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**STARTING A PROFICIENCY TESTING PROGRAM
FOR FLOW CYTOMETRY: ISTANBUL EXPERIENCE**

Gülderen Yanikkaya Demirel, Faruk Topbas

Centro Laboratuvarları, Gürsel Mah. Kağıthane Cad. No: 14/3 34400 Kağıthane
İstanbul / Turkey
gydemirel@centro.com.tr, ftopbas@centro.com.tr

Abstract

Aim: There are approximately 80 flow cytometry laboratories in Turkey. Participation to proficiency testing programs abroad is expensive for these laboratories and the samples are much affected during the transportation. This program is designed to meet the need for a national proficiency testing program for flow cytometry in Turkey.

Method: A questionnaire is sent to 32 delegated flow cytometry laboratories from different parts of Turkey in order to analyze the need for such a program. Analysis of these responses provided valuable information for design of the program. Two Samples for lymphocyte subset analysis were distributed for the first survey. Whole blood samples were obtained from a voluntary blood bank donor and homogenization of samples was accomplished by mixing the blood bag on unheated shakers. Homogenizations of samples were controlled by blood counts. Two samples were sent for the first survey from the same blood bag. The first was mixed with TransFix solution (membrane stabilization solution by Cytomark, UK) at manufacturer suggested quantities and the second was diluted from this first sample.

Results: 68% of the participating laboratories sent back their results by the announced time. 21% of the participants could not receive the samples in due time even though they were delivered to their institutions in time. 11% of the participants did not return their results in due time.

Conclusions: There were several lessons taken from this first distribution, which are: Samples should not be distributed in July and August due to vacations and excess heat, each participant should be called the day after distribution to confirm they received the sample and participants should be encouraged to attend this program through lectures and publications. This scheme for proficiency testing is planned to be distributed three times a year. Other schemes will be started in 2007.

Key words

Proficiency testing, flow cytometry, inter laboratory comparison, T cell subset

1. INTRODUCTION

To produce reliable and safe results, laboratories need to have quality assurance, traceability and evidence for good practice. “Proficiency Testing” programs were performed for analytical chemistry laboratories for a long time. In recent years, in almost all areas of laboratory practice, laboratories obtained proof for the quality of their work performance by participating to proficiency testing programs.

In most of the proficiency testing programs, provider sends sample/samples to participants, participants analyze the sample and return results to provider, provider analyzes the results by using the valid statistical methods, prepare and release reports to participants so that they can evaluate their performance and comparability to other laboratories in their specific fields.

By participating in such programs, a laboratory gains proof for reliability of their results. Results, which are not in acceptable ranges, provide the laboratory an opportunity to plan a corrective action and improvement.

1.1 Proficiency testing programs in Turkey

Very few proficiency testing providers were trying to create awareness and consciousness about the benefits of proficiency testing in Turkey until the “Proficiency Tests and Inter Laboratory Comparisons” project supported by EU-MEDA under the “Support to Quality Infrastructure in Turkey” has started. As stated on the web site of this project, *the key objectives and results of this project are to assist Turkish laboratories and stakeholders, especially the Turkish accreditation body, TÜRKAK, in use of proficiency tests and inter laboratory comparisons, in order to strengthen the laboratories’ provision of known, reliable and internationally recognized laboratory services.* Serial trainings and educational seminars organized by the project leaders have helped not only medical community but also textile, oil and such different areas to create their own proficiency testing and inter laboratory comparison schemes. Our program also has been developed and improved with the knowledge gained by these trainings and seminars [1,2,3].

2 DESIGN OF THE PROGRAM

2.1 Choice of scheme

Flow cytometry is a fluorescence measurement system, which is used both for routine and research applications. Immunophenotyping of cells is the most commonly used flow cytometry application. There are some proficiency testing providers for immunophenotyping in Europe and USA, but since the cell stabilization is not an easy task, the samples are either deteriorating on transport or arriving late [4]. This has led us to become a proficiency testing provider for flow cytometry.

2.2 Immunophenotyping panel

We have decided to start with a lymphocyte subset panel. This panel is used with almost the same antibody combinations by most of the routine laboratories in Turkey and the number of antibodies that are used is already somewhat standardized.

A table from the participation form is presented below. As will be seen on this table we have asked which system and what clone they were using but we did not make any evaluations with these parameters since the numbers in each group were not enough to obtain statistically meaningful evaluations.

Table 1 – Immunophenotyping Panel

Cells/Subsets	Markers	System*	Antibodies**	Result Sample A	Result Sample B	
1	Total T hucre	CD3 ⁺	BD/BC	Brand/clone	%	%
2	T helper	CD3 ⁺ CD4 ⁺	BD/BC	Brand/clone	%	%
3	T suppressor	CD3 ⁺ CD8 ⁺	BD/BC	Brand/clone	%	%
4	NK hucreler	CD3 ⁻ CD16 ⁺ 56 ⁺	BD/BC	Brand/clone	%	%
5	Total B hucre	CD19	BD/BC	Brand/clone	%	%
6	TOTAL T+B+NK Cells			%	%	

