Evaluation of a New Vacuum Blood Collection Tube for Cell-Free DNA (Insepack II-W)

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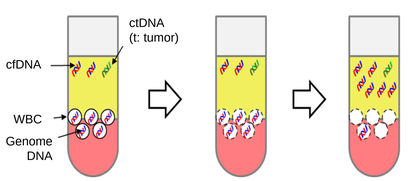
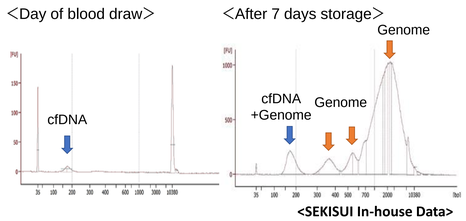
BACKGROUND & OBJECTIVES

Although tests using circulating tumor DNA (ctDNA) and cell-free fetal DNA (cffDNA) in circulating blood have been put to practical use, **contamination of white blood cell (leukocyte)-derived genomic DNA during sample storage must be suppressed** to maintain the integrity of the test [1,2,3]. To address this issue, we developed **a new dedicated blood collection tube for cfDNA testing containing a newly developed separation gel (Insepack II-W)**. We then compared the sample stability of Insepack II-W and general-purpose blood collection tubes at 4°C, 25°C, and 37°C.

[1] S.E. Norton et al. / Clinical Biochemistry 46 (2013) [2] Wim Ammerlaan et al. / Curr Pathobiol Rep (2019) 7:9–15 [3] Angela N. Barrett et al. / PLoS One, 6 (10) (2011), p. e25202

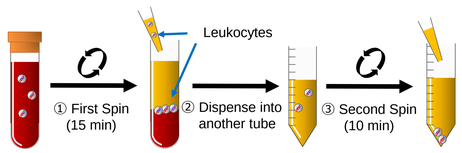




CHARACTERISTICS OF Insepack II-W

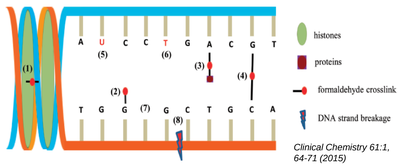
Commercially available cfDNA blood collection tubes contain cell-stabilizing agents to prevent cell rupture, which help maintain sample integrity. This is important because unexpected blood cell rupture can lead to the risk of contamination with genomic DNA. Despite the importance of cell-stabilizing agents, their use often requires a double-centrifugation operation to avoid contamination by leukocytes during plasma collection. Additionally, some cfDNA blood collection tubes utilize formaldehyde to fix cells and stabilize the sample. However, formaldehyde is known to denature nucleic acid, as evidenced by reports of nucleic acid denaturation in formalin-fixed paraffin-embedded (FFPE) samples.

Double-spin Procedure



- Double-centrifugation operation prolongs sample processing time.
- When transferring the specimen, risks such as mistaking specimens or contamination with external DNA may occur.

Nucleic Acid Denaturation by Formaldehyde



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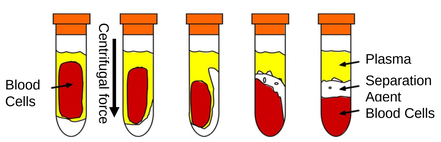
In FFPE, cross-linking and base substitution of DNA may occur.

Sekisui has developed a new type of cfDNA blood collection tube that utilizes a separation agent (separation gel) to create a physical barrier between the plasma and cellular layer. The separation agent has been improved to remove leukocytes in addition to erythrocytes. Since the sample is stored with the blood cells separated:

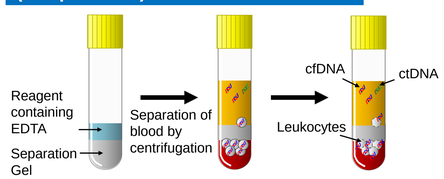
- **Contamination of genomic DNA in the plasma is minimized**, even if blood cells rupture unexpectedly.
- **Double-spin centrifugation operation is not necessary**, as blood cells can be removed in a single-spin.
- **Formaldehyde-free** to eliminate the risk of nucleic acid denaturation.

