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Quality Control in the Preanalytical Phase of Flow Cytometry

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Over the past years, flow cytometry has become widely used in the clinical laboratory. Recent developments in flow cytometry have facilitated its wider application [1]. Flow cytometric tests span the range from well-established to still emerging procedures. The quality of the analysis depends on the operator and the standardization of the procedure. In clinical chemistry, the standardization of the methods and defined standard operating procedures limit the influence of the preanalytical phase on sample collection and storage [2]; there is well-established internal and external quality control [3, 4]. However, for flow cytometry there are still preparation and system-specific differences in the results of routine analytes such as the determination of CD4 and CD8 lymphocytes [5]. Therefore, it is vital to keep the performance of flow cytometry highly accurate and reproducible within the laboratory. The preanalytical phase in these tests consists of the sample request, specimen collection, transport, identification, sample preparation and staining. 'When things go wrong, those who prepare the samples will always suspect instrument performance problems and those who operate the instrument will always suspect sample problems; about 50% of time, each will be right. ... My own bias is that in fact, sample related problems are probably more frequent, but less often recognized, than instrumental problems' [6]. Hence, the preanalytical phase is as important as the analytical stage. Sample collection should be performed in a standardized manner, e. g. after overnight fasting and under avoidance of physical stress. It is important for the determination of analytes such as platelet activation marker CD62p and CD63 that e.g. the patient refrains from smoking and is rested for at least 20 min before sample collection. For some analytes, e. g. CD4, CD8, and total lymphocytes, which show biochronological variation in peripheral blood cell counts [7], a standardized sample collection time, e. g. at 8.00 in the morning, is recommended. The medium for sample collection should be suited to the kind of cells to be analyzed. Platelets may be activated by heparin, and EDTA changes their shape from discoidal to spherical form. Platelet factor 4, released by platelets, neutralizes heparin and so the blood clots after a short time [8]. Prolonged stasis during venous puncture may activate platelets and may result in an absolute increase in blood cell counts. The same phenomenon may occur if the patient is upright instead of lying down during sample collection.

Samples should be transported to the laboratory immediately after collection. For some analyses, such as immunophenotyping, sample transport is less critical, others such as cells in cerebrospinal fluid continuously decrease in vitality. Temperatures of up to 50 °C can be observed in transport vehicles during summertime, and the temperature can drop below 0 °C during postal carriage in the winter. As a consequence samples can be affected by the variation in temperature.

Also pressure during transport by air tube and by plane can result in activation of cells. Upon arrival in the laboratory, the sample has to be identified to prevent sample mismatch. It should be visually inspected for hemolysis, clots, and the ratio of sample to anticoagulant. If the sample is stored in the refrigerator, 'refrigerator aids' may occur due to a decrease of CD4-positive lymphocytes. Data in our institute showed that samples may remain stable for 24 h [9]. Samples should be agitated gently or preferably rolled between both hands to mix before analysis. Separation gel or beads in the sample tube can absorb cells. Gradient centrifugation may cause a selective loss of CD8-positive cells and other leukocyte subpopulations. The amount of staining reagent must be adjusted, e. g. to the cell count in leukemia, and its quality has to be continuously monitored. It should contain epitope-specific antibodies and no free FITC or R-phycoerythrin. During storage, the reagent may become bleached and may be contaminated by other antibodies, bacteria, and staining reagents such as acridine orange. The incubation time, agitation, and temperature should be standardized. Lysis of the cells should be complete, and damage to leukocytes using additives should be avoided. All cells should be fixed, and the background fluorescence should be minimized [10].

These are only some of the factors that may affect the preanalytical phase of flow cytometry. Errors which occur in the preanalytical phase are difficult to identify and are often not recognized. So, quality control in this phase, in addition to quality control in the analytical and postanalytical phase, is necessary to obtain highly accurate and reproducible results despite the complexity of the system.

References

- Shapiro HM (ed): Practical Flow Cytometry. 3rd ed, New York, Wiley-Liss, 1994.
- Heins M, Heil W, Withold W: Storage of serum or whole blood samples? Effect of time and temperature on 22 serum analytes. *Eur J Clin Chem Clin Biochem* 1995;33:231-238.
- Neue Richtlinien der Bundesärztekammer. Qualitäts-sicherung der quantitativen Bestimmungen im Laboratorium. *Dtsch Arztebl* 1988;11:697-712.
- Centers for Disease Control: Regulations for implementing the clinical laboratory improvement amendments of 1988: A summary. *MMWR* 1992;1:41 (RR-2).
- Homburger HA, Rosenstock W, Paxton H, Paton ML: Assessment of interlaboratory variability of immunophenotyping; in Landay AL, Ault KA, Bauer KD, Rabinovitch PS (eds): Clinical Flow Cytometry. New York, The New York Academy of Sciences, 1993, pp 43-19.
- Muirhead KA: Establishing of quality control procedures in clinical flow cytometry; in Landay AL, Ault KA, Bauer KD, Rabinovitch PS (eds): Clinical Flow Cytometry. New York, The New York Academy of Sciences, 1993, pp 1-20.
- Bertouch JV, Roberts-Thomson PJ, Bradley J: Diurnal variation of lymphocyte subsets by monoclonal antibodies. *Br J Med* 1983;286:1171-1172.
- Narayanan S: An overview of cellular and plasma analysis: Preanalytical and analytical considerations Lecture presented in Osaka, 1994.
- Schauseil S, Grunewald RW, Rick W: Zur Bestimmung von Lymphocyten-Subpopulationen im peripheren Blut. *Internist* 1989;30:810-818.
- 10 Raffael A, Nebe T, Valet G: Grundlagen der Durchflußzytometrie; in Schmitz G, Rothe G (Hrsg): Durchflußzytometrie in der klinischen Zelldiagnostik. Stuttgart, Schattauer, 1994.

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