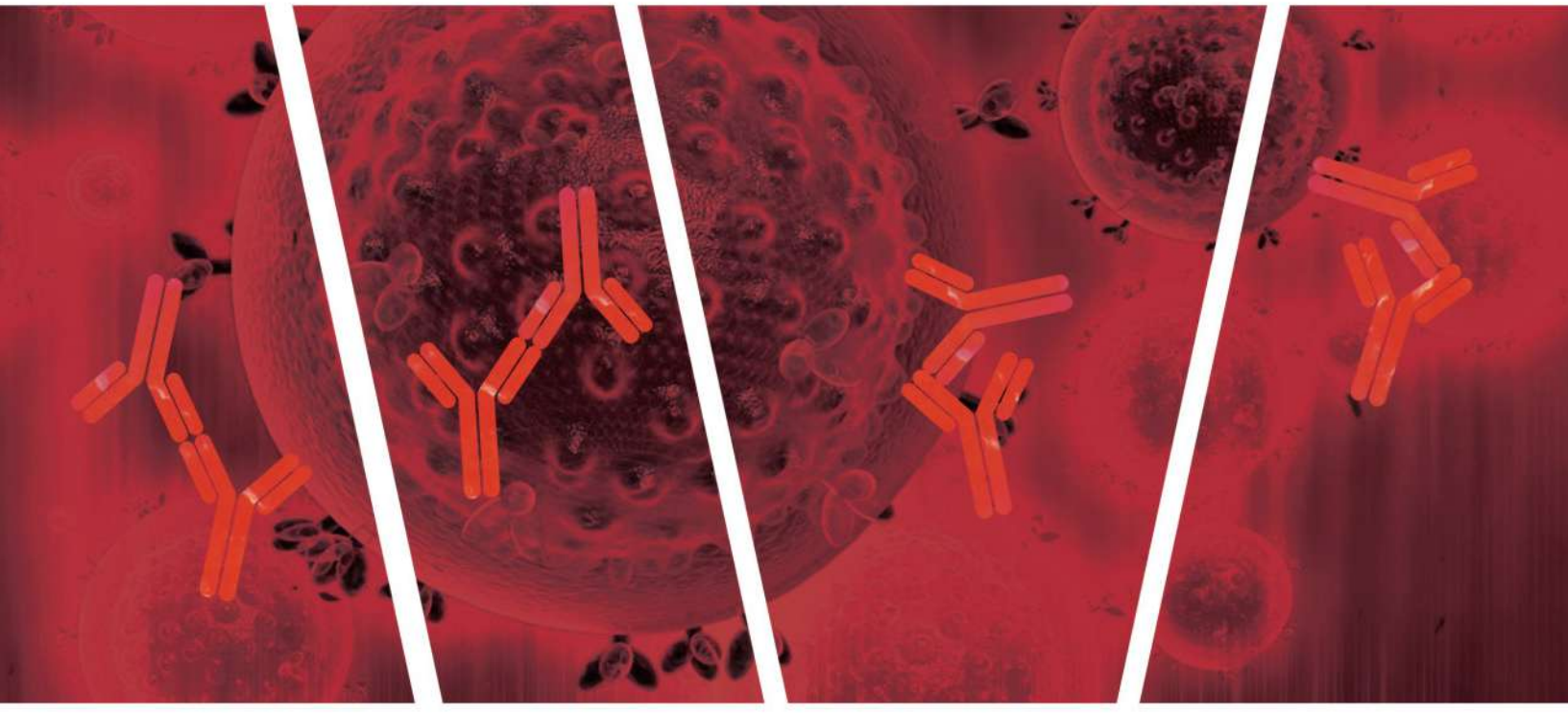
Anti-idiotypic Antibodies

Supporting Immunogenicity and Pharmacokinetics Analysis

Method Validated and Protocol Offered



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I.Background

An idiotope is the unique set of antigenic determinants (epitopes) of the variable portion of an antibody. An anti-idiotypic (Anti-ID) antibody binds to the idiotope of another antibody, usually an antibody drug, which makes it a very powerful tool for antibody drug development, especially for immunogenicity and PK/PD analysis.

