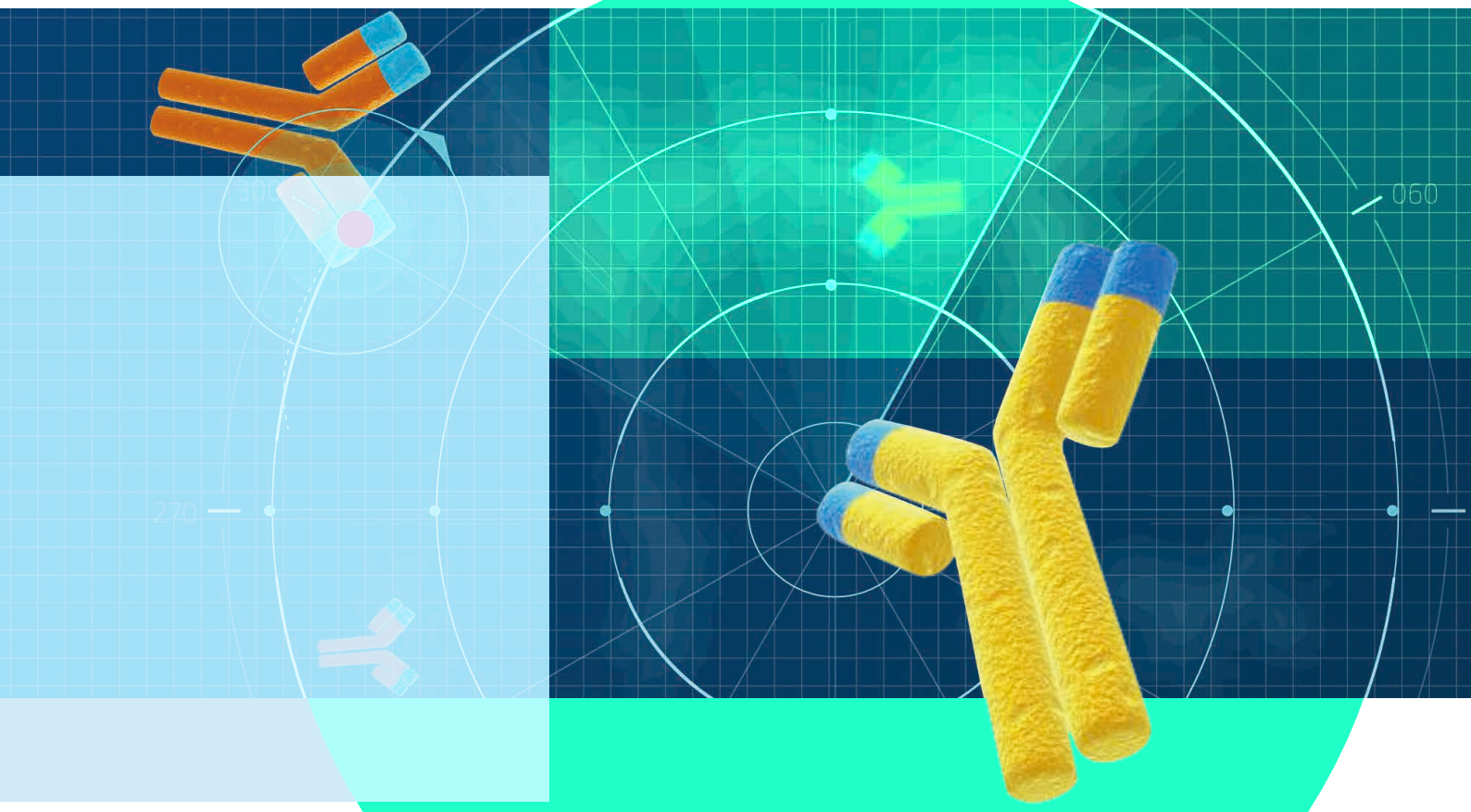
GenScript PROBIO

ANTI-IDIOTYPE ANTIBODY DEVELOPMENT AND APPLICATION



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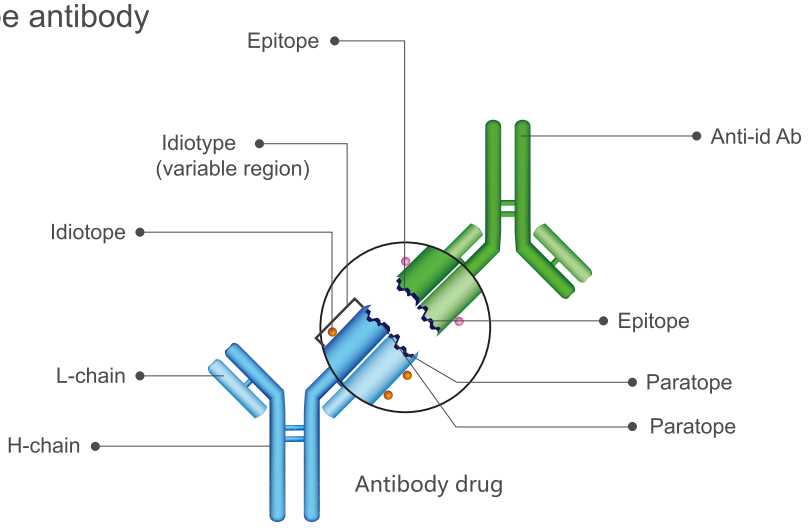
ANTI-IDIOTYPE ANTIBODY DEVELOPMENT AND APPLICATION

BACKGROUND

A What is anti-idiotype antibody

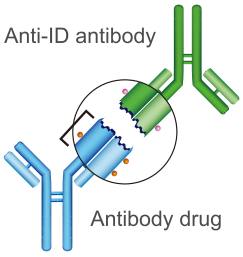


Anti-ID antibody schematic diagram

An anti-idiotype antibody(Anti-ID Ab) binds to the idiotype of another antibody, usually an antibody drug. An idiotype can be defined as the specific combination of idiotopes present within an antibody's complement determining regions. Since anti-ID Abs are capable of binding to antibody drugs within biological fluids, they are commonly used in preclinical setting for antibody drug pharmacokinetics (PK) and pharmacodynamics (PD). Due to the similarity between anti-ID Abs and anti-drug antibodies, anti-ID Abs are also commonly used as reference standard for antibody drug immunogenicity, anti-drug antibody (ADA) studies.

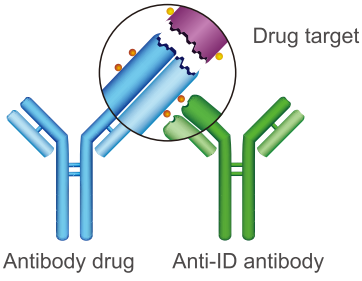
B The types of Anti-ID Ab

Antigen-blocking



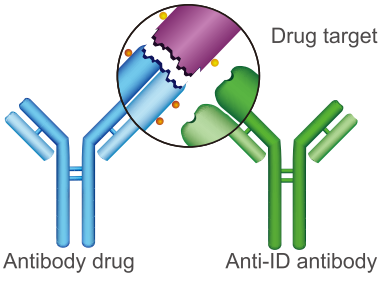
- Paratope-specific
- inhibitory
- Neutralizing
- Detects free drug

Non-blocking



- Not paratope-specific
- Not inhibitory
- Detects total drug(free, partially bound, fully bound)

Complex-specific



- Drug-target complex
- Not inhibitory
- Detects bound drug only

Due to the fact that Complex-specific antibody is usually difficult to develop, the first two types (Antigen-blocking & Non-blocking) are usually applied, and the content of bound drug can be obtained by calculation.

C The application of anti-ID Ab in biologics development

Specifically binding to the idiotype of antibody drug, Anti-ID Ab is an indispensable tool in pharmacokinetic study for detecting and quantitating Ab drug in human and animal serum. Due to the good homogeneity and specificity of anti-ID monoclonal antibody, It is recommended for usage in the preclinical stage, pharmaceutical research and clinical stage. And for Anti-ID polyclonal antibody or antigen capture ELISA could be used in drug discovery stage. In terms of detection sensitivity, mouse anti-id mAb can be developed for ng-level detection. If the sensitivity is expected to reach the pg level, the development of rabbit anti-ID mAb is also the option.

