

E. coli
Salmonella
Haemophilus

mastassure™

Bacterial Agglutinating Antisera Guide

Shigella

Vibrio

Campylobacter

Bordetella

Clostridium

Legionella

Pseudomonas

Staphylococcus

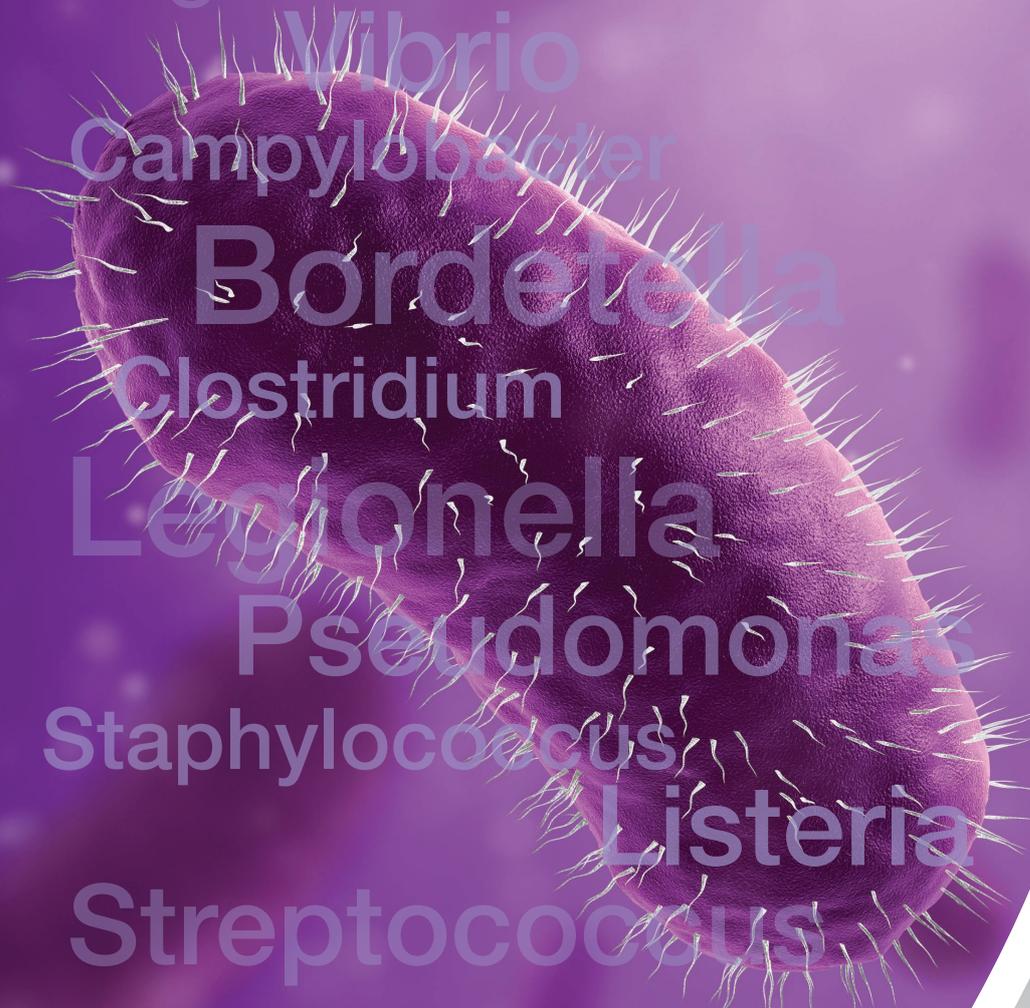
Listeria

Streptococcus

Yersinia

Proteus

Brucella



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References available upon request.

A scanning electron micrograph (SEM) showing several salmonella bacteria. The bacteria are rod-shaped and covered in a dense, green, fuzzy layer of flagella. They are positioned on a red, textured surface that appears to be a biological membrane or tissue. The background is out of focus, showing more of the same red surface and other bacteria.

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SALMONELLA ANTISERA

A. MAST ASSURE™ Salmonella Antisera

1. Introduction

a. Salmonella and Disease

Salmonella are Gram negative organisms belonging to the family *Enterobacteriaceae*. 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 Schema 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 family *Enterobacteriaceae* 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 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 E₁ was originally classified as O3, 10, Group E₂ as O3,15 and Group E₃ 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.

Table 2 lists all the MAST ASSURE™ Salmonella O Grouping Antisera (and Vi Antiserum) available to date together with the product codes, the Kauffmann-White alphabetical group equivalent and the actual antigenic agglutinins present. It is impractical to provide single factor sera for all known Salmonella antigens, however a wide range of sera is available in the MAST ASSURE™ range which is sufficient to identify with a reasonable degree of probability the majority of Salmonella types isolated.

