Preanalytical Quality Check of Blood Samples

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ABSTRACT

A spectral transmission measurement method and a module for non-contact classification of serum in centrifugated, labeled blood sample tubes was investigated in a study.

The results demonstrate that classification “OK” and “Not OK” (hemolytic, icteric) of blood sample tubes with attached paper labels is possible. For the spectral detection of lipemic samples, directing a laser beam through the serum and analyzing the scattered light pattern of a video camera image of the tube is suggested.

INDROTUCTION

Prior to diagnostics of centrifugated blood samples, it has to be observed, if the sample tubes *(Figure 1)* possess a hemolytic (H), icteric (L) or lipemic (I) distortion of the serum *(Figure 2)* beyond a threshold value, as these distortions interfere with diagnostics and require special attention. This classification can be performed by staff by visual inspection.

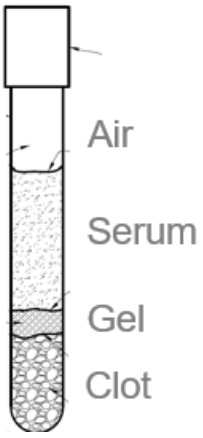

Figure 1: *Schematic of a centrifugated blood sample with characteristic compounds.*

Hemolytic and icteric blood samples exhibit characteristic colors in the visible region, significantly differing from “OK” spectra. On the other hand, lipemic samples appear cloudy, but do not change color.

In this work, we study the possibility to perform this classification by spectral absorbance measurement.

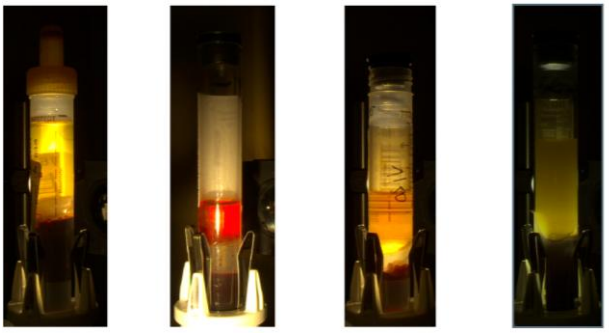

Figure 2: *From left to right „OK“, hemolytic, icteric and lipemic centrifugated blood samples (camera images of some samples investigated).*

PROBLEM STATEMENT

Classification shall be possible, if paper labels, applied to blood sample tubes in laboratories, cover up the serum content.

TECHNICAL SOLUTION

In this work, we study the possibility to perform the classification of serum automatically, in-line, non-contact by spectral absorbance measurement. An optical proof-of-concept module according to the suggested technical solution is indicated in *figure 3* and has been set up for the measurement of spectral absorbance of serum.

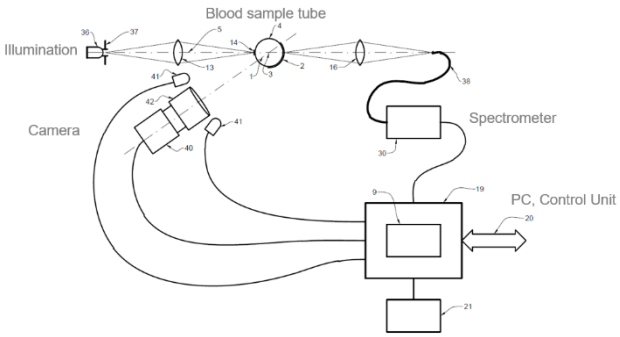

Figure 3: *Experimental setup for proof-of-concept testing.*

The setup also incorporated a camera for documentation purposes *(Figure 3)*. The results of one of our studies on the classification by spectral absorbance measurement are presented below. The absorbance spectra of a set of tubes *(Table 1)* were evaluated by PCA and additionally with a regression method (PLRS).

EXPERIMENTAL DATA

The setup utilized for the measurements is shown in *figure 4*.

