



Supplementary Figure 2: Proportion (percentage) of HCPs identified in the total protein sample covered by the HCP antibodies, the control antibodies, or bound to uncoated plates across the range of MW (A-C) and pI (D-F). Data are presented as mean +SEM (n=3. For the rabbit IgG, n=2). The coverage of HCPs was consistent for HCPs with a theoretical MW of 40-160 kDa varying between a mean of 70-105% of the number of HCPs identified in the total protein sample (A-C). The coverage of HCPs below 40 kDa was slightly decreased (mean of 63-78%) and only around 50% (45-56%) of the HCPs identified in the total protein sample with a theoretical MW below 20 kDa was covered by the HCP antibodies (A-C). This might reflect the known difficulties of eliciting effective antibody responses against low MW HCPs (Hanly, Artwohl, & Bennett, 1995). The coverage of HCPs with a MW above 160 kDa was slightly increased (>130%), but only four proteins were identified in this group. The coverage of HCPs by the HCP antibodies was consistent for HCPs with a theoretical pI between 5-10 with a coverage varying between a mean of 59-101% (D-F). The coverage of HCPs with a pI below 5 was slightly decreased with only 50% (43-57%) of the HCPs identified in the total protein sample covered by the HCP antibodies (D-F). Eleven HCPs identified in the early process sample with or without immunocapture have a pI above 11. The coverage of these was slightly increased (mean of 96-110%). The HCPs identified after immunocapture with the control antibody or bound to uncoated plates followed the coverage tendency of the HCP antibodies across the entire range of MW (A-C). For the HCPs with a pI from below 5 to 10, the share of covered HCPs identified after immunocapture with the isotype control or on uncoated plates followed the coverage tendency of the HCP antibodies (D-F). However, for the HCPs with a pI above 10 (50 protein identifications in the early process sample with or without immunocapture), the share of HCPs identified after immunocapture with the control antibodies or bound to the uncoated plate was highly increased (D-F).