A Lateral Flow Immunochromatographic Method For Anti-Drug Antibody Detection In Human Serum

Jian Li, Nathan Cheadle, Allen Schantz, Gopi Shankar

Biologics of Clinical Pharmacology, Janssen Research & Development, LLC., Radnor, PA 19087

Abstract

Purpose: To investigate the feasibility of a portable assay, the Nano-Intelligent Detection System (NIDS), a lateral flow immunochromatographic technology for detection of anti-drug antibodies (ADA) to an investigational human IgG1drug (CNTO X), and to address potential interference from samples bearing drug or drug target (cytokine X).

Methods: NIDS double antigen bridging ADA assay began by processing serum samples to minimize interferences. Target interference was addressed by pre-incubating samples with a biotin anti-cytokine X antibody and removing bound target via streptavidin-coated gold particles. Acetic acid dissociation of preformed ADA-drug complexes was included to improve drug tolerance. Acidified samples were incubated with hapten- and biotin-conjugated CNTO X in Tris base. The sample mixture was loaded onto the test strip. Drug complexes bridged by ADA were captured in situ as they flowed past a pre-coated anti-hapten antibody.

A bridging electrochemiluminescent ADA immunoassay (ECLIA) required lactic acid dissociation of serum samples which were then mixed with Biotin~CNTO X, rhuthenium~CNTO X, and goat anti-cytokine X antibody in Tris base. The sample mixtures were added to streptavidin-coated MesoScaleDiscovery (MSD) plates and ADAs were detected in a Sector 6000.

Results: Acid dissociation improved drug tolerance. The NIDS ADA method detected 250 ng/mL ADA in serum containing >12-fold molar excess of free drug and the ECLIA method detected 250ng/mL ADA in serum containing 50-fold molar excess of free drug. The sensitivities of the NIDS and ECLIA assays were 97.7 ng/mL and 48 ng/mL for a monoclonal ADA and 312 ng/mL and 98 ng/mL for a polyclonal monkey ADA, respectively.

Conclusion: The NIDS ADA assay was slightly less sensitive and drug tolerant than the ECLIA but more target tolerant. As a simple, portable detection device that required minimal laboratory equipment for use, the NIDS system has the potential to allow ADA detection at decentralized clinical sites.

Methods

CNTO X ADA NIDS Bridging Assay: 100 µL of serum sample was mixed with 100 µL 1M acetic acid pH2.5 for 30 min and then 120 µL of 1M Tris buffer pH 9.5 containing hapten- and biotin-CNTO X conjugates was added and incubated an additional 60 min at RT. A 100 µL aliquot of the sample mixture was transferred to a lateral flow strip that contained mobile streptavidin labeled gold particles on a loading pad and the immobilized mouse anti-hapten antibody in the capture zone. When ADA bridged labeled forms of the drug, the immune complex could be captured by the immobilized anti-hapten in order to yield a visible accumulation of gold particles that could be quantified by a portable SAR III Reader. The intensity of the test line was directly proportional to the concentration of the target analyte in the serum.

To reduce the target interference, serum samples were combined with streptavidin gold particles coated with biotin labeled goat-anti-cytokine X Ab and mixed for 30 min at RT on a shaker. After centrifugation, the gold particles and captured cytokine X were discarded and the processed serum was collected for ADA detection as per the screening method described above. Any residual cytokine X activity was further reduced by the addition of 100µg/mL polyclonal anti-target antibody in a solution containing 1M Tris buffer pH 9.5 containing hapten- and biotin-CNTO X conjugates.

CNTO X ADA ECLIA Bridging Assay: 20 µL of test serum was combined with 80 µL of 50mM lactic acid pH2.6 and incubated for 30 min at RT. After acid treatment, the samples were combined 1:1 with a master mixture of 0.5 µg/mL biotin labeled CNTO X and 0.5µg/mL BvTag labeled CNTO X prepared in Tris buffered saline, pH8.0, and incubated for 60 minutes at RT on a shaker. Streptavidin-coated 96 well plates were blocked by adding 200ul of StartingBlock™ per well. The blocking solution was discarded and 50 µL of the sample mixture was added to each well. After 60 minutes incubation, the plates were washed and 150 µL of MSD read buffer (Meso Scale Discovery, Inc., Gaithersbrug, MD) was added to each well. The ECL signal of each well was measured using a Sector® 6000 imager (Meso Scale Discovery).

