow producing yellow colonies but *Plesiomonas shigelloides* does not usually grow well on TCBS.

Technique

Streak the faeces or a subculture from an enrichment medium, e.g. alkaline peptone water, across the surface of Oxoid TCBS Cholera Medium and incubate for 18–24 hours at 35°C for clinical specimens or lower temperatures for environmental samples.

Cultures grown on TCBS should be examined soon after removal from an incubator as the yellow colonies of cultures of vibrios, e.g. *V. cholerae*, may revert to a green colour when left at room temperature.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive control:
Vibrio furnissii NCTC 11218 (a non-pathogenic strain[6])

Negative control:
Escherichia coli ATCC® 25922

Precautions

The identification of the various *Vibrio* spp. on TCBS medium is presumptive and further tests are required for confirmation.

Yellow colonies on TCBS medium will give unsatisfactory oxidase reactions.

Colonies taken from TCBS medium are 'sticky' and react poorly in slide agglutination tests. Subculture to nutrient agar is required before slide agglutination tests can be carried out.

References

1 Kobayashi, T., Enomoto, S., Sakazaki, R. and Kuwahara, S. (1963) *Jap. J. Bacteriol.* **18,** 10–11, 387–311.
2 Nakanishi, Y. (1963) *Modern Media* **9,** 246.
3 McCormack, W.M., DeWitt, W.E., Bailey, P.E., Morris, G.K., Socharjono, P. and Gangarosa, E.J. (1974) *J. Inf. Dis.* **129,** 497–500.
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10 Morris, G.K., DeWitt, W.E., Gangarosa, E.J. and McCormack, W.M. (1976) *J. Clin. Microbiol.* **4,** 133–136.
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13 Farmer, J.J. (1979) *The Lancet* **2,** 903.
14 Davis, B.R., Fanning, G.R., Madden, J.M., Steigerwall, A.G., Bashford, H.B., Smith, H.L. and Brenner, D.J. (1981) *J. Clin. Microbiol.* **14,** 631–639.

V. alginolyticus

V. hollisae strains generally do not grow on TCBS agar but may be isolated on MacConkey agar CM7 supplemented with salt.

Alkaline Bile Salt-Peptone-Tellurite Agar (Monsur)

Alkaline Bile Salt-Peptone-Tellurite Agar (Monsur)

A selective and differential medium for the isolation of *Vibrio cholerae.*

Formula

	grams/litre	Suggested Oxoid products
Tryptone	10	Tryptone L42
Sodium chloride	10	Sodium chloride L5
Sodium taurocholate	5	
Sodium carbonate	1	
Gelatin	30	Gelatin bacteriological L8
Agar	15	Agar bacteriological L11
Potassium tellurite	0.002	Potassium tellurite SR30
Water	1000 ml	

pH 8.5 ± 0.2

Description

A selective medium containing sodium taurocholate and potassium tellurite was used by Gohar[1] and Gohar and Makkawi[2] for isolation of *V. cholerae.* Smith and Goodner[3] recognised the value of detecting hydrolysis of gelatin by *V. cholerae* and incorporated this presumptive test in their vibrio medium. Monsur[4] combined the selective and differential features of the two media in a formula that enables *V. cholerae* present in heavily contaminated samples to be selected and presumptively identified by the characteristic appearance of colonies, together with the production, due to hydrolysis of gelatin, of halos surrounding the colonies. Incubation at 33°C for 24 hours yields colonies that appear almost flat and are transparent to grey at the centres, becoming almost black due to reduction of the tellurite. Halos are almost always present around the colonies. These characteristics become more pronounced if incubation is continued to 48 hours. Other species of *Vibrio* are able to grow on Monsur medium but the colony appearance is generally different.

Monsur medium has been modified by O'Brien and Colwell[5] so that *V. cholerae* and *V. parahaemolyticus* may easily be differentiated by the action of *V. cholerae* on 4-methylumbelliferyl-β-galactosidase. 4-methylumbelliferone is formed by *V. cholerae* but not by *V. parahaemolyticus.* This may be observed by fluorescence of cultures exposed to ultra-violet light.

Sucrose-fermenting variants of *V. parahaemolyticus* may mistakenly be discarded when growing on TCBS agar but can be detected on the modified Monsur medium. It is advisable that both TCBS agar and Monsur medium should be used in investigations to detect the presence of *V. parahaemolyticus.*

Modified Monsur medium may be useful also for differentiating other species of *Vibrio* when used with tests for gelatinase and tellurite reduction.

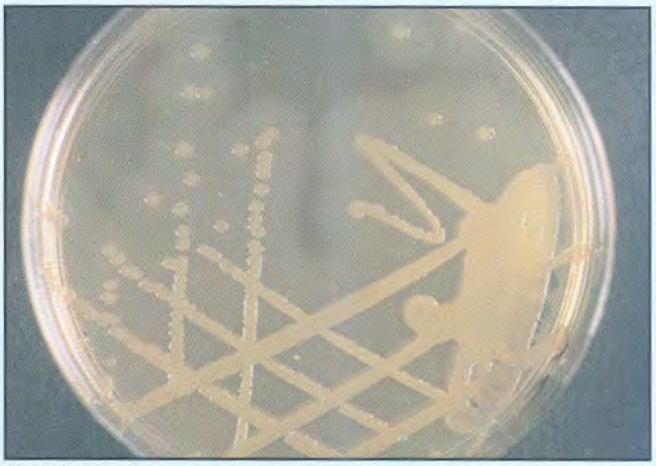
Monsur medium inhibits most non-*Vibrio* enteric organisms, although strains of *Proteus* spp. may produce small colonies. *Pseudomonas* spp. may grow but can usually be recognised by their intense pigmentation.

Furniss, Lee and Donovan[6] reported excellent performance of Monsur medium but cautioned against its occasional use because of the need to become familiar with colony appearance and the extensive quality control necessary to ensure the medium is working effectively.

References

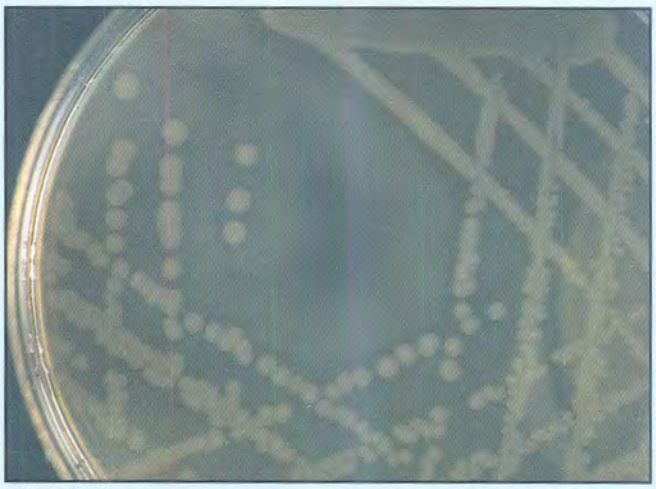
1 Gohar, M.A. (1941) *J. Trop. Med. Hyg.* **44,** 96.
2 Gohar, M.A. and Makkawi, M. (1947) *J. Roy. Egypt. Med. Ass.* **30,** 566.
3 Smith, H.L. Jnr. and Goodner, K. (1988) *J. Bact.* **76,** 662.
4 Monsur, K.A. (1961) *Trans. R. Soc. Trop. Med. Hyg.* **55,** 440–442.
5 O'Brien, M. and Colwell, R. (1985) *J. Clin. Microbiol.* **22,** 1011–1013.
6 Furniss, A.L., Lee, J.V. and Donovan, T.J. (1978) *The Vibrios* Public Health Laboratory Service Monograph Series number 11 page 5. HMSO London.

Appearance of major pathogenic *Vibrio* spp. on Monsur alkaline bile salt-peptone-tellurite agar:



V. cholerae

V. parahaemolyticus



V. vulnificus

Vibrio Agar

Vibrio Agar

A selective differential agar for the detection of *V. cholerae* when used with TCBS agar.

Formula

	grams/ litre	Suggested Oxoid products
Tryptone	4	Tryptone L42
Proteose peptone	3	Proteose peptone L85
Yeast extract	5	Yeast extract L21
Sucrose	20	
Sodium chloride	10	Sodium chloride L5
Sodium citrate: $2H_2O$	10	
Sodium thiosulphate: $5H_2O$	6.5	
Sodium deoxycholate	1	
Desiccated ox bile	5	
Sodium lauryl sulphate	0.2	
Water blue	0.2	
Cresol red	0.02	
Agar	15	Agar bacteriological L11
Water	1000 ml	
pH 8.5 ± 0.2		

Description

Vibrio agar[1] is a modification of a formula originally developed for the isolation of *V. parahaemolyticus*. Its selectivity lies between that of TCBS agar and alkaline bile salt agar.

Vibrio agar was developed to improve the poor inhibition shown by TCBS agar of *Proteus* and *Aeromonas* spp. and enterococci. Additionally, direct testing of colonies from plates of this medium is more satisfactory than direct testing from TCBS agar.

Alkaline bile salt agar is not a differential medium and its use requires a high degree of technical expertise to recognise colonies of *V. cholerae*. The authors recommend that *Vibrio* agar should replace alkaline bile salt agar for use with TCBS agar in the routine examination of faeces for *V. cholerae*. Little work appears to have been done on the usefulness of *Vibrio* agar in examining foods.

