

# A novel, automated approach providing standardized sample preparation for multiple Liquid Biopsy analyses

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## Introduction:

Preanalytical phase of liquid biopsy for cancer diagnostics is not standardized among different laboratories, being the cause of around 50% of analytical errors (1). Current clinical practice relies mostly on chemically stabilized blood samples shipped to clinical laboratories, posing the samples at risk of biological degradation or operators' manual errors in blood processing. Tethis presents **See.d**, a new pre-analytical platform for standardized and automated preparation of fresh blood samples *at the point of blood collection* for comprehensive analysis of biomarkers in cancer management.

## Methods:

Fresh whole blood samples collected in K2EDTA tubes from 20 healthy donors (HD) were processed by See.d according to the standardized preanalytical flow (Fig. 1). Firstly, the following steps were performed by See.d:

- plasma was separated from other blood components and collected in a separate vial;
- Red blood cells (RBC) fraction was gently lysed and discarded;
- White blood cells (WBC) fraction was resuspended in phosphate buffered saline (PBS), allowed to spontaneously adhere on proprietary, nanocoated SBS slides, and fixed with paraformaldehyde.

Further, after submission to storage and shipment mimicking conditions, plasma and WBC on SBS slides were analyzed as follows:

- cfDNA was extracted from plasma using QIAamp DSP Circulating NA Kit (Qiagen) and analyzed with Fragment Analyzer (Agilent) for cfDNA quantification and genomic contamination;
- WBC fraction on SBS slides was analyzed for different quality parameters (cell number per slide and homogeneity of cell distribution with a proprietary algorithm) and subjected to i) immunofluorescent staining with hematopoietic markers and DAPI and ii) to cytological staining with hematoxylin eosin (H&E).

## Results:

From each two blood draw tubes, 5 ml of plasma and 20 SBS slides containing a monolayer of WBC were obtained. In details:

- Fragment-size analysis has shown negligible contamination of genomic DNA in all plasma samples, even after submitting the samples to storage and shipment mimicking conditions (Fig. 2a)
- See.d processed plasma provides a comparable quantity of cfDNA compared to the one obtained through a manual clinical protocol (Fig. 2b).
- SBS slides contained around  $2,5 \times 10^6$  cells each with homogenous distribution across the adhesion area (Fig. 3).
- H&E and hematopoietic staining of WBC (Fig. 4) have shown intact cellular morphology of all blood cell types and good staining quality after slides conservation at the same conditions as tested for plasma samples (2).

## Conclusions:

See.d automates and standardizes the preanalytical step of liquid biopsy for blood samples: cells on SBS slides and plasma are stable and can be shipped to analytical laboratories for multianalyte tests in liquid biopsy (CTC, ctDNA, etc.). Tethis is currently planning the platform certification studies according to IVDR regulation.

