

**Immunology/ Serology Department
Clinical Laboratory
Nicosia General Hospital**

Validation steps according to ISO 15189

Architect / 2000 Serological markers for infectious diseases

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Immunology/ Serology Department

- Infectious Diseases
- Autoimmune diseases/Allergies

Why ISO 15189 is important for serology department?

- National Reference Laboratory for infectious diseases (HIV, HEP A, B, C, Syphilis, CMV etc)
- Public health laboratory, health and epidemiological surveillance
- Blood donors, organ donors, bone marrow donors
- Vaccine preventable diseases
- Prenatal tests
- Pre surgery

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Serology Department Nicosia General Hospital

- ✓ Daily quality control for all the examinations procedures
- ✓ Participation in external quality control schemes
- ✓ WHO accreditation for Measles and Rubella

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Validation of Methods according to ISO 15189

22 methods for infectious diseases

Tests performed on automated analyzer Architect i2000, Chemiluminescence (CMIA)

Guidelines for Laboratory Accreditation according to EN ISO 15189
(Hellenic Accreditation system)

- ✓ **REPEATABILITY**
- ✓ **REPRODUCIBILITY**
- ✓ **TRUNESESS (Recovery BIAS)**

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Validation of Methods according to ISO 15189

Repeatability

Control samples **of two levels of control** divided into aliquots
6 measurements at least **15 minutes** covering the whole day

Calculation of CVr

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Validation of Methods according to ISO 15189

✓ **Repeatability**

TEST	UNITS							AVERAGE	SD	CVr %
HIV Ag/Ab POSITIVE 1	S/CO	5.76	5.40	5.31	5.65	5.76	5.41	5.55	0.20	3.6
HIV Ag/Ab POSITIVE 3	S/CO	3.30	3.23	3.40	3.28	3.24	3.29	3.29	0.06	1.8
Anti-HCV NEGATIVE	S/CO	0.09	0.09	0.09	0.08	0.09	0.09	0.09	0.00	4.6
Anti-HCV POSITIVE	S/CO	4.00	4.47	4.46	4.05	4.22	4.33	4.26	0.20	4.7
HBsAg6pt POSITIVE 2	IU/ml	164.83	152.45	149.23	167.92	149.37	170.95	159.13	9.87	6.2
HBsAg6pt POSITIVE 1	IU/ml	0.19	0.21	0.18	0.20	0.19	0.20	0.20	0.01	5.4
Anti-HBcII NEGATIVE	S/CO	0.11	0.13	0.11	0.12	0.12	0.11	0.12	0.01	7.0
Anti-HBcII POSITIVE	S/CO	2.51	2.72	2.87	2.51	2.94	2.72	2.71	0.18	6.6
Anti-Hbe POSITIVE	S/CO	0.41	0.43	0.44	0.42	0.42	0.43	0.43	0.01	2.5
Anti-Hbe NEGATIVE	S/CO	1.82	1.84	1.85	1.91	1.98	1.90	1.88	0.06	3.1
Anti-HBs POSITIVE 1	mlU/ml	15.76	15.77	15.66	16.83	16.27	16.22	16.09	0.45	2.8
Anti-HBs POSITIVE 2	mlU/ml	81.04	80.15	81.47	81.80	81.39	81.80	81.28	0.62	0.8
HAVABIG NEGATIVE	S/CO	0.14	0.15	0.17	0.18	0.14	0.15	0.16	0.02	10.6
HAVABIG POSITIVE	S/CO	1.91	1.99	1.82	1.92	1.97	2.05	1.94	0.08	4.1
HAVAbIgM NEGATIVE	S/CO	0.13	0.15	0.10	0.17	0.17	0.14	0.14	0.03	18.5
HAVAbIgM POSITIVE	S/CO	2.06	1.89	1.97	2.19	2.03	1.99	2.02	0.10	5.0
HBcAB-IgM NEGATIVE	S/CO	0.05	0.05	0.05	0.06	0.05	0.05	0.05	0.00	7.9
HBcAB-IgM POSITIVE	S/CO	3.47	3.21	3.10	2.90	2.95	3.10	3.12	0.20	6.5
HBeAG POSITIVE	S/CO	3.49	3.51	3.73	3.54	3.73	3.56	3.59	0.11	3.1
HBeAG NEGATIVE	S/CO	0.34	0.40	0.40	0.40	0.41	0.37	0.39	0.03	6.7
Syphilis POSITIVE	S/CO	2.66	2.60	2.62	2.61	2.62	2.56	2.61	0.03	1.2
Syphilis NEGATIVE	S/CO	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.00	9.8