References

1 Tamura, K., Shimada, S. and Prescott, L.M. (1971) *Japan. J. Med. Sci. Biol.* **24,** 125–127.

Tellurite-Lauryl Sulphate-Salt Agar (TLS)

Tellurite-Lauryl sulphate-salt agar (TLS)

An alkaline medium for selective isolation of *Vibrio cholerae*.

Formula

	grams/ litre	Suggested Oxoid products
Blood agar base	40	Tryptose blood agar base CM233
Sodium chloride	15	Sodium chloride L5
Sodium carbonate	5	
Sucrose	10	
Sodium lauryl sulphate	0.1	
Water	1000 ml	
1% Potassium tellurite in defibrinated ox (or horse) blood	2 ml	
pH 9.6 ± 0.2		

Description

Alkaline-tellurite-lauryl sulphate-salt agar was formulated by Felsenfeld and Watanabe[1] to provide an improved selective medium for *V. cholerae* which could easily be prepared and used under field conditions. The existing media which depended solely on alkalinity for their selective activity, whilst adequately supporting growth of *V. cholerae*, were insufficiently inhibitory towards other enteric organisms. *Proteus* spp. and pseudomonads in particular were seen to spread and obscure colonies of *V. cholerae*. Additionally, there were difficulties for serological investigation of isolates because of the sharing of antigens by *V. cholerae* and some species of contaminating organisms.

Tryptose was chosen as a source of nitrogen because it particularly favours the growth of vibrios[2]. Potassium tellurite was included because of its successful use in a fluid enrichment medium by Gohar and Makkawi[3] and sodium lauryl sulphate because of its common use in liquid media for detection of coliforms. Sodium chloride was included at higher than usual concentrations because of the greater tolerance of vibrios towards it.

The medium included sodium carbonate to raise the pH and further enhance selectivity.

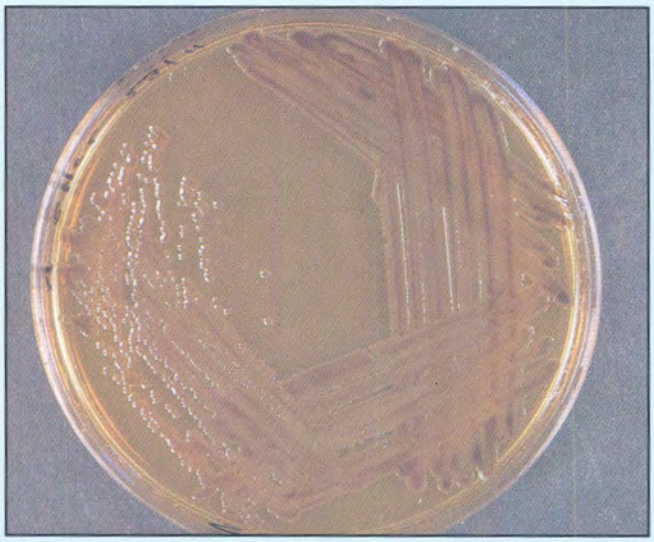
Tellurite-lauryl sulphate-salt agar is not entirely selective for *V. cholerae* at concentrations of potassium tellurite and sodium lauryl sulphate which do not adversely effect its growth. However, the grey colour of *V. cholerae* colonies due to reduction of potassium tellurite aids recognition of them when present in mixed culture.

For routine use the authors recommend that the medium should be used in conjunction with a nutrient medium of high pH and/or Aronson's medium[4].

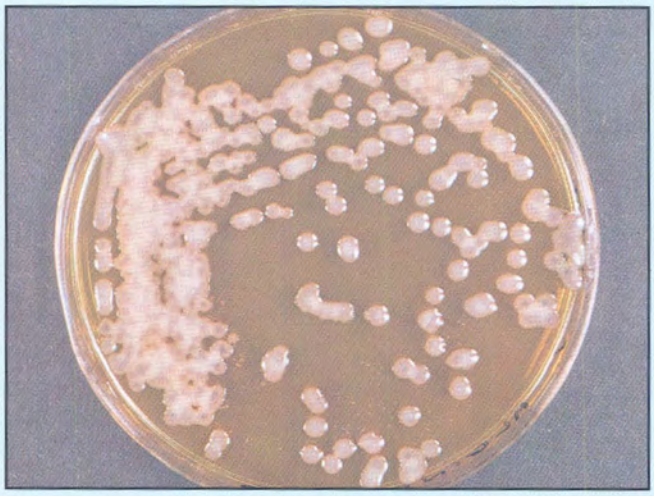
References

1 Felsenfeld, O. and Watanabe, Y. (1958) *United States Armed Forces Med. J.* **9,** 975–982.
2 Felsenfeld, O., Soman, D.W., Waters, T. and Ishihara, S.J. (1951) *J. Bact.* **62,** 175–180.
3 Gohar, M.A. and Makkawi, M. (1948) *J. Trop. Med.* **51,** 95–99.
4 Cited in Mackie and McCartney's *Handbook of Bacteriology*. Cruickshank (ed.) Tenth edition 1960. E. and S. Livingstone, Edinburgh.

Sucrose-Tellurite-Teepol (STT) Agar



Appearance of *V. cholerae* on TLS agar



Other species of *Vibrio* may grow on this medium e.g. *V. alginolyticus*

See page 25 for a description of TLS agar.

Sucrose-Tellurite-Teepol* (STT) Agar

A medium for *V. cholerae*.

Formula

	grams/ litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Meat extract	10	Lab-Lemco L29
Sodium chloride	5	Sodium chloride L5
Sucrose	10	
Potassium tellurite	0.005	
Bromothymol blue	0.05	
Agar	20	Agar bacteriological L11
Teepol*	2 ml	
Water	1000 ml	
pH 8.0		

Description

STT agar[1,2] is a selective differential medium in which Teepol replaces bile salts and the relatively low concentration of sucrose is claimed to permit oxidase testing directly from the medium. Colonies of *V. cholerae* from STT agar are easily emulsified, permitting slide agglutination tests to be performed without difficulty directly from the plate. In a comparative study[3] STT agar showed poorer selectivity than either TCBS agar or Monsur taurocholate-tellurite-gelatin agar. Although STT agar performed well for direct agglutination tests its reliability for direct oxidase testing could not be confirmed.

V. cholerae grows as yellow colonies and *V. parahaemolyticus* as blue-green colonies on STT agar.

References

1 Chatterjee, B.D., De, P.K. and Sen, T. (1977) *J. Infect. Dis.* **136,** 654–658.
2 Chatterjee, B.D., De, P.K. and Sen, T. (1977) *J. Infect. Dis.* **136,** 716: Erratum.
3 Morris, G.K., Merson, M.H., Huq, I. *et al.* (1979) *J. Clin. Microbiol.* **9,** 79–83.

**Sodium dodecyl sulphate may be substituted for Teepol.*

Polymyxin-Mannose-Tellurite (PMT) Agar

Polymyxin-Mannose-Tellurite (PMT) Agar

A selective medium for the differentiation of colonies of *Vibrio cholerae* 01 from those of *V. cholerae* non-01.

Formula

	grams/ litre	Suggested Oxoid products
Meat extract	5	Lab-Lemco L29
Mixed peptone	10	Peptone special L72
Sodium chloride	10	Sodium chloride L5
Mannose	20	
Sodium dodecyl sulphate	0.2	
Cresol red	0.04	
Bromothymol blue	0.04	
Agar	15	Agar bacteriological L11
Polymyxin B	180,000 i.u.	
Water	1000 ml	
pH. 8.4 ± 0.2		

Description

PMT agar was formulated by Shimada[1] *et al.* for use as an alternative to TCBS agar which may perform poorly when used in the detection of *Vibrio cholerae* 01 in seafood and water samples. Recognition of 01 strains on TCBS agar may be hindered in the presence of non-01 strains which are frequently present in much greater numbers. Colonies of 01 strains on PMT agar have a different colour from those of non-01 strains. 01 strains ferment mannose and form yellow colonies whilst the majority of non-01 strains do not ferment mannose and the colonies have a violet-purple appearance.

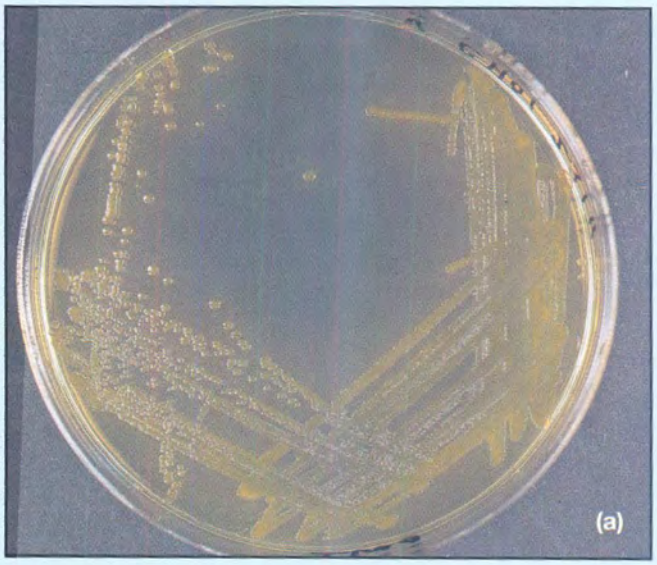
Colonies of *V. cholerae* 01 on PMT agar are larger than those that develop on TCBS agar and are reported to agglutinate more readily with 01 antiserum.

