New Developments in Materials for a Complete Quality Control

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Introduction

Control materials are essential to check for instrument performance during the operation of automated analytical devices. This presentation will describe the relationship between SYSMEX, a manufacturer of analytical devices and Streck, a manufacturer of control materials. The relationship started quite casually some twenty-five years ago when a gentleman who worked for SYSMEX US visited our then small Streck laboratory wishing to know (a) if we really existed and (b) if we knew how to make platelet control materials. Apparently we convinced him on both counts, and he brought in a couple of SYSMEX platelet instruments and from that our relationship developed. Over the years our collaboration has progressed to the extent that SYSMEX provides Streck with prototype analysers to support the development and fine-tuning of control materials so that these are available as soon as the first instruments are installed in the client laboratories.

Streck Laboratories is a manufacturer of haematology controls. We manufacture analogs, which, by definition, are similar to specific blood cells but are different in origin and structure. These are matched with SYSMEX measurement technologies and developed into controls for SYSMEX analytical devices. The various control materials available will be briefly described and some more detailed information will be presented about the specific analog developed for the reticulocyte (**figure 1**).

History of SYSMEX Haematology Controls

Product	Description	Date of Introduction
EIGHTCHECK	8-parameter	1984
EIGHTCHECK-3WP	3-part differential	1986
RETCHECK	Reticulocyte	1989
NE CHECK	5-part differential	1990
SE CHECK	5-part differential	1994
SF CHECK	5-part differential	1995
e-CHECK	5-part differential plus reticulocytes	2000

Figure 1
Control materials manufactured by Streck Laboratories for SYSMEX instruments.

The first control material developed by Streck for SYSMEX and sold under the SYSMEX label was EIGHTCHECK, an eight-parameter control material for red blood cell parameters, white blood cells and platelets. Later a three-part differential control was added to this (EIGHTCHECK-3WP) and then followed by unique five-part differential controls, as SYSMEX developed more complicated systems. And so NE CHECK, SE CHECK and SF CHECK were produced. Lastly a five-part differential control that includes a reticulocyte analog, called e-CHECK, has been developed for the XE-2100 instrument.

The manufacture of haematology control materials is the art of developing blood cell surrogates usually referred to as analogs. An analog mimics a specific cell type and the instrument recognises that analog as a particular white cell, maybe a platelet or a reticulocyte. The first analog used in the manufacture of haematology control materials was the chicken red cell. A chicken red cell contains a large nucleus and can be stabilised. It can then be counted by the instrument as a white cell, specifically a lymphocyte. The next analogs developed represented lysable platelets. These were manufactured from red cells and required the lysable characteristic to permit accurate white cell counts and haemoglobin measurement. The different control materials will now be described and the analogs identified.

EIGHTCHECK-3WP

EIGHTCHECK-3WP for use with the SYSMEX K-1000 analyser is illustrated in **figure 2**. In this product three different types of cells are used as leukocyte analogs: the lymphocytes are human red cells, the monocytes are turkey red cells, and the granulocytes are bovine in origin.

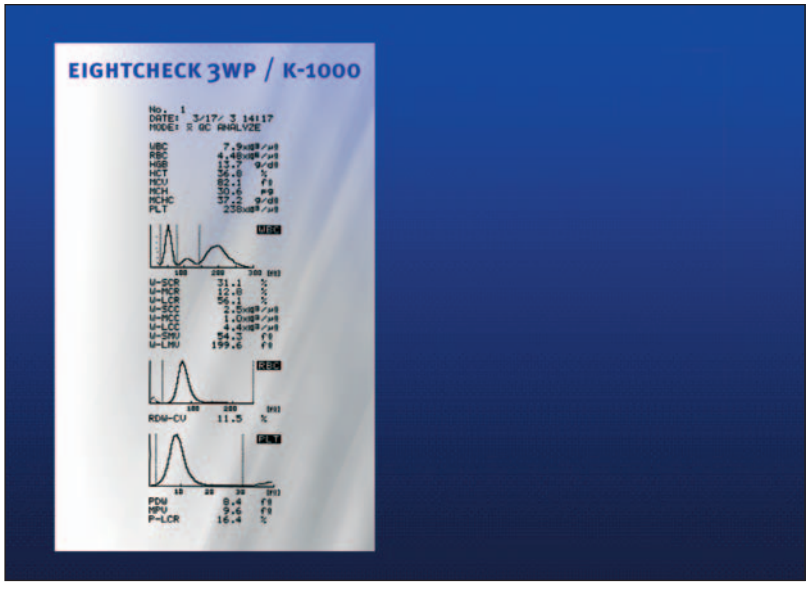

Figure 2
EIGHTCHECK-3WP for use on the SYSMEX K-1000. Note the three part differential leukocyte control.