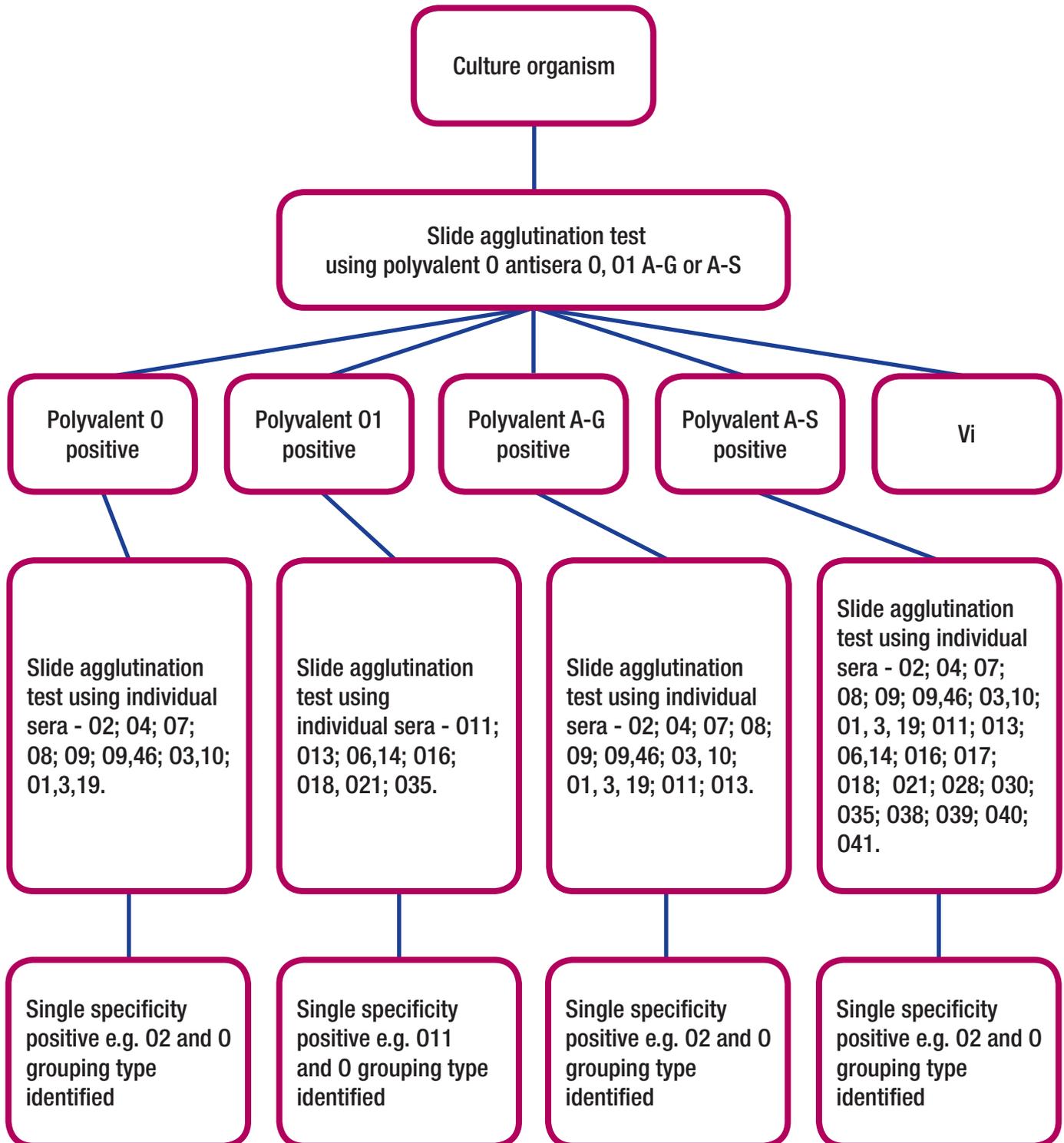
To determine the O-group of a Salmonella strain, polyvalent O antisera should be used initially to narrow down the range before specific grouping sera are used. This is illustrated schematically in Figure 1 and according to methods as detailed in section 4.

Table 2 - MAST ASSURE™ Salmonella O-Grouping Antisera Details

Mast Product Code	MAST ASSURE™ Salmonella O Antiserum	Kauffmann-White alphabetical grouping equivalent	Agglutinins present
M10308	Polyvalent O	A - E	O2, 3, 4, 5, 7, 8, 9, 10, 12, 15, 19, 34, 46.
M10309	Polyvalent O1	F - O excluding J, M and N.	O11, 13, 14, 16, 18, 21, 22, 23, 24, 35.
M14294	Polyvalent A-G	A -G	O2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 15, 19, 22, 34, 46.
M14300	Polyvalent A-S	A -S	O2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 28, 30, 34, 35, 38, 39, 40, 41, 46.
M10310	O2	A	O2
M10311	O4	B	O4, 5
M10312	O7	C1 + C4	O7
M10313	O8	C2 + C3	O8
M10314	O9	D	O9
M10315*	O9, 46	D2	O46
M10316	O3, 10	E1 + E2 + E3 *	O10, 15, 34
M10318*	O1, 3, 19	E4	O19
M10319*	O11	F	O11
M10320	O13	G1 + G2	O13, 22, 23
M10321	O6, 14	H	O14, 24, 25
M10322*	O16	I	O16
M10323*	O18	K	O18
M10324*	O21	L	O21
M10325*	O35	O	O35
M10326	Vi	-	Vi

* Denotes an 8 week lead time

Figure 1 - Summary of Salmonella O-Grouping Procedures



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References available upon request.

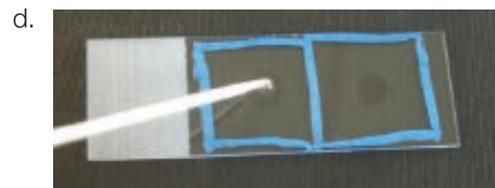
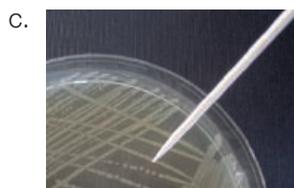
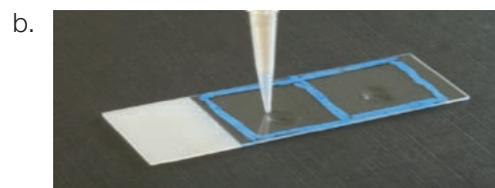
4. Procedures for O serotyping and Interpretation of Results

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.

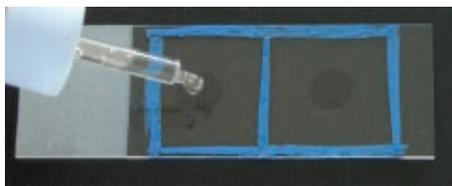
Cultures of organisms identified as *Salmonella* by their morphological and biochemical features may be serotyped by the following procedures. Also refer to figure 2 for a summary of the Salmonella O grouping procedure.

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.



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, z₁₅

G complex - contains g and one or more of f, m, p, q, s, t, u, z₅₁, (z₅₂, z₆₂, z₆₃)

L complex - contains l and one or more of v, w, z₁₃, z₂₈, (z₄₀)

z₄ complex - contains z₄ and one or more of z₂₃, z₂₄, z₃₂

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 z₆ 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. A list of the MAST ASSURE™ Salmonella H-Typing Antisera available is given in Table 3.

