Successful development of a key reagent and assay for detection of anti-drug antibodies for a therapeutic antibody

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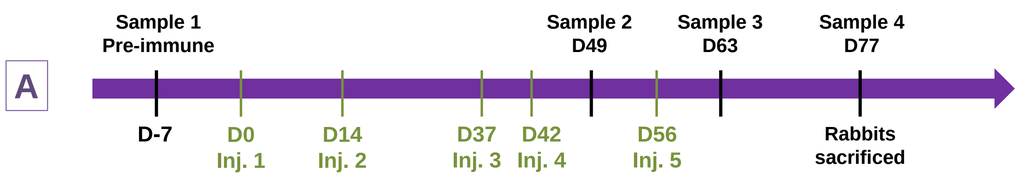
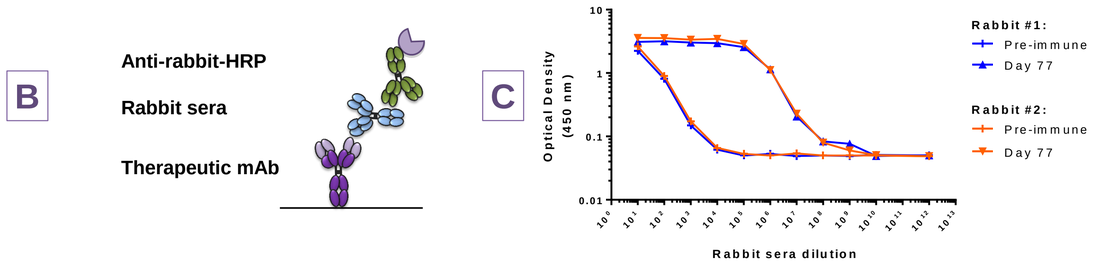
Introduction

During the development of a therapeutic monoclonal antibody (mAb), the level of the drug's immunogenicity must be evaluated. For this, a critical tool is a representative anti-drug antibody which is used as a positive control when setting up and running the immunogenicity assays. Ideally, the positive control should reflect the anticipated immune response that will occur in humans. In general, rabbit polyclonal antibodies (pAb), selected to target the variable part of the therapeutic mAb, are used.

A collaboration between AGRO-BIO (La Ferté Saint-Aubin, France) and NovImmune (Plan-les-Ouates, Switzerland) was established for the production of a positive control (rabbit polyclonal) for a therapeutic drug candidate, at NovImmune.

Immunization Design





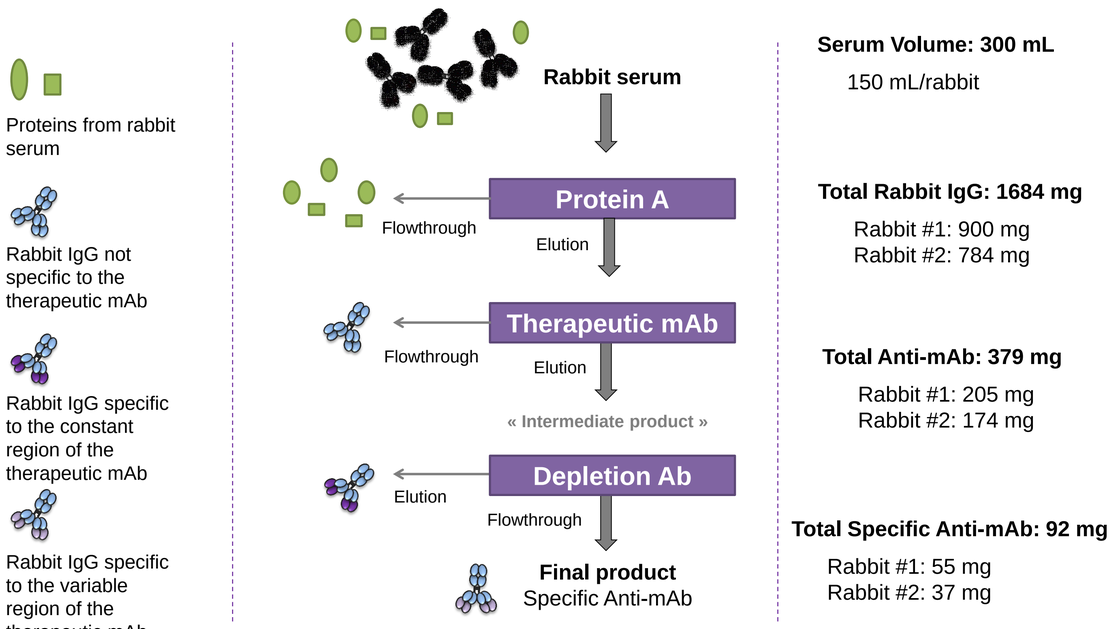
A. Two rabbits were immunized with 5 injections of the therapeutic mAb over a period of 77 days. Four samples per rabbit were collected during the immunization process.

