See.d®, a novel automated platform to standardize blood sample processing for cells on SBS® slides and plasma for Liquid Biopsy

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Introduction

The pre-analytical phase of liquid biopsy for cancer detection is not standardized among different laboratories, causing around 50% of analytical errors in diagnosis [1]. The current clinical practice relies mostly on chemically stabilized blood samples shipped to clinical laboratories, risking sample degradation or manual errors in blood processing.

With the aim of overcoming two main limitations of liquid biopsy adoption, namely lack of standardization and need of a comprehensive tumor snapshot [2], Tethis launches See.d®, a pre-analytical platform for standardized and automated preparation of fresh blood samples at the point of collection.

Fig. 1: Tethis pre-analytical standardization flow.

Methods

Fresh whole blood samples from 20 healthy donors (HDs) were collected in 10 mL K2EDTA tubes and automatically processed by See.d® obtaining two outputs:

a) plasma collected in a dedicated vial;
b) whole White Blood Cells (WBCs) fraction, including rare cells, adhered and fixed on proprietary nanocoated SBS® slides [3–5] (Fig. 1).

Genomic contamination and cfDNA quality of plasma samples were analysed, as well as different quality parameters of WBCs fraction upon bright field and immunofluorescence staining.

The analytical performance of See.d® was evaluated by spiking into HDs' blood both reference synthetic DNA and *in vivo* labelled model cancer cells.

Results

From each blood sample tube, See.d extracts 2.2 mL of plasma and produces 10 SBS® slides containing a monolayer of around 2.5x10⁶ WBCs each (Tab. 1).

	mean ± std dev	CV
Plasma volume recovery	2.2 ± 0.1 mL	3.0%
Cell count on SBS slides	2.65 ± 0.13 x 10⁶	4.8%

Tab. 1: Summary table of See.d® outputs results.

Plasma from See.d®, compared to a standard protocol, allowed

- to extract an equivalent amount of cfDNA/mL of plasma, with negligible contamination of genomic DNA (Fig. 2a,b);
- a comparable recovery of spiked synthetic DNA (Fig. 2c).

SBS® slides prepared by See.d® showed

- high cell adhesion efficiency and homogenous distribution (Fig. 3a);
- optimal WBCs morphology and staining, even after slides storage and shipment (Fig. 3b);
- high recovery rate and identification of spiked cancer cells (Fig. 3c,d).

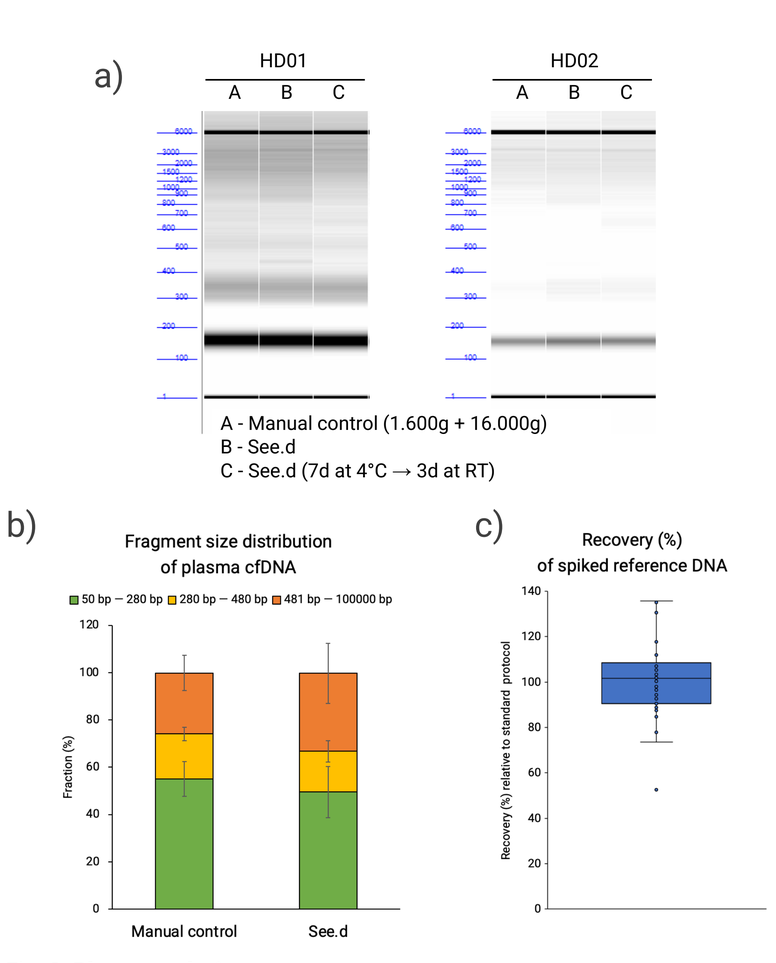

Fig. 2: Plasma analysis.
a) Examples of fragment-size analysis from two different HDs.
b) Quantification of three cfDNA fragment size ranges extracted from all 20 HDs plasma samples.
c) A synthetic DNA fragment of a non-human sequence has been spiked into HDs blood (n=20) for both See.d® and standard plasma preparation, followed by cfDNA extraction and dPCR analysis.

