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Significant, quantifiable differences exist between IgG subclass standards WHO67/97 and ERM-DA470k and can result in different interpretation of results ☆

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ABSTRACT

Objectives: Accurate measurement of IgG subclass (IgGSc) levels are essential to aid in the diagnosis of disease states such as primary immunodeficiencies. However, there is no single standardisation of nephelometric and turbidimetric assays for these analytes and two reference materials have been utilised. We expand on previous reports and present data from a multi-site analysis that both identifies and quantitatively defines the differences in calibration resulting from the use of different reference materials.

Design and methods: IgGSc antibodies in the serum specimens and reference materials were measured according to the manufacturers' instructions using commercially available IgGSc assays or components.

Results: Data from four independent sites showed that in spite of the different commercial suppliers of IgGSc assays calibrating to different reference materials, ERM-DA470k and WHO67 /97, the resulting calibrations were comparable for IgG1 and IgG2. However, for IgG3 and IgG4 the calibrations were significantly different. The use of assay specific normal ranges should compensate for these calibration differences, however, the two manufacturers' assays can give differing clinical classifications. The agreement between the different manufacturers' IgGSc assays was between 85.1% and 95.8% for all IgGSc assays, the discordance of sample classification for IgG1 and IgG2 assays was approximately 12% and 15% respectively, whilst that for IgG3 and IgG4 was 4% and 13% respectively.

Conclusion: We discuss the similarities and differences between assays that utilise the different reference materials.

Introduction

The measurement of IgG subclasses (IgGSc) is performed as part of an immune system evaluation where there is a continued clinical suspicion of an IgGSc imbalance, particularly in the background of normal total IgG levels. Deficiency in IgGSc levels has been found to be associated with a variety of immunodeficiency syndromes such as common variable immunodeficiency, ataxia telangiectasia and IgA deficiency as well as upper respiratory tract infections such as severe swine flu [1–6]. The measurement of all four IgGSc forms part of the accepted protocol for diagnosis of an IgG subclass deficiency. The concentration of IgGSc is age dependant and normal IgGSc concentrations change significantly as the immune system matures. In neonates, placental transfer plays an important part in determining IgGSc levels and the majority of IgG present at birth is derived from placental transfer from the mother. During the first 6 months of life levels decrease as the neonate develops the synthetic mechanisms to produce their own IgG. IgG1 and IgG3 levels increase most rapidly with near adult levels reached by the age of 12, adult levels of IgG2 and IgG4 are reached much more slowly. These significant differences between the IgGSc concentrations in children and adults have to be taken into account when interpreting IgGSc results. Both paediatric and adult normal ranges have been established for the IgGSc from the different commercial reagent suppliers to enable their use in disease diagnosis [7,8].

The standardisation of normal ranges for the measurement of IgGSc has proven difficult for several reasons: (1) different methods have been used for measurement, (2) study cohorts that have been used may differ in age, race, sex and number of subjects and (3) the use of different statistical analyses for data interpretation. Standardisation has been further hampered due to their being no single international reference material recognised for the determination for IgGSc. The three commercial sources of IgGSc assays use two different calibrations: in the case of The Binding Site (TBS) Certified Reference Material 470

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Table 1
Intra-assay precision and sample linearity of TBS and Siemens IgGSc assays.
For intra-assay precision: twenty seven replicates were assayed for each sample in each of the manufacturer's individual IgGSc assays and percent coefficient of variation (%CV) was calculated.
For linearity: the samples were diluted to 75%, 50% and 25% of its original concentration and the values of the dilution recorded. The percentage deviation from the expected value was determined as described in materials and methods. All individual dilutions were assayed in triplicate.

Manufacturer	Function		IgG1	IgG2	IgG3	IgG4
TBS	% CV		3.3	1.8	3.6	3.1
	mg/L		7971.3	4542.6	650.4	426.2
	Linearity:	75%	4.1	−1.9	1.8	−3.7
	% Deviation from	50%	−0.7	−2.7	2.8	−4.0
	expected value	25%	−1.1	−5.0	3.3	−7.0
Siemens	% CV		1.6	1.9	5.0	2.9
	mg/L		9364.9	4246.5	371.2	675.6
	Linearity:	75%	−0.1	−0.1	−2.2	2
	% Deviation from	50%	8.6	−3.3	−7.1	5.7
	expected value	25%	16.6	0.3	4.5	−0.7

(CRM470; now superseded with ERM-DA470k due to depletion of CRM470 stocks [7]) [9] and in the case of Sanquin and Siemens WHO67/97 [10] (later replaced by the commercial calibration material Sanquin M1590).

Bossuyt et al. [11] have previously reported the differences in calibration and thus data interpretation between TBS IgGSc assays and the Sanquin IgGSc assays. A commentary has recently been published highlighting the major difference between TBS and Siemens IgGSc assay calibration and concluded that this difference was due to the assays being standardised against two different reference materials [12].