To overcome target interference in the ECLIA method, a cytokine removal process was developed. 100 µL of biotin-labeled goat anti-cytokine X (100 µg/mL) was added to each well of a V bottom plate containing 100 µl of streptavdin beads and incubated for 60 minutes while shaking at 550 RPM at room temperature. The beads were washed with 200 µL of blocking buffer, then mixed thoroughly with a pipette, being careful to avoid creating air bubbles. The V bottom plate was placed on a magnetic separator for two minutes and supernatant was carefully discarded without disturbing the beads at the bottom of the plate. Controls and samples were diluted 1/2 with assay diluent. Diluted samples and controls were then added to wells containing goat anti-cytokine X Ab beads. A pipette was used to thoroughly mix the bead solution was mixed and ensure that the beads were in suspension. Plates were covered and incubated 60 minutes at room temperature while shaking at 550 RPM. V bottom plates were again placed on a magnetic separator for at least 2 minutes. Processed sera were carefully removed from the wells and tested in the ADA ECLIA method. Residual cytokine X activity was further reduced by adding polyclonal anti-cytokine X antibody (100µg/mL) into the master mix**.**

Results

Establishment of a screening cut point: The cut point for screening samples was calculated by adding the mean signal obtained from naive serum of three independent experiments and 1.645 times the standard deviation. To establish the screen cut point for the NIDS assay, 30 naïve human serum samples were randomly selected from a larger group and tested six times (three independent run by each of two operators). The screen cut point of the ANP assay was 474.1 units. To establish the screen cut point for the ECLIA method 50 naive human serum samples were tested in six independent experiments by three operators. The ECLIA cut point was 115 ECL units.

Comparison of Assay sensitivity: Comparable assay sensitivity was observed in NIDS and ECLIA using mAb ADA (1A, 1B) and polyclonal ADA (1C, 1D).

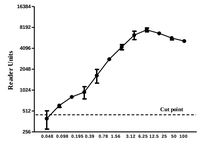

Figure 1A: NIDS assay sensitivity to a monoclonal ADA prepared in NHS was examined. Each data point represents the mean of four test strips and two independent experiments. The NIDS assay sensitivity was 97.7 ng/mL.

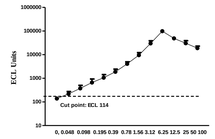

Figure 1B: ECLIA assay sensitivity to a monoclonal ADA prepared in NHS was examined. Each data point represents the mean of six independent experiments. The ECLIA assay sensitivity was 48ng/mL.

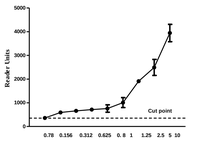

Figure 1C: NIDS assay sensitivity to a polyclonal IgG ADA prepared in NHS was examined. Each data point represents the mean of six test strips and three independent experiments. The NIDS assay sensitivity was 312ng/mL.

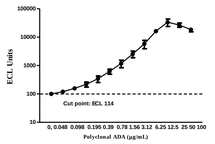

Figure 1D: ECLIA assay sensitivity to a polyclonal IgG ADA prepared in NHS was examined. Each data point represents the mean of six independent experiments. The ECLIA assay sensitivity was 98 ng/mL.

Comparison of Drug Tolerance: NIDS and ECLIA detected 250 ng/mL ADA in serum containing more than 12-fold and 50-fold molar excess of free drug, respectively (NIDS: Table 1, and ECLIA: Table 2).

NIDS ADA Assay Drug Tolerance (Table 1)

CNTO X (µg/mL)	Exp 1	Exp 2	Exp 3	Mean	Result
100	268	277	249	264.7	-
50	355	191.5	206.5	251	-
25	392.5	249.5	277	306.3	-
12.5	394.5	369	322	361.8	-
6.25	476	498.5	391.5	455.3	-
3.13	580	515	471.5	522.2	+
1.56	574.5	688.5	485.5	582.8	+
0.78	588.5	690	517	598.5	+
0.39	719.5	622	627	656.2	+
0	716.5	805	623	714.8	+

ECLIA ADA Assay Drug Tolerance (Table 2)

CNTO X (µg/mL)	Exp 1	Exp 2	Mean	Result
100	79	83	81	-
50	87	90	89	-
25	97	100	99	-
12.5	116	116	116	+
6.25	127	133	130	+
3.13	168	172	170	+
1.56	192	191	192	+
0.78	247	230	239	+
0.39	200	215	208	+
0	215	231	223	+

Comparison of Target Tolerance: The dimeric protein, cytokine X, generated false positive signal in NIDS and ECLIA CNTO X ADA assays. Drug tolerance was 400ng/mL and 50ng/mL of serum cytokine X for NIDS and ECLIA assays, respectively (Figure 2A and 2B).

