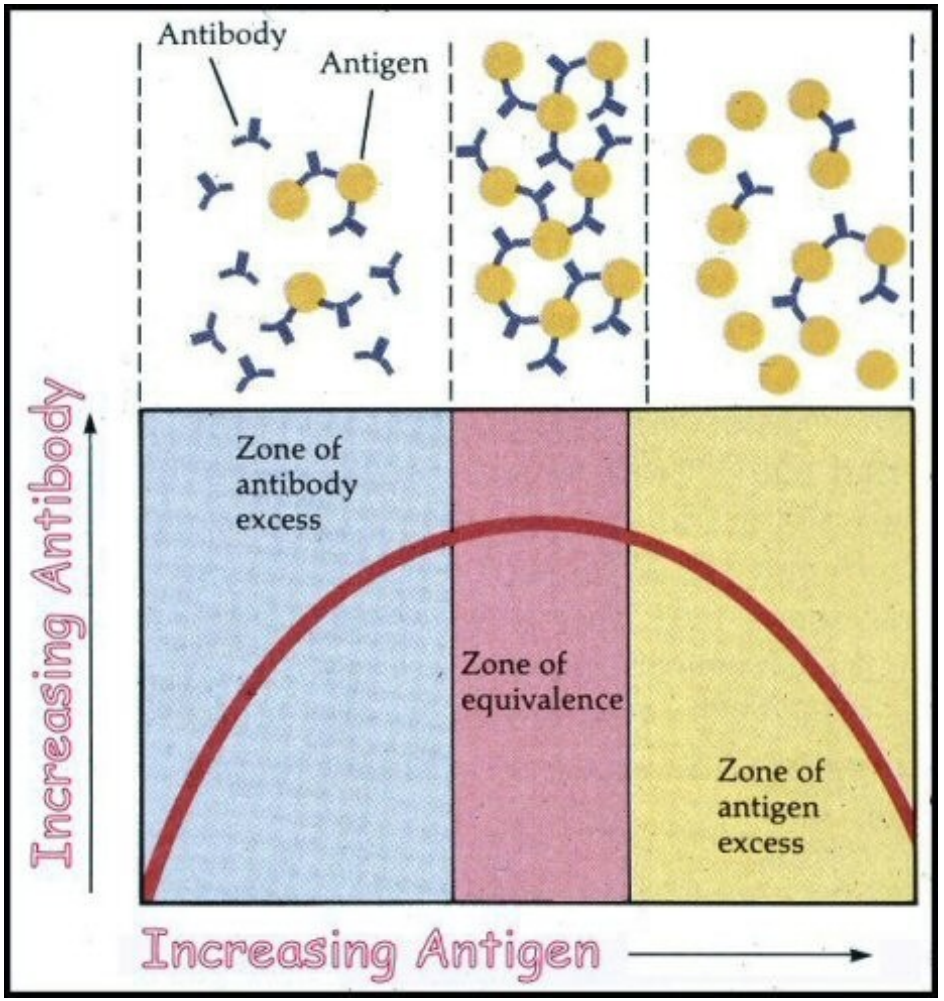
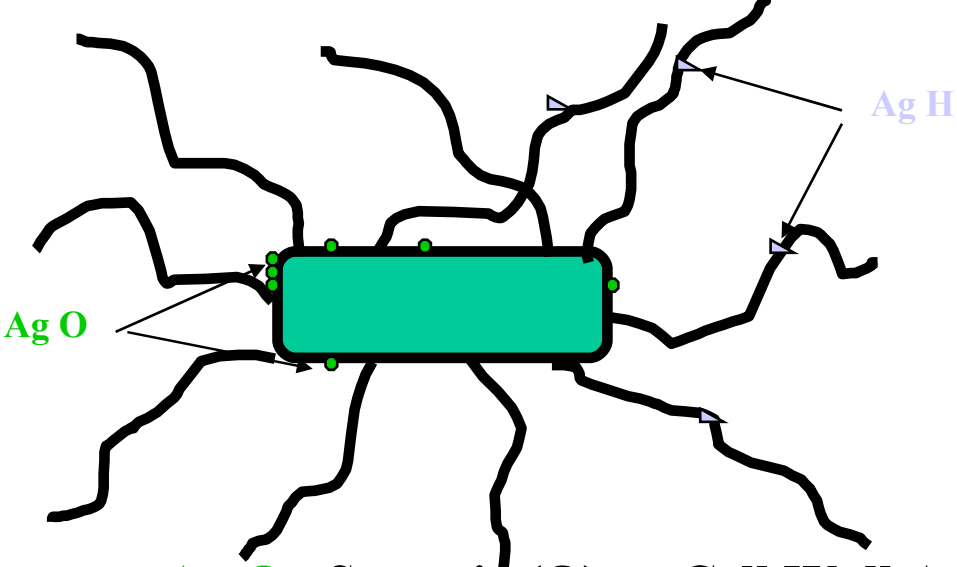
Salmonella Serotyping

Practical approach

Agglutination a network



If the correct proportion of sera and bacteria suspension is reached, you transform the suspension in an agglutination you can observe directly on a glass slide or plate



SALMONELLA serotyping = to detect 2 types of antigene on a strain

- **Ag O : Somatic (O) or Cell Wall Antigens**

LPS somatic antigens are heat stable and alcohol resistant. Denatured after formaldehyde treatment. Cross-absorption studies individualize a large number of antigenic factors, 67 of which are used for serological identification.

Ag H : Flagellar (H) Antigens

Flagellar antigens are heat-labile proteins but they resist to formaldehyde treatment. A few *Salmonella enterica*serovars (e.g., Enteritidis, Typhi) produce flagella with the same antigenic specificity. Such an H antigen is then called monophasic.