Despite the advantage of differentiating 01 and non-01 strains by differences in colony colour, checks of identity of purple colonies must still be carried out because some non-01 strains are able to ferment mannose. However, colour difference significantly reduces the number of colonies that require checking.

The authors recommend that both PMT agar and TCBS agar should be used when attempting to isolate *V. cholerae* from seafoods and water samples.

Reference

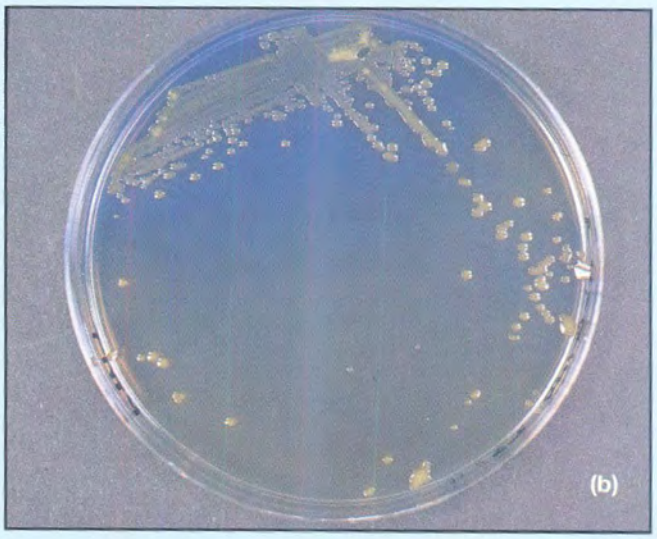
1 Shimada, T., Sakazaki, R., Fujimura, S. *et al.* (1990) *Jpn. J. Med. Sci. Biol.* **43,** 38–41.



Appearance of *V. cholerae* 01 on PMT agar (a).

Non-01 strains do not ferment mannose and the colonies, although similar, are violet-purple in colour.

Other species of *Vibrio* are able to grow on this medium, e.g. *V. alginolyticus* (b).



Vibrio vulnificus (VV) Agar

***Vibrio vulnificus* (VV) Agar**

An elective medium for improved growth of *Vibrio vulnificus*.

Formula

	grams/ litre	Suggested Oxoid products
Peptone	2	Peptone Bacteriological L37
Acid hydrolysed casein	0.5	Casein hydrolysate (Acid) L41
Ox bile	8	
Sodium chloride	10	Sodium chloride L5
Magnesium chloride $6H_2O$	2	
Potassium chloride	1	
Salicin	20	
Crystal violet	0.0015	
Potassium tellurite	0.0025	
Agar	15	Agar bacteriological L11
Tween 80 10% v/v aqueous	5 ml	
Water	1000 ml	

pH 8.6 ± 0.2

Description

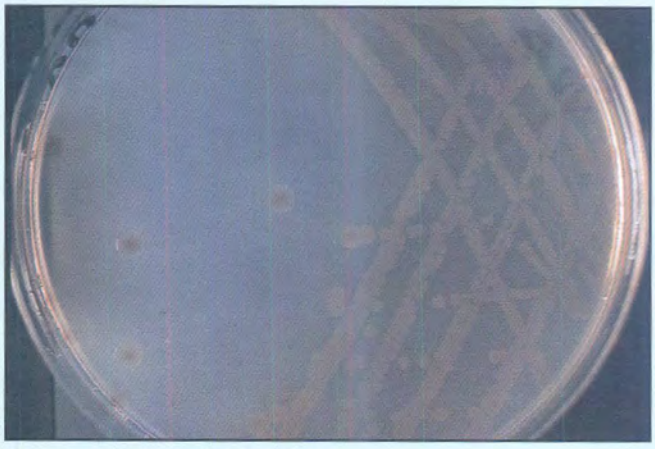
VV agar was formulated by Brayton and co-workers[1] as an alternative to TCBS agar which produces improved growth of *Vibrio vulnificus*. The formula of TCBS agar was taken as the starting point and made more suitable for this species by modifying the composition of the nutrients and inhibitory agents contained in TCBS agar. Salicin is included as the major source of carbon and only strains of *Vibrio* species that are capable of utilising salicin grow well on VV agar. Selection of *V. vulnificus* from a wide range of Gram-negative flora (including other *Vibrio* spp.) and Gram-positive flora is achieved using a high pH, bile, potassium tellurite and crystal violet. Tween 80 is present as a secondary source of carbon and, by reducing surface tension, assists the uptake of other nutrients. Reduction of the total nutrient content in TCBS agar by lowering the quantity of peptone and substituting acid-hydrolysed casein for yeast extract improved the growth of *V. vulnificus*. The concentration of sodium chloride was optimised for this halophilic species and addition of magnesium chloride and potassium chloride increased colony size.

Although salicin is utilised by *V. vulnificus*, fermentation by this species is weak and little or no colour change occurs in pH indicator dyes and consequently none were included. In addition to its inhibitory activity against Gram-positive organisms, crystal violet is present in the medium to impart a bluish-green tint found useful in aiding colony recognition.

After 24 hours incubation at 35°C, growth of *V. vulnificus* appears as large, light-grey, translucent colonies, raised and with a dark-grey or black centre caused by reduction of potassium tellurite. Colonies other than *V. vulnificus* appear opaque, pinpoint and fail to fix tellurite.

Reference

1 Brayton, P.R., West, P.A., Russek, E. and Colwell, R.R. (1983) *J. Clin. Microbiol.* **17,** 1039–1044.



Appearance of *V. vulnificus* on VV agar

Vibrio vulnificus Enumeration (VVE) Agar

***Vibrio vulnificus* Enumeration (VVE) Agar**

Formula

	grams/ litre	Suggested Oxoid products
Tryptose	10	Tryptose L47
Yeast extract	5	Yeast extract L21
Sodium chloride	20	Sodium chloride L5
Ox bile	1.0	
K_2HPO_4	1.0	
KH_2PO_4	1.0	
$MgSO_4 : 7H_2O$	0.1	
Cellobiose	5	
Lactose	0.1	
Agar	15	Agar bacteriological L11
Water	1000 ml	
pH 8.5 ± 0.2		

Supplementation for 1 litre of medium to be added following heat sterilisation of the medium base:

	gram
Sodium cholate	1.0
Sodium taurocholate	1.0
Potassium tellurite	0.002
Fe $Cl_3 : 7H_2O$	0.1
5-bromo-4-chloroindoxyl-β-D-galactopyranoside (x-gal)	0.1

The paper by Miceli, Watkins and Rippey[1] should be consulted for full details of preparation of the medium. The correct colour of cooled VVE medium is golden tan.

Description

VVE medium was developed by Miceli, Watkins and Rippey[1] for use in a procedure for enumerating *V. vulnificus* in oysters. Experimental work had shown that existing selective media allowed unacceptably low recovery levels. A satisfactory plating medium was desirable to use as an alternative to Most Probable Number (MPN) techniques which are relatively imprecise and need time-consuming measures to confirm and differentiate the organisms present in presumptive-positive tube tests.

Because much is still unknown about the significance of the number of *V. vulnificus* present in shellfish, accurate determination is necessary so that effective public health measures can be devised. Suspected *V. vulnificus* colonies on VVE medium are subcultured to a modified CSPS agar[2] as part of a biochemical identification procedure which confirms *V. vulnificus* by fermentation of cellobiose, positive oxidase test and positive tests for ornithine and lysine decarboxylase.

The scheme used to isolate and identify *V. vulnificus* is shown in Figure 1. VVE medium was found to be very productive, results showing recoveries of 92% of the level obtained on brain-heart infusion agar supplemented with 1% sodium chloride. Because VVE medium is only moderately selective, false-positive colonies must be expected and it is essential that presumed positive isolates are further tested to confirm their identity.

References

1 Miceli, G.A., Watkins, W.D. and Rippey, S.R. (1993) *Appl. Env. Microbiol.* **59,** 3519–3524.

2 Kaysner, C.A. and Tamplin, M.A. (1988) Isolation and identification of *V. vulnificus*. p. 42–59. In *Proceedings of the Workshop on Vibrio vulnificus and sanitary control of shellfish*. F.D.A. Washington D.C.

