The XS-1000i and the XS-800i, suitable for minimal sample throughput, are part of the Sysmex X-Class, whose outstanding feature is the unique system of fluorescence flow cytometry for white blood cell (WBC) differentiation. The two systems only differ in terms of sample loading, which can proceed open with the XS-800i and also closed with the XS-1000i. The XS-1000i can also be optionally equipped with a sample autoloader. We would like to present the experience gained with the XS-series with a focus on paediatric blood analyses.

- Which specifications are especially suitable in the measurement of paediatric blood analyses?
- What additional information do the measurement results deliver thanks to fluorescence technology?

**Further information beyond conventional WBC differentiation**

Neonates can present a major challenge in paediatrics, especially during their first days of life, on account of their immature immune system. Besides special physiological features in the blood count, such as normoblasts or elevated values for immature granulocytes, especially cases of sepsis in premature newborn, place high demands on the quality of the relevant laboratory tests. Hospital acquired infections are the most common cause of morbidity and mortality during the first days of life of a neonate. The mortality rates due to severe sepsis and septic shock are 25–30% and 40–70% respectively among these patients. In such cases, together with the clinical findings, only a combination of clinical chemistry and haematological parameters can provide a timely indication of the disease. Certain parameters from the XS-series can support very early recognition of a severe infection, together with the cytokines IL-6 and IL-8 and the value for the C-reactive protein (CRP).

Investigations of neonates have shown that the number of immature granulocytes rises significantly earlier than the CRP value, which only increases after approx. 12 hours. On the basis of fluorescence technology, the XS-series is capable of indicating immature granulocytes in the blood sample by means of a corresponding flag message. Moreover, the absolute and percentage value of the count is available as additional information.
As soon as metamyelocytes, myelocytes or promyelocytes are detected, the relevant flag message appears for immature granulocytes ('Imm Gran?'). In the presence of band cells, the XS-series displays a flag message with a reference to a 'left shift'.

Another advantage of the XS-series is the option of the specific sensitive detection of antibody-producing (reactive) lymphocytes such as lymphoplasmacytoid cells, immunocytes or plasma cells. Activated B-lymphocytes appear as a population in the highly fluorescent area and thus trigger the flag message for 'atypical lymphocytes'. Furthermore, the additional information can be extracted in the form of the research parameter 'Others' as an absolute or as a percentage value.

The following examples show typical cases of abnormalities in the DIFF scattergram in the analysis of paediatric blood:

**Example 1: Immature granulocytes (IG)**
This example displays a blood sample from a 17-day-old infant. In the DIFF scattergram the population of immature granulocytes is clearly identifiable, which triggers the corresponding flag 'Imm Gran?'. The additional information shows an IG value of 2.2%.

**Example 2: Atypical lymphocytes**
This example displays a blood sample of an 13-month-old infant with a blood count containing a conspicuously high number of atypical lymphocytes. The DIFF scattergram displays a lymphocyte population in the highly fluorescent area, which triggers the relevant warning 'Atypical Ly?'. This population is coloured violet in the scattergram comparable to the population of normal lymphocytes, deriving from the same cell type. Additionally the 'Other' parameter displays a value of 1%.
Fig. 2 The atypical lymphocytes in the highly fluorescent area generate the corresponding flag

Example 3: Normoblasts in neonates
The presence of normoblasts in the peripheral blood during the first days after birth is characteristic for neonates. It is therefore advantageous that the XS-series is capable of recognising these cells and referring to this population accordingly. Thereby the user is informed that a correction of the WBC should be undertaken if necessary. This also occurs with the specific flag message ‘NRBC?’ in the Diff scattergram.

Fig. 3 The flag message ‘NRBC?’ refers to the possible presence of normoblasts in the sample

Adapted specifications for measuring paediatric blood samples
Collecting blood is frequently a limiting factor with infants as often only a small volume of blood is available. In order to cope with this situation, the aspiration volume of the XS-series is just 20μL. Beside the outstanding quality of the measurement results, users also stress the functional advantages of the XS-series in the measurement of paediatric blood samples. Through the open sample aspiration, a volume of just over 20μL is quite sufficient to determine all 24 parameters, including a 5-part differential
analysis. The XS-series is also equipped with a special blood sensor that monitors the correct aspiration so as to avoid aspiration errors if the sample volume is too low.

Methodological aspects were also addressed in order to adapt the XS-series to the special considerations of paediatric blood analyses. Lyse-resistant red blood cells are an example of a typical interference that presents a high osmotic resistance. The white blood cell differentiation occurs in the DIFF channel. Here only cells or particles are stained which contain nucleic acids. As red blood cells do not have a nucleus, they therefore emit no fluorescence signal in this channel and hence remain ‘invisible’ in the ghost area of the scattergram.

Lipids in the blood or pronounced leukocytosis can cause difficulties for a haematological analyser in determining the haemoglobin value. The X-Class technology in the XS-series ensures dependable results here. The soap-like character of the reagent means that lipids are largely dissolved. Through the tremendously diluted sample any interference is minimised. In addition, the haemoglobin measurement proceeds in its own channel. The detergent used here (sodium lauryl sulphate) not only lyses the red blood cells, but also the white blood cells. So a pronounced leukocytosis is not a reason for having to dilute samples, as the haemoglobin value measured is reliable\[3\].

Attention was also paid to software programming in order to meet the special demands in paediatrics apart from the methodology. The reference values for 6 different patient groups are individually definable for all parameters, especially because they change significantly, particularly in the first days and weeks after birth\[4\]. These settings provide the relevant information on an increased or decreased cell count depending on the age of the patient. In a similar way, the flag messages for morphological abnormalities can be adapted to the individual needs of every laboratory. The specificity of the flag messages is not influenced, but rather their sensitivity in the detection of pathological samples.

**Literature**