Due to the similarity between anti-ID and anti-drug antibody, it can also be used as a positive control of anti-drug antibody in immunogenicity study for the determination of total anti-drug antibody. Specifically, anti-ID polyclonal antibodies is recommended as the positive control of anti-drug antibody due to its high coverage property, which could simulate anti-drug antibody to the greatest extent. For the detection of neutralizing anti-drug antibody, anti-ID mAb
which could block the binding of drug to target shall be developed as control.

Pharmacokinetic and immunogenicity analysis cover the whole process of biologics development. As an essential tool antibody, anti-ID is not only one of the critical reagents for IND filing, but also widely used in the clinical stage.

D Timing for the developing anti-ID antibodies

In accordance with NMPA, samples from pilot production could be used for pharmacokinetic and immunogenicity analysis. Considering that the development time of anti-ID mAb for pharmacokinetic analysis is around half a year, the development time of anti-ID pAb for immunogenicity analysis is about 2-3 months and the ELISA format takes about 2 months to establish, anti-ID Ab development is suggested to start at the very beginning of CMC.

E Compliance Guidance for Anti-idiotype Antibodies in IND filing and clinical use

For ligand binding assay (LBA), the critical reagents of the method (such as binding proteins, aptamers, antibodies or coupling antibodies, enzymes, etc.) have a direct impact on the analytical results and therefore must be quality assured. If the batch of critical reagents is changed during method validation or sample analysis, it must be confirmed that the performance of the method does not change as a result, thus ensuring the consistency of results from batch to batch.

PK and ADA assays are important efficacy and safety assays in IND filing and clinical trials. Anti-idiotype antibodies are the main raw material for PK and ADA assays. Therefore, as a critical reagent, the quality of anti-idiotype antibodies can have a significant impact on the efficacy and accuracy of subsequent PK/ADA assays. Especially in long-term clinical trials, it is critical to ensure the consistency of the kits from batch to batch. Therefore, a strict critical reagent management strategy is needed to ensure the batch-to-batch consistency of the assay kits and thus the reliability of the results.

Compliance Guidance

Data management:

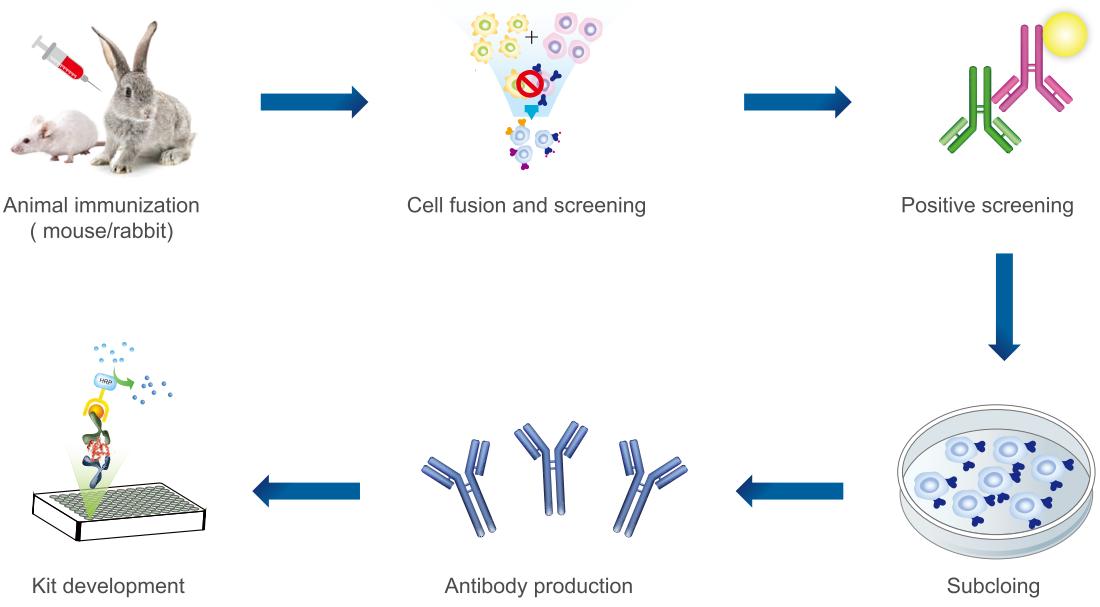
- Well-established operating procedures and management systems to regulate the recording and review requirements of data generated during the production process.
- Regular calibration and verification of data-generating measuring instruments to ensure data reliability.
- Original data are backed up in accordance with data management requirements.

