Use of Ella[®] to facilitate drug quantification and antidrug antibody detection in preclinical studies

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Aim: To explore the usability of the Ella[®] platform for preclinical analysis of therapeutic antibodies and antidrug antibodies (ADA). **Experimental:** Two well-established ELISAs for the measurement of human IgG and ADA were transferred to the Ella platform. ELISA and the Ella platform were compared using assay qualification data and results of preclinical sample analysis. **Results:** The performance and results of both assays on the Ella platform were comparable to those of ELISA. The Ella platform had several advantages, including time efficiency, low sample consumption and a high degree of automation. ADA were assessed on Ella for the first time. **Conclusion:** The Ella platform is a promising tool for the analysis of preclinical samples.

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ELISA remains the most widely used immunoassay format for pharmacokinetic (PK) and antidrug antibodies (ADA) analysis. It is a robust format with sensitivities in the nanogram per milliliter range. ELISA requires only standard laboratory equipment and a plate reader and hence incurs insignificant material costs. However, ELISA is labor intensive due to a long and sometimes very complicated assay conduct, which relies on well-trained personnel. It also requires a relatively large sample volume, thus being less suitable for rare matrices such as eye, synovial or cerebrospinal fluids [1–4]. Pipetting robots can help overcome some disadvantages of the ELISA format and improve data quality [5].

Ella[®] platform is a microfluidic cartridge-based immunoassay platform from ProteinSimple (part of Biotechne), which is widely used for the quantification of soluble biomarkers [6–9], but is not yet applied in the field of PK and ADA assays. It is a very robust platform with excellent precision, accuracy and reproducibility [6] owing to its high degree of automation. Different cartridge formats allow for measuring of either 72 samples in one assay or 32 samples in four parallel running assays; multiplexing is achieved by separating the sample into four separate channels, eliminating the possibility of a crosstalk between the assays [8]. Measures are performed in triplicates in three glass nanoreactors (GNRs) for each sample using a fluorescent substrate, which helps to achieve a wide dynamic measuring range. The sensitivity of the available biomarker assays is often in the lower picogram/milliliter range with a required sample volume between 2.5 and 50 µl.

Having recognized the potential advantages of the Ella technology for PK and ADA analysis of preclinical samples, we transferred two of our most frequently used assays, an antihuman IgG-specific assay (Figure 1) [10] and an immune complex ADA assay (Figure 2) [11], to the Ella platform. In this paper, we describe the performance of these two assays on the Ella platform and compare them to the corresponding ELISA methods. We further compare both assays executed on the Ella platform and as ELISA using samples from preclinical studies and discuss the potential use of Ella as a replacement for ELISA in preclinical sample analysis. For more in-depth questions about the assay principles used, please refer to the corresponding publications for these assays [10–12].

Figure 1. Schematic presentation of the assay principle for human IgG quantification. (A) In ELISA, biotinylated-capture antibody is immobilized on a streptavidin-coated microtiter plate. After binding to the capture antibody, drug antibody (human IgG) is detected using a digoxigenin-labeled detection antibody and antidigoxigenin Fab fragments coupled with horse radish peroxidase. Horse radish peroxidase-catalyzed color reaction is used for signal detection. **(B)** On the Ella[®] platform, capture antibody is directly coupled to the glass nanoreactor. The bound drug antibody is detected using a biotinylated detection antibody and a streptavidin-labeled fluorescent dye (DyLight650).

Bi: Biotinylated; Dig: Digoxigenin; HRP: Horse radish peroxidase; SA: Streptavidin.