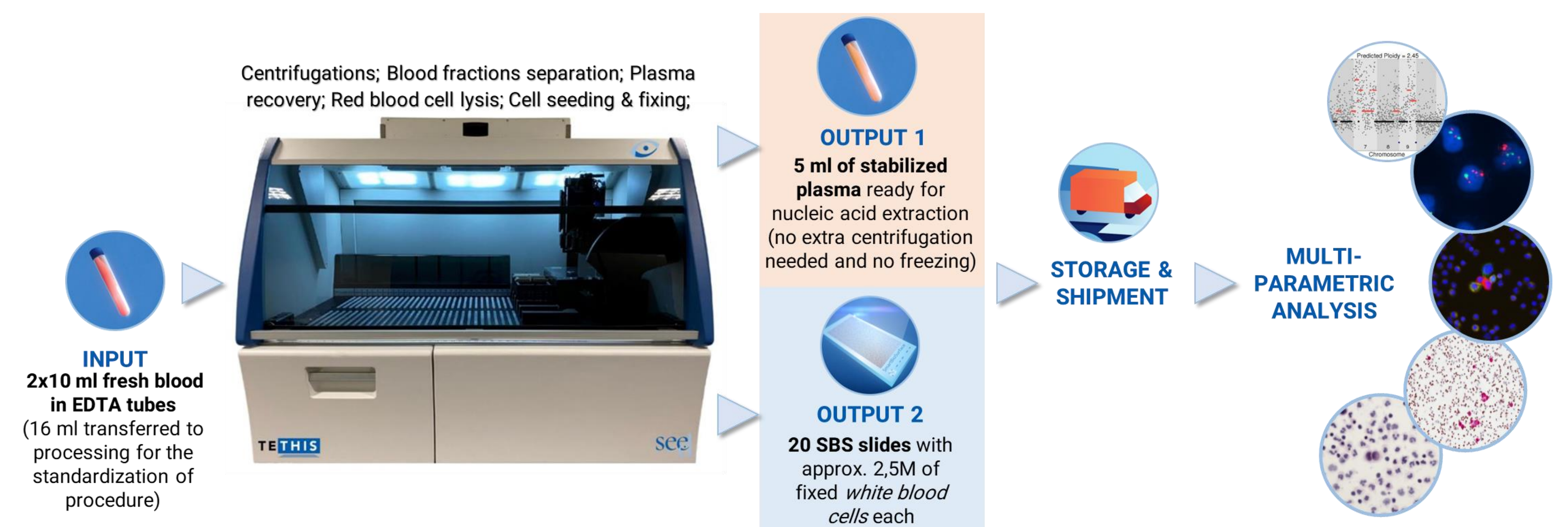


Fig. 1. Standardized preanalytical flow with See.d

Whole blood samples drawn in EDTA tubes are placed inside the device, which a) separates and collects plasma samples, b) lyses and discards RBC, c) resuspends WBC in PBS solution, d) seeds the WBC on the SBS slides, and fixes them. As a result, a set of 20 slides and 5 ml of high quality plasma from two 10 ml blood collection tubes are obtained. As prepared material, can be stored at 4 °C up to 7 days and shipped at RT to central laboratory for further downstream multiparametric analysis like: cytological, immunocytochemistry, immunofluorescence staining's, FISH and molecular analysis.

