K phenotyping using a PK-7200 automated analyzer

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K (Kell) is one of the most immunogenic of the red blood cell (RBC) antigens. In order to select K– RBC units, we developed K phenotyping on the Olympus PK-7200 equipment to save labor, time, and costs. The Olympus PK-7200 is fully automated equipment used primarily for blood typing and syphilis screening. We tested 3,587 blood donor samples in EDTA using a commercial anti-K serum diluted in HP Hemagen Power Solution^{R}(1:40). The equipment was set to prepare a 1.7% RBC suspension in bromelain and to dispense 25μL of the mixture (diluted serum and HP Hemagen Power Solution^{R}) in terraced microplates. After mixing, the microplates were incubated for 1 hour at 30°C. Reading was performed by a C.C.D. camera and the results were automatically transferred to the mainframe computer. We found 185 K+ blood samples and 3,402 K– samples. Four samples, K+ by the PK-7200, were confirmed as K– by tube test. The use of bromelain with the PK-7200 may have caused the falsely positive tests. The Olympus PK-7200, used for K phenotyping, saves labor time and costs. It also reduces handling and thus promotes less contamination risk for laboratory personnel. ***Immunohematology*** 1998;14:22–25.

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Anti-K (Kell) is the most common immune red blood cell (RBC) antibody outside the ABO and Rh blood group systems.[1] Many severe hemolytic transfusion reactions due to anti-K have been described, and it has been implicated in severe cases of hemolytic disease of the newborn.[2]

Kell blood group system antigen frequencies present important variations depending on ethnic origin.[3] The K antigen is present in approximately 9 percent of whites and in only 2 percent of North American blacks.[4] It is extremely rare in Mongoloid people of eastern Asia.[5] The highest K antigen frequency is observed among people of the Arabian and Sinai peninsulas, where up to 25 percent may be K+.[6]

The *K/k* gene frequency has been studied in different populations. In Brazilian blood donors, *K/k* gene frequencies are: in whites, $K = 0.0462$ and $k = 0.9574$; in blacks, $K = 0.0181$ and $k = 0.9819$; genotype frequencies are: *K/K* = 0.0021, *K/k* 0.0881, and *k/k* = 0.9097.[7] The K antigen is usually determined using an antiglobulin method.[8] However, there are unusual K and k phenotypes that require adsorption and elution studies in order to confirm true RBC phenotypes.[9] The weak expression of the K antigen is a rare event that occurs in the McLeod and Gerbich-negative phenotypes.[10] It can be detected also in a variety of phenotypes in which all high-frequency Kell and para-Kell antigens are depressed.[1]

There is a case report of a patient previously known to be K–k+ who became K+, as did transfused K– RBCs.[11] A gram-positive organism, *Streptococcus faecium*, was detected in the patient's blood culture. K– RBCs incubated in vitro with a culture containing *S.faecium* also were converted to K+. Because of its clinical significance, K phenotyping is performed in many blood banks for transfusion purposes.[7] The method used for K phenotyping depends on the number of donations per day and the need for K typing. The test can be performed by tube, microplate, gel test, capillary techniques, manually, or using automated equipment.[12,13]

Since 1963, when preliminary automated blood typing results were published by MacNeil et al.,[14] other equipment has been developed for routine blood banking applications. These are semi- or fully automated analyzers. All of them include a microprocessor or a microcomputer, giving greater flexibility and easier handling of data.[15,16] They can be used not only for ABO/Rh blood group typing but also for phenotyping other blood group antigens[17] and for antibody detection.[18]

We describe here our experience phenotyping for the K antigen using PK-7200 automated equipment. Basic characteristics include automated sample recognition, dispensing of reagents, incubation and settling, reading, interpretation, and printing of the results.[19]

Materials and Methods

Samples

From March to April 1996, 3,587 EDTA-anticoagulated blood donor samples were phenotyped for the K antigen in a PK-7200 automated analyzer (Olympus America,

Inc., USA) All blood samples were tested within 24 hours. Blood samples positive for diseases transmissible by blood according to Brazilian Standards for Collection, Processing, and Blood Transfusion were excluded.[20] Samples with positive screening for sickle cell trait[21] and samples with positive direct or indirect antiglobulin tests, or both, also were excluded.

