ABSTRACT
Proteins are key components of prophylactic vaccines against infectious diseases. Protein subunit based vaccine is an attractive alternative to the traditional detoxified bacterial or inactivated viral vaccine approach due to its highly purified and well characterized product nature. Purification of protein antigens to achieve consistent product purity and quality is an integral part of the protein subunit vaccine development process. Expression levels of the recombinant proteins in bacterial expression system may be extremely high following rapid technology advancement. The challenges and approaches used to develop purification processes for novel protein vaccine candidates expressed at g/L level are being discussed.

Introduction and Objective
• The concept of Platform Technology (capturing, purification and polishing) has been successfully applied to the Mab purification (Figure 1). The purity of in-process samples after 2 unit operation was typically >80% and for one protein was even >90% (Figure 3).
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• Binding and elution conditions of each protein including the buffers to purify these proteins on cation exchanger or HIC varied and were dependent on the individual characteristics of these proteins (data not shown).

Methods
• Chromatographic methods such as Anion or cation exchange, Hydrophobic interaction chromatography, mixed mode chromatography methods were used.
• AKTA Explorer was used for small-scale method development and processes were scaled-up using AKTA PILOT.
• In-Process samples were tested using inline monitoring by UV, conductivity etc.
• Fractions were analyzed by SDS-PAGE or BCA protein assay.

Results (1)
• Several protein purification processes were developed using different protein antigens expressed in E. coli expression systems.
• A trend was observed in most of these purification schemes i.e. strong anion exchange such as Q column worked well as primary capture step and had enough capacity to capture the target protein where the expression in upstream was 1-3 g/L (Figure 1).
• Binding and elution conditions of each protein including the buffers to purify these protein varied and were dependent on the individual characteristics of these proteins (data not shown).
• The purity of in-process fractions after 1st unit operation was typically >50%. (Figure 2)

Results (2)
• The 2nd unit operation selected was either a cation exchanger (such as SP) or Hydrophobic column chromatography (HIC) step.
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Results (3)
• The 3rd unit operation selected was almost always a mixed mode column chromatography step such as Ceramic Hydroxyapatite or Q membrane. The purity of in-process samples after 3 unit operation was typically >95% and met the criteria (Figure 4).
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• Binding and elution conditions of each protein including the buffers to purify these proteins on 3rd column were dependent on the individual characteristics of these proteins (data not shown).
• Three or two column purified material was used for final buffer exchange using Tangential Flow Filtration.

Conclusions
• Common theme or the standardized approach can be used to develop new purification processes for new recombinant E. Coli antigens (Figure 5).