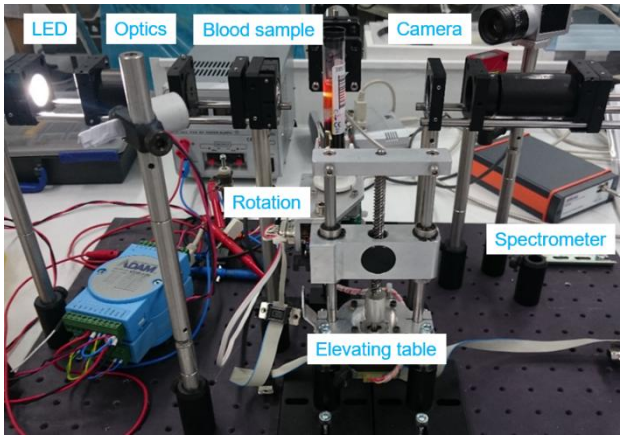

Figure 4: *Experimental setup.*

Measurements of 279 blood sample tubes (BD Vacutainer, 8,5ml; Sarstedt Monovette, 7,5ml) have been performed in a diagnostic laboratory with an experimental setup, as sketched in *figure 3*. (Spectrometer: AvaSpec-Mini2048CL-V25; Illumination Osram_dragoneye_led _spot_de1_-w4f-830_G3).

Hemolytic samples (low / medium / high): 36 / 24 / 21
Lipemic samples (low / medium / high): 24 / 27 / 30
Icteric samples (low / medium / high): 33 / 30 / 12
OK samples: 42

Table 1: *Investigated set of 279 centrifugated blood sample tubes, classification by laboratory staff.*

As a first step, the samples investigated were classified for reference by laboratory staff and the absorbance spectra were measured.

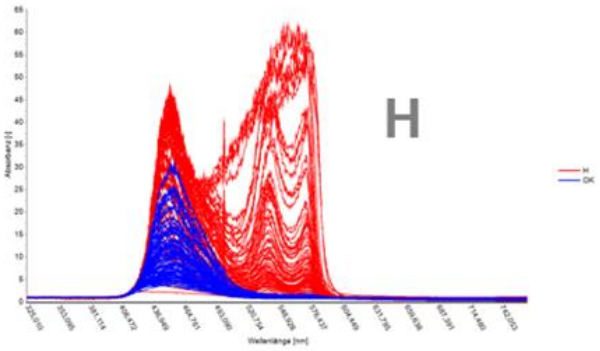

Figure 5: *Red: Absorbance spectra of investigated samples, classified as hemolytic "H" prior to measurements (varying grade). Blue: OK spectra.*

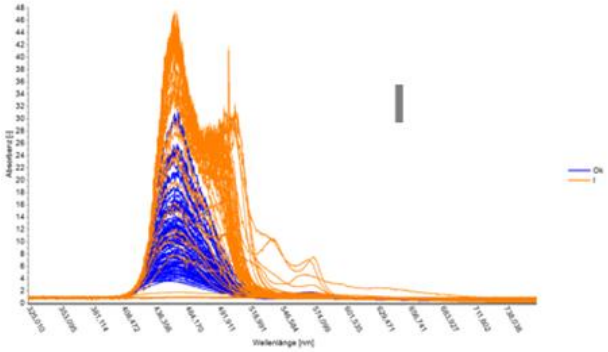

Figure 6: *Yellow: Absorbance spectra of investigated samples, classified as icteric "I" prior to measurements (varying grade). Blue: OK spectra.*

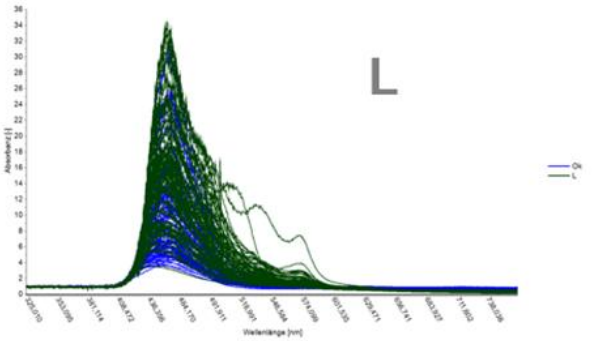

Figure 7: *Green: Absorbance spectra of investigated samples, classified as lipemic "L" prior to measurement (varying grade). Blue: OK spectra.*

The spectral indicates characteristic structure of absorbance spectra if hemolytic *(Figure 5)* and icteric *(Figure 6)*. They differ significantly from "OK" spectra (blue color). This is not the case for the spectra of lipemic samples in *figure 7*.