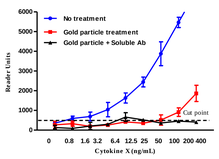

Figure 2A: NIDS assay target tolerance: Various concentrations of cytokine X were prepared in NHS. Data points represent the mean of four test strips from three independent experiments. Prior to analysis samples underwent no pre-tretment, or were pre-treated with anti-cytokine X coated gold particles with/without soluble goat anti-cytokine X antibody.

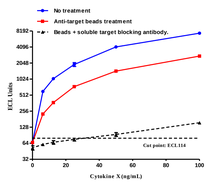

Figure 2B: ECLIA assay target tolerance: Various concentrations of cytokine X were prepared in NHS. Data points represent the mean of two independent experiments. Prior to analysis samples underwent no pre-tretment, or were pre-treated with anti-cytokine X coated gold particles with/without soluble goat anti-cytokine X antibody.

Analysis of mock samples: Mock serum samples were prepared with 50 ng/mL cytokine X, 250 ng/mL ADA and 50 ng/mL cytokine X + 250 ng/mL ADA then tested in the NIDS CNTO X ADA assay. Without sample pre-treatment, cytokine X produced a false positive ADA signal. Samples pre-treated with an anti-cytokine X Ab and gold particles were correctly identified for the presence or absence of ADA in each mock sample (Figure 3).

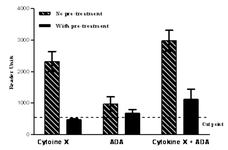

Figure 3: Mock serum samples spiked with 50ng/mL of cytokine X, 250ng/mL ADA, or 50ng/mL of cytokine X + 250ng/mL ADA were pre-treated to relax cytokine X interference or treated without pretreatment. Value represent the mean of two independent experiments.

***Reproduciblity of the NIDS ADA Assay*:** Precision was evaluated across 5 replicates per run with one run occurring on each of 3 days. Intra-assay precision was calculated as the mean of the percent coefficient of variance (CV) for each run, while inter-assay precision was calculated as the CV of the mean result for each run (Table 3).

Table 3: Precision of NIDS Assay

ADA (ng/mL)		mAb 1000	mAb 500	mAb 250	pAb anti-serum	Precision Range
Day 1 (n=5)	Mean	3173	2042	1138	1658	
	SD	399	164	61	239	
	CV	12.6	8	5.4	14.4	
Day 2 (n=5)	Mean	2499	1323	965	1531	
	SD	290	148	160	315	
	CV	11.6	11.2	16.6	20.6	
Day 3 (n=5)	Mean	2531	1722	974	1427	
	SD	335	375	186	283	
	CV	13.2	21.8	19.1	19.8	
Intra-day Precision	CV	12.5	13.7	13.7	18.3	12.5-18.3
Inter-day Precision	Mean (n=3)	2734	1696	1026	1539	
	SD	380	360	97	116	
	CV	13.9	21.2	9.5	7.5	7.5-21.2

Conclusion

We compared a NIDS double antigen bridging ADA assay to an established ECLIA method. Although the ECLIA was somewhat more sensitive and drug tolerant, it must be performed in a laboratory by a skilled analyst. The NIDS assay performed within generally accepted criteria (Shankar, 2008). Furthermore, the NIDS platform was compatible with the use of microscopic gold particles, which we were able to adapt for the purpose of reducing target interference. When testing must be conducted at decentralized locations, the NIDS technology may be useful. It is portable, comparatively low cost, can be performed by someone with minimal training and facilities, and permits flexible assay design.

References

Shankar, G, et al. *Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products*. J Pharm Biomed Anal 48 (2008) 1267-1281.

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