Records management:

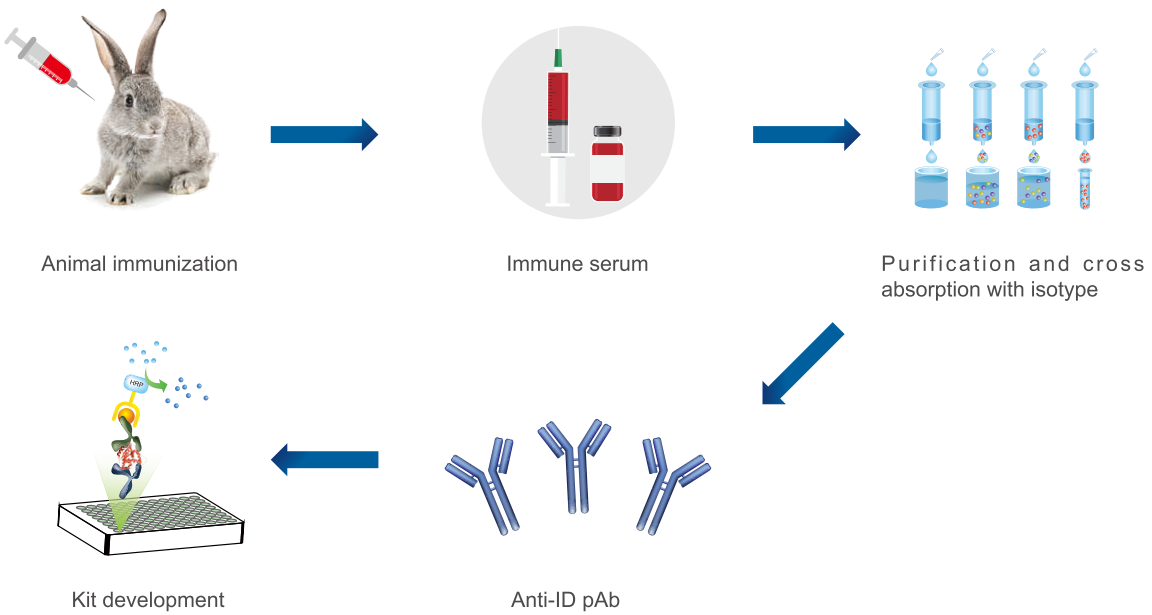
- Complete paper-based operating procedure documents and experimental record forms.
- A complete quality management system for documents and records, including processes for approval and review of documents and records, printing and issuance, record requirements, filing and preservation, copying and destruction, etc.
- Complete electronic record management system, including regular inspection of computerized system, time and time zone management, backup of system data, operation authority and user authority management, etc.

