



Information for use

SIT EnTy Kit Quick Identification Test for *in vitro* diagnostic of *Salmonella* Enteritidis and Typhimurium strains

Intended use

SIT EnTy Kit is an *in vitro* medical device used for quick identification of *Salmonella* Enteritidis and Typhimurium strains isolated from human or animal samples or other materials.

Description

The kit is composed of the absorbed polyclonal Antisera For Slide Agglutination Of *Salmonella*. The antisera included in the kit allow for serological identification of *Salmonella* Enteritidis and Typhimurium. Bacterial surface antigens form visible complexes with specific antibodies in the serum (agglutination reaction).

The ready for use antisera are sold in glass bottles with a dropper. The product is intended only for professional laboratory application by trained/qualified personnel observing the basic aseptic and antiseptic principles.

Composition

Antisera are produced from polyclonal antibodies obtained from animals vaccinated with inactivated bacterial strains. In order to contain only specific antibodies they undergo the process of absorption and are suspended in 0,85% NaCl. Quality control is carried out with a set of over 180 *Salmonella* strains. Antisera are preserved with 0,01% thiomersal

The SIT EnTy Kit is composed of 15 bottles of antisera:

- polyvalent antiserum HM - 5 ml (~125 tests),
- antisera for somatic antigens identification: O:4; O:9 and O:46, 3 ml each (~75 tests),
- antisera for flagellar antigens identification: H:g,m; H:m; H:q; H:s; H:t; H:i; H:2; H:5 and H:6 , 3ml each (~75 tests),
- antisera for phase inversion: H:i and H:2, 1 ml each (~5 reactions).

Method

Preparation of samples

Samples should be collected in sterile containers, transported to the laboratory and processed according to the guidelines for required type of samples. Clearly distant, typical colonies with no signs of contamination should be chosen to perform the slide agglutination assay.

Preparation of tested strains

Culture the tested strains at the required temperature, ranging from +34°C to +38°C for 24±3 hours on nutrient agar media (for identification of somatic antigens use 1,5% solid medium, for flagellar antigens use 0,5% semi-solid medium). (1,5% agar medium produced by Immunolab, catalog No. PG002, PGS02 and 0,5% Sven Gard swarm agar by Immunolab, catalog No. PG001, PGS01 are recommended). Test the *Salmonella* strains by the slide agglutination method

Performance of slide agglutination assay

Antisera should be brought to room temperature (from +18 to +25°C) prior to using them. If turbidity of antiserum is observed, it should be centrifuged.

In order to maintain the sterility of the product (the lack of which could make it impossible to perform the assay or make correct interpretation of the results), the test should be performed in conditions that prevent any contamination of the product.

First of all, before performing slide agglutination assay, a negative control of tested strain with 3% NaCl solution should be carried out. The occurrence of agglutination in a 3% NaCl solution indicates that the tested strain is in phase R (rough strain) and it is not suitable for serological identification in slide agglutination assay.

1. Place a drop of antiserum on the degreased glass slide.
2. With the use of a sterile wire loop/baguette, collect bacteria from the medium and place them next to the drop. While spreading the strain on the slide, mix it with the serum to form a homogeneous suspension.
3. While gently rocking the slide with circular motions for **10 - 30 seconds (max. till 1 minute!)**, observe the reaction. Be careful that the drop does not drip from the slides.

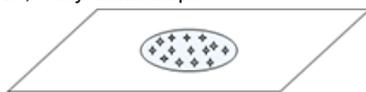
Interpretation of the results

To define the serotype of the tested strain, its antigenic formula needs to be determined with the use of antisera containing antibodies against somatic and flagellar antigens according to the White-Kauffmann Le Minor-classification scheme.

Nature of the reaction should be taken into account: agglutination visible as little lumps is characteristic for the somatic antigens and Vi antigen, as cloudlets for flagellar antigens. Presence of agglutination is a positive reaction. Lack of agglutination is a negative reaction.

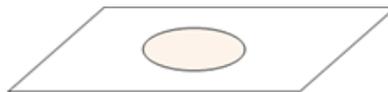
Interpretation of agglutination should be made according to the following scale:

- +++ agglutination present, distributed throughout the transparent drop or on its periphery,
- ++ agglutination present, distributed in a semitransparent drop,
- + small agglutination present on the drop's periphery or at the bottom in a milky white drop. Such results cannot be interpreted as positive in serological diagnostics. Such a situation occurs mostly with biphasic strains that have one strongly developed phase and mainly dominant phase is expressed. In order to determine antigens' composition, the dominant phase should be inhibited,
- (-) lack of agglutination, milky white drop.