Here, we expand on previous reports and present data from a multi-site analysis that both identifies and quantitatively defines the differences in calibration resulting from the use of different reference materials. Furthermore, the subsequent effect this has on classification of patient samples is also presented.

Materials and methods

Assay method

IgGSc antibodies in the serum specimens and reference materials were measured according to the manufacturers' instructions using commercially available IgGSc assays: SPA_{PLUS} IgGSc assays (IgG1–IgG4; NK006.S, NK007.S, LK008.S, LK009.S; The Binding Site, UK). Siemens BNII IgGSc assays were performed with the following components: N AS IgG1 (OQXI092), N AS IgG2 (OQXK092), N Latex IgG3 (OPAV032), and N Latex IgG4 (OPAU032), N-supplementary reagent (OQTD115), Siemens Cleaner SCS (OQUB195), and the N protein standard SL (OQIM135) (Siemens Healthcare Diagnostic Products, Germany).

The adult normal ranges stated for the TBS assays are IgG1: 3.82–9.29 g/L, IgG2: 2.42–7.00 g/L, IgG3: 0.22–1.76 g/L, IgG4: 0.04–0.87 g/L and for the Siemens assays IgG1: 4.1–10.1 g/L, IgG2: 1.7–7.9 g/L, IgG3: 0.11–0.85 g/L, IgG4: 0.03–2.0 g/L.

Precision

The precision of each assay was compared by running twenty seven replicates of the same sample on each assay on both the Siemens BNII and the TBS SPA_{PLUS} IgGSc assays. The sample consisted of pooled human serum with IgGSc levels within the standard measuring range for each assay on both manufacturers' assays.

Linearity

A serum sample was identified that gave a readable concentration towards the upper value of the measuring range for each IgGSc on both manufacturer's assays. Dilutions of the samples were prepared at 75%, 50% and 25% concentration of the original fluid. The linearity of the IgGSc assays was assessed by running each dilution in triplicate and comparing the mean result to the expected results. The

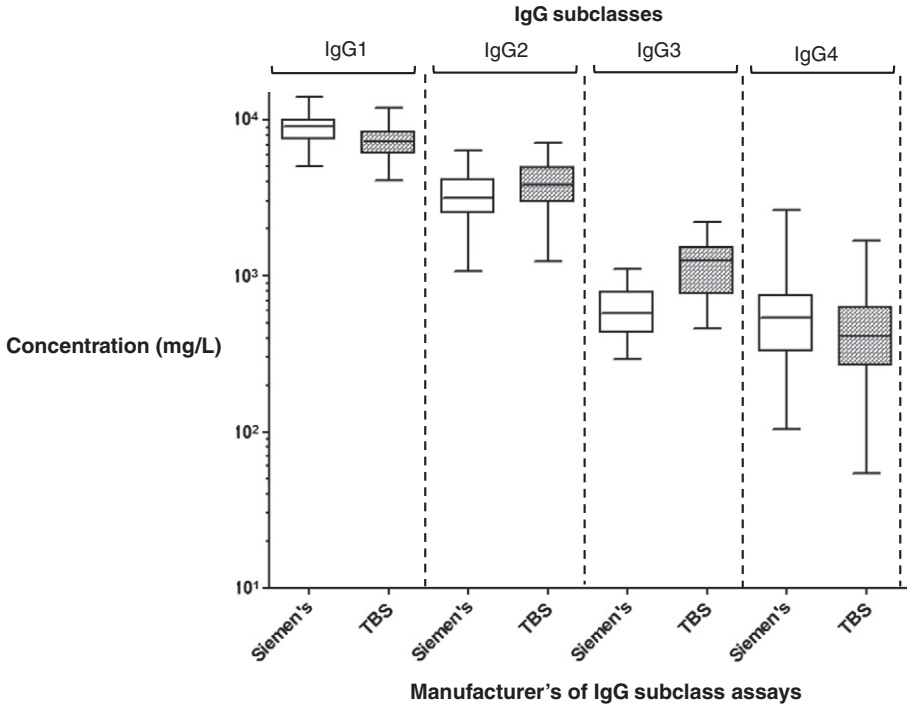

Fig. 1. Box and Whisker plot showing the comparison between IgG subclass assays and different manufacturers. There is a good agreement between the IgG1 and IgG2 assays. For IgG3 and IgG4 a significant difference between the manufacturers' assays is observed. The assays were run as described in the manufacturer's inserts and the methods and materials section. The line in the middle of the box represents the median value of the combined data set from all study sites, the upper edge of the box represents the value of the upper quartile, the lower edge the value of the lower quartile. The high error bar represents the highest value and the low error bar the lowest value in the sample population.