ARDENA

The challenges to overcome when developing a synthetic peptide Anti-Drug Antibody assay

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Therapeutic Peptides

- Peptides or polypeptides used for the treatment of diseases
- Mimic the functions of naturally occurring peptides (hormones, growth factors, neuro-transmitters, ion channel ligands, and anti-infectives)
- Peptide therapeutics are considered relatively safe and well-tolerated as peptides can be metabolized by the body

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Advantages versus Drawbacks

- Small size
- High specificity
- Good efficacy
- Good safety
- Low immunogenicity

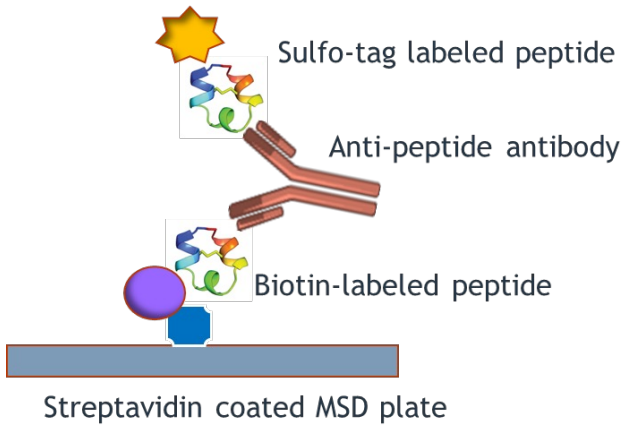
- Weak membrane permeability
- Poor in vivo stability

Authorities still require development of an ADA assay

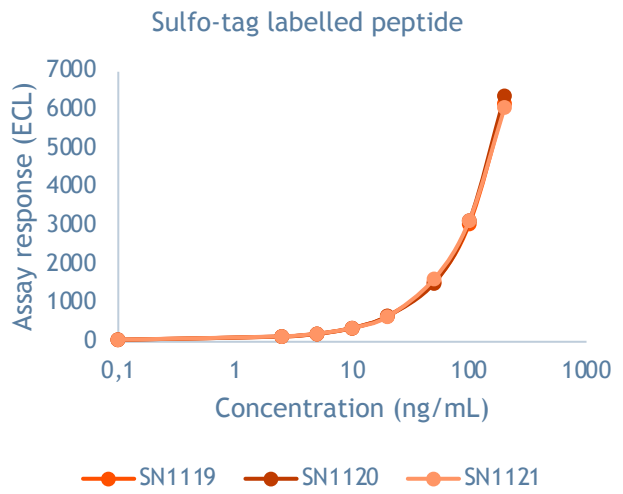
Methods tested - Standard bridging ECLIA assay

- Peptide = 31 aa (3410 g/mol) /biotin = 244.31 g/mol/ sulfo-tag = 1141 g/mol
- High binding plates were coated with antibodies SN1119, SN1120 and SN1121, followed by detection with sulfo-tag labelled peptide or biotin labelled peptide and sulfo-tag labelled streptavidin

