Evaluation of Gyrolab™ and Meso-Scale Discovery® platforms in the development of a clinical immunogenicity assay

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Purpose

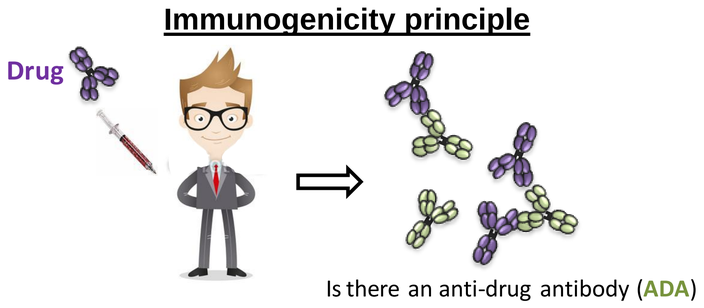
The development of an immunogenicity assay is a critical step in the safety assessment process for a new biotherapeutic drug. Key parameters which have to be considered include assay sensitivity, drug tolerance, and selectivity.

Drug tolerance, the binding of the free drug to the anti-drug antibodies (ADA), is evaluated in order to limit the risk of false-negative results. Selectivity, which evaluates the robustness of the assay in the presence of normal or disease matrix, is critical to ensure consistency when assessing different samples within a clinical study.

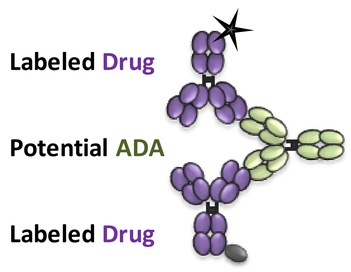
In order to obtain the most suitable assay, a homogenous bridging ADA assay was developed in parallel on both Gyrolab™ and Meso-Scale Discovery® (MSD®) platforms.

