ELISA-VIDITEST anti-VZV IgG and IgG avidity

Cat. No. ODZ-233

Instruction manual

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1. TITLE:

ELISA-VIDITEST anti-VZV IgG and IgG avidity - ELISA kit for the semiquantitative determination of IgG antibodies to varicella zoster virus in serum (plasma) and their avidity evaluation.

2. INTENDED USE:

ELISA-VIDITEST anti VZV IgG and IgG avidity assay is intended for in vitro diagnosis of VZV associated diseases, namely varicella and herpes zoster. The diagnostic kit can also be utilized for differential diagnosis of neuroinfections, infections of eye and skin exanthematous diseases. VZV IgG avidity determination can be used for differentiation between the primary VZV infection and reactivation or 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 reactivation or reinfection high-avidity antibodies preveal.

3. TEST PRINCIPLE:

ELISA-VIDITEST anti VZV IgG and IgG avidity assay is a solid-phase immunoanalytical test. The polystyrene strips are coated with a native antigen containing immunodominant epitopes of VZV. Serum samples are applied into two wells in parallel (eventually, into four wells) and the anti-VZV 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 8-well break-away strips coated with the antigen STRIPS Ag	1 microplate
1.3 mL High avidity control serum, r.t.u. * CONTROL HIGH AVID	1 vial
1.3 mL Low avidity control serum, r.t.u. CONTROL LOW AVID	1 vial
1.3 mL Standard 1, r.t.u. STANDARD 1	1 vial
1.3 mL Standard 2, r.t.u. STANDARD 2	1 vial
1.3 mL Negative control serum, r.t.u. CONTROL -	1 vial
15 mL Anti-human IgG antibodies labelled with horseraddish peroxidase, r.t.u. (Px-conjugate) CONJ	1 vial
125 mL Wash buffer, 10x concentrated WASH 10x	1 vial
15 mL Urea solution, r.t.u. UREA	1 vial

100 mL Dilution buffer, r.t.u. DIL	1 vial
15 mL Chromogenic substrate (TMB substrate), r.t.u. TMB	1 vial
15 mL Stop solution, r.t.u. STOP	1 vial
Sealable pouch for unused strips	
Instruction manual	
Quality Control Certificate	

* ready to use

5. MATERIAL REQUIRED BUT NOT PROVIDED WITH THE KIT:

1. Distilled or deionised water for dilution of the Wash buffer concentrate.
2. Appropriate equipment for pipetting, liquid dispensing and washing.
3. Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

6. PREPARATION OF REAGENTS AND SAMPLES:

a. **Allow all kit components to reach room temperature.**

b. **Vortex samples, the Standards and the Controls in order to ensure homogeneity** and mix all solution well prior use.

c. **Dilute serum (plasma) samples 101x in Dilution buffer** and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer). Do not dilute the Controls and Standards, they are ready to use.

d. Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionised water (e.g. 100 mL of the concentrated Wash buffer + 900 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 week if stored at +2 to +10° C.

e. Do not dilute Px-conjugate, TMB substrate, Urea solution and Stop solution, they are ready to use.

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

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, Control sera and serum samples to the wells according to the pipetting scheme in Figure 1 (page 3): fill first well with Dilution buffer (DIL) to determine reaction background. Fill the next two wells with STANDARD 1 (it serves as a calibrator). Fill the next well with Negative control serum (CONTROL -). The remaining wells fill with diluted tested samples (S1...). It is also suitable to apply positive control serum (STANDARD 2) for the test control. 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, STANDARD 1 as triplet. We also recommend applying internal reference positive control sample for verification of the calibration, continuity and variability of the test.

c. **Incubate 60 minutes (±5 min) at room temperature.**

d. 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.

e. Mix Px-conjugate r.t.u. (CONJ) well and then add 100 µL of Px-conjugate into each well.
f. **Incubate 60 minutes (±5 min) at room temperature**.
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.
i. **Incubate for 10 minutes (+/-5 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.
j. **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.
k. 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	**STANDARD 1**											
c	**STANDARD 1**											
d	**CONTROL -**											
e	**S1**											
f	**S2**											
g	**S3**											
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 absorbances of all other wells.

8.1 Processing of results for Qualitative interpretation

1. Compute the mean absorbance of STANDARD 1. If you applied STANDARD 1 into 3 wells and if any of the three STANDARD 1 absorbances falls out of the range +/-20% of the mean absorbance then exclude the deviating well from the calculation and compute a new STANDARD 1 mean using the values from the other two wells.
2. **Compute the Cut-off value** of the test by multiplication the STANDARD 1 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	+/-
1.11 - 2.50	+
2.51 - 5.00	++
5.01 - 8.00	+++
> 8.00	++++

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:

STANDARD 1 absorbances	= 1.111; 1.143
Mean STANDARD 1 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:

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 (page 5). 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 (CONTROL HIGH AVID) and two wells with Low avidity control serum (CONTROL LOW AVID). If you want to use qualitative interpretation for serum samples, fill next two wells with STANDARD 1. 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.

c. **Incubate 60 minutes (±5 min) at room temperature.**

d. 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.

e. Add **100 µL of Wash buffer (WASH)** into wells of even-numbered strips (i.e. column 2, 4, 6, 8, 10 and 12) and **100 µL of Urea solution (UREA)** into each well of odd-numbered strips (i.e. column 1, 3, 5, 7, 9 and 11).
f. **Incubate 10 minutes (±5 sec) at room temperature.**
g. Aspirate and wash 4x with 250 µl/well of Wash buffer. Tap the plate on an adsorbent paper.
h. Mix Px-conjugate r.t.u. (CONJ) well and then add 100 µL of Px-conjugate into each well.
i. **Incubate 60 minutes (±5 min) at room temperature**.
j. Aspirate and wash four times with 250 µl/well of Wash buffer. Tap the plate on an adsorbent paper.
k. Dispense 100 µl of TMB substrate into each well.
l. **Incubate for 10 minutes (+/-5 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.
m. **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.
n. 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:

	UREA	**WASH**	**UREA**	**WASH**	**...**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	DIL	DIL	S5	S5								
B	CONTROL HIGH AVID	CONTROL HIGH AVID	...									
C	CONTROL LOW AVID	CONTROL LOW AVID										
D	STANDARD 1	STANDARD 1										
E	S1	S1										
F	S2	S2										
G	S3	S3										
H	S4	S4										

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 absorbancies 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-VZV 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 STANDARD 1 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.

b. If you applied two duplicates, compute the mean absorbance of serum from wells on the same strip.

c. 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 (CONTROL HIGH AVID and CONTROL LOW AVID) are used for the internal validity test and must be involved in each run of the assay.

Formula:

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

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

11. VALIDITY, SPECIFICITY AND SENSITIVITY OF THE TEST:

11.1 Validity of the test

a. The background of the reaction (the absorbance of the Dilution buffer) is less than 0.100.
b. RAI of High avidity control serum (CONTROL HIGH AVID) is > 60 %.
c. RAI of Low avidity control serum (CONTROL LOW AVID) is < 40 %.
d. The mean STANDARD 1 absorbance should be in range that **is written in enclosed Quality control certificate**.
e. OD values of control sera should be:
STANDARD1 > STANDARD2 > CONTROL -.

11.2 Precision of the test

The intra-assay variability (within the test) and the inter-assay variability (between tests) were determined with samples of different RAI values.

Example of some positive sera which contain high avidity IgG antibodies (n=8)
RAI range: 63% - 100%

Example of some negative sera which contain low avidity IgG antibodies
RAI range: 17% - 35%

11.2.1 Intra-assay variality

The coefficient of intraassay variability is max. 5% (n = the number of parallels):

An example of intra-assay variabilty (n= number of paralel determinations in the same test)

n	A	±σ	CV
16	1.335	0.050	3.8 %
16	0.614	0.023	3.7 %

RAI intra-assay variability
Example of intra-assay variabilty of RAI (n= number of paralel determinations in the same test)

N	RAI (%)	SD(%)	CV%
16	76	2	2.6
16	61	4	6.6
16	43	2	4.7

11.2.2 Inter-assay variality

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 parallel determinations in the same test)

n	A	±σ	min – max	CV %
18	1.369	0.064	1.223-1.476	4.7%
18	0.463	0.060	0.337-0.569	12.9%

Example of inter-assay RAI variability (n=8):
Mean RAI (%):34 ± 4,8% e.i. min – max 24 – 41%
Mean RAI (%):87 ± 5,8% e.i. min – max 77 – 95%

11.2.3 Recovery test

Measured values of recovery test for every Lot are between 80-120% of expected values.

11.3 Diagnostic sensitivity and specificity

Evaluation was performed by the comparing the VIDITEST kit with two other commercial ELISA tests and with indirect immunofluorescence test.

VZVstatus	Total	Positive	Equivocal	Negative	
Negative	90	3	2	85	specificity: 96.9%
Positive	134	127	3	4	sensitivity: 96.6%

VZV IgG avidity	RAI in ELISA-VIDITEST anti VZV IgG and IgG avidity assay		
	<40%	40-60%	>60%
Low (n=14)	13	1	0
Indifferent (n=7)	0	5	2
High (n=21)	1	1	19

11.4. 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 50 mg/mL of triglycerides.

12. 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.

13. HANDLING PRECAUTIONS:

Manufacturer guarantees performance of the entire ELISA kit.

Follow the assay procedure indicated in the Instruction manual.

Controls, TMB substrate, Dilution buffer and Px-conjugate contain preservative ProClin 300®.

Wash solution, TMB substrate, 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

14. STORAGE AND EXPIRATION:

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. 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.

