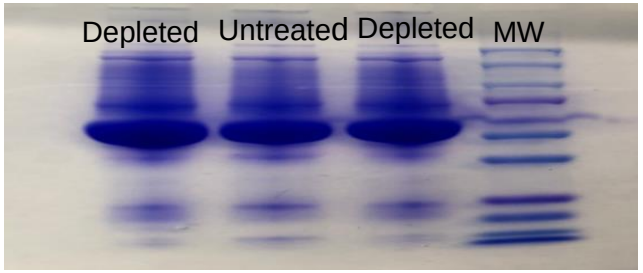
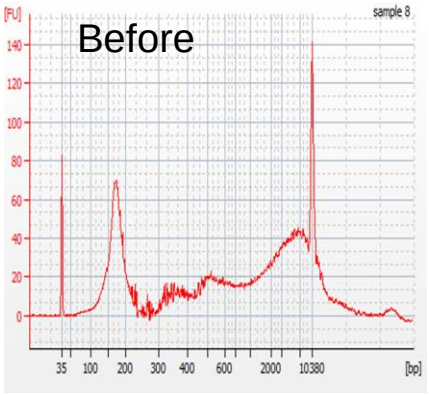
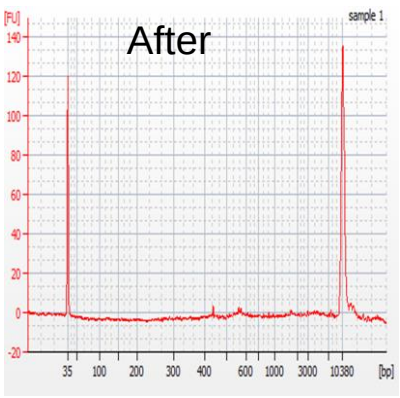
DNA-Depleted Plasma for Liquid Biopsy

Accurate Stable Traceable

Characterization: Removal of Background DNA Noise from Plasma or Serum While Keeping Everything Else Constant