Immunogenicity principle and assay

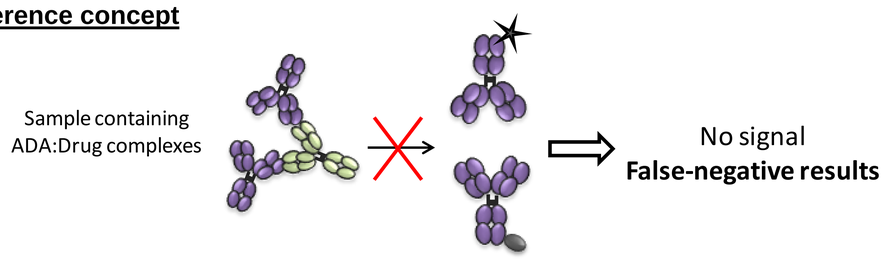
Immunogenicity principle



Bridging format assay

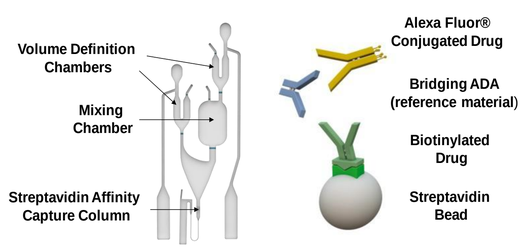


Drug interference concept



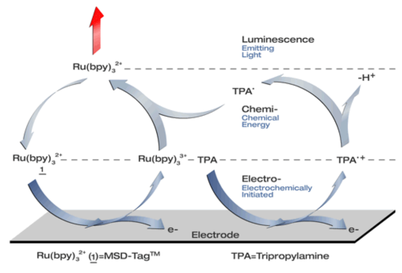
Technologies used

Gyrolab™



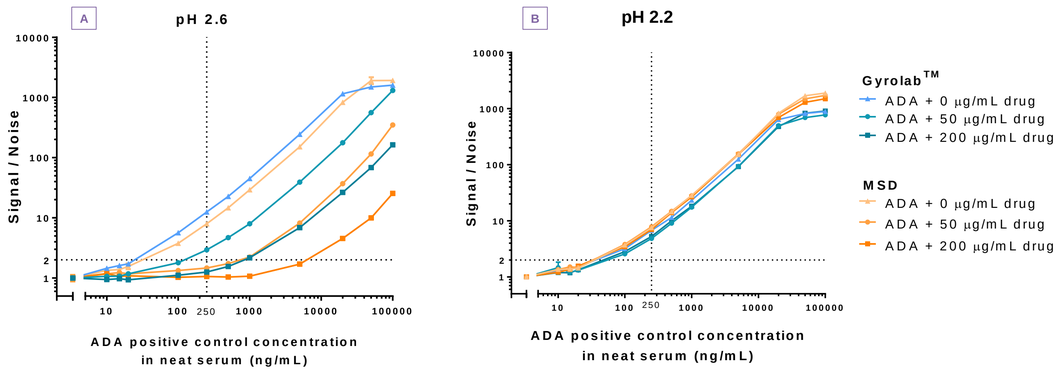
Gyrolab™ is a fully automated nano-liter scale platform. The system transfers samples and reagents from microplates to each microstructure within a Gyrolab™ CD. Results are obtained in less than an hour using high sensitivity, laser-induced fluorescence.

MSD®



MSD® is a plate reader based on electrochemiluminescence (ECL) detection technology using Sulfo-Tag labels. Multiple analytes can be measured in the same well.

Acid dissociation optimization and drug tolerance



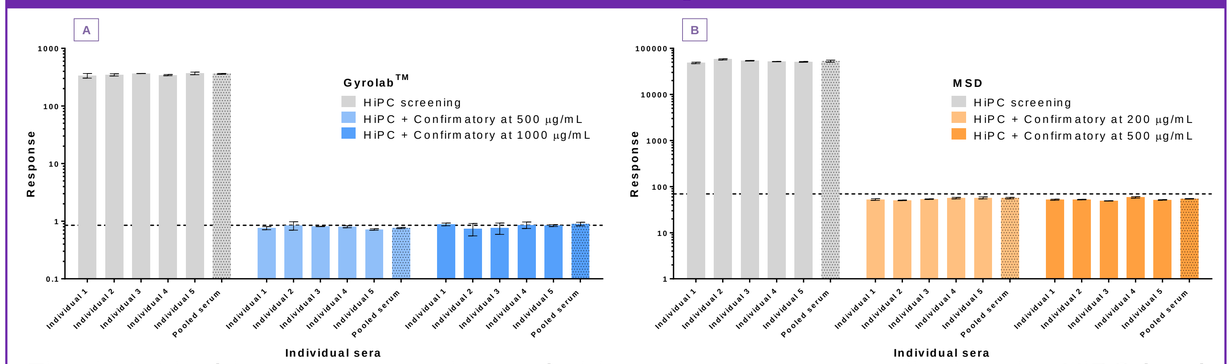
Concentration range of ADA positive control (rabbit polyclonal Ab) were prepared in blank matrix or in matrix containing 50 or 200 µg/mL of free drug. Acid treatment with 0.5M Glycine pH 2.6 (Figure A) or pH 2.2 (Figure B) were applied to these samples.

➡ **Loss of sensitivity with increasing free drug concentration.**

➡ **Free drug tolerance is pH dependent.**

The acid buffer 0.5M Glycine at pH 2.2 was selected.

Determination of confirmatory solution

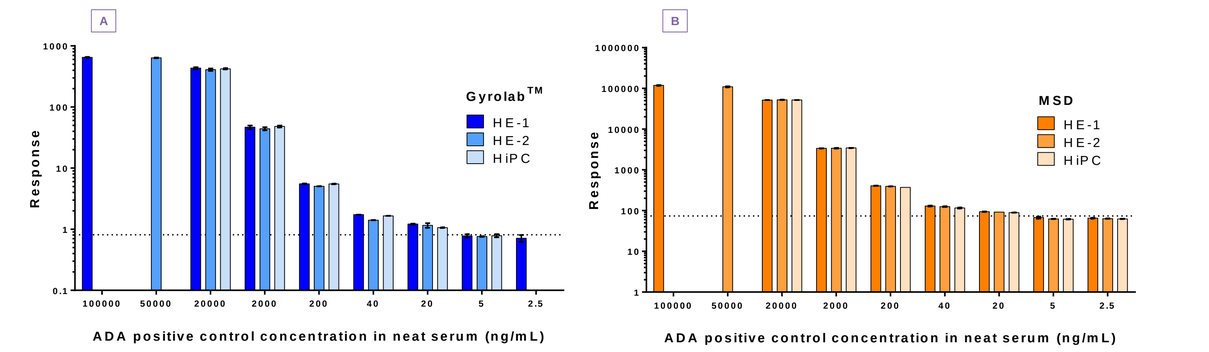


Five individual human sera and a pooled human serum were spiked at the HiPC level (20 000 ng/mL). Samples were screened and tested with a confirmatory solution containing free drug at 500 µg/mL or 1000 µg/mL for Gyrolab™ (Figure A); at 200 µg/mL or 500 µg/mL for MSD ® (Figure B).

