VIROTECH Validierung/Validation ELISA
(Validierung/Validation ELISA)

Order no: EC250.00

Pipettierkontrollen/Pipetting Control-Set

Order no: EN250K60

Color Coding: black

FOR IN VITRO DIAGNOSIS ONLY

Virotech Diagnostics GmbH
Waldstrasse 23 A2
63128 Dietzenbach, Germany

Tel.: +49(0)6074-23698-0
Fax.: +49(0)6074-23698-900
www.goldstandarddiagnostics.com

CE

Freigabedatum: 26.04.2022 20:51
REV 18 / VIROTECH Validierung / Validation ELISA EN

Contents

1. Intended Use

The Validation ELISA Testkit is intended for the regular check of your ELISA processor (fully- or semi- automated). It shall show the accumulated effect of unprecision during pipetting, washing, incubating and measuring on the photometer and therefore confirm the validity of results obtained. For this check of the ELISA processor always a complete microtiter plate has to be used. Processors, that process routinely more than one plate, must always be evaluated in full capacity. This means for a two-plate processor you have to use two plates, for a three-plate-processor 3 plates have to be tested in one run.
According to EG guideline 98/79 appendix 1, 3.1 it is required to ensure the functioning of a combination of two independent medical products (in this case ELISA processor and ELISA). The validation kit is designed to enable the user to do so.

The pipetting control is intended for checking fully automated ELISA processors with sample distributor. Like a patient serum sample, the control is diluted 1 + 100 with blue dilution buffer, pipetted and tested by the processor. The pipetting control can be used instead of the ready-to-use validation control contained in the validation kit (EC 250.00). Please note the validation kit's instructions for use.

2. Test Principle

The antibodies of the validation and/or pipetting control form an immune complex with the antigen coated on the validation microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added. The control serum has to be pipetted into all wells.

3. Package Contents

3.1 Validation-Kit

1. **1 Microtiter-Plate** consisting of 96 with proteins coated wells, lyophilised
2. **PBS-Washing Solution (20x concentrated), 50ml**, pH 7,2, with preservative and Tween 20
3. **Validation Control (containing IgG), 15ml,** human serum with protein-stabilizer and preservative, ready to use
4. **IgG-Conjugate (anti-human), 2 x 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
5. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 2 x 11ml**, ready to use
6. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

3.2 Pipetting Control Set

1. **1 Pipetting control, 2,8 ml**, human serum with protein stabilizers and preservative
2. **PBS dilution buffer (blue, ready-to-use), 2x50ml**, pH 7,2, with preservative and Tween 20

4. Storage and Shelflife of the testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.

Material	Status	Storage	Shelflife
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

5. Precautions and Warnings

1. Only material which has been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen is used as control sera. Nevertheless, conjugate and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If involved body parts are contacted, immediately wash under flowing water and possibly look up a doctor.
3. The disposal of the used materials is effected referring to the country-specific guidelines.

6. Material required but not supplied

1. Aqua dest./demin.
2. Paper towels or absorbent paper
3. Disposal box for infectious material
4. ELISA pipetting processor
5. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
6. Incubator

7. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

7.1 Preparation of Reagents

The ready to use validation control is **only** to be used with the plate lot number mentioned in the Quality Control Certificate.

1. Bring all reagents to room temperature before opening package of microtiter strips.
2. Shake all liquid components well before use.
3. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.

7.2 VIROTECH ELISA Test Procedure

1. For each test, pipette **100µl** of the **ready-to-use** validation control into all wells.
   If the pipetting process and the dilution process of the full automat are to be checked, use the pipetting control - such as a 1+100 diluted patient serum - rather than the ready-to-use validation control.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well.
4. Pipette 100µl of ready to use conjugate into all wells.
5. Incubation of conjugates: 30 min. at 37°C.
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB (substrate solution) into all wells.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into all wells. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to page 6 for Test Procedure Scheme

The extinctions shall be nearly identical and meet the ranges mentioned in the Quality Control Certificate.

8. Test Evaluation

The ready to use validation control serves for the assessment of the pipetting procedure as well as for all other working steps as incubation temperature, incubation time, measurement, washing process etc. on the processor. The method is suitable to

give notice towards irregularities during the treatment of an ELISA. The pipetting control is intended to check fully automated ELISA processors with sample distributor.