Agglutination „+++”

- ☉ agglutinates
- ☉ increasing transparency of the drops



No agglutination „-”

- ☹ no precipitates
- ☹ "milky" drop

Agglutination is more visible if the reaction is observed against a dark background with a magnifying glass with 5-fold magnification.

! Do not let the drop dry out, as it may be interpreted as a false negative result.

An instructional video with detailed information how to perform the test and how to interpret the obtained results is available on our website: <http://immunolab.com.pl/film>

Procedure

- Perform the slide agglutination tests according to the Flowchart of the slide agglutination tests sequence for SIT EnTy Kit..
 - 1.1. First test the strain with a 3% NaCl solution.
If the agglutination occurs, it means that the strain is rough and self-agglutinating. Such a strain cannot be identified serologically by the slide agglutination test.
 - 1.2. Perform the slide agglutination with polyvalent HM antiserum.
A positive reaction with HM antiserum confirms that the strain belongs to the Salmonella genus.
 - 1.3. Perform the slide agglutination with O:9 and O:4 antisera.
Positive reaction with one of these antisera allows for preliminary somatic group identification. The lack of reaction indicates the need to conduct tests with further antisera for group antigens. This means that the tested material does not contain S. Enteritidis and S. Typhimurium bacteria, but there may be other Salmonella strains.
 - 1.4. To perform comprehensive flagellar antigens identification, bacteria should be cultured on 0,5% Sven Gard swarm agar (34-38°C, 24±3 h).
Confirmation of specific flagellar antigens is not sufficient to confirm strain as Salmonella *Enteritidis* and Typhimurium. It is necessary to exclude antigens that differentiate Salmonella strains from *Salmonella* *Enteritidis* and Typhimurium strains.

The appropriate reaction results confirming the presence of *Salmonella Enteritidis* and *Typhimurium* are presented below (Table 1):

Table 1. The slide agglutination results for *Salmonella Enteritidis* and *Salmonella Typhimurium*.

Salmonella Enteritidis [1,9,12:g,m:-]										
					Phase I					Phase II
NaCl/antiserum	3% NaCl	HM	O:9	O:46	H:g,m	H:m	H:q	H:s	H:t	-
Result	-	+++	+++	-	+++	+++	-	-	-	-
Salmonella Typhimurium * [1,4,12:i:1,2]										
				Phase I			Phase II			
NaCl/antiserum	3% NaCl	HM	O:4	H: i			H:2	H:5	H:6	
Result	-	+++	+++	+++			+++	-	-	

+++ agglutination present, distributed throughout the transparent drop or on its periphery,
(-) lack of agglutination, milky white drop.

*Please note that there are also monophasic strains of *Salmonella Typhimurium* with the antigenic formula: 1,4,[5],12:i:- .

Additional information needed to identify *Salmonella Typhimurium*

If the agglutination with the H:i serum is positive and with the H:2 serum is negative (a first phase H:i of the strain is dominant), it means that H:i phase needs to be inhibited/inverted. The second phase is revealed by inhibiting the dominant phase by adding antibodies against dominant phase antigens to 0.5% Sven Gard swarm agar, in this case antiserum for H:i phase inversion, and inoculate the tested strain on the medium. If, after phase inhibition, confirmation of the H:2 phase is still not obtained, it should be assumed that we are dealing with a monophasic strain of *S. Typhimurium*.

Performance of phase inversion

Phase inversion should be performed by culturing tested *Salmonella* strain on 0.5% Sven Gard swarm agar containing antiserum for phase inversion according to the S.Gard method in the case when dominant phase interferes with the latent phase identification.

Phase inversion method

In the case of diphasic strains, when the tested strain gives a positive reaction with only one out of two phases (dominant phase) and the second phase cannot be determined (latent phase), it is necessary to perform phase inversion in order to impede the dominant phase and induce the latent phase.

In order to do that, the following steps should be taken:

- Prepare the semi-solid 0,5% Sven Gard swarm agar and allow it to cool to ~45°C,
- Place a couple of drops (100-200µl) of inhibitory antiserum into the middle of a small Petri dish (Ø approx. 5 cm). It is necessary to pay attention and not to touch the surface of a Petri dish with a dropper.
- Add approximately 10 ml of 0,5% Sven Gard swarm agar and mix gently with an antiserum by circular motions.
- Allow the medium to cool and solidify at room temperature. Do not dry the medium!
- With the use of a sterile wire loop, inoculate the strain to be inverted, at a single point in the center of a Petri dish. For inversion use bacteria previously grown on 0,5% Sven Gard swarm agar.
- Incubate the inoculated plate overnight, at temperatures ranging from +34 to +38°C. Do not place the Petri dish upside down!
- The swarmed strain from the peripheral parts of a plate can be used to identify the second phase in slide agglutination assay.