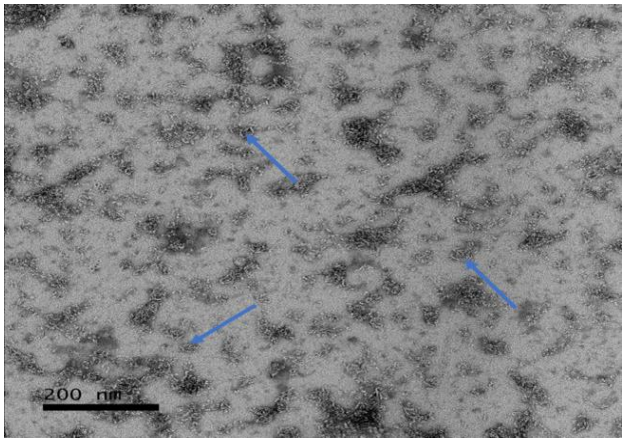


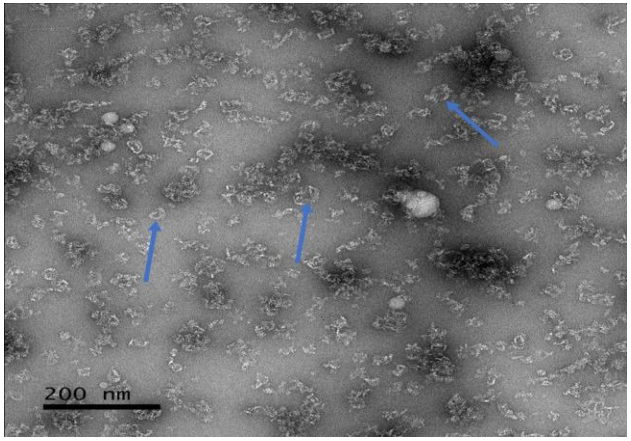




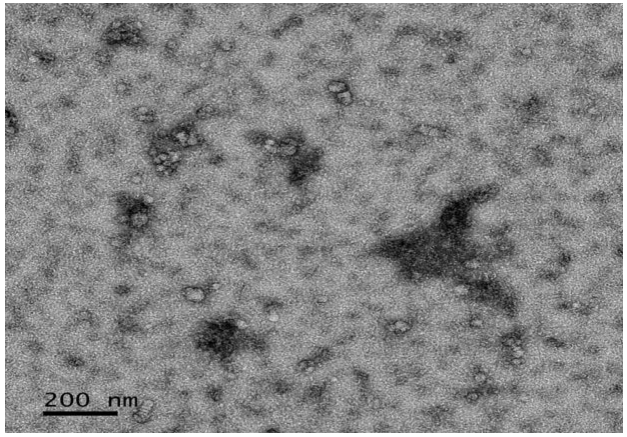
DNA: < 4 pg/ml (**as low as 0.06 pg/ml**)
Total protein: 5-7 g/dL
Immunoglobulin G: 500-1700 mg/dL
Immunoglobulin A: 90-400 mg/dL
Immunoglobulin M: 20-172 mg/dL
Cholesterol, Total: 50-199 mg/dL
Triglycerides: 50-149 mg/dL
HDL Cholesterol: > 20 mg/dL
VLDL Cholesterol: 10-40 mg/dL
LDL Cholesterol: 20-99 mg/dL
pH: 7.2-7.6
Negative for HIV, HBV, HCV, HTLV and Syphilis.

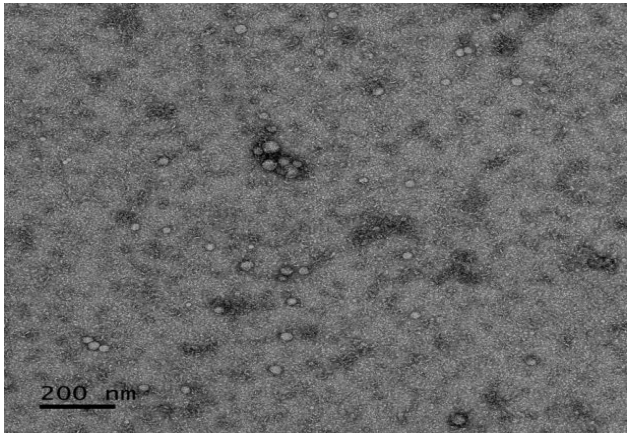
Exosomes and Non-exosomal Vesicles in DNA-depleted Plasma Are Comparable to Untreated





