FWD-AMR-RefLabCap 1st Training Course

Isolation and phenotypic identification of Salmonella incl. API, conventional serotyping

Tuesday, 17 May 2022
10:45 -11:30 CET at DTU Food

HaDEA Service Contract 20197409
Provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

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Salmonellosis Case Definition

Clinical Description:

- An illness of variable severity commonly manifested by diarrhea, abdominal pain, nausea, and sometimes vomiting.
  - Asymptomatic infections may occur and the organism may cause extra-intestinal infections.

Laboratory criteria for diagnosis:

- Isolation of Salmonella from a clinical specimen.

Isolate the organism → Identify the organism →

Case classification:

- Probable: A clinically compatible case that is epidemiologically linked to a confirmed case.
- Confirmed: A case that meets the laboratory criteria for diagnosis.
  - When available, O and H antigen serotype characterization should be reported.

→ Serotype the organism

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Salmonella Isolation from clinical samples

- Complicated by several factors:
  - Competing enteric flora: 10^{11} bacteria / Gram incl. 300-400 species
  - Stressed / Injured cells: Transport or antibiotics
  - Intermittent shedding: Issue with chronic carriers

- Necessary to utilize culture techniques which selectively reduce numbers of competing flora and increase population of target organism

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Salmonella Isolation – so what is the best procedure?

- There is no perfect procedure…
  - But ! There are good guidelines.
    - These guidelines are tailored to accommodate:
      - Media available in your facility
      - Staff (workload)
      - Sample volume
    - One Example…

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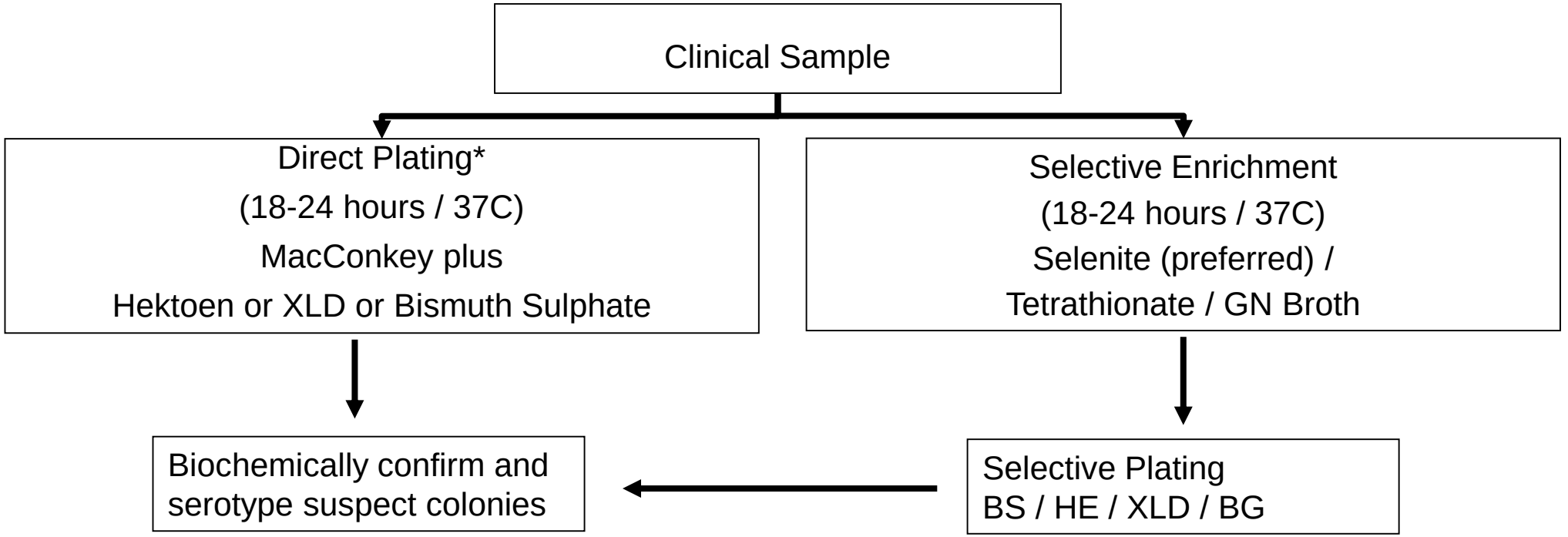
Salmonella Isolation – Culture Scheme



*Samples from sterile sites (e.g. blood or marrow) should also be plated to a non-selective media such as Sheep Blood agar or Chocolate agar.

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Salmonella Isolation – so what should we be doing??

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Salmonella Isolation – Day 1 (Arrival to the Lab)

- Suitable sample?
  - Unpreserved stool samples should be < 2 hours old.
  - Preferred stool transport medium is Cary-Blair
    - Samples should be refrigerated and arrive within 2 days of collection.
      - If this cannot be accomplished, samples should be frozen.

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Salmonella Isolation – Day 1 (Arrival to the Lab)

- Inoculation
  - Directly inoculate onto 2 selective plates: MAC and (XLD or HE or Desoxycholate, Citrate, Lactose, Sucrose agar (DCLS))
  - Incubate selective plates overnight (18-24 h) at 35-37C
  - Ideally selective enrichment broth should also be inoculated (more important w/ sub-clinical Salmonella carriers)

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Salmonella Isolation – Selection of media

Selective enrichment:

- Used to increase population of Salmonellae and allow stressed or injured Salmonellae to recover prior to plating on selective media.

- Can provide considerable increases in recovery:
  - Sensitivity Direct / Sensitivity post-enrichment
  - DCLS 22.7% / 81.8% (Selenite; Cassar; JCM 2003)
  - HE 85.4% / 98.4% (Selenite; Perez; JCM 2003).
  - CHROMagar Salmonella 76% / 99 % (Selenite; BD)

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Salmonella Isolation – Selection of enrichment broths

Selective Broth

- **Selenite** (preferred for most clinical samples)
  - Provides good selectivity for Salmonella spp. and is not inhibitory to Shigella spp.
  - Selectivity is lost if media is over-inoculated or incubated longer than 24 hours.
- **Tetrathionate**
  - Good suppression of E. coli (but also inhibits Shigella)
  - Good recovery of non-typhoidal Salmonellae
  - May be inhibitory to some typhoidal serovars (Typhi, Paratyphi A)
  - Highly selective

Selective Broth

- **Rappaport-Vassiliadis (RVS)**
  - Highly selective
  - Good recovery of non-typhoidal Salmonellae
  - Inhibitory to typhoidal serovars
  - Requires 42ºC incubator
- **MacConkey Broth and Gram-Negative Broth**
  - Provides suppression of Gram-positive organisms
  - Supports growth of virtually all Enterobacterales
  - Limited selectivity but useful if also testing for Shiga-toxin

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Salmonella Isolation – Selection of agar

Plating media

- MacConkey (**should always be included**):
  - Inhibits Gram-positives, supports growth nearly Enterobacterales including Salmonella and Shigella.
  - May provide recovery of injured cells.

- XLD & HE:
  - Utilise carbohydrate metabolism and H2S production to provide good differentiation of a variety of Enterobacterales.

Plating media

- Bismuth sulphate:
  - Works well for isolation of Salmonella Typhi.
  - Inhibits Shigella and some non-typhoidal Salmonellae
  - Temperature and light sensitive.

- Salmonella & Shigella Agar:
  - Similar to MAC.
  - Detects H2S production.
  - Inhibitory to S. dysenteriae Type 1.

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Salmonella Isolation – Selection of media

Medium	*Salmonella* (majority)	*Salmonella* Typhi	*Shigella spp*.
MacConkey Agar (MAC)*	Smooth, colourless colonies. 2-4 mm	Smooth, colourless colonies. 1-3 mm	Smooth, colourless colonies. 2-3 mm
Hektoen Enteric Agar (HE)*	Clear colonies with black centres. 2-4 mm	Clear colonies. Some may produce pinpoint black centres. 1-3 mm	Clear / green colonies 2-3 mm
Xylose Lysine Desoxycholate Agar (XLD)*	Clear colonies with black centres 2-4 mm	May be inhibitory. 1-3 mm clear colonies. Some with pinpoint black centres.	Red colonies 1-2 mm
Salmonella-Shigella (SS) Agar	Clear Colonies With black centres 2-4 mm	Clear colonies. Some may produce pinpoint black centres. 1-3 mm	May inhibit *S.d.* Type 1 Smooth, colourless colonies. 2-3 mm
Bismuth Sulphate Agar (BS)	Black colonies 2-4 mm	Black colonies w/ distinct metallic sheen	Significant or total inhibition
Brilliant Green Agar (BG)	White, pink, or red colonies surrounded by a red halo	Significant inhibition. May produce red colonies.	Significant or total inhibition

* The combination of MAC (HE / XLD) provide a wide spectrum of selectivity and support the growth of *Salmonella* and *Shigella*

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Salmonella Isolation – Day 2

- Selective plates are examined for the presence of Salmonella-like or Shigella-like colonies.
- Suspicious colonies (3-5) are selected for biochemical testing.
  - "Typical" colonies on selective are NOT diagnostic.
    - They just indicate the need for further testing.
- Selective enrichment broth (if used) is inoculated to selective plating media.

