ELISA-VIDITEST anti-TBEV IgG and IgG avidity

REF ODZ-170 96 tests 2°- 10 °C

Type of determination: IgG antibodies

Type of evaluation: Qualitative, Semiquantitative

Type samples: Serum/Plasma

Possibility of determination: Avidity of IgG antibodies

Processing: Manual

CE IVD

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ELISA-VIDITEST anti-TBEV IgG and IgG avidity

ODZ-170

Instruction manual

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1. TITLE

ELISA-VIDITEST anti-TBEV and avidity IgG - ELISA kit for measuring avidity of IgG antibodies to tick-borne encephalitis virus (TBEV) in human serum (plasma).

2. INTENDED USE

ELISA-VIDITEST anti TBEV IgG and IgG avidity is intended for in vitro diagnosis of TBEV- associated diseases (encephalitis, meningoencephalitis). Also it can be used for differential diagnosis of neuroinfections and for monitoring of the antibody response after vaccination against TBEV.
IgG avidity determination can be used for differentiation between primary TBEV infection and the reinfection. In primary infection antibodies with weak affinity to viral antigens (low-avidity) are produced and are subsequently replaced by antibodies that bind viral antigen strongly (high-avidity antibodies). In reinfection, high-avidity antibodies preveal. Determination of IgG avidity may aid to serological examination of TBEV especially in patients with atypical clinical course or in TBEV infections in vaccinated individuals.

The complementary examination to the detection of IgG and IgG avidity is anti-TBEV IgM detection (ELISA-VIDITEST anti TBEV IgM).

3. TEST PRINCIPLE

ELISA-VIDITEST anti TBEV IgG and IgG avidity is a solid-phase immunoanalytical test. The polystyrene strips are coated with a native antigen containing immunodominant epitopes of TBEV. Serum samples are applied into two wells in parallel (eventually, into four wells) and the anti-TBEV antibodies present in serum bind to the immobilized antigens. The next step is the incubation of one well with the wash buffer, the second respective well with the urea solution. Antibodies with low and high avidity remain bound to the antigen in the first well, whereas in the second well the low avidity antibodies are released due to the high concentration of urea and only the high avidity antibodies continue being in complexes with antigens. The bound antibodies are recognized by animal anti-human IgG antibodies labelled with horseradish peroxidase. The amount of the bound labelled antibodies is revealed by an enzymatic reaction that leads to a colour change. The presence of the low avidity antibodies is indicated by a drop of absorbance in wells where the urea solution was added. The ratio between the optical density of the well without urea (the one with the wash buffer) and the corresponding well with urea represents the relative avidity index (RAI).

4. KIT COMPONENTS

	ELISA break-away strips coated with specific antigens STRIPS Ag	1 microplate
1.3 mL	Standard A=negative control serum, r.t.u.[1)] STA/NC	1 vial
1.3 mL	Standard D=calibrator, r.t.u. STD/CAL	1 vial
1.3 mL	Standard E=positive control serum, r.t.u. STE/PC	1 vial
1.3 mL	High avidity control serum r.t.u. HIGH AVID	1 vial
1.3 mL	Low avidity control serum, r.t.u. LOW AVID	1 vial
13 mL	Anti-human IgG antibodies labelled with horseradish peroxidase r.t.u (Px-conjugate) CONJ	1 vial
55 mL	Wash buffer concentrate, 10x concentrated WASH 10x	1 vial
60 mL	Dilution buffer, r.t.u. DIL	1 vial
13 mL	Urea solution , r.t.u UREA	1 vial

13 mL Chromogenic substrate (TMB substrate), r.t.u. TMB 1 vial
13 mL Stop solution, r.t.u. STOP 1 vial
Instruction manual
Certificate of quality
[1)] r.t.u. ready to use

Notice: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Chromogenic substrate TMB is compatible and interchangeable between ELISA-VIDITEST kits which contain TMB and not with other Chromogenic substrates TMB-O, TMB-BF.