Exosomes



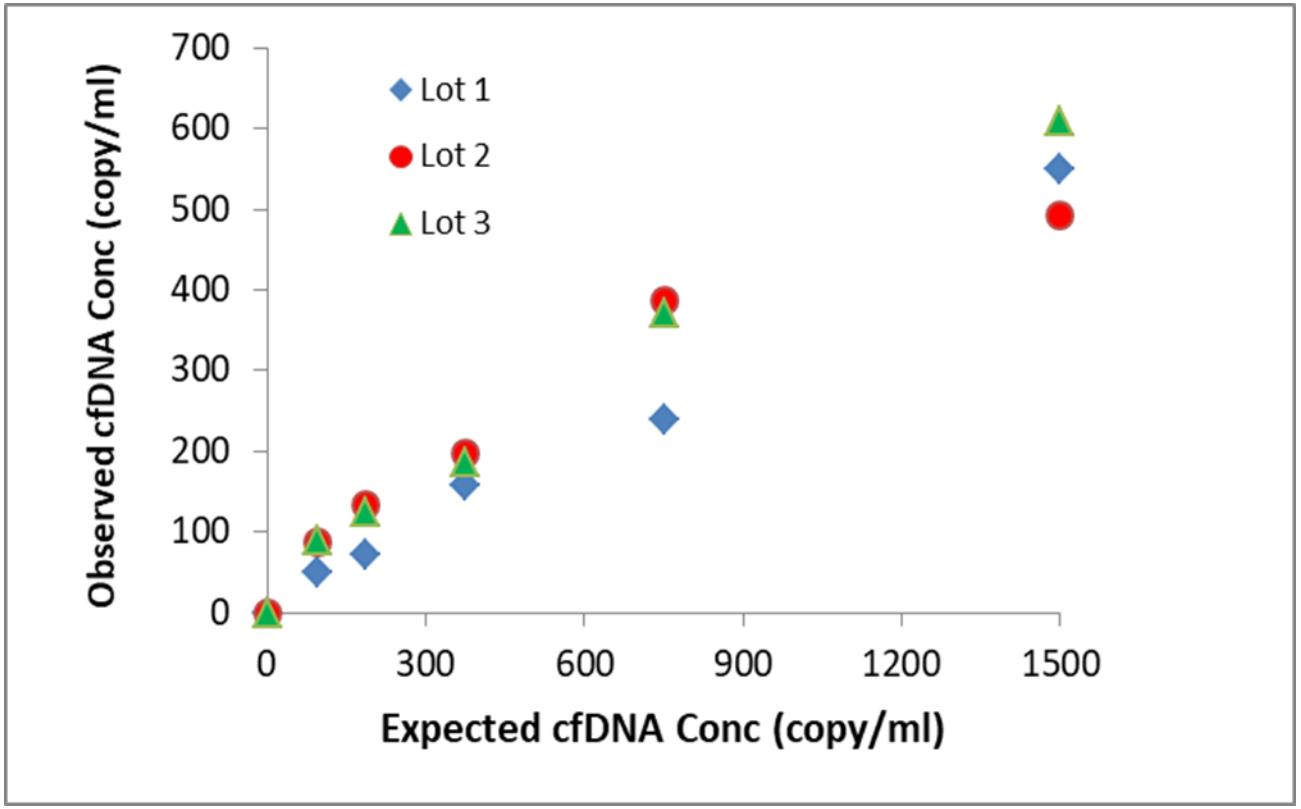


Non-exosomal Vesicles/aggregates

Normal plasma

DNA-depleted plasma

DNA Extraction Recovery from Different Plasma Lots



