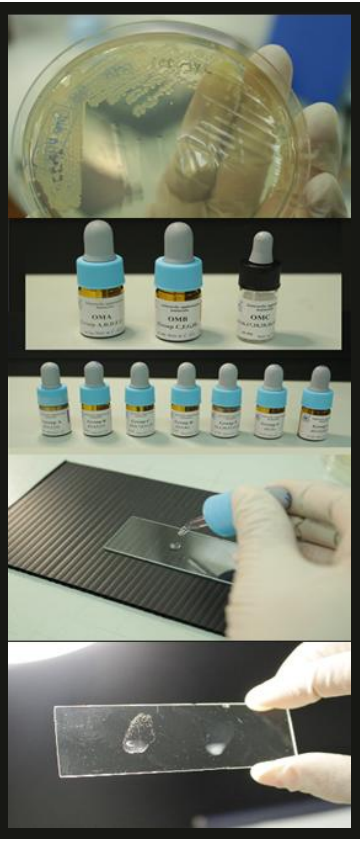
UKaid from the British people
The Fleming Fund Regional Grants
DTU
International Vaccine Institute
คณะสัตวแพทยศาสตร์
FACULTY OF VETERINARY SCIENCE
Chulalongkorn University

Salmonella serotyping using the Kauffman and White scheme

Prof. Rungtip Chuanchuen DVM, MS, PhD



Flow of *Salmonella* determination

Sample collection

↓

***Salmonella* isolation**

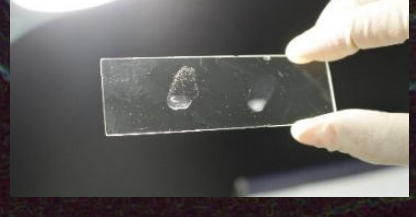
↓

***Salmonella* confirmation**

↓

***Salmonella* serotyping**

Antigen (*Salmonella*) + antiserum



ISO 6579-1:2017(E)

INTERNATIONAL STANDARD

ISO 6579-1

First edition
2017-02

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —

Part 1:
Detection of *Salmonella* spp.

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella —

Partie 1: Recherche des Salmonella *spp.*

ISO

Reference number
ISO 6579-1:2017(E)

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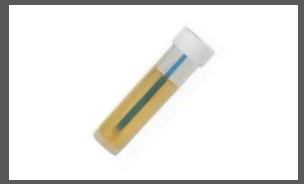
It is applicable to:

- products for human consumption & the feeding of animals

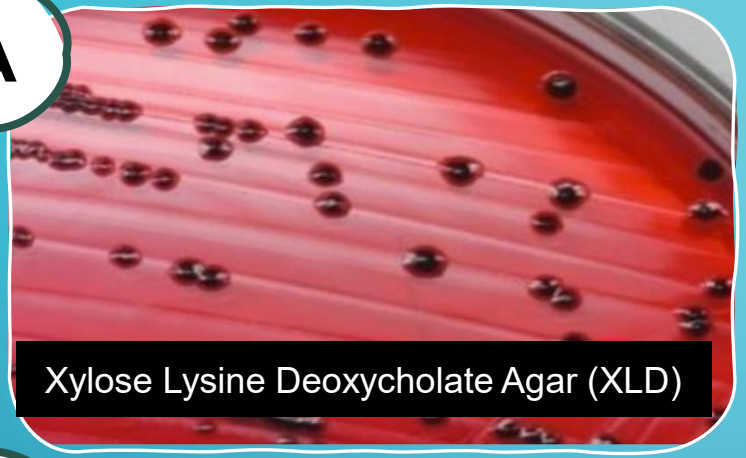


- environmental samples in the area of food production & food handling

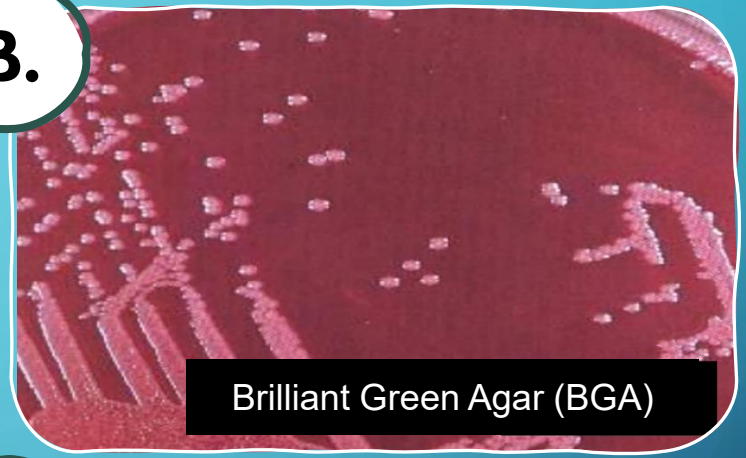
- samples from the primary production stage (e.g., animal faeces, dust & swabs).

2 + 3

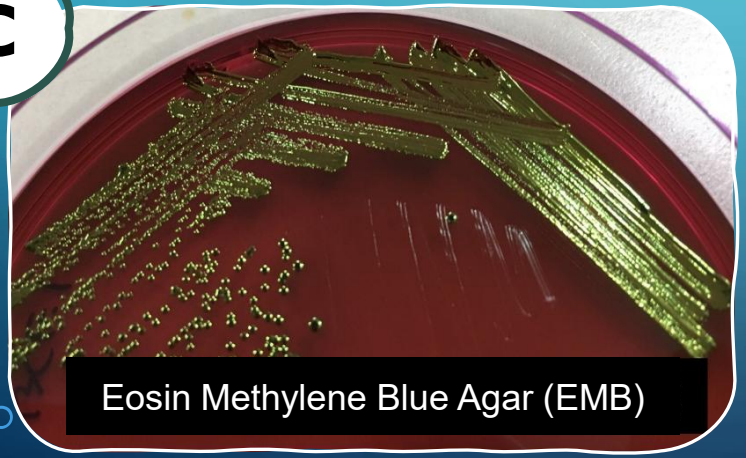
A


Xylose Lysine Deoxycholate Agar (XLD)

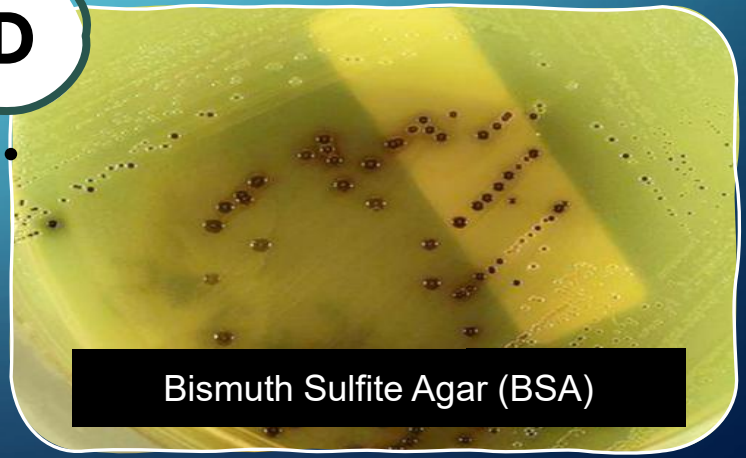
B.


