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Homologous Blood Transfusion Proficiency tests

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INTRODUCTION

Blood doping aims to improve oxygen transport capacity and therefore physical performance. The main doping methods to increase the total red blood cell mass are recombinant EPO and blood transfusions abuse [1;2]. Since 2001, recombinant EPO can be directly detected in urine. For that reason transfusions gained interest. Since 2004, homologous transfusion (also referred to as allogeneic blood transfusion) can be detected by anti-doping laboratories. Blood compatible for A, B and Rh(D) blood groups is transfused, but in most cases, differences exist for minor RBC antigens. Analyses of RBC antigenic expression pattern by flow cytometry are able to reveal the presence of mixed RBC populations in transfused recipients [3]. Other blood doping strategies can only be limited by using a no-start rule using haematological parameters [4].

Each of the participating laboratories validated their respective method, based on the same approach, following WADA International Standards for Laboratories and ISO17025 guidelines [5].

Beginning 2005, the organisation of an inter-laboratory proficiency test was necessary. Most of the laboratories were using a common methodological approach to identify a positive

sample, but there were some differences between the methods used. The aim of this proficiency test was to evaluate if these differences would lead to various interpretations and sensitivities. The Lausanne transfusion medicine department permitted the use of bloods from the Lausanne Red-Cross blood bank to prepare samples for this proficiency test.

Thus, double-blind, randomised multi-site proficiency study was organised for all laboratories. The Lausanne laboratory sent all samples to the other laboratories and collected all results. Then data from the Lausanne transfusion medicine were obtained and compared to the results returned by all five laboratories.

SAMPLE PREPARATION

For our proficiency test, blood samples completely phenotyped by the higher sensitivity agglutination test were used. None of these bloods expressed blood groups A, B or Rh(D). The healthy blood donors had agreed that their blood could be used for research purposes. The hospital transfusion department prepared, for each laboratory, the same eight samples. Five contained mixed blood from two donors (positive samples) and three single donor's blood (negative samples). Among the five positive samples, one contained only 0.5% of minor population, the others contained 1.5%, 3% (two samples) or 5% respectively (see table 1). This percentage is expressed as blood volume and not as RBC counts. As each healthy donor had an hematocrit value between 35% and 50%, comparison of sample using RBC count-based percentage was not possible.

Table 1 : Sample description. All bloods were O Rh(D) negative and were phenotyped by two independent users with two different methods. *: These numbers are those given by the Transfusion medicine department and were not the sample code received by the laboratories. †: These letters enabled us to access to the phenotype of each in order to predict the results. ‡: Blood volume percentage (not RBC counts percentage).

Sample # *	Type	Blood 1 †	Blood 2 †	Percent of blood 2 ‡
1	Positive	A	G	0.5
2	Positive	C	H	3
3	Positive	J	D	5
4	Positive	B	I	3
5	Positive	F	E	1.5
6	Negative	D	-	-
7	Negative	I	-	-
8	Negative	G	-	-

Before all results returned, none of the data presented in Table 1 was accessible to any of the laboratories.

Also, before sending the samples to the laboratories, the transfusion medicine department gave different codes to each samples, to each laboratory. In that way, this proficiency test was a double-blind, randomised, multi-site study.

SHIPMENT OF SAMPLES

All samples were sent by WorldCourier on Monday, 2005, 23rd of May. All packages were sent with refrigeration and isolation devices and the transporter ensured optimal temperature during shipment. The aim was to kept the samples between 2°C and 8°C. Except for Montreal's package, all arrived the day after (see table 2). Montreal received the package only on Friday, 2005, 27th of May, after 4 days of travel. Montreal were also the only laboratory to measure 8°C inside the package, all other laboratories having received a package with an inside temperature close to 4°C (see table 2).

Table 2 : Shipment delays and temperatures estimation on arrival of the parcel. The delivery to Lausanne laboratory was made directly by the transfusion medicine department's personal.
‡: The temperature were measured upon arrivals.

Laboratory	Delay [days] [†]	Temperature ‡
Athens	1	~4°C
Barcelona	1	~4°C
Lausanne	0	~4°C
Montreal	4	~8°C
Paris	1	~4°C

ANALYSES BY LABORATORIES

All laboratories stored the samples overnight in a refrigerator before starting the screening analyses. Screening analyses lasted between 1 and 4 days, with a mean \pm SD of 1.6 \pm 1.3 days. As five samples were positive, a lot of work was necessary for the confirmation. The overall confirmation processes took between 6 and 17 days, with a mean \pm SD of 9.8 \pm 4.2 days. As soon as the last results were obtained, Lausanne laboratory sent a formulary to centralise all interpretation results and asked for a common formulation of results in order to facilitate data management and information treatments.

Results were reported as the following : single or double population, with a minor peak expressing or not the corresponding antigens. For each sample, if no antigens presented a double population, the sample was negative. If only one antigen presented a double population, the sample was declared suspicious. If two or more antigens presented a double population, the sample was considered positive. Minimum threshold to consider that a minor peak represented a minor population varied among laboratories. More details about these differences between laboratories will be published elsewhere. Figure 1 shows different histograms obtained by different laboratories for the two types of mixed red blood cells (RBC) population. As can be seen, the area of the minor peak, the position and the background noise varied between laboratories. But for all of them a double population was observed, demonstrating that fine details of the experimental protocol used to obtain the result are less important than the global approach. Controls and flow cytometry principles are the same for all laboratories, but reagents and definition of minor population are, because of that, left to each laboratory through their own validation process.

