

Difference in hemoglobin %A2 between homozygous hemoglobin A and hemoglobin S-trait patients as measured by capillary electrophoresis

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Introduction/Objective: Quantitation by high-performance liquid chromatography (HPLC) of hemoglobin %A2 is often not used in evaluation for thalassemia of hemoglobin S-trait patients, due to analytical interference from glycosylated hemoglobin S1d to increase %A2. In contrast, an increase in %A2 for S-trait when measured by capillary electrophoresis (CE) has been reported, without known analytical interference. This observation has not been re-evaluated in modern versions of CE, however. For validation exercises associated with startup of CE at our institution, we compared distributions of %A2 among A patients and S-trait patients using Sebia “Capillaries® 2” CE.

Methods/Case Report: %A2 is provided in two Sebia “Capillaries® 2” methods: analysis of A1c (method 1, M1) and analysis of hemoglobin variants (method 2, M2). To minimize effect of potential preselection for thalassemia among M2 samples, we first evaluated distributions of %A2 for A and S-trait among M1 samples. We then evaluated correlation of A2 measurements between M1 and M2. Statistical analyses were conducted using R programming.

Results (if a Case Study enter NA): Using M1, %A2 for S-trait patients ($2.61 \pm 0.31\%$, $n=116$) was higher than for A patients ($2.11 \pm 0.27\%$, $n=108$) ($p < 0.001$), with difference = $0.42-0.57\%$ %A2 (95% confidence interval, CI). %A2 by M1 was consistently less than %A2 by M2, for both A and S-trait ($p > 0.25$): for A, $M1/M2 = 0.89 \pm 0.05$ ($n=35$); for S-trait, $M1/M2 = 0.88 \pm 0.05$ ($n=32$). Decreased %A2 by M1 compared to M2 may in part be due to separation in M1 of a glycosylated form of A2. Using M2, %A2 for S-trait patients ($3.05 \pm 0.29\%$, $n=32$) was higher than for A patients ($2.41 \pm 0.29\%$, $n=35$) ($p < 0.001$), with difference = $0.50-0.76\%$ %A2 (CI). M2 results were consistent with M1 data when combined with the observed M1/M2 ratios.

Conclusion: Results suggest a physiological increase in %A2 in S-trait patients compared to A patients, not likely to be attributable to thalassemia. The average increase is $\sim 0.6\%$ %A2 for hemoglobin variant analysis by CE.

Longitudinal Study of SARS-CoV-2 Antibody Characteristics Using Label-Free Immunoassays

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Introduction/Objective: Since the start of the COVID-19 pandemic, much research has focused on the kinetics and magnitude of humoral immune response. With the advantages of monitoring real-time immunoreactions, label-free immunoassay (LFIA) is becoming a powerful tool in serology studies. We have developed LFIA to measure SARS-CoV-2 antibody avidity and neutralization activity in a cohort of COVID-19 patients and determine if they correlate with antibody concentration. Serial serum samples collected from mild to severe COVID-19 patients were measured out to 8 months post-symptom onset to determine the durability of the neutralizing antibody response.

Methods/Case Report: Based on thin-film interferometry technology, we established a label-free IgG avidity assay and a label-free surrogate virus neutralization test (LF-sVNT). For measurement, sensing probes pre-coated with receptor-binding domain (RBD) of SARS-CoV-2 spike protein are applied to serum samples containing SARS-CoV-2 antibodies. The label-free IgG avidity assay measures the binding strength between RBD and IgG under urea dissociation. The LF-sVNT analyzes the binding ability of RBD to ACE2 after neutralizing RBD with antibodies.

Results (if a Case Study enter NA): IgG avidity indices and neutralizing antibody titers (IC50) were determined from serum samples ($n=246$) from COVID-19 patients ($n=113$). IgG concentrations were measured using a fluorescent immunoassay. The neutralizing antibody titers showed a weak correlation with IgG concentrations and no correlation with IgG avidity indices. Over the time course up to 8 months post-symptom onset, IgG concentrations and neutralizing antibody titers presented similar trends: an initial rise, plateau and then in some cases a gradual decline after 40 days. The IgG avidity indices, in the same cases, plateaued after the initial rise.

Conclusion: The results demonstrated that LFIA could be used an excellent solution in the determination of SARS-CoV-2 antibody characteristics. The study found that IgG concentration and neutralizing antibody titer declined over time, while IgG avidity index remained constant after reaching a plateau. The decline of antibody neutralization activity can be attributed to the reduction in antibody quantity rather than the deterioration of antibody quality, as measured by antibody avidity.

Proficiency Testing Performance for Point of Care Glucose Users in a Tertiary Hospital in Kenya

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