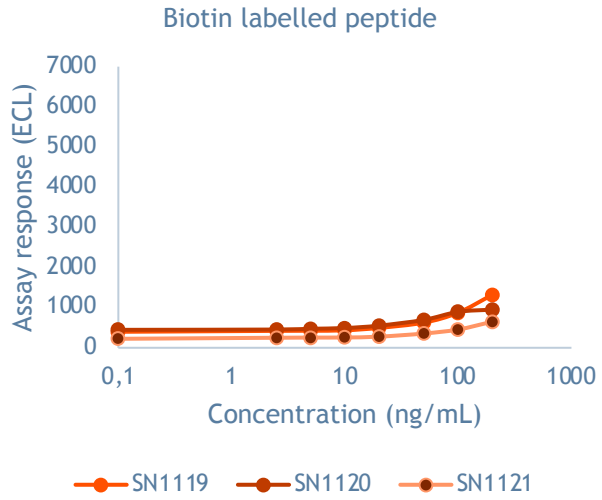
bridging ECLIA assay



sulfo-tag labelled peptide



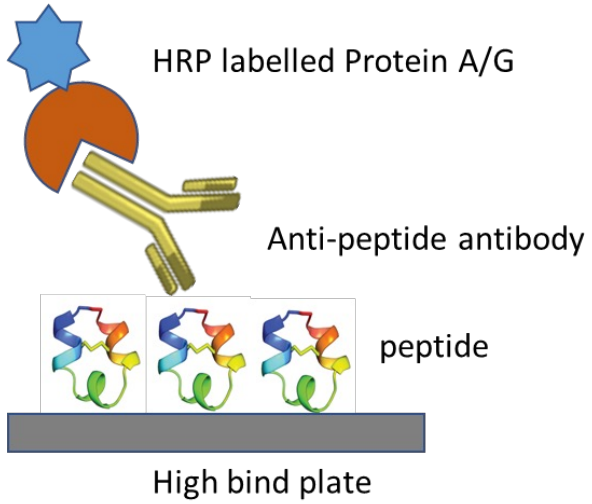
biotin labelled peptide



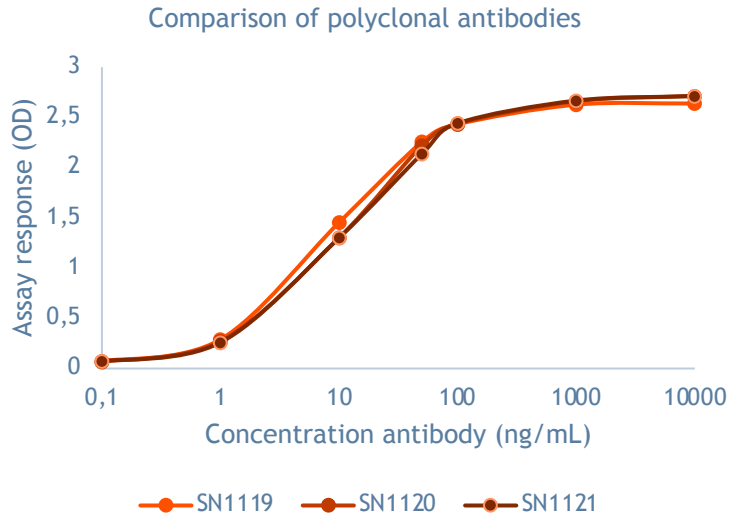
Methods tested

- Standard bridging ECLIA assay versus direct binding ELISA approach
  - For the direct binding ELISA approach, high binding plates were coated with peptide followed by incubation with serially diluted polyclonal antibodies SN1119, SN1120 and SN1121. Antibodies were detected using HRP labeled Protein A/G.

Direct binding ELISA



HRP labeled Protein A/G



The direct binding ELISA approach was selected as the preferred method as well as SN1120 as preferred PC.

Optimization ELISA

- Transfer of assay from buffer to matrix showed high CVs between duplicate wells.

Buffer vs Serum

	1	2	3	4	5	6	7	8	9	10	11	12	PC
A	OVRFLW	4.067	3.824	OVRFLW	3.671	3.793	3.34	3.335	0.26	0.31	0.371	0.392	10000 ng/mL
B	OVRFLW	OVRFLW	4.027	3.922	4.024	3.901	3.255	3.302	0.31	0.323	0.265	0.264	1000 ng/mL
C	3.451	3.552	3.067	2.896	2.98	2.275	1.612	1.882	0.325	0.226	0.24	0.308	100 ng/mL
D	2.967	2.937	2.536	2.374	1.69	1.248	0.798	0.638	0.837	0.704	0.189	0.253	50.0 ng/mL
E	1.206	1.199	0.973	0.958	0.772	0.632	0.549	0.889	0.568	1.042	0.414	0.384	10.0 ng/mL
F	0.194	0.19	0.427	0.43	0.449	0.437	0.696	0.509	0.76	0.817	0.479	0.564	1.00 ng/mL
G	0.059	0.06	0.366	0.397	0.354	0.39	0.496	0.462	0.252	0.196	0.459	0.607	0.100 ng/mL
H	0.046	0.045	0.455	0.343	0.401	0.381	0.401	0.577	0.286	0.281	0.047	0.047	0.000 ng/mL
Coating peptide	0.100 µg/mL												
Blocking	3% BSA in PBST												
PC dilutions	Assay buffer		5% matrix		10% matrix		20% matrix		Individual matrices (20%, 10% and 5%)				
Protein A/G HRP	1/1000.000												