8.1 Test function control

OD-values

The OD-values of the validation/pipetting control should be above the OD-values mentioned in the Quality Control Certificate. In case this requirement is not fulfilled, the test has to be repeated.

8.2 Calculation the coefficient of variation

a) Calculate the coefficient of variation of **all 96 OD-values**. It is obtained from the quotient of the standard deviation and the arithmetical average value multiplied by 100.
b) Detect now the number of wells with OD-values, that are more than 20% above or below the arithmetical average value (Hot Spot Analysis).
c) To facilitate evaluation, we offer evaluation software for the validation kit and for the pipetting control (Order No. S/250).

8.3 Interpretation of results

1. If the calculated coefficient of variation is under the value given in the certificate and if not more than three hot spots are found in the validation control or not more than five hot spots in the pipetting control, the instrument is working properly.
2. The test should be repeated if the obtained variation coefficient is higher than mentioned in the Quality Control Certificate. Confirming this result, considerably one of the pipetting steps and/or working steps have been proceeded non-uniform. In this case all the single working steps should be checked or the service company of your processor should be contacted.
3. If the calculated coefficient of variation is under the range given in the certificate, but more than three hot spots are found in the validation control or more than five hot spots in the pipetting control, this is an indication of imprecision in the instrument. If you perform an additional test as confirmation and establish that the hot spots reappear at the same site, then there is a systematic error. For example, the position of the needle may be inexact, the rinsing instrument is not working properly, or there is a programming error.
4. Processors which work with more than one plate routinely, have to be evaluated with full capacity. This means for a two-plate processor two plates would have to be used, for a three-plate-processor 3 plates have to be proceeded in one run.

8.4 Examples

As an illustration two examples are cited in the appendix.

9. Extended Application

To examine your ELISA processor (fully- or semi-automated) with the VIROTECH Diagnostics Validation Kit the incubation may also be proceeded at room temperature. The test procedure (7.2) has only to be modified with regard to the incubation temperature and the incubation time:

Incubation of the validation control:	40 minutes at room temperature
Incubation of the conjugates:	40 minutes at room temperature
Incubation of the TMB:	40 minutes at room temperature

The evaluation software of the validation kit or the pipetting control can also be used for this extended application.
The criteria of the Quality Control Certificate have to be fulfilled as for the procedure referring to point 7.2.

Preparation of the Washing Solution / Pipetting control

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.
▼ **Pipetting control:** 10 µl Pipetting control + 1000 µl Dilution buffer (1:101)