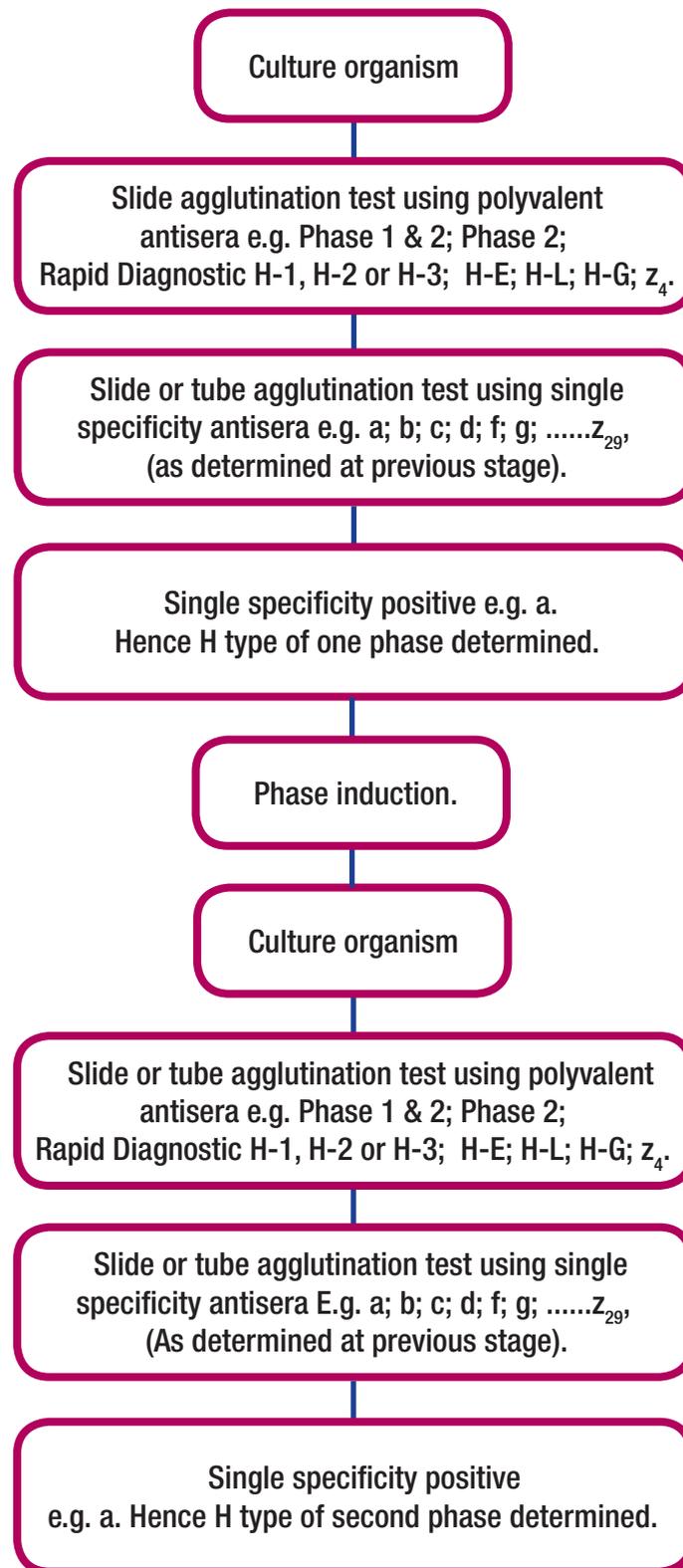
To determine the H-type of a Salmonella strain, polyvalent H antisera should be used initially to narrow down the range before specific typing sera are used. This is illustrated schematically in Figure 2 and according to methods as detailed in section 6.

Table 3 - MAST ASSURE™ Salmonella H-Typing Antisera Details

Mast Product Code	MAST ASSURE™ Salmonella H Antiserum	Agglutinins present	Comments
M14317	Polyvalent Phase 1 & 2	a-z ₂₉	
M10339	Polyvalent Phase 2 (H-1)	1, 2, 5, 6, 7, z ₆	
M14324	Rapid Diagnostic 1	b; d; E (n, x, h); r	
M14331	Rapid Diagnostic 2	d; E (n, x, h); k; L (l, v, w)	
M14348	Rapid Diagnostic 3	d; E (n, x, h); G (g, m, s, t); k	
M10327	H - a	a	
M10328	H - b	b	
M10329	H - c	c	
M10330	H - d	d	
M14335	H - E	n, x, h	
M10331	H - e, h	h	A factor of H - E
M10338*	H - e, n	n, x.	A factor of H - E
M10376*	H - x	x	A factor of H - E and H - e, n
M10377*	H - z ₁₅	z ₁₅	A factor of H - E and H - e, n
M10332	H - G	g, m, s, t	
M10364	H - f	f	A factor of H - G
M10365	H - m	m	A factor of H - G
M10366	H - p	p	A factor of H - G
M10367	H - q	q	A factor of H - G
M10368	H - s	s	A factor of H - G
M10369	H - t	t	A factor of H - G
M10370*	H - u	u	A factor of H - G
M10333	H - i	i	
M10334	H - k	k	
M10335	H - L	v, w,	
M10336	H - r	r	
M10337*	H - y	y	
M10378*	H - z	z	
M10379*	H - z ₄	z ₄ , z ₂₃ , z ₂₄	
M10372*	H - z ₂₃	z ₂₃	A factor of H - z ₄
M10373*	H - z ₂₄	z ₂₄	A factor of H - z ₄
M10374*	H - z ₃₂	z ₃₂	A factor of H - z ₄
M10380*	H - z ₁₀	z ₁₀	
M10381*	H - z ₂₉	z ₂₉	
M10344	H - 2	2	A factor of H - 1
M10345	H - 5	5	A factor of H - 1
M10346	H - 6	6	A factor of H - 1
M10348*	H - z ₆	z ₆	A factor of H - 1

* Denotes an 8 week lead time

Figure 2 - Summary of Salmonella H-Typing Procedures



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References available upon request.

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 be 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, z₆). 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 chimograph 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 floccular 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. A list of the MAST ASSURE™ Salmonella H Phase Induction Antisera available is given in Table 4.

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 37°C 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 37°C 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 37°C.
4. After incubation, remove the paper disc with sterile forceps and place it into glucose broth and incubate at 37°C 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 37°C 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.

Table 4 - MAST ASSURE™ Salmonella Phase Induction Antisera Details Non –CE marked, special order only, please check lead times

Mast Product Code	MAST ASSURE™ Salmonella H Antiserum	Agglutinins present
M10349/NCE	H - a	a
M10350/NCE	H - b	b
M10351/NCE	H - c	c
M10352/NCE	H - d	d
M10353/NCE	H - e, h	h
M10354/NCE	H - G	g, m, s, t
M10355/NCE	H - i	i
M10356/NCE	H - k	k
M10357/NCE	H - L	l, v, w
M10358/NCE	H - r	r
M10359/NCE	H - y	y
M10360/NCE	H - e, n	n, x
M10361/NCE	H - 1	1, 2, 5, z ₆
M10382/NCE	H - z	z
M10383/NCE	H - z ₄	Z ₄
M10384/NCE	H - z ₁₀	Z ₁₀
M10385/NCE	H - z ₂₉	Z ₂₉

9. Antisera Sets

MAST ASSURE™ Salmonella Antisera are also available as convenient sets as listed in Table 5 and comprise of individual bottles of stated antisera.