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

a. Distilled or deionised water for diluting of the Wash buffer concentrate.
b. Appropriate equipment for pipetting, liquid dispensing and washing.
c. Thermostat (set at 37°C) for ELISA plate incubation.
d. Spectrophotometer/colorimeter (microplate reader – wavelenght 450 nm).

6. PREPARATION OF REAGENTS AND SAMPLES

a. Allow all kit components to reach room temperature. Turn on the thermostat to 37°C.
b. **Vortex samples (sera/plasma) and Control sera in order to ensure homogeneity** and mix all solutions well prior use.
c. **Dilute serum (plasma) samples 1:100 in Dilution buffer** and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). **Do not dilute** the Controls (Standards), they are ready to use.
d. Prepare **Wash buffer** by diluting the Wash buffer concentrate (WASH) **10x** with an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to +32 to +37°C in a water bath. Diluted Wash buffer is stable for one month if stored at room temperature.
e. **Do not dilute** Px-conjugate, UREA, TMB substrate and Stop solution, they are ready to use.

7. ASSAY PROCEDURE FOR THE QUALITATIVE/ SEMIQUANTITATIVE DETECTION OF IgG ANTIBODIES IN SERUM SAMPLES

Manufacturer will not be held responsible for results if manual is not followed exactly.

a. Allow the vacuum-closed aluminium bag with strips to reach a room temperature. Withdraw the adequate number of strips and put unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
b. Pipette 100 µL of Dilution buffer, Standards, Controls and serum samples to the wells according to the pipetting scheme in Figure 1: fill first well with Dilution buffer DIL to determine reaction background. Fill the next two wells with Standard D ST D/CAL (it serves as a calibrator). Fill the next well with positive control serum ST E/PC and negative control serum ST A/NC. The remaining wells fill with diluted tested samples (S1...). It is satisfactory to apply one serum into one well (S1, S2, S3...). However, if you want to minimize a laboratory error, apply control sera and tested sera as doublets, ST D/CAL as triplet.

   Incubate 30 minutes (± 2 min) at 37°C.
c. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells 4x with 250 µL/well of Wash buffer. Avoid cross-contamination between wells!

   If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.
d. Mix Px-conjugate r.t.u. CONJ well and then add 100 µL of Px-conjugate into each well.

   Incubate 30 minutes (± 2 min) at 37°C.
e. Aspirate and wash four times with 250 µL/well of Wash buffer. Tap the plate on an adsorbent paper.

f. Dispense 100 µL of TMB substrate into each well. **Incubate for 15 minutes (+/- 30 seconds) in dark at room temperature.** The time measurement must be started at the beginning of TMB dispensing. Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.

g. **Stop the reaction** by adding 100 µL of Stop solution STOP. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.

h. Read the absorbance at **450 nm with a microplate reader within 10 minutes**. It is recommended to use reference reading at 620-690 nm.

Figure 1. Pipetting scheme for the detection of IgG antibodies:

	1	2	3	4	5	6	7	8	9	10	11	12
a	**DIL**											
b	**ST D/CAL**											
c	**ST D/CAL**											
d	**ST E/PC**											
e	**ST A/NC**											
f	**S1**											
g	**S2**											
h	**S…**											

8. PROCESSING OF RESULTS FOR THE DETECTION OF IgG ANTIBODIES

Begin the processing of results with subtraction of the background absorbance (absorbance of the DIL well) from the absorbance of all other wells. If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

8.1 Processing of results for Qualitative interpretation

1. Compute the mean absorbance of Standard D ST D/CAL. If you applied this control into 3 wells and if any of the three values absorbance falls out of the range +/-20% of the mean absorbance then exclude the deviating well from the calculation and compute a new mean value using the values from the other two wells.
2. **Compute the Cut-off value** of the test by multiplication the ST D/CAL mean by the Correction factor. **The correction factor value determined for this lot of the kit is stated in the Quality control certificate.**
3. Sera that have absorbance value < 90% cut-off are negative and sera with absorbance value > 110% cut-off are considered to be positive.

