A study to demonstrate that prolonged storage of blood in EDTA tubes is compatible with NIPT when using size selection to enrich fetal fraction

Yourgene Health

Programme ID P-94

Jessica Jefferies, Victoria Hutchinson, Daniel Ryan, Freya Sessions, Catherine Morrissey, Jonathan Bramwell, Ivan Sazanavets

Abstract

The study assesses the ability of Ranger® size selection to recover the fetal fraction (FF) from whole blood stored in EDTA blood collection tubes over a prolonged period of time prior to Non-Invasive Prenatal Testing (NIPT) using the IONA® Nx NIPT Workflow. Four 5 ml whole blood specimens were collected in BD Vacutainer™ K2EDTA Vacutainers from 74 patient volunteers (pregnant women >10+0 weeks' gestation) and were stored at room temperature until centrifugation at 4 different time points: 0-8 hours (Time Point 1), 72 hours (Time Point 2), 120 hours (Time Point 3), 168 hours (Time Point 4). The results of this study show that utilising size selection in NIPT workflows allows the use of EDTA blood collection tubes for prolonged periods over 8 hours. The data indicates that not only is fetal fraction maintained, but that the results of NIPT are concordant across the 7 days of the study.

Introduction

Yourgene's IONA® Nx NIPT Workflow utilises next generation sequencing to provide a fetal trisomy screening risk report for expectant mothers from samples derived from cfDNA present in maternal plasma. These cfDNA fragments are typically <200bp in size and can be of maternal or placental origin. The proportion of placentally derived cfDNA in the maternal blood sample is known as the fetal fraction (FF). Many NIPT tests utilize a 4% FF cut-off, however IONA® Nx uses a dynamic FF which, providing there are enough reads, will call <4% FF. Cell lysis due to sample instability can result in the release of maternally derived genomic DNA, the presence of which can cause dilution of the fetally derived cfDNA, leading to reduced FF. For this reason, sample stability and handling are regarded as crucial elements in the preservation of acceptable levels of FF and by extension the successful use of NIPT. Currently, the IONA® Nx test is validated for use with samples stored in Streck collection tubes for up to 120 hours and K2EDTA vacutainer tubes for up to 8 hours. Although Streck tubes have superior sample stabilisation properties, they are more expensive and are subject to IP licensing in some territories, therefore an extension to the usable timeframe of K2EDTA vacutainers would significantly decrease the costs of NIPT testing.

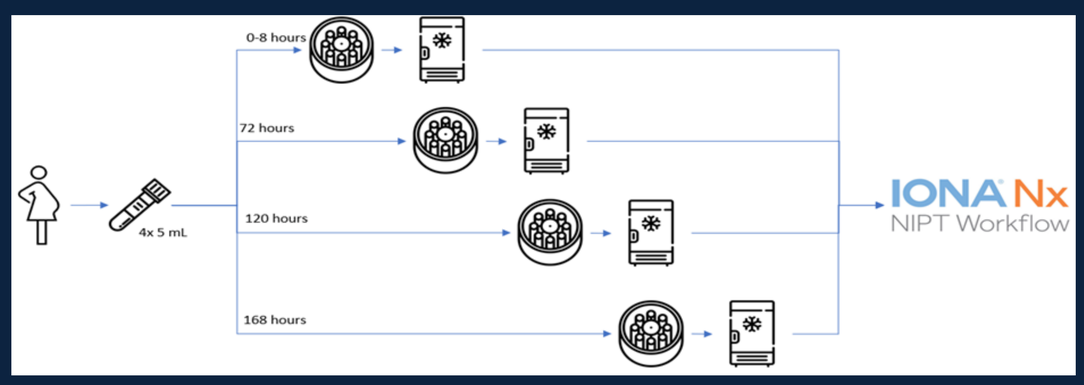
The IONA® Nx NIPT Workflow incorporates a unique size selection step, this step uses Yourgene Health's proprietary Ranger® Technology to allow collection of DNA library material within a defined size range. This step is intended to enrich the fetal derived cfDNA fragments in samples, which supports attaining the required sequencing depth and reduces failure rates. As maternal genomic DNA is typically larger in size than fetal cfDNA derived from the placenta, it is hypothesised that application of this size selection step may facilitate reduction/removal of this contaminant from pooled sample libraries, so allowing the use of K2EDTA collection devices outside of the recommended 0-8 hr limit. This study was a proof of concept to assess the performance of the IONA® Nx NIPT Workflow on samples stored in K2EDTA vacutainer tubes outside of the currently validated 8 hours.