Experimental

Chemicals, reagents & equipment

Roche Diagnostics GmbH (Penzberg, Germany) produced all antibodies. They were aliquoted and stored at -80°C . Murine monoclonal antibodies against human IgG and cynomolgus IgG were generated from mice immunized with purified human IgG or cynomolgus IgG. Antibodies from hybridoma supernatants were purified by protein A chromatography. Conjugates of cynomolgus monkey IgG with human IgG were generated in house [11]. Biotin (Bi)- and digoxigenin (Dig)-labeling was performed in house. Horse radish peroxidase (HRP)-labeled polyclonal anti-Dig antibody Fab fragments (pAb-Dig-S-Fab-HRP), phosphate-buffered saline (PBS), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS; ready to use solution) and Tween 20, were from Sigma Aldrich (MO, USA). Bovine plasma albumin and Roche Universal buffer were from Roche Diagnostics GmbH. Low Cross Buffer[®] was from Candor Bioscience GmbH, Wangen, Germany. Cynomolgus monkey serum (individual and pooled) was from Sera Laboratory International, Ltd (Haywards Heath, UK). Streptavidin (SA)-coated microtiter plates (SA-MTP) were from Microcoat Biotechnologie GmbH (Bernried, Germany). Antibodies and standards for the Ella cartridges were provided by Roche and labeled, immobilized and used by ProteinSimple, Biotechnne, MN, USA, to produce cartridges for the two assays.

Study samples

For Mab1, cynomolgus monkey serum samples were obtained from three animals from a preclinical study. Blood samples (3 ml) were taken predose, 12 (group 1 only), 24, 48, 72, 168 (all groups), 336 (groups 2 and 3 only) and 504 h (group 3 only) postdose.

For Mab2, cynomolgus monkey serum samples were obtained from three animals. One predose and 16 postdose blood samples (1.5 ml) were taken up to 504 h postdose.

Figure 2. Schematic presentation of the immune complex assay for antidrug antibody. (A) In ELISA, drug–ADA complex is captured by a biotinylated antihuman IgG capture antibody immobilized on a streptavidin-coated microtiter plate. The ADA in the captured immune complex is detected using digoxigenin-labeled anticynomolgus IgG detection antibody and antidigoxigenin Fab fragments coupled with horse radish peroxidase. Horse radish peroxidase-catalyzed color reaction is used for signal detection. **(B)** On the Ella[®] platform, capture antibody is directly coupled to the glass nanoreactor. The bound drug–ADA complex is detected using a biotinylated detection antibody and a streptavidin-labeled fluorescent dye (DyLight650). ADA: Antidrug antibody; Bi: Biotinylated; Dig: Digoxigenin; HRP: Horse radish peroxidase; SA: Streptavidin.

For Mab3, cynomolgus monkey serum samples were obtained from 11 animals. Animal 1 received no drug and served as a control animal. The other ten animals were divided into five groups. In each group, two animals received weekly injections with increasing quantities of Mab3 (animal 2/3: 0.03 mg/kg; animal 4/5: 0.1 mg/kg; animal 6/7: 0.3 mg/kg; animal 8/9: 1 mg/kg; animal 10/11: 3 mg/kg). Blood samples (1.5 ml) for ADA examination were taken before dosing (0 h), and 7, 15 and 29 days after dosing. In all studies, whole blood was collected in serum separator tubes. Blood was kept at ambient temperature until clotting and centrifuged to obtain serum, which was then kept at -70°C until use. For this study, we used leftover samples, resulting in slightly diverse time points per animal.

Drug quantification with ELISA

Drug levels in serum samples were determined using an ELISA with an assay range of 1.95–12.5 ng/ml and quality control (QC) samples at 4000 (high quality control), 1600 (medium quality control), 500 (low quality control) and 30 ng/ml (LLOQ quality control) in blank matrix (Figure 1A). Incubations were performed for 1 h at room temperature with shaking at 500 r.p.m. The assay buffer was Roche Universal Buffer and the wash buffer was PBS with 0.05% Tween-20.

