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Mast Group

MAST® ASSURE

Bacterial Agglutinating Antisera Guide

Salmonella
Escherichia coli
Haemophilus
Shigella
Vibrio
Campylobacter
Clostridium
Legionella
Pseudomonas
Staphylococcus
Listeria
Streptococcus
Yersinia
Proteus
Brucella

Contents

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References available upon request.

MAST® ASSURE
SALMONELLA ANTISERA

A. MAST® ASSURE Salmonella Antisera

1. Introduction

a. Salmonella and Disease

Salmonella are Gram negative organisms belonging to the order *Enterobacterales.* They are non-spore forming rods, and most of them are motile possessing flagella.

Salmonellae are widely distributed in nature, with all vertebrates and some invertebrates capable of harbouring salmonella in their gut. Most animal infections seem to be symptomless or to cause a self-limiting gastroenteritis of variable severity. Many serotypes e.g. *S. typhimurium* show a wide host range, while others e.g. *S. typhi* and *S. paratyphi* A, B and C are primarily human pathogens and are rarely isolated from animals other than humans. Others are particularly adapted to animal hosts include *S. cholera-suis* (pigs), *S. dublin* (cattle), *S. gallinarum-pullorum* (poultry), *S. abortus-equi* (horses) and *S. abortus-ovis* (sheep).

Salmonella infection in man can cause a wide spectrum of clinical illness however it mainly manifests itself in four syndromes. These are enteric fever, gastroenteritis, bacteraemia with or without metastatic infection, and the asymptomatic carrier state.

- Enteric fever is most usually caused by *S. typhi* and *S. paratyphi* A, B and C but can be caused by other Salmonella serotypes. The clinical features tend to be more severe with *S. typhi* (typhoid fever). Enteric fever is a systemic disease whose major symptoms are fever and headache and may be fatal if untreated.

- Acute gastroenteritis is characterised by vomiting, abdominal pain, diarrhoea and fever and is commonly seen as a result of food poisoning from infected poultry.

- Bacteraemia is a constant feature of enteric illness and may occur as a rare complication of any Salmonella infection. Transient bacteraemia may occur in gastroenteritis but in most cases organisms are cleared from the blood stream without ill effect.

- Carrier State. Most sufferers from a salmonella infection continue to excrete the organism in their stools for days or weeks after complete clinical recovery, but eventual clearance from the body is usual. A few patients continue to excrete Salmonellae for prolonged periods. Chronic carriers may excrete Salmonellae for a year or more and may have no symptoms of disease.

Laboratory diagnosis of salmonella infection usually depends upon the isolation and identification of the causal salmonella from a specimen of the patient's blood or faeces. Further antigenic analysis of the organism is often needed to identify the species or strain for epidemiological purposes.

b. Antigenic characterisation of Salmonellae

Salmonella possess two main types of antigen, the O (somatic or body) and the H (flagellar) antigens. The O antigens are heat stable and upon which grouping of the organisms are based. The H antigens are heat labile and are used for confirming and identifying the serotypes within the groups. The H antigen of an organism of a known O group may occur as two different serological entities, known as phases. Diphasic organisms may be separated into individual phases by growing the culture in the presence of a small amount of the specific phase antiserum of the phase already recognised, such that the only motile organisms isolated will be of the alternative phase. Occasionally a third antigen called the Vi antigen is also present in certain Salmonella strains. When present the Vi antigen may block the activity of the O antigens and must be inactivated before proceeding with the serological grouping of the organism.

As an example *Salmonella typhimurium* possess the O antigens 1, 4, [5], 12 and may possess either phase 1 antigen i or phase 2 antigen 1, 2. This may be written as 1, 4, [5], 12: i : 1, 2.

In the early 1920s White recognised the antigenic variation in Salmonella species and its importance in differentiating Salmonella on the basis of serotyping. Kauffmann later confirmed White's observations and greatly extended them to form the current Kauffmann-White Scheme for classification of types within the genus *Salmonella*. Today more than 2000 serotypes of Salmonella have been identified.

c. **MAST®** ASSURE **Salmonella Antisera: preparation and presentation.**

MAST® ASSURE Salmonella Antisera are prepared from rabbit's hyperimmunised with standard strains of Salmonella organisms possessing defined antigenic factors. All sera are heat inactivated at 56°C for 30 minutes, absorbed to remove cross-reacting agglutinins and filter sterilised. The **MAST®** ASSURE Salmonella Antisera provide a comprehensive range of O, H and Vi antisera and in addition Salmonella Phase Induction Antisera, for the induction of a hidden flagellar phase. Antigens are identified normally by qualitative slide agglutination or by quantitative tube agglutination tests or in the case of Salmonella Phase Induction Antisera using culture tubes (semi-solid medium) or agar plates (bridging method).

All **MAST®** ASSURE Salmonella Antisera except the Phase Induction Antisera are provided as 2ml (or 5ml) amounts in vials with dropper attachments and contain 0.1% sodium azide as preservative. Supplied ready to use. This is sufficient for 50 (125) slide agglutination tests or 20 (50) tube agglutination tests. The **MAST®** ASSURE Salmonella Phase Induction Antisera are provided sterile in 5 ml volumes and sealed in injection vials. Supplied ready to use with no preservative is added.

2. Culture of Salmonella - Preparation for Serology

Salmonella belong to the order *Enterobacterales* and there is much cross-reaction and antigenic relationships between Salmonella and other genera within this family. Hence it is important that organisms undergoing serological classification should be correctly identified as Salmonella by morphological and biochemical features first.

3. Salmonella O - Grouping Antisera

Classically Salmonella somatic O antigenic factors have been grouped together according to alphabetical designations as detailed in the Kaufmann-White Scheme. However these have become outdated as new antigenic factors were realised. Mast Group Ltd prefers to use the numbered designations as recommended by the WHO Collaborative Centre for Reference and Research on Salmonella in their **MAST®** ASSURE Antisera range.

Table 1 below indicates the correlation between the alphabetical and numerical systems.

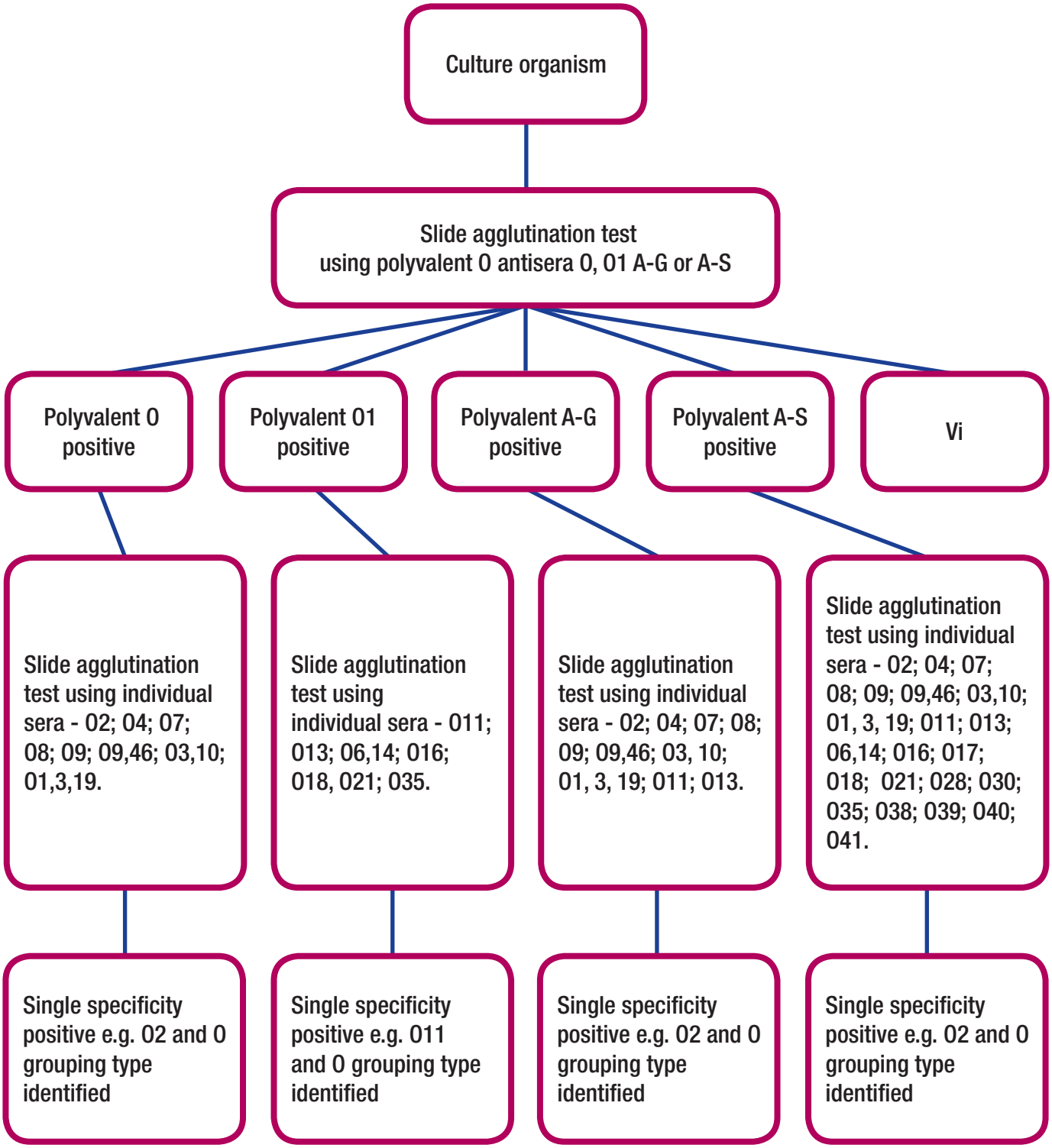
Table 1 - Correlation between the Alphabetical Grouping and Numerical O Factor Designation Systems for Salmonella O Grouping.