Table 1. Abbreviations: * BD : Becton Dickinson, BC: Beckman Coulter

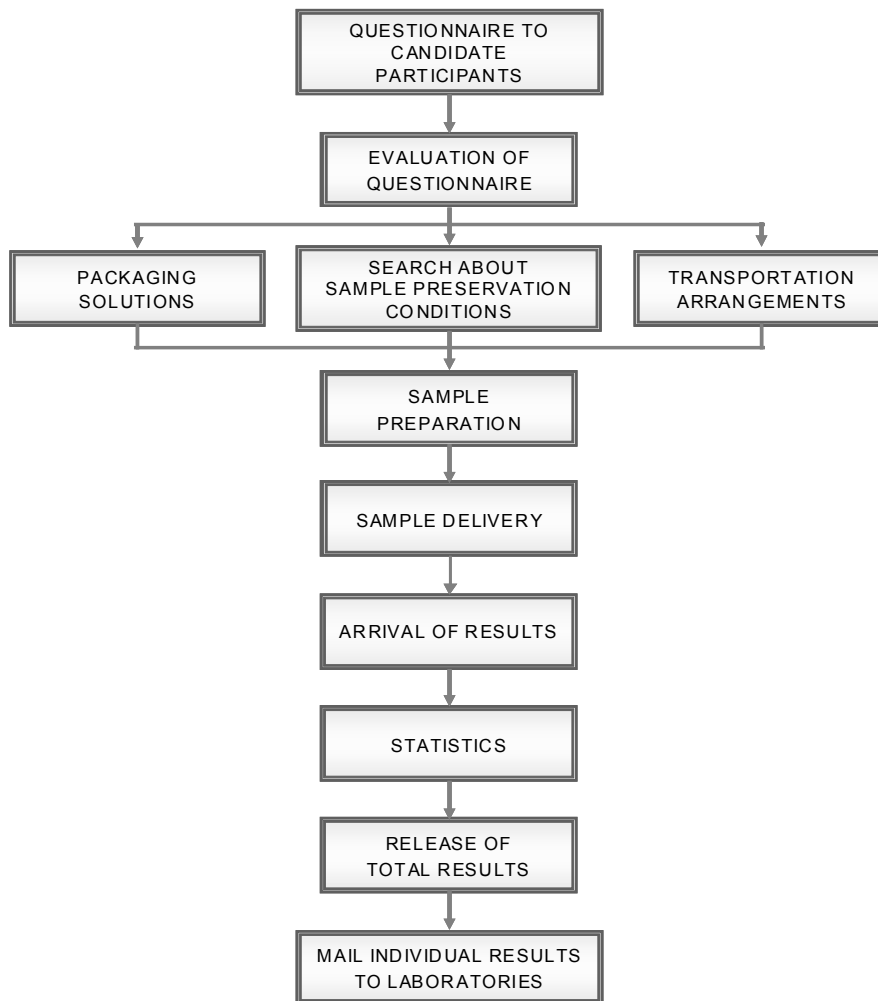


Figure 1 – The flow of the program

3 SAMPLE PREPARATION AND TRANSPORTATION

Fresh samples were prepared from blood samples in EDTA Vacutainer tubes taken from consented volunteers. At first distribution two samples were prepared, the first one was prepared by adding Transfix solution (Cytomark, UK) and mixing the blood at suggested concentrations by the manufacturer, second sample was prepared by dilution of the first sample with buffer solution at pH 7.2. At second sample distribution, samples were not fixed but transportation has been arranged to be realized in 24 hours that kept the samples near to native status. Analysis of samples were performed every day in our laboratory for three consecutive days in order to be able compare with the participants results and also to detect any changes or deteriorations occurring in time. All participants were asked to send a receivable e-mail after the delivery to be able to follow up the effective transportation. Samples to referral laboratories were also traced at transportation.

4 EVALUATION OF RESULTS

All of the results were sent to Centro Laboratuvarları via fax. For continuation of the anonymity and confidentiality, all of the results were recorded to excel files by the person who receives the result forms and does not have any role in programming of the scheme or the statistical analysis. After all of the results are received and recorded, the same person seals the results envelope and sends the computer files to program coordinator. Program coordinator completes the statistical analysis, calculates the assigned value from referral laboratories' results, two of which are accredited and recognized laboratories in Germany and the third one is the provider itself (also accredited for ISO 15189). All of the calculations and statistical analysis are performed according to ISO 13528 [5,7,8].

5 RELEASE AND DISSEMINATION OF RESULTS

Two reports are sent to each participant. First report is a compilation of all results, comparisons to assigned value and mean value of the participants. Second report is prepared separately for each laboratory; their individual results are compared to assigned value and mean value of all participants [7]. A part of the Centro Laboratuvarları web site will be used to publish the first report after the third distribution, which will be realized in May 2007.

6 EVALUATION OF THE PROGRAM

After each distribution an in house evaluation is performed. A questionnaire is sent to participants via e-mail, according to suggestions and recognized corrective actions; improvements are planned for the next distribution [6,7,9]. The program coordinator follows up corrective action forms and she also keeps result reports. All of the records are to be kept for 10 years.

7 FUTURE OF THE PROGRAM

This project, which has been started with support from EU-MEDA Project, will be run on regular basis for three times per year. New immunophenotyping panels will be initiated in late 2007. We also plan to establish new schemes on different test groups in near future.

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