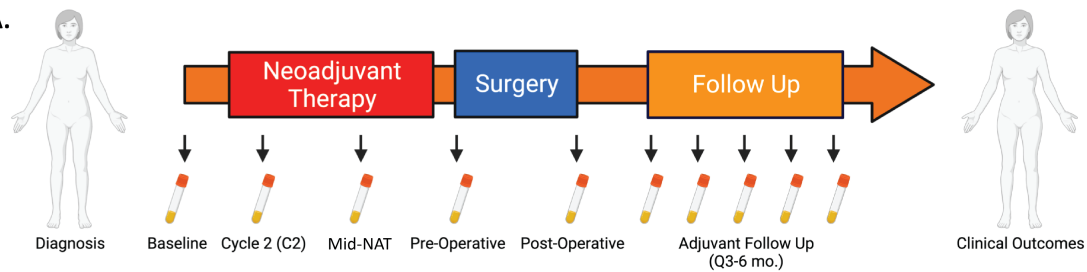
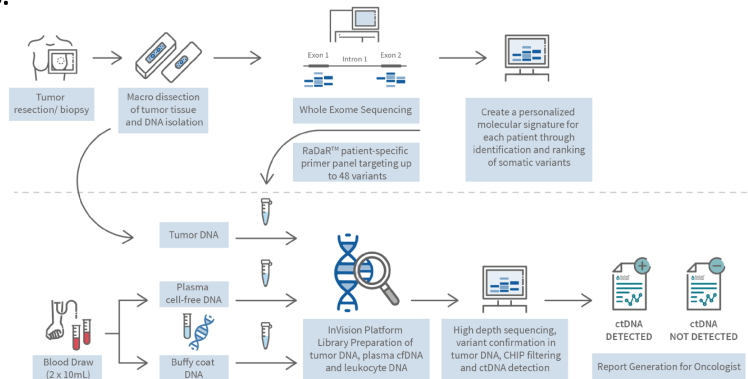


Supp. Figure 1.

A.



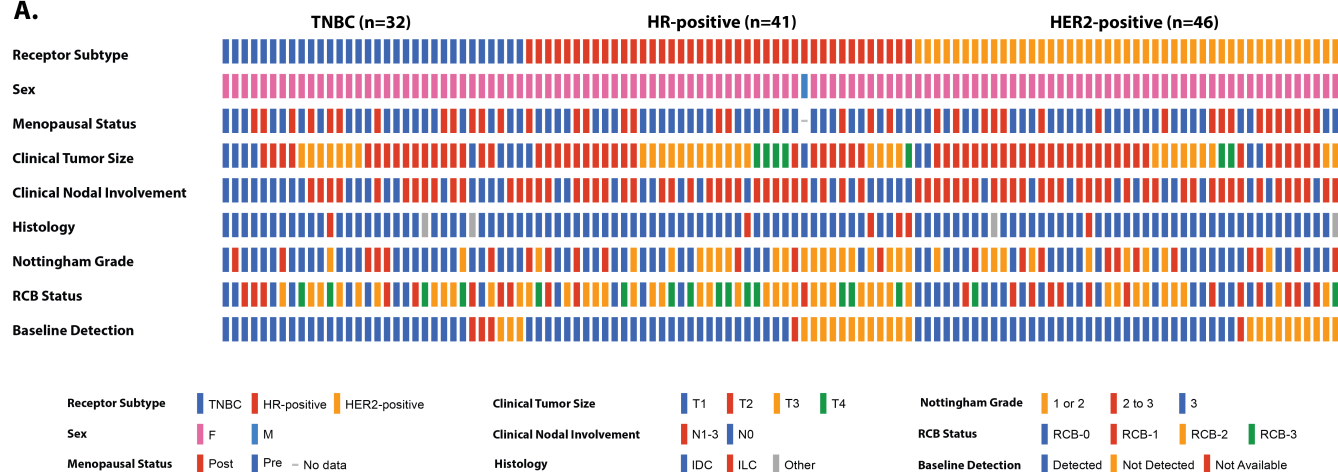
B.



