SIT *InHaVi* Kit
Quick Identification Test
For strains of *Salmonella* Infantis, Hadar and Virchow

Application
Antisera produced in Immunolab are rabbit polyclonal antisera. Antisera in prepared SIT InHaVi Kit allow for full serological identification of *Salmonella* Infantis, *Salmonella* Hadar and *Salmonella* Virchow according to EU regulations.

Composition
Antisera for agglutination of *Salmonella* rods are prepared from the rabbit sera. Rabbits are vaccinated with inactivated bacterial strains. Antisera are controlled with large sets of strains and prepared in such dilutions that allow for clear reaction in short time. Antisera are ready-to-use products diluted in 0,85% NaCl and preserved with 0,01% thiomersal.
The SIT InHaVi Kit includes necessary sera for detailed identification of the recommended strains of *Salmonella* Infantis, Hadar and Virchow (15 bottles of antisera with droppers: HM and O:C – 5 ml each and O:6,7; O:7; O:8; H:z_{10}; H:enx; H:x; H:nz_{15}; H:r; H:2; H:5, H:6, H:7 - 3 ml each) as well as antiserum for flagellar phase inhibition (1 bottle of antiserum with a dropper H:r - 2 ml). In addition we provide you with a bottle of 3% NaCl solution-5 ml and semi-solid medium by Garda for preparation of 5 x 0,5l of 0,5% media, which composition helps in flagellar development of *Salmonella*.

Detailed Instruction

Procedure
When the results of biochemical tests suggest that the tested strains belong to *Salmonella* genus follow the steps below:

1. Culture the tested strains at 37^{0}C for 20 hours on required medium (for the determination of the somatic antigens – 1,5% nutrient agar, flagellar antigens – semi-solid medium by Garda).

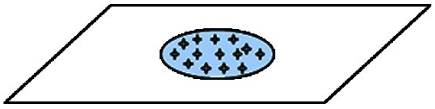
2. Perform the slide agglutination assay (see: Diagram for slide agglutination of *Salmonella* Infantis, Hadar and Virchow)
   - ☝ Before using, antisera should reach room temperature (18-24°C). *If turbidity of antiserum is observed, centrifuge at 4000-5000 RPM for 30 min.*
   - ☝ Serological identification should start from checking the strain with 3% NaCl solution.
     If agglutination occurs, it means that the strain is rough and auto-agglutinates. Such a strain cannot be serologically identified in a slide agglutination assay.
   - ☝ Next perform agglutination test with polyvalent antiserum HM.
     Positive reaction confirms that the tested strain belongs to Salmonella genus.

   Execution:
   i. On the degreased glass slide place a drop of NaCl or antiserum.

   ii. With heated up and then cooled down wire loop or sterile baguette collect bacteria from the medium and place next to the drop. While rubbing the strain on the slide mix it with the serum to form a homogeneous suspension.

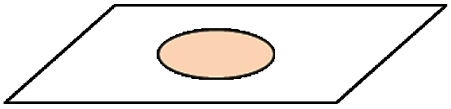
   iii. While gently swaying the slide with circular motions for **10 - 30 seconds (max till 1 minute!)**, watch the result of a reaction.

Be careful so that the drop does not drip off the slide.



Agglutination present „+++”

☺ clumps of bacteria joined by antibodies
☺ increasing transparency of the drop



Lack of agglutination „–”

☹ lack of clumps
☹ „milky” drop

Agglutination is more visible if the reaction is observed against a dark background.

DO NOT LET THE DROP DRY OUT AS IT GIVES FALSE POSITIVE RESULTS.

- ☝ Perform the test with antiserum O:C.
  Positive reaction allows for identification of serological group. [Negative reaction means that tested strain is not *S. Infantis, S. Hadar and S. Virchow, but it can be other strain of Salmonella, so the strain should be tested with other antisera for identification of somatic groups].*

- ☝ To carry out the complete identification of flagellar antigens, the tested strain needs to be cultured on semi-solid medium by Garda (0,5%) in 37±1^{0}C for 20±2 hours.