NE CHECK

In NE CHECK (**figure 3**), the lymphocyte and granulocyte analogs are human leukocytes but the monocytes are bovine in origin. Since Streck is located in Omaha, Nebraska obtaining bovine blood is very simple.

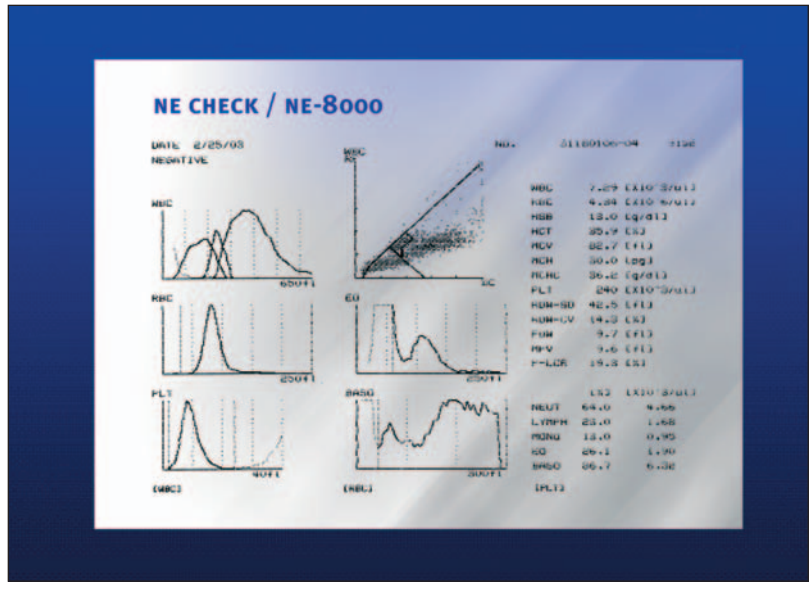

Figure 3
NE CHECK five-part leukocyte differential control material for use with the SYSMEX NE-8000.

SE CHECK

Next there is SE CHECK (**figure 4**) for use with the SE-9000 series analysers. All instruments developed by SYSMEX from this time on require human leukocytes in their control materials. This requirement of SYSMEX instruments means that Streck possesses more knowledge and experience than anyone else in the control material manufacturing industry on how to stabilise human leukocytes and maintain them in this state for a realistic shelf life. SF CHECK (**figure 5**) also contains human white cells.

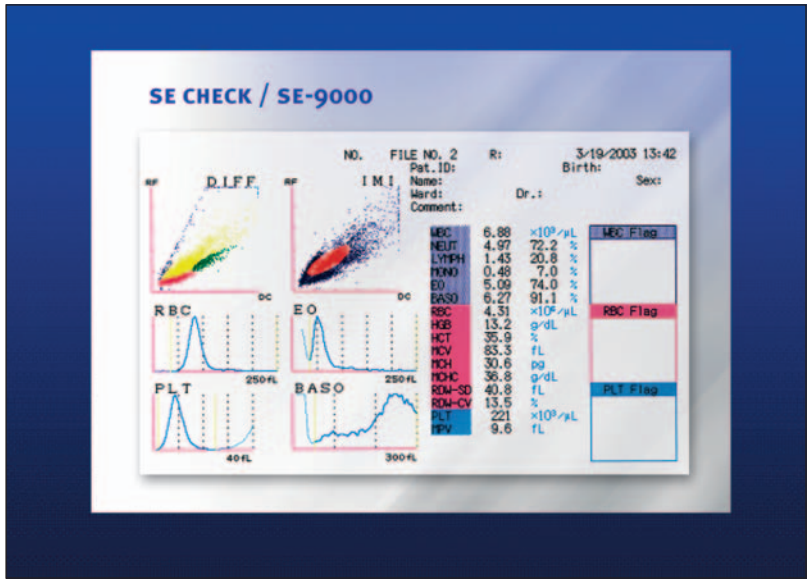

Figure 4
Five-part differential leukocyte control material for use on the SYSMEX SE-9000 series analysers. DIFF is a three part differential scattergram containing lymphocytes, monocytes and granulocytes. IMI is the Immature Myeloid Information scattergram.

