Purification of PSA from Human Semen

(From: Dr. Meera Venkatesh, India)

1. Human seminal plasma collected from many volunteers are pooled and passed through a column of phenyl sepharose equilibriated with 1.25 M ammonium sulphate. Elution is carried out with 1.25 M ammonium sulphate initially, to remove the bulk non-adsorbing proteins. Gradient elution of the absorbed proteins with 0.01 M Tris-HCl, 0.25 M NaCl, pH 7.0 buffer gives a sharp peak containing PSA. *

2. The absorbed protein peak containing PSA is then lyophilised, redissolved in Tris-HCl buffer and chromatographed in a Superdex-75 or Sephadex-75 column. The absorbed proteins elute out as multiple peaks and PSA is eluted as a sharp peak .*

3. Step 2 is repeated for better purity.

4. The PSA peak is lyophilised, dissolved in Tris-HCl buffer without NaCl and further purified on an ion exchange column (either anion or cation exchage columns such as DEAE Sephadex or CM-Sephadex or Mono Q). Gradient elution using Tris-HCl buffer without NaCl and Tris-HCl buffer with 0.25 M NaCl resulted in a sharp pure PSA peak (homogenous, sharp single band on SDS-PAGE).

This procedure is based on that reported by Wang et al., Oncology, 39,1,1982.

* At each stage, PSA has to be identified by an independant method such as immunodiffusion or an immunoassay.