SUMMARY

STABILITY STUDY

Salmonella

Transport study

AUTHOR: Jesper Broberg Schou (JBS)

PRODUCT GROUP(S):

The SSI Diagnostica product range of *Salmonella* antisera, are divided into the following categories:

- The Poly antisera (O and H) represented by **OMA**
- O group pool, O group and O factor antiserum, represented by **O:4**
- H phase pool, H phase, H factor and H phase inversion antiserum. Represented by **H:i**, **H:G**, **H:v** and **SG2**

By selecting the above-mentioned antisera, we represent the majority of the *Salmonella* product range. The results are therefore applicable for the entire range of *Salmonella* products.

DESCRIPTION OF STUDY:

The stability study has been performed from 2013 and reports the stability of the above-mentioned antiserum. The report is conducted according to ISO 23640:2015.

The chosen antisera must undergo different storage temperature (simulating shipping conditions) and were tested after 1, 3, 6, 9, 12, and 18 months, then 2, 3, and 4 years or until reactions are no longer positive. Minimum 3-5 strains were tested at the different time point. All the antisera were in ready-to-use products and there was not a single vial for each time point meaning that the same vial have been opened serval times. The phase-invasion antisera are not mixed with Schwörm agar before start of the individual tests (according to the IFU). All tests were made with non-sealed vials and no sterility testing were performed during

SSI Diagnostica A/S
Herredsvejen 2
3400 Hillerød
Denmark
T +45 4829 9100

ssidiagnostica.com

the duration of the study. All results are stored locally and a summery are reported here.

1. **NUMBER OF LOTS:** The ready-to-use antisera used in this study were from bulk antisera produced at different timepoints, but all were bottled in 2013. The study includes one lot antisera for each product. Each lot were bottled in tree lots.

2. **LOT NO.:** 876E-H sup (O:4), 579N-H sup (OMA), 842C-H sup (H:G), 726D-S sup (H:i), 698M-H sup (H:v) and 840K-H sup (SG 2).

3. **TEMPERATURE:** During this study the different storage conditions have been as follow:
   A. 37° C throughout the entire study
   B. 37° C for 2 weeks then at fridge (2-8° C) for the rest of study
   C. 37° C for 4 weeks then at fridge (2-8° C) for the rest of study
   D. 2-8° C throughout the entire study

4. **TEST:** Test has been performed every three months over the first year, every six months over the second year, and annually thereafter in 4 years. The test methods are performed according to the IFU.
   Salmonella O Group and O Factor and H Phase and H Factor antisera are intended for slide agglutination. 20µL antiserum and 3 representative colonies of the strain to be tested are mixed on a slide. The slide is tilted for 5-10 sec. A positive reaction is seen as a visible agglutination. A negative reaction is persistence of the homogeneous milky turbidity.
   Physiological saline pH 7.4 is used as a negative control and must be negative.
   All slide agglutination tests are performed with 3-5 representative positive strains for each specific antiserum.
   Salmonella Phase Inversion antisera (represented by SG2) are intended for inversion of the H phases. 100 µL of H antiserum for phase inversion is applied in the center of a 6 cm petri dish. 10 mL of the swarm agar are added. After solidification, the plate is inoculated at the center with a loop full of fresh bacterial culture. The plate is incubated overnight at 35-37°C. Culture from the

edge of the growth zone is tested for the inhibited phase using slide agglutination as described above.
A positive reaction is defined as an inhibition of the phase the phase-invasion serum was directed against, using slide agglutination as confirmation.
All slide agglutination tests are performed with 3-5 representative positive strains for each specific antiserum. All strains used are documented in the raw data of the study.

5. **RETEST:** If strains during the study do not react with homologue antisera or auto agglutinates, the strain is substituted with another strain with similar antigen definitions.

6. **HUMIDITY:** N/A

7. **TEST OF INTEGRITY DURING TRANSPORT:** The antisera product line might obtain bruises to their containers when handled roughly but it will not affect the performance of the products. For raw date see report "Drop-test for ImmuView, antisera and Culture Media".

8. **IN-USE STABILITY:** Equal to storage stability.

ACCEPTANCE CRITERIA:

The acceptance criteria for anitsera used for slide agglutination are met if a slide agglutination reaction is positive (visible agglutination) within 10 seconds of sliding.
The acceptance criteria for antisera used for phase inversion are met if a slide agglutination reaction, using material from the edge of a culture grown on swörm agar added the phase inversion antisera to be tested, are negative for the inhibited phase within 10 seconds.

- **Titer:** The titer for *Salmonella* antisera are not controlled during the stability study, as the antisera is tested in ready-to-use solution.

- **Long-term stability:** At the termination of the stability study, the antisera must show a positive reaction in ready-to-use solution for the reference strains.
- **Other:** Strains used as QC panel can be substituted with strains with similar antigen combination during the stability study.

RESULTS:

All results are recorded throughout the period of 4 years.

In table 1 the results are visualized, stating performance at the beginning and the end of the study, all raw data are available on request.

Table 1: Summarizing of results from transport study for *Salmonella* antisera over 4 years.

Antisera	Start of study				End of study			
	A	B	C	D	A	B	C	D
O:4	pos	pos	pos	pos	neg	pos	pos	Pos
OMA	pos	pos	pos	pos	neg	pos	pos	Pos
H:G	pos	pos	pos	pos	neg	pos	pos	Pos
H:i	pos	pos	pos	pos	neg	pos	pos	Pos
H:v	pos	pos	pos	pos	neg	pos	pos	Pos
SG 2	pos	pos	pos	pos	pos	pos	pos	Pos

(POS=Positive)

(NEG=Negative)

CONCLUSION:

Salmonella anti serum stored at 2-8° C results in no difference in performance and stability after 4 years of storage. The shelf life is therefore set to 4 years from date of manufacturing at a temperature range of 2-8° C. If antiserum is left in temperatures for up to 37° C for two to four weeks and then back in cold store the shelf life is not affected.

We have tested that antisera can be stored at 37° C for 12 month and then the antisera are still fully functional but shelf life after this cannot be guaranteed.

Transport: The antisera product line might obtain bruises to their containers when handled roughly but it will not affect the performance of the products.

In-use: The in-use stability is not affected by working with the antiserum on the bench throughout the day if it is stored at 2-8° C for no longer than 4 years form production date.

In conclusion the shelf life and performance of the antiserum are set to four (4) years after from the date of manufacturing. Also, the shelf life and performance are not affected by working with the antiserum on the bench throughout the day if it is stored at 2-8° C, overnight and for no longer than 4 years form manufacturing date

APPROVED:

DATE: 2020-09-08

SIGNATURE:

IVD TEAMLEAD

IVD MANAGER