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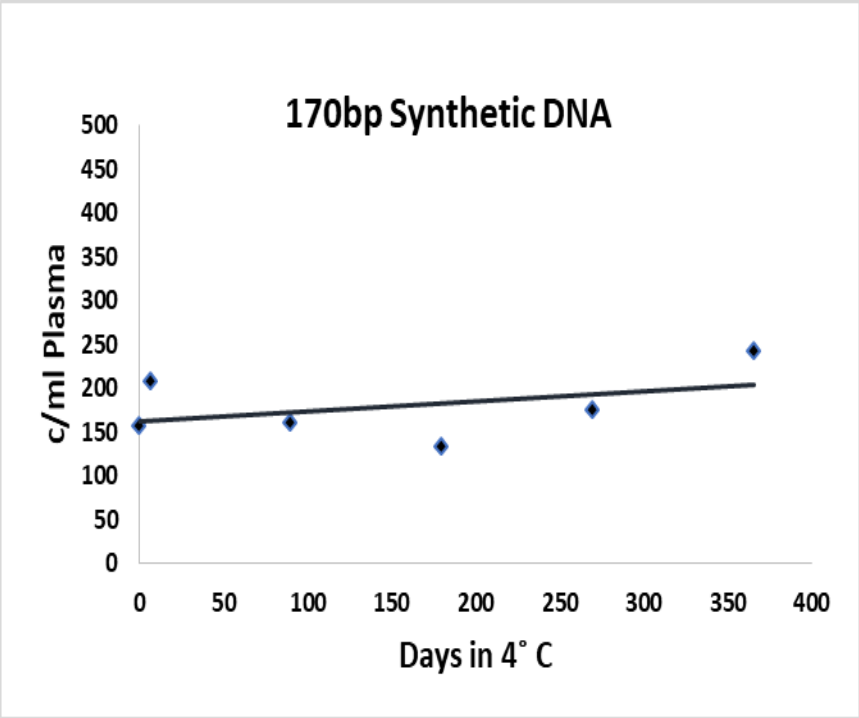
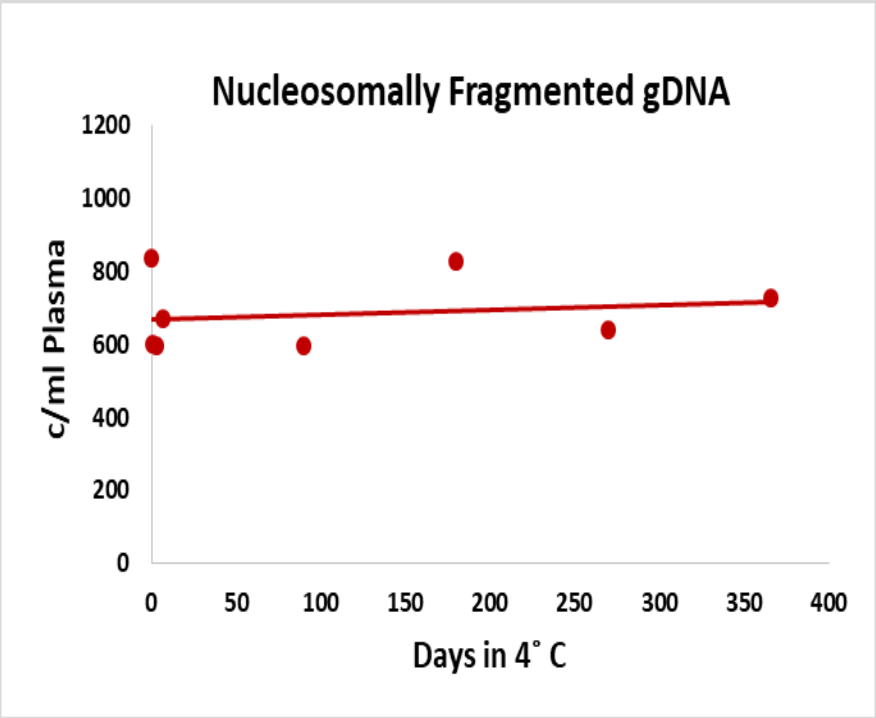
4˚C Real-Time DNA Stability in DNA-depleted Plasma (365Day)





