Development of an automated ADA immunoassay with integrated acid dissociation and neutralization

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Summary

- A new, drug-tolerant procedure for ADA analysis was established
- Integrated and automated sample pretreatment shortened time to result to one hour without the need for overnight incubation
- Working at nanoliter scale significantly reduced sample and reagent consumption
- Dedicated software streamlined the process from assay development through to confirmatory analysis

Objective

To integrate sample pretreatment (acid dissociation, incubation and neutralization) and immunoassay into a time-efficient, automated and integrated workflow when analyzing anti-drug-antibodies (ADA) in human serum. Parameters evaluated included drug tolerance, sensitivity, precision, reagent and sample consumption and time to result.

Methods

Instrumentation

Using two natural phenomena (centrifugal force and capillary action) and proprietary microfluidic technologies, sample pretreatment and the ADA immunoassay take place within the microstructures of a Gyrolab ADA CD under control of a Gyrolab workstation. Each microstructure generates one data point.

Fig 1. Gyrolab xP workstation.

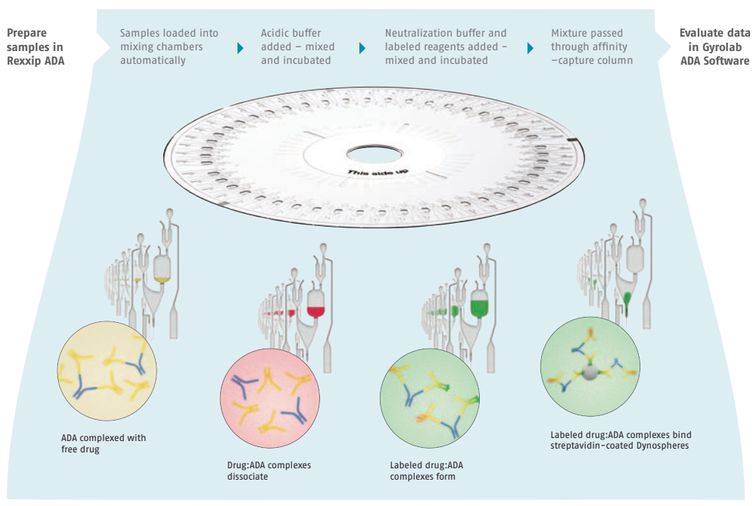

Fig 2. 48 samples per CD processed simultaneously in parallel, with every step controlled through an automated method. Illustration shows key steps in a bridging homogeneous assay format.

The optimized protocol for this ADA assay required a one minute incubation for acid dissociation and 10 minutes for neutralization. The entire run was completed within one hour.

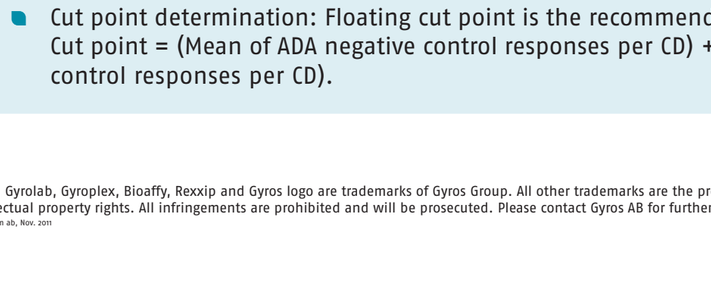

Fig 3. One microstructure:one data point in the Gyrolab ADA CD.

Experimental model

- The ADA assay was run in a bridging semi-sequential assay format
- Drug molecule analog: Recombinant human monoclonal antibody to HIV-1 gp120 (2G12, Polymun)
- ADA positive control: Murine monoclonal anti-idiotypic antibody to HIV-1 gp120 antibody 2G12, non-inhibiting (M1G1, Polymun)
- Matrix composition: normal human serum pool and samples diluted to 10% in Rexxip ADA buffer (Gyros)
- Cut point determination: Floating cut point is the recommendation for Gyrolab ADA analysis.
  Cut point = (Mean of ADA negative control responses per CD) + Correction Factor (CF) × (Standard deviation [SD] of ADA negative control responses per CD).

Results

Optimization of acid dissociation

Several parameters were evaluated to achieve optimal conditions for acid dissociation and neutralization prior to immunoassay in order to improve drug tolerance. Parameters included type of acidic buffer, pH, incubation times for acid dissociation and neutralization and minimal required dilution (MRD).