The biotinylated anti human κ -LC M-1.7.10 IgG antibody (I) was immobilized with 100 μ l per well on an SA-MTP. The diluted samples, QC samples and calibration standards (100 μ l each) were added in duplicates. QC samples were diluted 1:1000 (high quality control) or 1:500 (medium quality control/low quality control/LLOQ quality control). The 1:10 dilution was done with assay buffer; higher dilutions were done with assay buffer containing 10% pooled matrix. The bound drug was detected using Dig-labeled anti human CH1-HC M-1.19.31 IgG antibody and anti-Dig HRP conjugate with ABTS as substrate. At 405 nm (reference wavelength 490 nm), the color reaction was monitored and the amount of bound analyte was proportional to the signal obtained.

The standard curve fit and the recalculation of sample concentrations were performed in Microsoft Excel using a four-parameter fit algorithm in XLFit add-in.

Drug quantification with Ella

The same assay format was used as for the ELISA with adaptations to the Ella platform (Figure 1B). The same QC samples as for the ELISA assay were used for better comparability.

In brief, samples, QC samples and calibration standards were diluted with assay buffer. The QC samples were diluted one to ten; all other samples were diluted to a minimum of one to ten. Higher than one to ten dilutions were done with assay buffer containing 10% pooled matrix. Samples, QC samples and calibration standards were loaded onto an Ella cartridge and all relevant information such as sample names and dilutions were entered into the Simple Plex Runner software. The cartridge was inserted into the device and the assay was started. At the end of the assay, the results of triplicate measurements (corresponding to three GNRs) in relative fluorescence units, mean signal values, SD and coefficient of the variance (CV) were automatically displayed for each analyte. The mean signal values were transferred in Microsoft Excel and analyzed as described for ELISA.

Comparative sample analysis from preclinical studies

Drug concentration was measured on ELISA and Ella platforms in 19 cynomolgus serum samples from three different animals from a preclinical study with Mab1 and in 52 cynomolgus serum samples from three animals from a preclinical study with Mab2.

The correlation between the concentrations measured on both platforms was assessed using a spearman's rank correlation.

Immune complex ADA assay with ELISA

The immune complex assay was designed as a serial four-step ELISA (Figure 2A). Conjugates of cynomolgus monkey IgG and human IgG, which were spiked into an empty matrix, were used as QC samples (1200 [QC1], 640 [QC2], 380 [QC3] and 45 [QC4] ng/ml). An incubation time of 1 h at room temperature with shaking at 500 r.p.m. was applied to the reagents and samples. The assay buffer was Low Cross Buffer® and the wash buffer was PBS with 0.05% Tween 20. Capture antibody (Bi-labeled antihuman Fc M-R10Z8E9 IgG, 100 µl per well) was immobilized on an SA-MTP. The samples have been diluted 1:20 with an assay buffer that contains 1 µg/ml of the corresponding drug, incubated for 30 min and then transferred in duplicate together with QC samples to the SA-MTP (100 µl per well). The bound drug-ADA complexes were detected using Dig-labeled anticynomolgus monkey M1.7.10 IgG and anti-Dig HRP conjugate with ABTS as substrate. The color reaction, which is proportional to the amount of the bound drug-ADA complexes, was monitored at 405 nm (reference wavelength 490 nm).

In a statistical analysis, a floating cut point (CP) was evaluated with 16 male and 16 female individual blank serum samples according to Shankar *et al.* [13]. The plate-specific CP was obtained by multiplying the signal of a pooled plasma blank sample by the plate normalization factor. The samples with signals below the plate-specific CP were screening negative, and the samples with signals at or above the plate-specific CP were screening positive.

Immune complex ADA assay with Ella

The same assay format was used as for the ELISA with adaptations to the Ella platform (Figure 2B). The QC concentrations used in the assay were 600 (QC1), 300 (QC2), 75 (QC3) and 20 (QC4) ng/ml for the assay.