Separation Agent



- The separation agent is a gel-like substance that is pre-filled in the blood collection tube.
- During centrifugation, the separation agent moves between the plasma and the blood cells to form a gel barrier.

SEKISUI's cfDNA blood collection tube (Insepack II-W)



The gel barrier results in the separation of white blood cells from the plasma, minimizing genomic DNA contamination and therefore improving the quality of the test.

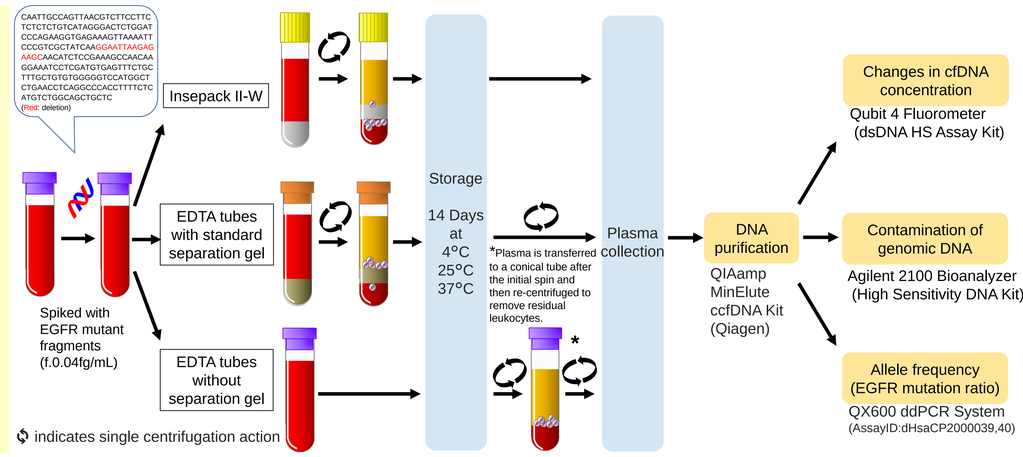
MATERIALS & METHODS

【Sample】
Pooled whole blood from healthy donors was spiked with EGFR mutant fragments (EGFR ΔE746-A750 Reference Standard).

【Blood Collection Tube】
Spiked whole blood was aliquoted into three tubes:
① Insepack II-W
② EDTA tubes without separation gel
③ EDTA tubes with Sekisui standard separation gel.

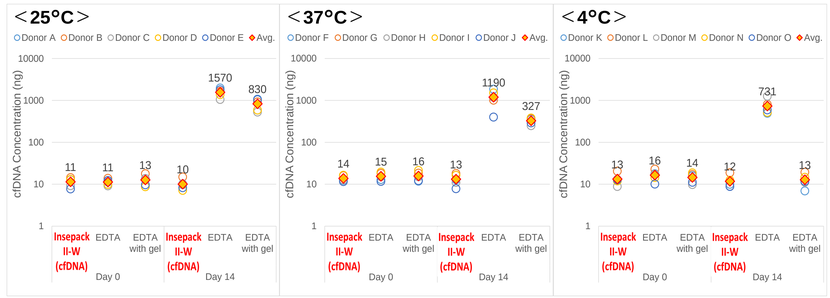
【Centrifugation and Sample storage】
Insepack II-W and EDTA with separation gel underwent centrifugation. Afterward, all samples were stored at 25°C for 14 days. After storage, the plasma was collected and DNA was purified from the plasma. EDTA tubes without separation gel were centrifuged once before DNA purification.

【Evaluation】
After DNA purification, the amount of the purified DNA was measured using qubit 4 Fluorometer (Invitrogen). Sample stability was assessed by comparing the changes in the amount of cfDNA recovered after storage to the day of blood collection. Additionally, the EGFR mutation ratio (mutant EGFR fragments/normal EGFR fragments) was also determined for each sample using ddPCR(BioRad, QX600 ddPCR System, assayID:dHsaCP2000039,40).

