NIDS® Rapid Assays for the Detection of Anti-Drug Antibodies to Various Polyethylene Glycol (PEG) Polymers

Jing Pan1, Thomas Small1, Dujie Qin1, Cindy Pauley2, Catherine Kaplanski2, Thorton Verch2, Dave Chen2, Ray Bakhtiar2, Yrene Naling1, Yli Remo Vallejo1, Ray Yin1

1 ANP Technologies Inc.®, 824 Interchange Boulevard, Newark, DE 19711   2 Merck Research Laboratory Merck & Co., Inc., Sumneytown Pike, West Point, PA. 19486

INTRODUCTION

Rapid double antigen bridging immunogenicity assays for the detection of anti-drug antibodies (ADA) to 40 kDa and 20 kDa Polyethylene Glycol (PEG) in human serum have been successfully developed.

The assays require no sample dilution and no washing steps which can perturb fragile complexes formed by low-affinity ADAs. No dilution improves the detection of low titer ADAs.

Principle of the Rapid Immunogenicity Assay

![Principle of the Rapid Immunogenicity Assay diagram]

**Handheld Readers and Rapid Assay Test Tickets**

The NIDS® handheld reader (left) is for on-site rapid testing, and the medical reader (right) is for quantitative Point of Care testing (POCT).

**Advantages of the NIDS® Rapid Immunogenicity Assay**

1. No sample dilution
2. No wash steps
3. Fixed Screening Cut-point
4. Fast
5. Accurate
6. Easy to use
7. Affordable

**Evaluation of Normal Human Serum Samples in the 40 kDa PEG Rapid Immunogenicity Assay**

Calculated Screening Cut-point = 104 reader units

Evaluation of Positive Samples in the Drug Depletion Assay

Assay Sensitivity = 125 ng/mL ADA

Evaluation of Positive Samples in the Drug Depletion Assay

**Rapid Bridging Immunogenicity Assay dose response curve with anti-PEG antibody (IgM) spiked into human serum pool**

**20 kDa PEG Rapid Immunogenicity Assay**

Calculated Screening Cut-point = 250 ng/mL ADA

**Evaluation of Normal Human Serum Samples in the 20 kDa PEG Rapid Immunogenicity Assay**

50 normal human serum samples were tested in the 20 kDa PEG Rapid Immunogenicity Assay. Using the calculated screening cut-point a 6% false positive rate was determined for the limited population tested. Using the fixed reader cut-off, two possible positive samples (no. 216 and 257) were identified and subsequently tested in a drug depletion assay.

**20 kDa PEG Depletion Assay in neat human serum**

**40 kDa PEG Depletion Curve, with 300 ng/mL ADA**

Assay Sensitivity = 250 ng/mL ADA

**20 kDa PEG Drug Depletion with 400 ng/mL ADA**

**40 kDa PEG Depletion Assay in neat human serum**

**20 kDa PEG Drug Depletion with 125 ng/mL ADA**

**Evaluation of Normal Human Serum Samples in the 40 kDa PEG Rapid Immunogenicity Assay**

50 normal human serum samples were tested in the 20 kDa PEG Rapid Immunogenicity Assay. Using the calculated screening cut-point a 6% false positive rate was determined for the limited population tested.

**40 kDa PEG Drug Depletion with 400 ng/mL ADA**

300 ng/mL of anti-PEG antibody (IgM) and increasing amounts of free 40 kDa PEG were spiked in a human serum pool and incubated at RT for 1 hour before testing

**40 kDa PEG Drug Depletion with 125 ng/mL ADA**

**40 kDa PEG Drug Depletion with 250 ng/mL ADA**

**40 kDa PEG Drug Depletion with 300 ng/mL ADA**

**40 kDa PEG Drug Depletion with 125 ng/mL ADA**