Keep in mind that the mere confirmation of the presence of the desired antigens is not enough. The possible presence of other antigens/factors that differentiate Salmonella Infantis, Hadar and Virchow from other *Salmonella* strains needs to be checked and ruled out.

The appropriate results confirming the presence of *Salmonella* Infantis (Table 1), Hadar (Table 2) and Virchow (Table 3) are presented below:

Table 1

***Salmonella* Infantis** [6,7:r:1,5]											
							Phase I	Phase II			
NaCl/antiserum	3% NaCl	HM	OC	O:6,7	O:7	O:8	H:r	H:2	H:5	H:6	H:7
Result	-	+++	+++	+++	+++	-	+++	-	+++	-	-

+++ positive reaction, agglutination visible to the naked eye
- negative reaction, lack of agglutination, milky suspension

Table 2

***Salmonella* Hadar** [6,8:z_{10}:enx]										
							Phase I	Phase II		
NaCl/antiserum	3% NaCl	HM	OC	O:6,7	O:7	O:8	H:z_{10}	H:enx	H:x	H:nz_{15}
Result	-	+++	+++	+++	-	+++	+++	+++	+++/++	+++/++

+++ positive reaction, agglutination visible to the naked eye
- negative reaction, lack of agglutination, milky suspension

Table 3

***Salmonella* Virchow** [6,7:r:1,2]											
							Phase I	Phase II			
NaCl/antiserum	3% NaCl	HM	OC	O:6,7	O:7	O:8	H:r	H:2	H:5	H:6	H:7
Result	-	+++	+++	+++	+++	-	+++	+++/++	-	-	-

+++ positive reaction, agglutination visible to the naked eye
- negative reaction, lack of agglutination, milky suspension

Additional information required for identification of *Salmonella* strains

If it is possible to detect only one phase of the tested strain, it means that the strain has a strong first phase, which must be inhibited. The second phase is revealed through the inhibition of the dominant (present) phase by adding antiserum with antibodies against antigens of a strong phase to semi-solid medium by Garda.

Inhibition of the dominant phase

Inhibition of the dominant phase is performed on plates with semi-solid medium by the method of S. Garda, when the strong presence of one of the phases makes it impossible to detect the second phase of the strain. Please, note that *S.* Virchow and *S*. Infantis differ only with one factor in phase II. For this reason it is very important to thoroughly identify phase H:1,2 (for *S.* Virchow) or H:1,5 (for *S*. Infantis). If the strain has strongly developed phase I - H:r and it is not possible to confirm phase II, then the inhibition of the dominant phase should be performed according to the procedure below.

Procedure:

1. Dissolve prepared earlier semi-solid medium by Garda (0,5%) in a microwave and allow for it to cool down to a temperature of approx. 45°C.
2. Place 5 drops of antiserum **H:r for phase inhibition** in the middle of little sterile Petri dish (Ø=5 cm).
3. To antiserum add approx. 10 ml of semi-solid medium by Garda 0,5% (of 45°C) and mix gently through cradling the Petri dish.
4. Allow the medium to solidify at room temperature. (Do not dry!)
5. Use a sterilized wire loop to collect bacteria from agar medium or culture broth and inoculate it in the middle of the plate on the surface of semi-solid medium.
6. Incubate overnight at 37±1°C.
7. The material from peripheral part of the Petri dish is suitable for slide agglutination assay.

☝ If there is still only dominant phase H detected, repeat the procedure.

(Material for the second inoculation should be taken from the culture that has been already subjected to phase inhibition).

Storage conditions and precautions

Antisera should be stored at temperature from **2°C to 8°C. DO NOT FREEZE!**
Protect from light.
Sometimes after a prolonged period of storage there is visible turbidity in antiserum due to precipitation of lipoproteins which can be removed by centrifugation of antiserum (4000-5000 RPM for 30 min).
Semi-solid medium by Garda should be stored at room temperature in a dry and dark place.
Do not use after the expiry date stated on the package.

CE

Manufacturer

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Diagram for slide agglutination of *Salmonella* Infantis, Hadar and Virchow