Adalimumab, a monoclonal antibody directed against TNFα, is a drug widely used for the treatment of inflammatory diseases (Rheumatoid Arthritis, Crohn’s disease...). Therapeutic Drug Monitoring is currently proposed to provide useful information to clinicians to improve the efficacy of the treatment. Theradiag has just developed the innovative i-TRACKER kits: fast quantification of Adalimumab (principals and biosimilar molecules) and Anti-Adalimumab antibodies are fully automated on the random access i-TRACK® chemiluminescent analyzer.

MATERIALS & METHODS

Adalimumab SPiked SAMPLES: 3 human serum matrices (from healthy donors) were used. The drug, Adalimumab pharmacological solution (50mg/ml), was spiked into these 3 matrices to reach 5 levels of concentration spanning the dynamic range of the assay (0.75, 2, 6, 12 and 18μg/ml). A total of 15 spiked samples were produced. % of recovery was calculated according to the following formula: (quantified concentration/spiked concentration) x 100.

CLINICAL SAMPLES: 32 serum samples from 80 patients (Inflammatory Bowel Disease patients) treated with Adalimumab were collected. They arrived frozen and were kept frozen until quantification at Theradiag. Additionally, 46 serum samples from IBD patients, pre-sampled for Anti-Adalimumab antibodies with LISA-TRACKER Anti-Adalimumab assay (RTCA OS, Theradiag) were used for correlation assessment.

1. TRACKER Adalimumab kit: composed of recombinant human TNFα coated magnetic beads, polyonal-anti-Adalimumab antibodies conjugated to acridinium ester, and sample dilution buffers. 1-TRACKER Anti-Adalimumab kit: composed of Adalimumab coated magnetic beads, Adalimumab conjugated to acridinium ester, and sample dilution buffer. Both types of kit contain 2 calibrators and 1 positive control dedicated to calibration processing (master curve) and validation of the run, respectively. Once performed, calibration is validated for 21 days.

1. TRACKER CHEMILUMINESCENT ASSAY: quantification of Adalimumab and Anti-Adalimumab antibodies were performed with the i-TRACK® chemiluminescent analyzer according to the technical insert of i-TRACK® kits (RTCA 002 and RTCA 003 respectively). Briefly, serum samples were diluted and added to the coated magnetic beads suspension. After incubation of 15 minutes at +37°C, beads were washed and acridinium ester (AE) conjugate was added. After 5 minutes of incubation at +37°C, beads were washed, and triggers were added. Instantly, a light emission (RLU) were detected and quantified by i-TRACK® chemiluminescent analyzer.

Concentrations of Adalimumab and Anti-Adalimumab antibodies were calculated according to the calibration curve provided with the kit (master curve). The lower and the upper limits of quantification are 0.5μg/ml and 24μg/ml for i-TRACKER Adalimumab assay, 10μg/ml and 2000ng/ml for i-TRACKER Anti-Adalimumab assay.

RESULTS (1/2)

ACCURACY & STANDARDIZATION (see figures below): on one hand, 15 Adalimumab spiked samples were quantified with i-TRACKER Adalimumab. The % of recovery were comprised between 77% and 118% (mean % of recovery was 96%). On the other hand, 10 spiked samples were prepared with the NIBSC/WHO Adalimumab international standard (KS7/336) and quantified. The % of recovery were comprised between 97% and 107% (mean % of recovery was 96%).

INTRA-RUN PRECISION (see figures on the right): for both assays, 5 clinical samples spanning the dynamic range of the respective assays were quantified 30 times within a run. The coefficients of variation (CV) were calculated for each sample: the CV ranged from 2.4% to 5.3% for Adalimumab assay and between 1.2% and 3.1% for Anti-Adalimumab assay.

INTER-RUN PRECISION (see figures on the right): for both assays, 5 clinical samples spanning the dynamic range of the respective assays were quantified on 6 independent runs. The coefficients of variation (CV) were calculated for each sample: the CV ranged from 3.0% to 10.6%. Similar results were obtained for the quantification of Adalimumab biosimilars (ABP801 and S85): CV ranged from 3.4% to 9.2%. For Anti-Adalimumab assay, CV ranged from 0.8% to 4.8%. The acceptance criteria (CV<20%) was met: high precision is reached with i-TRACKER Adalimumab assay and i-TRACKER Anti-Adalimumab assay.

RESULTS (2/2)

INTERFERENCES (see figures below): spiked samples (low and high level) were made with Adalimumab and Anti-Adalimumab antibodies with or without the presence of potential interfering agents, such as bilirubin, hemoglobin, triglycerides, rheumatoid factors (RF) and biotin. Adalimumab samples spiked with potential interfering agents were quantified with i-TRACKER Adalimumab kit and compared to results obtained with Adalimumab spiked samples. Same method was performed with Anti-Adalimumab antibodies spiked samples. The percentages of bias (% of variation between samples with/without interfering agents) were low (within +/- 10%).

CONCLUSION: i-TRACKER Adalimumab and i-TRACKER Anti-Adalimumab kits are innovative assays which exhibit fast, accurate and reproducible results. Excellent correlations agreements were observed with LISA-TRACKER assays. i-TRACKER kits are valuable tools for the monitoring of patients treated with Adalimumab (principals and biosimilars) and allowing rapid treatment adjustment.