The optimal pH for this ADA assay and its ADA positive control was found to be pH 3, providing a sufficiently low pH to dissociate ADA drug complexes while still maintaining ADA positive control performance.

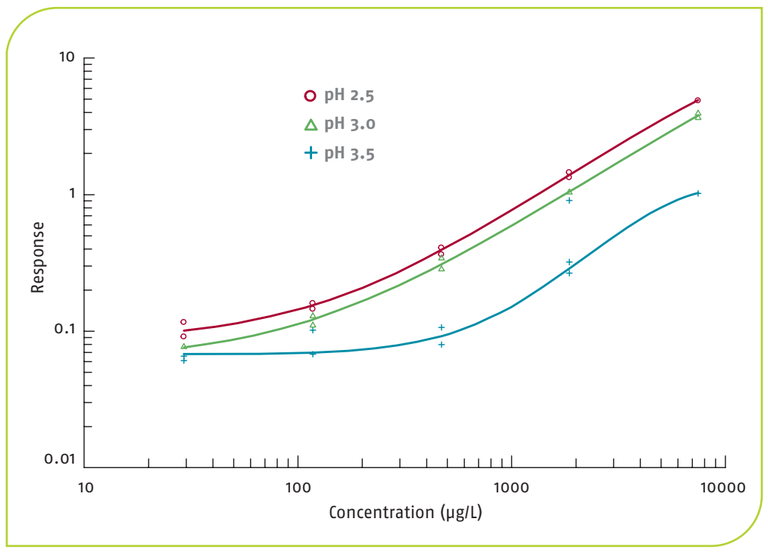

Fig 4. Effect of variation in acid dissociation pH on sample responses. Serial dilutions (in Rexxip ADA) of ADA positive controls in 10% serum were pre-incubated with 40 µg/mL free drug and subjected to dissociation conditions at various pH immediately followed by bridging assay in neutral pH within the CD. Acidic buffer (300 mM acetic acid) adjusted to pH levels 2.5, 3 and 3.5.

Precision

Parallel processing with precise control of mixing and incubation times ensured consistent assay performance.

	QC 1	QC 2	QC 3	QC 4
Concentration (ng/mL)	1000	200	100	50
Intra-CD CV (%) Run 1	3.7	5.6	10.3	15.1
Intra-CD CV (%) Run 2	4.7	5.3	5.2	17.4
Intra-CD CV (%) Run 3	6.2	22.1	10.0	2.8
Inter-CD CV (%)	5.0	19.1	11.7	21.2

Table 1. Assay precision determined using independent dilutions of 4 ADA positive control levels evaluated in triplicate in 3 consecutive runs. Inter-CD CV (%) was calculated using ANOVA.

Sensitivity

Limit of detection (LOD) for the assay was found to be 31 ng/mL. This ADA positive control was reproducibly recovered above cut point in three consecutive runs.

Expected Conc. (ng/mL)	Run 1 Av. Response	Run 2 Av. Response	Run 3 Av. Response	
0	0.023	0.025	0.049	
15.62	0.056	0.043	0.068	Cut point
31.25	**0.081**	**0.090**	**0.098**	
62.5	**0.139**	**0.131**	**0.154**	
125	**0.260**	**0.252**	**0.283**	
250	**0.479**	**0.469**	**0.477**	
500	**0.943**	**0.981**	**1.092**	
1000	**1.858**	**1.714**	**2.296**	

Table 2. Assay sensitivity determined using serial dilutions of ADA positive control from 1000 to 15.6 ng/mL in steps of 2 evaluated in 3 consecutive runs.

Cut point estimation in a model system

To test performance of Gyrolab ADA Software and determine a preliminary screening cut point, data from three consecutive runs of 21 drug-naive human serum samples were used to calculate a correction factor (CF=0.034) which was entered into the software.

Cut point = (Mean of ADA negative control responses per CD) + Correction Factor (CF) × (Standard deviation [SD] of ADA negative control responses per CD).

Screening results were generated by automatic data evaluation using user-defined acceptance criteria and a floating cut point (Fig 5). Positive samples were transferred to confirmatory analysis in which samples spiked with excess free drug (250 µg/mL) were assayed alongside unspiked samples (Fig 6).