Alphabetical group designation	Numerical O factor designation	Alphabetical group designation	Numerical O factor designation	Alphabetical group designation	Numerical O factor designation
A	O2	F	O11	Q	O39
B	O4	G_1	O13, 22	R	O40
$C_1 + C_4$	O6, 7	G_2	O13, 23	S	O41
C_2	O6, 8	H	O6, 14	T	O42
C_3	O8, [20]	I	O16	U	O43
D^1	O9, 12	J	O17	V	O44
D_2	O9, 46	K	O18	W	O45
D_3	O9, 46, 27	L	O21	X	O47
E_1	O3, 10 *	M	O28	Y	O48
E_2	O3, 15 *	N	O30	Z	O50
E_3	O3, 15, 34 *	O	O35	no code	O51 - O67
E_4	O3, 19	P	O38		

NOTE * = Group E_1 was originally classified as O3, 10, Group E_2 as O3,15 and Group E_3 as O3, 15, 34. It was later noted that Group E1 strains could be lysogenised by phage ε15 (O3, 10 → O3, 15) and then by phage ε34 (O3, 15 → O3, 15, 34). Because of this these strains are now classified under one common group.

To determine the O-group of a Salmonella strain, polyvalent O antisera should be used initially to narrow down the range before specific grouping sera are used. This is illustrated schematically in Figure 1 and according to methods as detailed in section 4.

Figure 1 - Summary of Salmonella O-Grouping Procedures



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References available upon request.

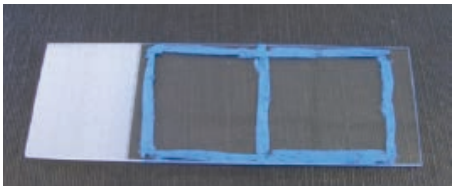
4. Procedures for O serotyping and Interpretation of Results

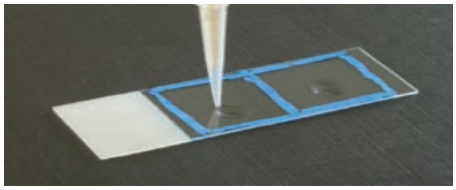
MAST® ASSURE Salmonella O Grouping Antisera are intended for use in the identification of O antigens by qualitative slide agglutination, although they may be used in quantitative tube agglutination tests for confirmatory purposes.

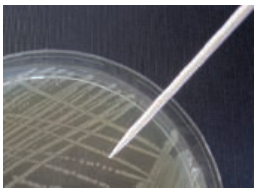
Cultures of organisms identified as *Salmonella sp* by their morphological and biochemical features may be serotyped by the following procedures. Also refer to figure 2 for a summary of the Salmonella O grouping procedure.

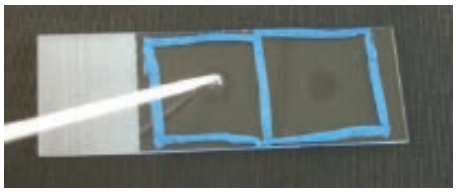
a. Slide Agglutination for O-antigen and Vi grouping.

1. Place two drops of sterile 0.85% saline solution (saline) onto a carefully cleaned microscope slide. The slide may be partitioned into several parts using a chinograph or glass pencil Picture a, b. With an inoculation loop or wire emulsify into each drop of saline a live cell colony from a fresh agar plate or slope culture to produce a distinct and uniform turbidity Picture c, and d.

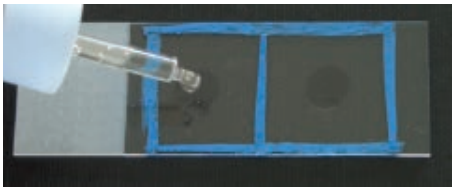
a. 

b. 

c. 

d. 

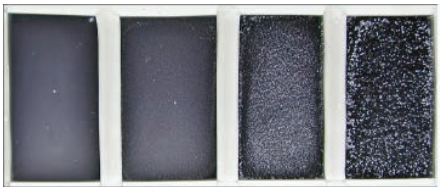
2. Place a drop of polyvalent antiserum onto one of the drops of emulsified isolate and to the other a drop of saline as a control.



Note: allow the antiserum to freefall from the dropper provided with the bottle. Do not contaminate the antiserum with organism.

3. Mix the reagents by tilting the slide back and forth for 60 seconds while viewing under indirect light against a dark background.

Distinct clumping or agglutination within this period, without clumping in the saline control (auto- agglutination) should be regarded as a positive result.



An isolate producing a distinct positive reaction with a polyvalent antiserum is assumed to be Salmonella bearing one or more of the O antigenic factors represented by that antiserum. Using this information, further testing of the isolate should be conducted, as described in steps 1 - 3, with specific O antisera to reveal the full O antigenic grouping of the isolate.

5. If no agglutination is found with any of the polyvalent sera and saline, repeat steps 1 - 3 above using the Vi antiserum. If a positive reaction is found with the Vi antiserum, prepare a dense cell suspension of the organism in 0.85% saline and heat the suspension to 100°C for 60 minutes or autoclave at 121°C for 15 minutes, then repeat the agglutination test using polyvalent and Vi sera on the heated cell suspension.

   If the live cell isolate produces a negative result with the polyvalent antisera and a positive result with the Vi antiserum, whilst the heated cell isolate produces a positive result with the polyvalent antisera and a negative result with the Vi antiserum the isolate should probably be regarded as *Salmonella typhi* (O9, 12, Vi) A few other organisms e.g. *Salmonella paratyphi* C (O6, 7, Vi) also contain the Vi antigen but may easily be distinguished by their O antigenic specificities.

b. Tube Agglutination method.

This method may be used for confirmatory testing only.

Live cell suspensions may be used as antigens, but care must be taken to handle cultures with care to avoid laboratory infection. Killed O antigens may be prepared by heating a saline suspension of organisms to 100°C for 10 minutes, centrifuging and resuspending the deposit in saline. The use of phenol or formalin in O antigen preparations should be avoided since these substances inhibit O agglutination in the presence of H antigens.

1. Remove colonies from a suitable agar or broth culture and prepare a fairly light suspension of bacteria of approximately 10^9 organisms per ml in sterile saline (0.85% saline).

2. Make serial dilutions of antiserum in 0.5ml volumes of saline from 1:10 to 1:640 or 1:1280. Round bottomed glass tubes approximately 9 x 85mm are most suitable.

3. To each tube add 0.5ml of antigen suspension.

Note: this doubles the dilution of the antiserum.

4. A control tube should be additionally set up containing 0.5ml of antigen suspension and 0.5ml of saline.

5. Shake the tubes thoroughly and incubate O factor tubes at 50°C for 4 hours or Vi factor tubes at 37°C for 2 hours then at 4°C for 18 hours.

Note: Vi factor tubes should be allowed to warm to room temperature before reading.

6. Examine the tubes for agglutination. Positive agglutination will be observed as obvious granular agglutination. In a negative reaction and in the saline control the appearance of the suspension should be unchanged, with a cloudy appearance, and show a typical swirl on agitation. The titre value is the dilution of the last tube showing agglutination. Titres at or near the stated value (available on request) indicate that the antigen is of the same serotype as the antiserum.

   If agglutination is observed in the saline control, the test is invalid. A new antigen solution should be prepared from a fresh culture preparation of the organism and retested.

