Vortex

Application Note

CTC Collection and Preparation for Papanicolaou Staining: A new paradigm in assessing tumour progression

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Overview

Traditional Cancer Diagnosis and Its Limitations

Cytology staining such as Papanicolaou (Pap) stain is a reliable and routinely used test for cancer diagnosis in human cytology [1]. The advantage of the Pap stain relies on its ability to yield detailed information of nucleoli and chromatinic patterns of cells. This information is often sufficient to determine whether cells are benign or malignant. Cytological investigation of bioptic material is an indispensable part of diagnosis, and clinical decision-making is often based on the cellular features of a biopsy as determined at the initial cytological assessment. Due to the invasive nature of excisional biopsy and the tumours often not being readily accessible, re-evaluation of the tumours during disease progression is not typically performed.

The Promise of Circulating Tumour Cells (CTCs)

Cytological analysis of circulating tumour cells (CTCs) that are continuously shed by tumours into the bloodstream could provide real-time and more representative information on tumour evolution, treatment effectiveness, and cancer metastasis risk.

Addressing Challenges with Vortex VTX-1

A need for cyto-technicians to learn new protocols or assess tumour cells among a large number of blood cells could reduce the value offered by this approach. Therefore, CTCs need to be enriched to high levels of purity to simplify assessments and be captured into a format that is common to cytological staining. The Vortex VTX-1 achieves both of these objectives making cytological investigation of CTCs a real possibility for ongoing disease monitoring.

Workflow

The preservation of morphology is a central tenet of histopathology. The Vortex VTX-1 benchtop platform provides intact, free-floating CTCs concentrated into a small liquid volume suitable for cytological staining [2].

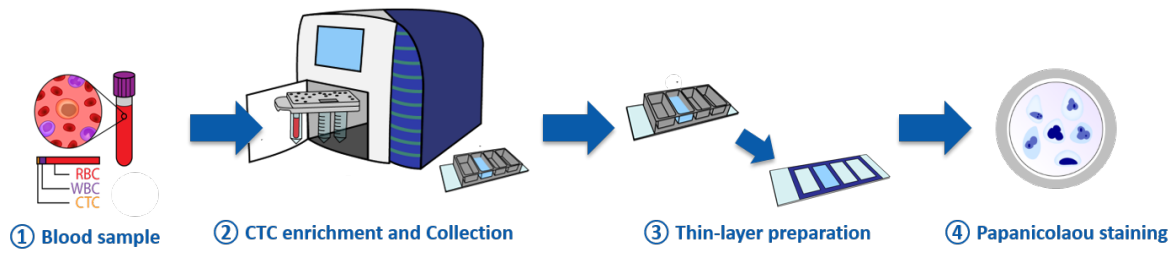

Figure 1. Workflow for CTC collection and Papanicolaou staining.

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① Patient blood is collected by peripheral venipuncture.

② Blood tube is inserted in the Vortex VTX-1 instrument for fully automated CTC isolation. Intact, free-floating cells are released in a small volume (300 µl) directly in a chamber slide.

③ Collected cells are fixed and a cellular monolayer prepared.

④ Slide is stained by conventional Papanicolaou staining procedure on a Sakura Tissue-Tek DRS 2000 (Sakura).

Results

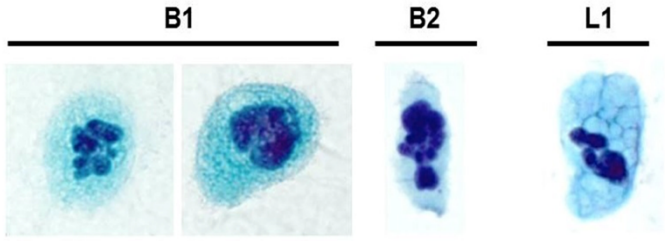

Figure 2: Cytological analysis of CTCs isolated from breast (B) and Non-Small Cell Lung Carcinoma (NSCLC) (L) metastatic cancer patients. Gallery of cells isolated with the Vortex VTX-1 system, and stained with a conventional Papanicolaou protocol. Cells were classified as malignant by a cytopathologist due to abundant granular cytoplasm with large pleomorphic, lobulated nuclei (B1), foamy cytoplasm and large pleomorphic hyperchromatic nuclei (B2), and enlarged nuclei with hyperchromasia and coarse chromatin (L1).

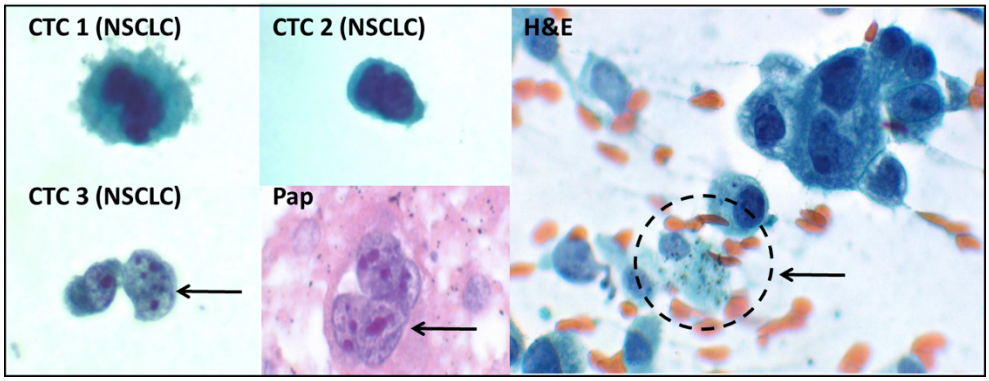

Figure 3: CTCs (CTC 1, 2, and 3) isolated from a NSCLC cancer patient. These cells have a very high nuclear-cytoplasmic ratio (N/C) and a distinct chromatin margination pattern that were recapitulated in cells from a Pap (P) and H&E stain (Q) of a fine needle aspirate.

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Conclusions

- Vortex VTX-1 shows promising results as a rapid sample preparation platform for blood samples to yield diagnostic cells for downstream cytopathology, expanding the range of clinical samples that can be analysed.
- The high purity of the CTCs isolated from blood biopsies with Vortex VTX-1 is particularly well suited for cytological studies, where high cellularity can sometimes confound analysis and hinder interpretation.
- The Vortex VTX-1 workflow directly interfaces with conventional protocols for cytological staining routinely used in the clinical laboratory, such as Papanicolaou staining.
- The Vortex VTX-1 workflow is compatible with existing automated laboratory equipment and doesn't require cyto-technicians to be trained on new protocols.
- Pap-stained CTCs have specific identifiable morphological features linked to their primary cancer origins, such as adenocarcinomas originated from the lungs (NSCLC) and breast.
- Cytological analysis of CTCs revealed that they share striking cytomorphological features with malignant cells from other body fluids such as pleural fluid but also fine needle aspirate (FNA), and core needle biopsy.

References

[1] Mach AJ, Adeyiga O, Di Carlo D. Review: Microfluidic sample preparation for diagnostic cytopathology. Lab Chip. 2013;21;13(6):1011-26.

[2] Dhar M, Pao E, Renier C, Go DE, Che J, Montoya R, Conrad R, Matsumoto M, Heirich K, Triboulet M, Rao J, Jeffrey SS, Garon G, Goldman J, Rao NP, Kulkarni R, Sollier E, and Di Carlo D. Label-free enumeration, collection and downstream cytological and cytogenetic analysis of circulating tumor cells. Sci Rep. 2016;14;6:35474.

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