Figure 1 – Procedure for isolation and enumeration of *V. vulnificus* using VVE medium

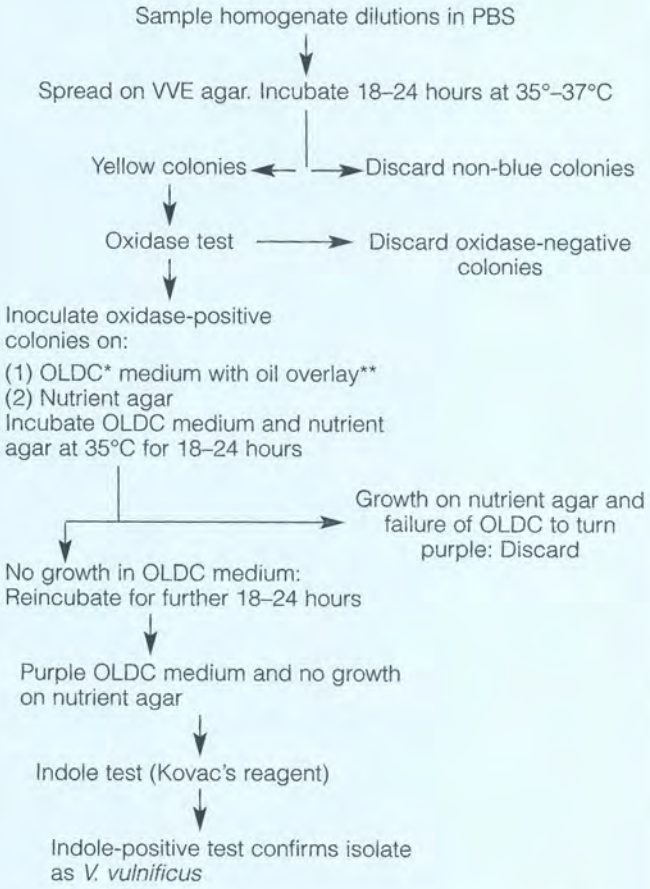


Figure modified from Miceli, G.A., Watkins, W.D. and Rippey, S.R. (1993) *Appl. Env. Microbiol.* **59,** 3519–3524.

*OLDC medium

Formula	grams/ litre	Suggested Oxoid products
Decarboxylase medium base (Moeller)	10.5	
L-ornithine	5.2	
L-lysine	5.2	
Sodium chloride	10	Sodium chloride L5
Water	1000 ml	
pH 6.0		

Sterilise by autoclaving.

** In use, pour sterile oil onto the medium immediately after inoculation.

Modified CSPS medium[2]

Formula	grams/ litre	Suggested Oxoid products
Proteose peptone	10	Proteose peptone L85
Meat extract	5	Lab-Lemco L29
Sodium chloride	20	Sodium chloride L5
Cellobiose	15	
Bromothymol blue	0.04	
Cresol red	0.04	
Agar	15	Agar bacteriological L11
pH 7.6		

Sterilise by autoclaving.

The correct colour of modified CSPS agar is blue to purple.

Cellobiose-Polymyxin B-Colistin Agar (CPC Agar)

Cellobiose-Polymyxin B-Colistin Agar (CPC agar)

A medium with enhanced selectivity for *Vibrio cholerae* and *V. vulnificus*.

Formula

	grams/ litre	Suggested Oxoid products
Solution 1		
Peptone	10	Peptone bacteriological L37
Meat extract	5	Lab-Lemco L29
Sodium chloride	20	Sodium chloride L5
Bromothymol blue	0.04	
Cresol red	0.04	
Agar	15	Agar bacteriological L11
Water	900 ml	
pH 7.6 ± 0.2		

Sterilise solution 1 by autoclaving at 121°C for 15 minutes.

Solution 2	
Cellobiose	15
Colistin	1,360,000 i.u.
Polymyxin B	100,000 i.u.
Water	100 ml

Filter-sterilise solution 2 using a 0.22 µ membrane filter.

Directions

Cool medium to 55°C.

Add solution 2 to solution 1, mix and pour into petri dishes.

When cool, the medium should be olive-green to light-brown in colour.

Description

Cellobiose-polymyxin B-colistin agar (CPC agar) was developed by Massad and Oliver[1] as a more satisfactory alternative to TCBS and VV agars when the primary requirement is the selection of *V. cholerae* and *V. vulnificus* from other members of the genus in the examination of natural environmental sources such as estuarine waters and edible shellfish.

The medium is very selective for *V. cholerae* and *V. vulnificus*. During development it was seen that, with the exception of one strain of *V. parahaemolyticus*, all other *Vibrio* species failed to grow. Marine *Pseudomonas* spp., *Photobacterium* spp. and *Flavobacterium* spp. are effectively inhibited.

Selectivity is based on the resistance of *V. cholerae* and *V. vulnificus* to the antibiotics colistin and polymyxin B. Incubation at 40°C enhances selectivity; at 37° some strains of *V. parahaemolyticus* and other *Vibrio* spp. are able to grow.

V. cholerae and *V. vulnificus* are differentiated on CPC agar by the inability of *V. cholerae* to ferment cellobiose. Colonies of *V. cholerae* are purple, surrounded by blue zones and those of *V. vulnificus*, which is able to ferment cellobiose, are yellow after 24–48 hours incubation.

While it may be advantageous in special circumstances to incubate CPC agar at 37° to detect *V. parahaemolyticus*, caution must be exercised in presumptively identifying species by the colour of the colonies they form. Some strains of *V. parahaemolyticus* are able to ferment cellobiose, making the presumptive distinction between *V. cholerae* and *V. vulnificus* less reliable.

CPC agar performance compares well with thiosulphate-citrate-bile salts-sucrose (TCBS) agar[2]. Although *V. vulnificus* recovers less well than *V. cholerae* on CPC agar, CPC agar is superior to TCBS agar for *V. vulnificus*.

Precautions

CPC agar must be used freshly prepared because the medium becomes more inhibitory on ageing, especially for *V. cholerae*.

References

1 Massad, G. and Oliver, J.D. (1987) *Appl. Env. Microbiol.* **53,** 2262–2264.

2 Bryant, R.G., Jarvis, J. and Janda, J.M. *Abstr. 20 Ann. Meeting Am. Soc. Microbiol.* 1987. Page 277.

Modified Cellobiose-Polymyxin B-Colistin Agar (mCPC)

Modified Cellobiose-Polymyxin B-Colistin Agar (mCPC)

Formula

	grams/ litre	Suggested Oxoid products
Solution 1		
Peptone	10	Peptone bacteriological L37
Meat extract	5	Lab-Lemco L29
Sodium chloride	20	Sodium chloride L5
Dye stock solution*	1 ml	
Agar	15	Agar bacteriological L11
Water	900 ml	
pH 7.6		
Solution 2		
Cellobiose	10	
Colistin	400,000 i.u.	
Polymyxin B	100,000 i.u.	
Distilled water	100 ml	

Sterilise by filtration. Add solution 2 to cooled solution 1. Mix and pour into petri dishes.

*Dye stock solution

Bromothymol blue	4 gm
Cresol red	4 gm
Ethanol 95%	100 ml

Description

This formula conforms to modified CPC agar specified in the U.S. F.D.A. Bacteriological Analytical Manual. The modified medium contains an increased quantity of colistin.

Reference

Food and Drug Administration *Bacteriological Analytical Manual.* Chapter 9 8th edition 1995.

Sodium Dodecyl Sulphate-Polymyxin B-Sucrose Agar (SPS)

Sodium Dodecyl Sulphate-Polymyxin B-Sucrose Agar (SPS)

A medium for detection of sulphatase activity by *V. vulnificus* and *V. cholerae* in environmental surveillance.

Formula

	grams/ litre	Suggested Oxoid products
Proteose peptone	10	Proteose peptone L46
Meat extract	5	Lab-Lemco L29
Sodium chloride	20	Sodium chloride L5
Sucrose	15	
Sodium dodecyl sulphate	1	
Bromothymol blue	0.04	
Cresol red	0.04	
Agar	15	Agar bacteriological L11
Polymyxin B	100,000 i.u.	
Water	1,000 ml	
pH 7.6		

Description

SPS agar is used following selective enrichment in starch-gelatin-polymyxin B broth (see page 17) for the detection of alkylsulphatase activity by demonstrating halo production around colonies of *V. vulnificus* and *V. cholerae* present in shellfish, sea and estuarine waters. The use of this combination of media is reported to be superior to a combination of alkaline polymyxin B broth and TCBS agar.

For environmental surveillance of *V. cholerae* 01, sodium chloride is omitted from the formula when examining seawater samples to prevent the growth of halophilic, sulphatase-positive marine vibrios including *V. anguillarum.*

The factors controlling the production of alkylsulphatases are complex and failure to produce a halo on SPS agar does not necessarily indicate the inability of a strain to produce the enzyme. The test protocol described by Kitaura *et al.* must be strictly followed to maximise positive results.

Reference

Kitaura, T., Doke, S., Azuma, I. *et al.* (1983) *FEMS Microbiology Lett.* **17,** 205–209.

Vibrio Parahaemolyticus (VP) Agar

Vibrio parahaemolyticus (VP) agar

A selective differential medium for *V. parahaemolyticus*, suitable also for *V. cholerae*.

Formula

	grams/ litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Yeast extract	5	Yeast extract L21
Sodium chloride	20	Sodium chloride L5
Sucrose	20	
Sodium citrate	10	
Sodium thiosulphate	10	
Sodium taurocholate	5	
Sodium lauryl sulphate	2	
Bromothymol blue	0.04	
Thymol blue	0.04	
Agar	20	Agar bacteriological L11
Water	1000 ml	
pH 8.6 ± 0.2		

Description

VP agar[1] was originally formulated as an inexpensive, easily prepared medium for *V. parahaemolyticus* but subsequently has been found to be suitable for isolating *V. cholerae*[2]. On VP agar *V. cholerae* and *V. parahaemolyticus* may readily be differentiated by the yellow colour of *V. cholerae* colonies due to sucrose fermentation. Colonies of *V. parahaemolyticus* are blue-green.