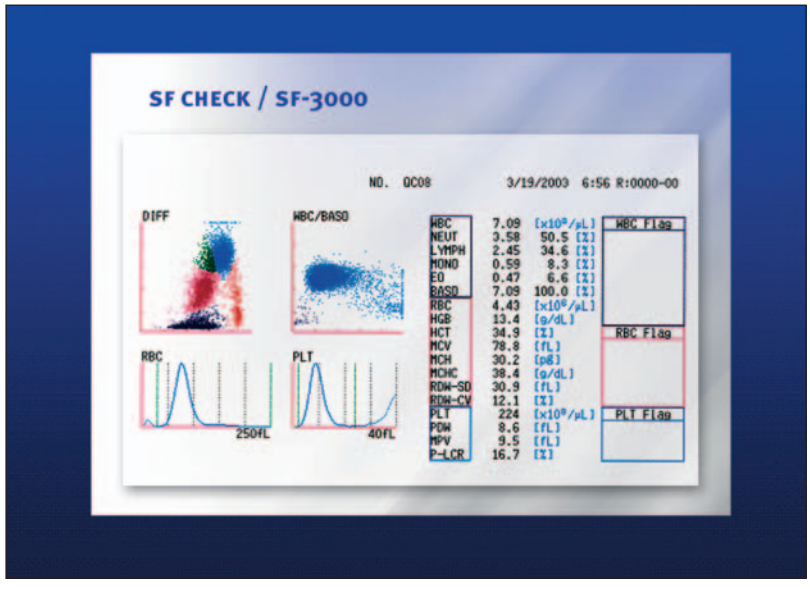

Figure 5
SF CHECK for use with the SYSMEX SF-3000 analyser.

e-CHECK

Finally there is e-CHECK (**figure 6**) for use with the SYSMEX XE-2100 analyser which adds a number of further cellular elements. This will be described in more detail later.

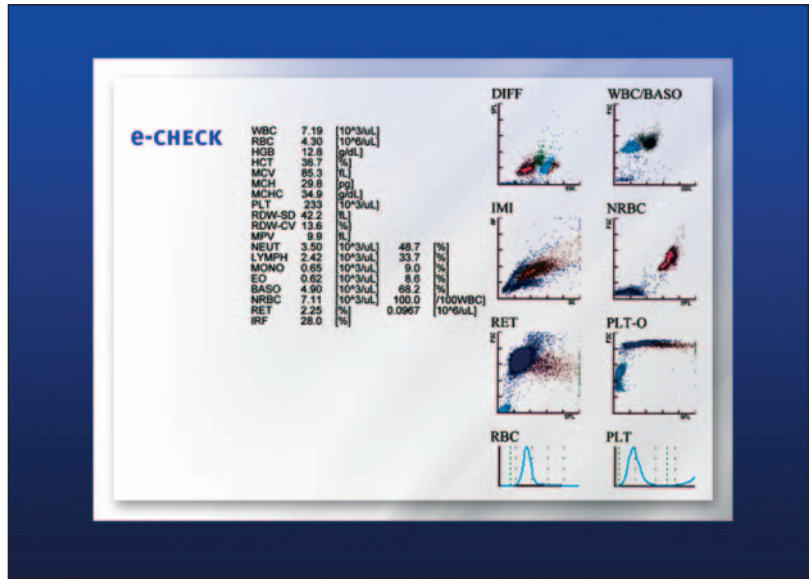

Figure 6
e-CHECK for use with the SYSMEX XE-2100.
NRBC = nucleated red blood cell scattergram,
RET = reticulocyte scattergram,
PLT-O = fluorescence (optical) platelet scattergram.