Brilliant Green Agar (BGA)

C


Eosin Methylene Blue Agar (EMB)

D


Bismuth Sulfite Agar (BSA)

A

Yellow slant
Yellow butt
+ Gas
+ H_2S

B.

Red slant
Yellow butt
- Gas
- H_2S

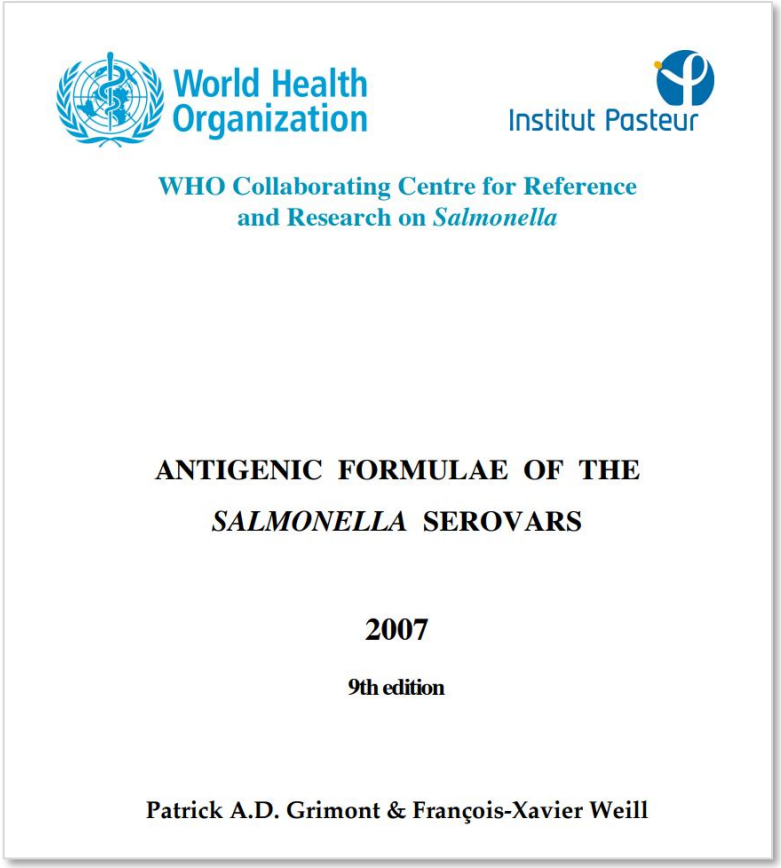
C

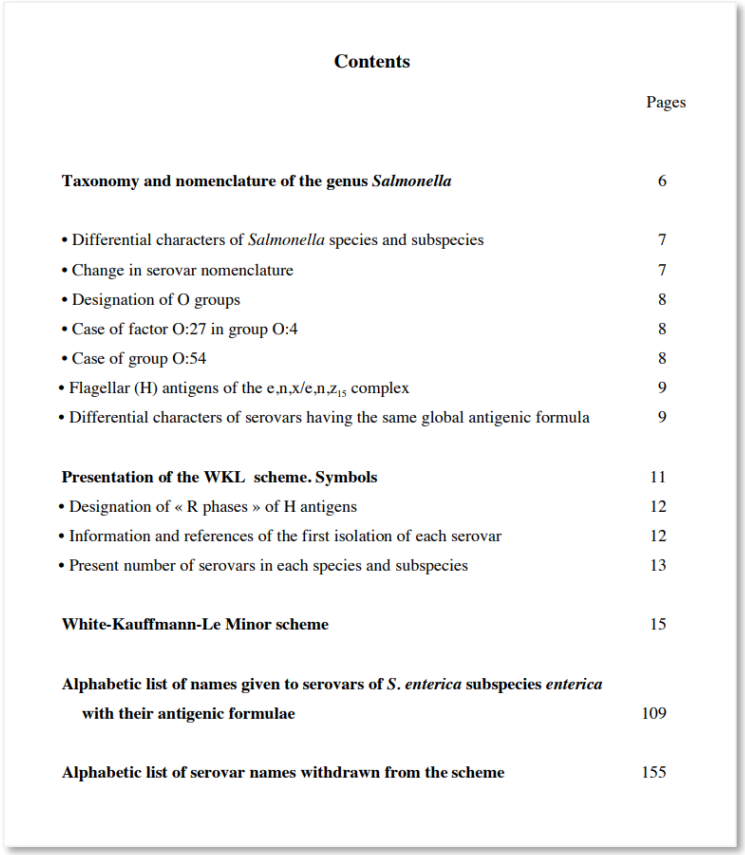
Red slant
Yellow butt
+ Gas
+ H_2S

D

Red slant
Red butt
- Gas
- H_2S

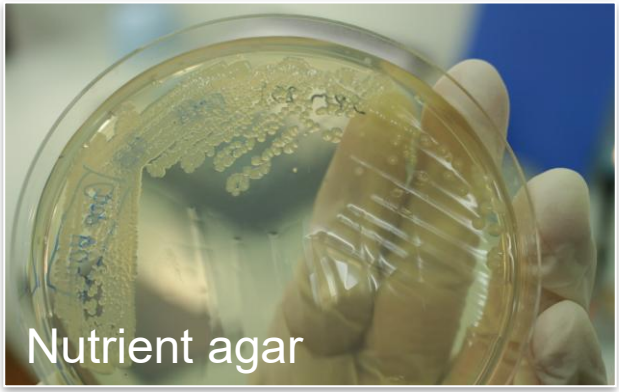
The Kauffman and White scheme

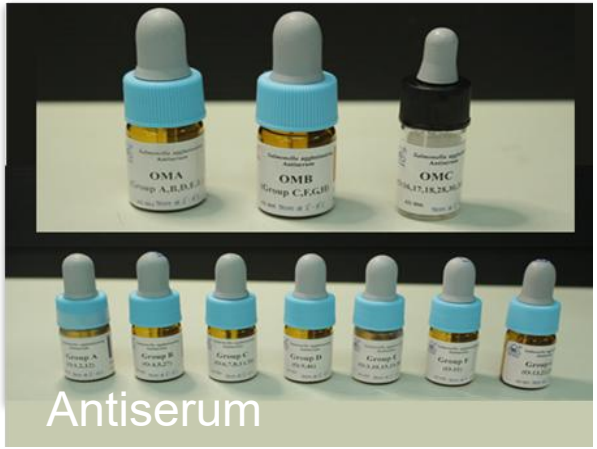




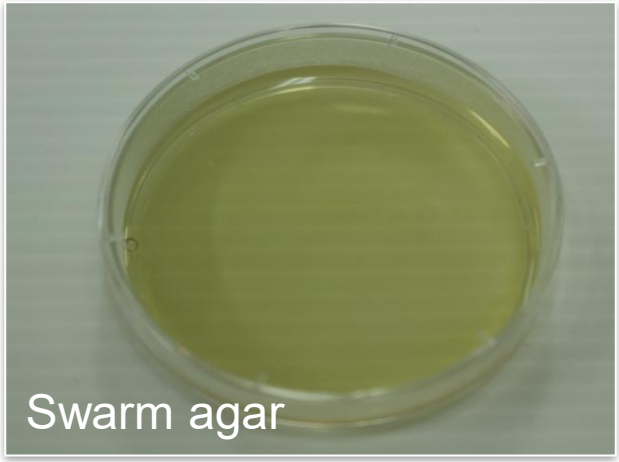
Contents

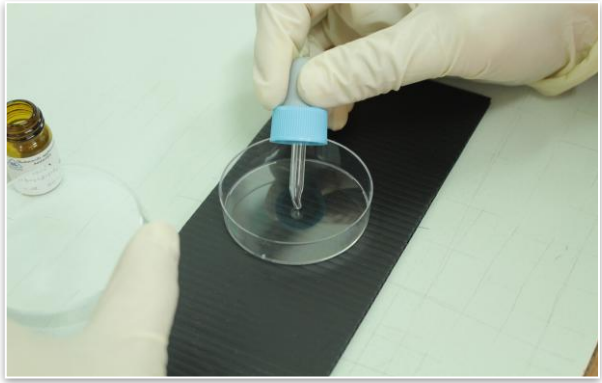
What do we need?


Nutrient agar



Antiserum


Swarm agar




Needle

What do we need?

There are 3 types:

- O antisera
- H antisera
- Vi antisera

Can be purchased from many microbiological reagent suppliers.

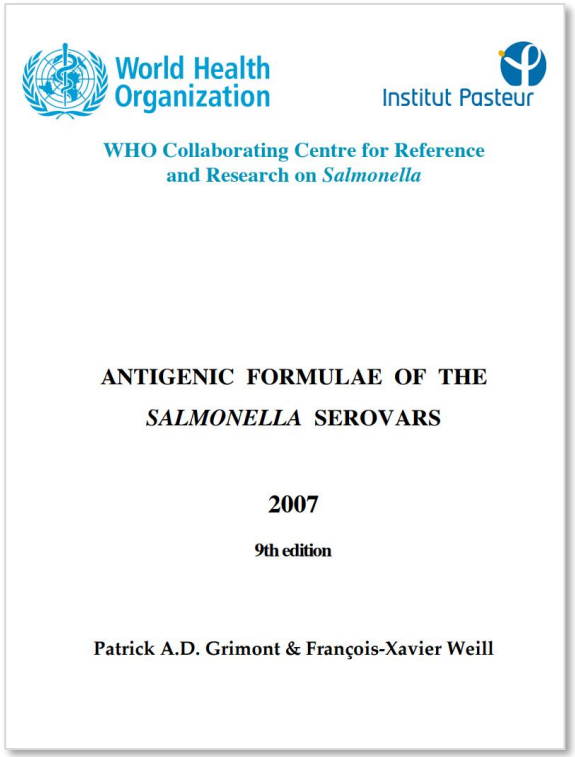
What do we need?