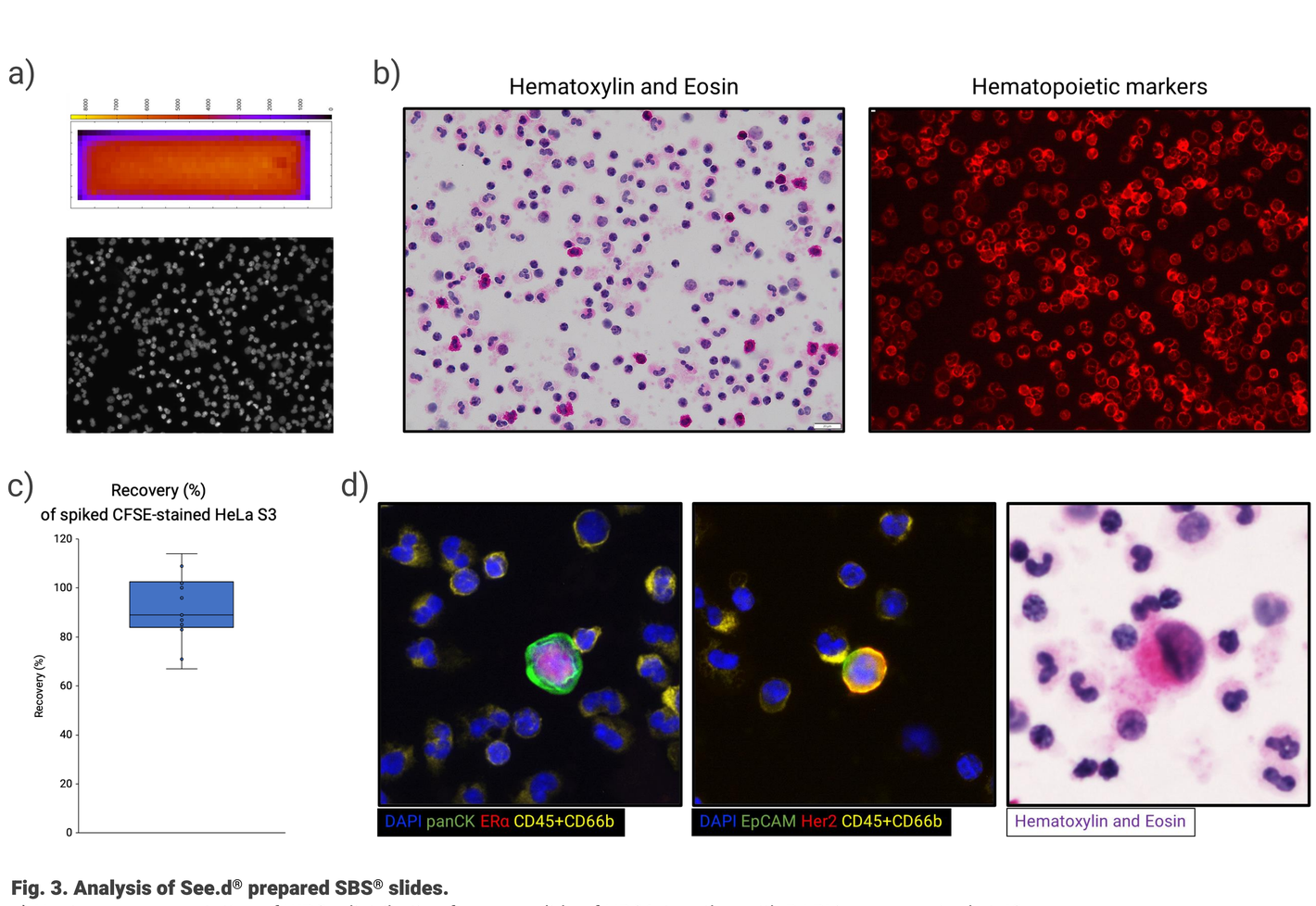

Fig. 3. Analysis of See.d® prepared SBS® slides.
a) Heat map representation of WBCs distribution from one slide of HD06, together with DAPI image acquired at 40x.
b) Evaluation of SBS® slides produced by See.d® subjected to storage for 7 days at 4 °C and shipment at RT. Representative images at 40x from HD06 are shown.
c) HeLa S3 cells were labelled with the vital dye CFSE and then spiked in a predefined number (1-20 cells for slide) into HDs blood (n=13). Spiked cells were identified and counted after 10x scans. Recovery was calculated according to blood volume analyzed and spiking efficiency obtained in simultaneous internal standard.
d) Model breast cancer cells were spiked into HDs blood processed by See.d® and then identified through immunofluorescence. Examples of 40x images of spiked MCF-7 (left) and SkBr-3 (middle) are shown. On the right panel it is shown a representative image of Hematoxylin and Eosin stain performed after spiking of the prostate carcinoma cell line 22Rv1 on HD blood processed by See.d®.

Conclusion

- See.d® automates and standardizes liquid biopsy pre-analytical steps
- Cells on SBS® slides and plasma are stable and can be shipped to analytical laboratories for multianalyte liquid biopsy assays (CTCs counting, cellular and soluble biomarkers, cfNA, etc.).

Outlook

- Current clinical trials (metastatic breast cancer [6, 7], prostate cancer [8])
- IVD certification
- Perfect monolayer of intact immune cells for digital pathology and biomarkers discovery (e.g. ADC) for personalized medicine

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