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WHY Using Plasma-based Validation Sample is Important for Assay Validation?

Problem: Lack of Appropriate Molecular Controls and Standards

- Human plasma is a complicated matrix of nucleic acids, vital proteins/enzymes, mineral salts, fats, hormones and vitamins.
- ctDNA is often present at very low levels (<1%) relative to total cell-free (cf) DNA levels.
- Actionable Molecular Assay Development requires:
  - High sensitivity for detection of rare ctDNA in the presence of predominantly cfDNA.
  - Quality standards/controls that mimic tissue characteristics.

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Result of Inappropriate Molecular Controls/Standards

1. Systemic lack of TRUE standards and controls results in:
   a) Poor Clinical sensitivity

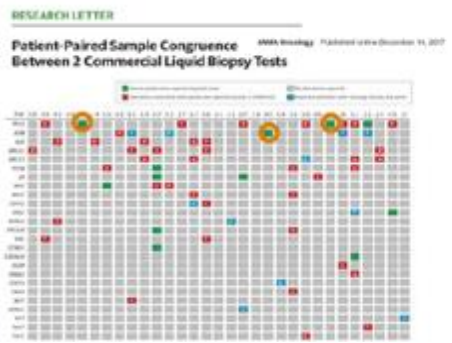
      (only 60-70%; ref)

   b) Discordance among assays - Despite enhancement in sequencing detection, there still remain rampant discordance among liquid biopsy assays. Assays developed in 2019 are no more sensitive than assays developed in 2017.