8.2 Semiquantitative evaluation

Determine the Positivity Index for each serum sample as follows:

1. Compute the cut-off value (see previous paragraph 8.1).
2. Compute the Positivity Index according to the following formula:

$$\text{Sample Positivity Index} = \frac{\text{Sample absorbance}}{\text{Cut-off value}}$$

3. Express the serum reactivity according to Table 1 (Semiquantitative interpretation of results).

Table 1: Semiquantitative interpretation of results

Index value	Evaluation
< 0.90	Negative
0.90 - 1.10	+/-
> 1.10	Positive*

* on the basis of the Positivity Index value it is possible to estimate semiquantitatively the amount of antibodies in the sample.

Note! An indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is again indifferent then it is recommended to use an alternative testing method or to obtain another sample from the patient, usually withdrawn 1-2 weeks later.

Example of calculation:

ST D/CAL absorbance	= 1.111; 1.143
Mean ST D/CAL absorbance	= 1.127
Sample absorbance	= 0.800
Correction factor	= 0.18
Cut-off value	= 1.127 x 0.18 = 0.203
Sample Positivity Index	= 0.800 / 0.203 = 3.94

9. ASSAY PROCEDURE FOR MEASURING AVIDITY OF IgG ANTIBODIES

Manufacturer will not be held responsible for results if manual is not followed exactly.

a. Allow the vacuum-closed aluminium bag with strips to reach a room temperature. Withdraw the adequate number of strips and put unused strips into the provided pouch and seal it carefully with the desiccant kept inside.

b. Pipette 100 µL of Dilution buffer, Controls and serum samples to the wells according to the pipetting scheme in Figure 2. First, fill wells in the first raw of the first two strips with Dilution buffer DIL to determine the reaction background. Then fill two wells with High avidity control serum HIGH AVID , two wells with Low avidity control serum LOW AVID. Fill the next two wells with Standard D STD/CAL and Standard E ST E/PC. Then pipette the diluted serum samples (S1, S2, S3…) in doublets into the remaining wells. It is satisfactory to use the "simple doublettes", however, if you want to minimize a pipetting error, apply serum samples as quadruplettes, i.e. two wells for each of the respective strip.

   Incubate 30 minutes (±2 min) at 37°C.

c. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells 4x with 250 µL/well of Wash buffer. Avoid cross-contamination between wells! If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last remaining drops.

d. Add **100 µL of diluted Wash buffer** WASH 1x into wells of odd-numbered strips (i.e. column 1, 3, 5, 7, 9 and 11) and **100 µL of Urea solution** UREA into each well of even-numbered strips (i.e. column 2, 4, 6, 8, 10 and 12).

   Incubate 10 minutes (±5 sec) at 37°C.

e. Aspirate and wash 4x with 250 µL/well of Wash buffer. Tap the plate on an adsorbent paper.

f. Mix Px-conjugate r.t.u. CONJ well and then add 100 µL of Px-conjugate into each well.

   Incubate 30 minutes (±2 min) at 37°C.

g. Aspirate and wash four times with 250 µL/well of Wash buffer. Tap the plate on an adsorbent paper.

h. Dispense 100 µL of TMB substrate into each well. **Incubate for 15 minutes (+/- 30 seconds) in dark at room temperature.**

   The time measurement must be started at the beginning of TMB dispensing.
   Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.

i. **Stop the reaction** by adding 100 µL of Stop solution STOP. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.

j. Read the absorbance at **450 nm with a microplate reader within 10 minutes**. It is recommended to use reference reading at 620-690 nm.