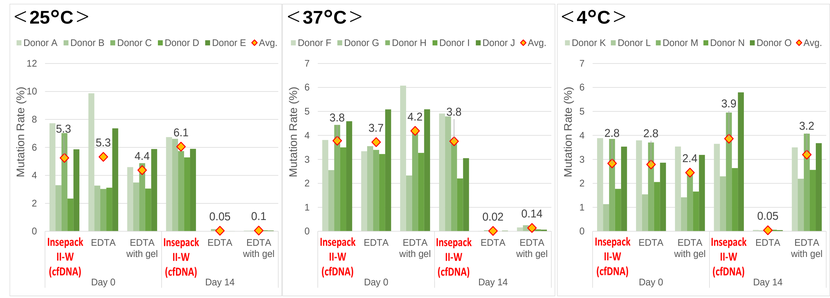


RESULTS

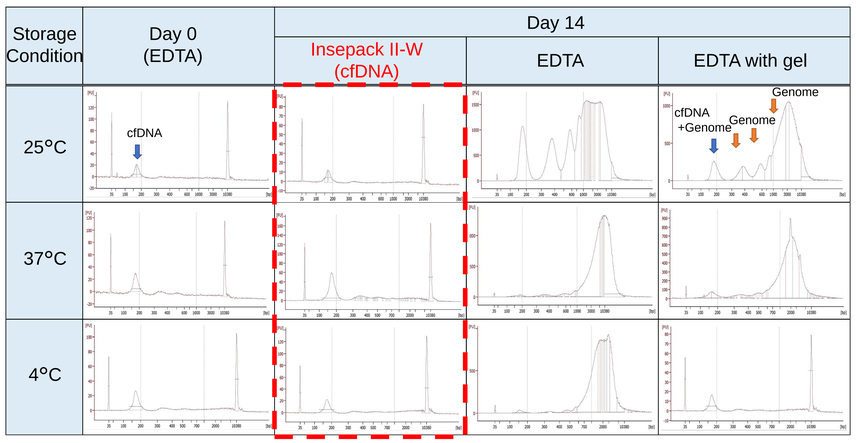
【Evaluating the Changes in cfDNA Concentration】
Under all storage conditions, **no increase in cfDNA was observed with Insepack II-W**. In contrast, EDTA tubes, with and without the separation gel, showed a significant increase in cfDNA when stored at 25°C or above.



【Evaluation of EGFR Mutation Ratio Before and After Storage】
In only **Insepack II-W, the EGFR mutation ratio remained consistent** with that of samples collected on the same day as blood collection and those stored for 14 days under all storage conditions.



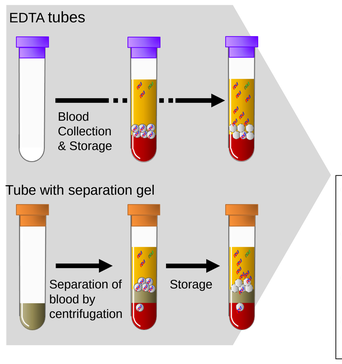
【Evaluating the Contamination of Genomic DNA from Leukocytes】
High molecular DNA fragments were observed in the plasma stored in EDTA tubes both with and without separation gel, suggesting that genomic DNA was released due to leukocyte rupture. **Insepack II-W prevented contamination with high molecular weight DNA fragments by effectively removing leukocytes.**



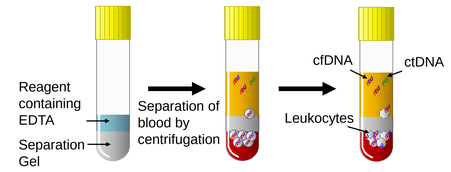
CONCLUSION & OUR SOLUTION

Insepack II-W has been demonstrated to improve the quality of cfDNA testing by reducing testing time with a single centrifugation operation and minimizing the contamination of leukocyte-derived genomic DNA during specimen storage by the removal of leukocytes using a separation gel.

Commercially Available Blood Collection Tubes



SEKISUI's cfDNA blood collection tube (Insepack II-W)



- Leukocytes are removed with a single-centrifugation operation.
- Samples are stable over a wide temperature range (4~37°C).
- No further sample processing is required for transport after centrifugation.
- PET tube and plastic caps ensure safety by reducing the risk of contact with patient blood.