References:

Wasmuth EH, Miller WJ. Sensitive enzyme-linked immunosorbent assay for antibody to varicella zoster virus using purified VZV glycoprotein antigen. J med Virol 1990; 32: 189-93.

Landry ML, Cohen SD, Mayo DR, Fong,CKY et al. Comparison of fluorescent antibody to membrane antigen test, indirect immunofluorescence assay and commercial enzyme linked immunoassay for determination of antibody to varicella zoster virus. J Clin Microbiol 1987; 25: 832-835.

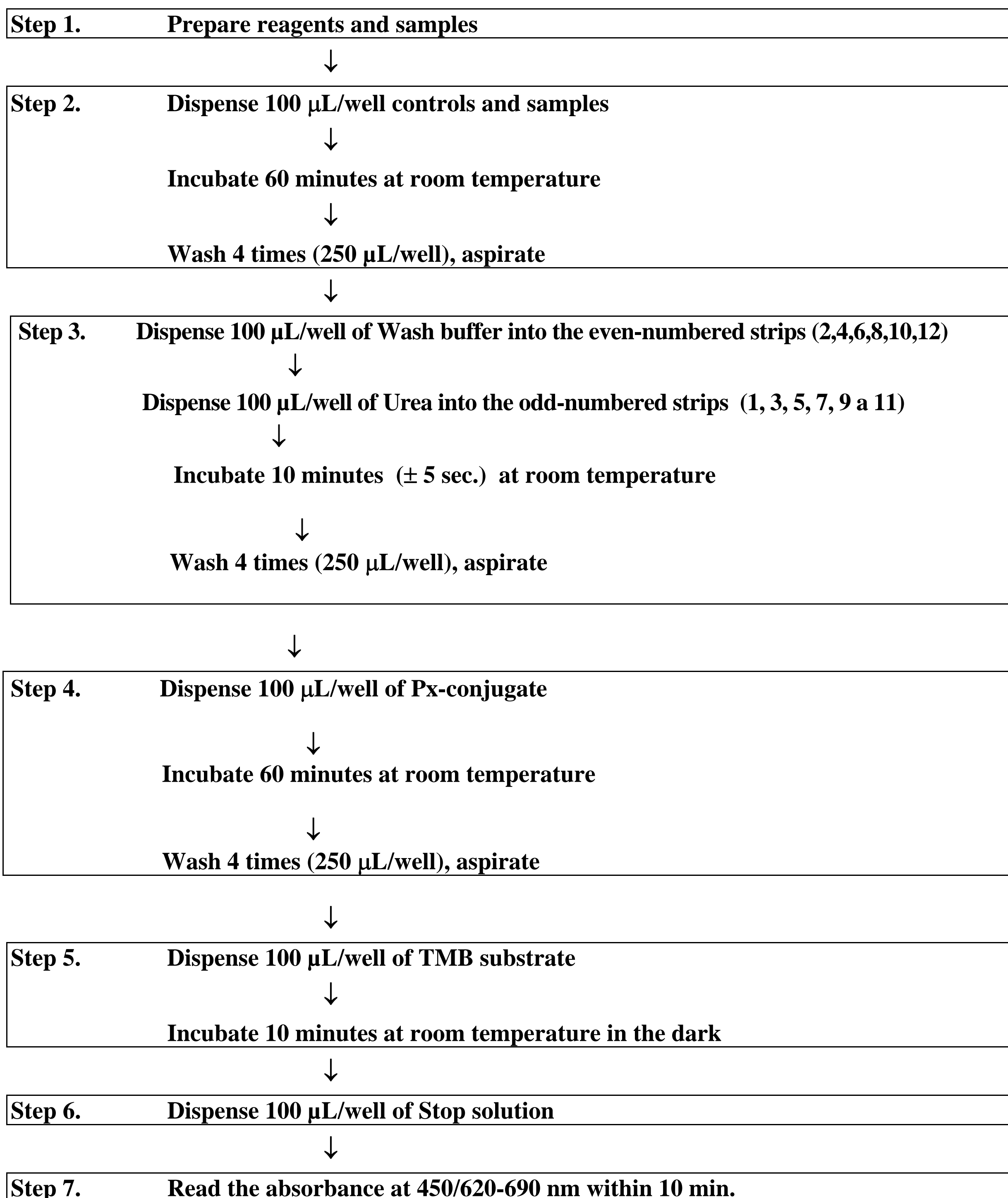
Gershon AA, Steinberg SP. Antibody response to varicella zoster and the role of antibody in host defense. Amer J Med Sci 1981;282: 12-17

Provost PJ, Krah DL, Kuter BJ, Morton DH et al. Antibody assays suitable for assesing immune response to live varicella vaccine. Vaccine 1991; 9: 111

Leung J, Harpaz R, Baughman AL, Heath K et al. Evaluation of laboratory methods for diagnosis of varicella. Clin Infect Dis 2010; 51: 23-32.

15. FLOW CHART:

If you do not determinate IgG avidity omit step 3



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