Figure 2. Pipetting scheme for measuring avidity of IgG antibodies:

	WASH	**UREA**	**WASH**	**UREA**	**...**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	DIL	DIL	S4	S4								
B	HIGH AVID	HIGH AVID	...									
C	LOW AVID	LOW AVID										
D	ST D/CAL	ST D/CAL										
E	ST E/PC	ST E/PC										
F	S1	S1										
G	S2	S2										
H	S3	S3										

10. PROCESSING OF RESULTS FOR MEASURING AVIDITY OF IgG ANTIBODIES

a. First, subtract the absorbance of the background (absorbance of the DIL well) from the absorbances of all other wells. Use the respective background value for wells incubated with the Urea solution and for wells incubated without the Urea solution.

   Important note: Avidity evaluation is possible in anti-TBEV IgG-positive serum samples only. If the tested serum sample is negative or indifferent, the relative avidity index (RAI) cannot be assessed. IgG-positivity can be checked using following calculation:

   Calculate cut-off value of the test: Multiply the absorbance value of ST D/CAL in the wells with Wash buffer (WASH) with correction factor. **The correction factor value determined for this lot of the kit is written in the Quality control certificate.**

   Samples incubated with Wash buffer with absorbance (OD) lower than the 90% cut-off value are considered negative and samples (incubated with WASH) with absorbance higher than the 110% cut-off value are considered positive.
   If you applied two duplicates, compute the mean absorbance of serum from wells on the same strip.

b. Calculate the relative avidity index value (**RAI**): divide the absorbance of a sample well incubated with the Urea solution by the absorbance of the sample well incubated with the Wash buffer, express in percent (i.e. multiply by 100).
   Both Controls (HIGH AVID and LOW AVID) are used for the internal validity test and must be involved in each run of the assay.
   Formula:

$$\frac{\textbf{absorbance with urea solution}}{\textbf{absorbance with wash buffer}} \textbf{ x 100 = RAI (\%)}$$

INTERPRETATION OF RESULTS:

RAI value in %	Interpretation
< 40 %	Presence of low avidity antibodies
40 % - 60 %	Indifferent result
> 60 %	Presence of high avidity antibodies

Example:

Absorbances of HIGH AVID in wells with Urea solution	= 1.770; 1.718
Mean absorbance of HIGH AVID in wells with Urea solution	= 1.744
Absorbances of HIGH AVID in wells with Wash buffer	= 1.845; 1.904
Mean absorbance of HIGH AVID in wells with Wash buffer	= 1.875
RAI (%)	= (1.744 x 100) / 1.875 = 93 %

11. INTERPRETATION OF THE RESULTS

Presence of anti-TBEV antibodies			Interpretation
IgG	IgM	IgG avidity	
-	-	-	Seronegative, sensitive to the infection
+	-	high	Anamnestic antibodies (past infection or the result of vaccination) Acute infection in vaccinated persons*: second serum sample collected in 1-2 weeks after the first one should be tested
-	+	-	Suspect early phase of acute infection: examination of the second serum sample taken in 1-2 weeks after the first one is recommended
+	+	low	Acute primary infection
+	-	low	Suspect acute or recent primary infection
+	+	high	Suspect recent infection, infection in vaccinated individuals or unspecific reactivity in IgM: examination of the second serum sample collected in two weeks and follow up of the antibody dynamics is recommended

***Important note:** Laboratory results can be interpreted only in the context with the patient´s symptoms and the clinical history. ELISA-VIDITEST anti-TBEV IgG kit may detect cross-reactive antibodies to other flaviviruses, i.e., Dengue virus, West Nile, yellow fever or Japanese encephalitis viruses. Presence of IgG anti-TBEV antibody does not ensure protective immunity against TBEV infection. Presence of protective antibodies must be confirmed by virus-neutralization test.

12. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST

12.1 Validity of the test

The test is valid if:
The background absorbance (the absorbance of the Dilution buffer) is less than 0.150
The mean absorbance values of standards/ control sera and the ratio between the absorbance values of ST E/PC / ST D/CAL are in the ranges stated in the Quality control certificate for this kit lot.