WORKFLOW OF ANTI-ID ANTIBODY DEVELOPMENT

A Anti-ID mAb Development Workflow



B Anti-ID pAb Development Workflow



DEVELOPMENT STRATEGIES

A Strategies against different format of antibody drugs

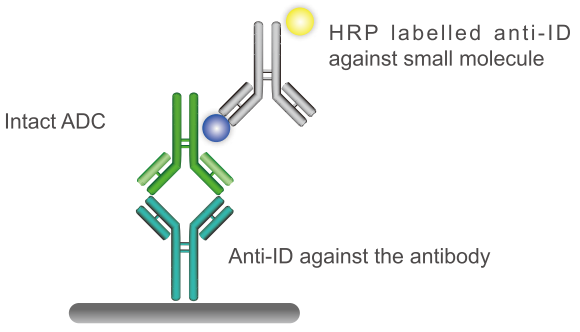
	Anti-ID mAb	Anti-ID pAb
IgG format	• Targeting variable region	• Targeting variable region
sdAb/ScFv	• Targeting full length (variable region)	• Targeting full length (variable region)
ADC	• Targeting small molecule • Targeting variable region of IgG	• Targeting the whole ADC molecule
Bi-specific antibody	• Targeting both variable region of bispecific antibody • Targeting either variable region of each mono-specific antibody	• Target both variable region of bispecific antibodies

B PK and ADA detection methods of traditional monoclonal antibody and single domain antibody drugs

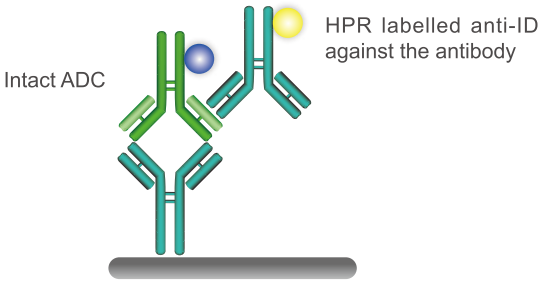
App.	Analyte	Assay Format
PK	Full Length Antibody	• ELISA (ligand binding assay, LBA) • Anti-ID against antibody drug
ADA	Full Length Antibody	• ELISA (ligand binding assay, LBA) • Anti-ID pAb against monoclonal antibody

C Anti-ID Development Strategy of ADC

Schematic diagram of PK assay



To detect intact ADC



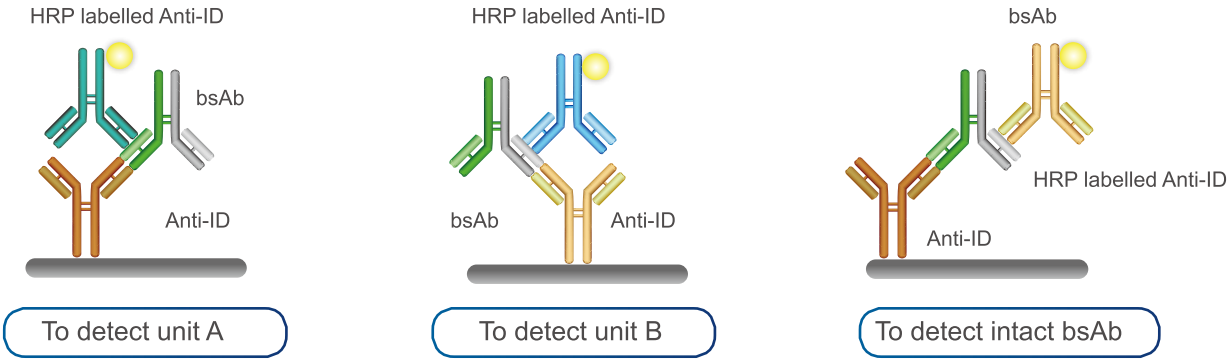
To detect antibody

PK and ADA assay format

Application	Target molecule	Assay format and reagent needed
PK	Intact ADC	• ELISA (ligand binding assay, LBA) • Paired Anti-ID mAb against the small molecule and antibody, respectively
	Antibody	• ELISA (ligand binding assay, LBA) • Anti-ID mAb against antibody
	Free small molecule	• LC-MS
ADA	Whole ADC	• ELISA (ligand binding assay, LBA) • Anti-ID pAb against the whole ADC

D Anti-ID Development Strategy for Bi-specific antibody(bsAb)

Schematic diagram of PK assay



PK & ADA Assay format

Application	Target molecule	Assay format and reagent needed
PK	Intact bsAb	• ELISA (ligand binding assay, LBA) • Paired Anti-ID mAbs against each of the mono-specific unit
	Mono-specific unit A	• ELISA (ligand binding assay, LBA) • Anti-ID against only unit A
	Mono-specific unit B	• ELISA (ligand binding assay, LBA) • Anti-ID against only unit B
ADA	Whole bi-specific antibody	• ELISA (ligand binding assay, LBA) • Anti-ID against the intact bsAb