CV %	1	2	3	4	5	6	7	8	9	10	11	12
A	NC		NC		2.3		0.1		12.4		3.9	
B	NC		1.9		2.2		1.0		2.9		0.3	
C	2.0		4.1		19.0		10.9		25.4		17.5	
D	0.7		4.7		21.3		15.8		12.2		20.5	
E	0.4		1.1		14.1		33.4		41.6		5.3	
F	1.5		0.5		1.9		21.9		5.1		11.5	
G	1.2		5.7		6.8		5.0		17.7		19.6	
H	1.6		19.8		3.6		25.5		1.2		0.0	

Optimization ELISA

Preparation of peptide coating in glass tube reduces duplicate well CV.

Tube comparison

Assay responses (OD)	coating: 30 ml tube				coating: normal 1.5 mLtube				coating: glass tube			
	1	2	3	4	5	6	7	8	9	10	11	12
A	2.65	2.66	0.35	0.35	2.801	2.716	0.311	0.321	2.575	2.595	0.355	0.337
B	2.996	2.732	0.17	0.169	2.705	2.728	0.174	0.178	2.803	2.802	0.172	0.176
C	1.619	1.598	0.107	0.108	1.549	1.53	0.122	0.125	1.677	1.67	0.113	0.112
D	1.138	1.167	0.52	0.535	1.101	1.079	0.525	0.531	1.197	1.199	0.458	0.462
E	0.372	0.45	0.277	0.316	0.409	0.396	0.338	0.349	0.445	0.447	0.321	0.321
F	0.164	0.169	0.107	0.107	0.179	0.185	0.123	0.128	0.17	0.168	0.112	0.111
G	0.164	0.164	0.306	0.328	0.173	0.181	0.349	0.354	0.162	0.157	0.294	0.292
H	0.145	0.14	0.168	0.177	0.149	0.159	0.195	0.199	0.147	0.145	0.222	0.196
Coating peptide	0.200 µg/mL new stock											
Blocking	0.1% Casein in PBST											
PC dilutions	10% matrix											
Protein A/G HRP	1/1000.000											

CV %	1	2	3	4	5	6	7	8	9	10	11	12
A	0.3		0.0		2.2		2.2		0.5		3.7	
B	6.5		0.4		0.6		1.6		0.0		1.6	
C	0.9		0.7		0.9		1.7		0.3		0.6	
D	1.8		2.0		1.4		0.8		0.1		0.6	
E	13.4		9.3		2.3		2.3		0.3		0.0	
F	2.1		0.0		2.3		2.8		0.8		0.6	
G	0.0		4.9		3.2		1.0		2.2		0.5	
H	2.5		3.7		4.6		1.4		1.0		8.8	

PC
10000 ng/mL
1000 ng/mL
100 ng/mL
50.0 ng/mL
10.0 ng/mL
1.00 ng/mL
0.100 ng/mL
0.000 ng/mL

Drug Tolerance

Drug tolerance determined for the direct binding ELISA approach.

ELISA

Average assay response		
Peptide (µg/mL)	iLPC (200 ng/mL)	HPC (5000 ng/mL)
15.0	0.141	0.146
7.50	0.594	1.176
3.75	0.263	1.250
1.88	0.281	1.371
0.938	0.294	1.404
0.469	0.301	1.444
0.234	0.349	1.393
0.117	0.341	1.367
Plate-specific cutpoint	0.223	0.223

Drug tolerance: the highest drug concentration at which the assay response is ≥ the plate-specific cut-point

- Only the highest concentration (15.0 µg/mL) peptide was able to completely inhibit LPC and HPC samples (back to negative control OD levels).
- All other peptide concentrations resulted in LPC and HPC responses that were approximately half of the responses when no drug was added.
- Rapid increase in signal inhibition between 7.50 and 15.0 µg/mL peptide raised the question whether this is caused by an unspecific solvent effect.