Table 5 - MAST ASSURE™ Salmonella Antisera Sets

Mast Product Code	MAST ASSURE™ Salmonella Antisera Set Components	Comments
M10303*	Salmonella Antisera Set 1 - This set consists of 17 O-Grouping + Vi antisera. i.e. Polyvalent O and O1, and O-Grouping sera O2; O4; O7; O8; O9; <u>O9, 46</u> ; <u>O3, 10</u> ; <u>O1, 3, 19</u> ; O11; O13; <u>O6, 14</u> ; O16; O18; O21; O35; and Vi	2ml × 18
M10304*	Salmonella Antisera Set 2 - This set consists of 17 H-Typing antisera. i.e. a; b; c; d; e, h; G; i; k; L; r; y; e, n; 1; z; z ₄ ; Z ₁₀ ; Z ₂₉	5ml × 17
M10386*	Salmonella Antisera Set 3 - This set consists of 4 H-L complex antisera. i.e. v; w; Z ₁₃ ; Z ₂₈	5ml × 4
M10387*	Salmonella Antisera Set 4 - This set consists of 5 H-1 complex antisera. i.e. 2; 5; 6; 7; z ₆	5ml × 5
M10388*	Salmonella Antisera Set 5 - This set consists of 7 H-G complex antisera. i.e. f; m; p; q; s; t; u	5ml × 7
M10389*	Salmonella Antisera Set 6 - This set consists of 3 H-z4 complex antisera and 2 H-e,n complex antisera. i.e. z ₂₃ ; Z ₂₄ ; Z ₃₂ ; X; Z ₁₅	5ml × 5
M10390*	Salmonella Antisera Set 7 - This set consists of O-Grouping O2 and O9 antisera, H-Typing antisera a and d, and Vi antiserum	2ml × 3 + 5ml × 2
M10391/NCE*	Salmonella H-sera for Phase Induction - This set consists of 17 H-Phase Induction antisera. i.e. a; b; c; d; e, h; G; i; k; L; r; y; e, n; 1; z; z ₄ ; Z ₁₀ ; Z ₂₉	5ml × 17

* Non CE marked, special order only, please check lead times

* Denotes an eight week lead time.

Bacterial Agglutinating Antisera

Order No

Product

Packsize

mastassure™

MAST ASSURE™ ANTISERA AVAILABLE FROM STOCK

SALMONELLA O ANTISERA - MONOVALENT

M10310	Salmonella O Factor O2	2ml
M10311	Salmonella O Factor O4	2ml
M10312	Salmonella O Factor O7	2ml
M10313	Salmonella O Factor O8	2ml
M10314	Salmonella O Factor O9	2ml
M10316	Salmonella O Factor O3,10	2ml
M10321	Salmonella O Factor O6,14	2ml
M10326	Salmonella O Factor Vi	2ml

SALMONELLA O ANTISERA - POLYVALENT

M10308	POLY O Factor O2, O4, O7, O8, O9, O9, 46, O3, 10 and O1,3,19	2ml
M10309	POLY O1 Factor O11, O13, O6, 14, O16, O18, O21, O35	2ml
M14294	POLY O A-G	2ml
M14300	POLY O A-S	2ml
M92537	Omnivalent (Kauffmann-White group A-067)	2ml

SALMONELLA H ANTISERA - MONOVALENT

M10327	Salmonella H Factor a	2ml
M10328	Salmonella H Factor b	2ml
M10329	Salmonella H Factor c	2ml
M10330	Salmonella H Factor d	2ml
M10331	Salmonella H Factor e, h	2ml
M14335	Salmonella H Factor G	2ml
M10332	Salmonella H Factor E	2ml
M10333	Salmonella H Factor i	2ml
M10344	Salmonella H Factor 2	2ml
M10345	Salmonella H Factor 5	2ml
M10346	Salmonella H Factor 6	2ml
M10364	Salmonella H Factor f	2ml
M10365	Salmonella H Factor m	2ml
M10366	Salmonella H Factor p	2ml
M10367	Salmonella H Factor q	2ml
M10368	Salmonella H Factor s	2ml
M10369	Salmonella H Factor t	2ml

SALMONELLA H ANTISERA - POLYVALENT

M14317	POLYVALENT PHASE 1&2 (a-z29)	2ml
M10339	POLYVALENT PHASE 2 (H-1)	2ml
M14324	RAPID DIAGNOSTIC 1 Factors b, d, E, r	2ml
M14331	RAPID DIAGNOSTIC 2 Factors b, E, k, l	2ml
M14348	RAPID DIAGNOSTIC 3 Factors d, E, G, k	2ml

mastassure™

**PATHOGENIC
ESCHERICHIA COLI ANTISERA**

A. MAST ASSURE™ Pathogenic Escherichia coli Antisera

1. Introduction

a. Escherichia coli and Disease

Escherichia coli (*E. coli*) is a group of Gram negative organisms belonging to the family *Enterobacteriaceae*. 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 pathogenic *E. coli* that cause diarrhoea, acute gastritis or colitis based on their pathogenic 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 (haemorrhagic 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:-

Pathogenic <i>E. coli</i> group	Common <i>E. coli</i> Serogroup
Enteropathogenic <i>E. coli</i> (EPEC)	O26, O44, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, O158
Enterotoxigenic <i>E. coli</i> (ETEC)	O6, O8, O15, O20, O25, O27, O63, O78, O85, O115, O148, O153, O159, O167
Enteroinvasive <i>E. coli</i> (EIEC)	O28, O29, O32, O42, O112, O124, O136, O143, O144, O152, O164
Verotoxigenic <i>E. coli</i> (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. Enterotoxigenic *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 diagnosis².

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™ Pathogenic *E. coli* Antisera: preparation and presentation.

MAST ASSURE™ Pathogenic *E. 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™ Pathogenic *E. coli* Antisera provide a comprehensive range of O and H antisera for the determination of O and H antigens of pathogenic *E. coli*. Antigens are identified normally by qualitative slide agglutination or by quantitative tube agglutination tests.