APPLICATION STRATEGY

A Pharmacokinetic(PK) study

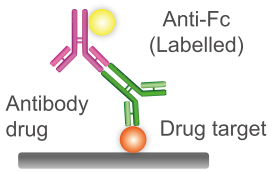
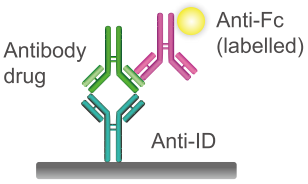
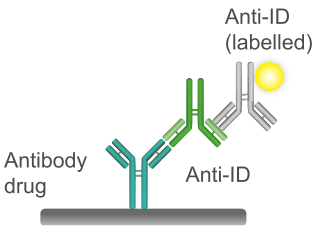
a.1 Detection of antibody drugs against membrane targets

For antibody drugs against the membrane targets, there is only one type of antibody drug in the serum, which is free antibody drug, with no special need to develop specific types of anti-ID antibodies. Therefore, both blocking and non-blocking anti-ID antibody can be used to detect the antibody drug.

a.2 Detection of antibody drugs against soluble targets

For antibody drug against soluble targets, there are free drug and bound drugs in the serum. Therefore, It is usually necessary to develop non-blocking anti-id antibody to detect total drug and blocking anti-id antibody to detect free drug respectively.

B PK Assay Format

	Antigen capture ELISA	Anti-ID capture ELISA	Sandwich ELISA
Format			
Coating	• Antigen	• Anti-ID	• Anti-ID
Analyte	• Antibody drug	• Antibody drug	• Antibody drug
Detect antibody	• Anti-human Fc antibody	• Anti-human Fc antibody	• Anti-ID
Advantages	• No need to develop Anti-ID • Simple format,saving time	• Only need one anti-ID • Saving cost	• Strong specificity • High sensitivity • Applicable to all antibody drug
Disadvantages	• Poor stability	• General Specificity, susceptible to matrix effect. • Only suitable for antibody with Fc region	• Great developing difficulties

Above are the classic PK assay methods. Due to the change of antigen's orientation after coating and stability issue, the accuracy and stability of antigen-capture ELISA are relatively low. As a result, it is only recommended for early stage pilot study. On the other hands, Anti-ID capture ELISA and sandwich ELISA are the most popular assay formats in clinical and preclinical PK analysis, which could be chosen based on the drug types and results of anti-ID development. In general, Sandwich ELISA is superior in specificity and sensitivity, which could be used in detection of all biologics.

C Pharmacokinetic Assay Kit Development

The kit can be developed once the type of pharmacokinetic analysis method has been selected.

First, a pre-developmental feasibility study is required, including antibody labeling, antibody pairing and confirmation, initial establishment of standard curves, matrix validation and preliminary sensitivity experiments. For sandwich ELISA, it is recommended to screen multiple antibody pairs in the Anti-ID antibody discovery and select the antibody pair with the best pairing result for methodological development to ensure the success of kit development. Matrix validation and sensitivity should be customized by setting up different criteria based on the characteristics of different samples to be tested.

Next, the reaction conditions and parameters of the kit need to be optimized and the standard curve need to be established.

After the development is completed, the performance of the kit need to be validated referring to the *Bioanalytical Method Validation Guidance for Industry*. The standard curve consistency, sensitivity and minimum detection limit, inter-batch difference, intra-batch difference, spiked recovery (80%-120%), stability, etc. will be validated. According to the development experience of GeneScript ProBio, the inter-batch and intra-batch variation should be controlled within 15% and 10% respectively.

Finally, the kits will be produced upon clients' request.

For the developed kits, the demander needs to validate the kits in a suitable experimental environment according to the relevant guidelines mentioned above, and clinical samples can be tested only after the validation is passed.