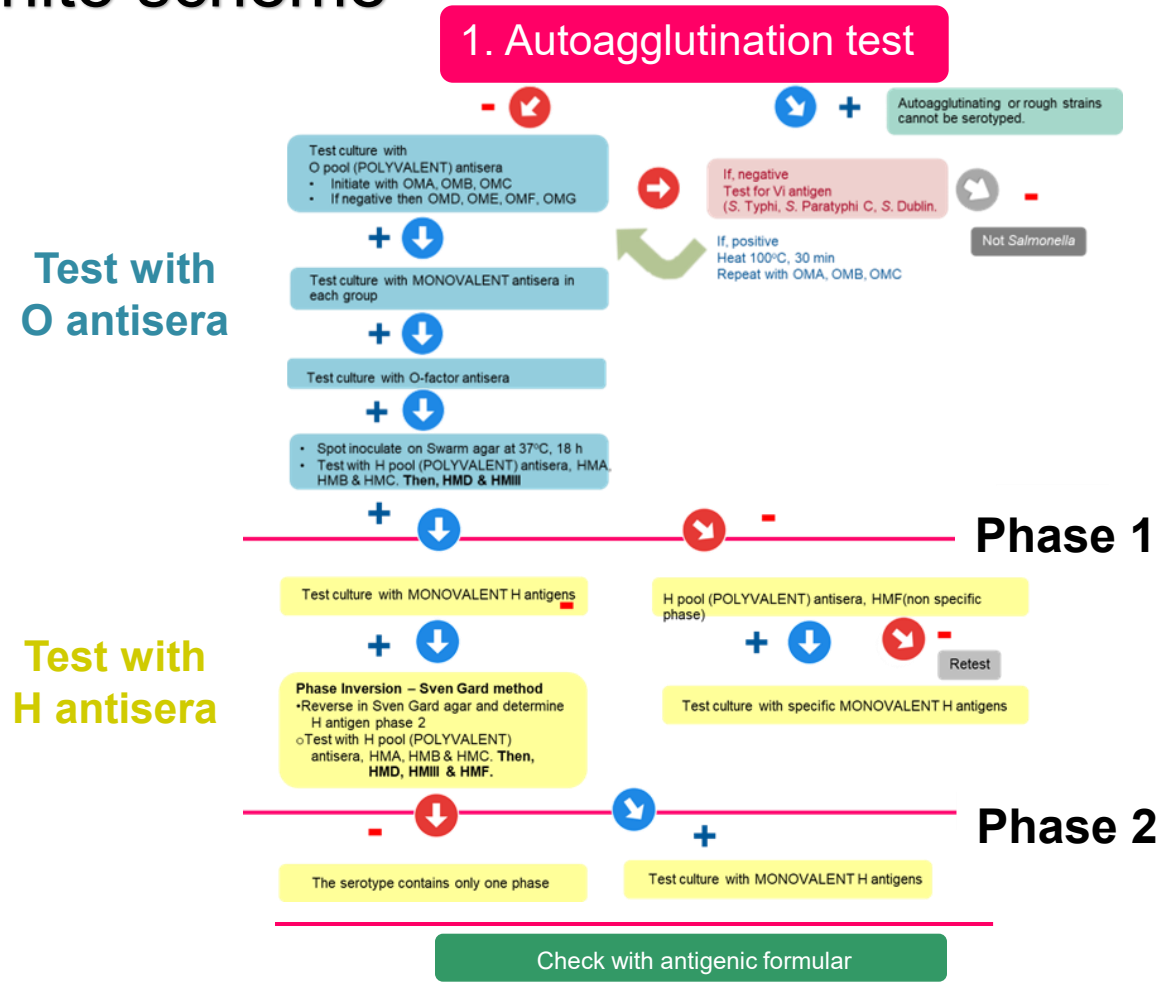
Chart of *Salmonella* pool antisera

OMA	=	O agglutinins of groups : 2 (A) – 4 (B) – 9 (D_1) – 9,46 (D_2) – 3,10 (E_1) – 1,3,19 (E_4) – 21 (L). (O:1,2,12; 4,5,12; 9,12; 9,46; 3,10; 1,3,19; 21).
OMB	=	O agglutinins of groups : 7 (C_1) – 8 (C_2 – C_3) – 11 (F) – 13 (G) – 6,14 (H). (O:6,7; 6,8; 8,20; 11; 13,22; 13,23; 6,14,24).
OMC	=	O agglutinins of groups : 16 , 17 , 18 , 28 , 30 , 35 , 38.
OMD	=	O agglutinins of groups : 39 , 40 , 41 , 42 , 43 , 44 , 45.
OME	=	O agglutinins of groups : 47 , 48 , 50 , 51 , 52 , 53 , 61.
OMF	=	O agglutinins of groups : 54 , 55 , 56 , 57 , 58 , 59.
OMG	=	O agglutinins of groups : 60 , 62 , 63 , 65 , 66 , 67.

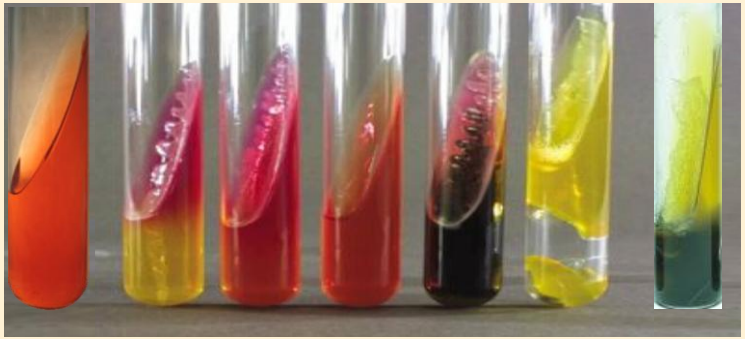
HMA	=	a, b, c, d, i, z_{10}, z_{29}
HMB	=	e,h; e,n,x; **G**
HMC	=	k, y, z, **L**, **Z_4**, r
HMD	=	z_{35}, z_{36}, z_{38}, z_{39}, z_{41}, z_{42}, z_{44}, z_{60}
HMII	=	z_{52}, z_{53}, z_{54}, z_{55}, z_{57}, z_{61} (H factors of subspecies III only).
H:H	=	Phase I , Phase II (all flagella)
H:**L**	=	Iv, Iw, Iz_{13}, Iz_{28}, $Iz_{13}Iz_{28}$
H:**G**	=	fg, fgt, gm, gms, gt, gpt, g……
H:E	=	eh, enx, enz_{15}, $enxz_{15}$, ……..
H:F	=	1,2; 1,5; 1,6; 1,7; z_6
H:1	=	1,2; 1,5; 1,6; 1,7