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Salmonella Identification – Approaches

Selective Plates

- Suspect colonies screened biochemically
- Short panel of tests can rule-in / rule-out key pathogens.
- Typically 3-5 colonies should be selected (from various plates)

Biochemicals

- TSI (triple sugar iron agar)
- LIA (lysine iron agar)
- Simmons' Citrate
- Motility-Indol-Ornithine (MIO)
- ONPG
- Urea
  - Panel can be modified based on available reagents

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Salmonella Identification – reactions

- Biochemical variation among the six subspecies of S. enterica and between S. enterica and S. bongori.
- Generally regarded as aerogenic, citrate +, motile, lysine and ornithine decarboxylase positive, indol negative, MR positive, VP negative, hydrogen sulphide positive.

- Some important exceptions…
  - Citrate: Virtually all Salmonellae positive
    - serovars Typhi, Paratyphi A, Gallinarum, & Pullorum are negative.
  - LDC: Virtually all Salmonellae positive;
    - serovar Paratyphi A are negative
  - ODC: Virtually all Salmonellae positive;
    - serovar Typhi & Gallinarum are negative.
  - Hydrogen sulphide: Virtually all Salmonellae positive;
    - serovar Paratyphi A are negative.
  - Dulcitol: Virtually all ssp. I Salmonellae positive;
    - serovars Typhi & Pullorum are negative.

Salmonella Identification – Triple Sugar Iron Agar (TSI)

- Three fermentable sugars (glucose, sucrose, lactose) and a hydrogen sulphide indicator.
- Sucrose and/or lactose + organisms acidify the butt and slant. Organisms which are glucose + but sucrose and lactose –, produce an alkaline slant and acid butt.
- Record acid (A) or alkaline (K) reactions for the slant and butt as well as hydrogen sulphide production and gas production.

K/Ag++

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Salmonella Identification – Lysine Iron Agar

- Small amount of glucose incorporated into broth which is quickly utilised causing an initial rise in pH turning the media yellow.
- LDC + organisms then attack lysine. Resulting amine causes a rise in pH and turns media purple.
- Hydrogen sulphide production will cause a blackening of the media.

Left to Right:
Uninoculated
Lysine negative (Shigella flexneri)
Lysine positive (Salmonella ser. Typhi)
Lysine deamination (Proteus mirabilis)
Lysine positive w/ H2S (Salmonella ser. Newport)

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Salmonella Identification – Urea

- Detects urease production.
- Small amount of glucose in medium is quickly fermented, acidifying the media and causing the development of a yellow colour.
- Organisms which are urease positive then degrade the available urea, producing ammonia, raises the pH, and changes the colour of the media from yellow to red/purple.
- Virtually all Salmonellae & Shigellae are urease negative. Most Proteus and some Citrobacter are urease positive

Left to Right:
Uninoculated
Urease positive (Proteus mirabilis)
Urease negative (Shigella flexneri)

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Salmonella Identification – Motility Indol Ornithine (MIO)

- Detects motility, ornithine decarboxylase activity, and indol production.
- Single stab into media
- Motile organisms diffuse through media. Non-motile organisms only grow along stab line.
- ODC positive organisms turn media light purple. ODC negative organisms turn media yellow.
- Indol test performed AFTER recording motility and ODC reactions.
  - +/- 0.5 mL Kovacs' reagent added to surface of media.
    - If indol was produced (positive) reagent will turn red/pink.
    - If negative, reagent will not change

Left to Right:
Uninoculated
nonmotile / ODC - / indol - (Shigella flexneri)
motile / ODC + / indol + (E. coli)
motile / ODC + / indol ND (E. coli)
Non-motile / ODC - / indol ND (S. flexneri)

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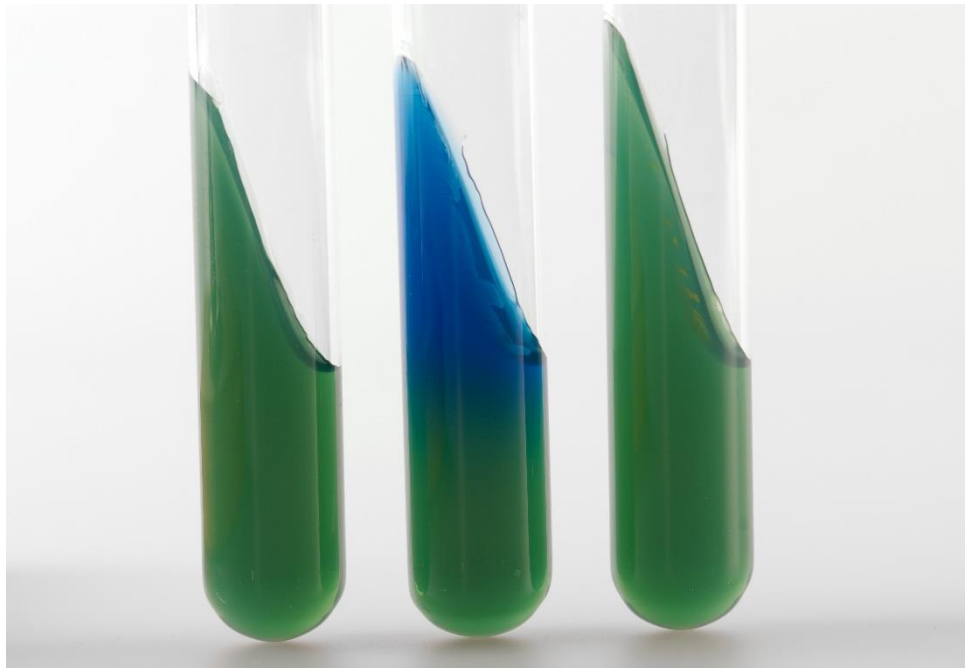
Salmonella Identification – Citrate (Simmons)

- Ability to utilise citrate as sole carbon source.
- No colour change with citrate negative organisms.
- Citrate utilisation produces ammonia, raises pH and turns media blue (positive reaction)
- Most non-typhoidal Salmonellae are positive.
- Shigella are negative



Left to Right:
Uninoculated
Citrate positive (Salmonella ser. Newport)
Citrate negative (E. coli)

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Salmonella Identification – ONPG

- Detects ß-galactosidase (enzyme associated with lactose fermentation)
- Substrate: colourless lactose analogue o-nitrophenyl-ß-D-galactopryanoside. Degradation by ß-galactosidase causes a colour change by cleavage of o-nitrophenol (yellow).
- May be performed using disks, broth, or agar.
- N.B.: S. enterica ssp. II (some) IIIa, IIIb, VI (some) and S. bongori are ONPG +.

Left to Right:
ONPG negatice
ONPG positive

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Salmonella Identification – S. enterica ssp. enterica

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Salmonella Identification – Salmonella serovar Typhi

S. enterica ssp. enterica

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Salmonella Identification – Shigella dysenteriae, S. flexneri, & S. boydii

S. enterica ssp. enterica

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Salmonella Identification – S. sonnei

S. enterica ssp. enterica

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Salmonella Identification – Remember Exceptions Prove the Rule

- Atypical results do occur and should always be confirmed with additional biochemical tests or molecular methods such as PCR, WGS etc..
- Biochemical tables and recommendations for ancillary tests may be found in the GFN - Protocol “Identification of *Salmonella spp.*”.
- This panel is very useful to rule-in / rule-out *Salmonella spp*. (Semi)-automated systems (e.g. API) may provide more definitive ID of non-*Salmonellae*.
- Polyvalent antisera may be useful for screening suspect colonies;
  - however, there is considerable O antigen cross-reactivity among members of the *Enterobacterales* (particularly *Salmonella, Escherichia, Citrobacter, & Hafnia*).
  - Reactivity to polyvalent sera must be confirmed both biochemically and with monospecific antisera.
- When feasible, all confirmed *Salmonella & Shigella* isolates should be serotyped.

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Salmonella Identification – API 20E system

- The API 20E System is a standardized, miniaturized micro-tube system consisting of 21 conventional “basic” and 6 supplementary biochemical tests used for the identification of Enterobacteriaceae and other non-fastidious Gram-negative bacteria.

- The API 20E System consists of micro-tubes containing dehydrated substrates.
  – The substrates are reconstituted by adding a bacterial suspension.

- When incubated, the organisms react with the contents of the tubes.

- The tubes are read when the various indicator systems are affected by the metabolites or added reagents, generally after 18 to 24 hours incubation at 35 - 37°C.


Service contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

SC 2019 74 09

Identification of Enterobacteriaceae Using API.