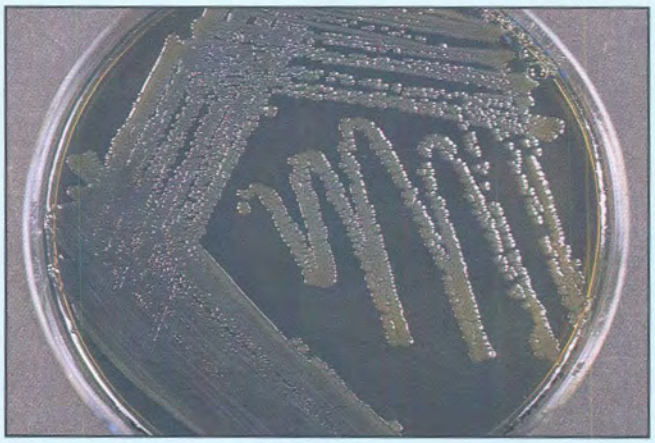
VP agar is unsuitable for oxidase testing directly from the medium.

Colonies taken directly from VP agar do not emulsify readily, making it unsatisfactory for direct serological testing.

References

1 De, S.P., Sen D., De, P.C. *et al.* (1977) *Ind. J. Med. Res.* **66,** 398–399.

2 Morris, G.K., Merson, M.H., Huq, I. *et al.* (1979) *J. Clin. Microbiol.* **9,** 79–83.



Appearance of *V. parahaemolyticus* on VP agar

Tryptone-Soya-Tetrazolium (TSAT) Agar

Tryptone-Soya-Tetrazolium (TSAT) Agar

A selective medium that differentiates *Vibrio parahaemolyticus* from *V. alginolyticus*.

Formula

	grams/ litre	Suggested Oxoid products
Tryptone soya agar	40	Tryptone soya agar CM131
Sodium chloride	25	Sodium chloride for L5
Sucrose	20	
Bile salts number 3	0.5	Bile salts number 3 L56
1% aqueous triphenyl-tetrazolium chloride	3 ml	
Water	1000 ml	
pH 7.1		

Description

TSAT agar was formulated by Kourany to assist the detection of *Vibrio parahaemolyticus* when present with large numbers of *V. alginolyticus* in seawater. The usefulness of TCBS agar in these circumstances is severely limited because the usual blue-green colony colour of *V. parahaemolyticus* on this medium may be altered to yellow due to fermentation of sucrose by adjacent *V. alginolyticus* colonies causing both species to look the same.

Triphenyltetrazolium chloride in TSAT agar causes a marked difference in the colony appearance of the two species. *V. parahaemolyticus* colonies are generally 3–4 mm diameter and dark-red in colour. *V. alginolyticus* colonies have a diameter of 2–3 mm and are white, sometimes with a pin-point pink centre.

Enteric bacteria resistant to the bile in the medium, with the exception of *Proteus* spp., are readily distinguished from *V. parahaemolyticus* by colony appearance. *Proteus* spp. may be distinguished from *V. parahaemolyticus* by their biochemical reactions on triple sugar-iron agar.

Non-vibrio halophilic bacteria may grow on TSAT agar, producing white or pinkish colonies of less than 0.5 mm diameter.

Reference

Kourany, M. (1983) *Appl. Env. Microbiol.* **45,** 310–312.

Mannitol-Maltose Agar

Mannitol-Maltose Agar

Formula

	grams/ litre	Suggested Oxoid products
Soya peptone	5	Soya peptone L44
Mixed peptones	5	Special peptone L72
Meat extract	5	Lab-Lemco L29
Mannitol	10	
Maltose	10	
Sodium chloride	20	Sodium chloride L5
Dye solution	1 ml	
Agar	13	Agar bacteriological L11
Water	1000 ml	
pH 7.8 ± 0.2		
Dye Solution		
Bromothymol blue	4 gm	
Cresol red	4 gm	
Ethanol 95%	100 ml	

Description

This medium is suitable for isolation of *V. hollisae* which is unable to grow on TCBS agar.

Reference

Nishibuchi, M., Doke, S., Toizumi, S., *et al.* (1988) *Appl. Env. Microbiol.* **54,** 2144–2146.

Thiosulphate-Chloride-Iodide (TCI) Agar

Thiosulphate-Chloride-Iodide (TCI) Agar

A selective medium for the recovery of pathogenic *Vibrio* spp.

Formula

	grams/ litre	Suggested Oxoid products
Columbia agar base	39	Columbia agar base CM331
Potassium iodide	46	
Sodium chloride	5	Sodium chloride L5
Sodium thiosulphate	5	
Water	1000 ml	
pH 7.2 ± 0.2		

Description

TCI agar was formulated by Beazley and Palmer[1] to overcome difficulties that may be encountered with TCBS agar due to the presence of bile in the formula and the inhibitory activity this may show towards pathogenic *Vibrio* species other than *V. cholerae.* An evaluation of TCI medium[2] broadly confirmed the findings of the authors but observed that it is unsuitable for *V. vulnificus* and, like TCBS agar, for *V. hollisae.* Performance with *V. mimicus* and *V. alginolyticus* is similar to that on TCBS agar.

Unusually for media formulated for detection of vibrios, TCI agar is not alkaline. The principal inhibitory agent is potassium iodide. The medium is a simple solution of salts in Columbia agar base and is easily made. The total sodium chloride content is suitable for halophilic species and has been set at 1% to avoid inhibition of *V. cholerae.* TCI agar does not contain carbohydrate and tests for oxidase can be conducted directly from the medium. However, TCI agar may also be used as a differential medium by incorporating a suitable carbohydrate for a particular *Vibrio* species and phenol red indicator. Direct oxidase testing may show false-negative results on this modification to the medium. Serological tests may be done directly from TCI agar. The medium is not heat-sensitive and is sterilised by autoclaving.

The potassium iodide in TCI agar inhibits Gram-negative flora including *Pseudomonas* spp. and *Aeromonas* spp. *Enterobacteriaceae* are inhibited with the exception of some strains of *Proteus* spp.

Gram-positive flora are inhibited by sodium thiosulphate although there may be some growth of enterococci and *Bacillus* spp.

Appearance of *V. cholerae* on TCI agar

Alkaline Peptone Agar

Precautions and limitation[2]

Colony size of vibrios on TCI agar is much smaller than on TCBS agar. A Gram-stain must be performed on any oxidase-positive colony showing a typical appearance for *Vibrio* spp. to eliminate the possibility of it being a species of *Bacillus*. The lack of sucrose in TCI agar prevents the separation of *Vibrio* spp. into the two major groups based on fermentation of this carbohydrate, thus removing a valuable identification feature from the primary culture.

References

1 Beazley, W.A. and Palmer, G.G. (1992) *Australian J. Med. Sci.* **13,** 25–27.

2 Abbott, S.L., Cheung, W.W.K.W. and Janda, J.M. (1993) *Med. Microbiol. Lett.* **2,** 362–370.

Alkaline Peptone Agar

Formula

	grams/ litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Sodium chloride	20	Sodium chloride L5
Agar	15	Agar bacteriological L11
Water	1000 ml	
pH 8.5 ± 0.2		

Description

A simple, non-differential, selective medium for growth of *Vibrio* spp. Adjustment of the sodium chloride content up to 3% makes the medium suitable for a wide range of halophilic species.

Reference

Elliot, E.L., Kaysner, C.A., Jackson, L. and Tamplin, M.L. (1998) Culture medium formula M8 in Revision A, of F.D.A. *Bacteriological Analytical Manual* 8th edition 1991. A.O.A.C. International, Gaithersburg, M.D.

Dieudonné's Alkaline Blood Agar

Dieudonné's Alkaline Blood Agar

Constituents

		Suggested Oxoid products
Nutrient agar	700 ml	Nutrient Agar CM3
Defibrinated ox blood	150 ml	
IN potassium or sodium hydroxide	150 ml	

Description

This medium[1] is made by adding a mixture of equal parts of ox blood and potassium or sodium hydroxide to the nutrient medium base. Time to allow ageing of the blood alkali medium is required otherwise *V. cholerae* may fail to grow.

The medium is now almost redundant. Details for preparation of the blood-alkali mixture are given in Mackie and McCartney's Handbook of Bacteriology[2].

References

1 Dieudonné, A. (1909) Blutalkliagar ein Elektivnahrboden für cholera vibrionen. *Centr. F. Bakt.* **50,** 107–108.
2 Dieudonné's Blood-Alkali Agar p. 225. Mackie and McCartney's *Handbook of Bacteriology*, tenth edition. Cruickshank, R. (ed). E. and S. Livingstone, Edinburgh (1960).

Aronson's Fuchsin-Sulphite Agar

Aronson's Fuchsin-Sulphite Agar

A selective differential medium for the detection of *Vibrio cholerae*.

Formula

	grams/ litre	Suggested Oxoid products
Peptone	10	Peptone bacteriological L37
Meat extract	10	Lab-Lemco L29
Sodium chloride	5	Sodium chloride L5
Agar	35	Agar bacteriological L11
Sodium carbonate, 10% solution	60 ml	
Sucrose, 20% solution	50 ml	
Glucose, 20% solution	50 ml	
Sodium sulphite, 10% solution	20 ml	
Basic fuchsin, saturated solution	4 ml	
Water	1000 ml	
pH not stated		

Description

Aronson[1] formulated his medium as shown above, adding a number of the constituents as concentrated solutions thus increasing the volume of water from 1 litre to 1174 ml. The medium was subsequently altered by others. Harvey[2] and Klimmer[3] substituted dextrin for glucose but later workers retained glucose[4].