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Validation of Methods according to ISO 15189

✓ Repeatability

TEST	UNITS							AVERAGE	SD	CVr %
CMV IgG POSITIVE 1	AU/ml	32.70	33.80	32.80	33.00	32.50	32.50	32.88	0.49	1.5
CMV IgG POSITIVE 2	AU/ml	161.70	153.90	153.20	151.90	160.70	146.10	154.58	5.82	3.8
CMV IgM POSITIVE 1	index	2.45	2.42	2.42	2.34	2.36	2.32	2.39	0.05	2.2
CMV IgM NEGATIVE	index	0.31	0.28	0.36	0.37	0.29	0.25	0.31	0.05	15.1
EBV EBNA - G NEGATIVE	S/CO	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.0
EBV EBNA - G POSITIVE	S/CO	2.63	2.60	2.51	2.56	2.55	2.55	2.57	0.04	1.6
EBV VCA -G POSITIVE	S/CO	2.81	2.86	2.90	2.72	2.80	2.84	2.82	0.06	2.2
EBV VCA -G NEGATIVE	S/CO	0.05	0.05	0.04	0.05	0.04	0.04	0.05	0.01	12.2
EBV VCA -M POSITIVE	S/CO	3.16	3.14	3.08	3.05	3.07	3.03	3.09	0.05	1.7
EBV VCA -M NEGATIVE	S/CO	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.00	0.0
HCV Ag POSITIVE 1	f mol/L	43.89	46.46	42.25	44.76	45.62	43.67	44.44	1.50	3.4
HCV Ag POSITIVE 2	f mol/L	279.72	280.29	283.90	274.16	293.37	292.98	284.07	7.71	2.7
Rubella G POSITIVE 1	IU/ml	27.40	26.40	27.40	25.90	27.20	26.30	26.77	0.65	2.4
Rubella G POSITIVE 2	IU/ml	337.80	317.50	301.90	325.50	304.50	315.20	317.07	13.36	4.2
Rubella M POSITIVE	index	3.31	3.48	3.38	3.39	3.29	3.68	3.42	0.14	4.2
Rubella M NEGATIVE	index	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.00	0.0
Toxo IgG POSITIVE 1	IU/ml	6.30	6.00	6.00	6.00	6.10	6.10	6.08	0.12	1.9
Toxo IgG NEGATIVE	IU/ml	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.00	0.0
Toxo IgM NEGATIVE	index	0.05	0.07	0.05	0.05	0.04	0.05	0.05	0.01	19.0
Toxo IgM	index	1.81	1.53	1.58	1.88	1.45	1.70	1.66	0.17	10.1
TOXO avidity pool sample	%	84.20	84.50	82.10	82.40	83.40	84.40	83.50	1.05	1.3
TOXO avidity pool sample	%	20.20	19.30	22.30	20.60	19.80	18.90	20.18	1.20	6.0

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Validation of Methods according to ISO 15189

✓ Reproducibility

The results of the **Quality control chart (QC)**
ten measurements covering **three months** for the **same lot number of control** and **two**
different **batches of reagents**.

The reproducibility was calculated for **all the levels of controls** of all the methods.