The measured absorbance spectra were then evaluated by PCA. The results are presented in *figure 8*.

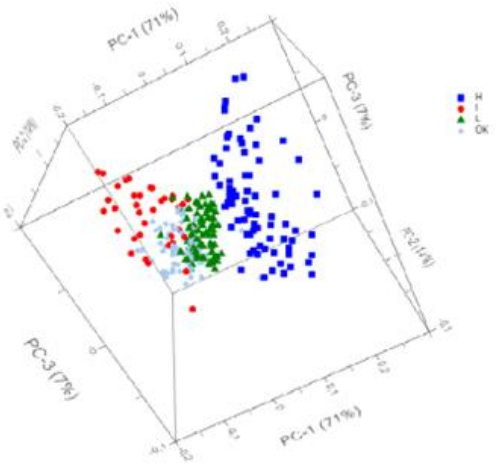

Figure 8: *Principal Components Analysis (PCA) of measured spectra of tube set of Table 1.*

By PCA, spectra of samples, classified as “hemolytic” (blue square) could be distinguished from OK spectra (light blue triangles).

Also, most spectra of icteric samples could be distinguished from OK spectra and hemolytic spectra. Lipemic samples could not be separated well from OK samples by PCA.

Alternatively, the spectra were evaluated by a regression method (PLRS) and classified by comparison to a threshold value *(Figure 9, Figure 10, Figure 11).*

In these figures, the predicted values are plotted vs. concentrations in the serum, which were obtained by chemical analysis.

The samples were then classified in relation to defined threshold values (H: >50mg/dl hemoglobin , I: >2,5 mg/dl bilirubin; L: Cholesterol, no threshold specified).

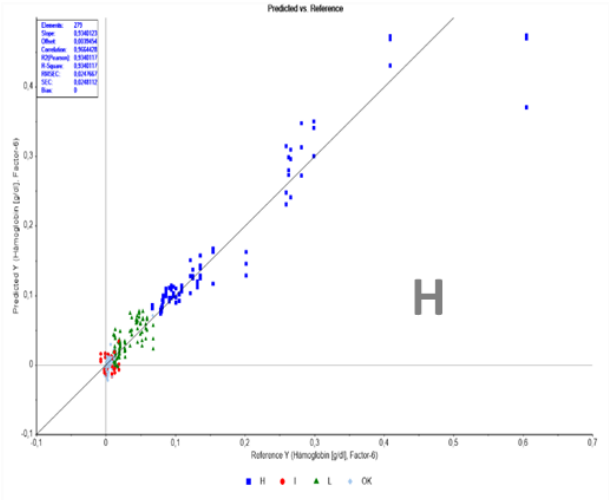

Figure 9: *PLRS: Hemolytic samples, plot of predicted vs. analytics value.*

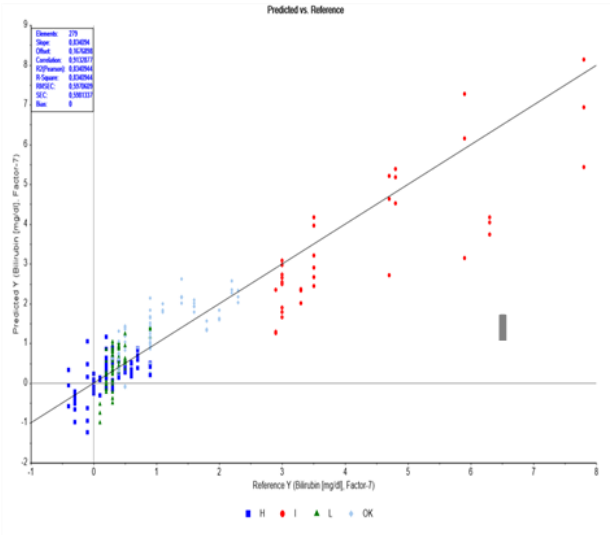

Figure 10: *PLRS; Icteric samples, plot of predicted vs. measured concentration.*