May 2022

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Salmonella Identification – API 20E system reagents

- NaCl 0.85 % Medium, 5 ml or API Suspension Medium, 5 ml
- API 20 E reagent kit
  - TDA
  - JAMES
  - VP1+VP2
  - NIT 1 +NIT2
  - Zn reagent
  - Oxidase
- Mineral oil

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S. Identification – Preparation of the inoculum

- Open an ampule of API NaCl 0.85 % Medium (5 ml) or use any tube containing 5 ml of sterile saline or sterile distilled water, without additives.
- Use colony from an isolation plate. It is recommended to use fresh cultures (18-24 hours old).
- Carefully emulsify to achieve a homogeneous bacterial suspension.
- This suspension must be used immediately after preparation.

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S. Identification – Preparation of the inoculum

- Dispense about 5 ml of tap water into the tray with a squeeze bottle.
  - Note: that the bottom of the tray has numerous depressions to accept the water.

- Vortex mix the saline suspension to get uniform dispersal

- Inoculate all the tubes on the test strip with the pipette by depositing the suspension into the micro-tubes as you tilt the API tray

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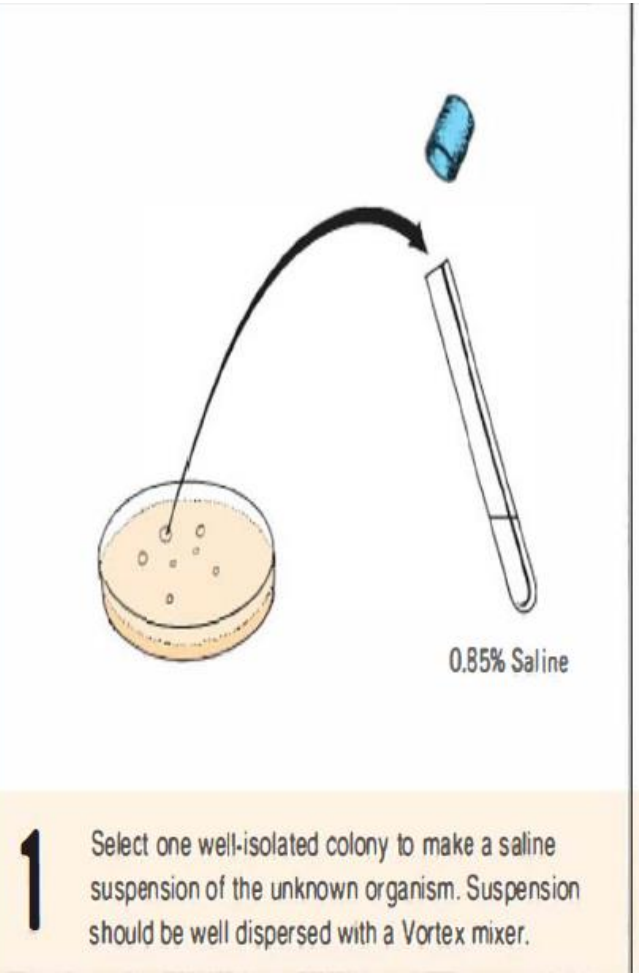
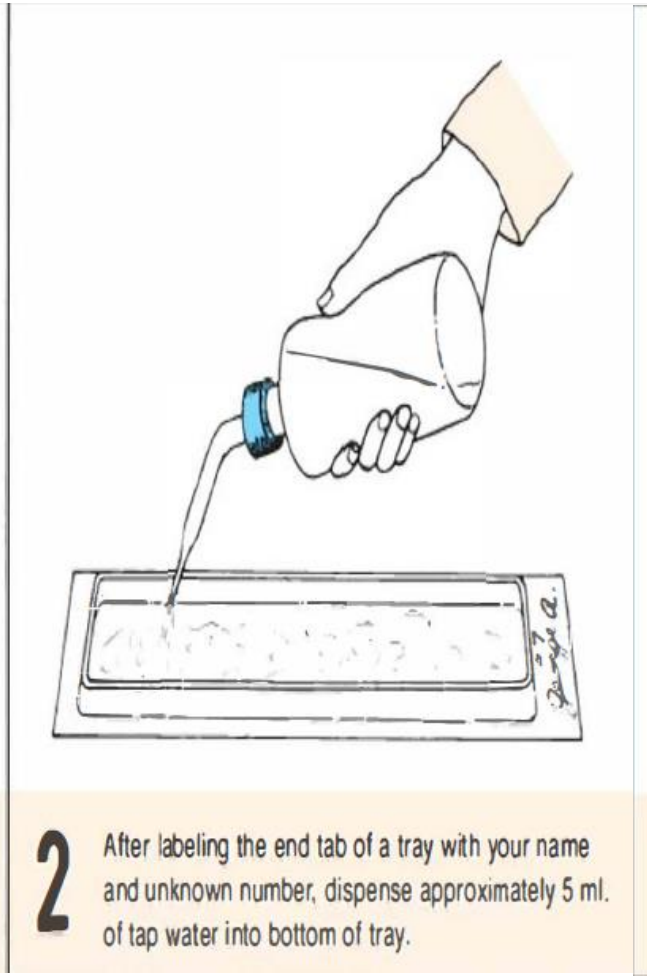
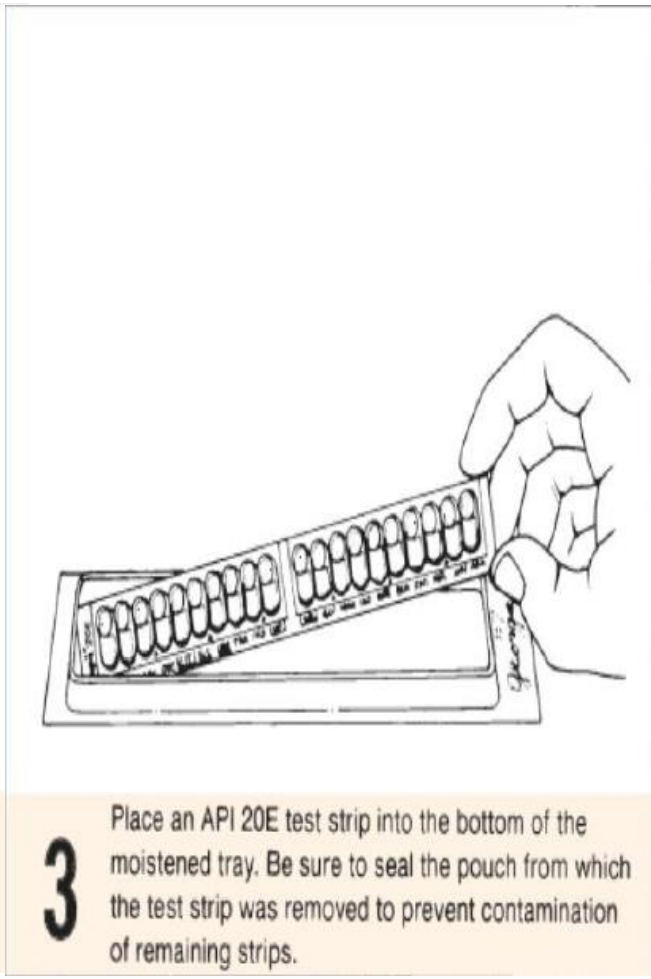
S. Identification – Preparation of the inoculum



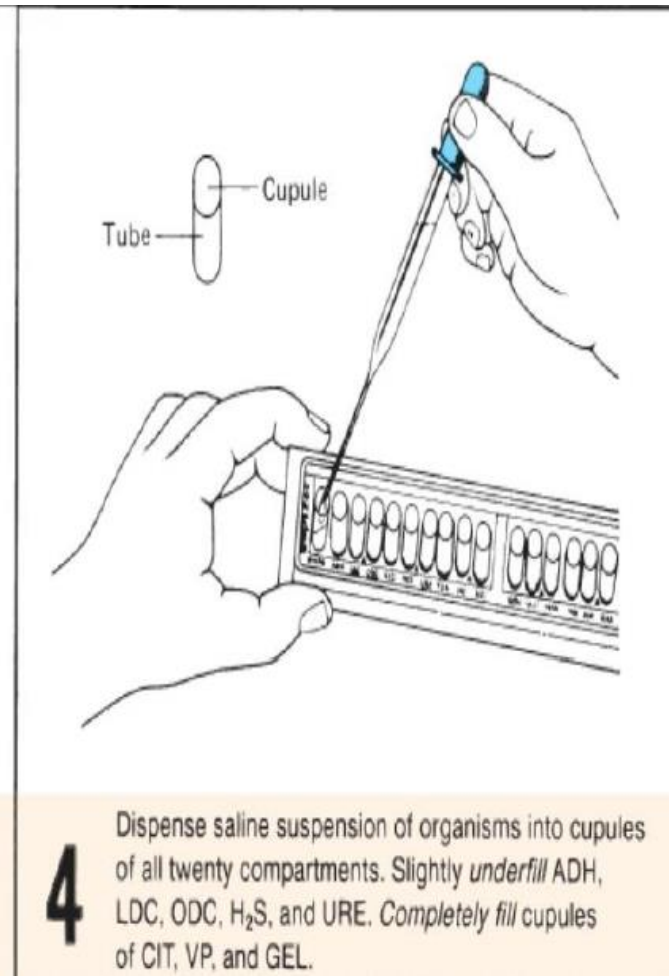
1 Select one well-isolated colony to make a saline suspension of the unknown organism. Suspension should be well dispersed with a Vortex mixer.