A heavy precipitate forms during the preparation of Aronson's medium; this must be left behind when pouring plates. Correctly poured medium should be clear and yellowish-brown in colour.

V. cholerae grows as large red colonies, visible in 12 to 24 hours. Growth of *Enterobacteriaceae* is inhibited but there may be some growth which shows as small pink colonies.

Furniss, Lee and Donovan[5] observed that Aronson's medium is less satisfactory for the growth of other *Vibrio* species than it is for *V. cholerae* and, because of inconsistent performance, were unable to recommend its use.

References

1 Aronson, H. (1915) *Deutsche med. Wchnschs.* **41,** 1027–1029.
2 Harvey, W.F. (1921) Bacteriology and Laboratory Technique, Section II *Ind. J. Med. Res.* **9,** 66–131.
3 Klimmer, M. (1923) *Technik und Methodik der Bakteriologie und Serologie.* Verlag von Julius Springer. Berlin.
4 Aronson's medium. P221. Mackie and McCartney's *Handbook of Bacteriology* 10th ed. Cruickshank, R. (ed) (1960) Livingstone, Edinburgh.
5 Furniss, A.L., Lee, J.V. and Donovan, T.J. (1978) *The Vibrios.* Public Health Laboratory Service Monograph Series number 11. HMSO London.

Some Tests used in the Identification of *Vibrio* species

Some Tests used in the Identification of *Vibrio* species

Colony appearance

Luxuriant growth on the highly selective culture media formulated for *Vibrio* spp. is the first indication that an isolate is a member of the genus. *Vibrio* colonies on TCBS agar are from 2 to 5 mm diameter but can vary in appearance and more than one type may be present in a culture. The colour of the colony is an initial indication of a species; species are divided into two major groups: those that ferment sucrose and form yellow colonies and those that do not and consequently the colonies retain the blue-green colour of the medium. The colour of colonies produced by different species is given in the description of TCBS agar on page 22. Information about the appearance of colonies produced on other media described in this monograph may be found on the appropriate page or, if required, the original publication should be read.

Microscopic appearance (see page 2)

Vibrios appear by Gram-stain as short Gram-negative rods. The curvature of the rods typical of vibrios may sometimes be absent and should not be relied upon as a diagnostic feature.

Wet preparations of *Vibrio* cultures are usually motile but motility may be absent or poor in strains grown under sub-optimal conditions. When present, the motility has a characteristic appearance.

Requirement for salt

The necessity for increased levels of salt in culture media is a key feature in identification because it distinguishes between two major groups known as halophilic and non-halophilic vibrios. Non-halophilic species are able to grow even when sodium chloride is absent whereas the halophilic species always require salt and very high concentrations are tolerated by some. The requirement for salt is probably most conveniently tested by culturing on CLED agar which is free of sodium chloride. Halophiles fail to grow on this medium. Tolerance of the different species towards a range of salt concentrations can be used as an identifying marker and may be determined by inoculating peptone water (most usefully, made from tryptone) containing sodium chloride concentrations of 0% to 10%.

Almost all diagnostic culture media require additional salt when used for identification of the species of *Vibrio*.

Oxidase test

Species of *Vibrio* that require only low quantities of salt may grow on the usual laboratory media for *Enterobacteriaceae*. Consequently the oxidase test is a key one to distinguish *Vibrionaceae* from other enteric pathogens.

Oxidase tests must not be conducted directly on media containing fermentable carbohydrates. Yellow colonies taken from TCBS agar give unreliable results and colonies should be subcultured to a carbohydrate-free medium before the test is done. Because of the inevitable delay in identification, faster procedures have been developed[1]. However, these tend to be technically laborious and still require a minimum time of 3 hours for growth to occur in a medium lacking carbohydrate. Further progress has been made in minimising the time required with the development of a 1-minute test in which colonies taken from TCBS agar are suspended in TRIS-HCl buffer in a tube and Kovac's reagent added to the contents[2].

Positive (left) and negative (right) oxidase tests using oxidase touch sticks BR64

Susceptibility to 0129 (2, 4-diamino-6,7-di-isopropyl-pteridine)

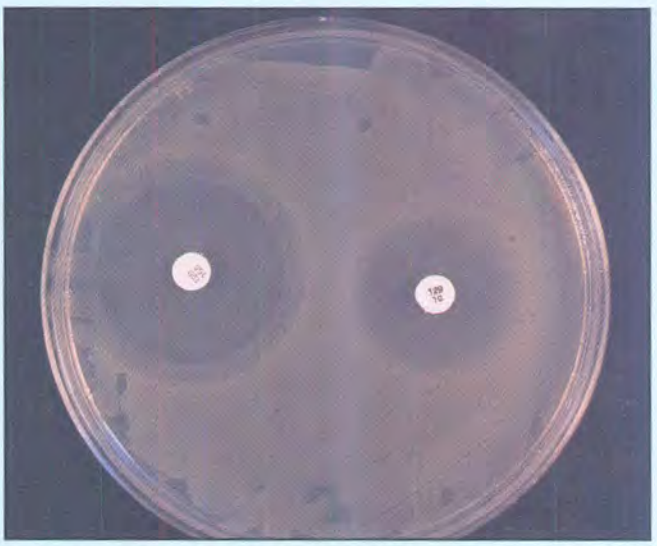
The susceptibility of vibrios to 0129 has long been recognised[3]. For practical reasons it is more usual now to use the water-soluble derivative 2–4-diamino-6,7-di-isopropyl-pteridine phosphate.

Oxoid diagnostic discs DD14 and DD15 are used in an agar diffusion susceptibility test to differentiate vibrios from *Aeromonas* spp. which are resistant to 0129.

The degree of susceptibility of different *Vibrio* species to 0129 may be used as an aid to their identification[4]. Paper discs impregnated with 10 µg and 150 µg are used. Sensitive strains show zones of inhibition around both discs and strains that are only partially sensitive show zones around the 150 µg disc only. Nutrient agar should be used in the test. Specialised susceptibility test media such as Isosensitest agar must not be used because their low sodium chloride content discourages growth of halophilic species and might encourage growth of *Enterobacteriaceae* resulting in false-positive tests.

Susceptibility to 0129 is not limited to Gram-negative organisms; *Bacillus* spp. and enterococci may be inhibited by the concentrations used although their different microscopic and colonial appearances should normally ensure that they are not tested. Incubation at 41.5° may be effective in preventing growth of non-vibrio, 0129-positive Gram-negative organisms and reduce the number of false-positive tests.

Strains of *Vibrio cholerae* resistant to 0129 have been reported[5], probably arising originally from clinical cases because strains resistant to 0129 are also resistant to trimethoprim. This cross-resistance has a genetic basis and is transferrable[6].



Susceptibility of *V. cholerae* to 0129 discs DD14 and DD15. 150 µg (left) and 10 µg (right).

Susceptibility to 0129 cannot now be considered an entirely reliable test for identifying vibrios. However, alternative simple tests have been suggested[5] to differentiate vibrios from aeromonads and pseudomonads which also are oxidase-positive.

Fermentation tests

Peptone water sugars used for these tests require supplementation with 1% of sodium chloride. Lactose, arabinose, mannose, cellobiose and raffinose are useful for distinguishing the species of *Vibrio* but other carbohydrates useful for distinguishing members of the *Enterobacteriaceae* have little or no value for identifying vibrios.

Indole test

Kovac's indole reagent is used to test 24 hour cultures. This test is probably best conducted on the culture showing best growth in the salt-tolerance test.

Haemolysis

Many species of *Vibrio* cause haemolysis on horse-blood agar. Haemolysis of sheep cells is a test used to distinguish classical and El Tor biovars of *V. cholerae*. The Kanagawa test is a specialised test in which human red blood cells are used to recognise *V. parahaemolyticus*.

Differing degrees of haemolysis by isolates can be used in their identification as species but unless tests are rigidly standardised and controlled the results are unreliable. The agar plate method devised by Sakazaki[8] is convenient and reproducible. Details of its application and the results that can be expected are given by Furniss and colleagues[4].

Chick red cells are used in a haemagglutination test to distinguish classical and El Tor biovars of *V. cholerae*.

The Kanagawa Reaction for Virulent *V. parahaemolyticus*

Results of this test are valid only if isolates are tested for haemolysis on Wagatsuma agar.

Positive results are indicated by clear zones of haemolysis around the colonies. Tests must be conducted strictly according to procedure and adequately controlled with a positive strain.

The Kanagawa Reaction. Photograph of a positive test indicating a virulent strain of *V. parahaemolyticus* reproduced with permission from *Foodborne Pathogens: An Illustrated Text*. Manson Publishing, 1996, © A. H. Varnam, M. G. Evans, 1991, 1996

Wagatsuma agar

Formula

	grams/ litre	Suggested Oxoid products
Yeast extract	5	Yeast extract L21
Tryptone	10	Tryptone L42
Sodium chloride	70	Sodium chloride L5
Mannitol	5	
Crystal violet	0.001	Agar bacteriological L11
Water	1000 ml	
pH 7.5		

Sterilise the medium by autoclaving and cool to 50°C. Add 10 ml of a 20% suspension of washed fresh human red blood cells to 100 ml of medium.