5. Salmonella H - Typing Antisera

Salmonella flagellar (H) antigens may occur as two different serological entities, known as phases. Antigens labelled alphabetically may appear in phases 1 or 2, but antigens labelled numerically only appear in phase 2. The alphabetically labelled antigens are designated as:- a-z, and z_1-z_{68} and the numerically labelled antigens are designated as:- 1, 2, 5, 6 and 7. For the alphabetically labelled antigens certain factors are always seen in association with others. These are listed below:-

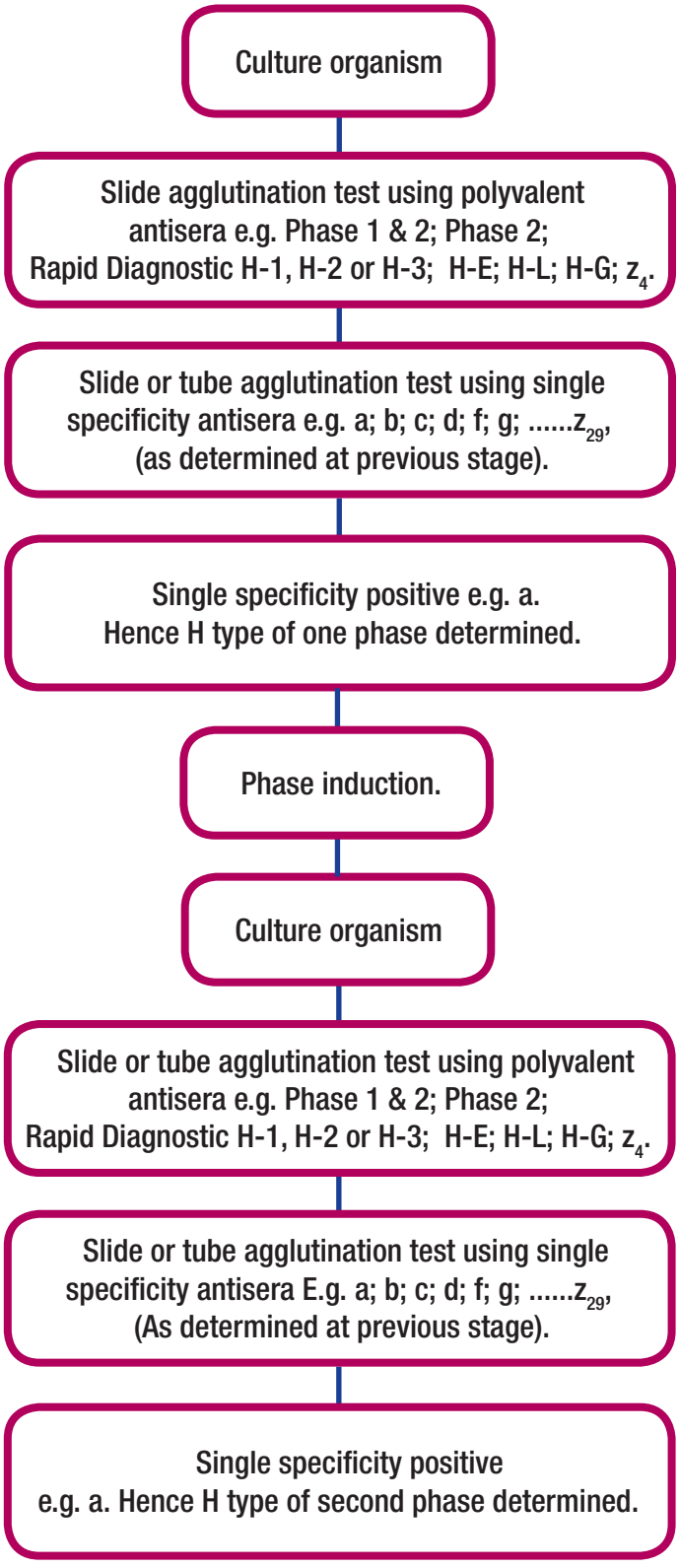
E complex - contains e and one or more of n, h, x, z_{15}
G complex - contains g and one or more of f, m, p, q, s, t, u, z_{51}, (z_{52}, z_{62}, z_{63})
L complex - contains l and one or more of v, w, z_{13}, z_{28}, (z_{40})
z_4 complex - contains z_4 and one or more of z_{23}, z_{24}, z_{32}

For the numerically labelled antigens factors 2, 5, 6 and 7 are always seen in association with factor 1, e.g. 1, 2 or 1,7. Antigen z_6 is also linked with this group.

Once the O - group of a Salmonella strain has been determined the organism may be identified further using **MAST®** ASSURE Salmonella H-Typing Antisera.

To determine the H-type of a Salmonella strain, polyvalent H antisera should be used initially to narrow down the range before specific typing sera are used. This is illustrated schematically in Figure 2 and according to methods as detailed in section 6.

Figure 2 - Summary of Salmonella H-Typing Procedures



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6. Procedures for H serotyping and Interpretation of Results

MAST® ASSURE Salmonella H Typing Antisera are intended for use in the identification of H (flagellar) antigens Polyvalent antisera should preferably be used in slide agglutination tests. Specific H-typing sera may also used in initial qualitative slide agglutination, although they may be used in quantitative tube agglutination tests for confirmatory purposes.

Normally an isolate is tested initially against the Polyvalent H phase 1 & 2 serum (specific and non-specific antigens). If this shows agglutination it should be tested against the Polyvalent H phase 2 serum (non-specific antigens i.e. 1, 2, 5, 6, 7, z_6). If it is not agglutinated by this serum the isolate is assumed to be in the specific phase and attempts should be made to determine the phase 1 antigens using the Rapid antisera, Polyvalent H - G, L or E complex antisera or monovalent H-antisera, as described below.

The commonly occurring Phase 1 antigen, with the exception of factor i, may be identified by slide agglutination tests using the Rapid Diagnostic Antisera, as illustrated below:-

Antigenic factor(s)	Rapid Diagnostic 1	Rapid Diagnostic 2	Rapid Diagnostic 3
B	+	+	-
d	+	-	+
E	+	+	+
G	-	-	+
k	-	+	+
L	-	+	-
r	+	-	-

For H-serotypes possessing common determinants (for example the G group: f,g; g,m; g,p; g,s,t) specific antisera should be used for characterising the antigenic group further e.g.
f, m, p, s or t.

Sometimes only one of the two phases possessed by a diphasic organism are detected in this test. To determine the antigens in the other phase, secure the culture in the other phase by phase induction (see section 8 - Phase Induction Procedures).

a. Slide Agglutination for H-antigen typing.

Cultures of organisms identified as Salmonella by their morphological and biochemical features and O-grouped as detailed in section 4 may be H-serotyped by the following procedures.

1. Place two drops of sterile 0.85% saline solution (saline) onto a carefully cleaned microscope slide. The slide may be partitioned into several parts using a chinograph or glass pencil. With an inoculation loop or wire emulsify into each drop of saline a live cell colony from a fresh agar plate or slope culture to produce a distinct and uniform turbidity.

2. Place a drop of polyvalent antiserum onto one of the drops of emulsified isolate and to the other a drop of saline as a control.

Note: allow the antiserum to freefall from the dropper provided with the bottle. Do not contaminate the antiserum with organism.

3. Mix the reagents by tilting the slide back and forth for 60 seconds while viewing under indirect light against a dark background.

4. Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination) should be regarded as a positive result.

An isolate producing a distinct positive reaction with a polyvalent antiserum is assumed to be a Salmonella bearing one or more of the H antigenic factors represented by that antiserum. Using this information, further testing of the isolate should be conducted, as described in steps 1 - 3, with specific H antisera to reveal the full H antigenic grouping of the isolate.

b. Tube Agglutination method.

1. Prepare a suspension of bacteria in 0.5% (v/v) formal saline or formalinised broth cultures may be used. Colonies taken from primary isolation media may be unsatisfactory for use in determining the H serotype due to poor motility of organisms. This may be improved by using organisms grown at 37°C for 6-8 hours in a broth culture, by subculture onto moist agar slopes, using 0.5% agar in a Petri dish or in 0.2% agar in a Craigie tube and picking the leading edge of the culture after incubation.

2. Make serial dilutions of antiserum in 0.5ml volumes of saline from 1:10 to 1:640 or 1:1280. Round bottomed glass tubes approximately 9 x 85mm are most suitable.

3. To each tube add 0.5ml of antigen suspension.

Note: this doubles the dilution of the antiserum.

4. A control tube should be additionally set up containing 0.5ml of antigen suspension and 0.5ml of saline.

5. Shake the tubes thoroughly and incubate tubes at 50-52°C for 1-2 hours.

6. Examine the tubes for agglutination. Positive agglutination will be observed as characteristic flocccular agglutination. In a negative reaction and in the saline control the appearance of the suspension should be unchanged, with a cloudy appearance, and show a typical swirl on agitation. The titre value is the dilution of the last tube showing agglutination. Titres at or near the stated value (available on request) indicate that the antigen is of the same serotype as the antiserum.