To support preclinical/clinical immunogenicity and PK analysis, ACROBiosystems has developed a series of high-affinity anti-idiotypic antibodies. Our pipeline covers five hot targets including adalimumab, rituximab, cetuximab, trastuzumab, and bevacizumab. To help the drug development process, we provide assay protocols which can be applied to different application scenarios.

II. Product List

Cat. No.	Antigen	Neutralizing Activity	Affinity K_D, nM	Application
ADB-Y19	Adalimumab F(ab')$_2$	Neutralizing Antibody	0.0013	ADA assay Neutralizing assay Indirect ELISA
CEB-Y28	Cetuximab F(ab')$_2$	Neutralizing Antibody	0.0015	ADA assay Neutralizing assay Indirect ELISA
CEB-Y31	Cetuximab F(ab')$_2$	Non-Neutralizing Antibody	0.421	ADA assay Indirect ELISA
RIB-Y35	Rituximab F(ab')$_2$	Neutralizing Antibody	0.03	ADA assay Neutralizing assay Indirect ELISA
BEB-Y12	Bevacizumab F(ab')$_2$	Neutralizing Antibody	0.0828	Neutralizing assay Indirect ELISA
BEB-Y9	Bevacizumab F(ab')$_2$	Neutralizing Antibody	1.92	ADA assay Indirect ELISA

III.Application

ADA Assay - Perfect as Calibrator

Therapeutic proteins such as monoclonal antibodies are currently essential in the treatment of cancer, autoimmune disease, and other diseases. Since protein has its intrinsic feature of immunogenicity owing to its structure containing potential B-cell and T-cell epitopes, therapeutic proteins have the potential to induce anti-drug antibodies (ADA) even if the protein has the same amino acid sequence as endogenous human proteins. The emergence of ADA in patients can potentially lead to loss of efficacy and/or adverse events. Therefore, immunogenicity risk assessment and risk-mitigating strategies are required during the development of therapeutic protein products.

Developing a mono/multi-clonal antibody in-house as a positive control for ADA assay is extremely time-consuming. To solve this problem, ACROBiosystems developed a series of anti-drug antibody standards for ADA assays.

Case 1 Anti-Adalimumab Antibodies (ADB-Y19)

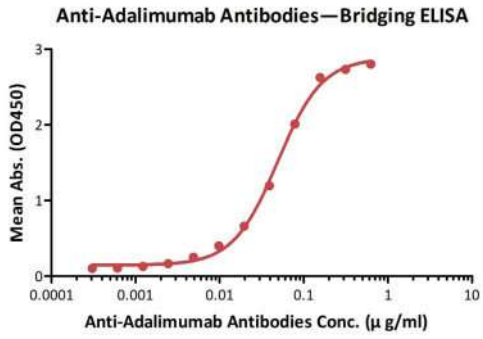


Figure 1. Anti-Adalimumab Antibodies bridging ELISA for Anti-Drug Antibody (ADA) assay development. *Immobilized adalimumab at 1 μg/ml, added increasing concentrations of Anti-Adalimumab Antibodies (Cat. No. ADB-Y19, 10% human serum) and then added biotinylated adalimumab at 5 μg/ml. Detection was performed using HRP-conjugated streptavidin with a sensitivity of 0.6 ng/mL.*

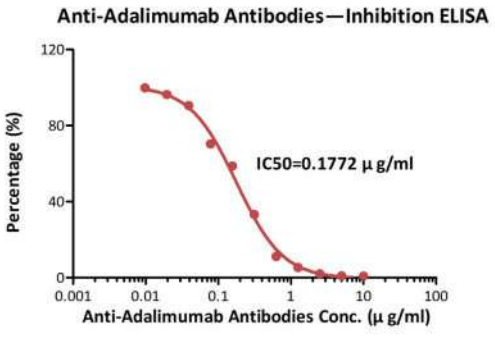


Figure 2. Measured by its neutralizing ability in a functional ELISA. *Immobilized adalimumab at 0.5 μg/mL (100 μL/well) can bind pre-mixed Anti-Adalimumab Antibodies (Cat. No. ADB-Y19) and Biotinylated Human TNF-alpha (Cat. No. TNA-H82E3) with an inhibition rate of 100%.*

Case 2 Anti-Rituximab Antibodies (RIB-Y35)

