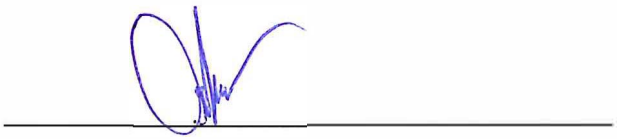
Validation Report

Nivolumab Antibodies

Cat. No.: NIV-QNS-OPD

This report has been reviewed by the undersigned and to the best of our knowledge is complete, accurate, and in compliance with the standards below:

- Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. G Shankar et al. J Phar Biom Analysis 48(2008): 1267-1281
- Validation of immunoassays used to assess immunogenicity to therapeutic monoclonal antibodies. D Geng et al. J Phar Biom Analysis 39(2005): 364-375
- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures (2014)
- CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach (2003)
- CLSI EP09-A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples (2013)
- CLSI EP10-A3: Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures (2006)
- CLSI EP15-A3: User Verification of Precision and Estimation of Bias (2014)
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures (2012)
- CLSI EP28-A3C: Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory (2010)
- Bioanalytical method validation, Guidance for industry, US Department of Health and Human Services, FDA, CDER, CVM, Draft (2013).



November 22, 2023

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All assays are studied according to assay procedure.

Performance studies and acceptance criterias

Performance study	Acceptance Criteria	Results
Calibration Curve (Linearity, Dilutional linearity)	>3 calibrations. Bias <%20 r^2 >0,9 for all calibrations	Bias <%20 r^2 >0,9 for all calibrations
Sensitivity	LLOQ CV <30% Recovery <70-130%	CV<30% Recovery <70-130%
Specificity (Selectivity, Interference)	Recovery <70-130%	Recovery <70-130%
Accuracy (Trueness)	Specificity study data	WHO study results Recovery <100±30%
Cut-off (cut-point)	Not included	
Precision	Inter-assay CV <20% Intra-assay CV <20%	Inter-assay CV <20% Intra-assay CV <20%
Robustness	Not included	
Ruggedness	Not included	
Stability	Four weeks stability study bias 80-120%	Four weeks stability study bias 80-120%

Performance Study Data

1. **Calibration curves**
2. **Control studies**

Standard curves

	Standard 1 (500 ng/mL)		Standard 2 (250 ng/mL)		Standard 3 (125 ng/mL)		Standard 4 (62,5 ng/mL)		Standard 5 (31,25ng/mL)		
	Concentration	Bias (%)	Concentration	Bias (%)	Concentration	Bias (%)	Concentration	Bias (%)	Concentration	Bias (%)	r^2
	503,836	0,76	256,922	2,76	125,309	0,24	63,729	1,96	33,520	7,26	0,999
	506,179	1,23	255,366	2,14	124,414	-0,45	63,545	1,67	31,151	-0,31	0,999
	499,759	-0,04	244,908	-2,03	128,160	2,52	62,074	-0,68	32,050	2,56	0,999
	506,282	1,25	252,052	0,82	127,655	2,12	61,747	-1,20	30,368	-2,82	0,998
	501,666	0,00	245,421	-1,83	122,347	-2,12	59,614	-4,61	32,523	4,07	0,999
	505,068	1,01	250,298	0,11	123,765	-0,98	63,750	2,00	31,141	-0,34	0,999
	500,721	0,14	250,473	0,18	122,554	-1,95	62,807	0,49	30,258	-3,17	0,999
Mean	503,279		250,777		124,886		62,466		31,573		
SD	2,650		4,207		2,135		1,379		1,099		
CV (%)	0,526		1,677		1,710		2,209		3,483		

Precision

	Control 1 (93,75 ng/mL)		Control 2 (375 ng/mL)	
	Intra-assay	Inter-assay	Intra-assay	Inter-assay
	89,566	96,751	370,703	376,831
	87,592	95,311	363,687	387,828
	94,906	89,407	363,140	392,150
	86,199	91,702	362,510	375,990
	96,691	98,370	367,185	370,180
	98,446		378,520	
Mean	92,233	94,308	367,624	380,595
SD	4,666	3,295	5,629	8,120
CV (%)	5,059	3,493	1,531	2,133

Anti-Nivolumab concentrations in serum

Spiked Standard (ng/mL)	Mean of 2 runs (ng/mL)	Recovery (%)
320	322,881	99,99
160	167,252	99,99
80	86,675	99,99
40	41,413	99,99

Sample: Spiked anti-Nivolumab in human serum

Anti-Nivolumab concentrations in serum

Standard	Mean of 4 runs	SD	CV%
500 ng/mL	495,901	4,38	5,0
250 ng/mL	244,511	3,49	1,4
125 ng/mL	125,394	1,83	1,5
62,5 ng/mL	62,266	0,69	1,1
31,25 ng/mL	30,842	2,03	6,9

Sample: Spiked anti-Nivolumab in human serum

LLOQ (Lower Limit of Quantification)

	20 ng/mL
Run 1	18,790
Run 2	21,531
Run 3	19,109
Mean	19,810
SD	1,223
CV (%)	6,178

Stability data

	Control 1 (93,75 ng/mL) Low Control	**Control 2 (375 ng/mL) High Control**
	Intra-assay	Intra-assay
Day 1	96,751	398,520
Day 15	94,905	370,703
Day 30	95,797	376,831
