The Association for Clinical Biochemistry & Laboratory Medicine

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TOP TIPS FOR THE MEASUREMENT 25 HYDROXY VITAMIN D METABOLITES

ACB Liquid-Chromatography Tandem Mass Spectrometry – Special Interest Group

- Always use commercial calibrators and controls preferably aligned to an international standard.
- Be aware that the 3-epimer of 25-OH vitamin D3 will be detected by standard chromatography and can be high in infants. These samples should be reanalysed using a method designed to separate the 3-epimer to give a corrected result.
- Ensure isobaric interferences in the 25-OH vitamin D3 scan are minimised and this can be improved using 2 Dimensional HPLC techniques.
- Use deuterated internal standards. Similarities in mass and chromatographic behaviour between the vitamin D metabolites means a single internal standard is usually sufficient e.g. deuterated 25-OH vitamin D3. Always check the purity of new batches of deuterated internal standard to ensure it is not contaminated with unlabelled molecule.
- Monitor column performance carefully before and after each analytical run. This is particularly important if the assay is run weekly on a dedicated analytical column.
- Sample preparation may involve a number of liquid extraction stages or it could be the simple addition of acetonitrile containing the internal standard. If the latter, always either store this at -20oC and add to plasma straight from the freezer or introduce an incubation stage in the fridge. (Otherwise secondary precipitation can occur when extracts are stored in chilled autosamplers which can effect analysis and column life.)
- Always participate in the DEQAS quality control scheme for Vitamin D.