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✓reproducibility

TEST	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
HIV Ag/Ab NEGATIVE	0.16	0.14	0.10	0.23	0.15	0.14	0.13	0.10	0.15	0.19	26.18	0.04	0.15
HIV Ag/Ab POSITIVE 1	5.05	5.04	5.23	4.98	5.37	5.19	5.45	5.19	4.89	4.94	3.60	0.18	5.13
HIV Ag/Ab POSITIVE 2	3.42	3.29	3.38	3.48	3.49	3.87	4.21	3.83	3.62	3.79	7.79	0.28	3.64
HIV Ag/Ab POSITIVE 3	3.20	3.14	3.32	3.00	2.81	3.32	3.28	3.23	2.94	3.19	5.48	0.17	3.14
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
HBSAG NEGATIVE	0.01	0.03	0.02	0.01	0.00	0.01	0.02	0.00	0.00	0.00	N/A		
HBSAG POSITIVE 1	0.25	0.24	0.26	0.26	0.28	0.26	0.24	0.23	0.21	0.25	7.79	0.02	0.25
HBSAG POSITIVE 2	189.00	178.41	166.90	174.64	165.87	174.15	175.80	172.68	175.54	170.78	3.71	6.48	174.38
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
HCV NEGATIVE	0.08	0.07	0.08	0.09	0.10	0.08	0.07	0.09	0.09	0.10	12.71	0.01	0.09
HCV POSITIVE	3.63	3.31	3.25	3.39	3.11	3.78	3.90	4.02	4.15		10.21	0.37	3.62
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
ANTI HBE NEGATIVE	1.93	1.97	2.01	1.84	1.95	1.94	1.99	1.98	2.03	1.95	2.67	0.05	1.96
ANTI HBE POSITIVE	0.46	0.47	0.47	0.45	0.47	0.45	0.49	0.50	0.48	0.49	3.60	0.02	0.47
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
HbcAB-IgM NEATIVE	0.06	0.05	0.05	0.06	0.07	0.05	0.06	0.05	0.06	0.05	12.49	0.01	0.06
HbcAB-IgM POSITIVE	3.75	4.03	3.88	4.02	3.81	3.54	3.49	3.85	3.36	3.21	7.58	0.28	3.69
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
HBEAG NEGATIVE	0.30	0.31	0.33	0.33	0.39	0.41	0.37	0.40	0.42	0.39	11.85	0.04	0.36
HBEAG POSITIVE	3.57	3.43	3.39	3.33	3.53	4.06	3.69	3.43	3.65	3.70	5.89	0.21	3.58
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
HAVA IGM NEGATIVE	0.17	0.14	0.11	0.13	0.15	0.12	0.11	0.16	0.17	0.18	17.99	0.03	0.14
HAVA IGM POSITIVE	2.22	2.16	1.84	2.27	1.86	2.25	2.01	2.00	2.18	1.99	7.63	0.16	2.08

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✓reproducibility

TEST	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
ANTI-HCII NEGATIVE	0.20	0.18	0.17	0.20	0.18	0.13	0.11	0.15	0.17	0.14	18.30	0.03	0.16
ANTI-HCII POSITIVE	2.53	2.62	2.65	2.56	2.79	2.82	2.84	2.83	2.74	2.80	4.33	0.12	2.72
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
SYPHILIS NEGATIVE	0.04	0.04	0.05	0.06	0.03	0.04	0.04	0.04	0.04	0.04	18.78	0.01	0.04
SYPHILIS POSITIVE	2.62	2.90	2.86	2.79	2.91	3.16	2.89	2.68	2.87	2.54	6.23	0.18	2.82
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
ANTI HBS NEGATIVE	0.00	0.00	0.00	0.00	0.04	0.30	0.08	0.11	0.19	0.22	N/A		
ANTI HBS POSITIVE 1	14.64	14.81	15.70	13.89	13.77	12.67	15.15	15.17	15.16	15.34	6.30	0.92	14.63
ANTI HBS POSITIVE 2	81.81	88.74	89.84	99.05	90.97	81.72	85.50	86.27	76.58	86.58	7.06	6.12	86.71
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
TOXO IGM NEGATIVE	0.05	0.03	0.01	0.04	0.05	0.04	0.05	0.06	0.07	0.04	37.42	0.02	0.04
TOXO IGM POSITIVE	1.30	1.32	1.46	1.50	1.36	1.43	1.57	1.54	1.62	1.50	7.34	0.11	1.46
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
TOXO IGG NEGATIVE	0.00	0.00	0.00	0.01	0.00	0.10	0.00	0.10	0.00	0.00	N/A		
TOXO IGG POSITIVE 1	6.80	7.20	7.70	7.20	7.10	6.40	6.60	7.30	7.10	7.60	5.75	0.41	7.10
TOXO IGG POSITIVE 2	134.90	113.30	136.10	129.60	126.30	121.50	120.30	128.30	130.00	120.20	5.69	7.17	126.05
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
RUBELLA IGG NEGATIVE	0.00	0.00	0.20	0.00	0.10	0.10	0.00	0.10	0.00	0.10	N/A		
RUBELLA IGG POSITIVE 1	24.30	24.60	25.60	22.90	23.60	29.60	32.60	30.00	27.40	28.70	11.97	3.22	26.93
RUBELLA IGG POSITIVE 2	289.90	282.40	290.50	289.50	313.70	345.80	345.00	331.10	323.80	311.60	7.60	23.74	312.33
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
EBV VCA G NEGATIVE	0.06	0.05	0.07	0.06	0.06	0.04	0.04	0.05	0.03	0.04	24.94	0.01	0.05
EBV VCA G POSITIVE	3.09	3.14	3.17	3.31	2.97	2.55	2.52	2.60	2.49	2.40	12.13	0.34	2.82