In brief, samples and calibration standards were diluted 1–20 with assay buffer containing 1 µg/ml of the corresponding drug; QC samples were diluted 1–20 in assay buffer without drug. After incubation for 30 min, samples, QC samples and calibration standards and wash buffer were loaded onto an Ella cartridge and measured in the same way as described for drug quantification. The results of triplicate measurements (corresponding to three GNRs) in relative fluorescence units, mean signal values, SD and CV were transferred in Microsoft Excel and analyzed as described for ELISA. The CP factor was evaluated as described for the ELISA platform. The identical 32 individual blank serum samples were used.

Table 1. Qualification parameters for drug quantification assay.[†]

Parameter	ELISA				Ella [®]			
	HQC	MQC	LQC	LLOQQC	HQC	MQC	LQC	LLOQQC
QC (matrix concentration) (ng/ml)	4000	1600	500	30	4000	1600	500	30
QC dilution factor	1:1000	1:500	1:500	1:50	1:10	1:10	1:10	1:10
QC (assay concentration) (ng/ml)	4	3.2	1	0.6	400	160	50	3
Intra-assay precision (%)	3	7	4	3	4	3	6	2
Intra-assay accuracy (recovery in %)	99	88	103	95	99	99	94	85
Interassay precision (%)	6	7	10	8	11	6	5	14
Interassay accuracy (recovery in %)	102	97	101	92	94	96	94	95
Dynamic range (ng/ml assay concentration)	0.19–12.5				1.56–800			
LOD (ng/ml)	0.19				1.56			
Minimal dilution	1:10				1:10			
Sensitivity at minimal dilution (ng/ml)	1.9				15.6			
Sample volume at minimal dilution (μl)	25				2.5			

[†] The QC concentrations used were chosen to be measurable on both platforms.

HQC: High quality control; LLOQQC: LLOQ quality control; LQC: Low quality control; MQC: Medium quality control; QC: Quality control.

Results & discussion

Drug quantification assay

Drug quantification assay showed comparable qualification data with very good accuracy and precision both as ELISA and on the Ella platform (Table 1). The dynamic range for this assay differed markedly between the platforms. ELISA had a relatively small assay range (0.19–12.5 ng/ml) as compared with a wider assay range for the Ella (1.56–800 ng/ml; Table 1 & Figure 3). Thus, the assay was approximately eight-times more sensitive when executed as ELISA. To improve the comparability of the two platforms, the QC concentrations were chosen to be measurable on both platforms.

The moderate sensitivity of the assay on the Ella platform would likely have little impact in practice because the LLOQ of 15.6 ng/ml is sufficient for most preclinical samples with therapeutic human IgGs. In contrast, an increased dynamic range would be beneficial because samples would not require high dilutions to be in the assay range. In addition, lower sample consumption of the Ella platform (2.5 vs 25 μl in ELISA) would reduce the difference in sensitivity for samples with limited availability such as mouse samples or rare sample matrices like eye, synovial or cerebrospinal fluids [1–4]. For example, if only 2.5 μl is available, the sensitivity of the Ella assay would be still 15 ng/ml, whereas the sensitivity of ELISA would be decreased to 19 ng/ml.

In the Ella assay, the standard curves on different cartridges and from different days showed little deviation from each other (Figure 3 and online Supplementary Table 1). In addition, we found hardly any variations between two operators (CV = 8%) and two instruments (CV = 9%). The close similarity of the standard curves can simplify the assay conduct, allowing for the use of a master curve provided with each cartridge. The absence of calibration standards (only QC samples would need to be prepared and measured) would increase the capacity of the cartridge and also reduce errors in the generation of the standard curve. Indeed, ProteinSimple has already implemented this approach in commercial assays, where the standard curve is digitally stored on the cartridge as a barcode and QC samples are provided as a set of lyophilized controls.

