

**SUMMARY**  
**STABILITY STUDY**  
*E. coli* antiserum  
Transport study

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**PRODUCT GROUP(S):**

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

- O single and pool serum represented by O1, O90, O pool II and antiserum
- OK O single and pool serum represented by OK O55, OK O114, and OK O pool I antiserum
- H single and pool serum represented by H11, H26 and O Pool D antiserum

By selecting the above-mentioned antisera, we represent the majority of the *E. coli* product range. The results are therefore applicable for the complete product range within *E. coli* antisera.

**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, and 9 months, then 2, 3, and 4 years or until reactions are no longer positive. Two positive strains were tested at the different time points. All the antisera were ready-to-use products and there was not a single vial for each time point meaning that the same vial have been opened several times. All results are stored locally and a summary 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.
2. **LOT NO.:** O1A1-4S (O1), O90D1-H (O90), OPIIK1-S (O pool II), HPDJ1-SH (O pool D), OKO55L1-1H (OK O55), OKO114F1.1H (OK O114), OKOP1L1-2H (OK O pool 1), H11D1-2S (H11), H26D1-S (H26).
3. **TEMPERATURE:** During this study, the 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

**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.

O antisera are used for agglutination of boiled cultures in round bottom microtitre plates. Equal quantities (80 µL) of antiserum and culture are mixed, and the result is read after overnight incubation at 50-52 °C. A positive reaction is seen as a “grey carpet”, covering the bottom of the well, often in a clear fluid. When the reaction is negative the bacterial suspension is seen as a small white spot in a clear or a milky turbidity centered in the well.

H antisera are used for agglutination of formalin-killed cultures (final formalin concentration of 0.48%) in test tubes. Equal quantities (180 µL) of antiserum and culture are mixed, and the result is read after 1½-2 hours' incubation at 50-52 °C. A positive reaction is seen as a loose and fluffy cloud in a clear fluid. A negative reaction is seen as a homogenous milky turbidity.

All tests are performed with 2 positive strains for each specific antiserum. All strains used are documented in the raw data of the study.

OK O 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.

4. **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.
5. **HUMIDITY:** N/A
6. **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 data, see report "Drop-test for ImmuView, antisera and Culture Media".
7. **IN-USE STABILITY:** Equal to storage stability.

#### **ACCEPTANCE CRITERIA:**

The acceptance criteria for a positive agglutination is as described in the IFU. For O antisera a gray carpet in the microtiter well, for OK O antisera grains in a clear fluid on a glass slide and for H antisera a fluffy cloud in a clear fluid.

- **Titer:** The titer for *E. coli* antiserum are controlled during the stability study.
- **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 (4 years), all raw data are available on request.

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

E. coli	Start of study				End of study			
	A	B	C	D	A	B	C	D
H11	POS	POS	POS	POS	NEG	POS	POS	POS
H26	POS	POS	POS	POS	NEG	POS	POS	POS
O1	POS	POS	POS	POS	NEG	POS	POS	POS
O90	POS	POS	POS	POS	NEG	POS	POS	POS
OKO55	POS	POS	POS	POS	NEG	POS	POS	POS
OKO114	POS	POS	POS	POS	NEG	POS	POS	POS
O Pool II	POS	POS	POS	POS	NEG	INV *	POS	POS
H Pool D	POS	POS	POS	POS	NEG	NEG**	NEG**	NEG***
OKO pool 1	POS	POS	POS	POS	NEG	POS	POS	POS

(POS=Positive)

(NEG=Negative)

(INV=Invalid)

\* Due to laboratory error it was not possible to determine suitable result. This result shows that the same pool is positive at year 4 if it left at 37 degrees for 4 weeks (study C). Therefore, is there less or no concern for the invalid result in study B

\*\* Only positive for up to 2 years

\*\*\* Only positive for up to 3 years

## CONCLUSION:

*E. coli* antiserum 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.

This is valid for all *E. coli* products except for H pools. These products lose their titer faster than the other products. We will therefore have a shelf life for H pool that is 3 years if stored at 2-8°C and a shelf life of 2 years if the product have been stored at temperatures up to 37° C for up to 4 weeks, this is worst case.

We have tested that antisera can be stored at 37° C for 3 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, three (3) years for H pools, 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, 3 years for H polls, form manufacturing date

**APPROVED:**

**DATE: 2020-11-05**

**SIGNATURE:**

  
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IVD TEAMLEAD  
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IVD MANAGER