Management of Immunoassay Reagent for Bioanalytical Methods, an example with isatuximab antibody reagent (PK and ADA assays)

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Introduction

The stability of critical reagents over a long period is essential for the reproducibility and accuracy of bioanalytical data. Indeed, the antibodies that are qualified as critical reagents serve as reference values for these methods and are therefore essential, Anti-idiotypic and anti-drug antibodies are mainly used as critical reagents for pharmacokinetic, immunogenicity (ADA control antibodies) and neutralizing ADA assays. They can either be monoclonal or polyclonal.

Once characterized and validated in function of their final application, these critical reagents enter in a phase of long term stability analysis to ensure their suitability through the clinical studies of the biotherapeutic compound. This stability analysis is more and more regulated by health authorities that notably draw attention to the fact that the critical reagents have a direct influence on the sensibility and accuracy of the results of the bioanalytical methods. For example, as part of isatuximab clinical development, the antibodies critical reagent of bioanalytical method for immunogenicity and pharmacokinetic analysis, was characterized and re-evaluated for Life Cycle Management.

Management of critical Reagent

The strategies for the management of critical reagents that is offered by Agro-bio have been particularly designed to follow the critical reagents over a long periods (> 10 years) and be aligned to the guidance of the health authorities.

The first step consist of thoroughly characterizing the reagents following predefined parameters. These parameters will be the main quality controls over the years. The second steps includes the determination of the acceptance of variability for each parameter. Thirdly, a detailed Certificate of Analysis (CoA), resuming the quality control and the retest date will be edited. Lastly the conditions of storage of the critical reagents (temperature and aliquoting) will be defined.

Below are two different examples of *scenarii* for the long term stability analysis of critical reagents. Scenario could be based either on :

- Planned uses PK, ADA, Neutralization ADA, IHC, Flow Cytometry, ELISA
- Purposes after approval : like need for a companion diagnostic, analytical kit, potency test, specific-HCP ELISA

Scenario 1

Selected quality control:
- ELISA titration
- Purity analysis by SDS Page
- Determination of the concentration

Edition of a CoA :
Including all the date regarding the critical reagent:
- Titration/Purity/concentration
- Conservation parameters
- Retest date Y+1

Securing :
Storage of sufficient aliquots of critical reagents to perform stability analysis regularly from client needs (Y+1/+2/+3/,,,),

Scenario 2

Selected quality control:
- Specificity control by WB
- Purity analysis by SDS Page
- Determination of the concentration
- Affinity measurement by BIACORE

Edition of a CoA :
Including all the date regarding the critical reagent:
- Specificity/Purity/Affinity /Concentration
- Conservation parameters
- Retest date Y+5

Securing :
Storage of sufficient aliquots of critical reagents to perform long-term stability analysis

Isatuximab case study anti-CD38 mAb

Isatuximab is an investigational Sanofi monoclonal antibody (mAb) targeting a specific epitope on the CD38 receptor. It is designed to trigger multiple, distinct mechanisms of action that are believed to directly promote programmed tumor cell death (apoptosis) and immunomodulatory activity. CD38 is highly and uniformly expressed on multiple myeloma cells and is a cell surface receptor target for antibody-based therapeutics in multiple myeloma and other malignancies.

In view of bioanalytical method development for immunogenicity and pharmacokinetic analysis of isatuximab, rabbit polyclonal antibodies have been generated by Agro-bio. These polyclonal antibodies have been selected as critical bioanalysis reagents and routinely used during the clinical studies of isatuximab. Agro-bio has implemented its strategy for the management of the critical reagent, comprising:

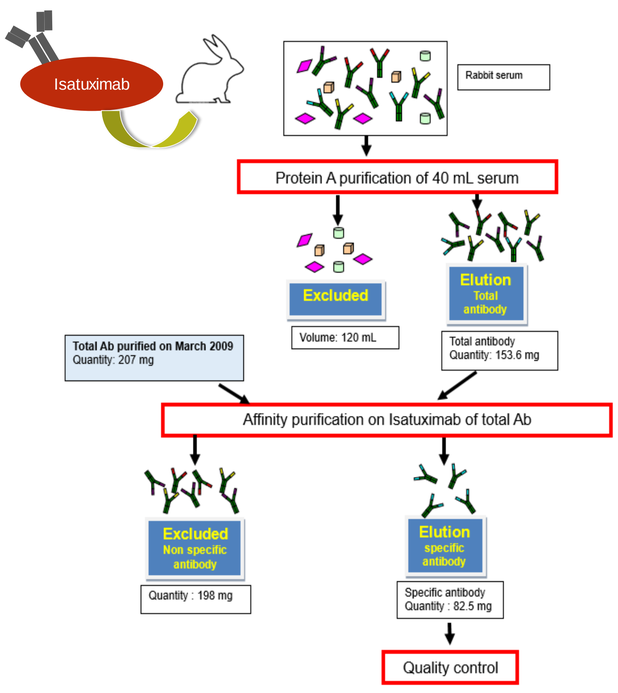
1/ delivery of sufficient quantities for the entire clinical development and reagents characterization (avoiding time-consuming bridging assays),

