The intra-assay precision was determined from 2 plates performed each day for 3 days by 2 analysts. The inter-assay precision was calculated from all accepted plates (Table 1).

### VALIDATION RESULTS

The screening cut point were determined from the individual samples analyzed each at 3 separate days. Individual male and female serum samples were included on each plate. A floating screening cut point factor was adapted to calculate the plate specific cut point (Table 2).

### MATERIALS AND METHODS

The neutralizing anti-Adalimumab antibody in human serum detection assay was developed in WEHI-13VAR cell line, which has high sensitivity to TNF-α. Adalimumab blocks the TNF-α induced cell cytotoxicity. The neutralizing antibodies to Adalimumab bind to the drug and restore the TNF-α induced cytotoxicity. In this homogenous assay, samples and controls are pre-incubated with Adalimumab and TNF-α, then the mixture is incubated with cells to initiate the TNF-α induced cytotoxicity. The presence of neutralizing antibodies inhibit the function of Adalimumab. The screening cut point was determined from 50 drug naive individual lots.

Drug tolerance was evaluated by pre-incubating the anti-Adalimumab polyclonal antibodies with the innovator drug or PF-06410293 at room temperature before being analyzed. The positive samples (last reading below the plate cut point) were highlighted (Table 3).

### CONCLUSIONS

- Two independent cell-based assays to detect the neutralizing anti-Adalimumab innovator and anti-Adalimumab biosimilar (PF-06410293) antibodies were validated in human serum to support ongoing clinical studies.
- Screening cut points were determined for both in normal populations.
- Two anti-Adalimumab innovator antibodies (one monoclonal, one polyclonal) and one anti-PF-06410293 monoclonal antibody were evaluated in both innovator EU and biosimilar NAB assays for cross-reactivity and sensitivity.
- The two assays were found to have comparable precision, drug tolerance and assay sensitivity.