MAST ASSURE™ Pathogenic *E. 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 family Enterobacteriaceae 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

Table 1 lists the MAST ASSURE™ Polyvalent Pathogenic *E. coli* O-Grouping Antisera available together with the product codes, Table 2 lists the MAST ASSURE™ single factor Pathogenic *E. coli* O-Grouping Antisera available and Table 3 lists the MAST ASSURE™ Pathogenic *E. coli* H-Typing Antisera available. It is impractical to provide single factor sera for all known *E. coli* antigens, however a wide range of sera is available in the MAST ASSURE™ range which is sufficient to identify with a reasonable degree of probability the majority of *E. coli* types isolated.

To determine the O-group of a pathogenic *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™ Pathogenic *E. coli* Antisera are also available as convenient sets as listed in Table 4 and comprise of individual bottles of stated antisera.

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

MAST ASSURE™ Pathogenic *E. 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 Pathogenic *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 100°C for 60 minutes or autoclave at 121°C 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 pathogenic *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 37°C 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 50-52°C 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 Enterobacteriaceae may occur.

Table 1 - MAST ASSURE™ Pathogenic *E. coli* Polyvalent O Antisera Details

Mast Product Code	MAST ASSURE™ Pathogenic <i>E. coli</i> O Antisera	O serotypes detected
M12005*	<i>E. coli</i> O - Polyvalent D1	O1; O26; O86a; O111; O119; O127a, O128;
M12006*	<i>E. coli</i> O - Polyvalent D2	O44; O55; O125; O126; O146; O166
M12007*	<i>E. coli</i> O - Polyvalent D3	O18; O114; O142; O151; O157; O158
M12008*	<i>E. coli</i> O - Polyvalent D4	O6; O27; O78; O148; O159; O168
M12009*	<i>E. coli</i> O - Polyvalent D5	O20; O25; O63; O153; O167
M12010*	<i>E. coli</i> O - Polyvalent D6	O8; O15; O115; O169
M12011*	<i>E. coli</i> O - Polyvalent D7	O28ac; O112ac; O124; O136; O144
M12012*	<i>E. coli</i> O - Polyvalent D8	O29; O143; O152; O164
M14263	<i>E. coli</i> O - Polyvalent 2	O26; O55; O111; O119; O126
M14270	<i>E. coli</i> O - Polyvalent 3	O86; O114; O125; O127; O128
M14287	<i>E. coli</i> O - Polyvalent 4	O44; O112; O124; O142

* Denotes an eight week lead time.

Table 2 - MAST ASSURE™ Pathogenic *E. coli* Single Factor O-Grouping Antisera

Mast Product Code	<i>E. coli</i> O Serotype
M12013*	O1
M12014	O26
M12015*	O86a
M12016	O111
M12017	O119
M12018	O127a
M12019*	O128
M12020*	O44
M12021*	O55
M12022*	O125
M12023*	O126
M12024*	O146
M12025*	O166
M12026*	O18
M12027*	O114
M12028*	O142
M12029*	O151
M12030	O157
M12031*	O158
M12032*	O6
M12033*	O27
M12034*	O78
M12035*	O148
M12036*	O159
M12037*	O168
M12038*	O20
M12039*	O25
M12040*	O63
M12041*	O153
M12042*	O167
M12043*	O8
M12044*	O15
M12045*	O115
M12046*	O169
M12047*	O28ac
M12048*	O112ac
M12049*	O124
M12050*	O136
M12051*	O144
M12052*	O29
M12053*	O143
M12054*	O152
M12055*	O164

* Denotes an eight week lead time.

Table 3 - MAST ASSURE™ Pathogenic *E. coli* Single Factor H -Typing Antisera

Mast Product Code	<i>E. coli</i> H Serotype
M12056*	H-2
M12057*	H-4
M12058*	H-5
M12059*	H-6
M12060*	H-7
M12061*	H-9
M12062*	H-10
M12063*	H-11
M12064*	H-12
M12065*	H-16
M12066*	H-18
M12067*	H-19
M12068*	H-20
M12069*	H-21
M12070*	H-27
M12071*	H-28
M12072*	H-34
M12073*	H-40
M12074*	H-41
M12075*	H-42
M12076*	H-45
M12077*	H-51

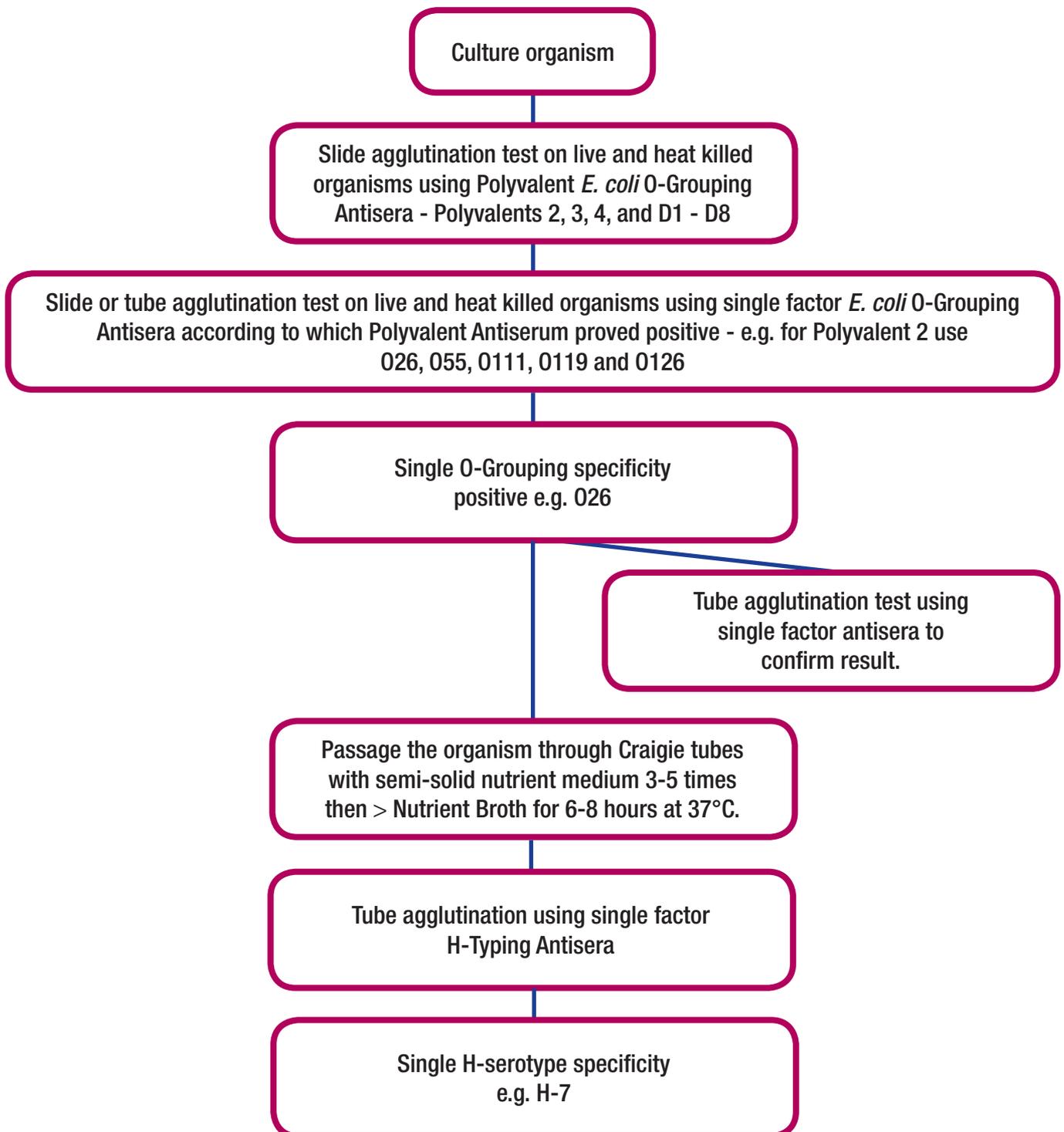
* Denotes an eight week lead time.