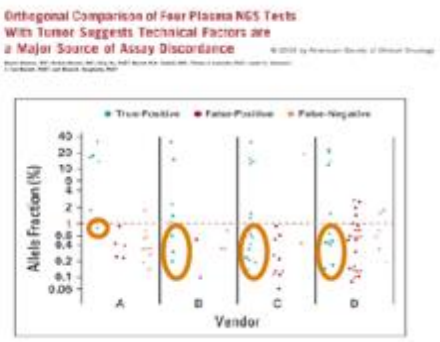
      (Variable levels of False positives & false negatives)

JAMA Oncol. 2018 Jun 1;4(6):868-870. doi: 10.1001/jamaoncol.2017.4027 .
Patient-Paired Sample Congruence Between 2 Commercial Liquid Biopsy Tests

2017



2019



JCO Precision Oncology. 2019 Feb 3(3):1-9. doi:10.1200/PO.18.00191

Orthogonal Comparison of Four Plasma NGS Tests With Tumor Suggests Technical Factors are a Major Source of Assay Discordance

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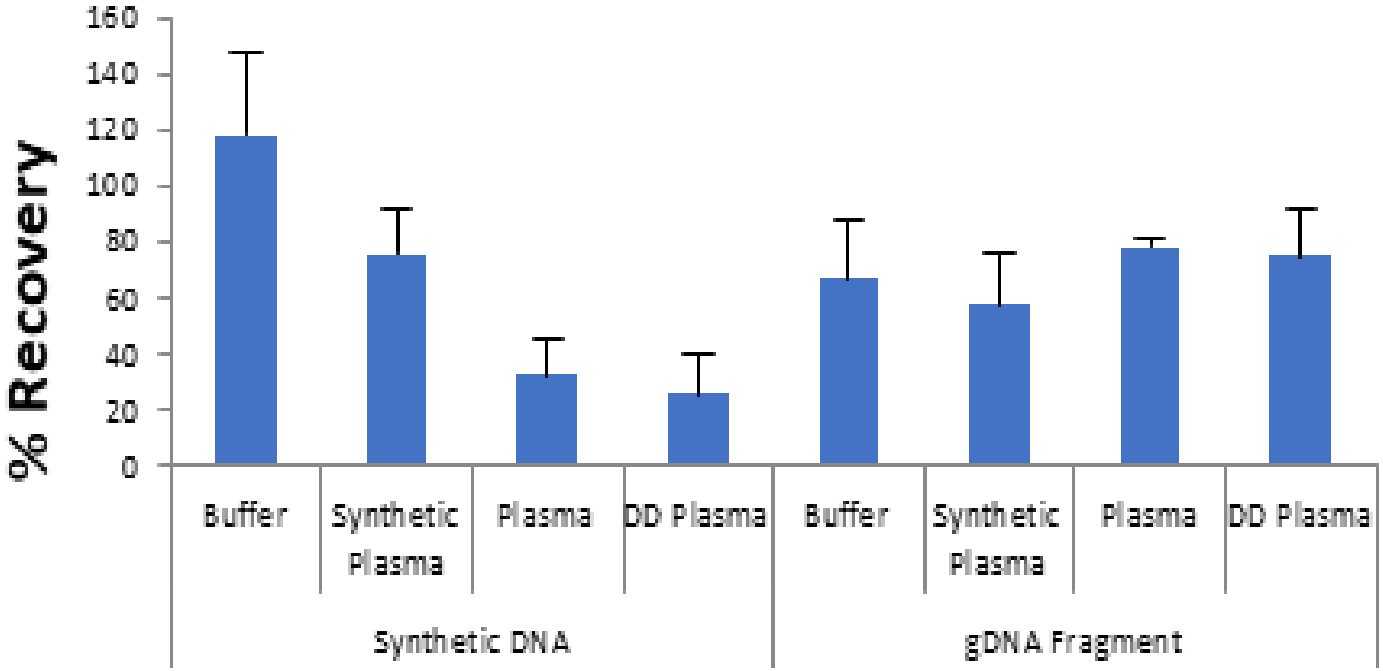
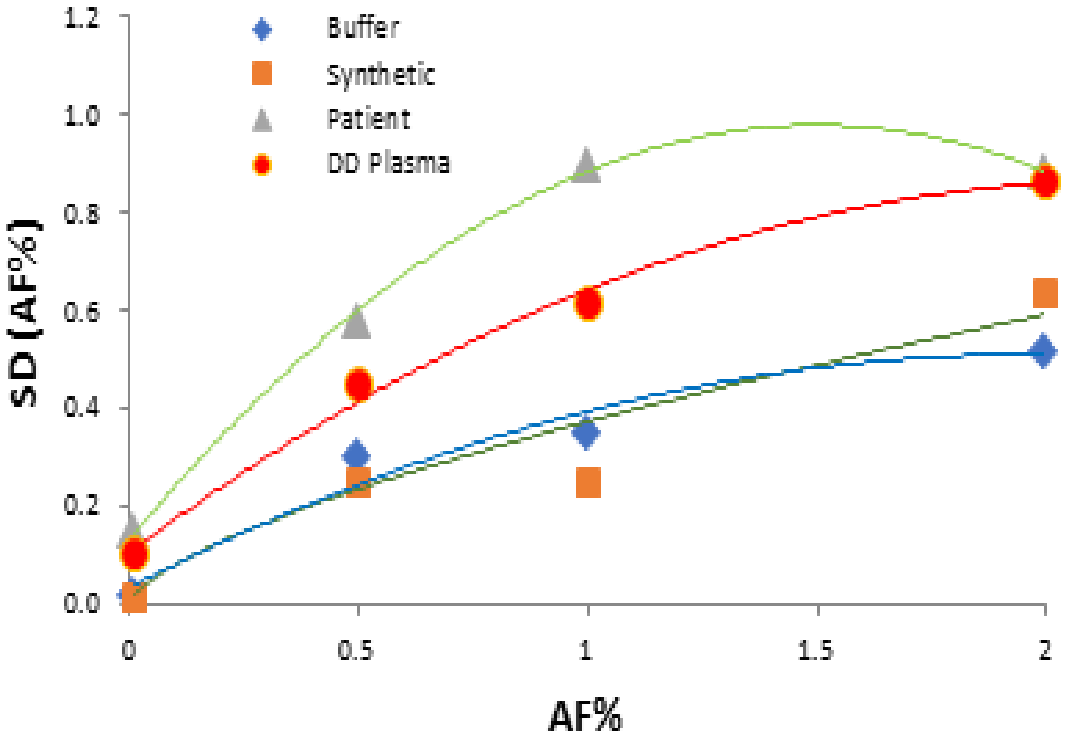
Matrix affects cfDNA extraction and recovery