H:**Z_4**	=	z_4, z_4, z_{23}, z_4, z_{24}, z_4, z_{32}, z_4 ……

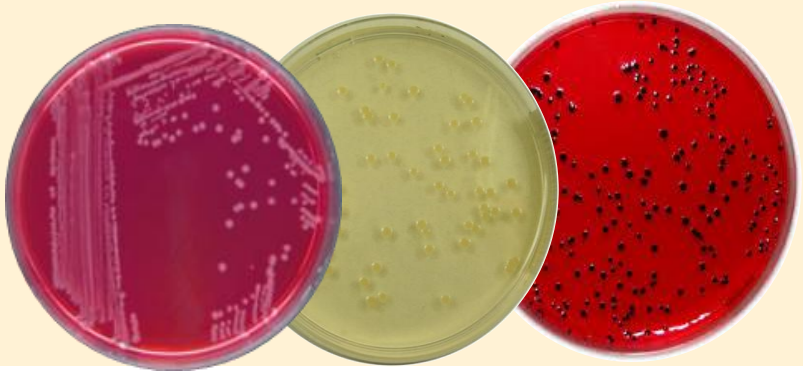
The Kauffman and White scheme





Before start, make sure that





- The isolates are verified by confirmatory biochemical tests.
- The isolates are well purified and there is no contamination.

Flow of the Kauffman and White scheme

1

1. Test culture for **auto-agglutination** in normal saline

\- → Autoagglutinating or rough strains cannot be serotyped. ← +

2

Test culture with
O pool (POLYVALENT) antisera
- Initiate with OMA, OMB, OMC
- If negative then OMD, OME, OMF, OMG

If, negative
Test for Vi antigen
(*S.* Typhi, *S.* Paratyphi C, *S.* Dublin.

\- → Not *Salmonella*

If, positive
Heat 100°C, 30 min
Repeat with OMA, OMB, OMC

\+

3

Test culture with MONOVALENT antisera in each group

\+

Test culture with O-factor antisera

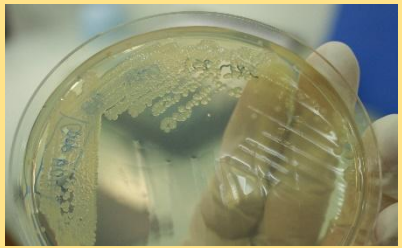
\+

4

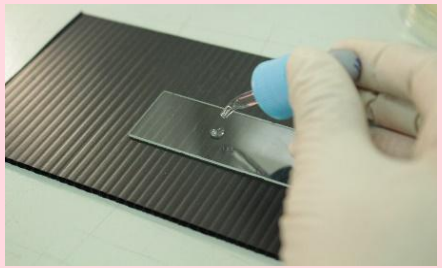
- Spot inoculate on Swarm agar at 37°C, 18 h
- Test with H pool (POLYVALENT) antisera, HMA, HMB & HMC. **Then, HMD & HMIII**

Phase 1

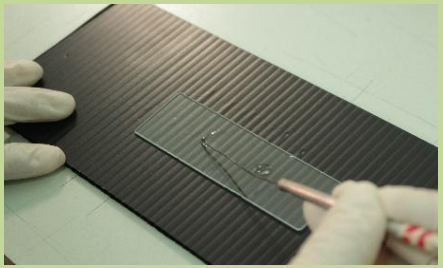
1 Auto-agglutination test



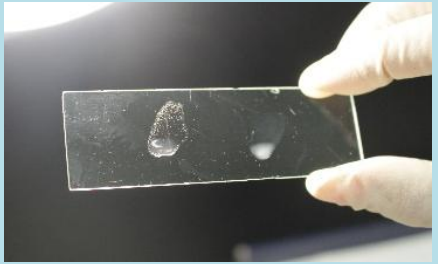
After confirmation, the colonies are purified.