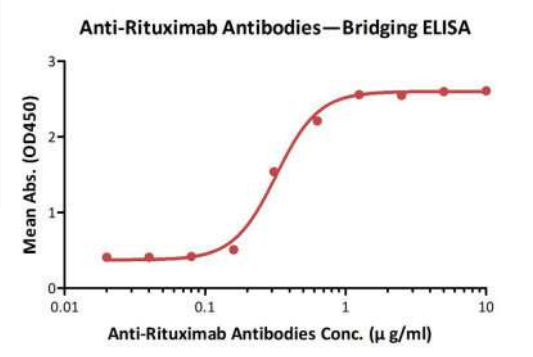


Figure 3. Anti-Rituximab Antibodies bridging ELISA for Anti-Drug Antibody (ADA) assay development. *Immobilized rituximab at 5 μg/ml, added increasing concentrations of Anti-Rituximab Antibodies (Cat. No. RIB-Y35, 10% human serum) and then added biotinylated rituximab at 5 μg/ml. Detection was performed using HRP-conjugated streptavidin with a sensitivity of 20 ng/mL.*

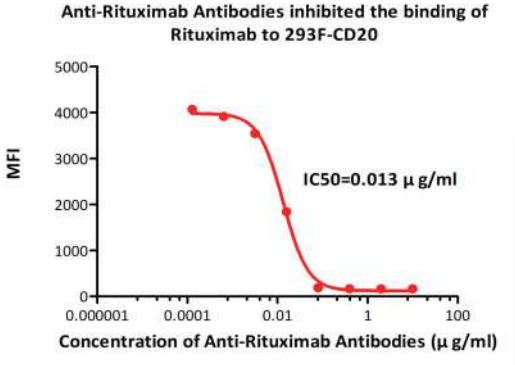


Figure 4. Measured by its neutralizing ability in FACS. *The data shows that the binding of rituximab to 293F overexpressing CD20 was inhibited by increasing concentrations of Anti-Rituximab Antibodies (Cat. No. RIB-Y35). The concentration of rituximab used is 10 ng/ml. The IC50 is 0.013 μg/ml.*

PK Assay - Quantitative Analysis of Therapeutic Antibody in Matrix

ELISA is the most commonly used method for quantitative analysis of therapeutic antibody in matrix, and we compare the three most representative ones in the following table:

Method	Coating	Sample	Detection
Antigen capture ELISA	CD20	Sample	Goat anti-human IgG
Anti-idiotypic capture ELISA	Anti-Rituximab Antibodies	Sample	Goat anti-human IgG
Bridging ELISA by anti-idiotypic antibodies	Anti-Rituximab Antibodies	Sample	Biotinylated Anti-Rituximab Antibodies

>>> Antigen Capture ELISA

Instrument:
BMG CLARIOstar microplate reader
Sample:
Rituximab Serum Sample
Main Reagents:
Human CD20 Protein
Peroxidase AffiniPure Goat Anti-Human IgG, Fcγ Fragment Specific (Jackson, Cat.No. 109-035-098)
Procedures:
1. Coat 0.5 μg/well human CD20 to the 96-well plate;
2. Serial dilute rituximab to human serum. Make sure the final serum concentration is 0.1%;
3. Add diluted rituximab sample from step two to the 96-well plate;
4. Add Peroxidase AffiniPure Goat Anti-Human IgG as secondary antibody;
5. Add TMB substrate solution.
Results:
Serum samples are diluted for 1000-fold to avoid non-specific background. The assay is not able to detect the level of rituximab in serum because of low affinity of CD20 and rituximab (Figure 5).