12.2 Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) were determined with samples of different RAI values.

12.2.1 Intraassay variability

The coefficient of intraassay variability is max. 8%.

Example: (n = number of parallels on the same plate)

n	Absorbance	±σ	CV%
16	1.254	0.064	5.1

12.2.2 Interassay variability

The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison of the OD values of the same serum sample in several consecutive tests.

Example: (n = number of an independent examination of the same serum sample)

n	A	±σ	min – max	CVrepro
10	0.674	0.090	0.567-0.779	13.4%
9	0.832	0.061	0.729-0.927	7.3%
7	1.116	0.069	1.048-1.221	6.2%

12.3 Diagnostic sensitivity and specificity

The diagnostic sensitivity was determined with the samples with expected positivity for IgG anti-TBEV (vaccinated people, patients with acute or past TBEV infection). The diagnostic sensitivity of the test is 98.5%.Agreement with another commercial test was 98.5%

The Diagnostic specificity was determined using anti-TBEV IgG negative serum samples from unvaccinated blood donors. The specificity of the test was 100% and agreement with an alternative commercial test was 94.8%.

12.4 Interference

Haemolytic and lipemic samples have no influence on the test results up to concentration of 50 mg/mL of haemoglobin, 5 mg/mL of bilirubin and 80 mg/mL of triglycerides.

13. SAFETY PRECAUTIONS

All ingredients of the kit are intended for laboratory use only.

Controls contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However they should be regarded as contagious and handled and disposed of according to the appropriate regulations.

Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine. Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.

Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.
Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

14. HANDLING PRECAUTIONS

Manufacturer guarantees performance of the entire ELISA kit.

Follow the assay procedure indicated in the Instruction manual.

Calibrator and control sera contain preservative ProClin 300® (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-iothiazol-3-one (3:1)).

Wash solution, Stop solution and Dilution buffer are compatible and interchangeable between different ELISA-VIDITEST sets except those with explicite statement in their Instruction manuals.

Avoid microbial contamination of serum samples and kit reagents.

Avoid cross-contamination of reagents.

Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.

Variations in the test results are usually due to:

* Insufficient mixing of reagents and samples
* Inaccurate pipetting and inadequate incubation times
* Poor washing technique or spilling the rim of well with sample or Px-conjugate
* Use of identical pipette tip for different solutions

15. STORAGE AND EXPIRATION

The ELISA kit should be used within three months after opening.

Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light. Store unused strips in the sealable pouch and keep the desiccant inside.

Store undiluted serum samples at +2 to +10°C up to one week. For longer period make aliquots and keep them at -20°C. Avoid repeated thawing and freezing.

Do not store diluted samples and a diluted Px-conjugate. Always prepare fresh.

Kits are shipped in cooling bags, the transport time up to 72 hours have no influence on expiration. If you find damage at any part of the kit, please inform the manufacturer immediately.

Expiration date is indicated at the ELISA kit label and at all reagent labels.