2 After labeling the end tab of a tray with your name and unknown number, dispense approximately 5 ml. of tap water into bottom of tray.



3 Place an API 20E test strip into the bottom of the moistened tray. Be sure to seal the pouch from which the test strip was removed to prevent contamination of remaining strips.



4 Dispense saline suspension of organisms into cupules of all twenty compartments. Slightly *underfill* ADH, LDC, ODC, H_2S, and URE. *Completely fill* cupules of CIT, VP, and GEL.

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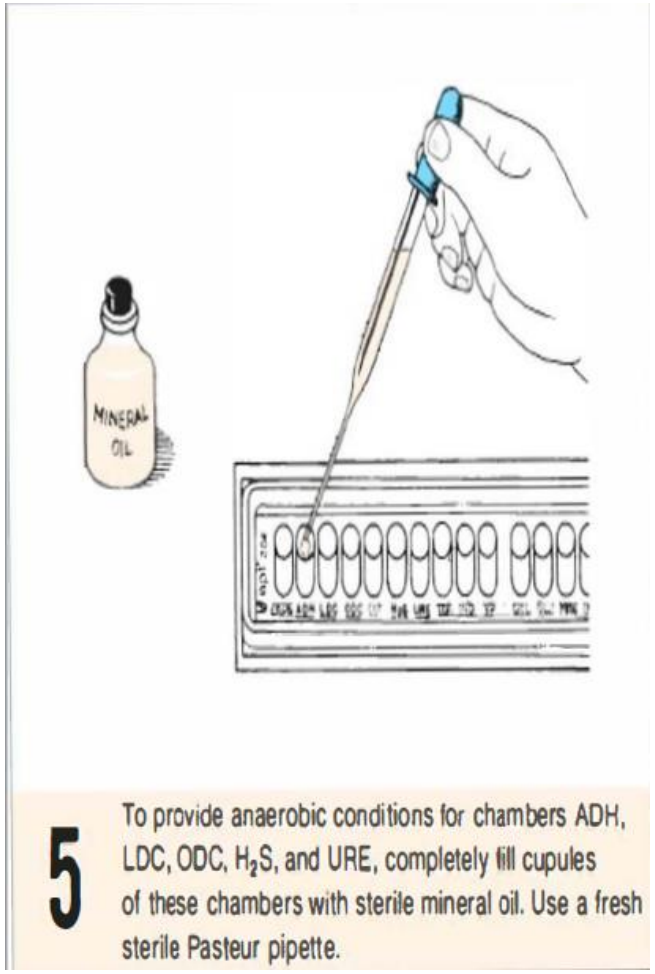
S. Identification – Preparation of the tests

- Create anaerobiosis in the tests ADH, LDC, ODC, H2S and URE by overlaying with mineral oil.
- Since the media in CIT, VP, and GEL compartments require oxygen, completely fill both the micro-tube and tube of these compartments.
- Fill only the tube (and not the micro-tube) of the other tests.
- Close the incubation box. Incubate at 36°C ± 2°C for 18-24 hours.
  - NOTE: The indole production test must be performed last since this reaction releases gaseous products which interfere with the interpretation of other tests on the strip.



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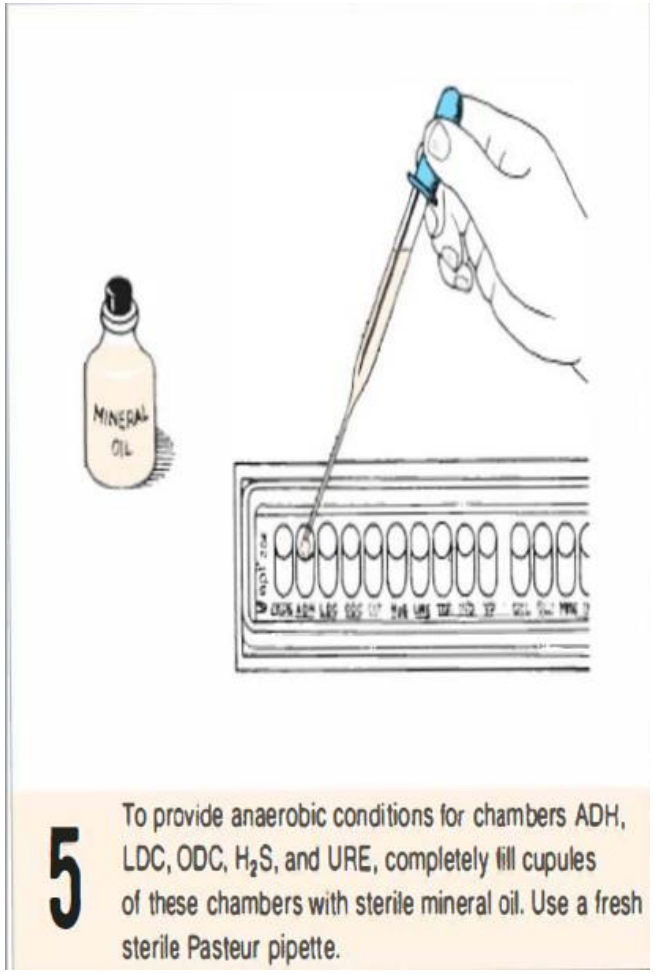
S. Identification – Preparation of the tests

- The plastic incubation lid should not be replaced after the addition of the reagent.
  - If the number of positive tests (including the GLU test) before adding the reagents is less than 3:
    - Re-incubate the strip for a further 24 hours (± 2 hours) without adding any reagents.
    - Reveal the tests requiring the addition of reagents



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S. Identification – Interpretation

- Add 1 drop each of NIT 1 and NIT 2 reagents to the GLU tube. Wait 2 to 5 minutes.
  - A red color indicates a positive reaction (N02) . A negative reaction (yellow) may be due to the reduction to nitrogen (as sometimes evidenced by gas bubbles):

- Add 2 to 3 mg of Zn reagent to the GLU tube. After 5 minutes, if the tube remains yellow this indicates a positive reaction (N2) to be recorded on the result sheet.
  - If the test turns orange, this is a negative reaction:
    - the nitrates still present in the tube have been reduced by the Zinc.
    - This reaction is useful when testing Gram-negative, oxidase positive rods.

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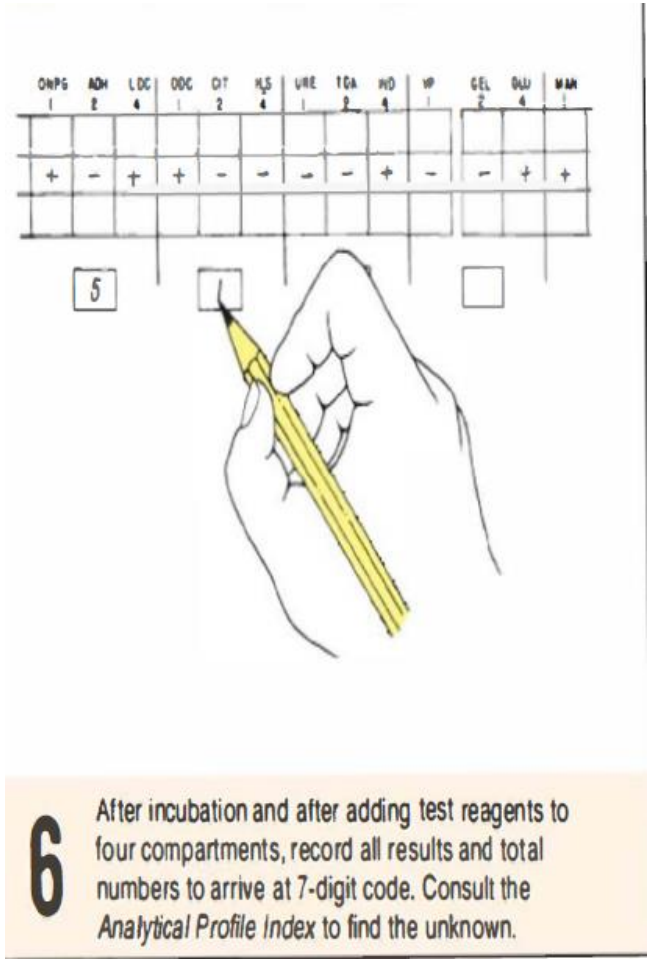
S. Identification – Interpretation

- Determination of the numerical profile :
  - On the result sheet, the tests are separated into groups of 3 and a value 1, 2 or 4 is indicated for each.
  - By adding together the values corresponding to positive reactions within each group, a 7-digit profile number is obtained for the 20 tests of the API 20 E strip.