Place one drop of **normal saline** onto a slide for agglutination



Add a pure colony and mix thoroughly



Rock the slide in the circular motion for 30 sec and observe agglutination

If isolate show positive reaction (agglutination), it cannot be serotyped

Note

- Agglutination should appear between 1-10 sec.
- If agglutination occurs >60 sec, the antigens cannot be identified correctly.

Flow of the Kauffman and White scheme

1

1. Test culture for auto-agglutination (in normal saline)

\- → 2

\+ → Autoagglutinating or rough strains cannot be serotyped.

2

Test culture with
O pool (POLYVALENT) antisera
- Initiate with OMA, OMB, OMC
- If negative then OMD, OME, OMF, OMG

If, negative
Test for Vi antigen
(*S.* Typhi, *S.* Paratyphi C, *S.* Dublin.)

\- → Not *Salmonella*

If, positive
Heat 100°C, 30 min
Repeat with OMA, OMB, OMC

3

Test culture with MONOVALENT antisera in each group

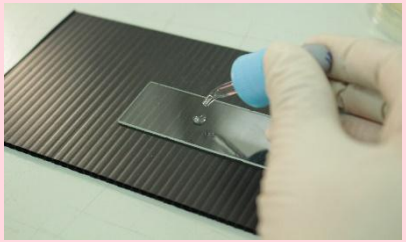
Test culture with O-factor antisera

4

- Spot inoculate on Swarm agar at 37°C, 18 h
- Test with H pool (POLYVALENT) antisera, HMA, HMB & HMC. **Then, HMD & HMIII**

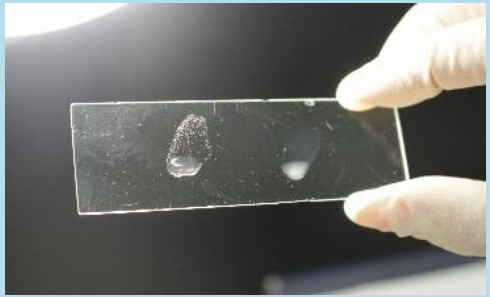
Phase 1

2 O antigen testing



Place one drop of **O antiserum** onto a slide for agglutination

Add a pure colony and mix thoroughly



Rock the slide in the circular motion for 30 sec and observe agglutination

If isolate show positive reaction (agglutination), continue testing with monovalent and O factor antisera.

Note

- Agglutination should appear between 1-10 sec.
- If agglutination occurs >60 sec, the antigens cannot be identified correctly.

Flow of the Kauffman and White scheme

1

1. Test culture for auto-agglutination (in normal saline)

\- → Autoagglutinating or rough strains cannot be serotyped.

\+

2

Test culture with
O pool (POLYVALENT) antisera
- Initiate with OMA, OMB, OMC
- If negative then OMD, OME, OMF, OMG

\+

If, negative
Test for Vi antigen
(*S.* Typhi, *S.* Paratyphi C, *S.* Dublin.

\- → Not *Salmonella*

If, positive
Heat 100°C, 30 min

3

Test culture with MONOVALENT antisera in each group

\+

Test culture with O-factor antisera

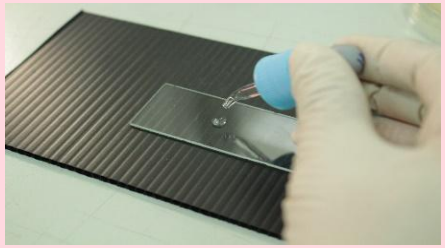
\+

4

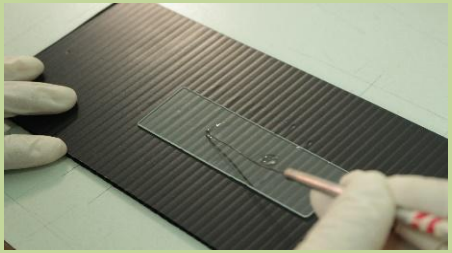
- Spot inoculate on Swarm agar at 37°C, 18 h
- Test with H pool (POLYVALENT) antisera, HMA, HMB & HMC. **Then, HMD & HMIII**

Phase 1

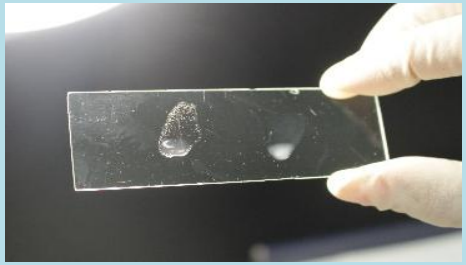
3 Testing with MONOVALENT and O factor antisera



Place one drop of **O factor antiserum** onto a slide for agglutination



Add a pure colony and mix thoroughly



Rock the slide in the circular motion for 30 sec and observe agglutination

Note

- Agglutination should appear between 1-10 sec.
- If agglutination occurs >60 sec, the antigens cannot be identified correctly.

Flow of the Kauffman and White scheme

1

1. Test culture for auto-agglutination (in normal saline)

\- ↙

↘ + Autoagglutinating or rough strains cannot be serotyped.

2

Test culture with
O pool (POLYVALENT) antisera
- Initiate with OMA, OMB, OMC
- If negative then OMD, OME, OMF, OMG

→ If, negative
Test for Vi antigen
(*S.* Typhi, *S.* Paratyphi C, *S.* Dublin.