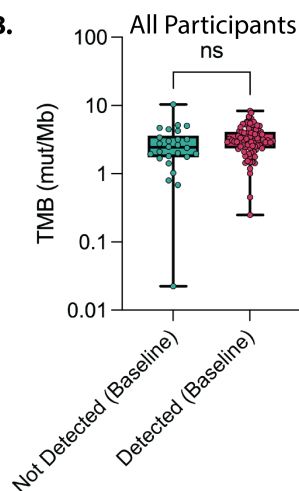
Supplementary Figure 1. Cohort information. (A) A participant's timeline with targeted plasma collections (B) ctDNA assay protocol schema. Q3-6 mo.: every 3-6 months.

Supp. Figure 2.

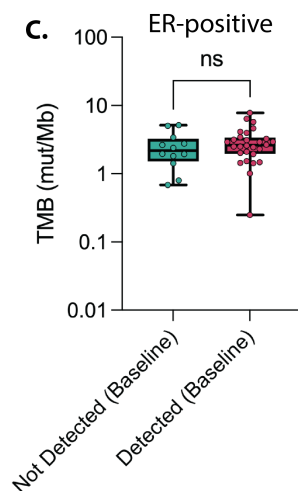
A.



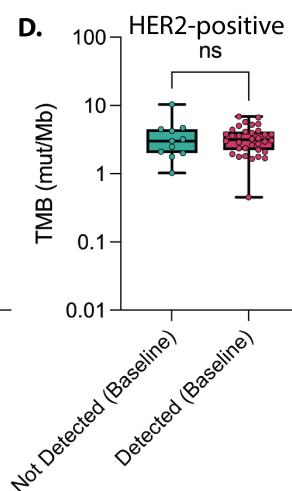
B.



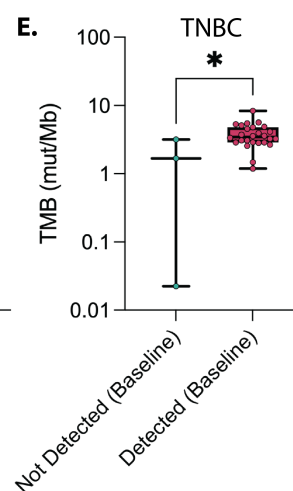
C.



D.