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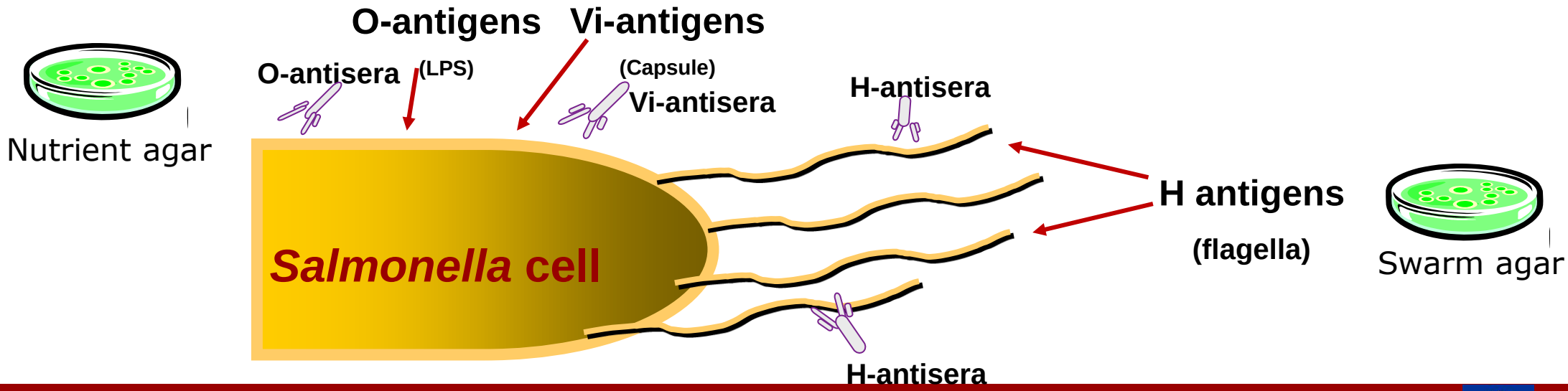
Salmonella serotyping

- The best bacterial phenotyping method ever made
- Established in 1929 (White) then Kauffmann
- Phenotypic characterization of strains based on the immunologic reactivity of two surface structures:
- Lipopolysaccharide (O antigen)
- Flagellin protein (H antigen)
- +/- Vi antigen (capsule)



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Salmonella antisera brands

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O – Antisera

OMA = 2 (A) - 4 (B) - 9 (D_1) - 9,46 (D_2) - 3,10 (E_1) - 1,3,19 (E_4) -21 (L)

OMB = 7 (C_1) - 8 (C_1-C_2) - 11 (F) - 13 (G) - 6,14 (H)

OMC = 16 + 17 + 18 + 28 + 30 + 35 + 38

OMD = 39 + 40 + 41 + 42 + 43 + 44 + 45

OME = 47 + 48 + 50 + 51 + 52 + 53 + 61

OMF = 54 + 55 + 56 + 57 + 58 + 59

OMG = 60 + 62 + 63 + 65 + 66 + 67

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H – Antisera

HMA = a + b + c + d + i + z10 + z29

HMB = E -complex + G -complex

HMC = k + y + z + L -complet + Z4 -complex + r

HMD = z35 + z36 + z38 + z39 + z41 + z42 + z44 + z60

HME = z52 + z53 + z54 + z54 + z55 + z57 + z61

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O – Antisera

O: 10	**O : 46**					
O : 9	**O : 34**	**O : 20**		**Conc. H : z_{10}**		
O : 5	**O :27**	**O : 14**		**Conc. H : i**		
O : 4	**O : 19**	**O : 8**		**Conc. H : d**	**Conc. H : r**	
O : 2	**O : 15**	**O : 7**		**Conc. H : c**	**Conc. H : L**	**Conc. H : z29**
O : 1	**O : 12**	**O : 6**		**Conc. H : b**	**Conc. H : G**	**Conc. H : z_6**
OMA		**OMB**	**O: Vi**	**Conc. H : a**	**Conc. H : E**	**Conc. H : 1 complex**

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H – Antisera

H : z10	**H : f**			**H : 7**		
H : i	**H : G complex**	**H: p**		**H : z6**		
H : d	**H : z15**	**H : t**		**H : 6**		
H : c	**H : x**	**H : s**	**H : r**	**H : 5**		
H : b	**H : h**	**H : q**	**H : w**	**H : 2**		
H : a	**H : E complex**	**H : m**	**H : L complex**	**H : 1 complex**	**H : z29**	
HMA	**HMB**		**HMC**	**HMD**	**HME**	

Complex:

H : E complex = H:eh , H:enx , H:enz$_{15}$

H : G complex = H:gm , H:gmx , H:gp , H:fg , H:gst , H:fgs

H : L complex = H:lv , H:lw

H : 1 complex = H:1,2 , H:1,5 , H:1,6 , H:1,7

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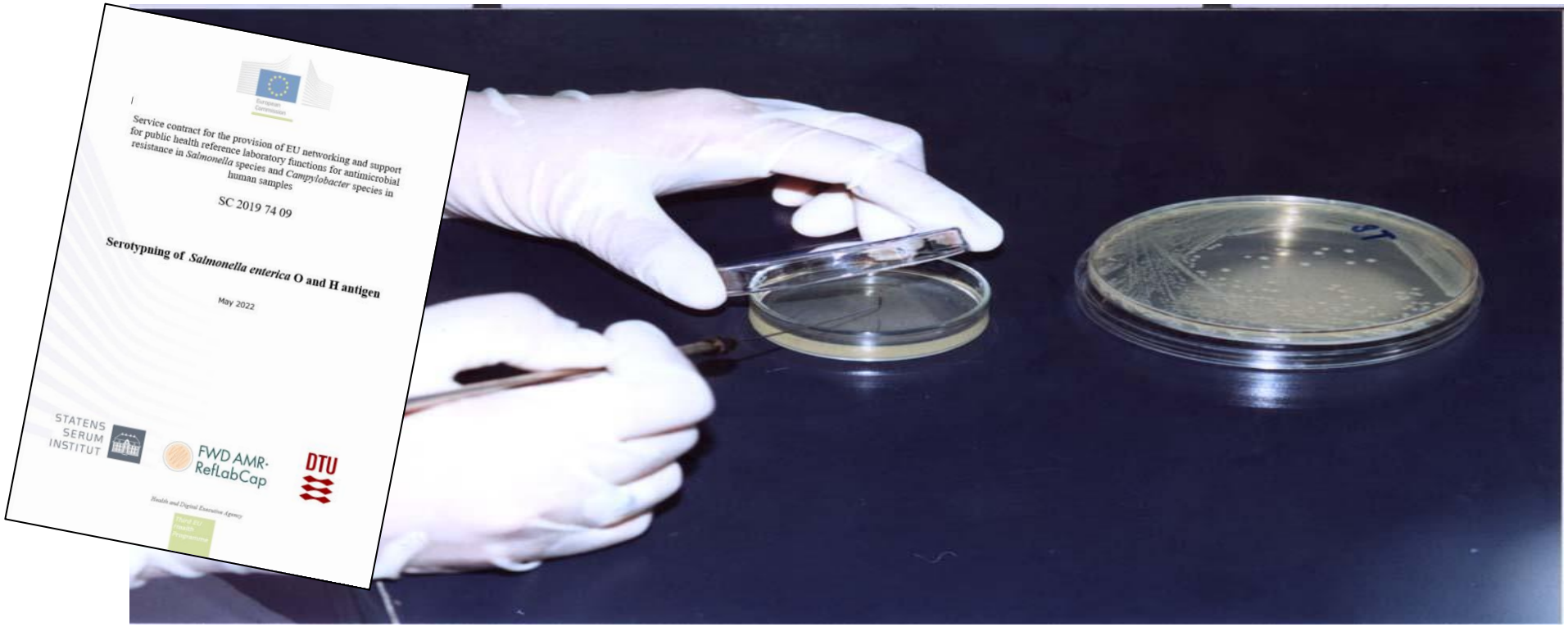
Confirmation of Salmonella – Day 1

- Inoculate the strain onto a Nutrient agar plate and a swarm agar plate
- Incubate over night at 37ºC