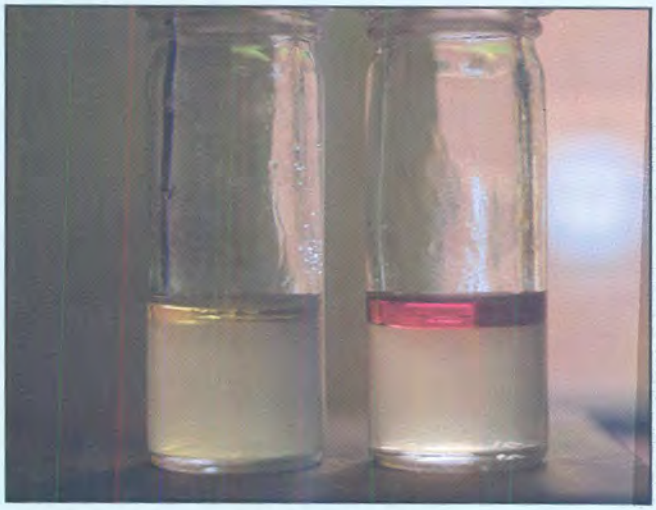
Procedure[7]

1. Inoculate plates of Wagatsuma agar with overnight broth cultures. Multiple tests may be conducted on a plate and a positive control strain must be included.
2. Incubate at 37°C for 18–24 hours. The incubation time must not exceed 24 hours.
3. Positive tests are indicated by clearly haemolytic zones. α-Haemolysis or discoloration of the medium must not be recorded as a positive result.

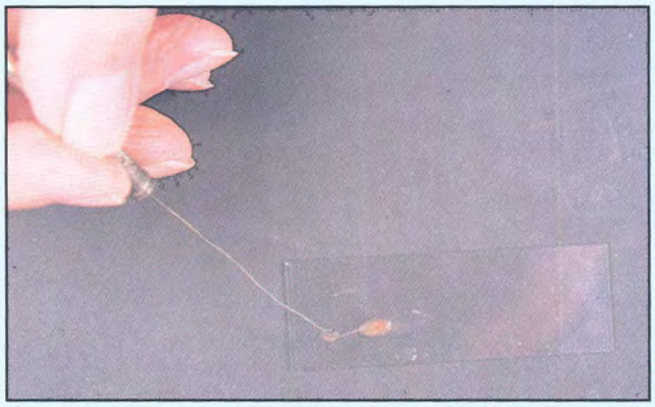
The String test

This simple test[9] is useful for presumptive identification of both 01 and non-01 serovars of *V. cholerae*.

A colony is emulsified in a drop of 5% sodium deoxycholate in 0.85% sodium chloride solution. A mucoid mass is formed by *V. cholerae*. Strings of the mucoid mass are seen when a loopful is lifted.



The indole test. Positive (right) and negative (left). This test is best done in tryptone water on the culture showing the heaviest growth in the salt tolerance test.



The string test for presumptive identification of *V. cholerae*. Note the 'string' of mucoid cellular material connecting the emulsified colony and the inoculating loop (see page 37)

Other tests

The tests described above are not a complete list of those available for identifying vibrios and distinguishing the species. Tests which detect enzymatic activity e.g. gelatinase, DNAse, lipase and lecithinase are used. Biochemical tests include nitrate reduction and the VP test for detection of acetylmethylcarbanol. Patterns of susceptibility to antibiotics (antibiograms) may be useful.

Specialised texts should be consulted for more information. Application of a variety of tests is discussed by Furniss and colleagues[4] and detailed methodology is given in the FDA Bacteriological Analytical Manual[10] and national and international standard methods.

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Table 4 – Differentiation of vibrios, pseudomonads, aeromonads and *Plesiomonas* from *Enterobacteriaceae*

Test	Vibrio	Aeromonads	*Plesiomonas*	*Pseudomonas*	*Entero-bacteriaceae*
Growth on salt-deficient medium	+ or –	+	+	+	+
Oxidase	+	+	+	+	–
Oxidation/fermentation (O/F)	F	F	F	O	F
Susceptibility to 0129 (150 µg)	S	R	S	R	R

Table 5 – Differentiation of *Vibrios* from *Aeromonas* and *Plesiomonas* spp. *Aeromonas* and *Plesiomonas* may also be separated by these characteristics

Test	*Vibrios*	*Aeromonas*	*Plesio-monas*
Requirement for sodium chloride by many species	+	–	–
DNAse	+	+	–
0129 susceptibility	S	R	S
Acid from inositol	–	–	+

Table 6 – Minimal Distinguishing Characteristics of the Major Food-borne Pathogenic vibrios

Characteristics	*V. parahaemolyticus*	*V. vulnificus*	*V. cholerae*
% Salt tolerance			
0	–	–	+
3	+	+	+
6	+	+	–
8	+	–	–
10	– or ±	–	–
Voges-Proskauer (VP) test	–	–	Classical – El Tor +
ONPG test	–	+	+
Sucrose fermentation	–	– or rarely +	+
Arabinose fermentation	+ or variable	–	–
Appearance on TSI			
Slant	Alkaline	Alkaline or rarely acid	Acid
Butt	Acid	Acid	Acid
Gas	–	–	–
H_2S	–	–	–
Susceptibility to 0129			
10 µg	R	S	*S
150 µg	S	S	*S

Source FDA *Bacteriological Analytical Manual* *Resistant strains have been reported[1]

1 Nath, G. and Sanyal, S.C. (1992) *The Lancet* 340. August 8th. 366–367.

Table 7 – Major Distinguishing Characteristics of Food-borne Pathogenic *Vibrio* species

	V. cholerae	*V. mimicus*	*V. parahae-molyticus*	*V. vulnificus*	*V. fluvialis*	*V. furnissii*	*V. hollisae*	*V. algino-lyticus*	*V. damsela*
Growth in: % NaCl									
0	+	+	–	–	–	–	–	–	–
1	+	+	+	+	+	+	+	+	+
6	+ or –	+ or –	+	+ or –	+	+	+	+	+
8	–	–	+	–	+ or –	+	–	+	–
Lactose	–	–	–	+	–	–	–	–	–
Sucrose	+	–	–	+ or –	+	+	–	–	+
Cellobiose	–	–	–	+	+ or –	–	–	–	–
Salicin	–	–	–	+	–	–	–	–	–
Indole	+	+	+	+	+ or –	–	+	+	–
Gelatinase	+	+ or –	+	+ or –	+	+	–	+	–
Arginine dihydrolase	–	–	–	–	+	+	–	–	+
Lysine decarboxylase	+	+	+	+	–	–	–	+ or –	–
Ornithine decarboxylase	+	+	+	+ or –	–	–	–	+ or –	–
DNAse	+	+ or –	+	+ or –	+	+	–	+	+ or –
Inhibition by 0129 150 µg	+	+	+ or –	+	+ or –	–	+ or –	+ or –	+

Key: + >0.90% of strains positive; + or –10–90% of strains positive; Negative, but up to 10% of strains may be positive.

Source: *Food Microbiology. Fundamentals and Frontiers.* Doyle, M.P., Beuchat, L.R. and Montville, T.J. Jnr. (eds). (1997). ASM Press, Washington D.C.

Table 8 – Differentiation of Classical and El Tor biovars of *Vibrio cholerae* 01

Test	Classical Biovar	El Tor Biovar
Haemolysis	–	+ or –
Haemagglutination of chick cells	–	+
Voges-Proskauer	–	+
Susceptibility to polymyxin 50 µg	+	–
Phage susceptibility:		
Classic phage IV	+	–
El Tor phage 5	–	+

Toxins and other Virulence Factors of *Vibrio* species

Toxins and other Virulence Factors of *Vibrio* species

Introduction

Widespread and common occurrence of cholera and the severity of the symptoms of infection by *V. cholerae* have ensured that the virulence mechanisms of this species are the most understood amongst the pathogenic vibrios. Much has still to be learnt about how the other species associated with food-borne infection cause disease and it is still unclear how *V. parahaemolyticus* causes the intense cholera-like diarrhoea that characterises infection by the species. *V. vulnificus* has undergone intensive study since the recognition in the 1970's of its role in extremely serious infection. *V. vulnificus* is the leading cause of reported deaths from food-borne disease in Florida[1]. The remaining species associated with food-borne disease possess a variety of features that might be virulence factors. Little is currently known for certain about their relative significance in initiating disease.

V. cholerae

Unlike many of the organisms that cause diarrhoea, *V. cholerae* does not invade the gut mucosa. Chemotactic attraction to the gut wall and reduction of mucus viscosity aid the motile cells to make contact with, and adhere to, microvilli of small intestine epithelial cells.

Production of enterotoxin, which causes great fluid loss into the gut due to interference with sodium, potassium chloride and bicarbonate functions in controlling intracellular fluid and ion balance, leads to intense diarrhoea.

Although cholera toxin (CT) is the major toxin, other enterotoxins distinct from cholera toxin are produced by 01 and non-01 strains. These include a heat-stable toxin similar to ST toxin of enterotoxigenic *E. coli* and one similar to Shiga toxin of *Shigella*. A thermostable haemolysin identical to that of *V. parahaemolyticus* is also produced.