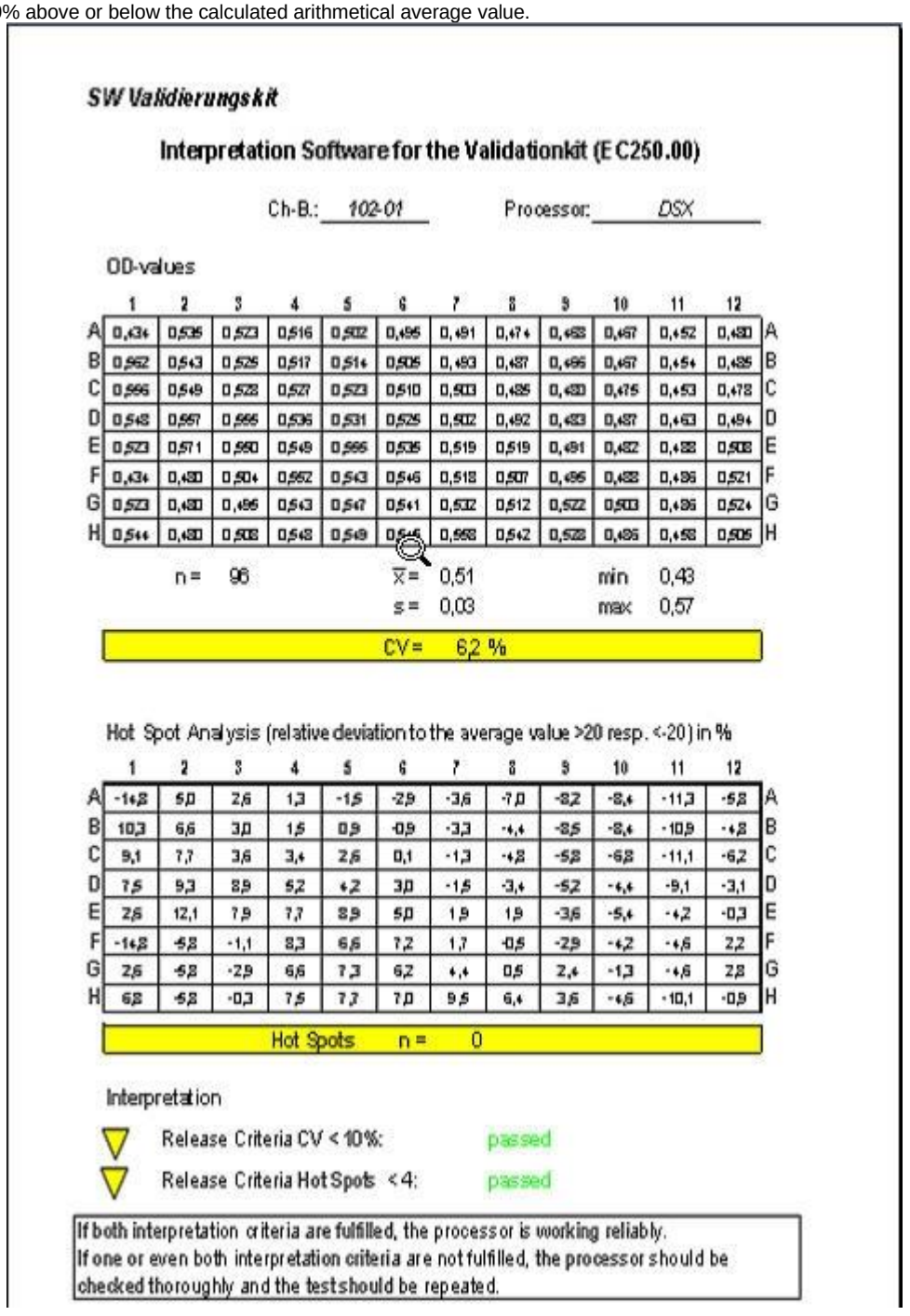
Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Validation Control** **100 µl Diluted pipetting control**
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgG
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇩		
Stopping		**50 µl Stopping Solution** shake carefully
⇩		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**

11. Appendix

11.1 Example 1 (Validation control)

This requires error-free processing with the validation control. The coefficient of variation is below 10% and there are no OD-values >20% above or below the calculated arithmetical average value.



SW Validierungskit

Interpretation Software for the Validationkit (EC250.00)

Ch-B.: 102-01 Processor: DSX

OD-values

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,434	0,535	0,523	0,516	0,502	0,496	0,491	0,474	0,483	0,467	0,452	0,480	A
B	0,562	0,543	0,525	0,517	0,514	0,505	0,493	0,487	0,496	0,467	0,454	0,485	B
C	0,566	0,549	0,528	0,527	0,523	0,510	0,503	0,485	0,480	0,475	0,453	0,478	C
D	0,548	0,557	0,565	0,536	0,531	0,525	0,502	0,492	0,483	0,487	0,463	0,494	D
E	0,523	0,571	0,560	0,549	0,566	0,535	0,519	0,519	0,491	0,482	0,488	0,508	E
F	0,434	0,480	0,504	0,562	0,543	0,546	0,518	0,507	0,496	0,488	0,496	0,521	F
G	0,523	0,480	0,496	0,543	0,547	0,541	0,532	0,512	0,522	0,503	0,496	0,524	G
H	0,544	0,480	0,508	0,548	0,549	0,545	0,558	0,542	0,528	0,485	0,458	0,505	H