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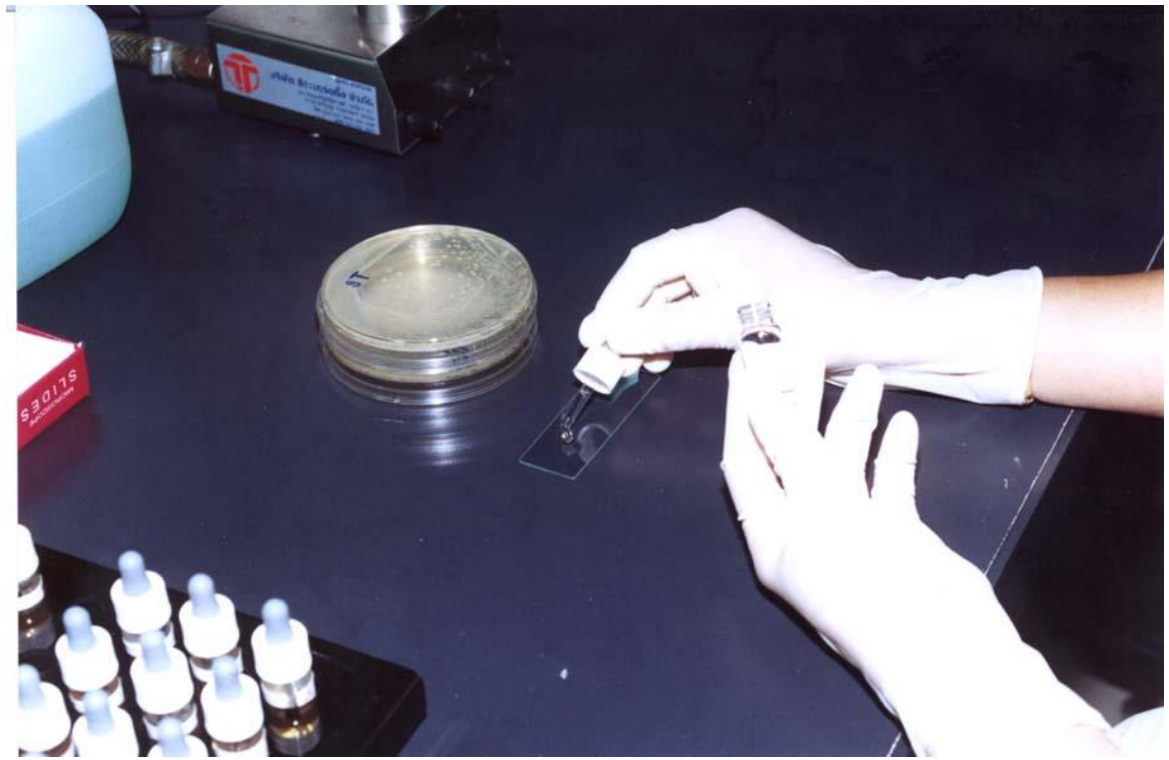
Confirmation of Salmonella – Day 2

- Add a drop of antisera on a slide.
- Colonies from the Nutrient agar plate or swarm agar plate are mixed with the antiserum.
- Rock the slide gently for about 30 sec



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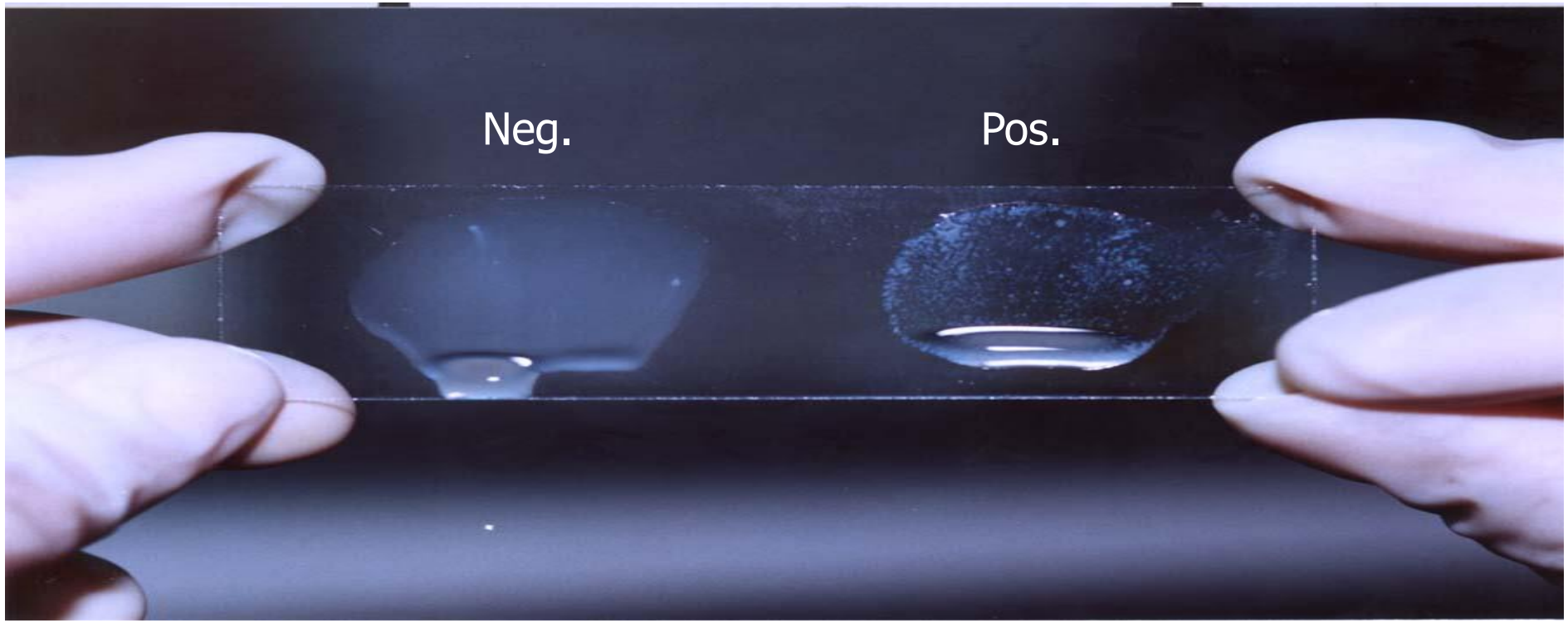
Confirmation of Salmonella – day 2

- Positive reaction → visible clumping = agglutination.
- Negative reaction → homogenous milky turbidity.
- Control = Saline or a negative reaction in other antisera.



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Slide agglutination – Test for O-Antigen – Day 2

O: 10	**O : 46**					
O : 9	**O : 34**	**O : 20**		**Conc. H : z_{10}**		
O : 5	**O :27**	**O : 14**		**Conc. H : i**		
O : 4	**O : 19**	**O : 8**		**Conc. H : d**	**Conc. H : r**	
O : 2	**O : 15**	**O : 7**		**Conc. H : c**	**Conc. H : L**	**Conc. H : z29**
O : 1	**O : 12**	**O : 6**		**Conc. H : b**	**Conc. H : G**	**Conc. H : z_6**
OMA		**OMB**	**O: Vi**	**Conc. H : a**	**Conc. H : E**	**Conc. H : 1 complex**

- Test the isolate from the Nutrient agar plate in the polyvalent O- antisera.

Slide agglutination – Test for O-Antigen – Day 2

O: 10	**O : 46**					
O : 9	**O : 34**	**O : 20**		**Conc. H : z_{10}**		
O : 5	**O :27**	**O : 14**		**Conc. H : i**		
O : 4	**O : 19**	**O : 8**		**Conc. H : d**	**Conc. H : r**	
O : 2	**O : 15**	**O : 7**		**Conc. H : c**	**Conc. H : L**	**Conc. H : z29**
O : 1	**O : 12**	**O : 6**		**Conc. H : b**	**Conc. H : G**	**Conc. H : z_6**
OMA		**OMB**	**O: Vi**	**Conc. H : a**	**Conc. H : E**	**Conc. H : 1 complex**

- Test the factor sera represented in the positive pool until you find a positive reaction

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Slide agglutination – Test for O-Antigen – Day 2

O: 10	O : 46					
O : 9	O : 34	O : 20		Conc. H : z_{10}		
O : 5	O :27	O : 14		Conc. H : i		
O : 4	O : 19	O : 8		Conc. H : d	Conc. H : r	
O : 2	O : 15	O : 7		Conc. H : c	Conc. H : L	Conc. H : z29
O : 1	O : 12	O : 6		Conc. H : b	Conc. H : G	Conc. H : z_6
OMA		OMB	O: Vi	Conc. H : a	Conc. H : E	Conc. H : 1 complex

- Positive reaction = O: 6,7
- After having completed the O-typing, continue with H-typing.

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Slide agglutination – Test for H-Antigen – Day 2

H : z10	**H : f**			**H : 7**		
H : i	**H : G complex**	**H: p**		**H : z6**		
H : d	**H : z15**	**H : t**		**H : 6**		
H : c	**H : x**	**H : s**	**H : r**	**H : 5**		
H : b	**H : h**	**H : q**	**H : w**	**H : 2**		
H : a	**H : E complex**	**H : m**	**H : L complex**	**H : 1 complex**	**H : z29**	
HMA	**HMB**		**HMC**	**HMD**	**HME**	

- Test the isolate from the Swarm agar plate using the polyvalent H- antisera

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Slide agglutination – Test for H-Antigen – Day 2

H : z_{10}	**H : f**			**H : 7**		
H : i	**H : G complex**	**H: p**		**H : z_6**		
H : d	**H : z15**	**H : t**		**H : 6**		
H : c	**H : x**	**H : s**	**H : r**	**H : 5**		
H : b	**H : h**	**H : q**	**H : w**	**H : 2**		
H : a	**H : E complex**	**H : m**	**H : L complex**	**H : 1 complex**	**H : z29**	
HMA	**HMB**		**HMC**	**HMD**	**HME**	

- Test the isolate from the Swarm agar plate using the polyvalent H- antisera

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Slide agglutination – Test for H-Antigen – Day 2

H : z_{10}	**H : f**			**H : 7**		
H : i	**H : G complex**	**H: p**		**H : z_6**		
H : d	**H : z15**	**H : t**		**H : 6**		
H : c	**H : x**	**H : s**	**H : r**	**H : 5**		
H : b	**H : h**	**H : q**	**H : w**	**H : 2**		
H : a	**H : E complex**	**H : m**	**H : L complex**	**H : 1 complex**	**H : z29**	
HMA	**HMB**		**HMC**	**HMD**	**HME**	

- Positive reaction = H : r

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Slide agglutination

- Result
  – 6.7 : r : ?
- Next step is to detect the 2. phase of the H-antigens by phase-inversion

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Slide agglutination phase-inversion – Day 2

Plate preparation

- Add 10µl of concentrated antisera in a small petri dish
  - corresponding to the phase whish has been identified
    - Ex. H:r
- Add approximately 10ml of melted swarm agar.
- Leave the plate for solidification.