Percent recovery from extraction of either spiked synthetic ctDNA (left) or fragmented gDNA (right) from 4 matrices are shown, including Anchor's DNA-Depleted Plasma (DD Plasma)

Matrix affects cfDNA extraction precision



Replicate Standard Deviation of the calculated allele frequency is shown

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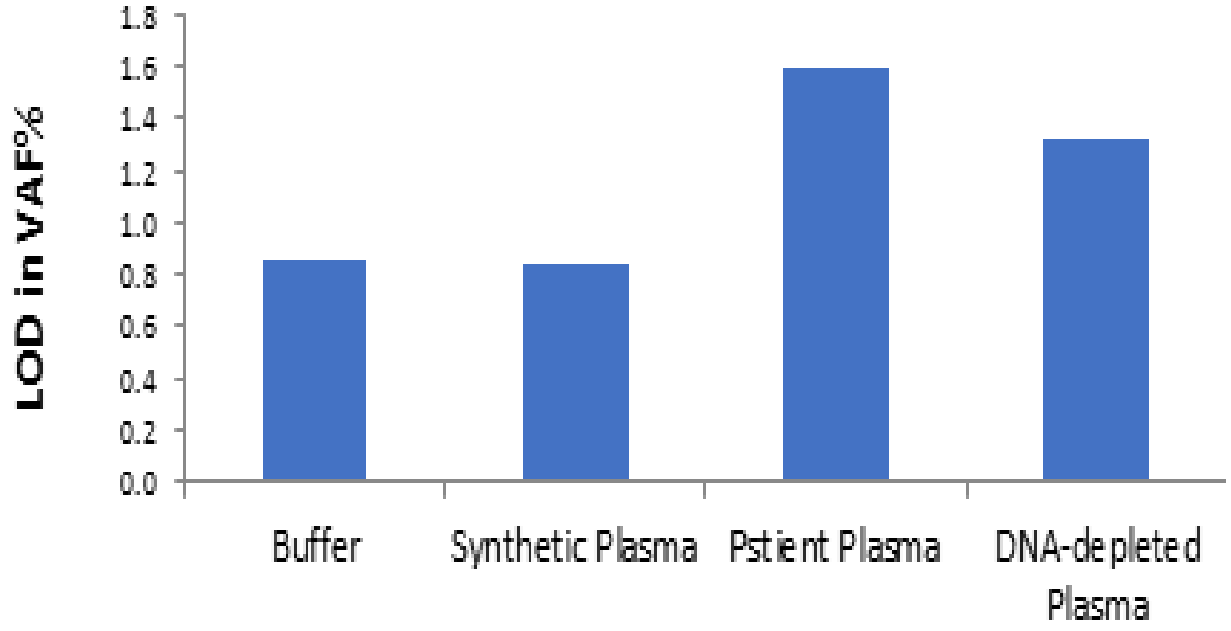
Underestimation of assay LOD when Buffer or Synthetic Plasma were used as sample matrix



