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Instructions For Use

Serazym®
Anti-Francisella tularensis

REF E-049

Σ 96

CE

IVD

In-vitro-Diagnostic device

Enzyme immunoassay for detection of IgG, IgA, IgM antibodies to *Francisella tularensis* in human serum samples

REF	Catalogue-No.	LOT	Lot-No.
	Storage temperature		Manufacturer
	Notice advices		Use by
		Σ	Number of determinations
	Consult Instructions for use		Biohazard

Introduction

Francisella tularensis is the causative agent of tularaemia (deer fly fever) and is transferred to man directly from animals (especially rodents) or their ectoparasites. Infections via contaminated food or water and aerosols may also occur. After a 3 to 5 days lasting incubation time the onset of disease shows rather uncharacteristic common symptoms like headache, rheumatism, fatigue and fever. In dependence of the place of infection the clinical course manifests as ulcero-glandular type (primary affect, swelling of lymph nodes), as oculo-glandular type (conjunctivitis) or as inner type of tularaemia. The pulmonary course of infection is characterised by pneumonia and pleuritis, the abdominal course of infection by diarrhea, splenomegaly and intermittent fever.
Direct detection of *Francisella tularensis* is possible after culture via microscopy and immunofluorescence. Detection of specific antibodies built in infected persons about two weeks after infection becomes more and more important for the diagnosis of tularaemia in the routine laboratory (indirect hemagglutination, complement-fixation, enzyme immunoassay).

Intended Use

The *Serazym*® Anti-Francisella tularens is an in vitro diagnostic kit for detection of IgG, IgA and IgM antibodies to the lipopolysaccharide (LPS) of *Francisella tularensis* in human serum.

Principle Of The Test

The *Serazym*® Anti-Francisella tularensis is an immuno enzymometric two-step assay.

Specimens and controls are pipetted into the wells, coated with the lipopolysaccharide of *Francisella tularensis*.
After an incubation time of 30 minutes at 37 °C unbound components are removed from the wells by washing them 5 times with wash solution.

Then anti-human-IgG-/anti-human-IgA-/anti-human-IgM-HRP-conjugate is added and incubated for 15 min at 37 °C.

After a second wash cycle as described above, substrate (tetramethylbenzidine and hydrogen peroxide) is added to the wells for enzymatic reaction. After 10 minutes at room temperature protected from light the reaction is stopped by addition of stop solution (sulphuric acid) to the wells. The blue colour produced by the enzyme action on the chromogen/substrate solution turns to yellow after reaction stop.

The absorbances are measured with a microplate reader at 450 nm (reference filter 620 nm). The results are interpreted referring to the absorbances of the controls.

Preparation And Storage Of Samples

Serum, plasma or other biological fluids can be investigated for anti - *Francisella tularensis* IgG+IgA+IgM antibodies with the *Serazym*® Anti-Francisella tularensis.
Serum or plasma samples have to be diluted 1:51 with sample diluent, e. g. 10 µl sample + 500 µl sample diluent (3).
Pay attention to a sterile sample collection. Samples can be stored at 2...8 °C for a maximum time of 48 hours. For longer storage times samples have to be kept at - 20 °C. Frozen samples have to be warmed to room temperature quickly and mixed well before starting the test run. Repeated freezing and thawing of samples has to be avoided.

Test Components For 96 Wells

1 **WELLS**	**Microtitaton plate** 12 single breakable 8-well strips (in all 96 wells) coated with purified LPS of *Francisella tularensis*	1 vacuum-sealed with desiccant
2 **WASHBUF CONC 10X**	**Wash buffer, 10-fold** for 1000 ml solution	100 ml concentrate, white cap
3 **DIL**	**Sample diluent**	100 ml ready to use, black cap
4 **CONTROL +**	**positive control**	0.5 ml ready to use, red cap
5 **CONTROL WEAK +**	**weak positive control**	0.5 ml ready to use, white cap
6 **CONTROL -**	**negative control**	0.5 ml ready to use, green cap
7 **CONJ HRP**	**HRP-conjugate** HRP-labelled, polyclonal anti-human-IgG/IgA/IgM antibodies (sheep)	12 ml ready to use, red cap
8 **SUBSTR TMB**	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml ready to use, blue cap
9 **STOP**	**Stop solution** 0.25 M sulphuric acid	15 ml ready to use yellow cap