Methods and Materials

The collection of samples, ethics, approval and informed consent were the responsibility of Tommy's Research Centre Team, Maternal and Fetal Health Research Centre, St Mary's Hospital, Manchester. Samples were collected from a cohort of low-risk pregnant volunteers (>10+0 weeks gestation), where fetal abnormalities intended for detection by IONA® Nx NIPT Workflow were not anticipated.

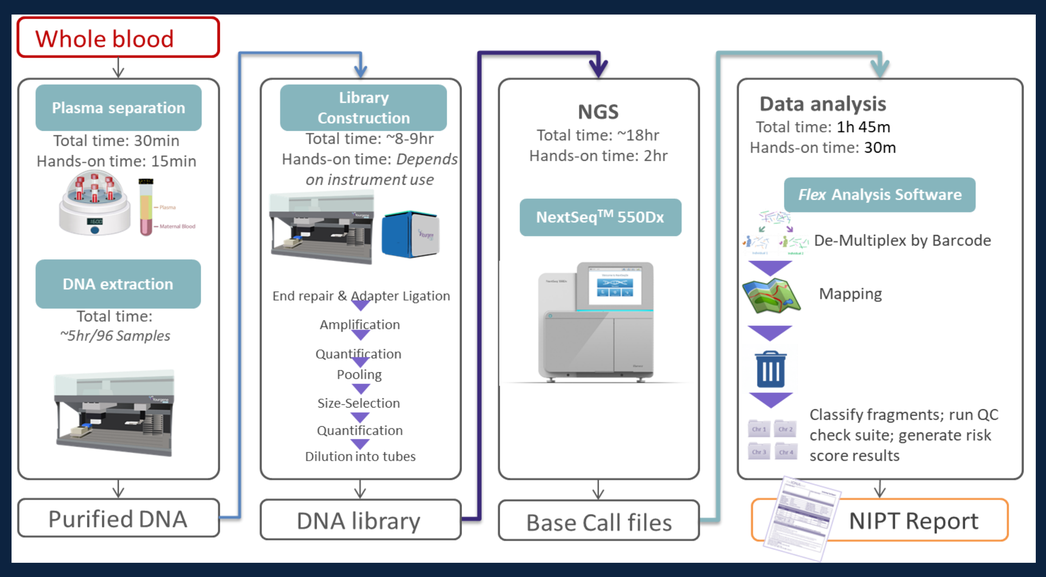
Up to four 5ml blood specimens were collected from 74 pregnant women. The whole blood was stored within vacutainers at room temperature (6-37°C) for the specified time points (+/- 8 hours) prior to centrifugation to retrieve the plasma fraction (Figure 1). Samples underwent a double spin procedure for plasma separation, plasma samples were then stored at -80°C until processing.

Figure 1. Study schematic of blood storage time points for each matched patient sample.



As per Figure 2, plasma samples were then processed through the IONA® Nx NIPT workflow using IONA® Software v2.0.2 and results analysed for Trisomy 13, 18 & 21 and fetal fraction. Further to this, metrics relating to library, sequencing and overall validity were calculated. One-way ANOVA analysis was carried out to assess variability in fetal fraction across timepoints. Results from time points 2-4 were compared with time point 1 for the relevant matched sample to establish concordance for Trisomy 13, 18 and 21 testing.

Figure 2. Schematic of IONA® Nx NIPT workflow.



