to purify five mouse IgG2a samples in parallel on these columns. The total quantity of mouse IgG2a obtained with either resin following purification is summarized in Fig. 5. For all tested mouse IgG2a, the quantity of purified protein obtained from Protein A was at least 50% higher than those obtained from Protein G purification.

3.3.2. Development of a post-load wash step to improve HCP removal during protein A purification

Removing impurities post-Protein A chromatography still represents a significant challenge to purification process development in order to achieve the required drug substance specifications suitable for patient administration (HCP < 100 ppm). Using a post-load wash step is a key means to achieve HCP clearance, since it has been demonstrated that HCP associates with antibodies and co-elute during the elution step. Basic pH and wash additives such as arginine seem to improve HCP removal during the Protein A chromatography wash step by disrupting interactions between the antibody and the HCPs [13]. Here we evaluated both Tris and phosphate-based arginine wash buffers at different pH values (7 to 9) in comparison to conventional wash buffer (DPBS or citrate pH 5.0) using 1 mL HiTrap MabSelect SuRe columns. These various wash conditions (Table 1) were tested in parallel with 3 different antibodies (CHO supernatants) using the Protein Maker. Yield and HCP levels obtained for each wash condition are presented in Fig. 6. The use of basic wash buffers containing arginine showed improved HCP removal (1.7 to 2.4-fold) compared to the conventional DPBS wash for the three antibodies tested, with detrimental effects on purification yields only for one antibody (mAb3).

3.3.3. Stability hold of clarified harvest for a mAb

In a series of experiments, a clarified mAb harvest was held at either 2–8 °C or 19–23 °C and sampled at various time points corresponding to t = 0, 1, 2, 3, 5 and 7 days before purification using MabSelect SuRe followed by buffer exchange to DPBS in order to evaluate changes in the product using an analytical assay panel. The purification performance at time points, t = 0 and t = 7 days, is shown in Table 2. Purification recoveries based on the measured product titer were at least 85%. Analysis of charge variants and glycosylation profiles at each time point at either temperature revealed a significant decrease in acidic charge variants upon storage at 19–23 °C, corresponding to a drop in the sialic acid content of the mAb (results not shown). This assessment made it possible to define the manufacturing hold time duration necessary for process control during GMP manufacturing.

4. Discussion

During early-stage therapeutic antibody R&D projects, it is often necessary to purify large numbers of samples that will be used as reagents for *in vivo* or *in vitro* screening assays. The nature of the purification strategy employed is determined by the quantity and final concentration of purified protein required; the number of samples that need to be purified per unit time (throughput), the supernatant volume and initial product titer, as well as the availability, if any, of automated liquid handling instrumentation. While clearly there is more than one possible solution to achieve the purification objectives,

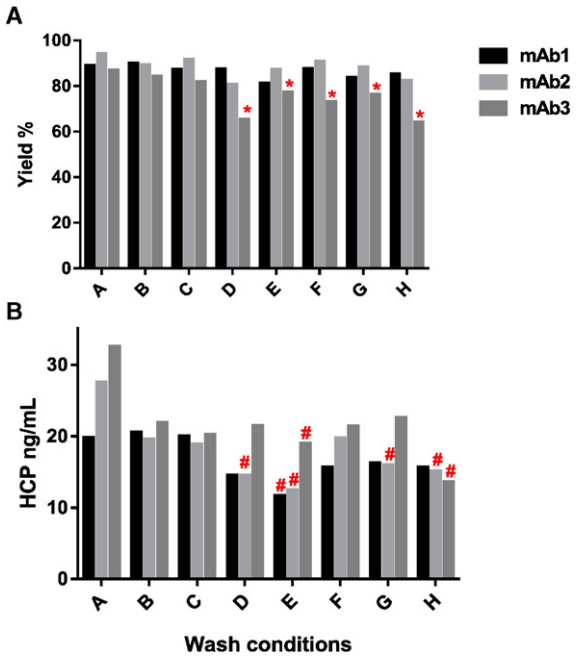

Fig. 6. Effect of wash buffers on (A) purification yields and (B) HCP levels. Wash conditions — A: PBS; B: 100 mM Citrate, 50 mM NaCl pH 5.0; **C**: 50 mM Tris, 100 mM Arginine, 50 mM NaCl pH 7.0; D: 50 mM Tris, 100 mM Arginine, 50 mM NaCl pH 8.0; E: 50 mM Tris, 100 mM Arginine, 50 mM NaCl pH 9.0; F: 10 mM Phosphate, 100 mM Arginine, 50 mM NaCl pH 7.0; G: 10 mM Phosphate, 100 mM Arginine, 50 mM NaCl pH 8.0; H: 10 mM Phosphate, 100 mM Arginine, 50 mM NaCl pH 9.0. *: Yields below 80% — #: HCP reduction *vs* PBS greater than 1.7-fold.

we selected the Protein Maker as a platform using HiTrap columns, including Protein G (Protein G HP), Protein A (MabSelect SuRe) and IMAC (Ni Sepharose Excel), and have applied these to feeds from either hybridoma or CHO supernatants ranging from 10 mL to 200 mL volumes. A key outcome has been for purification of small quantities (<1 mg) of purified mAbs using the Protein G method described here, with greater than 1000 mAbs purified and utilized as reagents for *in vitro* screening assays.

For the development of platform-based purification methods on the Protein Maker, several parameters that influence the overall purification outcome required evaluation. One of the most critical parameters is the residence time used in the binding step, as most of the overall time required to execute the method involves binding of the product to the packed bed. Through several purification campaigns utilizing Protein G HP with murine IgG's, we have established that a three-minute residence time ensures capture of the product, as revealed by non-reducing SDS-PAGE analysis. Another critical parameter is to establish the elution volume range for the product. The elution volume will depend on the column volume and the quantity of product bound to the packed bed, with larger elution volumes being required as one approaches the dynamic binding capacity limit of the column. The optimal elution volume is a balance between the desired product concentration, yield and recovery of the purified product. One rule of thumb

Table 1
Wash conditions tested for Protein A post-load HCP removal.

	Wash 1 (5 CV)	Wash 2 (5 CV)	Wash 3 (5 CV)
1	PBS	PBS	PBS
2	PBS	100 mM Citrate, 50 mM NaCl pH 5.0	PBS
3	PBS	50 mM Tris, 100 mM Arginine, 50 mM NaCl pH 7.0	PBS
4	PBS	50 mM Tris, 100 mM Arginine, 50 mM NaCl pH 8.0	PBS
5	PBS	50 mM Tris, 100 mM Arginine, 50 mM NaCl pH 9.0	PBS
6	PBS	10 mM Phosphate, 100 mM Arginine, 50 mM NaCl pH 7.0	PBS
7	PBS	10 mM Phosphate, 100 mM Arginine, 50 mM NaCl pH 8.0	PBS
8	PBS	10 mM Phosphate, 100 mM Arginine, 50 mM NaCl pH 9.0	PBS