   If agglutination is observed in the saline control, the test is invalid. A new antigen solution should be prepared from a fresh culture preparation of the organism and retested.

7. Salmonella H Antigen Phase Induction

Sometimes it is necessary to isolate the second phase of a diphasic organism for complete serological identification.

These antisera are provided sterile in sealed injection vials and contain no preservative. For use, the required volume may be withdrawn under sterile conditions using a syringe and needle.

8. Salmonella H Antigen Phase Induction Procedures

Phase induction may be done according to the following procedures:-

a. Craigie Tube Method

1. Add 0.1ml of the H antiserum by which the organism is agglutinated, to about 3ml of semi-solid nutrient agar held at 50°C in a water bath. Mix the contents taking care to avoid frothing.

2. After mixing the serum and agar, aseptically place a previously sterilised Craigie tube vertically into the medium, with the upper end projecting well above the agar surface.

3. After the medium has cooled and solidified, inoculate the organism using a straight wire into the agar inside the Craigie tube. Incubate the culture at 37°C overnight (16-18 hours).

4. After incubation, remove organisms from the agar outside the Craigie tube, place into glucose broth and incubate at 37ºC for 6-8 hours. By this time there should be enough growth to assess the H antigens of the induced phase.

5. Determine the H antigens of the induced phase using **MAST®** ASSURE Salmonella Antisera.
   For method see separate instructions.

   If the organism fails to appear outside the Craigie tube at 37ºC overnight (16-18 hours), the cultures should be left a further 24 hours, or reduce the volume of serum used for phase induction by half to 0.05ml. If such procedures fail to work, it should be assumed that the organism has flagella of only one phase.

B. Bridging Method

1. Cut a 50 × 20mm ditch in a well dried nutrient agar plate.

2. Soak a strip of previously sterilised filter paper (approximately 36 × 7mm) in the H antiserum by which the organism is agglutinated and place this strip across the ditch at right angles. At one end of the filter paper strip place a sterilised filter paper disc (approximately 7mm in diameter) so that half of it is on the serum strip and half is on the agar.

3. Inoculate the agar at the opposite end of the paper strip to the disc with organisms from a 6-8 hour nutrient broth culture of the organism and incubate overnight (16-18 hours) at 37ºC.

4. After incubation, remove the paper disc with sterile forceps and place it into glucose broth and incubate at 37ºC for 4 hours. By this time there should be enough growth to assess the H antigens of the induced phase.

5. Determine the H antigens of the induced phase using **MAST®** ASSURE Salmonella Antisera.
   For method see separate instructions.

   If the organism fails to appear on the paper disc after incubation at 37ºC overnight (16-18 hours), repeat the test. If the procedures fail to work again, it should be assumed that the organism has flagella of only one phase.

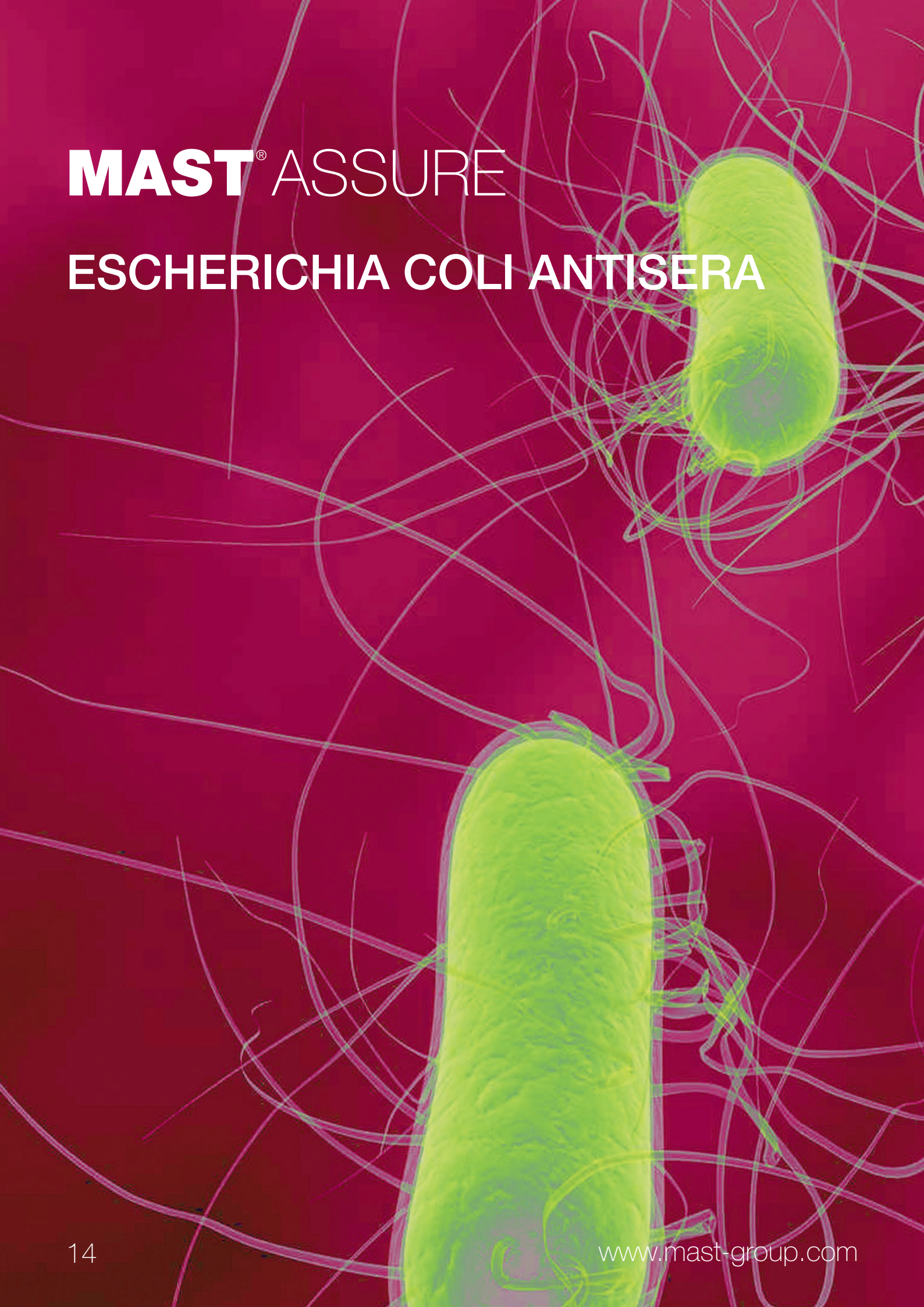
Note:- ensure that the surface of the agar plate is dry before use and that the filter paper strip is not over saturated with antiserum. If moisture is present on the surface of the plate organisms may swarm round the side of the ditch and be recovered on the filter paper disc giving erroneous results.

9. Antisera Sets

MAST® ASSURE Salmonella Antisera are also available as convenient sets and comprise of individual bottles of stated antisera.

MAST® ASSURE

ESCHERICHIA COLI ANTISERA



A. **MAST®** ASSURE **Escherichia coli Antisera**

1. Introduction

a. *Escherichia coli* and Disease

Escherichia coli (*E. coli*) is a group of Gram negative organisms belonging to the order *Enterobacterales*. They are non-spore forming rods, and most are motile possessing flagella.

Many strains of *E. coli* occur naturally in human and animal intestine where they are the predominant organism in the aerobic commensal flora. Some strains however may be associated with human infection, causing urinary tract infections (the most common cause of acute, uncomplicated urinary tract infection), diarrhoea and gastroenteritis, suppurative lesions, abscesses in a variety of wounds, neonatal septicaemia and meningitis, or with animal infections causing mastitis, pyometria in bitches, coli granulomata in fowls and white scours in calves.

There are four groups of *E. coli* that cause diarrhoea, acute gastritis or colitis based on their mechanisms:- Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC) and Enterohaemorrhagic (EHEC) or now referred to as Verotoxigenic (VTEC).

- The Enteropathogenic *E. coli* (EPEC) cause infantile enteritis especially in tropical countries, where outbreaks often occur in hospitals resulting in high mortality rates. Such outbreaks in industrialised countries have become uncommon. Symptoms include diarrhoea, stomach ache, fever and vomiting.

- The Enterotoxigenic *E. coli* (ETEC) produce a heat-labile (ST) or a heat-stable (LT) enterotoxin or both. In addition they possess colonisation factors that are specific for the host animal species and which enables the organisms to adhere to the epithelium of the small intestine. Symptoms include diarrhoea, stomach ache, fever and vomiting.

- The Enteroinvasive *E. coli* (EIEC) cause an illness identical to Shigella dysentery in patients of all agents. Symptoms include diarrhoea (with mucus and blood), stomach ache and fever.

