Analytical interference of paraprotein, albumin and gamma-globulin with the Elecsys Anti-SARS-CoV-2 Immunoassay.

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1. Introduction

- Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2), shown in Figure 1, is the large, enveloped, single stranded RNA Coronavirus responsible for the ongoing global pandemic [1]. Infection with SARS-CoV-2 triggers the onset of Coronavirus disease 2019 (COVID-19) and can present asymptomatically, with mild respiratory irritation or severe disease which can lead to lifelong complications and death [2].
- Reverse transcriptase polymerase chain reaction is used to diagnose SARS-CoV-2 infection [3].
- Serological antibody testing is a useful tool in epidemiological studies and monitoring antibody production in response to vaccination programmes [4].
- The Elecsys Anti-SARS-CoV-2 assay measured antibody titre using a recombinant protein which represents the nucleocapsid antigen in a double-antigen sandwich assay [5]
- The rapid implementation of SARS-CoV-2 serological antibody assays prevented the completion of adequate technical method validation, including the assessment of analytical interferences [6].
- Immunoassays are susceptible to interferences which can falsely elevate or depress measured analyte concentration [7]. The Hook effect can influence immunoassay performance by producing falsely low results, whilst the presence of paraprotein, albumin and gamma-globulin in samples possess the potential to interfere with measured analyte concentration [8].

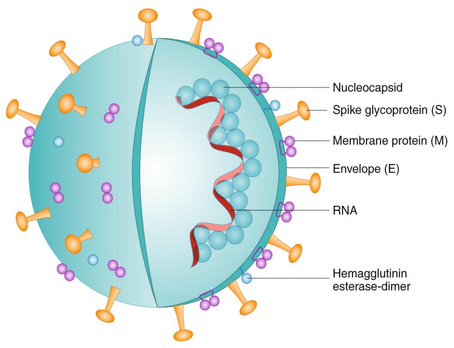

Figure 1 : Diagrammatic representation of SARS-CoV-2 structure. The RNA virus has four structural proteins, including spike and nucleocapsid proteins which are targets for humoral immune response [9].

Aim:

- The aim of this study was to evaluate the analytical performance of the Roche Elecsys Anti-SARS-CoV-2 immunoassay by assessing the interference of paraprotein, albumin and gamma-globulin.

2. Materials and Methods

Sample Collection: 18 paraprotein samples, 24 positive and 15 negative SARS-CoV-2 antibody samples were selected. A pool of negative sera was prepared.

Laboratory Measurements: SARS-CoV-2 antibody titre was measured using the Elecsys Anti-SARS-CoV-2 immunoassay on the Roche Cobas e 801 module. Results are given as a single result (cut off index (COI)). Total protein and albumin measurements were completed on the Roche c 702 module.

Linearity Assessment: A doubling dilutions series using SARS-CoV-2 antibody positive samples (n=3) producing neat, 1/2, 1/4, 1/8, 1/16, 1/32 dilutions.

Paraprotein Interference: 1/5 dilutions of SARS-CoV-2 antibody positive (n=15) and negative (n=15) samples with paraprotein and negative diluents.

Dilution with Paraprotein: Doubling dilutions series using SARS-CoV-2 antibody positive (n=3) samples in paraprotein and negative diluents producing neat, 1/2, 1/4, 1/8 and 1/16 dilutions.

Albumin and Gamma-Globulin Interference: 100 g/L stock solutions of albumin and gamma-globulin were prepared. Solutions of increasing protein concentration were produced, to which SARS-CoV-2 antibody positive serum was added.

Precision Study: Six 1/6 dilutions of SARS-CoV-2 antibody positive serum and 0.9% sodium chloride.

Data Analysis: Dependent *T*-test determined the significance between groups in paraprotein interference study and factorial repeated measures ANOVA evaluated paraprotein isotypes; completed on SPSS. Coefficient of variation was calculated as standard deviation divided by mean and the *F* values were determined using an *F*-test in Microsoft Excel.

3. Results

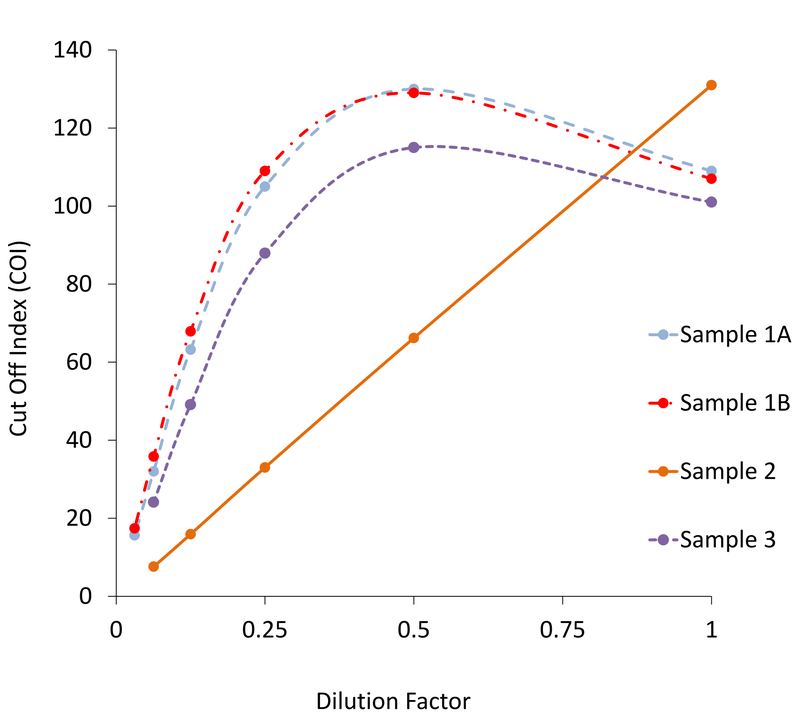

Figure 2: Dilution curve profiles for three SARS-CoV-2 antibody positive serum samples.