Most *Salmonella* serovars can alternatively produce flagella with two different H antigenic specificities. The H antigen is then called diphasic. Antiflagellar antibodies can immobilize bacteria with corresponding H antigens.

One cell is monophasic, a culture is biphasic (H1/H2 ration changes in the culture)

Serotyping practical aspects

- **According to Kauffmann and White?**
- **Ag O** : some are **major** (67provide more than 50 **groups**)
  others are **accessory**
  - They differ from strain to strain in a same group : Diagnostic
  - Some are strongly associated with major O, they do not present an interest for diagnostic
  - Could come from
    - chromosome : they are (ex. : O:[5])
    - Associate with bacteriophage
    - Associated with plasmid

Underlined noticed (ex. : O:1)

Example *Salmonella* Typhimurium : 1,4,[5],12 :i :1,2

Serotyping practical aspects

Step by step strategy, by successive elimination

Ag O OMA, OMB, OMC, OMD… are pool of group of sera.

Example : A strain was negatively tested with OMA (pool of sera), then positively with OMB.

You can stop with other O sera… but you have to further characterise inside OMB

OMB : 6,7 + 6,8 + 11 + 13,22 + 13,23 + 6,14,24+ (8),20

group of sera

O : 11 O : 6,7,8 O : 13,22,23 O : 6,14,24

You start with O:11, no agglutination. You tested O:6,7,8 : you obtain agglutination so you don't have to test the next ones

To finish you have to test O:7 and O:8

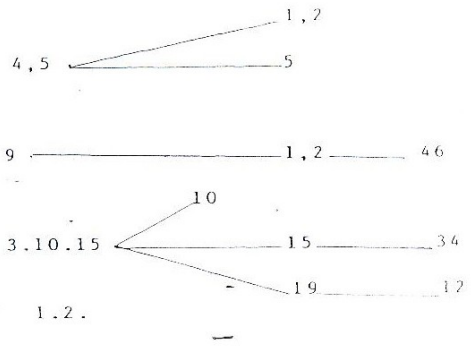
Strong agglutinations on slide or plate

SHEMA POUR AGGLUTINATION SUR LAME DE SALMONELLA

SERUMS O
"mélanges"

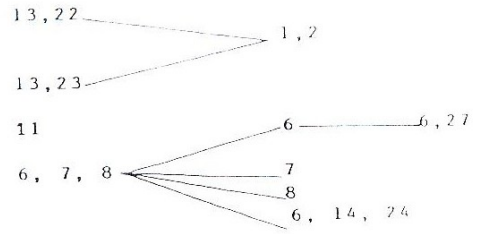
ANTIGENE SOMATIQUE

OMA



ABDEL

OMB

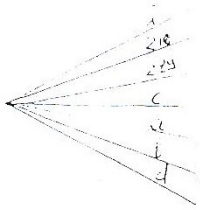


CFGH

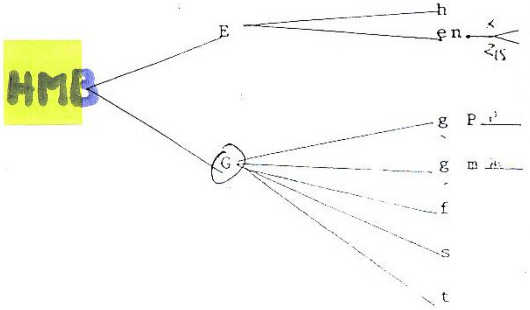
OMC

IJKMNOP

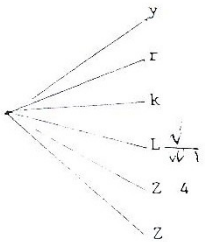
HMA



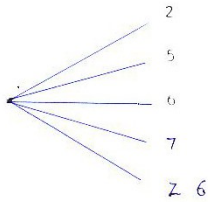
HMB



HMC

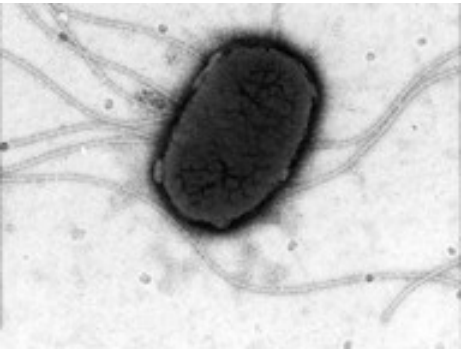


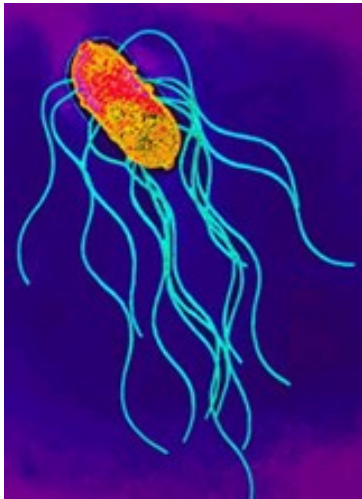
H:I



Salmonella Serotyping

- AgH : Mixing salmonella cells with flagella-specific antisera gives a characteristic pattern of agglutination (bacteria are loosely attached to each other by their flagella and can be dissociated by shaking).
- Use the same strategy for AgH identification





Salmonella Serotyping

- First test auto agglutination in 2% NaCl in water
- Then use successive agglutinations to conclude for the O antigenicity
- Then try to identify the H variability… test the second one
- Phase inversion

Phase inversion : how to obtain

- Physically fix the first phase bacteria
- Introduce anti first phase H in a semisolid agar
- Inoculate in surface
- Incubate… the migrating bacteria produce the second phase H, the will migrate
- Test the second phase on these bacteria