

## Information for the User

### SET OF ANTISERA FOR SLIDE AGGLUTINATION OF *SALMONELLA*

#### Description

The set of antisera for slide agglutination assay of *Salmonella* is a medical device for *in vitro* use. The antisera set is used for identification of *Salmonella* serotypes isolated on bacteriological media from human samples or other materials. The set of antisera is composed of the absorbed polyvalent and monovalent rabbit antisera, which contain antibodies against group and single factors of somatic, flagellar and capsular Vi antigens. The set of antisera allow for serological identification of *Salmonella* occurring in Poland and in the world.

#### Application

Antisera are designed for serological diagnostics of bacteria belonging to *Salmonella* genus. Antisera allow for the complete serological identification of strains most frequently isolated in Poland and in the world.

#### Composition

Antisera for agglutination of *Salmonella* rods in drops are prepared from the rabbit sera. Rabbits are vaccinated with inactivated bacterial strains. Antisera obtained from these rabbits require a number of absorptions to select specific antibodies. Antisera are controlled with large sets of strains and prepared in such dilutions that allow for clear reaction in a short time. Antisera are diluted in 0,85% NaCl and preserved with 0,01% thimerosal.

#### Properties

Identification of the *Salmonella* strain is based on determining the antigenic structure of the tested strain such as detection of somatic antigens "O" and flagellar antigens "H". Strains of *S. Typhi* usually have capsular antigen "Vi", which is detected in agglutination with antiserum Vi.

The set of antisera for slide agglutination of *Salmonella* consists of: antisera for the identification of somatic antigens: A(O:2); B(O:4,5); C(O:6,7,8,20); D(O:9,46); E(O:3,10,15); E<sub>4</sub>(O:1,3,19); G(O:13,22,23); H(O:14); O:4; O:6,7; O:7; O:8; O:8,20; O:9; O:10; O:11; O:13(OG); O:13,22; O:13,23; O:14,24; O:15; O:18; O:19; O:20; O:21; O:22; O:23; O:28; O:35; O:41; O:46, antiserum Vi, antisera used for identification of flagellar antigens: H:a; H:b; H:c; H:d; H:i; H:g,m; H:g,p; H:f,g; H:m; H:p; H:f; H:q; H:s; H:t; H:u; H:e,h; H:h; H:e,n,x; H:n; H:x; H:k; H:l,v; H:v; H:l,w; H:w; H:r; H:y; H:1,2,5; H:2; H:5; H:6; H:7; H:z; H:z<sub>6</sub>; H:z<sub>10</sub>; H:z<sub>13</sub>; H:n,z<sub>15</sub>; H:z<sub>4,z<sub>23</sub></sub>; H:z<sub>23</sub>; H:z<sub>24</sub>; H:z<sub>28</sub>; H:z<sub>29</sub>; H:z<sub>35</sub>; H:z<sub>36</sub>; H:z<sub>38</sub>; H:z<sub>39</sub>; H:z<sub>42</sub>; H:z<sub>52</sub>; H:z<sub>53</sub>; H:z<sub>57</sub>, polyvalent antisera: HM (containing antibodies against all known flagellar antigens), OMA (O:A;B;D<sub>1</sub>;D<sub>2</sub>;E<sub>1</sub>;E<sub>4</sub>), OMB (O:C<sub>1</sub>;C<sub>2</sub>;C<sub>3</sub>;11;13;6;14), H:E (H:e,h;e,n,x;e,n,z15), H:G (H:f,g;f,g,s;g,m;g,m,q;g,m,t;g,p;g,p,u;g,t;g,s,t;g,q) H:L (H:l,v;l,w;l,z<sub>13</sub>;l,z<sub>28</sub>), H:Z<sub>4</sub> (H:z<sub>4,z<sub>23</sub></sub>;z<sub>4</sub>,z<sub>24</sub>) and H:1 (H:1,2;1,5;1,6;1,7), concentrated antisera for flagellar antigen inhibition/phase inhibition: H:i; H:e,h; H:e,n,x; H:r; H:2; H:z<sub>10</sub> as well as ready-to-use kits for identification of specific strains: SIT EnTy Kit (identification of *S. Enteritidis* and *S. Typhimurium*) and SIT InHaVi Kit (identification of *S. Infantis*, *S. Hadar* and *S. Virchow*).

#### How to use

Culture the tested strains at 37±1°C for 20±2 hours on agar media (for identification of somatic antigens – 1,5% solid medium, flagellar antigens – 0,5% semi-solid medium). 5 ml of ready-to-use antiserum is contained in dark glass bottles of 5 ml with a dropper. Before using antisera should have room temperature (18-24°C). If turbidity of antiserum is observed, centrifuge at 4000-5000 RPM for 30 min.

#### Performance of reaction

1. On the degreased glass slide place a drop of antiserum.
2. 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.
3. While gently swaying the slide with circular motions for 10 - 30 seconds (max till 1 minute!), watch the result of a reaction. Agglutination is more visible if the reaction is observed against a dark background with a magnifying glass with 5-fold magnification. 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.

#### Interpretation of the results

To define the serotype of the tested strain, its antigenic formula needs to be determined through usage of antisera containing antibodies against somatic and flagellar antigens according to the Kauffmann-White 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 drop's periphery or at the bottom in a milky white drop. Such results cannot be interpreted as positive in serological diagnostics. Such situation occurs mostly with biphasic strains that have one phase strongly developed and mainly dominant phase is expressed. In order to determine antigens' composition, the dominant phase should be inhibited.

(-) Lack of agglutination, milky white drop.

#### 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 if the tested strain belongs to the *Salmonella* genus.

#### The content of the package unit

1 bottle with dropper contains:

5 milliliters of antiserum, which allows to perform approx. 100 tests or

3 milliliters of antiserum, which allows to perform approx. 60 tests or

1 milliliter of antiserum, which allows to perform approx. 20 tests.

#### Storage conditions and precautions

Antisera should be stored at temperature from 2°C to 8°C.

DO NOT FREEZE!

Protect from light. If turbidity occurs in antiserum, centrifuge.

Do not use after the expiry date stated on the package.

#### Manufacturer

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