↘ - Not *Salmonella*

If, positive
Heat 100°C, 30 min
Repeat with OMA, OMB, OMC

\+ ↓

3

Test culture with MONOVALENT antisera in each group

\+ ↓

Test culture with O-factor antisera

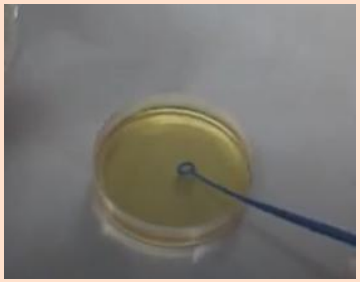
\+ ↓

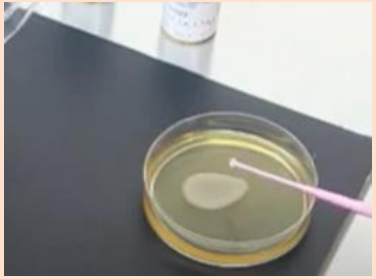
4

- Spot inoculate on Swarm agar at 37°C, 18 h
- Test with H pool (POLYVALENT) antisera, HMA, HMB & HMC. **Then, HMD & HMIII**

Phase 1

4 H antigen testing using polyvalent & monovalent antisera

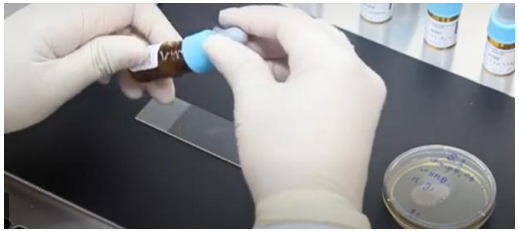


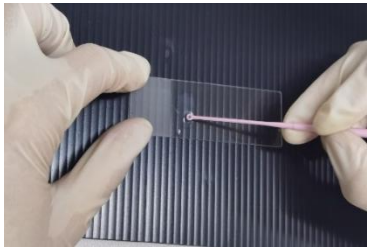


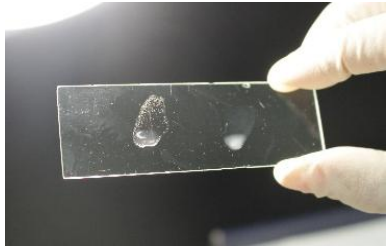
Spot a colony on to swam agar at 37˚C, 18 hours

Phase 1 testing

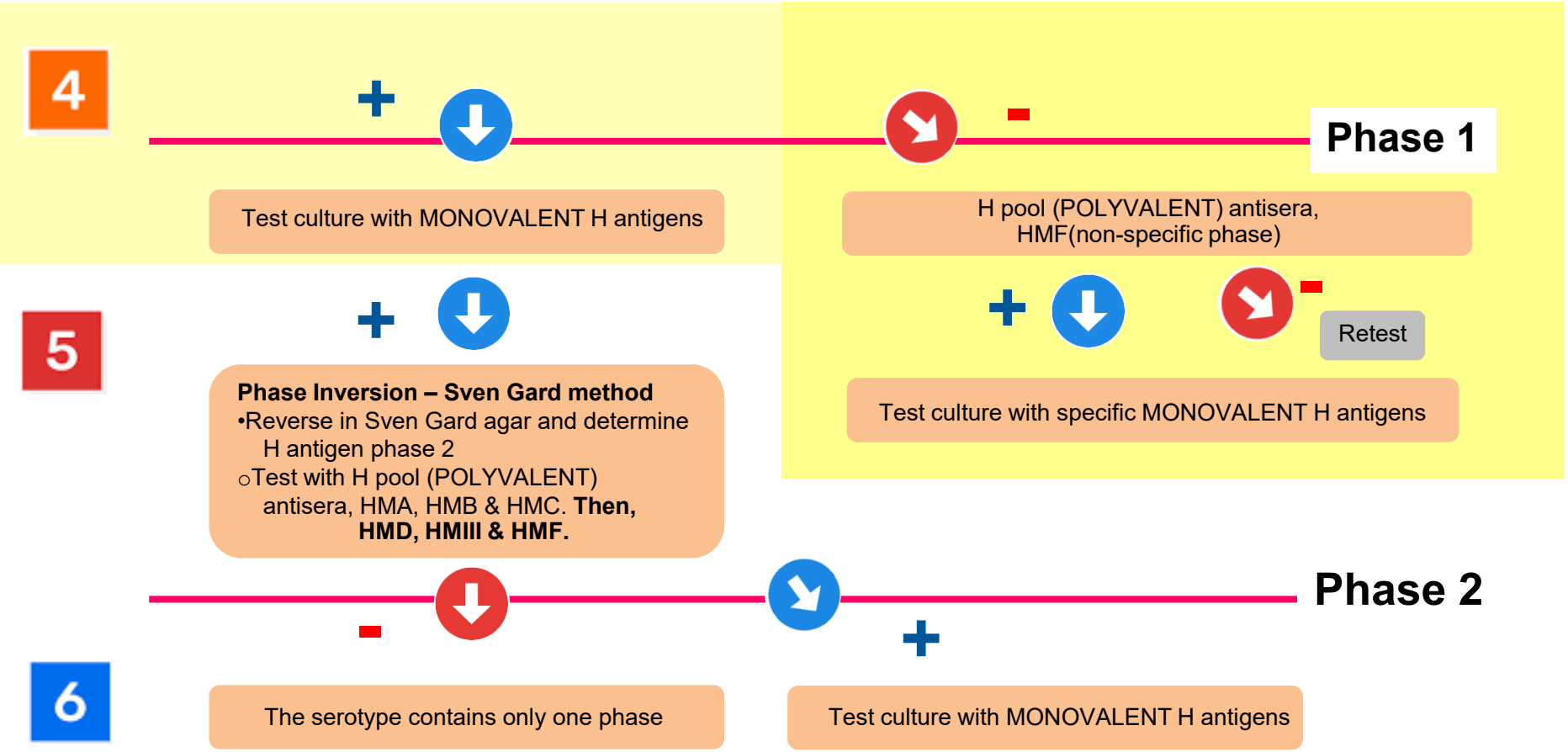
- Test culture with H Pool POLYVALENT antisera (HMA, HMB, HMC, HMD)
  - If negative, test culture with HMF(non-specific phase) antisera
  - If positive, test culture with MONOVALENT antisera in each group