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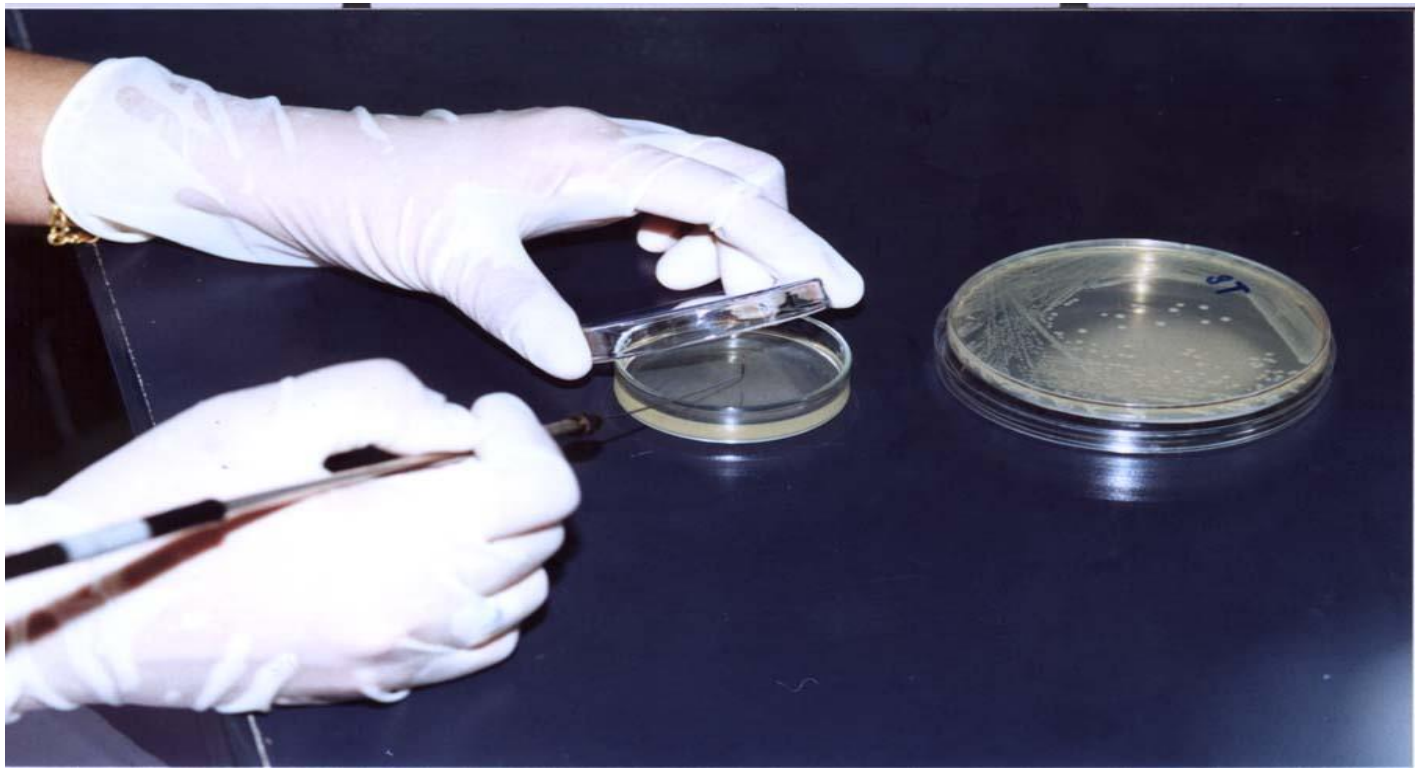
Slide agglutination phase-inversion – Day 2

Inoculation of plate for phase inversion

- Inoculate the plate in the centre with a loop
- Incubate over night at 37ºC.



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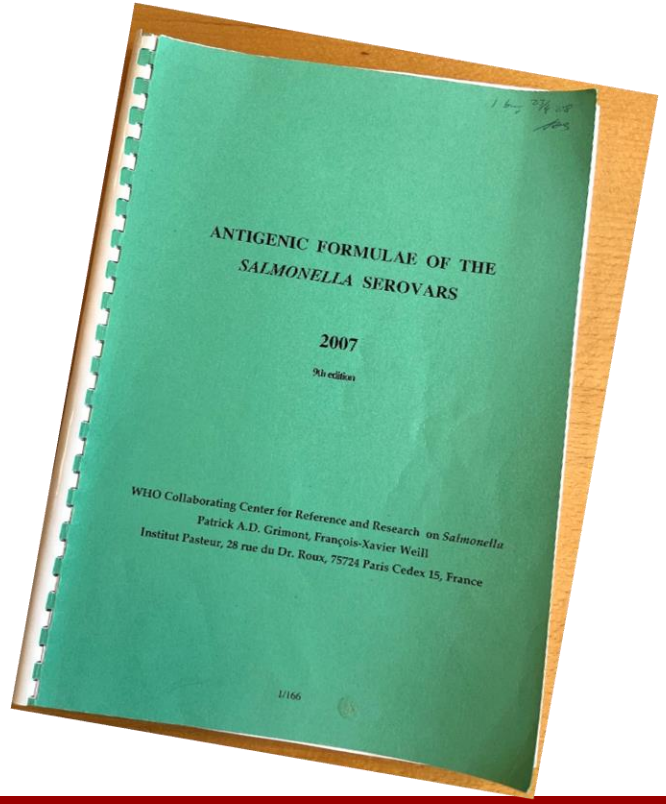
Slide agglutination phase-inversion – Day 3

Detection of the 2. phase of the H-antigen

- Use culture from the edge of the growth zone for slide agglutination.
- Select the relevant H antisera, by using the Kauffmann- White Scheme.



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Kauffmann-White Scheme – Day 3

Strathcona	6,7	l,z_{13},z_{28}	1,7
II	6,7	l,z_{28}	1,5:[z_{42}]
II	6,7	l,z_{28}	e,n,x
II	6,7	l,z_{28}	z_6
Virchow	6,7,14	r	1,2
Infantis[1]	6,7,14	r	1,5
Nigeria	6,7	r	1,6
Colindale	6,7	r	1,7
Papuana	6,7	r	e,n,z_{15}
Grampian	6,7	r	l,w
Richmond	6,7	y	1,2
Bareilly	6,7,14	y	1,5
Oyonnax	6,7	y	1,6
Gatow	6,7	y	1,7
Hartford	6,7	y	e,n,x:[z_{67}]
Mikawasima[2]	6,7,14	y	e,n,z_{15}
Chile	6,7	z	1,2

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Kauffmann-White Scheme – Day 3

Strathcona	6,7	l,z_{13},z_{28}	1,7
II	6,7	l,z_{28}	1,5:[z_{42}]
II	6,7	l,z_{28}	e,n,x
II	6,7	l,z_{28}	z_6
Virchow	6,7,14	r	1,2
Infantis[1]	6,7,14	r	1,5
Nigeria	6,7	r	1,6
Colindale	6,7	r	1,7
Papuana	6,7	r	e,n,z_{15}
Grampian	6,7	r	l,w
Richmond	6,7	y	1,2
Bareilly	6,7,14	y	1,5
Oyonnax	6,7	y	1,6
Gatow	6,7	y	1,7
Hartford	6,7	y	e,n,x:[z_{67}]
Mikawasima[2]	6,7,14	y	e,n,z_{15}
Chile	6,7	z	1,2

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Kauffmann-White Scheme – Day 3

Strathcona	6,7	l,z_{13},z_{28}	1,7
II	6,7	l,z_{28}	1,5:[z_{42}]
II	6,7	l,z_{28}	e,n,x
II	6,7	l,z_{28}	z_6
Virchow	6,7,14	r	1,2
Infantis[1]	6,7,14	r	1,5
Nigeria	6,7	r	1,6
Colindale	6,7	r	1,7
Papuana	6,7	r	e,n,z_{15}
Grampian	6,7	r	l,w
Richmond	6,7	y	1,2
Bareilly	6,7,14	y	1,5
Oyonnax	6,7	y	1,6
Gatow	6,7	y	1,7
Hartford	6,7	y	e,n,x:[z_{67}]
Mikawasima[2]	6,7,14	y	e,n,z_{15}
Chile	6,7	z	1,2