16. USED SYMBOLS

number of tests

Conformité Européenne – product meets the requirements of European legislation

diagnostika *in vitro*

±σ standard deviation

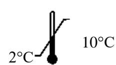
CV coefficient of variation

OD optical density

manufacturer

expiration

Lot of kit



storage at +2°C - +10°C

°C Celsius degree

% percentage

n number of tested samples

A valuea of tested sample

read usage instructions

catalog number

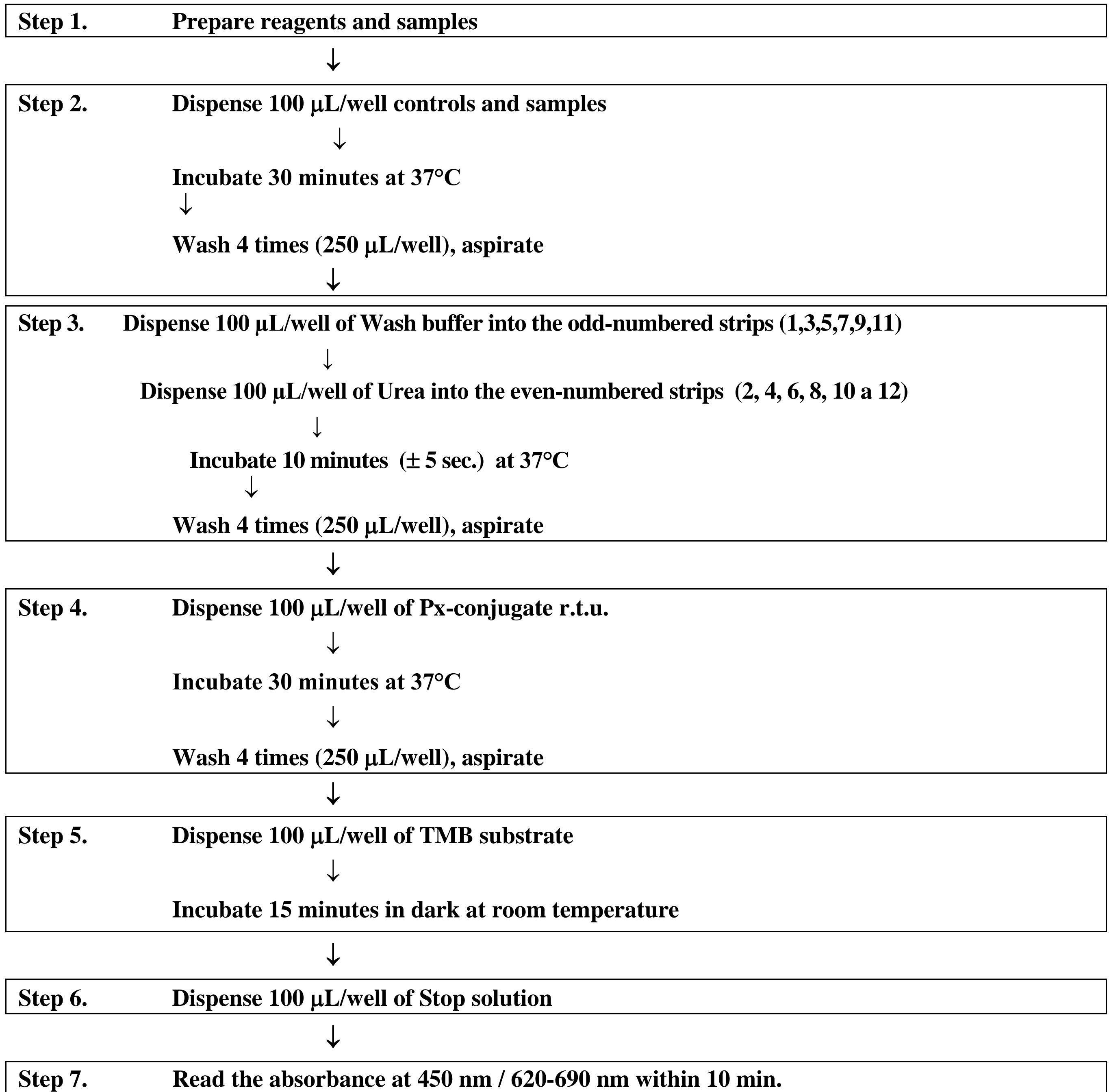
17. REFERENCES

Dumpis U., Crook D., Oksi J.: Tick-Borne Encephalitis. Clinical Infectious Diseases, 1990; 28 (April): 882-890.

Gassmann, C., Bauer G.: Avidity determination of IgG directed against tick-borne encephalitis improved detection of current infection. J. Med. Virol. 51, 1997; 242-251.

18. FLOW CHART

If you do not determinate IgG avidity omit step 3



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