Materials Required But Not Provided

- micropipettes
- multi-channel pipette or multi-pipette
- Reagent container for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water
- glassware
- tubes (2 ml) for sample preparation

Preparation And Storage Of Reagents

Kit size and expiry

One kit is designed for 96 determinations.

The expiry date of each component is reported on its respective label, the expiry date of the complete kit is stated on the outer box label.
Upon receipt, all test components have to be kept at 2…8 °C, preferably in the original kit box.
After opening all kit components are stable for at least 2 months, provided proper storage.

Reagent preparation

Allow all components to reach room temperature prior to use in the assay.

The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the 10fold concentrated wash buffer 1 + 9 with distilled or de-ionized water.
For Example:
10 ml wash buffer concentrate + 90 ml distilled water.
This ready to use wash solution is stable for at least 30 days when stored at 2…8 °C.

Make sure the soak time of the wash solution in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!

Avoid light exposure of the TMB substrate solution!

Assay Procedure

- **Dilute samples with sample diluent *(3)* 1 + 50, e.g. 10 µl serum + 0.5 ml sample diluent *(3)* before starting the assay**
- **Avoid any time shift during dispensing of reagents and diluted samples.**

Working steps

1. Warm all reagents to room temperature before use. Mix gently without causing foam.
2. Add **100 µl *DIL*** (ready-to-use sample diluent) ***(3))*** to all wells
3. **Dispense**
   20 µl prediluted samples,
   20 µl *CONTROL +* (ready-to-use positive control ***(4)),***
   20 µl *CONTROL WEAK +* (ready-to-use weak positive control ***5)***),
   20 µl *CONTROL -* (ready-to-use negative control***(6))*** resp.
   to the intended wells and mix thoroughly
4. Cover plate and incubate for **30 min** at 37°C.
5. Aspirate, then wash each well **5x** with **300 µl** wash solution (diluted from ***(2)***) and tap dry onto absorbent paper.
6. Dispense
   100 µl *CONJ HRP (7)*
7. Cover plate and incubate for **15 min** at 37°C.
8. Aspirate, then wash each well **5x** with **300 µl** wash solution (diluted from **(2)**) and tap dry onto absorbent paper.
9. Dispense
   100 µl *SUBSTR TMB (8)*.
10. Incubate for **10 min** at room temperature protected from light.
11. Dispense
    100 µl *STOP (9)* mix gently.
12. Read absorbances at **450 nm** (reference filter 620 or 690 nm) with a microplate reader within 30 min after reaction stop.

Result Interpretation

Quality control

Test results are valid if:

- the absorbance of the negative control is ≤ 0.20 and
- the absorbance of the positive control is ≥ 1.50

If the above mentioned quality criteria are not met, repeat the test and make sure the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Reference Values

Serazym® Anti-Francisella tularensis	Absorbance
Negative	**< 0.20**
Positive	**> 0.25**
Grey zone	**0.20 - 0.25**

Limitations of the procedure

A result interpretation should always consider clinical findings. In certain cases repeated investigations of patient sera collected with a distance of several weeks may be helpful.
Microbial contaminations of reagents or samples as well as cross contaminations of test kit components and samples can cause false results.
Incorrect washing and incorrect incubation times and temperatures can cause false results.