- The Verotoxigenic *E. coli* (VTEC) produce one or two Vero cytotoxins (VT1 and VT2). VT1 is closely related to the so-called Shiga toxin produced by strains of *Shigella dysenteriae* 1 and is sometimes called Shiga-like toxin. VTEC organisms cause a range of symptoms from mild, watery diarrhoea to a severe diarrhoea with large amounts of fresh blood in the stool (heamorrhagic colitis). An important complication in children is the Haemolytic uraemic syndrome. Symptoms include diarrhoea (with blood), stomach ache and fever.

The most common groups of diarrhoea causing *E. coli* are given below:-

E. coli group	Common E. coli Serogroup
Enteropathogenic *E. coli* (EPEC)	O26, O44, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, O158
Enterotoxigenic *E. coli* (ETEC)	O6, O8, O15, O20, O25, O27, O63, O78, O85, O115, O148, O153, O159, O167
Enteroinvasive *E. coli* (EIEC)	O28, O29, O32, O42, O112, O124, O136, O143, O144, O152, O164
Verotoxigenic *E. coli* (VTEC)	O157

b. Antigenic characterisation of *Escherichia coli*

Members of the species *E. coli* possess three main types of surface antigen, the O (somatic), the K (capsular) and the H (flagellar) antigens. The O antigens are heat stable and upon which grouping of the organisms are based. The H and K antigens are heat labile and normally only the H antigens are used for identifying the serotype of an organism further. The term K antigen was originally used collectively for surface or capsular antigens and were divided into three classes L, A and B according to the effect of heat on the agglutinability, antigenicity and antibody binding power. The designations L, A and B are now no longer used and indeed the determination of K antigens is no longer deemed necessary for identification of *E. coli* serotype. Many of the antigens thought to be K antigens are now known not to be K antigens. K antigens are only used diagnostically for example with certain toxigenic *E. coli* of veterinary importance. These toxigenic *E. coli* have pili antigens which are antigenically different from those of other *E. coli*. The

pili are important not only for attachment and infection of the organism to the intestinal mucous epithelial cells but also associated with the organism's ability to produce enterotoxin. The K88 and 987P antigens occur in strains of *E. coli* associated with diarrhoeal disease in swine, and the K99 antigen with diarrhoeal disease in lambs and calves. Enertotoxigenic *E. coli* (ETEC) in humans also have colonisation factor antigens (CFA I, II , and E8775) and there may be others, but they are not routinely used for clinical diagnosis[2].

In 1947 Kauffmann first proposed a scheme for classifying *E. coli* on the basis of their O antigens. Currently more than 160 O, 90 K and 50 H antigens have been described, and this forms the basis for current serological testing of pathogenic *E. coli*.

c. MAST® ASSURE **Escherichia coli, Antisera: preparation and presentation.**

MAST® ASSURE Escherichia coli Antisera are prepared from rabbits hyperimmunised with standard strains of *E. coli* organisms possessing defined antigenic factors. All sera are heat inactivated at 56°C for 30 minutes, absorbed to remove cross-reacting agglutinins and filter sterilised. The **MAST®** ASSURE *Escherichia coli* Antisera provide a comprehensive range of O and H antisera for the determination of O and H antigens of E. coli. Antigens are identified normally by qualitative slide agglutination or by quantitative tube agglutination tests.

MAST® ASSURE Escherichia coli Antisera are provided as 2ml (or 5ml) amounts in vials with dropper attachments and contain 0.1% sodium azide as preservative. Supplied ready to use. This is sufficient for 50 (125) slide agglutination tests or 20 (50) tube agglutination tests.

2. Culture of *E. coli* - Preparation for Serology

E. coli belong to the order *Enterobacterales* and there is much cross-reaction and antigenic relationships between *E. coli* and other genera within this family particularly *Shigellae*. Hence it is important that organisms undergoing serological classification should be correctly identified as *E. coli* by morphological and biochemical features first.

3. Pathogenic *E. coli* O -Grouping and H -Typing Antisera Products

To determine the O-group of a *E. coli* isolate, polyvalent O-Grouping antisera should be used initially to narrow down the range before specific grouping sera are used. This is illustrated schematically in Figure 1 and according to methods as detailed in section 4.

MAST® ASSURE Escherichia coli Antisera are also available as convenient sets and comprise of individual bottles of stated antisera.

4. Procedures for Serogrouping and Typing, and Interpretation of Results

MAST® ASSURE Escherichia coli Antisera are intended for use in the identification of O antigens by qualitative slide agglutination, although they may be used in quantitative tube agglutination tests for confirmatory purposes.

Cultures of organisms identified as *E. coli* by their morphological and biochemical features may be serotyped by the following procedures. Also refer to figure 6 for a summary of the *E. coli* O-grouping and H-Typing procedures.

A. O-Antigen Grouping

a. Slide Agglutination of live organisms

1. Place two drops of sterile 0.85% saline solution (saline) onto a carefully cleaned microscope slide. The slide may be partitioned into several parts using a chinograph or glass pencil. With an inoculation loop or wire emulsify into each drop of saline a live cell colony from a fresh agar plate or slope culture to produce a distinct and uniform turbidity.

2. Place a drop of polyvalent antiserum onto one of the drops of emulsified isolate and to the other a drop of saline as a control.

Note: allow the antiserum to freefall from the dropper provided with the bottle. Do not contaminate the antiserum with organism.

3. Mix the reagents by tilting the slide back and forth for 60 seconds while viewing under indirect light against a dark background.

4. Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination) should be regarded as a positive result. An isolate producing a distinct positive reaction with a polyvalent antiserum is assumed to be an *E. coli* bearing one or more of the O antigenic factors represented by that antiserum. Using this information, further testing of the isolate should be conducted, as described in steps 1 - 3, with single factor O antisera to reveal the full O antigenic grouping of the isolate.
Always confirm the O grouping by slide agglutination on heat killed organisms (see below).

   In general the serogroups covered by polyvalent O antisera D1, D2 and D3 are considered to be enteropathogenic, serogroups covered by polyvalent sera D4, D5 and D6 are considered to be enterotoxigenic, and serogroups covered by polyvalent sera D7 and D8 are considered to be enteroinvasive. These represent the most common O antigenic groups encountered.

b. Slide Agglutination for heat killed organisms

If the live organisms give a positive reaction with a particular single factor O antiserum, prepare a dense cell suspension of the organism in 0.85% saline and heat the suspension to 100ºC for 60 minutes or autoclave at 121ºC for 15 minutes then repeat the agglutination test as above using single factor O antiserum on the heated cell suspension. This should be done to identify the O antigen type as distinct from the K antigen.

c. Interpretation of Results

If both live and heat killed cells of the bacterial isolate, which has been previously morphologically characterised as an *E. coli*, positively agglutinate with a single factor antiserum the organism should be regarded as a *E. coli* belonging to the sero-group represented by single factor antiserum. However if the live cells give a positive agglutination result while the heat killed cells do not, the O sero-group cannot be determined as represented by that single factor antiserum.

d. Tube Agglutination

Quantitative tube agglutination tests may be carried out according to the following method:-

1. The cell suspension should be prepared by removing colonies from a suitable pure agar culture or from a centrifuged and washed broth culture, and resuspending them in sterile saline or 0.5% formal saline to give a fairly light suspension (about 7.5×10^8 organisms per ml).

2. Heat the suspension for 1 hour at 100°C to obtain an O antigen suspension. Alternatively a heat treated 4-6 hour broth culture may be used.

3. Make serial dilutions of antisera in 0.5ml volumes of saline, from 1:10 to 1:1280. Round bottomed glass tubes approximately 9 × 85mm are most suitable.

4. To each tube add 0.5ml of the antigen suspension.

Note: this doubles the dilution of the antiserum.

5. A control tube should additionally be set up containing 0.5ml of antigen suspension and 0.5ml of saline.

6. Shake the tubes thoroughly and incubate the tubes at 50°C for 16-24 hours.

7. Examine the tubes for agglutination. Positive agglutination will appear as a clearing of the fluid with sediment that rises in a granular mass when the tube is flicked with the finger. The control tube should be cloudy and any sediment should resuspend on flicking. Agglutination titres of 1:20 are not counted as significant. Titres at or near that stated for the antiserum indicate that the antigen is of the O group stated by the antiserum that caused the agglutination.

B. H - Antigen Serotyping

The majority of *E. coli* strains are poorly motile when first isolated; hence it is normally necessary to passage them serially through several tubes of semi-solid agar motility medium to enhance motility and H antigen development. The number of passages required may vary with individual cultures.