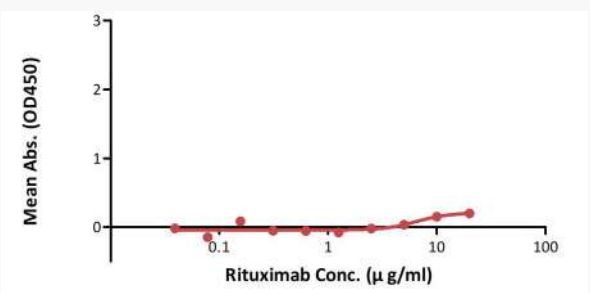


Figure 5. *Detection of rituximab by antigen-capture ELISA (0.1% serum).*

>>> Anti-idiotypic Capture ELISA

Instrument:
BMG CLARIOstar microplate reader
Sample:
Rituximab Serum Sample
Main Reagents:
Anti-Rituximab Antibodies (ACROBiosystems, Cat. No. RIB-Y35)
Peroxidase AffiniPure Goat Anti-Human IgG, Fcγ Fragment Specific (Jackson, Cat.No. 109-035-098)
Procedures:
1. Coat 0.05 μg/well Anti-Rituximab Antibodies （ACROBiosystems, Cat. No. RIB-Y35） to the 96-well plate;
2. Serial dilute rituximab to human serum. Make sure the final serum concentration is 0.1%;
3. Add diluted rituximab to the 96-well plate;
4. Add Peroxidase AffiniPure Goat Anti-Human IgG to the plate as secondary antibody;
5. Add TMB substrate solution.
Results:
Linear detection range is 0.156-10 μg/ml and the detection limit is 0.156 μg/ml(Figure 6).

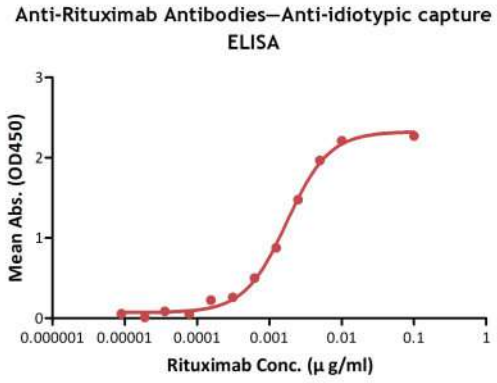


Figure 6. *Detection of rituximab by anti-idiotypic capture ELISA (0.1% serum).*

>>> Bridging ELISA by Anti-idiotypic Antibodies

Instrument:
BMG CLARIOstar Plate Reader
Sample:
Rituximab Serum Sample
Main Reagents:
Anti-Rituximab Antibodies (ACROBiosystems, Cat. No. RIB-Y35)
Biotinylated Anti-Rituximab Antibodies
Streptavidin Protein, HRP (Thermo, Cat.No. 21126)
Procedures:
1. Coat 0.2 μg/well Anti-Rituximab Antibodies (ACROBiosystems, Cat. No. RIB-Y35) to the 96-well plate;
2. Serial dilute rituximab to human serum. Make sure the final serum concentration is 20%;
3. Add diluted rituximab to the plate;
4. Add Biotinylated Anti-Rituximab Antibodies;
5. Add Streptavidin Protein, HRP as secondary antibody;
6. Add TMB substrate solution.
Results:
Linear detection range is 0.195-1.56 μg/ml and the detection limit is 0.195 μg/ml(Figure 7).

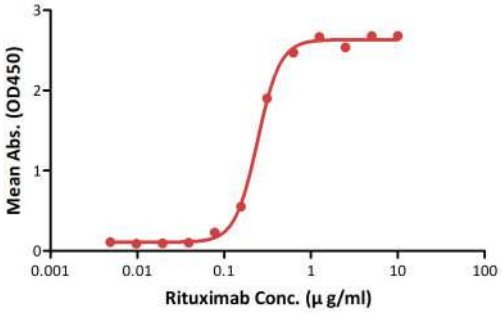


Figure 7. *Detection of rituximab by anti-idiotypic bridging ELISA (20% serum).*

Methods Comparison

Method	Linear Detection Range (μg/mL)	Sensitivity (μg/mL)	Advantage	Disadvantage
Antigen capture ELISA	—	—	Simple, Universal	High background, No bioactivity
Anti-idiotypic capture ELISA	0.156-10	0.156	Wide detection, High sensitivity	High background, Only suitable for rituximab biosimilar
Bridging ELISA by anti-idiotypic antibodies	0.195-1.56	0.195	High sensitivity, Low background	Narrow detection, Only suitable for rituximab biosimilar

Among the aforementioned three assay designs, the antigen-capture ELISA is not able to detect the level of rituximab in serum because of low affinity of CD20 and rituximab, indicating that it's not a solution for the application. The anti-idiotypic capture ELISA and the bridging ELISA by anti-idiotypic antibodies use anti-rituximab antibodies to replace CD20. The linear detection range for the anti-idiotypic capture ELISA is the best. However, the use of goat anti-human IgG as secondary antibody results in high background due to unspecific binding, and therefore require pre-dilution before analyses (Figure 8A). On the other hand, the bridging ELISA by anti-idiotypic antibodies uses HRP-conjugated streptavidin for secondary detection, which minimize the background interference (Figure 8B).