Performance of a phase inversion - an alternative method

- Prepare a small Petri dish (Ø approx. 5 cm) with approx. 10 ml of sterile, semi-solid 0,5% Sven Gard swarm agar. Store the plate with the lid up. Do not dry the medium!
- Add a couple of drops (100-200µl) of inhibitory antiserum into the middle of a plate with a nutrient medium. It is necessary to pay attention and not to touch the surface of a medium with a dropper.
- Spread the antiserum over the surface with a sterile (glass) spatula.

4. With the use of a sterile wire loop, inoculate the strain to be inverted at a single point in the center of a dish. For inversion use bacteria previously grown on 0,5% Sven Gard swarm agar.
5. Incubate the inoculated plate overnight, at temperatures ranging from +34 to +38°C. Do not place the Petri dish upside down!
6. The swarmed strain from the peripheral parts of a plate can be used to identify the second phase in slide agglutination assay.

! Phase inversion is not always achieved at the first attempt. When necessary, the procedure should be repeated before concluding that the organism has no alternative phase 2 (monophasic strains). *The bacteria for further inoculation should be collected from the plate used during the first attempt.*

User quality control

In exceptional cases, there is a possibility of cross-reactions with other species of Enterobacteriaceae (e.g. strains of *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp., *E. coli* or *Shigella* spp.) due to their high similarity between antigens or their phylogenetic relationship. For this reason, biochemical tests are necessary to determine whether the tested strain belongs to the *Salmonella* genus. Only pure cultures of *Salmonella* should be used for serological diagnostics.

Limitations of the method

In exceptional cases, there is a possibility of cross-reactions with other species of Enterobacteriaceae (e.g. strains of *Citrobacter* spp., *Hafnia alvei*, *Proteus* spp., *E. coli* or *Shigella* spp.) due to their high similarity between antigens or their phylogenetic relationship. For this reason, biochemical tests are necessary to determine whether the tested strain belongs to the *Salmonella* genus. Only pure cultures of *Salmonella* should be used for serological diagnostics.

Materials required to perform the assay, which are not included in the set

Dark background, inoculation loop, magnifying glass, glass slides, solution of 3% NaCl, container for contaminated materials, sterile (glass) spatula, Petri dish, the White-Kauffmann-Le Minor classification scheme, an instructional video (available on our website: <http://immunolab.com.pl/film>).

Storage conditions and precautions

- Antisera should be stored at temperatures ranging from 2°C to 8°C.
- Transport up to 3 days in temperatures not exceeding 25°C does not deteriorate the properties of the product.
- **DO NOT FREEZE!**
- Protect from light.
- Do not use after the expiration date stated on the package.
- The antiserum is a reusable product.
- The expiration date applies to the product in intact packaging, stored in accordance with the instructions, and the product after opening, used in accordance with the intended use, in the conditions described in the instructions and stored after opening in accordance with the instructions.
- Do not use antiserum with altered physico-chemical characteristics indicating microbiological contamination.
- Do not sterilize antiserum prior to use.
- Turbidity and/or precipitates not caused by microbiological contamination may appear in the antiserum. The antiserum is still suitable to be used, but must be centrifuged (8000rpm, 15 min) in order to give correct agglutination results.

Precautions

The product does not pose a health threat to humans and the environment, when used according to the instructions. Aseptic working conditions must be observed and precautions against microbiological hazards should be taken during all procedures. The instructions for use must be strictly followed.

Any serious incident involving the device should be reported to the manufacturer and the competent authority of the Member State in which the user resides or has his place of business.

Precautions when working with infectious material:

- Rabbit antisera contain material of animal origin and should be handled as potentially infectious.
- Performing a slide agglutination assay involves working with pathogenic bacteria, therefore all necessary rules applicable when working with infectious microbiological material must be observed. Wear protective clothing and gloves. Samples collected to perform the assay as well as the material from the slides should be protected against access by third parties, due to the possibility of infection. Slides used in the assay containing antiserum and infectious material, as well as other contaminated materials, should be autoclaved or handled in accordance with applicable laboratory rules



Manufacturer

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E-mail: info@immunolab.com.pl
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Explanation of the symbols used



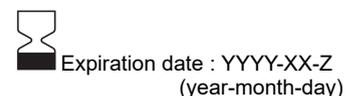
Manufacturer



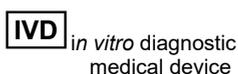
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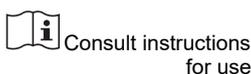
Catalogue number