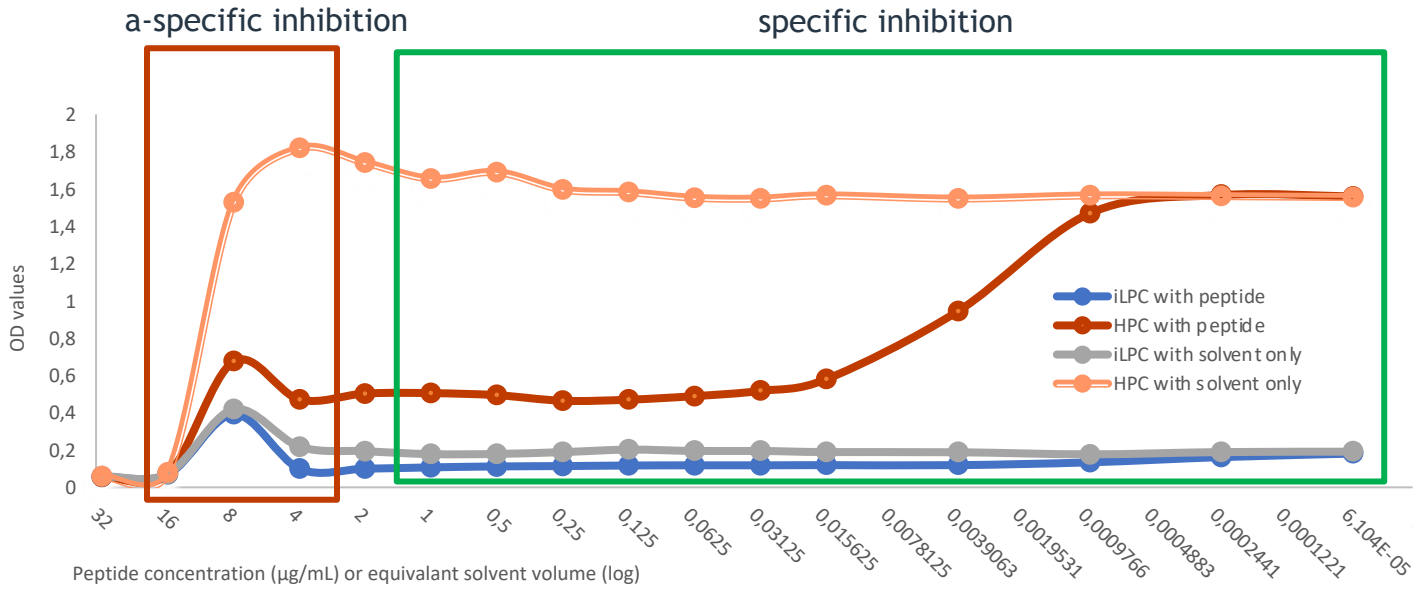
Solvent effect

- Solvent buffer content:
  - 10.0 mg/mL BSA
  - 1.00% Tween-80
  - Ortho-Phosphoric acid 85%
  - Acetonitrile
  - Ultra pure water

- Peptide stock solution 30x diluted in Assay Buffer (0.1% Casein in PBS + 0.05% Tween-20) before use

Solvent effect

Comparison solvent and buffer



Signal Inhibition - iLPC

PC (ng/mL)	Assay buffer 1.50 µg/mL	Assay buffer 0.500 µg/mL	Assay buffer 0.150 µg/mL	Solvent buffer 1.50 µg/mL	Solvent buffer 0.500 µg/mL	Solvent buffer 0.150 µg/mL
10000	38.7	36.3	34.9	1.1	0.6	-0.3
5000	45.7	40.4	40.1	1.6	1.3	2.2
3500	48.1	43.8	39.7	1.4	2.3	2.8
2500	52.3	46.1	43.3	0.8	3.0	4.7
200	59.4	51.3	44.8	0.2	4.6	5.6
0	13.8	13.4	11.5	9.7	12.0	8.8