The manufacture of three and five-part leukocyte differential control materials is really an art, and it becomes more and more difficult as different cells require to be added for the three-part and five-part white cell differentials. Each analog has to be sized and positioned very carefully and accurately. One analog must not interfere with other analogs in the control material. It is necessary to manufacture three levels of control material, low, normal and high, each with differing percentages of these different white cells. Also there is a requirement for different total white counts. The analogs that we develop have to be stable for 120 days to cover manufacture, finishing of the product, assay, shipping and still have a realistic shelf life when they reach the client laboratory.

The National Committee for Clinical Laboratory Standards (NCCLS) definition [**1**] states 'The control material should have characteristics which enable it to provide information about what is going on with the testing process. A material whose composition is similar to or identical with the patient sample matrix being analyzed is generally best.' Since they are derived from human blood, Streck control materials largely conform to this definition. This is always the goal but it is often difficult to make stable preparations that also behave exactly as the human sample. Nevertheless these human derived cells are analogs and when being developed they must be shown to act like the cell they represent when exposed to the instrument and its reagent subsystem in terms of lysing, shrinking, membrane stripping, fixing and staining. Each instrument type is different and requires a dedicated control material that will react appropriately. If the control material is either partially or completely insensitive to the reagents, then an adequate control system does not exist.

Reticulocytes

One cell that Streck has spent a great deal of time attempting to refine, as an analog, is the reticulocyte. The first reticulocytes manufactured by Streck in 1986 were for manual counting. They required to appear like reticulocytes microscopically. Initially animal blood was used but, as time went on, it was felt to be inappropriate since the animals had to be rendered anaemic to produce sufficient reticulocytes for harvesting. Another problem with animal reticulocytes was their instability. We therefore developed a new process for the manufacture of reticulocytes. The procedure is described in 'The encapsulation of ribose nucleic acid (RNA) in human red blood cells for use as a reticulocyte quality control material for flow cytometry', an article by Ebrahim and Ryan [**2**]. This is an interesting and unique process. By means of a hypertonic solution, the pores in the red cell membrane are enlarged and the cells then incubated with RNA. As they incubate, the RNA seeps through the pores. The pores are then sealed and the RNA captured within the cell. By controlling the quantity of RNA entering the red cells, the size and position of the cells in the scattergram can be located correctly. As a result of this procedure Streck has the only five-part differential control material with reticulocytes on the market. Patents exist to protect this product. The result of staining this product by New methylene blue is shown in **figure 7**. This is actually e-CHECK.

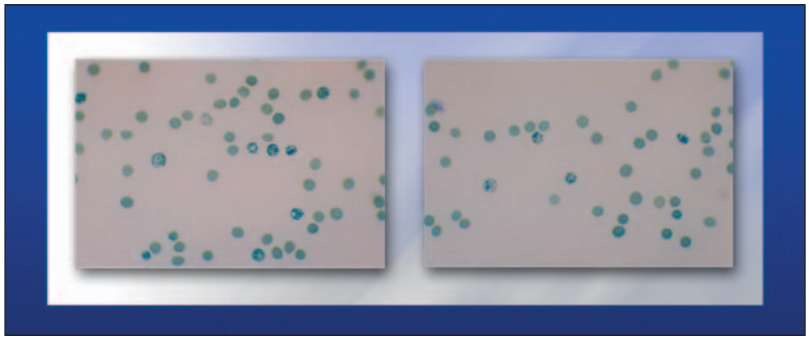

Figure 7
Encapsulated reticulocytes stained by New methylene blue.

Conclusions

On behalf of Streck Laboratories, I welcome this opportunity to describe our quality control materials at this SYSMEX European Symposium. The partnership between Streck and SYSMEX has been very effective and has allowed Streck to see the new instruments as they are being developed, to be in a unique position to know what type of instruments are going to be on the market, and thus be in a position to develop control materials appropriately.

References

[1] **NCCLS** (1998)
Terminology and Definitions For use in NCCLS Documents; Approved Standard. NCCLS Publication NRSCL8-A. NCCLS 940 West Valley Road, Suite 1400, Wayne Pennsylvania 19087–1898 USA

[2] **Ebrahim A., Ryan W.**, (1996)
The encapsulation of ribose nucleic acid (RNA) in human red blood cells for use as a reticulocyte quality control material for flow cytometry. Cytometry 25, 156–163