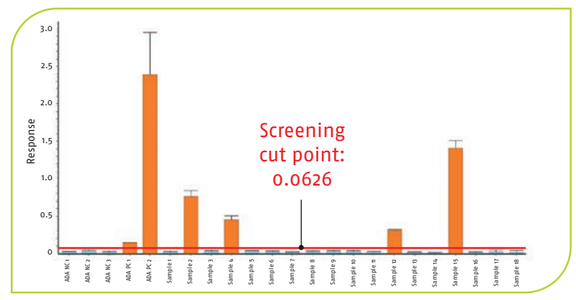

Fig 5. Sample responses from the screening assay are evaluated against screening cut point.

Positive samples transferred to confirmatory analysis

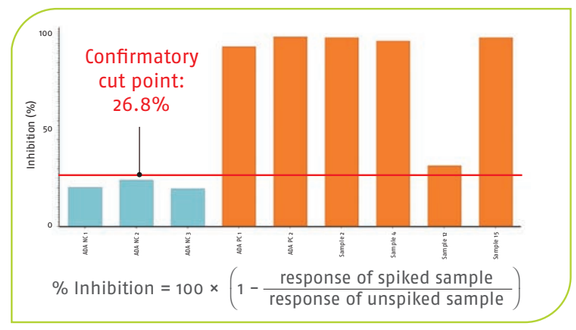

Fig 6. The degree of inhibition (%) reflects the loss of response signal when an excess amount of free drug is added to the positive sample identified in the screening assay. Inhibition values above confirmation cut point indicate true positive samples.

Drug tolerance

Acid treatment enables detection of 160 ng/mL ADA positive control in presence of up to 160 µg/mL of free drug, a ratio of 1:1000. Despite a short acid dissociation time (1 min), sensitivity in presence of excess amount of free drug exceeded minimum regulatory requirements (>250 ng/mL for clinical assays).

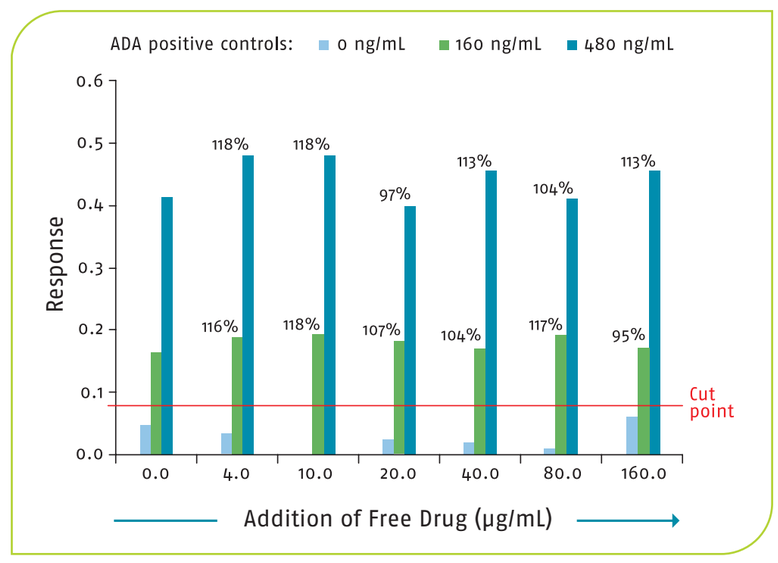

Fig 7. Drug tolerance. ADA positive controls are displayed as data series (0, 160 and 480 ng/ml) with addition of increasing levels of free drug (0, 4, 10, 20, 40, 80 and 160 ug/ml) along the x-axis. The floating cut point in this experiment is 0.081 (Mean of ADA negative controls + 0.034 × SD of ADA negative controls).

Sample/reagent consumption and time to result

Using the nanoliter-scale format significantly reduced consumption of samples, ADA positive controls and labeled reagents. The automated, integrated sample pretreatment shortened overall assay time.

Sample	Neat sample volume	Volume loaded in instrument
Unknown sample	3 µL	5 µL
ADA positive control	3 µL	5 µL

Table 3. Required volumes to produce two data points (one sample in duplicate). Samples assumed to be diluted to 50% in Rexxip ADA.

	For one Gyrolab ADA CD (48 data points)
Biotin-labeled drug consumption	5 µg – 50 µL
Alexa-labeled drug consumption	50 ng – 50 µL
Time to result	1 hour

Table 4. Reagent consumption and time to result for one full Gyrolab ADA CD.

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