B. The immune response against the therapeutic mAb was monitored using a sandwich ELISA.

C. A serum dilution of the Pre-immune and the Day 77 samples were performed for each rabbit.

At the end of immunization process, an appropriate immune response was observed for both rabbits

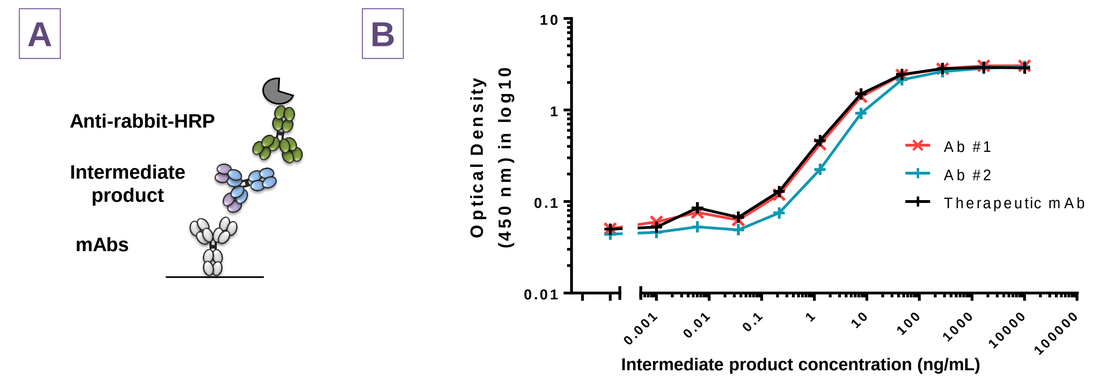
Purification Process



The purification process was composed of three steps: protein A, therapeutic mAb affinity purification and then a depletion step.

No difference was observed between rabbits and a good yield produced for both (33mg/ 100mL = 100mg/300mL)

Depletion Antibody Selection



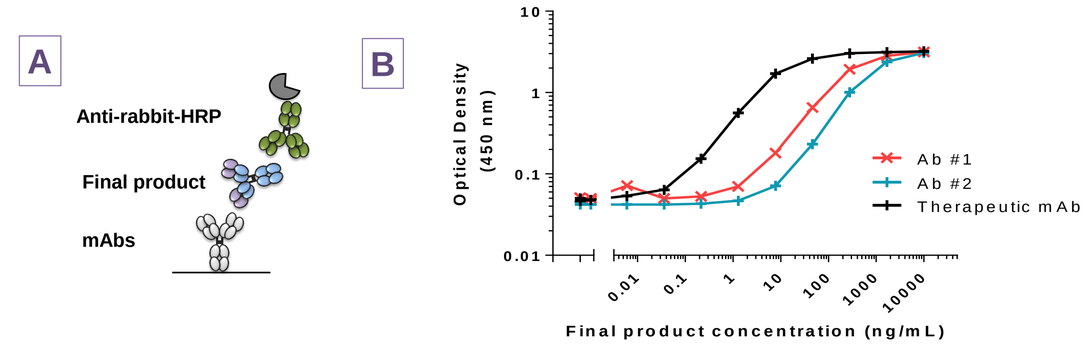
A. Assay format used to select the antibody used for the depletion step.