2/ stability analysis.

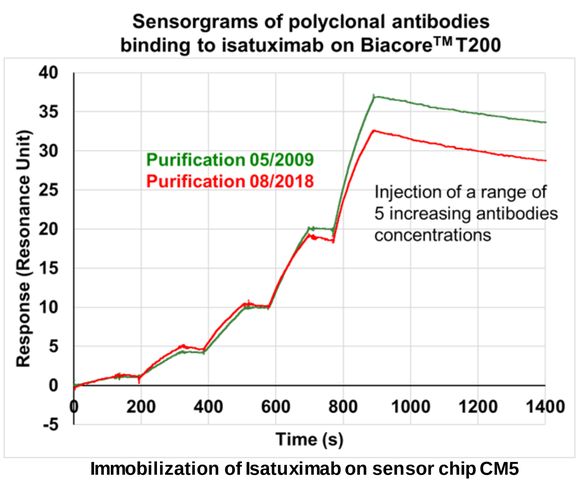
The following quality controls have been used as reference methods to validate the stability of the polyclonal antibodies:
- Purity analysis by electrophoresis SDS-PAGE
- Affinity measurement by BIACORE™ T200

The critical reagents have been generated, validated and characterized in 2009. The last re-test date was performed in 2018 to validate the stability **over a 10-year period.** The latest technologies in production in a comparative manner showed successful stability evaluation.

Antibody Protocol Design



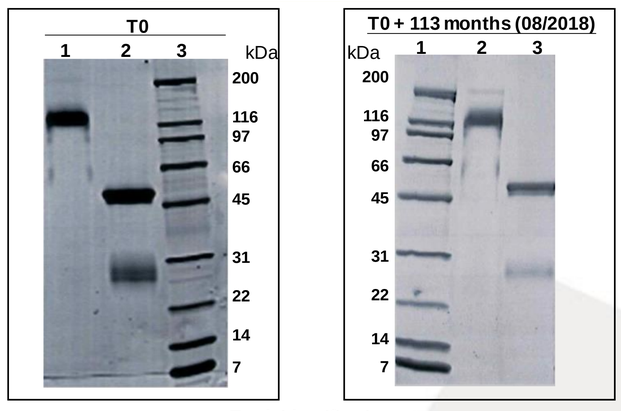
Affinity



	Rabbit serum containing anti-isatuximab pAb	
Immunisation	03/2009	
Process Purification	**05/2009**	**08/2018**
Storage	Below -25°C since purification	- na -
QC analysis	08/2018	08/2018
*K_D determination**	**5.70 nM**	**5.81 nM**
Electrophoresis	**Purity > 99%**	**Purity > 99%**
Bioanalytical use	**Identical Bioanalytical Performances (e.g. LLOQ)**	

* Affinity values are apparent due to polyclonal antibodies.

SDS-PAGE Data



Track identification

Track1: native conditions
Track 2: reduced conditions
Track 3: Standard

Track1: Standard
Track 2: native conditions
Track 3: reduced conditions

Conclusion

The management of critical reagents of bioanalytical methods is of primary importance for the accuracy of the bioanalytical method. The characterization and stability analysis of these reagents is recommended by the health authorities and will ensure the validity of the results obtained during the various clinical phases. The methods described in this poster allow the sponsor to ensure the stability and relevance of the critical reagents.

The implementation of the long-term management of these critical reagents must be adapted to the specific constraints of the sponsor and be aligned to the guidance of the health authorities. The chosen quality controls should be selected so that potential modifications of the critical reagents are monitored and therefore the relevance of their use. Particular attention should be placed on their selection in order to not generate any irrelevant additional data.