n = 96 $\bar{x}$ = 0,51 min 0,43

s = 0,03 max 0,57

CV = 6,2 %

Hot Spot Analysis (relative deviation to the average value >20 resp. <-20) in %

	1	2	3	4	5	6	7	8	9	10	11	12	
A	-14,2	5,0	2,6	1,3	-1,5	-2,9	-3,6	-7,0	-8,2	-8,4	-11,3	-5,2	A
B	10,3	6,6	3,0	1,5	0,9	-0,9	-3,3	-4,4	-8,5	-8,4	-10,9	-4,2	B
C	9,1	7,7	3,6	3,4	2,6	0,1	-1,3	-4,2	-5,2	-6,2	-11,1	-6,2	C
D	7,5	9,3	8,9	5,2	4,2	3,0	-1,5	-3,4	-5,2	-4,4	-9,1	-3,1	D
E	2,6	12,1	7,9	7,7	8,9	5,0	1,9	1,9	-3,6	-5,4	-4,2	-0,3	E
F	-14,2	-5,2	-1,1	8,3	6,6	7,2	1,7	-0,5	-2,9	-4,2	-4,6	2,2	F
G	2,6	-5,2	-2,9	6,6	7,3	6,2	4,4	0,5	2,4	-1,3	-4,6	2,8	G
H	6,8	-5,2	-0,3	7,5	7,7	7,0	9,5	6,4	3,6	-4,6	-10,1	-0,9	H