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Kauffmann-White Scheme – Day 3

Final Results

- Antigenic formula: 6,7 : r : 1,2
- *Salmonella enterica* subspecies *enterica* serotype Virchow

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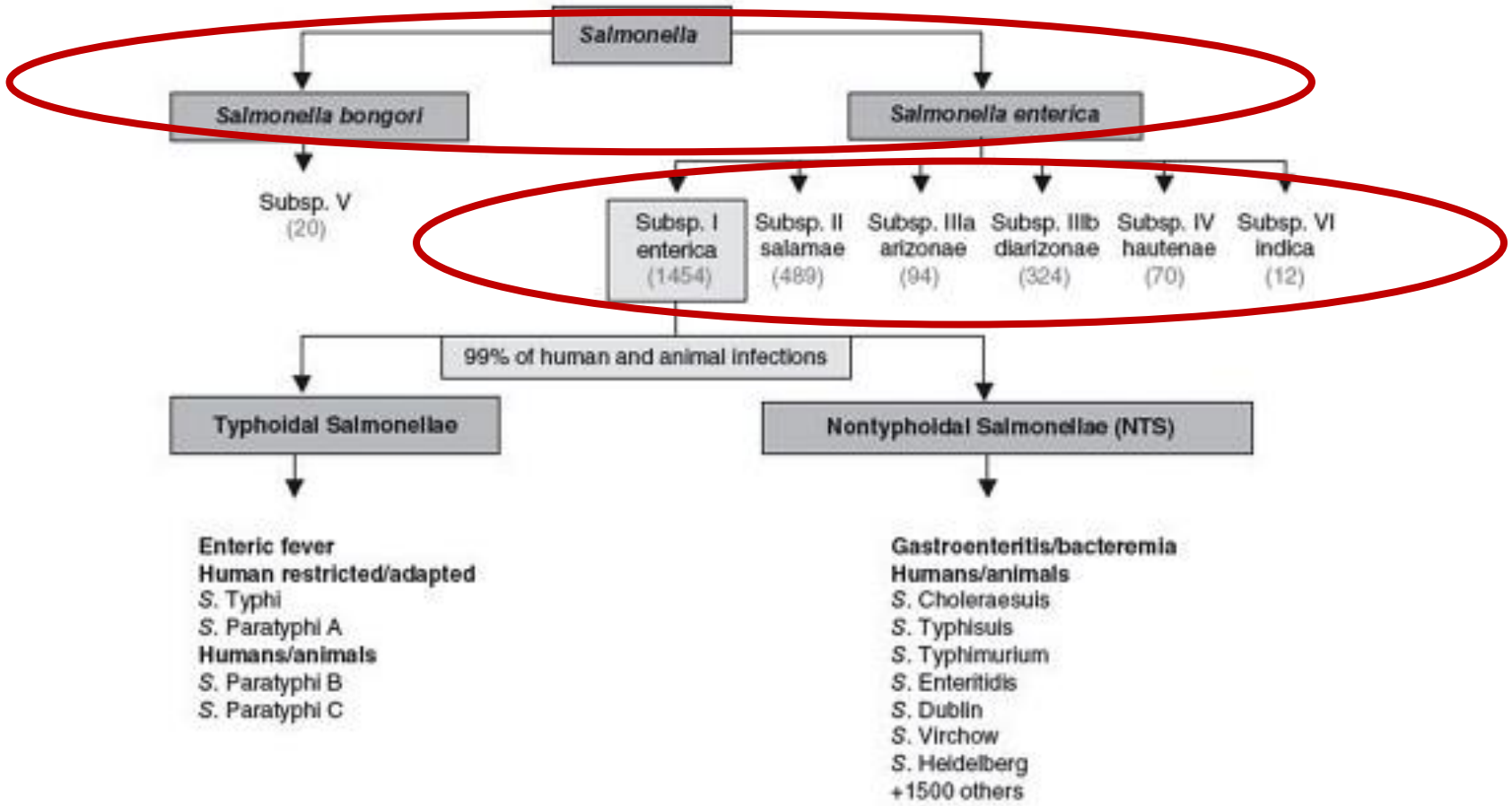
Salmonella Species and subspecies



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Salmonella Species and subspecies

- Some serotypes have the same antigenic formula
  - Should be separated by biochemical tests
    - Consult page 13 in the Kauffmann-White Scheme

Kiambu	1,4,12	z	1,5
II	1,4,12,27	z	1,5
Loubomo	4,12	z	1,6
Indiana	1,4,12	z	1,7
II	4,12	z	1,7

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Salmonella Species and subspecies

DIFFERENTIAL CHARACTERS OF *SALMONELLA* SPECIES AND SUBSPECIES(1)

Species	*S. enterica*						*S. bongori*
Subspecies	*enterica* I	*salamae* II	*arizonae* IIIa	*diarizonae* IIIb	*houtenae* IV	*indica* VI	
Characters							
Dulcitol	+	+	–	–	–	d	+
ONPG (2 h)	–	–	+	+	–	d	+
Malonate	–	+	+	+	–	–	–
Gelatinase	–	+	+	+	+	+	–
Sorbitol	+	+	+	+	+	–	+
Culture with KCN	–	–	–	–	+	–	+
L(+)-tartrate(a)	+	–	–	–	–	–	–
Galacturonate	–	+	–	+	+	+	+
γ-glutamyltransferase	+(*)	+	–	+	+	+	+
ß-glucuronidase	d	d	–	+	–	d	–
Mucate	+	+	+	– (70%)	–	+	+
Salicine	–	–	–	–	+	–	–
Lactose	–	–	– (75%)	+ (75%)	–	d	–
Lysis by phage O1	+	+	–	+	–	+	d
Usual habitat	Warm-blooded animals		Cold-blooded animals and environment				

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Salmonella Species and subspecies

DIFFERENTIAL CHARACTERS OF *SALMONELLA* SPECIES AND SUBSPECIES(1)

Species	*S. enterica*						*S. bongori*
Subspecies	*enterica* I	*salamae* II	*arizonae* IIIa	*diarizonae* IIIb	*houtenae* IV	*indica* VI	
Characters							
Dulcitol	+	+	–	–	–	–	+
ONPG (2 h)	–	–	+	+	–	–	–
Malonate	–	+	+	+	–	–	–
Gelatinase	–	+	+	+	+	–	–
Sorbitol	+	+	+	+	+	–	+
Culture with KCN	–	–	–	–	+	–	+
L(+)-tartrate(a)	+	–	–	–	–	–	–
Galacturonate	–	+	–	+	+	+	+
γ-glutamyltransferase	+(*)	+	–	+	+	+	+
ß-glucuronidase	d	d	–	+	–	d	–
Mucate	+	+	+	– (70%)	–	+	+
Salicine	–	–	–	–	+	–	–
Lactose	–	–	– (75%)	+ (75%)	–	d	–
Lysis by phage O1	+	+	–	+	–	+	d
Usual habitat	Warm-blooded animals		Cold-blooded animals and environment				

- Separate them by biochemical tests.
- Choose one to five tests, depending on which one you have to distinguish between.

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Salmonella Species and subspecies

- Here you have to distinguish between
  - S.enterica subsp **enterica (S.Indiana)** and S.enterica subsp **salamae**.
- Consult the KW scheme

Kiambu	1,4,12	z	1,5
II	1,4,12,27	z	1,5
Loubomo	4,12	z	1,6
Indiana	1,4,12	z	1,7
II	4,12	z	1,7

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Salmonella Species and subspecies

DIFFERENTIAL CHARACTERS OF *SALMONELLA* SPECIES AND SUBSPECIES(1)

Species	*S. enterica*						*S. bongori*
Subspecies	*enterica* I	*salamae* II	*arizonae* IIIa	*diarizonae* IIIb	*houtenae* IV	*indica* VI	
Characters							
Dulcitol	+	+	–	–	–	d	+
ONPG (2 h)	–	–	+	+	–	d	+
Malonate	–	+	+	+	–	–	–
Gelatinase	d	d	–	+	–	d	–
Sorbitol	+	+	+	– (70%)	–	+	+
Culture with KCN	–	–	–	–	+	–	–
L(+)-tartrate(a)	–	–	– (75%)	+ (75%)	–	d	–
Galacturonate	+	+	–	+	–	+	d
γ-glutamyltransferase							
ß-glucuronidase							
Mucate							
Salicine							
Lactose							
Lysis by phage O1							
Usual habitat	Warm-blooded animals		Cold-blooded animals and environment				

- Ex Malonat.
  - Malonat ÷ : S. Indiana
  - Malonat + : S. enterica subsp. Salamae - S.II 4.12: z : 1,7
-

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Acknowledgement

Curtesy to
Microbiologist Matthew Mikoleit
National Enteric Reference Laboratory
Team, Enteric Diseases Laboratory Branch,
Centers for Disease Control and Prevention,
Atlanta, GA, USA

for sharing some slides

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Thank you for your attention

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