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Cell count in broncho-alveolar lavage fluid (BALF) of rats with the XN-1000V hematology analyzer

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Introduction

Differential cell count in BALF of rats is an obligatory investigation in inhalation toxicity studies. The manual differential cell count is a laborious work, and the question is if an automated, differential cell count in BALF can be used as screening method.

Objectives of the present evaluation were: 1. automated counting of total cells in bronchoalveolar lavage fluid (BALF) of rats; 2. estimation of the ratio of mononuclear cells (MN: macrophages, monocytes, lymphocytes) and polymorphonuclear cells (PMN: mainly neutrophils) in BALF

Material and Methods

BALF generation

Male and female, 10- to 19-week-old Crl:Wi(Han) rats of regulatory studies performed according to the German Animal Welfare Act and in an AAALAC-accredited facility were sacrificed under ketamine/ xylazine anesthesia and exsanguinated from the abdominal aorta and vena cava. The thorax of the fixed rats lying on the back was excavated and a plastic vein catheter was fixed in the right main bronchus and connected via a three-way valve with two electrical pumps. After ligation of the left main bronchus 2 to 5 mL physiological NaCl solution depending on the body weight was instilled with a flow rate of 6 mL/min. After 30 seconds the lavage is re-aspirated via a second pump with the same infusion rate. This procedure is done twice and the BALF is pooled.

Cell counts in the BALF

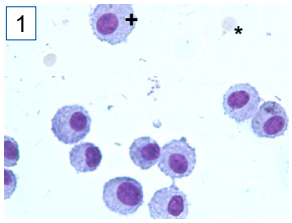
15 BALF samples of healthy rats and 5 BALF samples with neutrophilia were measured in the body-fluid-mode (BF) of the hematology instrument XN-1000V, Sysmex Corporation, Kobe, Japan. The scattergram gate setting was optimized. With these gates 43 BALF samples of healthy rats and 24 BALF samples of rats with local neutrophilia (total cells >400/µL or PMN >30%) were evaluated. The results were compared with manual total cell counts (TC) in a Neubauer counting chamber, and microscopic differential cell counts of cytocentrifugated samples after a WRIGHT stain as reference methods.

Discussion

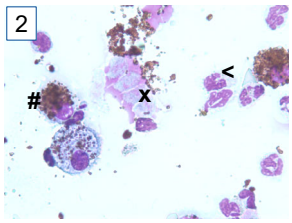
- Total cell counts (TC) in BALF of healthy rats measured with the XN-1000V in BF mode correlated well with the reference method
- TC in BALF with local neutrophilia measured with the XN-1000V tended to be a bit higher compared to the reference method
- PMN counts (in %) in BALF of healthy rats and with local neutrophilia resulted in a bit higher values probably due to a difficult separation from degraded cells
- Optimized gate setting in the BF mode of the XN-1000V in test BALF samples could be used for validation samples without further adjustment

Results

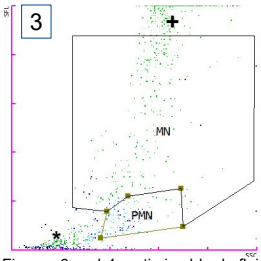
BALF of healthy rats

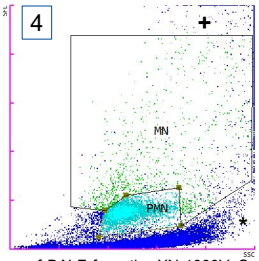


BALF of rats with local neutrophilia

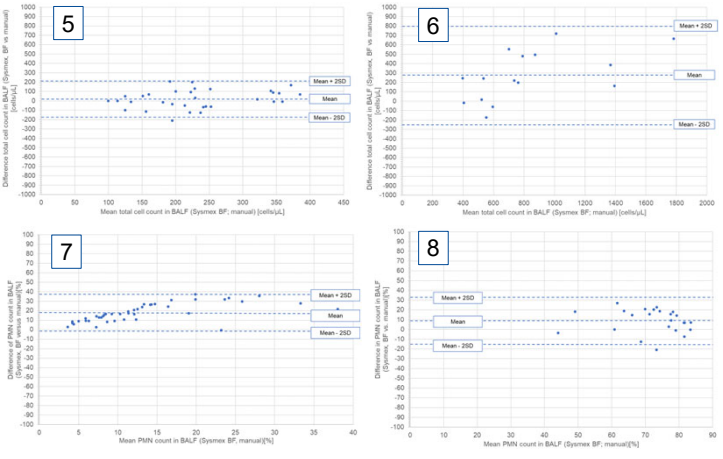


Figures 1 and 2: Microscopic photos of BALF cytocentrifuge slides stained according to WRIGHT; 1000x magnification; + macrophage, * erythrocyte, # macrophage with inhaled compound particles, x epithelial cell group, < neutrophil cell





Figures 3 and 4: optimized body fluid (BF) scattergram of BALF from the XN-1000V, Sysmex; y-axis: side fluorescence light signal (SFL), x-axis: side-scattered light signal (SSC); PMN polymorphonuclear cells (mainly neutrophils); MN mononuclear cells (macrophages, monocytes, lymphocytes); + high fluorescence events (epithelial cells, cell conglomerates); * no leukocytes (cell detritus , compound particles, other cells)



Figures 5 – 8: Bland-Altman charts of total cell counts (5 (N = 32) and 6 (N = 15) in cell/µL) and polymorphonuclear cell counts (PMN; 7 (N = 43) and 8 (N = 24) in %) in BALF; y-axis: difference between counts of XN1000V versus reference method; x-axis: for each sample mean of XN-1000V and reference method count

Conclusions

The BF mode of the XN-1000V instrument can be used to measure total cell counts in rat BALF samples including a categorization of the cells in mononuclear and polymorphonuclear cells as screening method.