Tube Agglutination Test

1. Allow the organism to pass through Craigie tubes with semi-solid nutrient medium 3-5 times. Then inoculate the passaged organism into a suitable nutrient broth tubes and incubate at 37ºC for 6-8 hours.

2. After incubation make a 1:2 dilution by adding an equal volume of 0.85% saline containing 1% (v/v) formalin to the culture.

3. Add 2 drops of the required type specific H serum into a small test tube, and add 0.45 - 0.5ml of the treated organism suspension it. As a control prepare a similar tube which contains only the antigen suspension.

4. Shake the contents of the test tube thoroughly and allow the tubes to stand in a water bath at 50-52ºC for 1 hour.

5. Observe the tubes for spontaneous and distinct agglutination seen easily with the naked eye. Do not shake them as this will disturb the agglutination pattern. An isolate producing a distinct positive reaction is assumed to be an *E. coli* bearing the H antigenic factors represented by that antiserum.

Quantitative tube agglutination tests may be performed as described in section d above
(O - Antigen Grouping, Tube Agglutination Procedure).

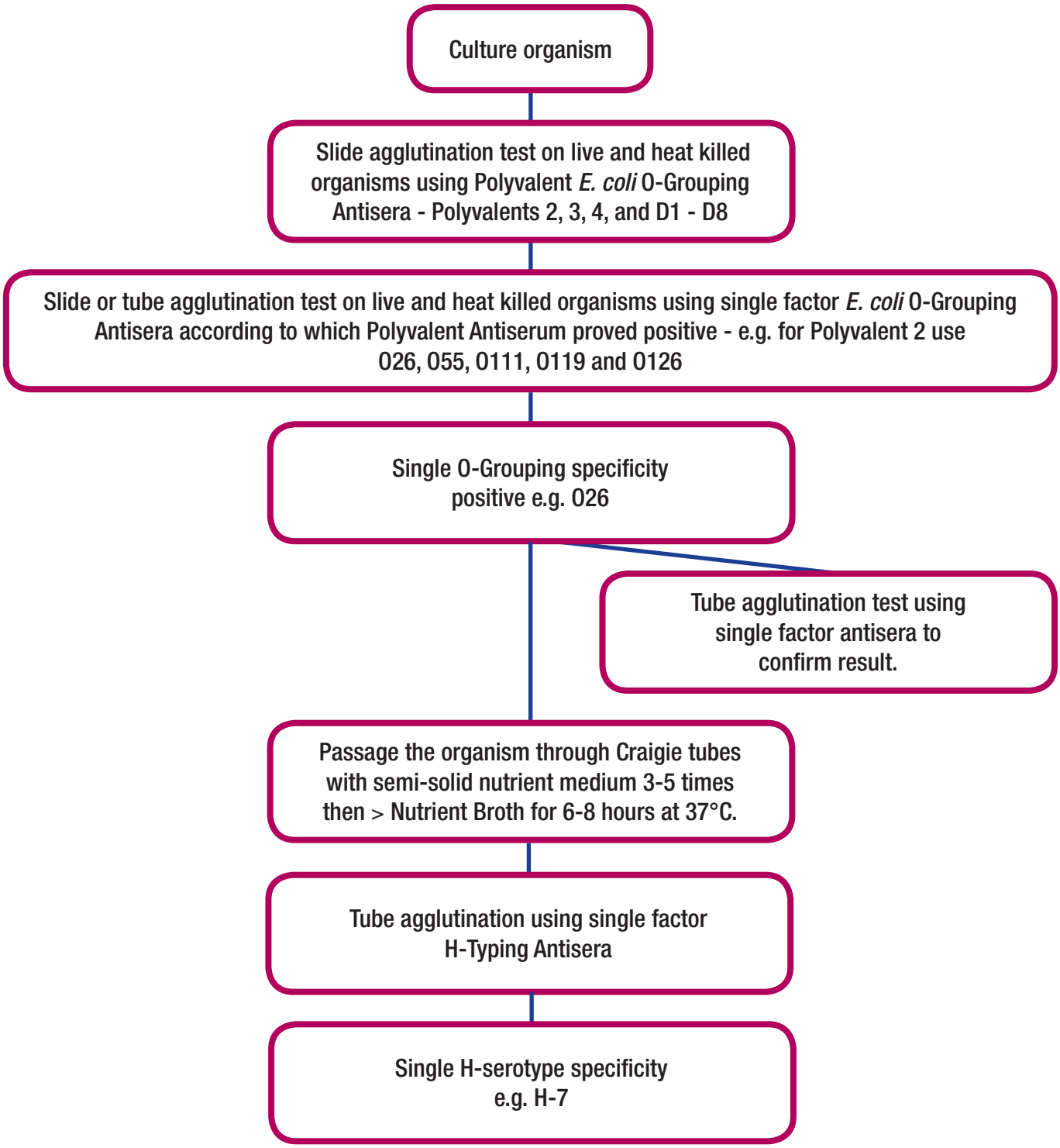
Limitations of the Test

1. The test results obtained when using these sera cannot determine whether a particular organism is pathogenic. To determine pathogenicity it is necessary to test for factors associated with pathogenicity e.g. toxins.

2. In general the serotypes covered by polyvalent sera 1, 2 and 3 are considered to be enteropathogenic, serotypes covered by polyvalent sera 4, 5 and 6 are considered to be enterotoxigenic, and serotypes covered by polyvalent sera 7 and 8 are considered to be enteroinvasive. However, it must be noted that there are other pathogenic *E. coli* that are not covered by these antisera.

3. Organisms must be morphologically and biochemically characterised as being an *E. coli* before testing with the antisera as cross-reactions with closely related members of the Enterobacterales may occur.

Figure 1- Summary of Pathogenic *E. coli* O-Grouping and H-Serotyping Procedures



This document does not replace the IFU's intended for use with these products. It is primarily aimed at being a resource for education, training and a guide for a laboratory writing Standard Operating Procedures looking at the more traditional methods associated with bacterial agglutinating sera

References available upon request.

MAST® ASSURE

SHIGELLA ANTISERA

A. **MAST®** ASSURE **Shigella Antisera**

1. Introduction

a. Shigella and Disease

The genus *Shigella* is a group of Gram negative organisms belonging to the order *Enterobacterales* and is closely related to the genus *Escherichia*. They are non-spore forming rods that possess no flagella and hence are non-motile.

Shigellae are the causative agents of classic bacilliary dysentery. The disease is usually spread via the faecal-oral route, though occasional epidemics have been traced to unchlorinated water supplies. The disease is endemic in most warm-climate countries and is also prevalent in many countries with a temperate climate. In the UK and other countries outbreaks are common in institutions and schools where more lax sanitation allows spread of organism.

The disease varies in severity according to species and serotype of the causal organism, but is characterised by severe blood stained, mucopurulent diarrhoea accompanied by fever and malaise. A brief episode of watery diarrhoea often precedes the severe bloody flux. In the more virulent forms such as that caused be *Shigella dysenteriae* symptoms such as severe water and electrolyte loss from the small intestine are caused by toxin production, and indeed the infectious dose of organisms required to produce disease is small. After reaching the large intestine the organisms multiply in the gut lumen where many adhere to and invade epithelial cells of the gut mucosa. Organisms then multiply within the epithelial cells and spread to adjacent cells and patches of necrotic epithelial cells slough off and ulcers form. A cellular immune response occurs resulting in the presence of large numbers of polymorphonuclear phagocytes, which can be readily observed on microscopic examination of the stool.

Laboratory diagnosis of Shigella infection usually depends upon the isolation and identification of the causal organism from a specimen of the patient's blood or faeces. Microscopic examination of the specimen is normally conducted to be able to distinguish bacterial dysentery from amoebic dysentery and to note the cellular exudate. Further antigenic analysis of the organism is often needed to identify the species or strain for epidemiological purposes.

b. Antigenic characterisation of Shigellae

Modern classification of Shigellae owe much to Ewing who built on the foundation of Murray, Andrews and Inman and Boyd whose recognition of type specific and group specific antigens introduced some system into the serological analysis of Shigellae. Shigellae are currently differentiated into four subgroups on the basis of their O (somatic) antigens and further differentiated into serotypes as detailed below:-

Group A - *Sh. dysenteriae*, contains 12 distinct antigenic serotypes.

Group B - *Sh. flexneri*, contains 6 serotypes (I-VI) that can be divided further into sub serotypes according to their possession of group factors designated 3,4; 4; 6; 7; and 7, 8 (see Table1).

Group C - *Sh. boydii*, contains 18 distinct antigenic serotypes.

Group D - *Sh. sonnei*, contains only one distinct serotype that may occur in two forms, phase I (smooth) and phase II (rough).