However, these standard curves are only to be applied to the respective production lot. We had the possibility to test two different production lots for this assay and found differences in the standard curve regarding the signal heights, but with a unchanged linear range and the recovery of all QC samples was between 98 and 113%. The use of a digitally stored master curve is an acceptable approach for exploratory studies. However, its application in a regulated environment requires further thorough investigations. At least it is possible to compare the current standard curve with the standard curves from different cartridges if the results obtained on a particular cartridge appear unusual.

All samples from preclinical studies with Mab1 and Mab2 were successfully quantified using both ELISA and the Ella platform. The measurements on both platforms showed an excellent correlation (Mab1: $\rho = 0.97$, $p < 0.0001$; Mab2: $\rho = 0.98$, $p < 0.0001$; Figure 4A). The absolute values were closely comparable for Mab1; however, for Mab2

Figure 3. Average of four independent standard curves on the Ella[®] platform. The figure shows an average of four standard curves for Mab2 quantification generated on four different days. Error bars depict standard deviation. RFU: Relative fluorescence unit.

Table 2. Qualification parameters for immune complex assay.								
Parameter	ELISA				Ella®			
	QC1	QC2	QC3	QC4	QC1	QC2	QC3	QC4
QC (matrix concentration) (ng/ml)	1200	640	380	45	12,000	6000	1500	400
QC (assay concentration) (ng/ml):	60	32	19	2.25	600	300	75	20
Intra-assay precision (% CV)	6	4	3	10	4	2	2	2
Interassay precision (% CV)	7	9	20	12	4	2	4	5
Minimal dilution	20 (5% serum)				20 (5% serum)			
Sample volume at minimal dilution (μl)	12.5				2.5			
CV: Coefficient of variation; QC: Quality control.								

ELISA tended to show higher concentrations than the Ella platform (about 13% higher on average), especially at lower concentrations (Figure 4B).

It is known that assay platforms can differ in absolute values despite good linear correlations due to differences in assay format and sample processing [14]. Although we tried to keep the assay format as similar as possible, it was impossible to have absolutely identical procedures for all steps. Various factors such as different labels, incubation times, physical stress or sample dilutions can explain the observed discrepancies between measured concentrations on both platforms.

Immune complex assay

We have successfully transferred the immune complex assay to the Ella platform (Table 2). All control samples were tested positive in all runs both as ELISA and on the Ella platform. The assay showed better precision on the Ella platform than as ELISA with an especially pronounced difference for the interassay precision (Ella: 2–5% vs

Figure 4. Human IgG quantification in cynomolgus monkey PK study samples. (A) Correlation of drug concentrations measured using ELISA and Ella[®] platform in 19 cynomolgus serum samples with Mab1 and 52 cynomolgus serum samples with Mab2. Spearman's rho correlation coefficient (ρ) and p-values obtained using Spearman rank correlation for values (n) within assay dynamic range of both platforms. **(B)** Comparison of the measured drug concentrations using the Bland-Altman method. For each sample, the ratio of individual values obtained on in ELISA and Ella is plotted against their mean. Circles above the solid line at 1.0, which indicates no difference, correspond to the samples where drug concentrations measured by ELISA were higher than those measured on the Ella platform. Dotted lines show the 95% CI.

ELISA: 7–20%). The required sample volume was markedly lower for Ella than for ELISA (12.5 vs 2.5 µl for the Ella platform).

Since an antispecies IgG antibody is used as detection antibody, unspecific binding of immunoglobulins can lead to increased blank values. These can vary between different individuals. To evaluate this on both platforms, we have tested 32 blank cynomolgus serum samples on both platforms in parallel. The distribution of the individual samples is very similar on both platforms (Figure 5). The pairwise correlation of the data is poor with a ρ factor of 0.177 and a p-value of 0.166. This can have different reasons, for example, different sample processing or different surface materials of the two platforms resulting in different unspecific binding of antibodies in the matrix. However, neither of the two platforms seems to be significantly advantageous here. This dataset was used to calculate a CP factor according to Shankar *et al.* and a factor of 1.89 on the Ella platform and 2.05 for the ELISA is obtained. By multiplying the pool blank with this factor, a plate or cartridge-specific CP can be determined.