- iLPC and HPC samples incubated with the volume of solvents that is normally present at 15.0 µg/mL peptide show similar signal inhibitions compared to samples incubated with solvent only.
- This finding confirms that the rapid increase in signal inhibition between 7.50 and 15.0 µg/mL peptide is caused by an unspecific solvent effect in the drug interference experiment.
- Lower concentrations of peptide were tested for use in the confirmatory assay → 1.50 µg/mL can be used without detectable solvent effects (peptide stock 300x diluted).

Validation

Screening cut-point	
• Cut-point type	Floating cut-point
• Cut-point factor	1.56
• Plate-specific cut-point	Mean response of NC samples on plate x 1.56
Confirmatory cut-point	16.3 %
Assay sensitivity	
• Sensitivity of screening assay	24.2 ng/mL (anti-peptide antibodies)
• Sensitivity of confirmatory assay	14.2 ng/mL (anti-peptide antibodies)
Determination of the low positive control (LPC) concentration	
• LPCs	60.0 ng/mL (anti-peptide antibodies)
• LPCc	33.4 ng/mL (anti-peptide antibodies)

Selectivity	
• Individual human serum samples	In the screening assay, all LPCs samples were positive and all NC and unspiked samples were negative. In the confirmatory assay, all LPCc samples were positive.
• Haemolysed human serum sample	No interference by haemolysed human serum was observed.
• Lipemic human serum sample	No interference by lipemic human serum was observed.

Precision	Screening	Confirmation
Within-run Precision (%CV)	NC: 7.8% LPCs (60.0 ng/mL): 10.2% LPCc (33.4 ng/mL): 4.3% MPC (1000 ng/mL): 7.7% HPC (5000 ng/mL): 3.9% HPCc (2500 ng/mL): 2.6%	LPCc (33.4 ng/mL): 13.8% HPC (5000 ng/mL): 6.6% HPCc (2500 ng/mL): 5.6%
Between-run Precision (%CV)	NC: 7.8% LPCs (60.0 ng/mL): 10.2% LPCc (33.4 ng/mL): 16.2% MPC (1000 ng/mL): 12.9% HPC (5000 ng/mL): 7.8% HPCc (2500 ng/mL): 8.2%	LPCc (33.4 ng/mL): 19.3% HPC (5000 ng/mL): 12.6% HPCc (2500 ng/mL): 10.0%

Study drug interference	
(Drug tolerance)	Drug tolerability at iLPC level (200 ng/mL): up to 1.50 µg/mL peptide Drug tolerability at HPC level (5000 ng/mL): up to 1.50 µg/mL peptide
Stability	
• Bench-top Stability	21 hours at room temperature.
• Freeze/thaw Stability	9 freeze/thaw cycles.
End-point titre	All end-point titres are within the median end-point titre of 128 ± 1 titre value.
Prozone effect	No prozone effect was observed.

Conclusions

The direct binding ELISA showed good performance with high precision, sensitivity and selectivity.

- Preparation of coating in glass tube reduces duplicate well CV.
- During the drug tolerance experiments, a solvent effect was observed as only the highest concentration (15.0 µg/mL) peptide was able to completely inhibit LPC and HPC samples.
- A comparison was performed between solvent only and peptide, demonstrating that the volume of solvents that is normally present at 15.0 µg/mL peptide showed similar signal inhibitions compared to samples incubated with solvent only, confirming that the rapid increase in signal inhibition between 7.50 and 15.0 µg/mL peptide is caused by an unspecific solvent effect.
- Lower concentrations of peptide were tested for use in the confirmatory assay. Here, it was concluded that lower concentrations of peptide (1.50 µg/mL) can be used without detectable solvent effects.
- Overall, the validated Anti-peptide Antibody assay has been successfully implemented in the bioanalysis of human samples from a Phase 2a clinical trial.

ARDENA

Thank You

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