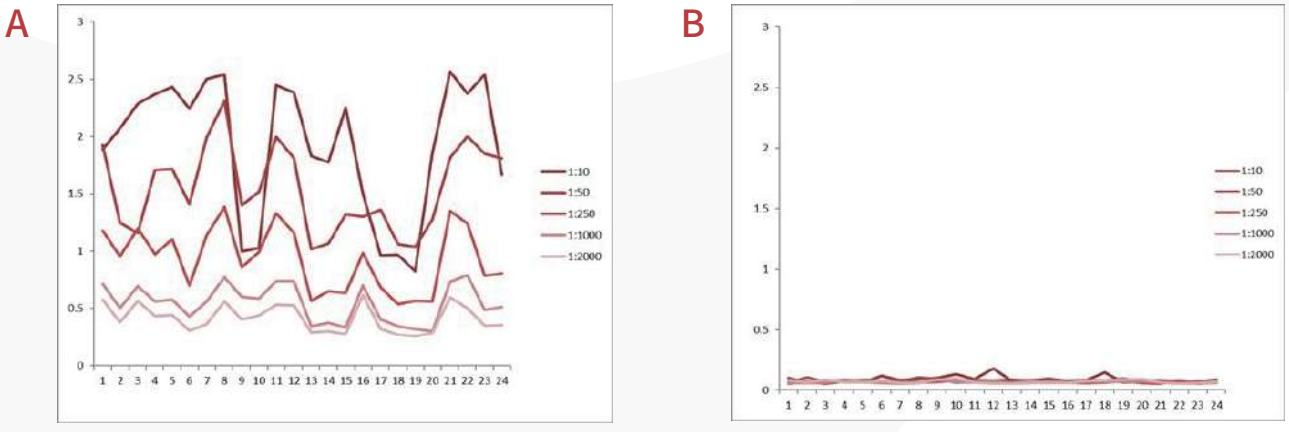


Figure 8. *Comparison between anti-idiotypic capture ELISA and anti-idiotypic bridging ELISA for rituximab detection in patient samples. Left: anti-idiotypic capture ELISA; Right: anti-idiotypic bridging ELISA.*

Anti-idiotypic antibody method has considerable advantages over the traditional detection methods in preclinical/clinical studies. To support these efforts, ACROBiosystems has developed a series of anti-idiotypic antibodies, which are proved to have high affinities and high specificities.

IV. Features

High Affinity

Affinity measures the strength of interaction between an antigen and an antibody, which can be described as K_D. The smaller K_D represents the stronger affinity. We measured the affinity of all the anti-idiotype antibodies using the SPR method. This piece of data helps to predict the sensitivity in assay development.

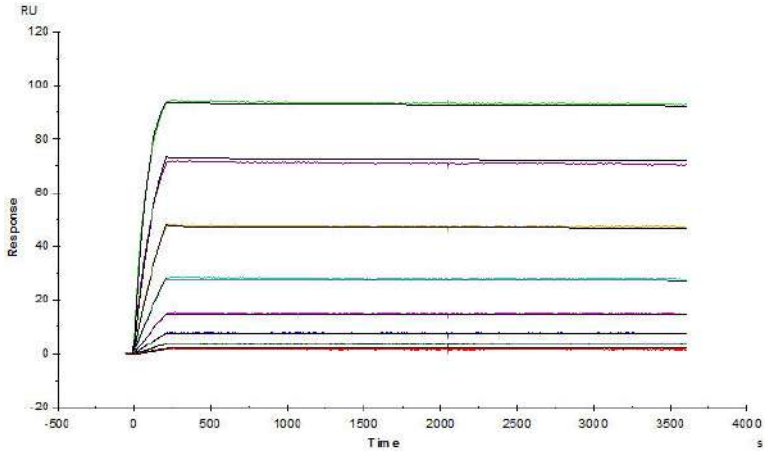


Figure 9. *Anti-Adalimumab Antibodies (mouse IgG1, Cat. No. ADB-Y19) captured on CM5 chip via anti-mouse antibodies surface, can bind human adalimumab with an affinity constant of 1.36 pM.*

High Specificity

We ensured the specificity of each anti-idiotype antibody product before it goes on the market.

