

Introduction

Immunogenicity assays for long-lived therapeutics have the limitation that free drug in the sample could bind to the produced antibodies and thus inhibit detection of antibodies that have been produced to that drug. Therefore, maximizing drug tolerance and sensitivity is a great concern.

To achieve this goal, a sensitive anti-drug antibody (ADA) assay was developed with an adequate level of tolerance to free circulating drug. We have successfully developed and validated a novel capture-elution method utilizing chemiluminescence (ECL) technology based on the Meso-Scale Discovery (MSD) platform. There are several advantages to this approach in addition to increased sensitivity and drug tolerance. First, it is not species specific and therefore, one species can be used as a control for another without the need for a second detection system. Secondly, there is no requirement for the anti-drug antibody to be functionally divalent, as the biotinylated and ruthenium labeled drug do not have to be bound to the ADA at the same time, as in a typical bridging ELISA. This enables this assay to detect all subclasses of ADA, including any which may be functionally monovalent (IgG4).

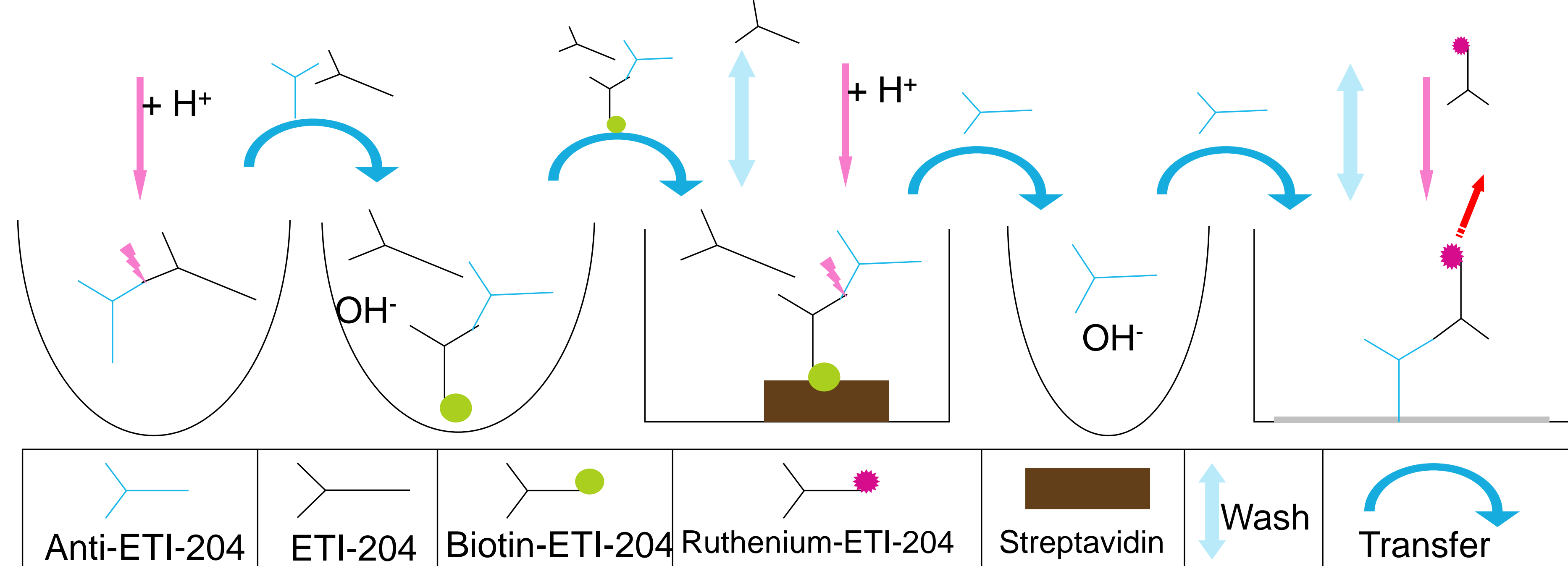
ETI-204 is a therapeutic monoclonal deimmunized humanized antibody of IgG1 isotype. Clinical safety assessment of this antibody requires assessment of its immunogenicity in human subjects. Clinical samples from a clinical safety trial with ETI-204 were analyzed using the validated assay.