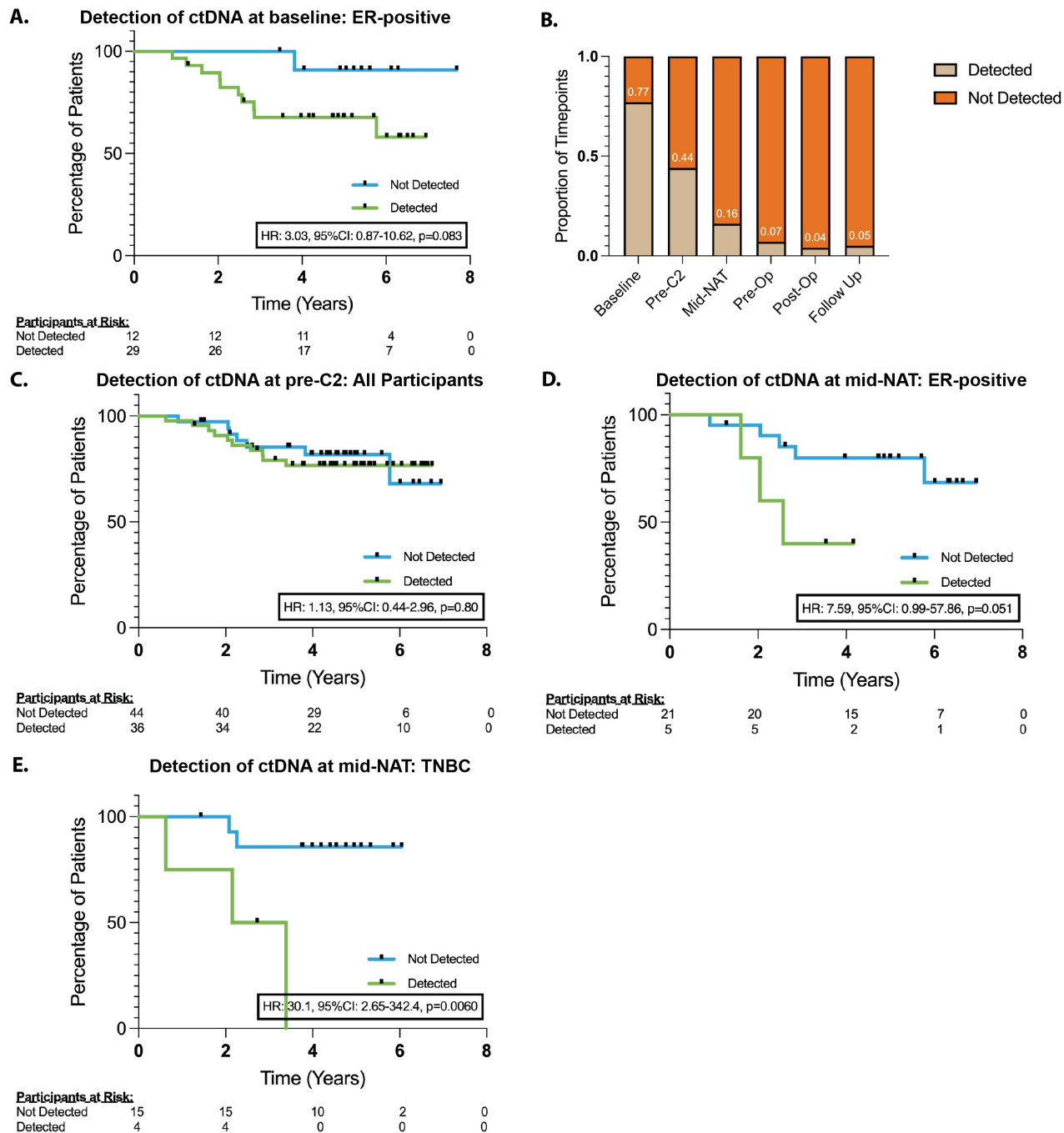


E.