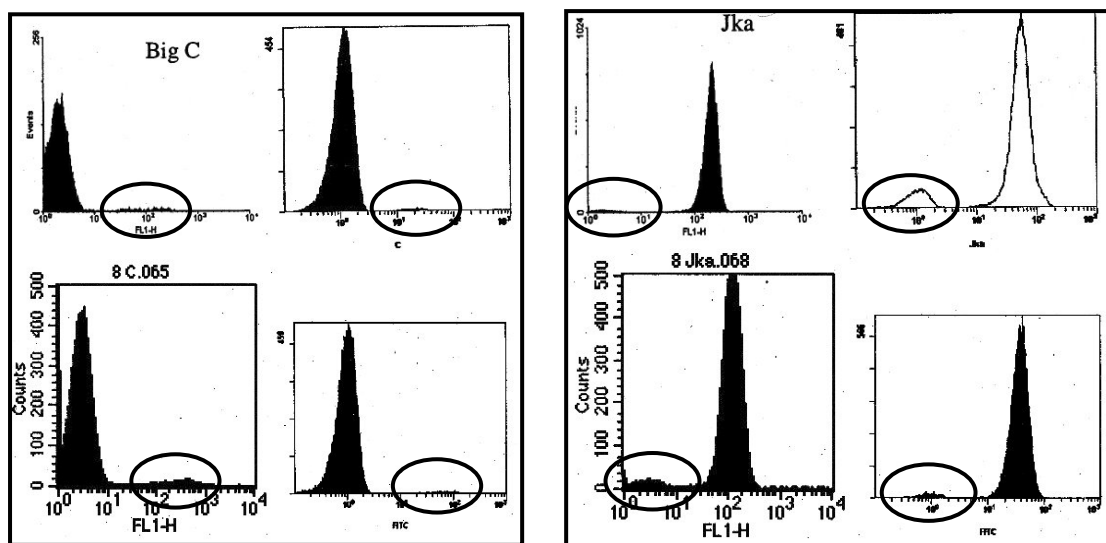


Figure 1 : Same antigens from same samples gave different peak shape and positions in the corresponding histograms, but a major and a minor peak were observed in all histograms.

RESULTS CENTRALISATION AND TREATMENT

The first part of this work was to identify all results and the correspondent sample. Using the data of each donor blood used to prepare sample, results were predicted for each samples.

All data were entered in Excel and double-checked. Then automatic comparison was made. Each laboratory's results were classified compared to the predicted results. Using the graphical results and this results, sources of some differences between laboratories could be identified as due to practical cause or to interpretation.

PROFICIENCY TEST RESULTS

All 47 single population antigens were interpreted correctly by all 5 laboratories (absence of false positive), so the specificity was 100%. Neither, none of the three negative samples were interpreted as positive or suspicious by any of the laboratories. All samples were, in that way, properly identified and there was also a specificity of 100%.

The mean sensitivity (absence of false negatives) at the sample level was equal to 72%, based on 5 positive samples. At the level of individual antigens, the sensitivity was equal to 76.5% (17 double population antigens). The antigen for which a double population was always detected was antigen E. 70-90% of double population findings was accomplished for antigens C, c, Fy^a, Fy^b, Jk^a, and Jk^b.

The most difficult sample was sample #1, (positive sample with 0.5% minor RBC population). Results were interpreted as negative by one laboratory because their criteria to classify a double population was more restrictive than for the others. Three other laboratories interpreted correctly the corresponding individual antigens for this 0.5% sample, but decided to render the sample only as suspicious due to the small size of the minor peaks. The fifth laboratory unambiguously identified the sample as positive. When the percentage of second blood population was between 1.5 and 3%, four out of five laboratories unambiguously returned the sample as positive while a fifth laboratory declared it suspicious. When the second population was 5%, all five laboratories returned a positive identification.

Two laboratories used different antibodies coming from different provider which were not able to give clear proof of identification capabilities for Fy^a antigen. Unfortunately, that antigen exhibited a double population in some of the positive samples. For that reason false negative results were returned.

From the analytical point of view, differences between laboratories appeared mostly to be due to different types of flow cytometers. Some of these devices produced more background noise, rendering interpretation of results more difficult (see Figure 1).

CONCLUSION AND FURTHER DEVELOPPEMENTS

Full confidence to detect real negative samples and to identify most positive blood transfusion samples was verified. This inter-laboratory proficiency test clearly demonstrated that robust results were obtained even without a common experimental protocol. A clear positivity criteria combined with a description of confirmation principles appears to be appropriate. In order to obtain maximum sensitivity, each laboratory would have to optimise its method specifically for the reagents and the flow cytometer available. Of course, in order to register this procedure in the scope of their 17025 ISO accreditation and to finalise their validation, each laboratories has to characterise his detection limit, sensitivities and will have to prove his specificity.

An other inter-laboratory test will be organised to include other laboratories who wish to implement this method in the close future. End of 2006, apart from the authors's laboratories, the following laboratories implemented a method to detect homologous blood transfusion doping : DoCoLab (Ghent, Belgium), Laboratory for Doping Analysis (Cologne, Germany) and Mitsubishi Kagaku Bio-Clinical (Tokyo, Japan).

Later, other laboratories could also start to implement a method to detect homologous blood transfusion. In order to help them, the heads of the laboratories using flow cytometer have decided to produce a technical document for WADA under the coordination of WAADS.

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