Table 1 - Antigenic Structure of *Sh. flexneri*

Serotype	Sub serotype	Type antigen	Group antigen*
1	1a	I	4
1	1b	I	4,6
2	2a	II	3,4
2	2b	II	7,8
3	3a	III	(3,4),6,7,8
3	3b	III	(3,4),6
4	4a	IV	3,4
4	4b	IV	6
5	5a	V	3,4
5	5b	V	7,8
6		VI	(4)
X variant		-	7,8
Y variant		-	3,4

*** Not all group antigens are listed.**

c. **MAST®** ASSURE **Shigella Antisera: preparation and presentation.**

MAST® ASSURE Shigella Antisera are prepared from rabbit's hyperimmunised with standard strains of Shigella organisms possessing defined antigenic factors. All sera are heat inactivated at 56°C for 30 minutes, absorbed to remove cross-reacting agglutinins and filter sterilised. The **MAST®** ASSURE Shigella Antisera are a comprehensive range of polyvalent and single specificity antisera for the agglutination of specific O serotype and group antigens of Shigellae. Antigens are identified normally by qualitative slide agglutination. Positive results may be confirmed by tube agglutination tests.

All **MAST®** ASSURE Shigella Antisera are provided as 2ml amounts in vials with dropper attachments and contain 0.1% sodium azide as preservative. Supplied ready to use. This is sufficient for 50 slide agglutination tests or 20 tube agglutination tests. Supplied ready to use with no preservative is added.

2. Culture of Shigella - Preparation for Serology

Shigella belongs to the order Enterobacterales and there is much cross-reaction and antigenic relationships between *Shigella* and other genera within this family especially between the genus *Escherichia*. Hence it is important that organisms undergoing serological classification should be correctly identified as Shigella by morphological and biochemical features first.

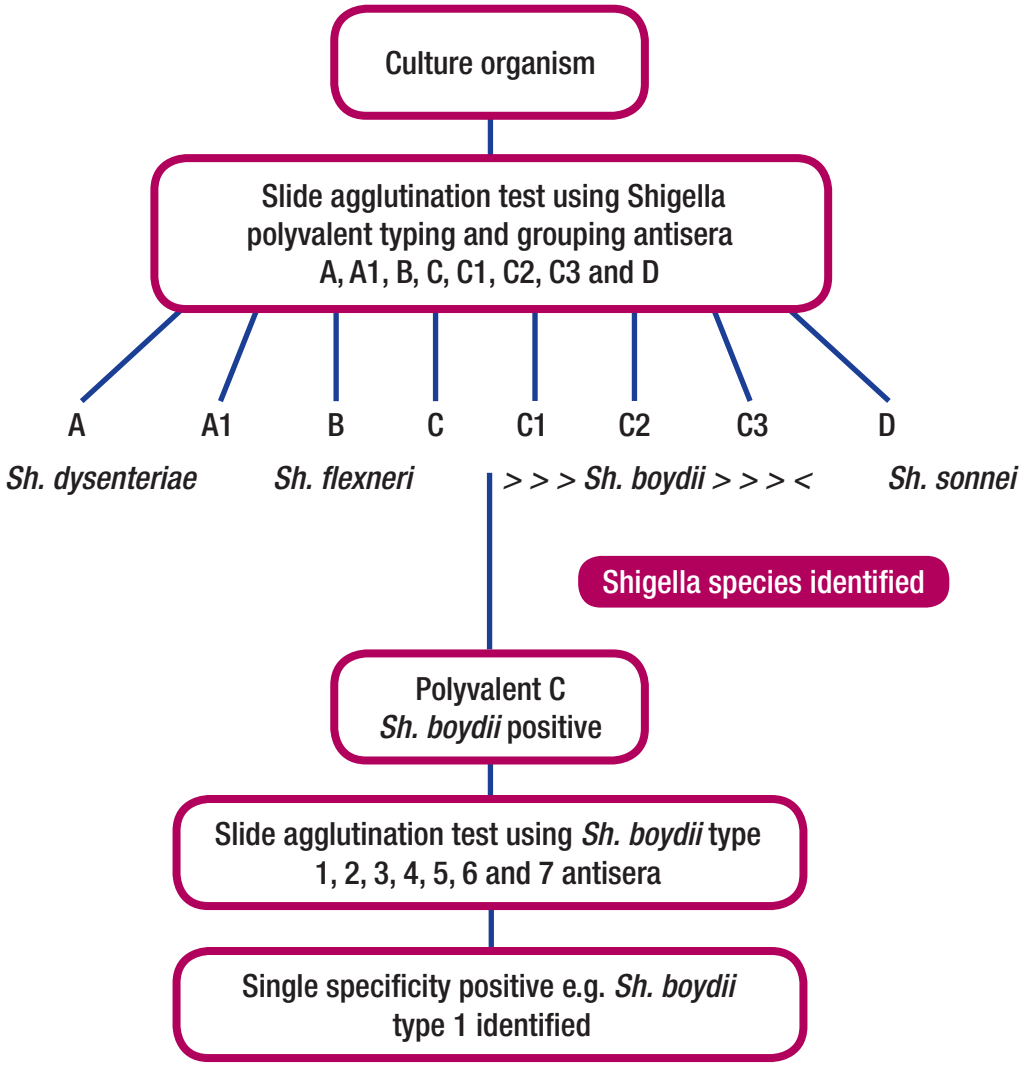
3. **MAST®** ASSURE **Shigella Typing and Grouping Antisera Products**

A wide range of **MAST®** ASSURE Shigella Typing and Grouping Antisera are available, including monovalent and polyvalent antisera. Antisera are also available as convenient sets and comprise of individual bottles of antisera.

MAST® ASSURE Shigella Typing and Grouping Antisera are intended for use in the identification of O antigens by qualitative slide agglutination.

Cultures of organisms identified as *Shigella sp* by their morphological and biochemical features may be serotyped by the following procedures. To determine the Type, Group or Phase of Shigella species isolate, polyvalent antisera should be used initially to narrow down the range before specific sera are used. This is illustrated schematically in Figure 1.

Figure 1 - Summary of Shigella Sero-grouping and Typing Procedures



4. Procedure for Serogrouping and Typing

1. Place two drops of sterile 0.85% saline solution (saline) onto a carefully cleaned microscope slide. The slide may be partitioned into several parts using a chinograph or glass pencil. With an inoculation loop or wire emulsify into each drop of saline a live cell colony from a fresh agar plate or slope culture to produce a distinct and uniform turbidity.

2. Place a drop of polyvalent antiserum onto one of the drops of emulsified isolate and to the other a drop of saline as a control.

Note: allow the antiserum to freefall from the dropper provided with the bottle. Do not contaminate the antiserum with organism.

3. Mix the reagents by tilting the slide back and forth for 60 seconds and while viewing under indirect light against a dark background.

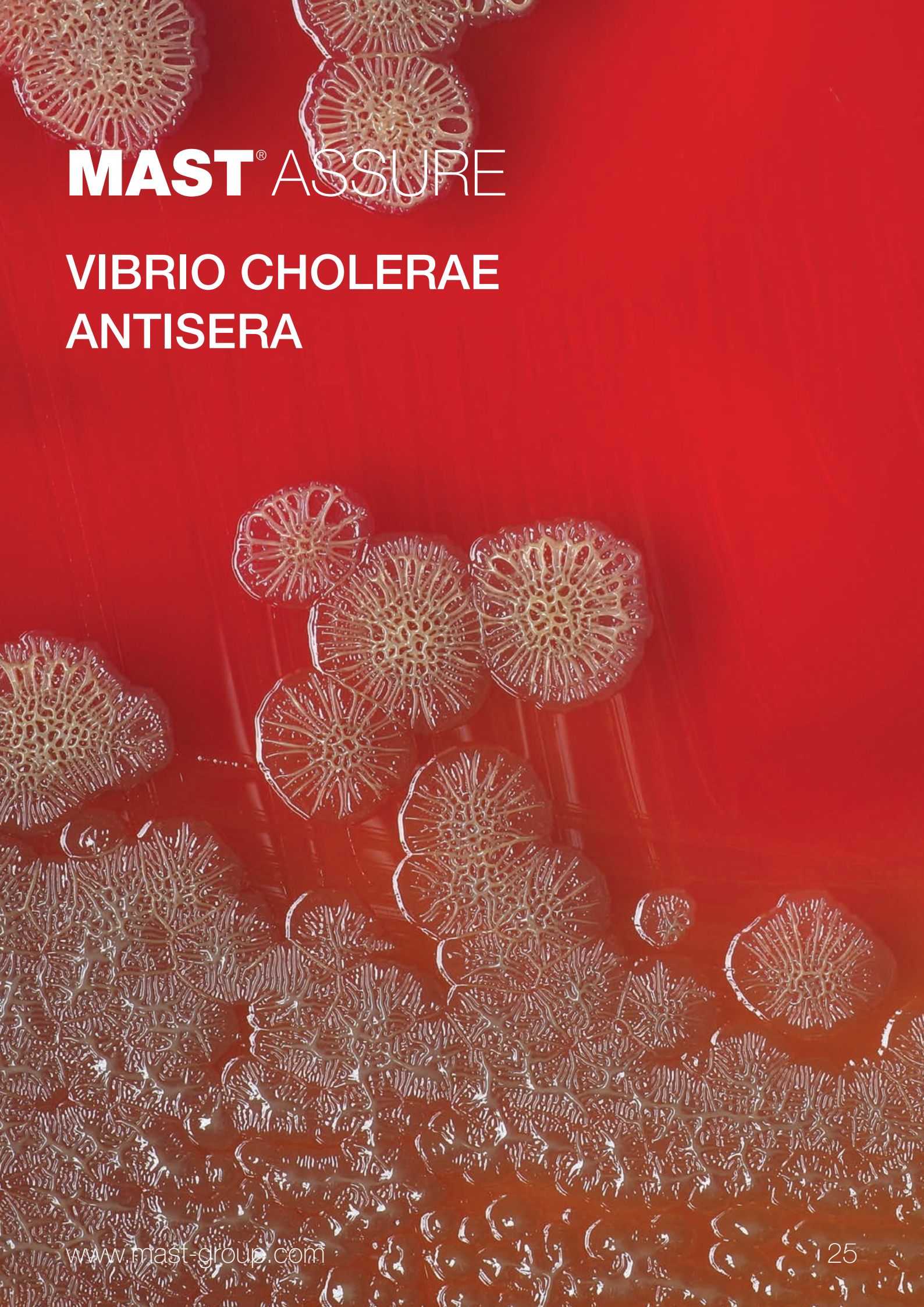
4. Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination) should be regarded as a positive result. Weak agglutination should be counted as negative.

