Glucocorticoids such as cortisol (CL) and cortisone (CN) are steroid hormones that contribute to the hypothalamic-adrenal-pituitary feedback system. Diseases such as Cushing’s syndrome, Addison’s disease and Apparent mineralocorticoid excess can be diagnosed by measuring concentrations of cortisol in blood and urine. Prediction of an effective balance between 11β-hydroxysteroid dehydrogenase (type 1 and type 2) mimics the action of cortisol and is used as part of a diagnostic algorithm for the above diseases. Simultaneous measurement of dexamethasone with cortisol has been found to assist with efficient diagnosis of Cushing’s syndrome.

**Enzyme Preparation**
- To develop a sensitive and fast assay for measuring the 3 analytes in serum samples using an Agilent 1200 series HPLC pump and oven, CTC PAL Autosampler, an API 4000 Triple Quadrupole Mass Spectrometer (AB Sciex) and Ultra HPLC Aroma 100 s x 3.2 x 3um column (Restek).
- The method could be useful for monitoring patients during the dexamethasone suppression test.
- To compare distribution of concentrations of cortisol, cortisone and their ratios in samples from intensive care (ICU) and non-intensive care unit patients by measuring the estimated Glomerular Filtration Rates (eGFR).

**Sample Preparation**
- Calibration curve prepared in 0.1% BSA
- 200 ul, patient sample s and controls
- 100 ul, stable isotope labeled internal standards
- 700 ul, deionized water
- Shake for 15 min, centrifuge for 2min at 2500 rpm
- Condition the adsorbent
- Apply the samples on Phenomenex Strata X -33u
- Solid Phase Extraction plate
- Elute the analytes with 15% IPA-EA solution
- Dry the eluate under nitrogen
- Reconstitute in 100 ul, of 3:1 water: methanol containing 10mg/L estriol
- Vortex at low speed for 1 min, centrifuge at 3500 rpm for 1 min
- Analyze samples

**Conclusion**
A sensitive and selective tandem mass spectrometry method for analyzing the 3 related analytes was developed. High CCRs determined from samples collected from ICU patients evidenced the possible alteration in activity of 11βHSD in modulation of systemically available cortisol.