Method

During initial setup of the Olympus equipment, we generated files in the basic software of the system to perform specific liquid-handling and data processing tasks according to our manual microplate protocol for K antigen phenotyping. Subsequent daily start-up operation (approximately 30 minutes) included turning on the components of the system and following instructions on the monitor of the computer. Routinely, blood samples were centrifuged (Sorvall, Dupont, USA), for 10 minutes at 2,000 rpm (1630 × g) to separate cells and plasma. Clean terraced microplates were used for testing 10 blood samples per plate. For each batch of tests, up to 10 blood samples were placed in a rack, which was moved into a sampling station. Sample and microplate identification were accomplished through bar code labels that were read and interpreted by two different built-in laser scanning devices. At the first sample dilution station, bromelain at 0.05% was added to each cup of cells. The final RBC concentration was 1.7%. We dispensed 50 μL of a RBC bromelain suspension into each microplate well. A mixture of 25μL of the HP Hemagen Power Solution^R (Hemagen Diagnostics Inc., USA) and a polyclonal anti-K (Gamma Biologicals, USA) was added into the wells with the RBC bromelain suspension. (HP Hemagen Power Solution^R [Hemagen Diagnógsticos, Brazil] is a native polymer supplemented with bovine albumin in a phosphate-buffered saline solution using thimerosal as preservative.) The anti-K was used in a concentration of 1:40. The working dilutions of antisera were determined by testing serial twofold dilutions of antisera against cells with the K+k+ phenotype. The working dilution was chosen to be two dilutions less than that which caused the last strong agglutination reaction. After all reagents and RBCs were added to the plates, they were incubated for 1 hour at 30°C. Microplate reading was performed automatically by a C.C.D. camera interpreted by the computer, and the results were stored on a floppy disk. All computer interpretation results were reviewed visually by one technologist. PK-7200 performed 240 K typings per hour. The results, once confirmed visually, were automatically transferred to the mainframe computer.

The automated results were compared to results compiled by our institution using a tube test.[22] For the tube test, polyclonal anti-K and anti-k (Gamma) were used simultaneously.

Four discrepant samples were retested using three different anti-K reagents (Ortho Diagnostics Inc.; Biotest, Germany; and DiaMed, Brazil). The four samples were also tested after treatment with 0.01 M DTT.[22]

Results

From 3,587 red cell samples studied, we found 185 K+ samples and 3,402 K– samples using the PK-7200. No discrepancy was observed when the operator compared the computer reading with the visual results. We found 181 samples K+ and 3,406 K– samples by the tube test. The results of the four samples that tested as K+ by PK-7200 but K– by tube test were confirmed as K– using three additional anti-K reagents. When the same samples were DTT-treated, they tested as K– by PK-7200. As a control, the DTT-treated cells tested as k+. There were no falsely negative results.

Discussion

In the last 5 years, quality assurance (QA) in blood banking has become a major issue in blood transfusion.[23] Therefore, it is essential that blood establishments implement effective control over manufacturing processes and systems. Automated blood grouping and phenotyping provide an important improvement in the blood bank. It helps a QA program significantly by decreasing errors, ensuring the credibility of test results, implementing effective system controls, and promoting standardization. Automation reduces human error and increases efficiency of reagents.[24,25]

Some papers have been published regarding ABO/Rh typing in semi- or fully automated equipment.[26,27] On the other hand, there is scarce information regarding phenotyping for other RBC antigens.[17,27] Phenotyped RBC units are needed for sensitized patients or to prevent alloimmunization in a selected group of patients.[28] We report here our experience using a PK-7200 automated equipment for K phenotyping. This equipment uses specially shaped microplates to detect hemagglutination reactions. It is a flexible system, making it easier to adjust dilutions and to use different commercial reagents. Computer proficiency is an asset but not a requirement, and after a short training course, the equipment is operable.

The Olympus PK-7200 automated analyzer was validated for K phenotyping in our institution after software documentation and validation for correct installation, correct sample processor volumes, correct reagent dispensing, correct bar code transfers, and correct result interpretation in accordance with our validation plan. The PK-7200 equipment saves time, performing 240 tests per hour. It also saves costs, using 1 mL of a commercial anti-K to test 1,600 samples. For the tube method, 1 mL of a commercial anti-K is sufficient to perform no more than 20 blood samples, and it takes almost 1 hour to complete testing whenever an antiglobulin phase is indicated. Compared to the tube method, K phenotyping using the PK-7200 automated analyzer offers the advantages of requiring smaller volumes of reagents, reducing handling, and promoting less contamination to the laboratory personnel, thus contributing to biosafety. It also reduces clerical errors, as the results are automatically transferred.

The falsely positive results by PK-7200 testing could have been caused by cold agglutinins present in the red cell samples. This supposition is supported by the facts that when the cells were DTT-treated, they tested as K–, and bromelain, known to enhance cold agglutinin reactivity, is added to each PK-7200 test. Further study is indicated to clarify the reason for a rare falsely positive test using the PK-7200.

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