Summary of References

Bioanalytical Method Validation Guidance for Industry

D Immunogenicity analysis

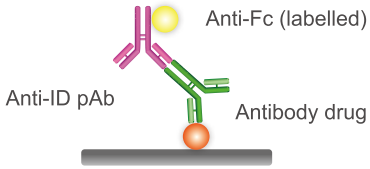
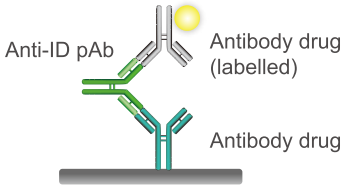
d.1 Anti-drug antibody detection

In immunogenicity analysis, it is required to detect and quantitate total anti-drug antibody. Because of the similarity between anti-ID antibody and anti-drug antibody, anti-ID pAb is often used as positive control.

d.2 Neutralizing anti-ID antibody analysis

Neutralizing antibodies can block the binding of the antibody drug to target and neutralize the activity of the antibody drug. In preclinical and clinical immunogenicity analysis, it is often required to analyze neutralizing antibodies produced in animals and human to assess their effect on pharmacodynamics. Neutralizing anti-id antibody that can block the binding between antibody drug and drug target in the competitive environment can be developed as a positive control, and neutralization antibody can be quantitatively analyzed by combining with the pharmacodynamics model.

E ADA Assay Format

	Capture ELISA	Bridging ELISA
Format		
Coating	• Antibody drug	• Antibody drug
Analyte	• Anti-ID	• Anti-ID
Detect antibody	• Anti-Fc antibody	• Antibody drug
Advantages	• Easy to develop	• High accuracy
Disadvantages	• Need to replace the detect antibody, accuracy is general • Susceptible to the matrix effect	• Narrow linear range • Development difficulty

Two ELISA formats are used to detect anti-drug antibody: capture ELISA and bridging ELISA. For capture ELISA, detection antibody type needs to be changed according to the Fc type, which will have a certain impact on the accuracy of the data. Generally, bridging ELISA is more accurate, but is more difficult to develop.

F Immunogenicity Assay Kit Development

The development of the kit can start once the type of immunogenicity assay has been selected.

Like the process of the pharmacokinetic assay kit development, immunogenicity assay kit development includes pre-developmental feasibility study (antibody labeling, antibody pairing and confirmation, initial establishment of standard curves, matrix validation and preliminary sensitivity experiments), optimization of kit reaction conditions and parameters, determination of standard curve and performance validation of the kit, and finally kit production.

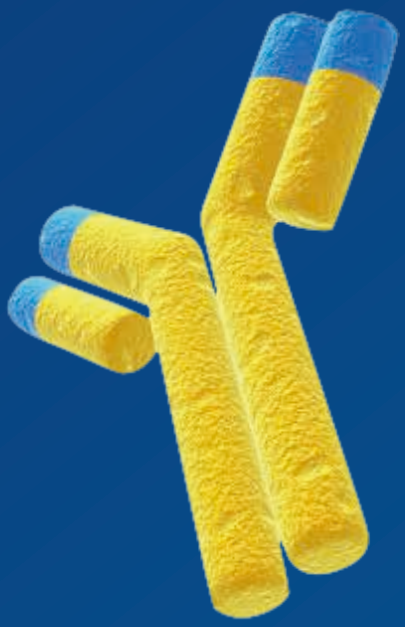
Similarly, different standards should be set for matrix verification and sensitivity based on the characteristics of different samples to be tested, and the design should be customized. Also, the inter-batch and intra-batch variation should be controlled within 15% and 10% respectively according to GenScript ProBio's developmental experience.

The reference regulations and guidelines for the developmental process are summarized below.

For developed kits, the demander needs to validate the kits in a suitable experimental environment according to the relevant guidelines mentioned below, and clinical samples can be tested only after the validation is passed.

Summary of References

Immunogenicity Testing of Therapeutic Protein Products-Developing and Validating Assays for Anti-Drug Antibody Detection



Anti-Idiotpe Antibody

Development

Application

860 Centennial Ave.
Piscataway, NJ 08854 USA
Tel: +1-732-885-9188
Toll-Free: +1-877-436-7274
Fax: +1-732-210-0262
Email: cdmo@genscript.com