Methods

An ECL assay for immunogenicity was developed using the drug, ETI-204 monoclonal antibody, as capture and detection reagents (Figure 1). To improve drug tolerance levels, acetic-acid assisted disruption of drug: ADA complexes in samples was used. The released Anti-ETI-204 was then bound to a Biotinylated-ETI-204 molecule in excess amounts of a neutralization buffer. The Biotin-ETI-204:Anti-ETI-204 complex is then captured on a streptavidin plate. This plate is washed to remove any unbound molecules, especially any unbound free ETI-204. Acetic acid treatment is once again used to release the Anti-ETI-204 from the complex. The released Anti-ETI-204 is then passively absorbed on ECL plates. Detection of coated Anti-ETI-204 was accomplished using Ruthenium-labeled ETI-204. Finally, ECL values were measured using the Sector Imager® 2400 and MSD software (Meso Scale Discovery). Cut-point, the level above which samples are classified as positive, was established using sera from 51 healthy volunteers with the desired false positive rate of 5%.

Immunogenicity assessment was performed on serum samples collected from healthy human volunteers treated with ETI-204 (Study AH-102). Anti-ETI-204 antibody presence was tested in samples prior to and 6 weeks following the drug administration. For ADA-positive subjects, testing was then performed on 8 and 10 week samples.

Figure 1. Method Schematic



References

- Smith HW, et al. "Detection of antibodies against therapeutic proteins in the presence of residual therapeutic protein using a solid-phase extraction with acid dissociation (SPEAD) sample treatment prior to ELISA. Regul Toxicol Pharmacol. 2007 Dec; 49(3):230-7
- Bourdage JS, et al. "An Affinity Capture Elution (ACE) assay for detection of anti-drug antibody to monoclonal antibody therapeutics in the presence of high levels of drug". J Immunol Methods. 2007 Oct 31; 327(1-2):10-7
- Patton A, et al. "An Acid dissociation bridging ELISA for detection of antibodies directed against therapeutic proteins in the presence of antigen". J Immunol Methods. 2005 Sep; 304 (1-2):189-95
- Mire-Sluis AR, et al. "Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products" J Immunol Methods. 2004 Jun; 289 (1-2):1-16
- Shankar G, et al. "Scientific and regulatory consideration on the immunogenicity of biologics". Trends Biotechnol. 2006 Jun; 24(6):274-80
- Shankar G, et al. "Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products". J Pharm Biomed Anal. 2008 Dec 15; 48(5):1267-81

Results – Assay Validation

Typical standard curve and free drug interference pattern are shown in Figures 2 and 3, respectively. Sensitivity of the anti-ETI-204 detection assay was 27 ng/mL. The drug tolerance level, 100 µg/mL, was sufficient to provide adequate immunogenicity testing in study subjects treated with therapeutic doses of ETI-204. Cut-point was determined to be 300 using Box-Cox power transformation statistical methodology.