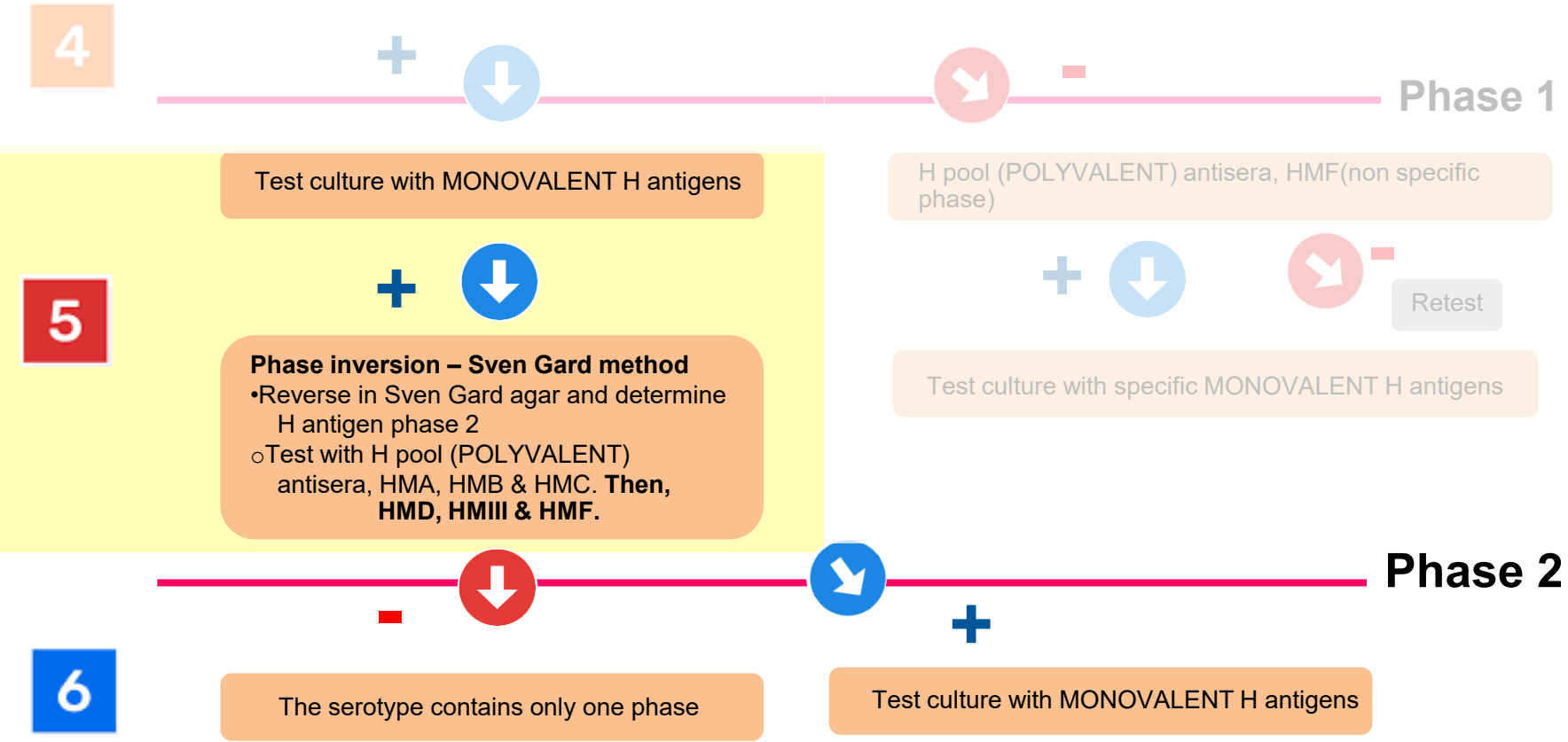




Flow of the Kauffman and White scheme



Flow of the Kauffman and White scheme

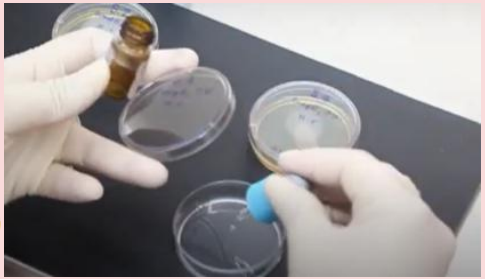


5 H antigen testing - Phase inversion by Sven Gard method)

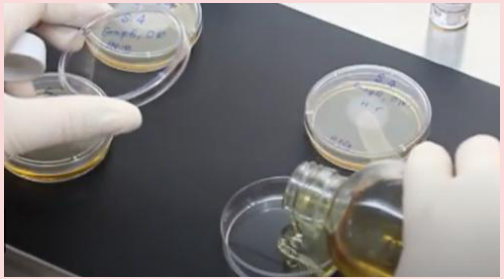
Test culture with MONOVALENT H antisera

Anti-H phase inversion sera

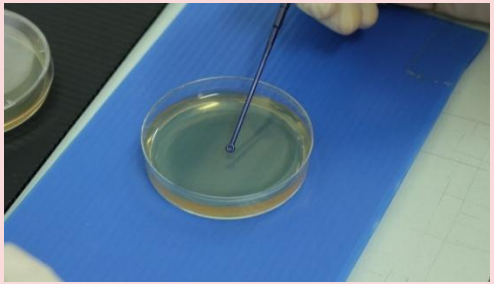
Sven Gard Method



Add **phase inversion antisera (**Anti-H phase inversion sera**)** into the petridish



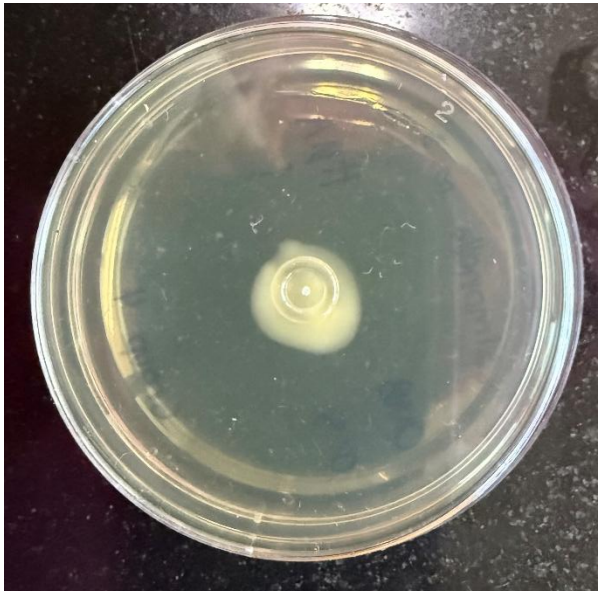
Pour the swarm agar into petridish and mix gently



When solidified, spot culture on the agar and incubate at 37˚C for 18 hrs

Swarm agar = a low-concentration agar medium (~0.3–0.5%) (ex., In 1000 mL, BHI 19 g, Tryptose 5g, Agar 5 g)