Table 4 - MAST ASSURE™ Pathogenic *E. coli* Polyvalent O-Grouping and H-Typing Antisera Sets

Mast Product Code	MAST ASSURE™ Pathogenic <i>E. coli</i> Antisera Set components	Comments
M12001*	<i>E. coli</i> O Antisera Set 1 - This set consists of 8 O-Grouping Polyvalent Antisera and 43 single factor O-Grouping antisera. i.e. Polyvalent. D1 - D8, and single factor sera O1; O26; O86a; O111; O119; O127a, O128; O44; O55; O125; O126; O146; O166; O18; O114; O142; O151; O157; O158; O6; O27; O78; O148; O159; O168; O20; O25; O63; O153; O167; O8; O15; O115; O169; O28ac; O112ac; O124; O136; O144; O29; O143; O152; O164.	2ml × 51
M12002*	<i>E. coli</i> O Antisera Set 2 - This set consists of 22 H-Typing Antisera. i.e. 2; 4; 5; 6; 7; 8; 9; 10; 11; 12; 16; 18; 19; 20; 21; 27; 28; 34; 40; 42; 45; 51	5ml × 22

* Denotes an eight week lead time.

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.

Bacterial Agglutinating Antisera

Order No

Product

Packsize

mastassure™

MAST ASSURE™ ANTISERA AVAILABLE FROM STOCK

E.COLI O ANTISERA - MONOVALENT

M12014	<i>Escherichia coli</i> Factor O26	2ml
M12016	<i>Escherichia coli</i> Factor O111	2ml
M12017	<i>Escherichia coli</i> Factor O119	2ml
M12018	<i>Escherichia coli</i> Factor O127a	2ml
M12030	<i>Escherichia coli</i> Factor O157	2ml

E.COLI O ANTISERA - POLYVALENT

AVAILABLE FROM STOCK

M14263	<i>E. coli</i> POLY 2 Factors O26, O55, O111, O119, O126	2ml
M14270	<i>E. coli</i> POLY 3 Factors O86, O114, O125, O127, O128	2ml
M14287	<i>E. coli</i> POLY 4 Factors O44, O112, O124, O142	2ml



mastassure™

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 family *Enterobacteriaceae* 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 bacillary 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 by *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 family *Enterobacteriaceae* 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

Table 2 lists the MAST ASSURE™ Shigella Typing and Grouping Antisera available, including monovalent and polyvalent antisera, together with the product codes. Antisera are also available as convenient sets and comprise of individual bottles of antisera as stated.

Table 2 - Mast Assure™ Shigella Grouping and Typing Antisera

Order Code	Product	Serotype
M10109	<i>Sh. dysenteriae</i> Polyvalent A	Types 1, 2, 3, 4, 5, 6 and 7
M10110	<i>Sh. dysenteriae</i> Polyvalent A1	Types 8, 9, 10, 11 and 12
M10116	<i>Sh. dysenteriae</i> type 1	Type 1
M10117*	<i>Sh. dysenteriae</i> type 2	Type 2
M10118*	<i>Sh. dysenteriae</i> type 3	Type 3
M10119*	<i>Sh. dysenteriae</i> type 4	Type 4
M10120*	<i>Sh. dysenteriae</i> type 5	Type 5
M10121*	<i>Sh. dysenteriae</i> type 6	Type 6
M10122*	<i>Sh. dysenteriae</i> type 7	Type 7
M10123*	<i>Sh. dysenteriae</i> type 8	Type 8
M10124*	<i>Sh. dysenteriae</i> type 9	Type 9
M10125*	<i>Sh. dysenteriae</i> type 10	Type 10
M10152*	<i>Sh. dysenteriae</i> type 11	Type 11
M10153*	<i>Sh. dysenteriae</i> type 12	Type 12
M10111	<i>Sh. flexneri</i> Polyvalent B	Types I, II, III, IV, V, and VI and groups (3) 4, 6 and 7 (8)
M10126	<i>Sh. flexneri</i> type I	Type I
M10127	<i>Sh. flexneri</i> type II	Type II
M10128*	<i>Sh. flexneri</i> type III	Type III
M10129*	<i>Sh. flexneri</i> type IV	Type IV
M10130	<i>Sh. flexneri</i> type V	Type V
M10131	<i>Sh. flexneri</i> type VI	Type VI
M10132*	<i>Sh. flexneri</i> group (3)4	Group (3) 4
M10133*	<i>Sh. flexneri</i> group 6	Group 6
M10134*	<i>Sh. flexneri</i> group 7(8)	Group 7 (8)
M10112	<i>Sh. boydii</i> Polyvalent C	Types 1, 2, 3, 4, 5, 6, and 7
M10113	<i>Sh. boydii</i> Polyvalent C1	Types 8, 9, 10 and 11
M10114	<i>Sh. boydii</i> Polyvalent C2	Types 12, 13, 14 and 15
M10154	<i>Sh. boydii</i> Polyvalent C3	Types 16, 17 and 18
M10135*	<i>Sh. boydii</i> type 1	Type 1
M10136	<i>Sh. boydii</i> type 2	Type 2
M10137*	<i>Sh. boydii</i> type 3	Type 3
M10138*	<i>Sh. boydii</i> type 4	Type 4
M10139*	<i>Sh. boydii</i> type 5	Type 5
M10140*	<i>Sh. boydii</i> type 6	Type 6
M10141*	<i>Sh. boydii</i> type 7	Type 7
M10142*	<i>Sh. boydii</i> type 8	Type 8
M10143*	<i>Sh. boydii</i> type 9	Type 9
M10144*	<i>Sh. boydii</i> type 10	Type 10
M10145*	<i>Sh. boydii</i> type 11	Type 11
M10146*	<i>Sh. boydii</i> type 12	Type 12
M10147*	<i>Sh. boydii</i> type 13	Type 13
M10148*	<i>Sh. boydii</i> type 14	Type 14
M10149*	<i>Sh. boydii</i> type 15	Type 15
M10155*	<i>Sh. boydii</i> type 16	Type 16
M10156*	<i>Sh. boydii</i> type 17	Type 17
M10157*	<i>Sh. boydii</i> type 18	Type 18
M10115	<i>Sh. sonnei</i> Polyvalent D	Phase I and II