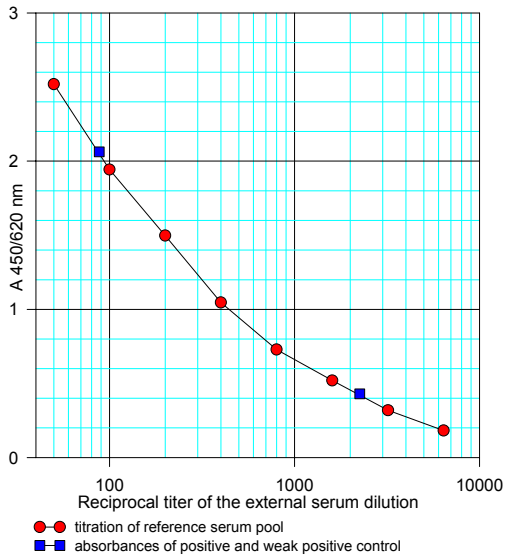
Performance Characteristics

Precision

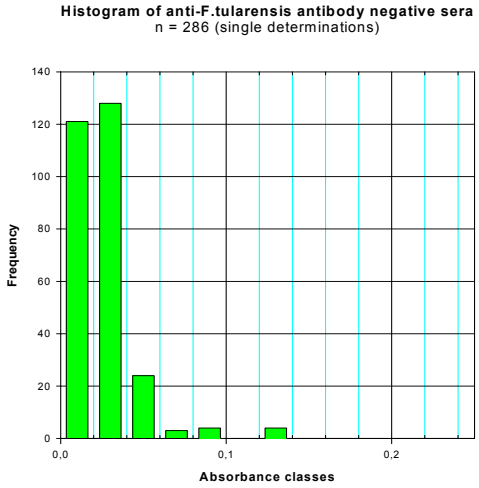
Intra-assay coefficient of variation:

Pool of antibody positive serum samples (titer of external predilution)	Mean absorbance	Standard deviation	Coefficient of variation (%)
1:50	2.713	0.071	2.62
1:100	2.286	0.099	4.32
1:200	1.971	0.065	3.29
1:400	1.606	0.070	4.33
1:800	1.172	0.037	3.19
1:1600	0.800	0.036	4.46
1:3200	0.511	0.030	5.97
1:6400	0.334	0.029	8.61

Titration curve of an anti-Francisella tularensis reactive pooled serum sample



Frequency distribution



Clinical evaluation

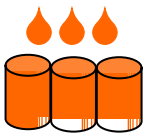
A total of 402 serum samples has been tested with the anti-Francisella tularensis antibody ELISA of the German reference laboratory (InstMikroBio San Ak Bw) and with the *Serazym*® Anti-Francisella tularensis.

		Anti-Francisella tularensis ELISA Reference laboratory (InstMikroBio SanAk Bw)	
		-	**+**
***Serazym*® Anti-Francisella tularensis**	**-**	**312**	**0**
	+	**1**	**89**

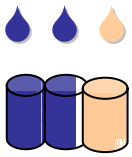
Sensitivity: 100 %
Specificity: 99.7 %

Incubation Scheme

Serazym® Anti-Francisella tularensis

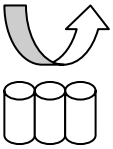


100 µl ***DIL (3)*** to all wells



20 µl ***CONTROL + (4)***
20 µl ***CONTROL WEAK* + (5)**
20 µl ***CONTROL* - (6)**
20 µl **diluted serum sample (1:51)**

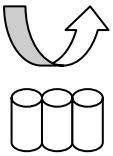
30 min incubation at 37 °C



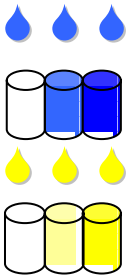
5 X Wash with wash solution

100 µl ***CONJ HRP (7)***

15 min incubation at 37 °C



5 X Wash with wash solution



100 µl ***SUBSTR TMB (8)***

10 min incubation at room temperature, protected from light

100 µl ***STOP (9)***

Read absorbances at 450/620 nm

Common Advices and Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only.

The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.

Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.

Do not use reagents from other manufacturers.

Avoid time shift during dispensing of reagents.

All reagents should be stored at 2…8 °C and warmed to room temperature before use.

Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes.

Handle all components and all patient samples as if potentially hazardous.

Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Note safety precautions of the single test components.