Figure 2. Sensitivity

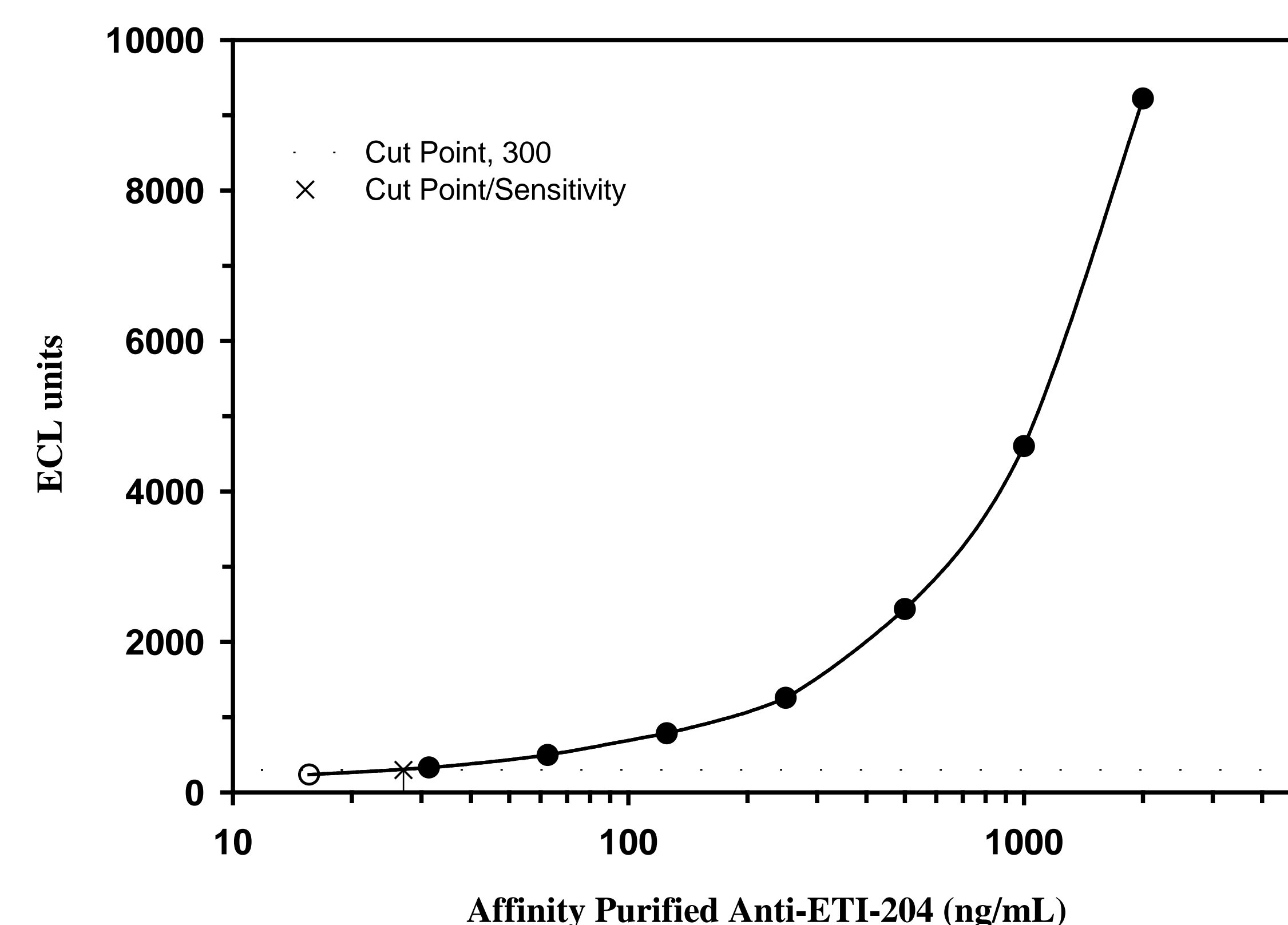
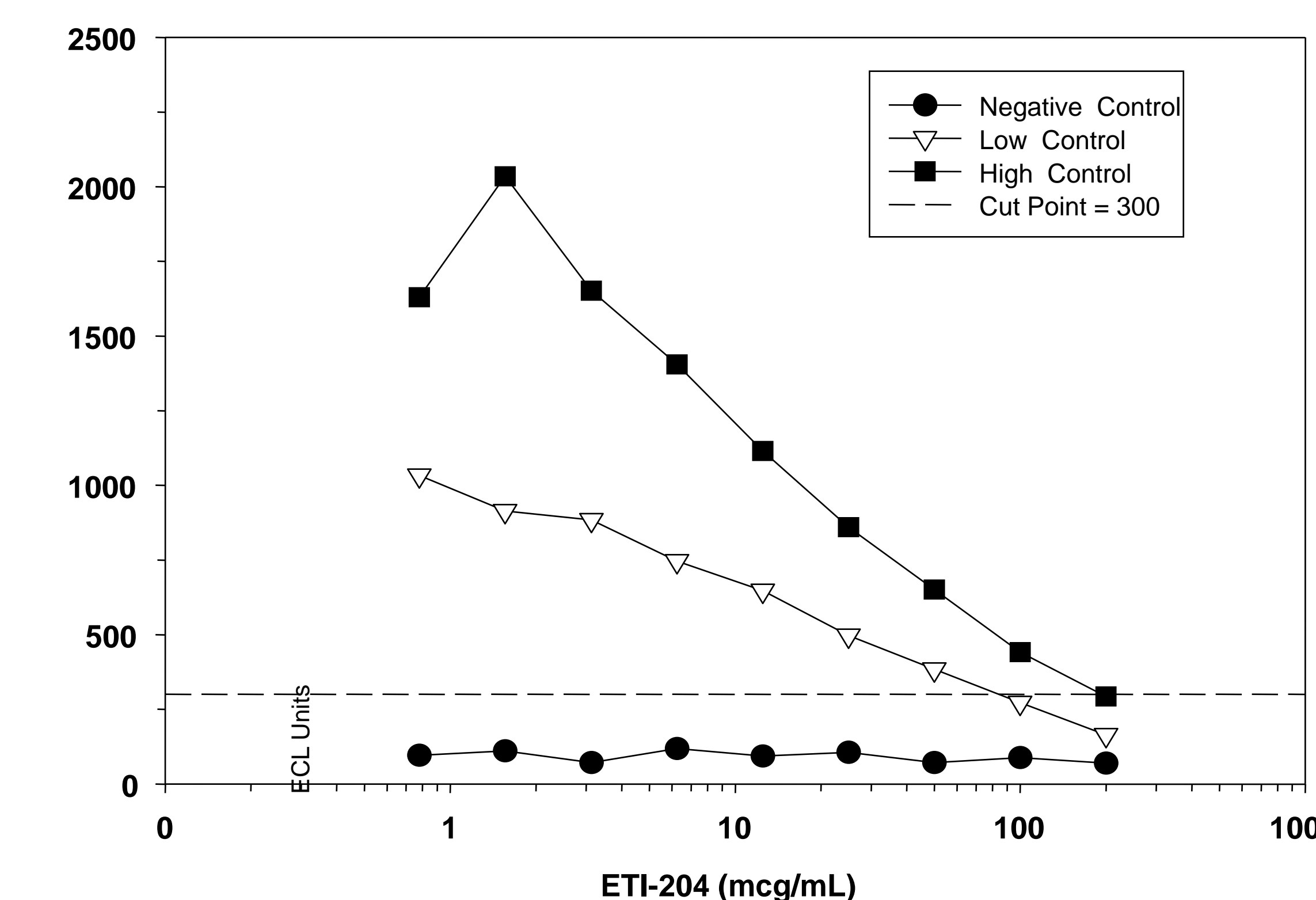


Figure 3. Drug Tolerance



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Results – AH-102 Serum Sample Testing

The rate of false positivity in study subjects, i.e. presence of anti-drug antibodies in healthy subjects prior to treatment was 8.9%. Transient low-titer positivity 6 weeks post-dosing was detected in 1 of 36 subjects treated with ETI-204 (Table 1). This study subject (01-115) had a subsequent ADA negative result at week 8 (Figure 4).

Conclusion

A sensitive anti-ETI-204 antibody detection method was developed with an adequate tolerance level to free ETI-204 to provide immunogenicity testing in clinical studies. Results from safety study AH-102 suggest that ETI-204 did not induce significant immunogenicity in healthy volunteers.

Table 1. Assessment of Immunogenicity ADA Positive

Study Subjects	N
Total Number	45
ETI-204 Group	36
Placebo Group	9
Positive prior to Treatment	4 (8.9%)
Converted to Positive Post Treatment	1 (2.8%)

Figure 4. Examples of AH-102 Sample Testing