* Denotes an eight week lead time.

Table 2 - Mast Assure™ Shigella Grouping and Typing Antisera (CONTINUED)

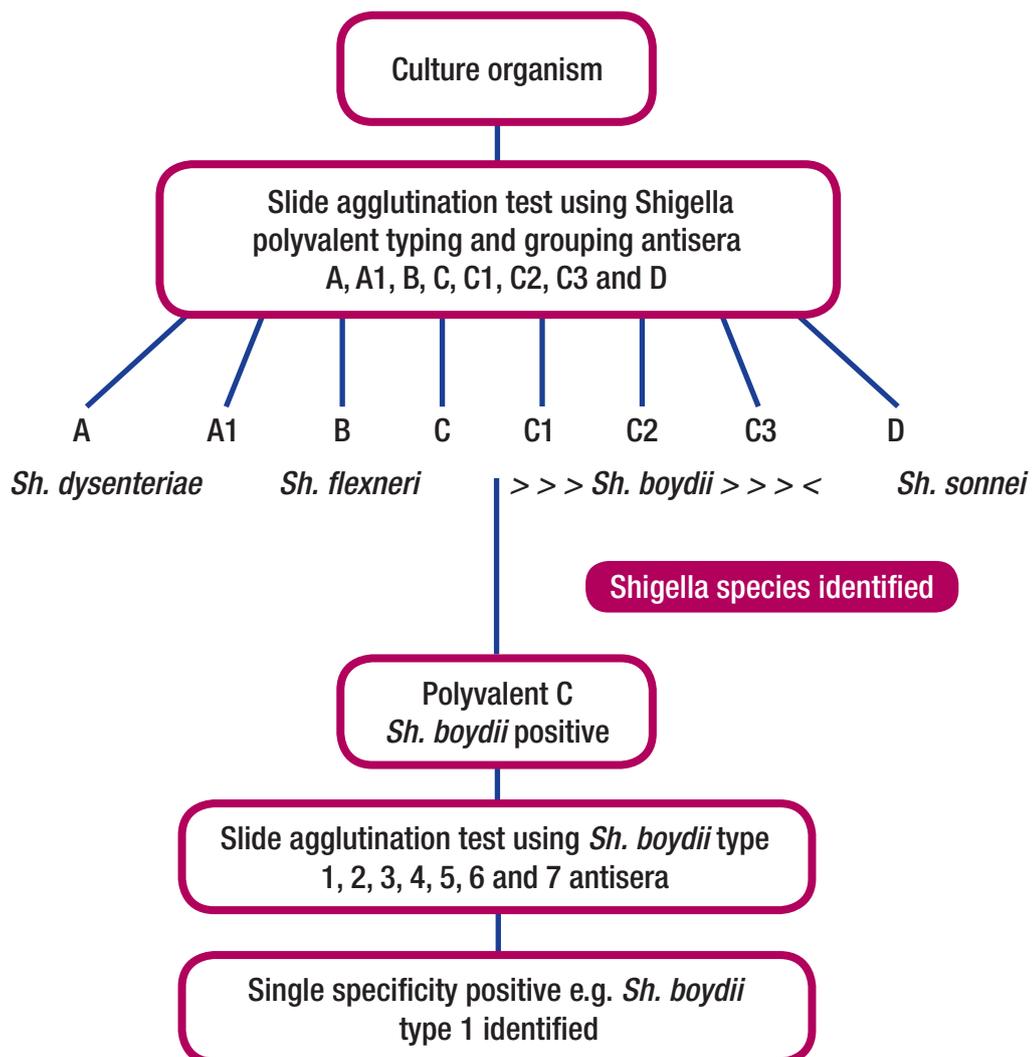
Order Code	Product	Serotype
M10150	<i>Sh. sonnei</i> phase I	Phase I
M10151	<i>Sh. sonnei</i> phase II	Phase II
M10104*	Shigella Antisera Set 1	8 Polyvalent - A, A1, B, C, C1, C2, C3, and D, and 41 monovalent sera constituting the above polyvalent sera.
M10105*	Shigella Antisera Set 2	8 Polyvalent - A, A1, B, C, C1, C2, C3, and D, and 11 monovalent sera covering all <i>Sh. flexneri</i> types and groups and <i>Sh. sonnei</i> phase I and II
M10106*	Shigella Antisera Set 3	8 Polyvalent sera - A, A1, B, C, C1, C2, C3, and D.

* Denotes an eight week lead time.

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* 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



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 chimograph 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* 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.

