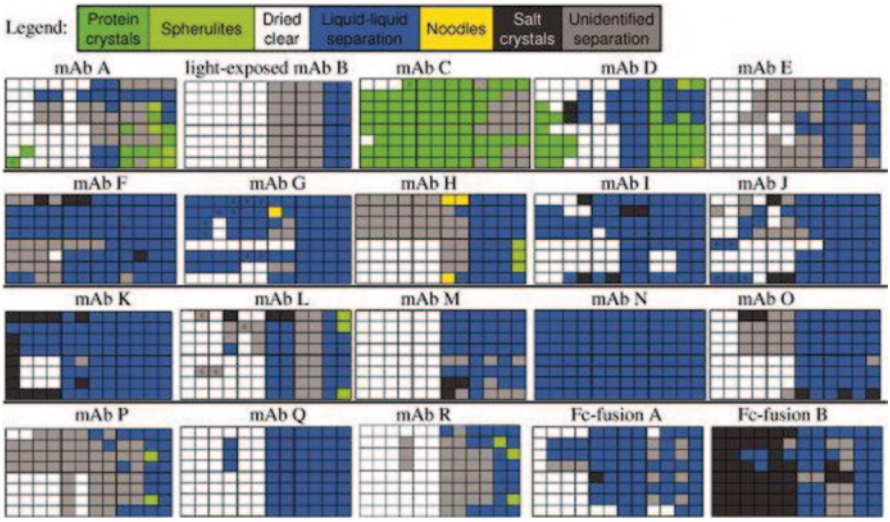

Fig. 7 Evaporative screen results using conditions in the study. Letter C in some wells stands for "clear" and indicates that, although there was phase separation at some point during drying, only a single phase was observed toward the end. *mAb* monoclonal antibodies (Refer to the study for details on screening conditions. Adapted with permission from [47])

bulk storage applications [5]. Crystallization parameters such as crystal morphology, crystal size distribution, and yield were optimized at laboratory scale by varying the salt concentration, pH, temperature and anti-solvents. Finally, the developed crystallization method was implemented into the manufacturing process.

Trilisky et al. examined the crystallization and liquid–liquid phase separation (LLPS) behavior of a set of 20 monoclonal antibodies and 2 Fc-fusion proteins, employing both vapor diffusion and evaporative screening-based methods [47]. Screening was performed using a set of about 100 conditions using reagents that are generally regarded as safe (GRAS), thus allowing the possibility of employing the crystallization condition as a purification step in process development and also as a protein formulation tool. While a small fraction of the tested proteins, could be crystallized in the preliminary screening, four of the IgG2s produced diffraction-quality crystals and three of these IgG2s could be crystallized with inexpensive GRAS reagents. In general, all the tested proteins exhibited LLPS which can be implemented as a concentration or purification step. Figure 7 describes the results of evaporative screen for the 20 tested proteins, depicting different morphologies (crystals, LLPS, spherulites, etc.) formed under different crystallization conditions.

Hebel et al. describe a stirred batch crystallization-based approach for obtaining crystals of a therapeutic antibody fragment FabC225 [20]. Vapor diffusion-based crystallization conditions already identified during the structure determination of FabC225 served as a starting point for development and optimization of the microbatch crystallization process at 10 µl scale. Figure 8 describes the crystals for FabC225 under microbatch conditions with varying concentrations of protein and ammonium sulfate. Figure 9 describes a phase diagram obtained for FabC225 from