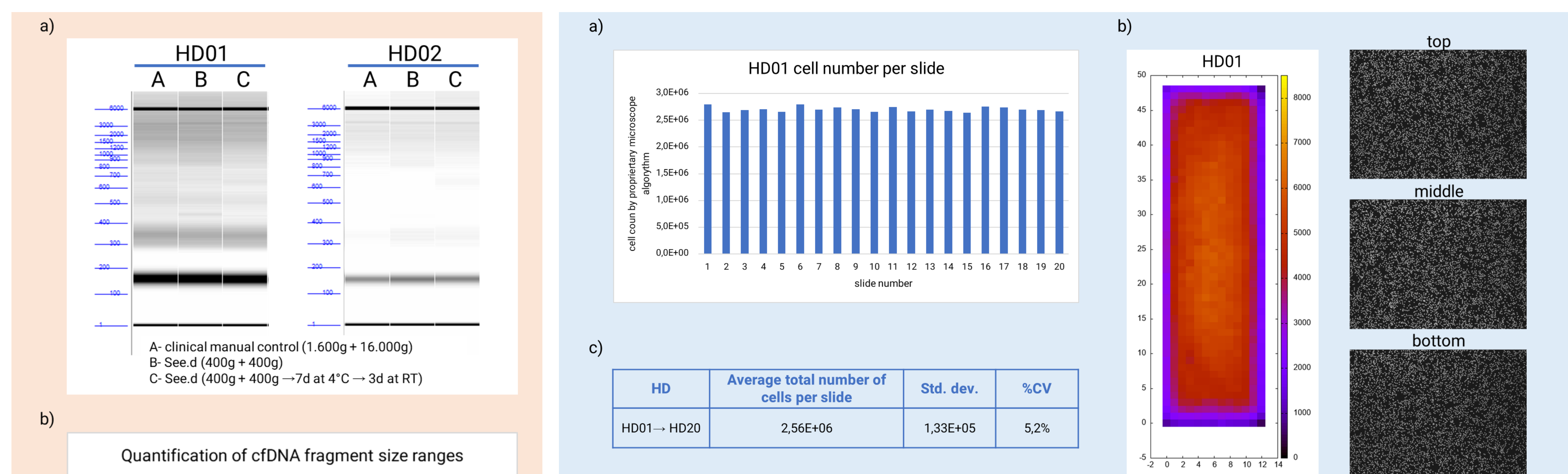


Fig. 3. Analysis of See.d prepared SBS slides containing HD WBC

a) Example of WBC count for all 20 slides from HD01 b) heat map representation of WBC distribution across slide 1 from HD01 together with DAPI images acquired at 10x from the top, middle and bottom area of the SBS slide c) Table with average number of WBC per slide for all 20 HD.

