Application of the Euroimmun anti-SARS-CoV-2-S1-IgG ELISA antibody test to dried blood spots

The qualitative Euroimmun anti-SARS-CoV-2-S1-IgG ELISA antibody test has been most commonly used in the analysis of serum samples since early 2020. In the population-based seroepidemiological study 'CORONA-MONITORING bundesweit' (RKI-SOEP study) [1], however, it is used to analyse dried blood spots (DBS). A method study was therefore conducted comparing serum with dried blood, embedded in the 'CORONA-MONITORING lokal' study [2]. This method study comprised 276 individuals who had participated both in the baseline survey in May/June 2020 and in the follow-up survey of the study 'CORONA-MONITORING lokal' in October 2020. The sample was made up of individuals who either had a positive or indeterminate IgG test result in serum measurements at the time of the baseline survey (n = 265) or had a negative test result but reported a positive PCR test before the baseline survey in the questionnaire (n = 11).

Study execution and laboratory methods

During the follow-up survey, the study team collected both a venous blood sample, which was processed into serum, and a capillary blood sample, which was processed into dried blood. Both samples were tested for IgG antibodies using the anti-SARS-CoV-2-S1-IgG ELISA (trade name “Anti-SARS-CoV-2 ELISA (IgG)“, Euroimmun AG, Lübeck, Germany, batch E200518BC). The results of this test are semi-quantitative ratio values which were classified for serum samples using the manufacturer-supplied cutpoints (positive: ratio ≥ 1.1; indeterminate: 0.8 ≤ ratio < 1.1, negative: ratio < 0.8).

Statistical analysis

The aim of the analysis was to examine the test characteristics of the IgG test based on DBS compared to serum samples and, if appropriate, to derive a cutpoint adapted to dried blood so that the seroprevalence based on dried blood is comparable to a seroprevalence based on serum samples. The categorization used was 'positive' versus 'non-positive' (negative or indeterminate). Results of the serum measurement using the manufacturer-supplied cutpoints were regarded as the gold standard for the present analysis.

On the one hand, the adapted cutpoint was determined by minimizing the misclassification rate. To this purpose, cutpoints in the range 0.7−1.1 were used to classify the dried blood ratio values. This range was chosen since first analyses showed that dried blood spot samples yielded somewhat lower ratio values than serum samples. For each cutpoint, the proportion of misclassified DBS test results in comparison to serum results was determined, i.e. the proportion of all dried blood samples that were classified differently from the corresponding serum sample. Confidence intervals for the proportion of misclassified DBS test results were calculated using the Wilson score method [3,4].

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On the other hand, a correction formula was estimated to predict serum ratio values from DBS ratio values, and the cutpoint was converted using this formula. The correction formula was estimated via piecewise linear regression, with the ranges for the piecewise regression defined by examining residual plots.

Results

The measurements performed with dried blood (mean value 1.52; range 0.09–6.97) yielded slightly lower ratio values compared to the results from serum (mean value 1.68; range 0.11-6.72). Half of the serum samples collected in the follow-up survey were IgG positive (Table 1).[1] Overall, the proportion of DBS samples misclassified was 5.1% compared to the corresponding serum sample, applying the manufacturer-supplied cutpoint to the DBS samples (14 of 276 dried blood samples were misclassified, 95% CI: 3.0–8.3%) (see Table 1). All misclassifications were false negative categorizations (10.1% of 138 positives in serum were categorized as negative in the DBS sample, 95% CI: 6.1–16.3%).

Result of serum sample	Result of dried blood spot sample		
	Positive (≥ 1.1)	Non-positive (< 1.1)	**Total**
Positive (≥ 1.1)	124 (89.9%)	14 (10.1%)	138
Non-positive (< 1.1)	0 (0%)	138 (100%)	138
Total	124	152	276

Table 1: *Categorized IgG measurement in serum vs. categorized IgG measurement in dried blood spot using the manufacturer-supplied cutpoint (number, row percentage)*

The minimum misclassification over all cutpoints tested was 2.9% (8 of 276 samples misclassified, 95% CI: 1.5–5.6%). It was reached with a cutpoint of 0.94 and 0.95, respectively (see Table 2; the categorizations for these two cutpoints were identical). With this cutpoint, false positive and false negative misclassifications occurred with equal frequency.

[1] Differences to the baseline IgG categorization may be explained by two factors: (1) waning of antibodies between baseline and follow-up; (2) use of a different test batch.