The higher imprecision in both untreated patient plasma and DNA-depleted plasma is associated with higher Limit of Blank (LOB) and Limit of Detection (LOD) for AF% determination

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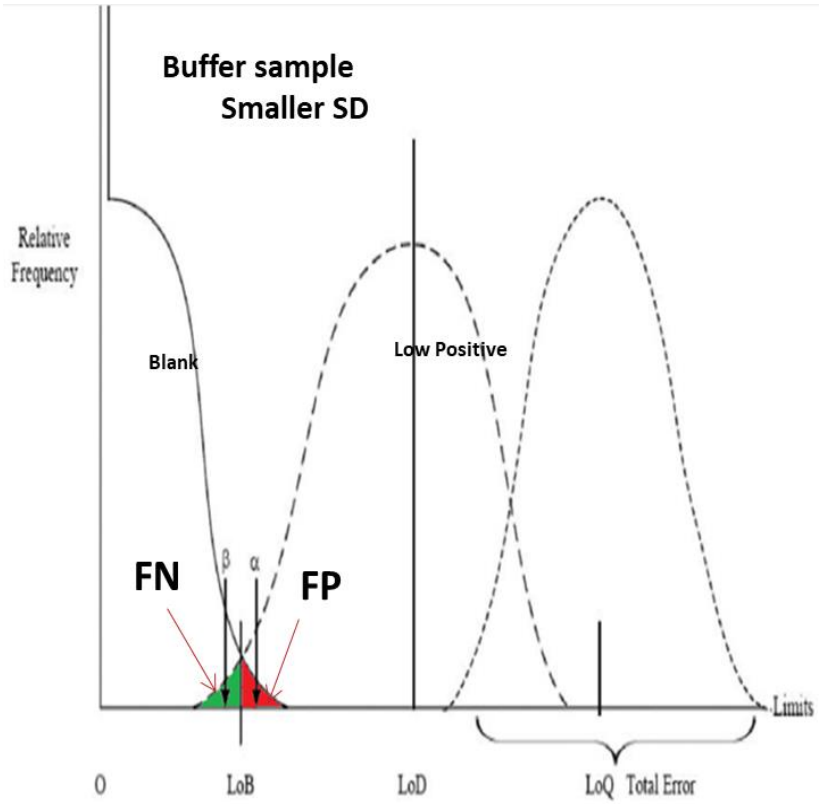
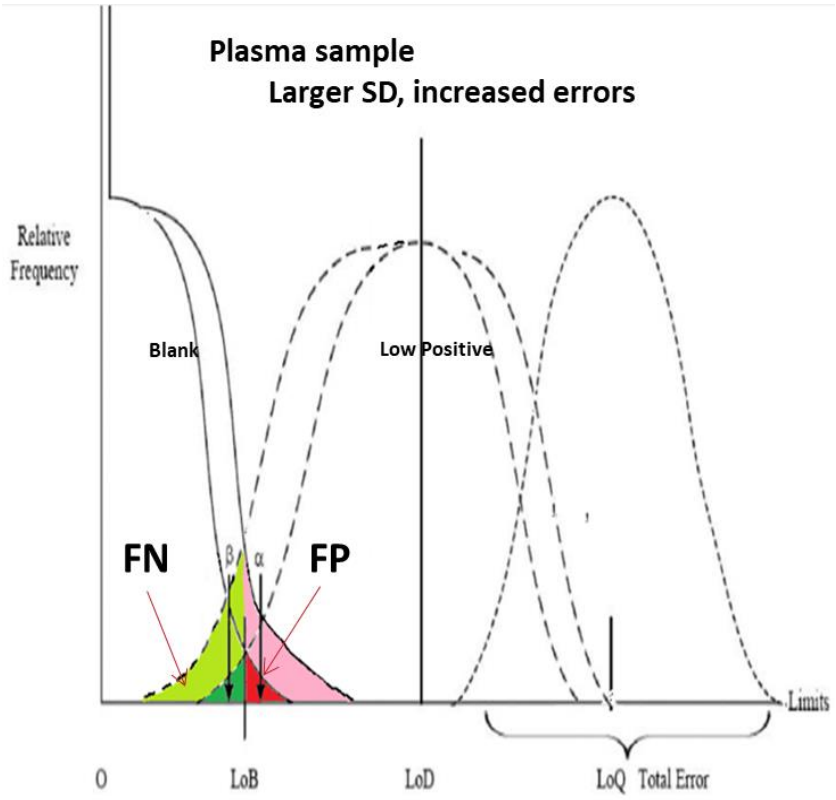
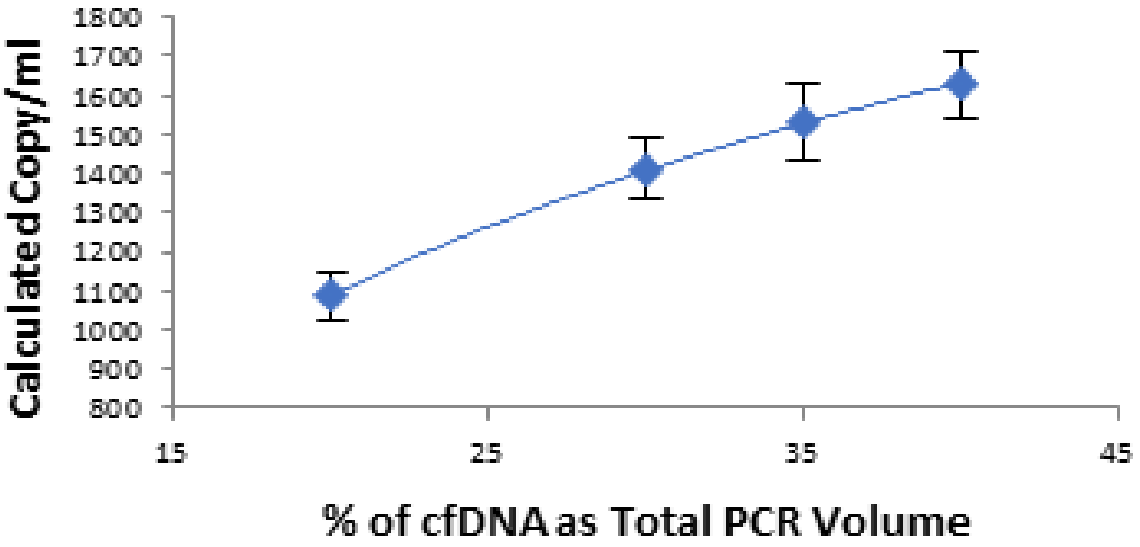
Increased SD Results in High FP and FN





If cut-off is determined based on buffer based sample (left), the cut-off will lead to higher FP and FN for real sample (right).

Drift in qPCR quantification caused by co-eluted plasma protein



The same **extracted gDNA** from plasma sample was quantitated with four qPCR assays with sample loading at 20, 30, 35 and 40% of the total PCR volume. The copy/ml increased with increasing sample loading. The drift was abolished by prior proteinase K treatment of the sample (data not shown).

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