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✓reproducibility

	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
EBV VCA M NEGATIVE	0.08	0.08	0.09	0.04	0.08	0.08	0.08	0.09	0.09	0.08	18.34	0.01	0.08
EBV VCA M POSITIVE	2.95	2.96	3.00	2.98	2.85	2.98	3.03	3.06	3.09	3.08	2.39	0.07	3.00
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
EBV EBNA -G NEGATIVE	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.01	37.16	0.00	0.01
EBV EBNA -G POSITIVE	2.80	2.82	3.33	2.86	2.78	3.06	3.00	2.99	3.03	3.02	5.55	0.16	2.97
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
CMV IGM NEGATIVE	0.20	0.19	0.20	0.17	0.19	0.21	0.29	0.31	0.30	0.27	22.80	0.05	0.23
CMV IGM POSITIVE	1.84	1.81	1.95	1.87	1.95	1.91	2.45	2.47	2.24	2.78	15.83	0.34	2.13
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
CMV IGG NEGATIVE	0.10	0.00	0.20	0.10	0.10	0.20	0.10	0.10	0.10	0.10	N/A		
CMV IGG POSITIVE 1	35.60	37.70	37.30	36.40	34.20	35.40	35.10	36.30	36.80	34.20	3.37	1.21	35.90
CMV IGG POSITIVE 2	184.50	194.80	186.50	187.60	168.00	165.30	162.30	165.60	164.40	171.50	6.84	11.98	175.05
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
RUBELLA IGM NEGATIVE	0.08	0.08	0.09	0.10	0.10	0.11	0.10	0.08	0.09	0.10	11.39	0.01	0.09
RUBELLA IGM POSITIVE	3.10	3.42	3.37	3.41	3.43	3.61	3.46	3.62	3.18	3.38	4.78	0.16	3.40
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
HCV AG NEGATIVE	0.23	0.72	0.93	1.46	0.00	0.00	0.42	2.54	0.36	0.00	N/A		
HCV AG POSITIVE 1	51.10	52.50	50.27	53.36	45.80	52.13	50.54	51.78	50.43	49.98	4.07	2.07	50.79
HCV AG POSITIVE 2	295.99	289.32	300.68	268.96	334.03	346.34	335.41	347.49	303.06	289.06	8.85	27.53	311.03
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
HAVA IGG NEGATIVE	0.12	0.13	0.15	0.12	0.13	0.14	0.16	0.15	0.14	0.13	9.76	0.01	0.14
HAVAbIGG POSITIVE	2.21	2.10	2.14	2.15	2.16	2.50	2.35	2.42	2.14	2.11	6.39	0.14	2.23
	BATCH 1					BATCH 2					CVR%	SDR	AVERAGE
TOXO AVIDITY LEVEL 1	21.10	28.10	25.00	26.20	20.30	21.80	23.50	23.90	18.00	21.30	13.13	3.01	22.92
TOXA AVIDITY LEVEL 2	76.90	76.40	76.80	76.00	75.60	75.00	78.30	76.00	78.20	77.50	1.42	1.09	76.67

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Trueness:**Recovery experiments – calculation of % Recovery and bias**

Pool samples were divided into aliquots and were measured three times then were mixed with calibrators of known concentration and been measured six times in the same day

Recovery experiments were performed for both quantitative (concentration units) and qualitative methods (S/CO)

