Comparison of two spectrophotometers with DNA concentration measures from total blood

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Introduction

The Etablissement Français du Sang manages the French transfusion network. One of its sectors is the HLA Laboratory (for Human Leukocyte Antigen). This Laboratory studies the transplant compatibility: bone marrow donation, hepatic and heart transplants. This training period consisted in comparing two spectrophotometers by means of DNA concentrations. DNA was extracted from total blood, collected with EDTA, which is an anticoagulant.

Materials and Experimental Conditions

The first spectrophotometer was an "Amersham Bioscience GeneQuant Pro", a classic UV-visible spectrophotometer. The second one was a Nanodrop 2000c, designer Thermo Science.
The DNA optical density was measured at a wavelength of 260 nm, wavelength which corresponded to the highest absorbance of DNA. Only the first spectrophotometer required a tenth dilution to perform the DNA absorbance measures: 15 µl of DNA added to 135 µl of elution buffer.
In a Falcon tube, 2 mL of total blood collected with ETDA and 2.5 mL of lyse buffer (its aim is to attack the cellular membrane) were added to 300 µL of proteinase K (protein's aim is to dissolve the nuclear membrane). After agitation and incubation at water bath (56°C) during 5 minutes, 2.5 mL of absolute ethanol were added to the blend. The last step consisted in introducing this blend in the extractor. By pressure and piston system, the technician got back the DNA sample.

Statistical approach

In the first instance, both spectrophotometers were compared with five different DNA. Then, reproducibility of both spectrophotometers was studied during one week with the same DNA sample.
Before analysing the results, the experimental data needed to be corrected. Indeed, during the tests, some errors were visible.
With a statistical test, these absurd values could be deleted, especially with Box and Whiskers Plot. All the values which were not in the Box and Whiskers plot were absurd.

Statistical approach

According to Fig.1, the HLA averages were not really away from the Nanodrop averages. To see if both methods were equivalent, statistical tests (Fisher, Cochran and Standard Normal Law) were performed. According to the average and variance comparisons, both methods were completely different. The Nanodrop 2000c is more efficient than the Amersham GeneQuant Spectrophotometer because it is more reproducible (Fig.2). The Amersham standard deviations were higher than the Nanodrop standard deviations.

Moreover, the reproducibility study over one week, with the same sample, confirmed the initial conclusion (Table1).
CV_R^{HLA} was more important than CV_R^{Nano}, and CV_R^{HLA} was higher than 10% so a day effect existed with the Amersham Spectrophotometer. Between each day of the study, the DNA sample was kept on refrigerator.

Fig 1. *A bar chart about the Averages comparison obtained with five different DNA.*

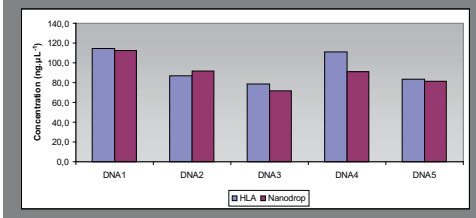

Fig 2. *Standard deviation comparison of both spectrophotometers.*

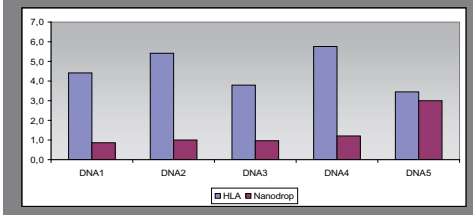

Table 1. Detection of the day effect.

	S^2_R	S^2_g	S^2_r	CV_R (%)
HLA	215.51	187.7	29.82	16.9
Nanodrop	0.689	0.353	0.336	0.91

S^2R: Variance of intermediate accuracy
S^2g: Variance of intermediate "inter-day"
S^2r: Variance of repeatability
CV_R: Coefficient of variation of intermediate accuracy

Conclusion

To sum up, the Nanodrop 2000c Spectrophotometer was more efficient than the Amersham Spectrophotometer in terms of reproducibility. The DNA concentration is really important because with this DNA, technicians make numerous of experimentation to determine the transplant compatibility, so it is important to have a good value of concentration. I recommended to the HLA Laboratory to change their Amersham Spectrophotometer for the Nanodrop 2000c Spectrophotometer.

ÉTABLISSEMENT FRANÇAIS DU SANG

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