➡ **Confirmatory solution reduced signal to below the cut-point.**

Confirmatory solutions were selected at 500 µg/mL for Gyrolab™ and at 200 µg/mL for MSD®, respectively.

Hook effect and titer assessment

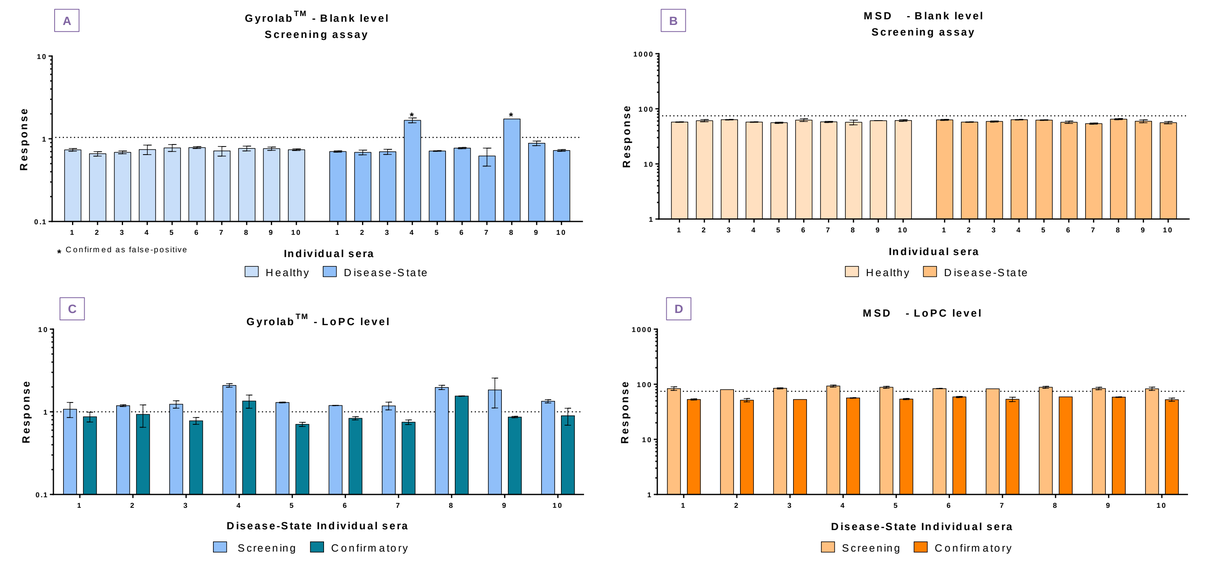


Two Hook Effect samples (HE-1 and HE-2) at a concentration of 100 000 ng/mL and 50 000 ng/mL, respectively, and the HiPC (20 000 ng/mL) were tested neat and diluted by serial dilution on Gyrolab™ (Figure A) and MSD ® (Figure B).

➡ **Dilution profile is linear.**

No hook effect observed.
All of the samples at 5 ng/mL were below the cut-point.

Evaluation of the impact of disease matrix



Ten healthy and ten disease-state individual human sera were tested on Gyrolab™ and MSD®, blank in screening assay (Figures A and B) and spiked at the LoPC level (15 ng/mL) in screening and confirmatory assays (Figures C and D).

➡ **More variability observed on Gyrolab™ platform.**

➡ **Similar results between both technologies except for two individual sera.**

The disease-state matrix had no impact on either assay.

Conclusion

- Both Gyrolab™ and MSD® platforms demonstrated excellent performance in assay parameters such as sensitivity, drug tolerance, titer and selectivity.
- Both assays were suitable for validation and clinical sample testing.
- Automation of the Gyrolab™ platform resulted in less sample handling procedures and a shorter assay time, but a limited number of samples per assay CD. In addition, some variability was observed for the low response level.
- The MSD® platform was more labour intensive, but showed lower variability and a higher throughput per assay plate.