Expiration date : YYYY-XX-Z
(year-month-day)



in vitro diagnostic
medical device



Consult instructions
for use

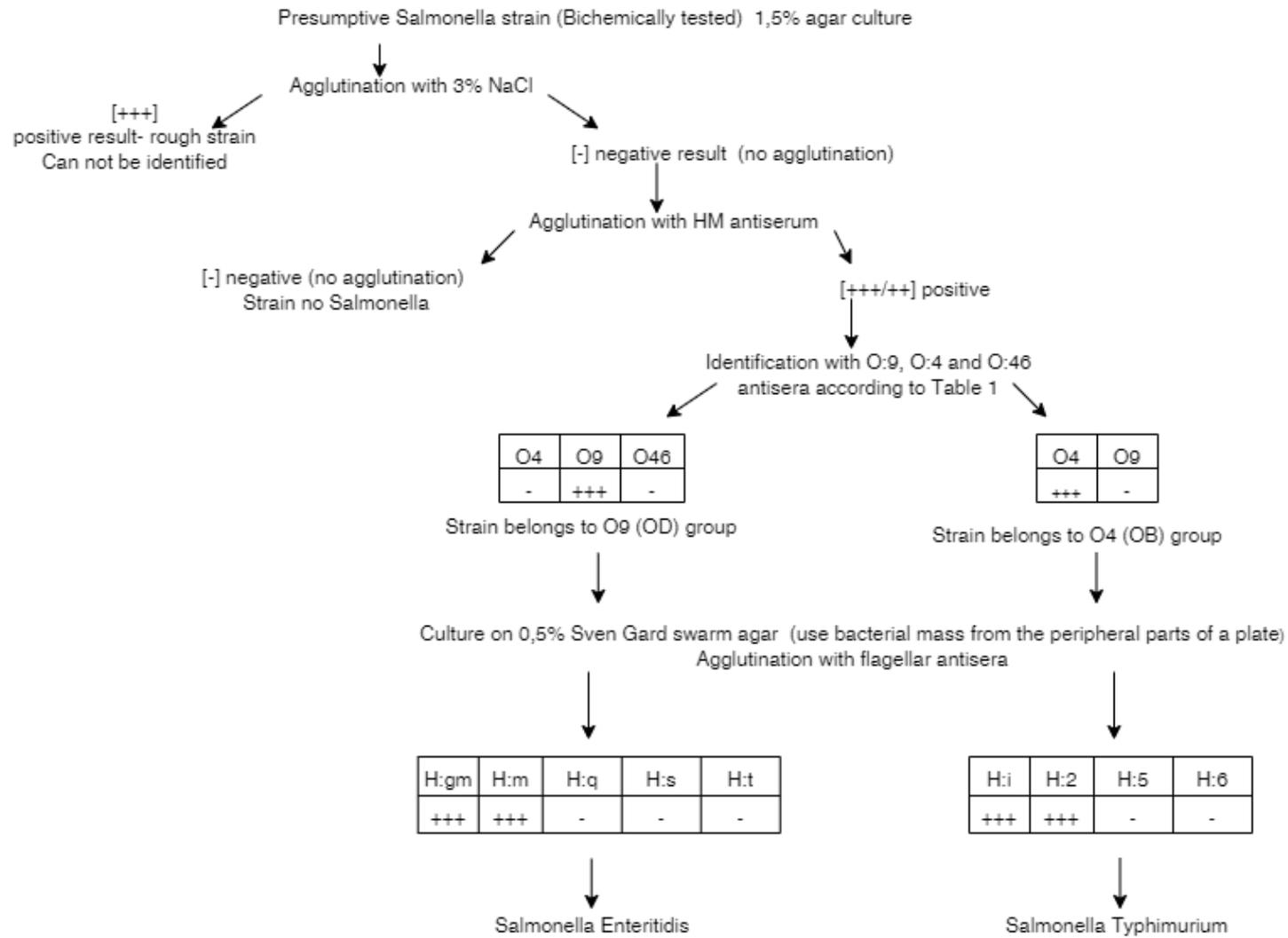


Storage
temperature limit



Transport
temperature limit up to 3 days

Flowchart of the slide agglutination tests sequence for SIT EnTy Kit.



Please note that there are also monophasic strains of Salmonella Typhimurium with the antigenic formula: 1,4,[5],12:i:-

O4	O9	H:i	H:2	H:5	H:6
+++	-	+++	+++	-	-