V. parahaemolyticus

No obvious enterotoxin is produced by *V. parahaemolyticus* and there is very little evidence about adherence to the gut wall which is generally an essential preliminary to pathogenesis. A number of haemolysins are produced, one of which, Vp-TDH responsible for the Kanagawa reaction, appears to be the principal virulence factor. However, evidence of its role in inducing diarrhoea is not very strong and the situation is complicated by Kanagawa-negative strains now occurring more commonly in infection.

Other toxins are produced including the neurotoxin tetrodotoxin but their role is mostly speculative.

V. vulnificus

This species produces at least two haemolysins in infected subjects and *in vitro* tests show the haemolysins to be produced by strains obtained from both clinical and environmental sources. However, virulence appears not to be diminished in non-haemolytic mutants. The presence of a cell capsule appears essential to pathogenicity.

Proteases are important in initiating wound infection and various enzymes active against lipids, fibrinogen, DNA and other substances important in tissue structure and defence probably also contribute.

Enterotoxin distinct from haemolysins and proteases is produced but its ability to cause diarrhoea apparently requires the presence of other virulence factors.

Iron acquisition is very important in the pathogenesis of *V. vulnificus*. Unusually, this organism is able to overcome the iron-binding activities of the host to obtain iron from haemoglobin in erythrocytes.

V. mimicus

V. mimicus does not adhere as tightly to mucosal epithelial cells and this may account for its reduced virulence relative to *V. cholerae*. The factors that contribute to adherence of *V. mimicus* are not known.

V. mimicus does not possess a unique enterotoxin but does possess toxins which are also found in other species of *Vibrio*. These include *V. cholerae* CT toxin and thermostable enterotoxin of non-01/0139 *V. cholerae* serovars.

Haemolysins are produced by *V. mimicus* and at least one of them is also proteolytic.

Other food-borne pathogenic vibrios

Little is certain about the virulence factors possessed by most of the species of *Vibrio* associated with food-borne infection, although adhesins, haemolysins and toxins have been described. The available information for the less frequently isolated species has been reviewed by Oliver and Kaper[2].

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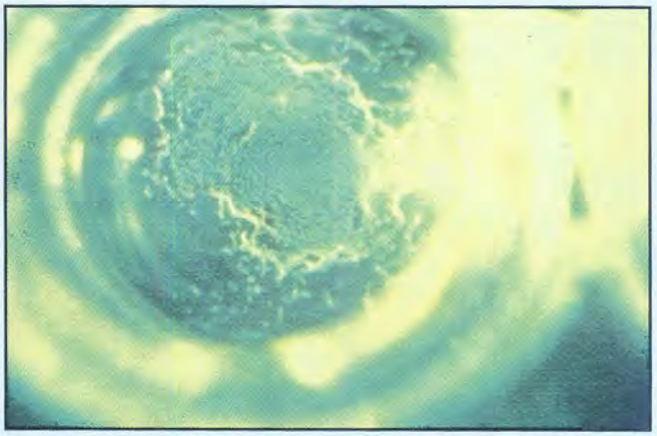
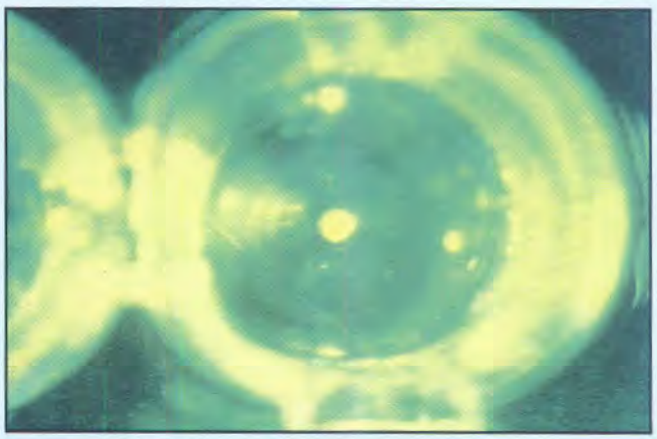


VET-RPLA cholera toxin (CT) detection test. Positive test (above). Negative test (below) (see page 41)



VET-RPLA Cholera Toxin Detection Kit

VET-RPLA Cholera Toxin Detection Kit

Code TD920

For the detection of *Vibrio cholerae* enterotoxin (CT) in culture filtrates

Description

Vibrio cholerae produces an enterotoxin which causes the intense diarrhoea which is so typical of the disease cholera. The action of the toxin is to disrupt the fluid retention mechanism of intestinal epithelial cells. Diarrhoea is so profuse that death may occur because of severe fluid and electrolyte loss.

Cholera is generally spread by poor sanitation resulting in contaminated drinking water supplies, but the disease may be food-borne, particularly from eating raw shellfish harvested from faecally-polluted coastal waters.

V. cholerae (CT) toxin is chromosomally-mediated and closely similar to the plasmid-borne heat-labile (LT) toxin of *Escherichia coli*. Because the antigenic structures of both enterotoxins are similar, antiserum prepared by immunising rabbits with CT will react with both CT and LT toxins in a reversed-passive-latex agglutination test (RPLA). The sensitivity of the test is 1–2 ng/ml in culture filtrates.

Test Principle

The test is performed in V-well microtitre plates.

Latex particles are sensitised with purified antiserum taken from rabbits immunised with purified *V. cholerae* enterotoxin. In the presence of *V. cholerae* enterotoxin (CT) or *E. coli* heat-labile enterotoxin (LT), the latex particles will agglutinate forming a clearly visible lattice structure.

If the enterotoxins are absent, or present at a concentration below the assay detection level, a lattice structure is not formed and the latex particles settle in a tight button in the base of the well.

Procedure

Sample preparation

V. cholerae and *E. coli* for testing may be recovered from clinical samples and identified by standard methods.

V. cholerae enterotoxin production

V. cholerae may be tested for CT production following growth in Peptone Water (Oxoid CM9) at 30°C for 24 hours. After incubation, the test sample may be extracted by means of centrifugation.

Assay Method

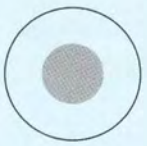
1. Shake the reagents thoroughly.
2. Position a V-well microtitre plate so that each row consists of 8 wells. (Each sample requires 2 such rows.)
3. Dispense 25 µl of diluent in each well of the 2 rows, except for the first well in each row.
4. Add 25 µl of test sample to the first and second well of each row.
5. Pick up 25 µl from the second well and perform doubling dilutions along each row, ending at the seventh well. The last well should contain diluent only.
6. Add 25 µl of sensitised latex (TD921) to each well in row 1.
7. Add 25 µl of control latex to each well in row 2.
8. Agitate the plate by hand or micromixer to mix the contents of each well.
9. Cover the plate and leave undisturbed for 20–24 hours.
10. Examine each well for agglutination against a black background. Dispose of all items in hypochlorite solution (1.3% w/w).

Interpretation of results

Agglutination should be assessed by comparison with the following illustration. For more detailed procedure and interpretation information, refer to the pack insert.

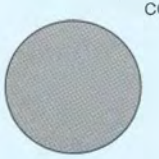
(–) Negative
Results classified as (–) and (±) are considered to be negative.

(±)



(+)

(++) Positive
Results classified as (+), (++) and (+++) are considered to be positive.



(+++)

Components of the kit

TD921 Sensitised Latex

TD922 Latex Control

TD923 Enterotoxin Control

TD924 Diluent

Serological Classification of *Vibrio* species

Serological Classification of *Vibrio* species

Vibrio cholerae

Polyvalent antisera are used in slide agglutination tests to distinguish 01 and 0139 serogroups from the remaining 137 which collectively are known as non-01/0139 serogroups. Oxidase-positive isolates that agglutinate in one or other antiserum may be reported as presumptive *V. cholerae* 01 or 0139 and sent to a reference laboratory for confirmation. Examination of colonies taken from TCBS agar may be unreliable. A co-agglutination test[1] may give better results.

Serological identification of non-01/0139 isolates is rarely carried out but any that appear to be implicated in sporadic cases of diarrhoeal illness should be referred to a specialist laboratory.

Identification of the flagellar antigens of *V. cholerae* is not useful.

Vibrio parahaemolyticus

Isolates of *V. parahaemolyticus* may be serotyped by identifying the somatic O and capsular K antigens[2]. However, although the O antigen of most strains isolated from clinical cases can be typed, some clinical and many environmental isolates are untypeable by their K antigens.

At least 12 O and more than 50 K antigens are known. There is no apparent link between serotype and virulence amongst *V. parahaemolyticus* strains.

Vibrio vulnificus

Serotyping of *V. vulnificus* isolates is not routinely done.

A study[3] using polyclonal antibodies to identify lipopolysaccharide and capsular antigens differentiated strains into at least 10 types, all of which were associated with human infection. Two of the types accounted for approximately 1/3 of the isolates. A much higher proportion of strains that had been isolated from the environment were of two other types.

A species-specific test that uses anti-flagellar antisera has been used to confirm the identity of isolates[4] and an immunodiffusion test for the same purpose has been described.

Other Species

Passive agglutination may be used to identify *V. mimicus.*

Serological classification of other pathogenic *Vibrio* species has yet to be established.

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Notes

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