Hot Spots n = 0

Interpretation

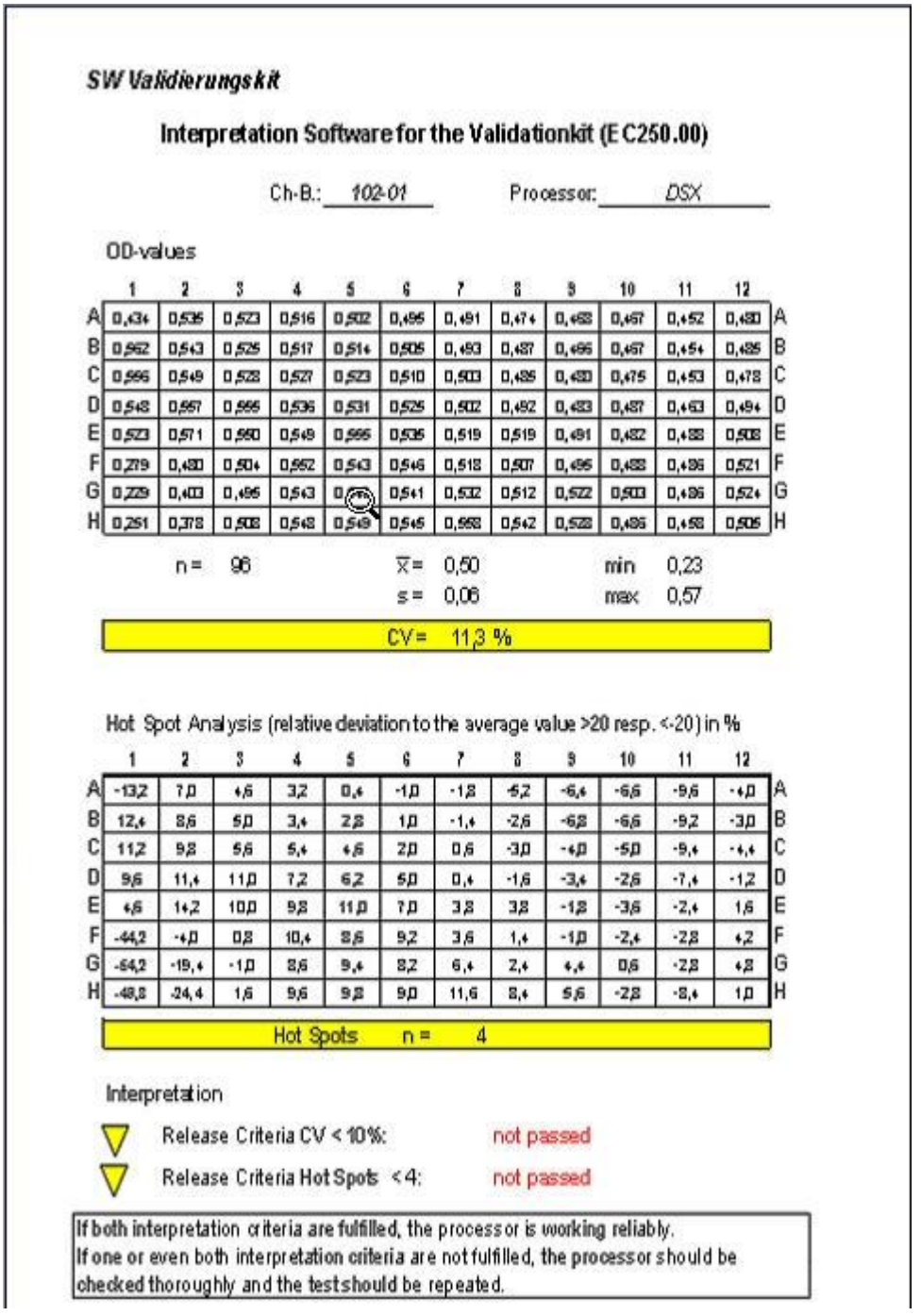
Release Criteria CV < 10%: passed

Release Criteria Hot Spots < 4: passed

If both interpretation criteria are fulfilled, the processor is working reliably.
If one or even both interpretation criteria are not fulfilled, the processor should be checked thoroughly and the test should be repeated.

11.2 Example 2 (Validation control)

There are errors in the processing of the validation control (CV >10% and 4 outliers). These four hot spots are in the bottom left corner of the microtiter plate as you can clearly see from the analysis.



SW Validierungskit

Interpretation Software for the Validationkit (EC250.00)

Ch-B.: 102-01 Processor: DSX

OD-values

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,434	0,535	0,523	0,516	0,502	0,496	0,491	0,474	0,468	0,467	0,452	0,480	A
B	0,562	0,543	0,525	0,517	0,514	0,505	0,493	0,487	0,496	0,467	0,454	0,485	B
C	0,566	0,549	0,528	0,527	0,523	0,510	0,503	0,485	0,480	0,475	0,453	0,478	C
D	0,548	0,557	0,566	0,536	0,531	0,525	0,502	0,492	0,483	0,487	0,463	0,494	D
E	0,523	0,571	0,560	0,549	0,566	0,535	0,519	0,519	0,491	0,482	0,488	0,508	E
F	0,219	0,480	0,504	0,552	0,543	0,546	0,518	0,507	0,496	0,488	0,496	0,521	F
G	0,229	0,403	0,496	0,543	0,[illegible]	0,541	0,532	0,512	0,522	0,503	0,496	0,524	G
H	0,251	0,378	0,508	0,548	0,549	0,545	0,558	0,542	0,528	0,486	0,458	0,505	H

n = 96 $\bar{x}$ = 0,50 min 0,23

s = 0,06 max 0,57

CV = 11,3 %

Hot Spot Analysis (relative deviation to the average value >20 resp. <-20) in %

	1	2	3	4	5	6	7	8	9	10	11	12	
A	-13,2	7,0	4,6	3,2	0,4	-1,0	-1,8	-5,2	-6,4	-6,6	-9,6	-4,0	A
B	12,4	8,6	5,0	3,4	2,8	1,0	-1,4	-2,6	-6,8	-6,6	-9,2	-3,0	B
C	11,2	9,8	5,6	5,4	4,6	2,0	0,6	-3,0	-4,0	-5,0	-9,4	-4,4	C
D	9,6	11,4	11,0	7,2	6,2	5,0	0,4	-1,6	-3,4	-2,6	-7,4	-1,2	D
E	4,6	14,2	10,0	9,8	11,0	7,0	3,8	3,8	-1,8	-3,6	-2,4	1,6	E
F	-44,2	-4,0	0,8	10,4	8,6	9,2	3,6	1,4	-1,0	-2,4	-2,8	4,2	F
G	-54,2	-19,4	-1,0	8,6	9,4	8,2	6,4	2,4	4,4	0,6	-2,8	4,8	G
H	-48,8	-24,4	1,6	9,6	9,8	9,0	11,6	8,4	5,6	-2,8	-8,4	1,0	H

Hot Spots n = 4

Interpretation

Release Criteria CV < 10%: not passed

Release Criteria Hot Spots < 4: not passed

If both interpretation criteria are fulfilled, the processor is working reliably.
If one or even both interpretation criteria are not fulfilled, the processor should be checked thoroughly and the testshould be repeated.