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Result of serum sample	Result of dried blood spot sample		
	Positive (≥ 0.94)	Non-positive (< 0.94)	**Total**
Positive (≥ 1.1)	134 (97.1%)	4 (2.9%)	138
Non-positive (< 1.1)	4 (2.9%)	134 (97.1%)	138
Total	138	138	276

Table 2: *Categorized IgG measurement in serum vs. categorized IgG measurement in dried blood spot using the cutpoint that minimizes the overall misclassification rate (number, row percentage)*

As another way to establish a cutpoint, a correction formula was derived to convert the DBS values into serum values. This resulted in a good model fit when using piecewise linear regression:

(1) For DBS values < 0.19 (n = 8):

predicted serum ratio value = DBS value

(2) For DBS values from 0.19 to 2.2 (relevant range for the categorization into positive/negative), the following applies (n=201):

predicted serum ratio value = 0.074 + 1.093 × DBS ratio value

The explained variance (R^2) in this range is 95.5%. The intercept (0.074) has a standard error of 0.0169, and the slope parameter (1.093) has a standard error of 0.017.

(3) For DBS values > 2.2 the following applies (n=67):

predicted serum ratio value = 0.166 + 1.013 × DBS ratio value

The explained variance (R^2) for these high DBS values is 92.1%. The intercept (0.166) has a standard error of 0.015 and is therefore not significantly different from zero. The slope parameter (1.013) has a standard error of 0.037 and is not significantly different from 1.

Figure 1 (left panel) shows the data points together with the estimated regression line. The right panel of the figure examines the agreement between measured serum ratio values and the serum ratio values predicted from the regression on DBS ratio values, using a Bland-Altman plot [5]. For this plot, the difference between the measured value and the predicted value is plotted against the mean of the two values. The plot indicates a uniform distribution of differences around zero throughout the range of values with only a small number of outliers, indicating a good model fit.

According to the correction formula (2), a DBS ratio value of 0.94 corresponds to a serum ratio value of 1.1 (by inverting the above regression equation: $(1.1 - 0.074)/1.093 = 0.939$). Thus, this method yields an adjusted cutpoint of 0.94 for dried blood spot samples.

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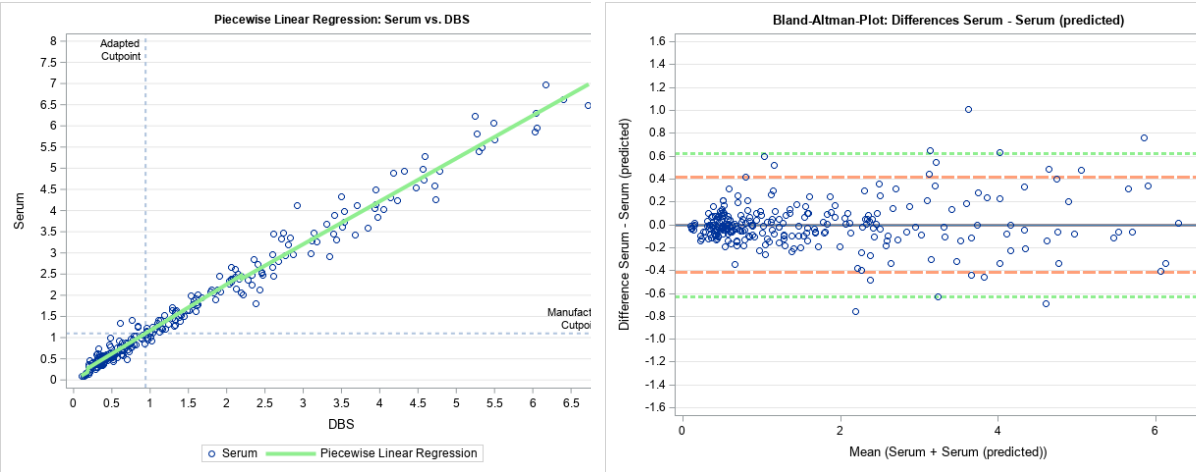

Figure 1: Left panel: *Data points and piecewise regression line for the regression of serum IgG ratio values on DBS IgG ratio values.* ***Right panel:*** *Bland-Altman plot of the difference between the measured serum IgG ratio value and the value predicted by the regression model against the mean of the two values. The lines show the limits of agreement (red, dashed line: ± 2 standard deviations; green, dotted line: ± 3 standard deviations).*

Implementation in the analysis of the seroprevalence study

Both using the correction formula and by minimizing the misclassification rate, 0.94 is obtained as the adapted cutpoint for classifying dried blood spot samples as IgG positive. This cutpoint was therefore used in the evaluation of the RKI-SOEP seroprevalence study [1] to classify the ratio values of the Euroimmun IgG antibody test in dried blood spot samples.

References

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