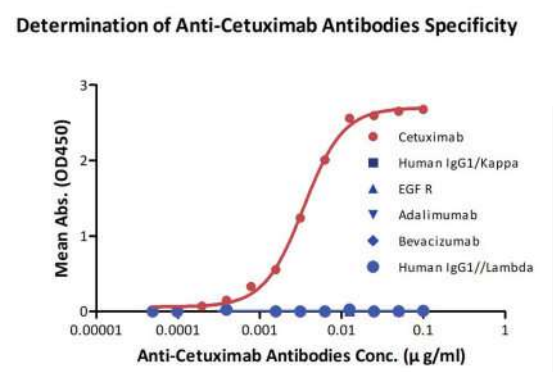


Figure 10. *Demonstration of the specificity of Anti-Cetuximab Antibodies (Cat. No. CEB-Y28) to the cetuximab.*

High Stability

ACROBiosystems performed accelerated stability tests and freeze-thaw tests to ensure the stabilities of our products before they go on the market.

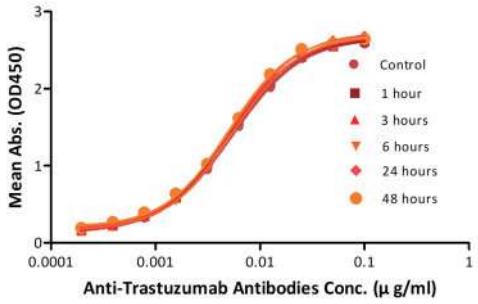


Figure 11. Accelerated stability test. *Reconstituted Anti-Trastuzumab Antibodies were diluted to 0.4 mg/ml, aliquoted and placed at 37°C. Aliquots were removed from 37°C at every time point and placed at 4°C along with the control. No significant loss of activity was observed.*

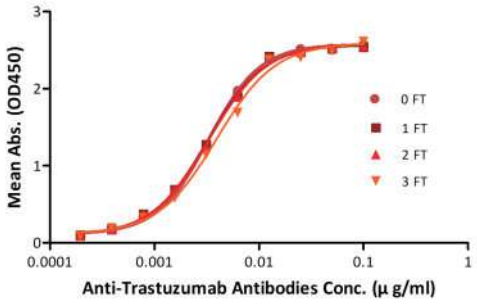
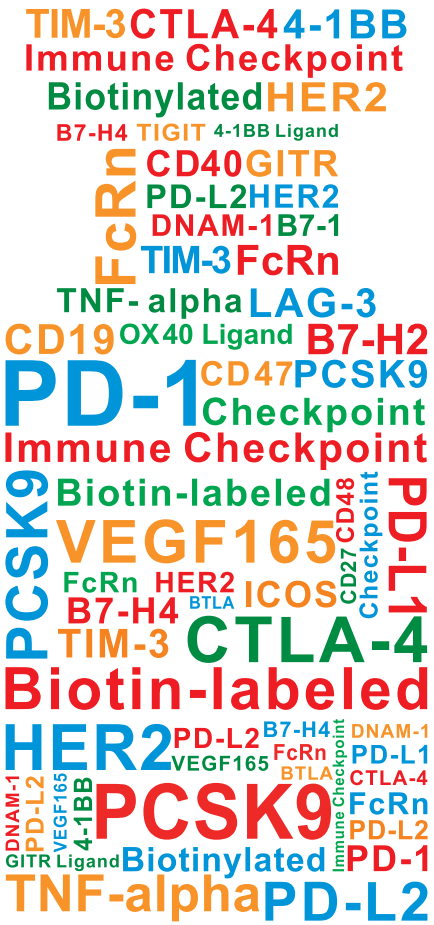


Figure 12. Freeze-thaw test. *Anti-Trastuzumab Antibodies were subjected to the indicated number of freeze-thaw cycles (FT). No significant loss of activity was observed.*



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