Results

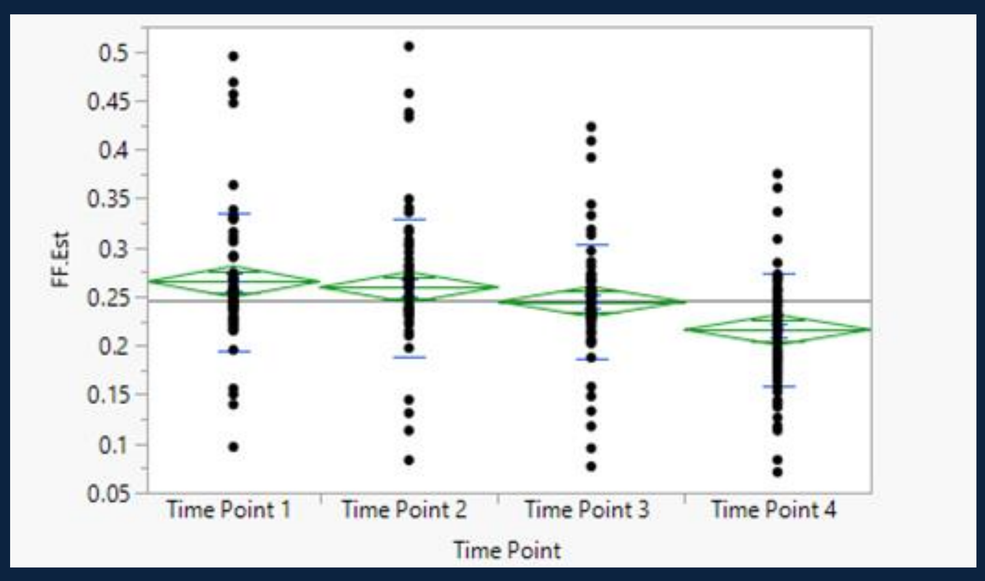
Overall, 268 samples gave a valid result after sequencing, closer inspection showed a trend towards increased library validity with increased storage time (100% of samples at time points 3 and 4 returning valid libraries). This trend was reversed when looking at sequencing validity where 100% of valid libraries produced valid sequencing results at time points 1 and 2. Overall system validity (the number of samples valid after sequencing/number of plasma samples) was >90% for each time point, with time points 2, 3 and 4 all outperforming the baseline (Time Point 1) (Table 1).

Table 1. Summary table of the library validity, sequencing validity and overall system validity rate across each time point. *= One sample was included in sample pool for size selection due to being close to meeting the library validity criteria (2.49µL).

	No. of Plasma Samples	No. of Valid Libraries	Library Validity Rate	No. of Samples Valid After Sequencing	Sequencing Validity	Overall System Validity – Core IONA® Nx
Time Point 1	70	63*	90%	64	100.00%	91.43%
Time Point 2	68	66	97.06%	66	100.00%	97.06%
Time Point 3	72	72	100%	69	95.83%	95.83%
Time Point 4	74	74	100%	69	93.24%	93.24%

Statistical analysis on valid samples showed that FF decreased with increasing storage time prior to centrifugation (Figure 3) . Although one-way ANOVA analysis indicates that this difference is statistically significant ($p<0.0001$), mean FF far exceeds 4% (Mean FF for time point 1 – 26.5% (0.265) vs 21.6% (0.216) for time point 4) for each time point suggesting that this decrease is insufficient to negatively affect the success of the NIPT workflow.

Figure 3. One-way ANOVA of the raw fetal fraction estimate for the 268 valid samples after sequencing, by time point. The mean for all time points are shown by the horizontal line, where green represents ANOVA and blue represents ± standard deviation.



Comparison of the T13/18/21 status result assigned by the IONA® Nx workflow showed 100% concordance with the time point 1 matched samples for each timepoint (Table 2).

Table 2. Concordance analysis for T13/18/21 status comparative to the valid baseline result (Time Point 1) for the 55 patient samples that returned a valid result for all time points.

	Time Point 1	Time Point 2	Time Point 3	Time Point 4
Valid T13/18/21 Result	55	55/55	55/55	55/55
# Valid for Concordance Analysis	N/A	55	55	55
Concordance	N/A	100%	100%	100%

Conclusion

The results of this study support the hypothesis that utilising size selection capability of Yourgene's proprietary Ranger® Technology in our NIPT workflow allows the use of EDTA blood collection tubes for prolonged periods over 8 hours. The data indicates that not only is fetal fraction maintained, but that the results of NIPT are concordant across the 7 days of the study. The use of EDTA blood tubes in NIPT could offer a considerable saving in the cost of the stabilising blood tube, and in the US where there is IP in the use of stabilising blood collection tubes, avoid costly licensing fees.

The results greatly improve the feasibility of EDTA tube usage, particularly in situations where delayed transport of samples between collection site and analysis locations may occur, which will be very beneficial and gives greater flexibility and choice to labs and clinicians.

Acknowledgements

We would like to thank: Jessica Morecroft, Rachel Eastham & Sarah Lee (Tommy's Charity, St Marys Hospital, Manchester Foundation Trust) for recruiting patients and collecting samples.
Alice Robins, Martha Kelly, Elisabeth Veso, Divya Annathurai, Alice Clements, Flora Averill-Spence & Lora Dobreva (Yourgene Health) for processing samples.

Scan the QR code to learn more about Ranger® Technology