INTRODUCTION
Developing highly sensitive and accurate LC-MS methods for large molecule therapeutic quantification is quite challenging. This is especially true for protein quantification workflows that commonly employ the bottom-up approach, or surrogate peptide approach, using enzymatic digestion to break down the prolamins into smaller peptides. Method development of this entire process is time consuming and complex. Performing many steps and various reagents which often require high levels of expertise and can add significantly to the complexity, for extreme sensitivity and selectivity needs, protein level clean up of the complex biological matrices can be employed at different specificities to capture certain species and remove many or most endogenous contaminants. Thus, there is a strong need for a more simplified and standardized sample preparation workflow.

METHODS Sample Preparation Whole Plasma Samples
Using the Waters ProteinWorks™ (ProteinWorks) Auto-
exPress Direct Digest and µEulsion SPE Clean-Up Kits with their included protocols, rat plasma samples (35 ìL) containing various concentrations of the therapeutic mAbs, infliximab and cetuximab, sample digestion and subsequent peptide level clean up was performed using the Hamilton Microlab STAR (STAR). Affinity Purified Plasma Samples
Performed by a Hamilton Microlab STAR, 25 ìL of streptavidin beads were charged with 33 ìL (comparability study) or 50 ìL (quantification study) Goat anti-human Biotinylated IgG antibodies for 2 hours. 50 ìL spiked or blank rat plasma were allowed to capture with 50 ìL spiked or blank rat plasma samples were automatically affinity purified using the Waters ProteinWorks™ Auto-
exPress Low 5 ìL. Subsequent peptides were injected directly without further clean up.

LC-MS/MS Conditions
LC-MS/MS quantification of resulting peptides was performed using a Waters Xevo TQ-TOF quadrupole mass spectrometer (ESI+). Chromatographic separation was achieved using an ACQUITY UPLC® BEH C18 Column, 300A, 1.7 ìm, 2.1 mm ×50 mm, flow rate of 0.3 mL/min using a linear gradient with 0.1% formic acid in water and acetonitrile on an ACQUITY UPLC® I-Class UPLS. Signature tryptic peptides and their respective manually prepared samples. The %RSD bars on either side represent that %RSD of the manually prepared (blue) and surrogate peptides representing etanercept, infliximab, and trastuzumab. The Automated Area Normalized bar summarizes in Table 1.

RESULTS I. COMPARABLE AUTOMATED VS. MANUAL PROTEIN DIGESTION
Automated Protein Digestion Compared to Manual Protein Digestion
Automated new area counts of each peptide were normalized to the respective manually prepared samples.

II. ACCURATE AND REPRODUCIBLE PROTEIN QUANTIFICATION USING STANDARDIZED DIGESTION KITS AND AUTOMATED LIQUID HANDLER LINEAR & ACCURATE ETANERCEPT QUANTIFICATION
Table 2. Representative standard curves for signature peptides used to quantify cetuximab, manually digested and digested using Waters ProteinWorks™ Auto-exPress LC-Elution SPE Clean KIT and respective protocols on the Hamilton STAR.

III. COMPARABLE AUTOMATED VS. MANUAL AFFINITY PURIFICATION PERFORMANCE USING MAGNETIC BEADS
Table 3. Representative standard curves for signature peptides used to quantify cetuximab, manually digested and digested using Waters ProteinWorks™ Auto-exPress Low 5 ìL digested kit.

APPLICATION HIGHLIGHTS
A comparability test using infliximab surrogate peptides, ICT samples compared to blank plasma. All automated affinity purified and digested on the Hamilton STAR.

CONCLUSION
Automating the complex and time consuming sample preparation for protein quantification workflows streamlines the process, maximizes productivity, reduces errors, and ensures analytical reproducible workflows, while also productivity, eliminating the costly time spent of high level scientists at the lab bench.

Figure 1. Comparable automated (STAR) vs. manual digestions performance using the ProteinWorks Auto-
exPress Direct Digest and µEulsion SPE Clean-Up Kits.

Figure 3. Comparable automated (STAR) vs. manual sample capture performance (peak areas and %RSDs) of surrogate peptides representing etanercept, infliximab, and trastuzumab. The Automated Internal standard bar represents the raw area counts of the automated samples normalized to the raw area counts of the manually prepared samples. The %RSD bars on either side represent that %RSD of the manually prepared (blue) and automated (green) samples.

Figure 4. Example chromatograms of cetuximab surrogate peptide, DILL, LCID at 100 ng/mL, compared in the same aliquot sample. Both samples were automatically digested and SPE extracted using the Hamilton STAR.

Figure 5. Q C statistic for signature peptides used to quantify etanercept, manually digested and digested using Waters ProteinWorks™ Auto-exPress Low 5 ìL digested kit.