ELISA and Ella showed very similar results for preclinical samples from studies with Mab1 and Mab3. Qualitative results for all Mab1 samples were equal on both platforms (Figure 6 and online Supplementary Table 2). Samples up to 168 h postdose were tested ADA negative in all three animals as expected for the timeline of ADA formation. For Mab3, results differed only in two of 38 measured samples (Animal 2 at D7; Animal 3 at D15, Table 3). The samples with discordant results were both slightly above the CP. The Animal 2 D7 sample was detected positive in the ELISA and negative on the Ella platform, and the Animal 3 D15 sample was negative in the ELISA and

Figure 5. Scatter plot of background signals from 32 individual sera in the cyno immune complex assay on the Ella[®] platform and in the ELISA. The lines represent the mean and the standard deviation. AU: Absorbance unit; RFU: Relative fluorescence unit.

positive on the Ella platform. Importantly, later samples from these animals were consistently tested positive on both platforms.

Thus, the immune complex assay can be performed on the Ella platform to detect ADA. However, Ella is not suitable for the most commonly used ADA bridging assays, which require a preincubation step with two labeled drug molecules. This is currently not possible on the Ella platform because it is optimized for sequentially performed sandwich assays. In principle, ADA bridging assay can be done sequentially, yet this would result in poor drug tolerance. In contrast, the immune complex assay has sequential design very similar to the sandwich assays and thus it is well suited to the Ella platform. The additional advantages of the assay are the excellent drug- and oligomeric target tolerance, good sensitivity and applicability to many different projects and species (cynomolgus, mouse, minipig) [1,11,15]. Since the drug tolerance of this assay depends on the capacity of the surface used, we have also investigated it on the Ella platform. In a similar experiment as described in [1], we were able to detect a true ADA-positive sample in the presence of up to 10,000 µg/ml drug in the matrix as ADA positive (Supplementary Table 3). This corresponds approximately to the drug tolerance of the ELISA.

Overall discussion

We have used a straightforward approach to transfer ELISA drug quantification and ADA assays to the Ella platform, introducing only absolutely necessary changes. In particular, we have not performed any additional antibody screening. Overall, differences in the assay conduct were relatively small. In the Ella, the capture antibody is directly coupled to the GNR and the detection antibody is biotinylated. The immobilization of the capture antibody and the biotinylation of the detection antibody were carried out by Biotechnie, who also carried out feasibility tests. All other characterizations were performed by us. The development of these two assays at Biotechnie took about 4–6 weeks. Due to the simple, data-driven approach, we did not encounter any major problems in assay transfer, but we are aware that the successful development of customized assays depends on the expertise of the staff involved. The fact that there is significantly reduced flexibility in the development of assays compared with open systems such as ELISA, and the dependence on a single vendor with all inherent risks such as production quality and long-term supply of reagents must certainly be taken into account when assessing Ella.

Both assays showed good and comparable results as ELISA and on the Ella platform, including good sensitivity, accuracy and precision. The Ella platform provides a wider dynamic range than ELISA, but ELISA assays, which usually use colorimetric substrates, can also be performed with special substrates such as 3-(4-hydroxyphenyl)propionic

Figure 6. Detection of antidrug antibody in samples from a preclinical study with Mab1. Dotted lines represent the cut-off values. Red bars indicate antidrug antibody-positive results.

AU: Absorbance unit; RFU: Relative fluorescence unit.