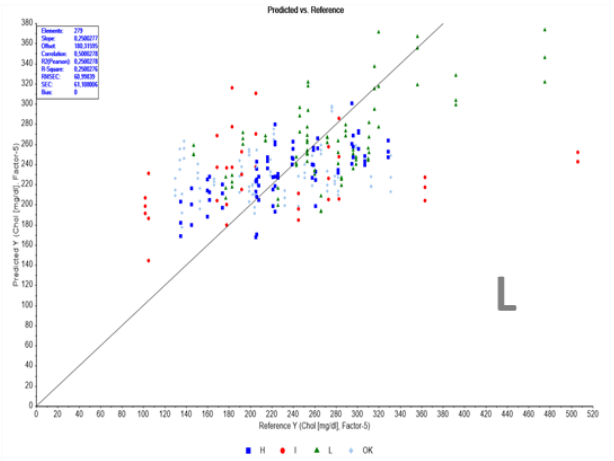

Figure 11: *PLRS; Lipemic samples, plot of predicted vs. measured concentration.*

Results of PLSR regression with threshold value:

It was possible to classify tubes wrapped in up to 2 layers of labels (e.g., two paper layers on the front side, two layers on the back side of the tube). Integration time, mean value: no layer: 120ms, 2 layers: 850ms).

94% of hemolytic samples were classified correctly (threshold). 95% of icteric samples were classified correctly.

And as it was already found for PCA analysis, it was not possible to distinguish lipemic samples from “OK” samples by PLSR.

Alternatively, it is suggested to direct a laser beam onto the sample and to evaluate the scattering patterns by image analysis.

As sketched in *figure 12 (A)*, a laser beam is directed at an angle to the sample tube through the serum. This requires a gap in the labelling.

B: tube without labels, clear serum, laser point when entering and exiting the tube visible in camera image;

C: label at front, classification not possible;

D: label at back, clear serum;

E: Lipemic sample, laser is scattered when entering and exiting the tube and in between by cloudy serum (*Figure 12*, Image on the right: experimental setup with a dummy cloudy sample tube).

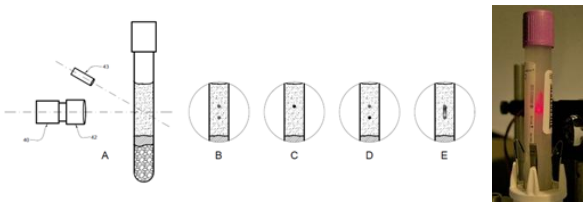

Figure 12: *Setup for automatic detection of lipemic samples (gap between labels on tube is required).*

A possible layout of an in-line module for classification of centrifugated blood samples, utilizing the presented measuring principle, is sketched in *figure 13.*

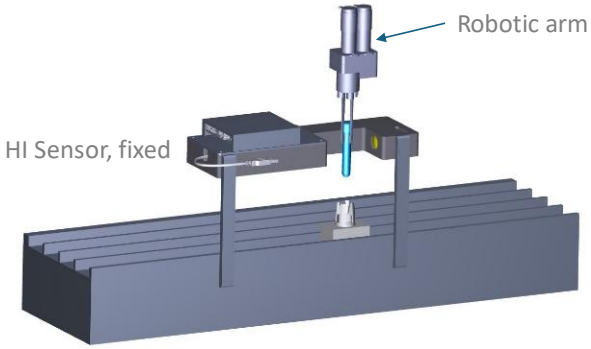

Figure 13: *In-line module for the classification of centrifugated blood samples (3D-study).*

Still to be determined are methods and strategies to diminish the influence of individually colored labels, of barcodes and handwritings on labels. Possible solutions are the correction for absorbance spectra of labels, stored in a data base and/or taking the mean value of absorbance spectra, measured at different heights of the labels.

CONCLUSION

In this study, the classification of centrifugated blood samples concerning their hemolytic and icteric distortion was possible by the presented setup utilizing spectral absorbance measurements and standard chemometric analysis methods with an accuracy of up to 95% of correctly classified hemolytic and icteric samples.

The best result was achieved by evaluation of the measured absorbance spectra by PLRS versus a threshold value.

To identify lipemic samples, it is suggested to analyze the scattering pattern of a laser beam, directed onto the serum.

In this study, it was possible to classify tubes with up to 4 layers (2 at the front, 2 at the back of the tubes) of paper labels.