   An isolate producing a distinct positive reaction with a polyvalent antiserum is assumed to be a *Shigella sp* from the letters (A-D) represented by the antiserum. Using this information, further testing of the isolate should be conducted, as described in steps 1 - 4, with monovalent antisera. If the organism is identified as *Sh. flexneri* it should be typed and grouped separately.

5. If the live cells do not produce positive agglutination, prepare a dense cell suspension of the organism in 0.85% saline and heat the suspension to 100°C for 60 minutes or autoclave at 121°C for 15 minutes then repeat the agglutination tests as described in steps 1 - 3. Some Shigellae strains possess heat labile capsular (K) antigens, which mask the presence of the heat stable somatic (O) antigens.

MAST® ASSURE

VIBRIO CHOLERAE ANTISERA



A. **MAST®** ASSURE **Vibrio cholerae Antisera**

1. Introduction

a. *Vibrio cholerae* and Disease

Vibrio cholerae belongs to the genus Vibrio which are Gram negative aerobic or facultative anaerobic comma shaped rods, they are fermentative and nearly all are oxidase positive. They are motile possessing a single polar flagellum.

Cholera is typically characterised by the sudden onset of effortless vomiting and profuse watery diarrhoea. Although vomiting is a common feature the rapid onset of dehydration and hypovalaemic shock, which may cause death in 12 - 24 hours, are related mainly to the profuse, watery, colourless stools with flecks of mucus and a distinctive fishy smell ('rice-water' stools) which contain little protein and are very different from the mucopurulent blood-stained stools of bacillary dysentery. Anuria develops, muscle cramps occur and the patient quickly becomes weak with loss of skin turgor, low blood pressure and an absent or thready pulse. Symptoms vary in severity and milder cases cannot be easily distinguished from other secretory diarrhoeas. Symptomless infections are also common.

Laboratory diagnosis involves culturing the vibrios from stool specimens in alkaline peptone water followed by surface inoculation onto a suitable solid medium such as thiosulphate-citrate-bile salts-sucrose agar (TCBS). On this medium *Vibrio cholerae* strains appear as yellow sucrose-fermenting colonies. Colonies may subsequently be assessed for oxidase production, biochemical confirmatory tests and agglutination by antisera.

b. Antigenic characterisation of *Vibrio cholerae*

Taxonomically *Vibrio cholerae* is a homogeneous species comprising of organisms that are similar to each other biochemically, share a common H (flagellar) antigen and are closely related genetically. The serology of *Vibrio cholerae* is based on the Somatic O antigen scheme of Sakazaki et al. Now more than 80 Serogroup have been identified within the species. Serogroup O1 organisms comprise the causal agents of most epidemic and pandemic cholera outbreaks. A series of pandemic outbreaks of O1 cholera originating in the Bengal basin ravaged the word in the 19th and early 20th centuries. The strain responsible for the seventh outbreak was labelled the El Tor biotype as it was isolated from pilgrims at the quarantine station known as El Tor. Other outbreaks have been attributed to the classical biotype strains.

Strains *of Vibrio cholerae* serotype O1 may be subdivided with absorbed antisera into serovars called Ogawa, Inaba and Hikojima. These serovars share three somatic antigens - a, b and c. Absorption of O1 antisera with Ogawa organisms produces a serum which agglutinates Inaba and Hikojima strains. Similarly, absorption of O1 antisera with Inaba organisms produces a serum which agglutinates Ogawa and Hikojima strains. Unabsorbed serum (containing a, b and c) called 'polyclonal *Vibrio cholerae* antiserum agglutinates all three O1 variants.

Non-O1 *Vibrio cholerae* cause mild, sometimes bloody diarrhoea, often accompanied by abdominal cramps. Symptoms may occasionally be severe in which case the disease resembles cholera. Also some non-O1 strains produce virulence factors including toxins. Hence it may be important for epidemiological purposes to serotype the strain in an isolate.

In the early 1990's a new serotype of *Vibrio cholerae* was found to be the causative agent of a pandemic in India and Bangladesh, and was named serotype O139. It gives rise to an infection as serious as those caused by serotype O1.

c. **MAST®** ASSURE **Vibrio cholerae Antisera: preparation and presentation.**

MAST® ASSURE Vibrio cholerae Antisera are prepared from rabbits hyperimmunised with standard strains of *Vibrio cholerae* O1 Inaba type and Ogawa type or O139 Bengal type organisms. All sera are heat inactivated at 56ºC for 30 minutes, absorbed to remove cross-reacting agglutinins and filter sterilised.

MAST® ASSURE Vibrio cholerae Antisera are a set of antisera for the agglutination of specific *Vibrio cholerae* O1 antigens and the O139 (Bengal) antigen. Antigens are identified normally by qualitative slide agglutination. Positive results may be confirmed by tube agglutination tests.

All **MAST®** ASSURE Vibrio cholerae Antisera are provided as 2ml amounts in vials with dropper attachments and contain 0.1% sodium azide as preservative. Supplied ready to use. This is sufficient for 50 slide agglutination tests or 20 tube agglutination tests. Supplied ready to use with no preservative is added.

2. Culture of *Vibrio cholerae* - Preparation for Serology

It is important that organisms undergoing serological classification should be correctly identified as *Vibrio cholerae* by morphological and biochemical features first.

3. MAST® ASSURE **Vibrio cholerae Antisera Products**

A selection of monovalent, polyvalent and sets of **MAST®** ASSURE Vibrio Cholerae Antisera are available.

4. Procedures for O Antigen Typing *Vibrio cholerae* Isolates and Interpretation of Results

Cultures of organisms identified as *Vibrio cholerae* by their morphological and biochemical features may be serotyped by the following procedures.

1. Place two drops of sterile 0.85% saline solution (saline) onto a carefully cleaned microscope slide. The slide may be partitioned into several parts using a chinograph or glass pencil. With an inoculation loop or wire emulsify into each drop of saline a live cell colony from a fresh agar plate or slope culture to produce a distinct and uniform turbidity.

2. Place a drop of polyvalent antiserum onto one of the drops of emulsified isolate and to the other a drop of saline as a control.

Note: allow the antiserum to freefall from the dropper provided with the bottle. Do not contaminate the antiserum with organism.

3. Mix the reagents by tilting the slide back and forth for 60 seconds while viewing under indirect light against a dark background.
4. Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination) should be regarded as a positive result.

5. Specimens that show agglutination only with Inaba-type serum should be reported as *Vibrio cholerae* O1 serovar Inaba and specimens that show agglutination only with Ogawa-type serum should be reported as *Vibrio cholerae* O1 serovar Ogawa. Specimens that show agglutination both types of serum should be reported as *Vibrio cholerae* O1 serovar Hikojima. Specimens that show agglutination only with O139 Bengal serum should be reported as *Vibrio cholerae* O139 Bengal.

Note:- it should be remembered that El Tor biotype of *Vibrio cholerae* O1 cannot be distinguished from the classical biotype by serological means.

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