For color figures see online at: <https://www.future-science.com/doi/full/10.4155/bio-2018-0288>

acid (HPPA) to achieve a wide dynamic range [16]. So the assay performance per se cannot justify switching to the Ella platform. However, we argue that Ella offers several advantages over ELISA. These are time-saving conduct, robustness and simple implementation by external partners. The time required for the measurement of 72 samples including dilution of the standard curve, QC and study samples was about 2 h as compared with 5–6 h for ELISA. The robustness of the Ella method has been demonstrated here and in reports on the measurement of soluble proteins [14,17]. Although data presented in this report do not indicate any major advantage over ELISA in terms of robustness, our experience suggests that ELISA is susceptible to human errors because all its steps are performed manually. Owing to the high sensitivity of ELISA, even small differences, for example, in liquid handling, can have a major impact on assay performance. Finally, the simplicity of the Ella protocol may be a decisive factor in assay transfer to external partners. In our experience, both human IgG quantification and immune complex assays are particularly challenging. The former requires significant effort to avoid cross contamination with human IgGs, with

Table 3. Detection of antidrug antibody in samples from a preclinical study with Mab3.

Sample/Subject	Time point (Day)	Signal IC ELISA (AU)	Cut off (NF = 2.05) *Pool value	Positive/negative	Signal IC ELLA® (RFU)	Cut off (NF = 1.89) *Pool value	Positive/negative	Drug level (ng/ml)
Cyno pool serum		0.1			17.3			
Animal 1	D0	0.18	0.21	Negative	12.3	32.7	Negative	BLQ
	D7	0.14	0.21	Negative	15.5	32.7	Negative	BLQ
	D15	0.15	0.21	Negative	15.5	32.7	Negative	BLQ
	D29	0.13	0.21	Negative	13.4	32.7	Negative	BLQ
Animal 2	D0	0.05	0.21	Negative	8.5	32.7	Negative	BLQ
	D7	0.15	0.21	Negative	39	32.7	Positive	19
	D15	1.37	0.21	Positive	120.5	32.7	Positive	n.a.
	D29	2.96	0.21	Positive	235.5	32.7	Positive	24
Animal 3	D0	0.11	0.21	Negative	15.4	32.7	Negative	BLQ
	D7	0.18	0.21	Negative	15.9	32.7	Negative	11
	D15	0.21	0.21	Positive	22.8	32.7	Negative	n.a.
	D29	3.15	0.21	Positive	314.1	32.7	Positive	16
Animal 4	D0	0.04	0.21	Negative	5.3	32.7	Negative	BLQ
	D7	0.41	0.21	Positive	43.9	32.7	Positive	52
	D15	0.93	0.21	Positive	80.9	32.7	Positive	n.a.
	D29	3.29	0.21	Positive	575.2	32.7	Positive	31
Animal 5	D0	0.15	0.21	Negative	12.4	32.7	Negative	BLQ
	D7	0.18	0.21	Negative	21.6	32.7	Negative	42
	D15	1.34	0.21	Positive	108.1	32.7	Positive	n.a.
	D29	3.29	0.21	Positive	682.6	32.7	Positive	55
Animal 6	D0	0.07	0.21	Negative	10.1	32.7	Negative	BLQ
	D7	0.16	0.21	Negative	14.1	32.7	Negative	48
	D15	1.63	0.21	Positive	124	32.7	Positive	n.a.
	D29	1.33	0.21	Positive	110.6	32.7	Positive	133
Animal 7	D0	0.11	0.21	Negative	17.9	32.7	Negative	BLQ
	D7	1.1	0.21	Positive	60.4	32.7	Positive	180
	D15	2.46	0.21	Positive	166	32.7	Positive	n.a.
	D29	1.26	0.21	Positive	93.8	32.7	Positive	289
Animal 8	D0	0.08	0.21	Negative	6.5	32.7	Negative	BLQ
	D29	2.97	0.21	Positive	423.9	32.7	Positive	142
Animal 9	D4	0.18	0.21	Negative	16.3	32.7	Negative	291
	D29	3.16	0.21	Positive	925.9	32.7	Positive	BLQ
Animal 10	D7	0.07	0.21	Negative	13.4	32.7	Negative	714
	D15	0.1	0.21	Negative	7.3	32.7	Negative	n.a.
	D29	0.16	0.21	Negative	13.9	32.7	Negative	2195
Animal 11	D7	0.14	0.21	Negative	29.8	32.7	Negative	818
	D15	0.81	0.21	Positive	49.2	32.7	Positive	n.a.
	D29	0.7	0.21	Positive	40.5	32.7	Positive	989