Bacterial Agglutinating Antisera

Order No	Product	Packsize
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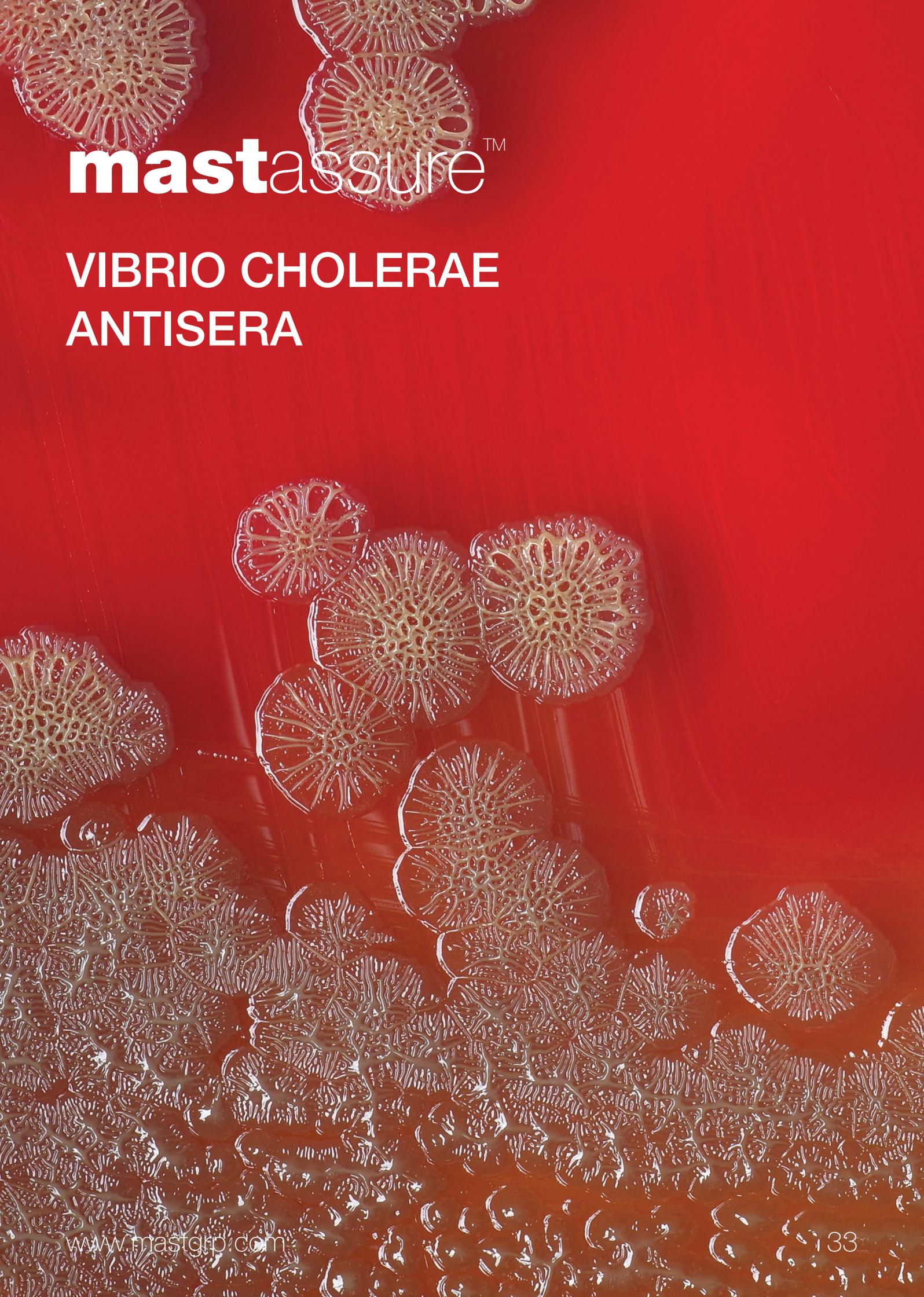
MAST ASSURE™ ANTISERA AVAILABLE FROM STOCK

SHIGELLA ANTISERA - MONOVALENT

M10116	<i>Shigella dysenteriae</i> Type 1	2ml
M10126	<i>Shigella flexneri</i> Type I	2ml
M10127	<i>Shigella flexneri</i> Type II	2ml
M10130	<i>Shigella flexneri</i> Type V	2ml
M10131	<i>Shigella flexneri</i> Type VI	2ml
M10136	<i>Shigella boydii</i> Type 2	2ml
M10150	<i>Shigella sonnei</i> Phase I	2ml
M10151	<i>Shigella sonnei</i> Phase II	2ml

SHIGELLA ANTISERA - POLYVALENT

M10109	<i>S.dysenteriae</i> POLY A Types 1, 2, 3, 4, 5, 6, 7	2ml
M10110	<i>S.dysenteriae</i> POLY A1 Types 8, 9, 10, 11, 12	2ml
M10111	<i>S.flexneri</i> POLY B Types I, II, III, IV, V, VI, (Groups (3)4, 6 & 7 (8)	2ml
M10112	<i>S.boydii</i> POLY C Types 1, 2, 3, 4, 5, 6, 7	2ml
M10113	<i>S.boydii</i> POLY C1 Types 8, 9, 10, 11	2ml
M10114	<i>S.boydii</i> POLY C2 Types 12, 13, 14, 15	2ml
M10154	<i>S.boydii</i> POLY C3 Types 16, 17, 18	2ml
M10115	<i>S.sonnei</i> POLY D Phase I & II	2ml



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**VIBRIO CHOLERAEE
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 hypovolaemic 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 world 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 56°C for 30 minutes, absorbed to remove cross-reacting agglutinins and filter sterilised.

MAST ASSURE™ - VIBRIO CHOLERAEE 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 CHOLERAEE 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

Table 1 lists the MAST ASSURE™ *Vibrio cholerae* Antisera available, including monovalent and polyvalent antisera, together with the product codes.

Table 1 - Mast Assure™ *Vibrio cholerae* Antisera

Order Code	Product
M11002	<i>Vibrio cholerae</i> Polyvalent (O1) - Serovar Inaba and Ogawa
M11003	<i>Vibrio cholerae</i> (O1) - Serovar Inaba
M11004	<i>Vibrio cholerae</i> (O1) - Serovar Ogawa
M15001	<i>Vibrio cholerae</i> O139 "Bengal"
M11001*	<i>Vibrio cholerae</i> (O1) Antisera Set consisting of 1X Polyvalent antiserum - Serovar Inaba and Ogawa 2X Monovalent antisera - Serovar Inaba and serovar Ogawa

* Denotes an eight week lead time.

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 chimograph 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.

IVD solutions through partnership

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Bacterial Agglutinating Antisera
and Febrile Antigens

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Haemophilus

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Bordetella
Clostridium
Legionella
Pseudomonas
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Streptococcus
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