

Resolution of Spurious Immunonephelometric IgG Subclass Measurement Discrepancies by LC-MS/MS

Running Title: IgG Subclass Measurement by Mass Spectrometry

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Objective:

Immunonephelometry is a precise method to measure plasma proteins such as the IgG subclasses. However, this methodology has limitations some of which are common to other immunoassays including errors of antigen excess and cross reactivity. In the province of British Columbia, the Binding Site immunonephelometric (BSIN) IgG subclass reagents (IgG1, IgG2, IgG3, IgG4) are used to diagnose or monitor patients for immunodeficiency as well as IgG4 related disease (IgG4RD). Analytic errors were noted in patients with extreme elevations in IgG4: i) antigen excess errors, and ii) total IgG concentrations (Siemens) that were much less than the sum of the individual BSIN IgG subclasses. These samples were also noted to have an unexpectedly high IgG2 concentration. The objective of this study was to evaluate these IgG4 related BSIN method anomalies with a mass spectrometric method.

Method:

Samples were reanalyzed with tryptic digest LC-MS/MS to assess the total and sum subclass ($\text{sumIgG} = \text{IgG1} + \text{IgG2} + \text{IgG3} + \text{IgG4}$) measurements in high IgG4 concentration patients.

Results:

The LC-MS/MS sumIgG approximately equalled the total IgG measurement in all patients including those with high IgG4. Further, the sum of the LC-MS/MS IgG2 and IgG4 ($\text{sum}_{2_4} = \text{IgG2} + \text{IgG4}$) measurements matched the BSIN IgG2 measurement. This latter finding implied total cross reactivity of the BSIN IgG2 reagents with sample IgG4 immunoglobulins.

Discussion:

This apparent cross reactivity of BSIN IgG2 reagent antibodies for IgG4 should be recognized so that apparent IgG2 elevations in IgG4RD are acknowledged as spurious.