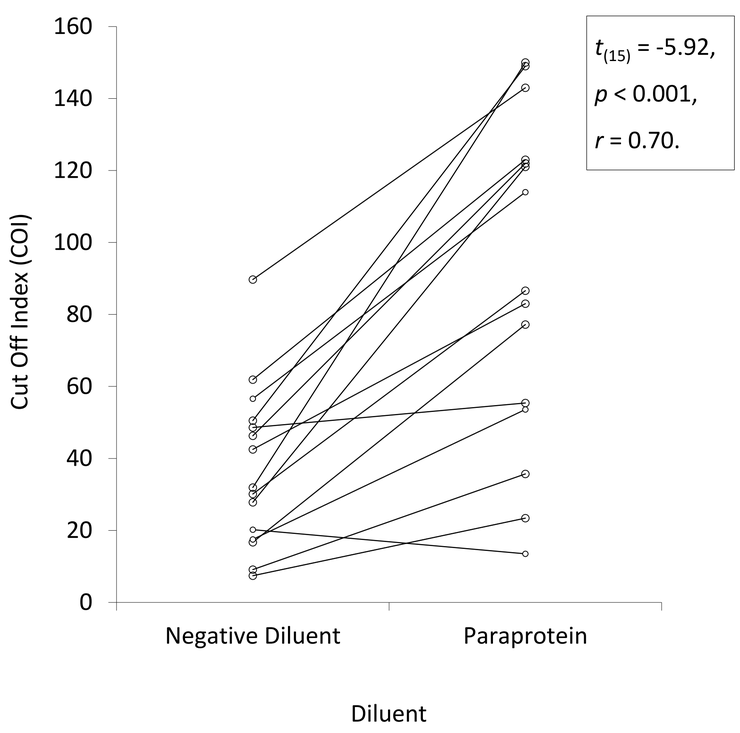

Figure 3: COI measurements for SARS-CoV-2 antibody positive samples (n=15) when negative diluent and paraprotein were added.

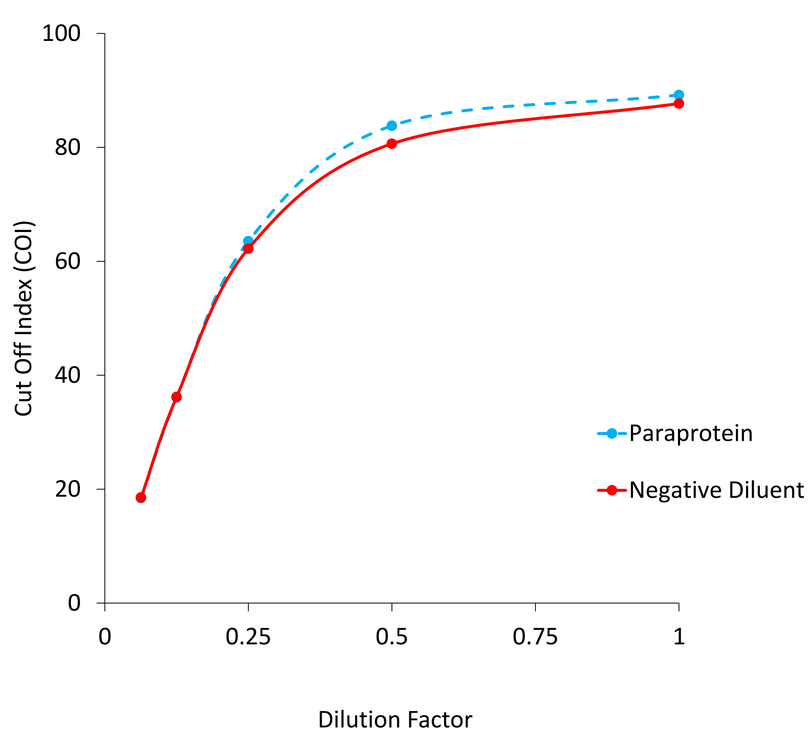

Figure 4: Mean COI of three SARS-CoV-2 antibody positive serum samples diluted in negative diluent and paraprotein.

4. Conclusions

- The Elecsys Anti-SARS-CoV-2 immunoassay did not produce a linear dilution pattern.
- Lau *et al*., [10] identified linearity in the Elecsys Anti-SARS-CoV-2 assay for COI values between 1.0 and 90.8, after which a curvilinear dilution pattern was observed.
- Not all paraproteins elicited analytical interference.
- It is likely that mechanisms behind paraprotein interference are specific to the unique properties of each paraprotein, as suggested by Kemble, Lamothe and Uhl [11].
- There was no evidence of interference with albumin or gamma-globulin. Comparison to precision study suggested an alternative unidentified source of interference may be present in samples.

Limitations:

- Not all components of serum were controlled.
- Limited sample availability.
- Change in reagent lot number resulted in lack of consistency in results.

Further Experiments:

- Gel filtration chromatography can be used to separate paraproteins from samples, controlling all other components of serum.
- Evaluation of other possible interferants, such as calcium or other antibodies which may be present in the serum.
- Investigate the same analytical interferences on an alternative analyser.

5. References

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