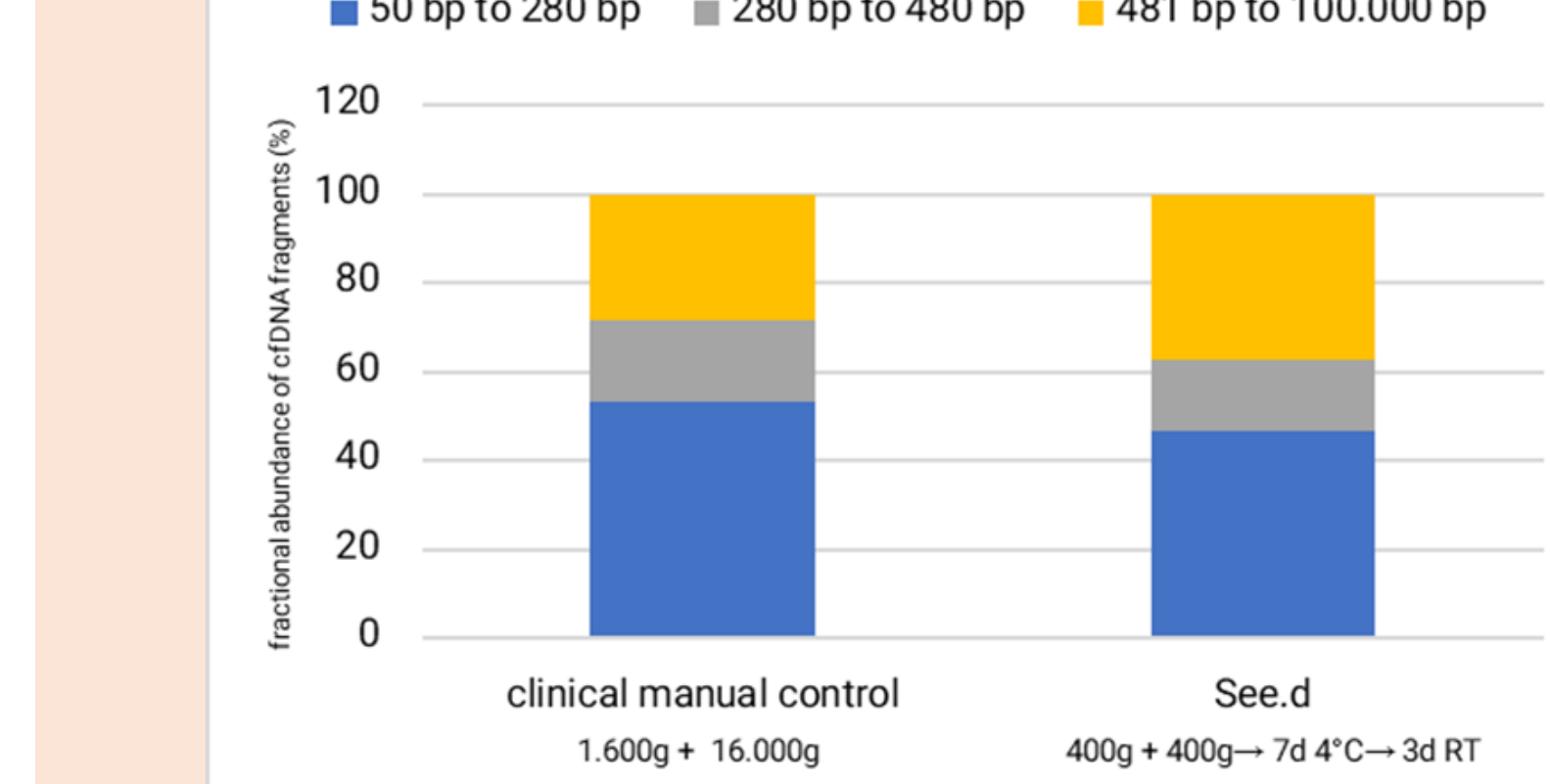


Fig. 2. Plasma analysis

a) Examples of fragment-size analysis from two different HD comparing the quality of plasma collected manually (lane A) and by See.d (lane B). See.d collected plasma is stable even after several days of storage at 4 °C and RT (lane C).

b) Quantification of three cfDNA fragment size ranges extracted from all 20 HD plasma samples which were collected according to the clinical manual protocol (1.600g + 16.000g) or in automation by See.d after double 400g centrifugation and storage at 4 °C and RT.

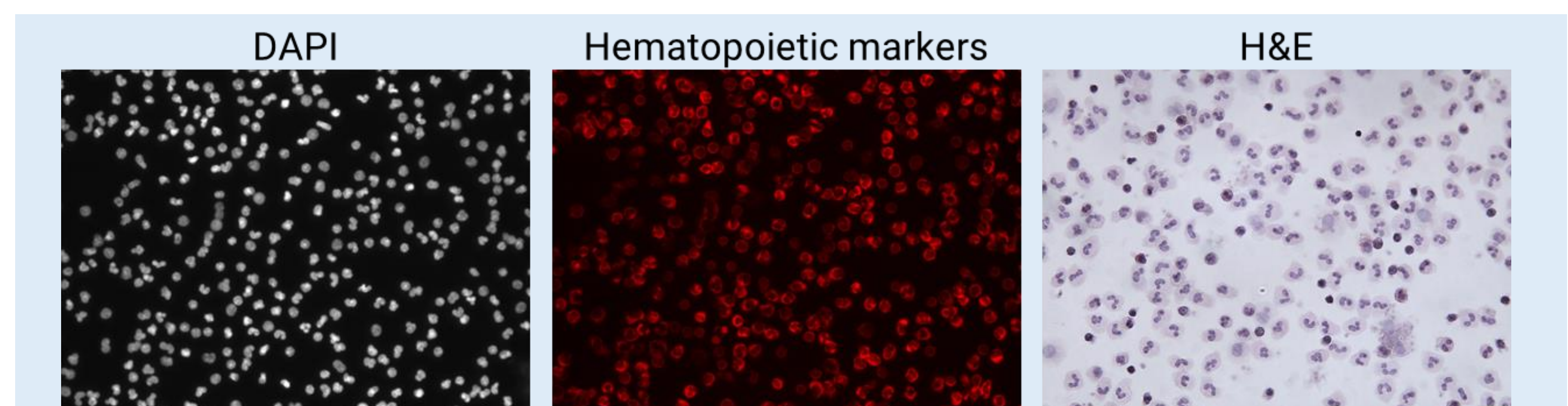


Fig. 4. Staining of See.d prepared SBS slides containing HD01 WBC after their storage at 4 °C and RT.

Example of images acquired at 40x showing good nuclei morphology after DAPI staining (left), hematopoietic staining (middle) and cell morphology after H&E staining (right).