B. The intermediate product (after therapeutic mAb affinity purification) was tested against different mAbs (the therapeutic mAb and 2 irrelevant mAbs). These two mAbs were selected on their germline similarity to the therapeutic mAb: Ab#1 with the closest germline to the therapeutic mAb, and Ab#2, with the germline furthest from the therapeutic mAb.

In order to obtain a highly specific product, Ab#1 was selected as the tool to be used for the depletion step; with the known risk that the antibody yield may be compromised by this choice

Characterization of the Final Product

SPECIFICITY OF THE FINAL PRODUCT

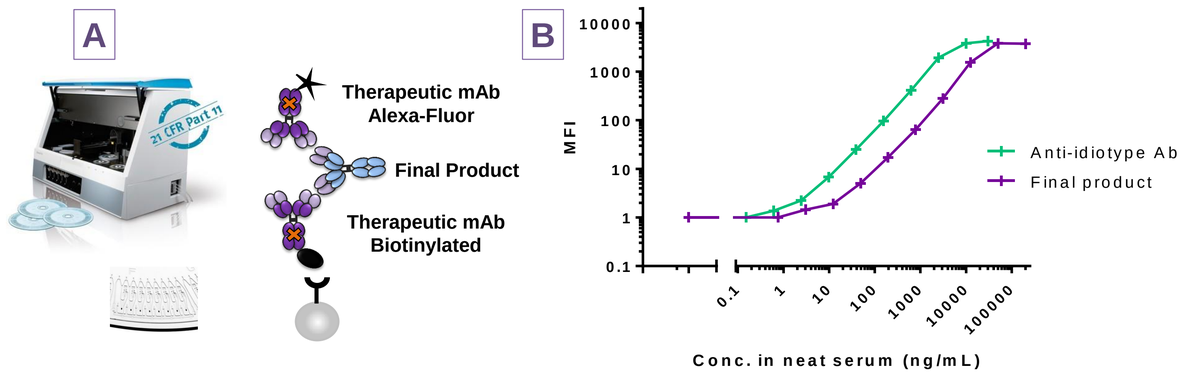


A. Assay format used to characterize the final product.

B. The final product (specific anti-mAbs) was tested against different mAbs (the therapeutic mAb and 2 irrelevant mAbs).

➡ **The final product exhibits an appropriate specificity for the therapeutic Ab after the purification process.**

FINAL PRODUCT PERFORMANCE IN ADA ASSAY



A. Bridging assay format used to evaluate the final product in the ADA assay.

B. The final product (specific anti-mAbs) were tested in the ADA assay, developed on the Gyrolab technology. The final product was compared to a mouse monoclonal anti-idiotype for evaluation of performance.

➡ **The final product exhibits a sensitivity around 50-100 ng/mL.**

The final product is specific for the therapeutic mAb and suitable for use in ADA assays

Conclusion

We have generated *de novo* 100 mg of a suitable positive control, within a period of 5 months, for a new therapeutic mAb program. Classical immunization of rabbits was carried out with the therapeutic mAb using 5 injections over 77 days. Rabbit sera was collected and sequentially purified on a protein A-coupled column and then a therapeutic mAb- coupled column. A key depletion step was next employed to obtain only the fraction of rabbit pAb specific to the variable region of the therapeutic mAb. With this reagent, an immunogenicity assay was successfully developed, exhibiting a sensitivity of 50-100 ng/mL, fulfilling current regulatory guidelines requiring a minimum assay sensitivity < 250 ng/mL. The assay is now fit for purpose and is currently being used for clinical sample testing.