AU: Absorbance unit; BLQ: Below limit of quantification; D: Day; IC: Immune complex; n.a.: Not available; NF: Normalization factor; RFU: Relative fluorescence unit.

particularly striking examples being the carry-over effects caused by automated washing stations used for human samples or unintentional introduction of human material from lab personal. The latter causes problems mainly because of the understanding of the different nature of the assay principle compared with conventional ADA assays. We are confident that Ella, an automated and closed system, can eliminate many of these problems. The application shown here is new, so we think that the current area of application is in a less regulated environment. These are early preclinical or exploratory studies. The experience gained here will show whether this can be extended later.

Conclusion

In this paper we showed the successful transfer of two ELISA-based generic assays to the ProteinSimple Ella platform. Hereby we demonstrated the applicability of Ella for PK and ADA analyses besides the quantification of biomarkers. We compared the performance of the Ella platform and ELISA in the measurement of different therapeutic antibodies in preclinical cynomolgus samples and we showed for the first time that ADA can be detected using an immune complex assay on the Ella platform. Both platforms demonstrated good performance and provided consistent results for drug quantification and immune complex assays. The advantages of the Ella platform over ELISA are low sample consumption, high dynamic range and high degree of automation, which allow for greatly simplified assay transfer.

Future perspective

After the successful transfer of two of our most frequently used ELISA assay formats, we plan to extend the use of the Ella platform to other assay formats and to use this platform in upcoming early preclinical studies. In addition to generic assays, compound-specific assays can also be transferred to the Ella platform, although this would require a case-by-case decision based on the expected sample number, reagent supply, required sensitivity and sample volume. Overall, we believe that the Ella platform has a great potential to simplify a wide range of bioanalytical applications and to facilitate assay transfers between laboratories.

Another major advantage of the Ella platform is its multiplexing capability. The two described assays can be combined with many other assays, including commercially available ProteinSimple biomarker assays. For example, a combination of an ADA assay with a PK assay and soluble target assays would enable a complete analysis of a single sample in a single experiment. This would provide advantages over four separate assays, including time efficiency and low sample consumption. In addition, all correlated values would refer to the same dilution of the same sample.

The development of this approach will certainly face challenges. The use of the same sample dilution in all assays may lead to a reduced sensitivity of some individual assays and the addition of free drug as required for the ADA assay may impact other assays on the same Ella cartridge. Despite these challenges, the combination of different assays on a single cartridge seems to be a very attractive approach worth of further exploration.

Executive summary

- A human therapeutic IgG quantification assay was successfully transferred from ELISA to the Ella[®] platform. The assay showed excellent precision and accuracy both as ELISA and on the Ella platform.
- The new Ella assay enabled a fully automated IgG quantification of two therapeutic antibodies in preclinical cynomolgus monkey samples with a good correlation with ELISA data.
- The standard curves in the Ella human IgG quantification test showed very small deviations between different days and cartridges. This may justify the use of a digitally stored master standard curve.
- An antidrug antibody (ADA) immune complex assay was successfully transferred to the Ella platform. ADA detection on the Ella platform was demonstrated for the first time.
- In 55 of 57 preclinical samples, qualitative ADA results were identical in ELISA and on the Ella platform.
- Both Ella assays were superior to the corresponding ELISA assays in terms of sample consumption, dynamic range and degree of automation, and slightly inferior regarding assay sensitivity.
- The novel use of Ella assays for the quantification of drugs and the detection of ADA in preclinical studies will simplify assay transfer to external laboratories.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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