Salmonella on Sven Gard agar

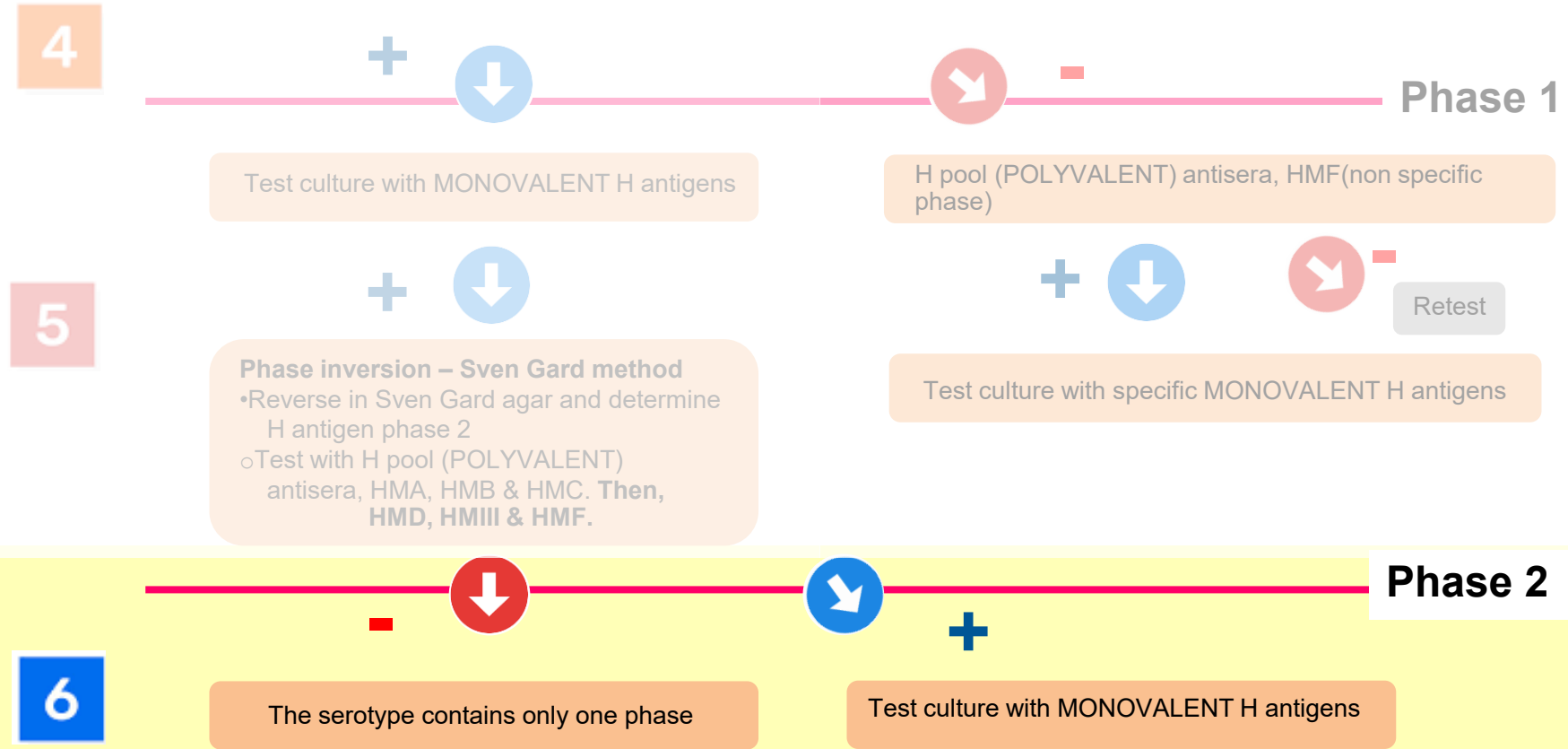


Monophasic *Salmonella*

Diphasic (Biphasic) *Salmonella*

Source: https://www.youtube.com/watch?v=ROeJJ47hLuk

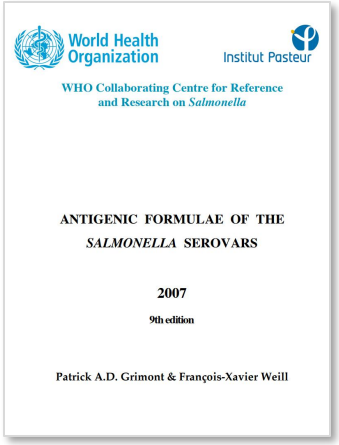
Flow of the Kauffman and White scheme



6 H antigen testing - Phase 2

Test culture with
MONOVALENT H antisera

7 Check with Antigenic Formulae of the *Salmonella* serovars


World Health Organization
Institut Pasteur
WHO Collaborating Centre for Reference and Research on *Salmonella*

ANTIGENIC FORMULAE OF THE *SALMONELLA* SEROVARS

2007

9th edition

Patrick A.D. Grimont & François-Xavier Weill

Group O:13 (G)

Groups formerly designated O:13,22 (G_1) and O:13,23 (G_2) were lumped together in a group which characteristic factor is O:13.

Type	Somatic (O) antigen	Flagellar (H) antigen		
		Phase 1	Phase 2	Other
Chagoua	1,13,23	a	1,5	
II	1,13,23	a	1,5	
Mim	13,22	a	1,6	
II	13,22	a	e,n,x	
Wyldegreen	1,13,23	a	l,w	
Marshall	13,22	a	l,z_{13},z_{28}	
II	1,13,23	a	z_{42}	
Ibadan	13,22	b	1,5	
Mississippi	1,13,23	b	1,5	
Oudwijk	13,22	b	1,6	
II	1,13,23	b	[1,5]	z_{42}
Bracknell	13,23	b	1,6	
Rottnest	1,13,22	b	1,7	
Vaertan	13,22	b	e,n,x	
Ullevi	1,13,23	b	e,n,x	
Bahati	13,22	b	e,n,z_{15}	
Durham	13,23	b	e,n,z_{15}	

The Kauffman and White scheme antigenic notation

Antigenic formular: **O:13(G)<u>1</u>,13,22:z:1,6 [Z_{44}][Z_{59}]**

Serotype	Group	O antigen	H antigen		
			Phase-1	Phase-2	Others
Poona	O:13(G)	<u>1</u>, 13, 22	z	1,6	[Z_{44}][Z_{59}]

O antigen: in arabic numerals
Phase-1 H antigen: a to z and then z1 to z83
Phase-2 H antigen: in arabic numerals

__ = Underlined O factors are determined by phage conversion

{ } = Exclusive O-factors. In a given serovar, factors in curly brackets cannot coexist with other factors in curly brackets. Some factors may be phage-determined (underlined).

[ ] = O (not underlined) or H factor that may be present or absent without relation to phage conversion.

( ) = O or H factor weakly agglutinable.

The Kauffman and White scheme antigenic notation

Antigenic formular: **O:13(G)1,13,22:z:1,6 [Z_{44}][Z_{59}]**

Group O:13 (G)

Groups formerly designated O:13,22 (G_1) and O:13,23 (G_2) were lumped together in a group which characteristic factor is O:13.

Type	Somatic (O) antigen	Flagellar (H) antigen		
		Phase 1	Phase 2	Other
Tunis	1,13,23	y	z_6	
Winslow	13,22	z	1,5	
II	1,13,23	z	1,5	
IIIb	13,23	z	1,5	
Poona	1,13,22	z	1,6	[z_{44}],[z_{59}]
Farmsen	13,23	z	1,6	
Bristol	13,22	z	1,7	
Ivrysurseine	1,13,23	z	z_6	
Tanzania	1,13,22	z	e,n,z_{15}	
Worthington	1,13,23	z	l,w	[z_{43}]
II	1,13,23	z	z_{42}	
II	13,22	z	–	
Ried	1,13,22	z_4,z_{23}	[e,n,z_{15}]	

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Thank you

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