$$bias = 1 - \frac{C_{final} - \frac{CO}{2}}{\frac{C_{standard}}{2}}$$

$$Recovery = 100 - bias$$

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✓Recovery – Bias

	RUBELLA IGG	ANTI HBS	HCVAG	CMV IGG	HBSAG	TOXO IGG
	IU/ml	mU/ml	fmol/L	AU/ml	IU/ml	IU/ml
CONCENTRATION CALIBRATOR						
Cstandard	75.00	50.00	600.00	75.00	100.00	5.00
C Before 1	139.40	267.53	196.01	175.10	66.60	61.50
C Before 2	128.40	280.93	211.89	169.30	51.13	64.70
C Before 3	122.60	269.16	200.17	171.90	68.65	64.00
AVERAGE BEFORE	130.13	272.54	202.69	172.10	62.13	63.40
C After 1	102.80	159.33	328.18	129.20	80.56	35.20
C After 2	99.80	164.92	330.48	131.30	77.17	35.90
C After 3	101.50	153.53	337.33	133.20	68.65	35.80
C After 4	106.00	152.17	340.02	133.20	67.02	35.80
C After 5	100.20	170.00	326.13	136.50	66.90	37.70
C After 6	96.60	172.91	335.83	132.40	71.42	38.00
AVERAGE AFTER	101.15	162.14	333.00	132.63	71.95	36.40
Recovery	0.99	1.01	0.83	1.07	0.89	1.06
%Recovery	98.62	100.54	82.97	107.35	88.76	106.43
Bias	0.01	-0.01	0.17	-0.07	0.11	-0.06
% Bias	1.38	-0.54	17.03	-7.35	11.24	-6.43

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✓Recovery – Bias

	HIV	ANTI HCV	CMV IgM	NTI HBCIG	ANTI HbCIG	ANTI HBE	HBEAG	TOXO M
	S/CO	S/CO	S/CO	S/CO	S/CO	S/CO	S/CO	INDEX
standard(s/co)	2.50	13.51	1.00	3.09	9.93	2.00	0.38	1.00
C Before 1	3.90	13.43	4.04	0.05	0.47	0.72	0.34	0.70
C Before 2	3.92	13.73	4.09	0.05	0.47	0.72	0.33	0.71
C Before 3	3.90	13.93	4.00	0.05	0.46	0.72	0.33	0.78
AVERAGE BEFORE	3.91	13.70	4.04	0.05	0.47	0.72	0.33	0.73
C After 1	3.48	14.27	2.71	1.66	4.75	1.21	0.41	0.92
C After 2	3.50	13.77	2.47	1.67	4.74	1.25	0.40	0.84
C After 3	3.40	13.94	2.55	1.69	4.72	1.22	0.42	0.82
C After 4	3.51	13.73	2.54	1.60	4.80	1.23	0.40	0.72
C After 5	3.43	13.43	2.80	1.68	4.73	1.19	0.41	0.87
C After 6	3.47	13.92	2.85	1.68	4.72	1.25	0.40	0.85
AVERAGE AFTER	3.47	13.84	2.65	1.66	4.74	1.23	0.41	0.84
Recovery	1.08	1.02	1.05	1.06	0.91	0.90	1.15	0.97
%Recovery	108.17	101.75	105.22	105.94	91.25	90.07	114.66	96.72
Bias	-0.08	-0.02	-0.05	-0.06	0.09	0.10	-0.15	0.03
% Bias	-8.17	-1.75	-5.22	-5.94	8.75	9.93	-14.66	3.28

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✓Recovery – Bias

	EBV VCA IGM S/CO	SYPHILIS S/CO	TOXO AV %	HAV G S/CO	HAV A S/CO	RUBELLA IGM S/CO
standard (s/co)	0.50	5.00	36.50	3.45	3.64	3.60
C Before 1	3.54	2.50	84.50	1.99	1.02	0.08
C Before 2	3.52	2.55	82.20	1.98	1.03	0.08
C Before 3	3.50	2.50	84.30	1.95	1.01	0.08
AVERAGE BEFORE	3.52	2.52	83.67	1.97	1.02	0.08
C After 1	2.07	4.02	60.20	2.65	2.24	1.69
C After 2	2.12	4.34	61.20	2.62	2.23	1.68
C After 3	2.07	4.24	60.30	2.75	1.22	1.72
C After 4	2.05	4.22	59.90	2.80	2.32	1.73
C After 5	2.04	4.12	59.82	2.65	2.42	1.63
C After 6	2.20	4.20	58.98	2.66	2.23	1.63
AVERAGE AFTER	2.09	4.19	60.07	2.69	2.11	1.68
Recovery	1.04	1.11	1.00	0.99	0.91	0.91
%Recovery	104.06	111.49	99.97	99.17	90.63	91.30
Bias	-0.04	-0.11	0.00	0.01	0.09	0.09
% Bias	-4.06	-11.49	0.03	0.83	9.37	8.70