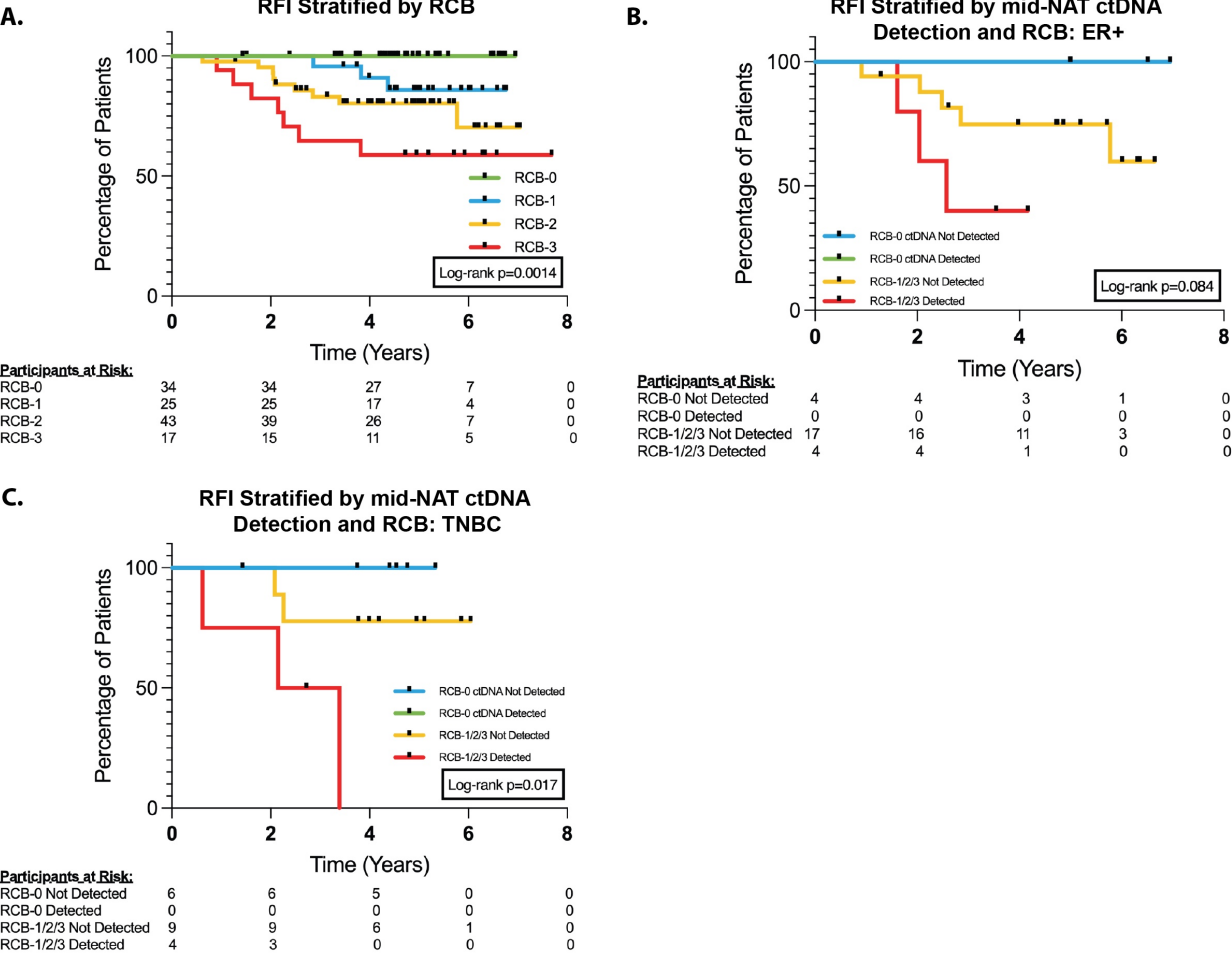
Supplementary Figure 2. Baseline participant clinical demographics and eVAF association with clinical variables. (A) OncoPrint outlining baseline clinical variables for participants included in this study. TMB in (B) all participants (t-test, $p=ns$) (C) ER+ (t-test, $p=ns$) (D) HER2+ (t-test, $p=ns$) and (E) TNBC (t-test, $p=0.024$) with and without ctDNA detected at baseline. All p -values are 2-sided, with $p<0.05$ considered statistically significant. ns=not significant.

Supp. Figure 3.



Supplementary Figure 3. Prognostic value of ctDNA detection. (A) ctDNA detection at baseline and association with RFI in ER+ EBC (Log-rank (Mantel-Cox) p=0.083). (B) Proportion of timepoints with ctDNA detected. (C) Pre-C2 detection of ctDNA and association with RFI in all participants (Log-rank (Mantel-Cox) p=0.80). Mid-NAT ctDNA detection and association with RFI in participants with (D) ER+ (Log-rank (Mantel-Cox) p=0.051) and (E) TNBC (Log-rank (Mantel-Cox) p=0.0060).

Supp. Figure 4.



Supplementary Figure 4. RCB Outcomes and Improved Prognostic Value of mid-NAT ctDNA Detection. (A) RFI outcomes in all participants stratified by RCB-score (Log-rank (Mantel-Cox) $p=0.0014$). Stratified assessment of RFI including RCB outcome and mid-NAT ctDNA detection in (B) ER+ (Log-rank (Mantel-Cox) $p=0.084$) and (C) TNBC participants (Log-rank (Mantel-Cox) $p=0.017$). Note: no participants were in the RCB-0 Detected category and thus the curve is not represented.

Supplementary Methods:

Tumor Whole-Exome Sequencing Analysis:

Deduplicated and filtered BAM files were obtained from NeoGenomics. Tumor whole-exome sequencing analysis to identify somatic mutations was performed independent of the RaDaR® ctDNA workflow (described below). Base quality score recalibration (BQSR) was performed using GATK (v3.8) with the BaseRecalibrator and PrintReads tools.^{31,32} The recalibrated BAM files were sorted and indexed using Samtools (v1.3.1).³³ Variant calling was performed with Mutect2 with the tumor-only option in GATK (v4.1.8.1). Variants were called against the human reference genome (Homo sapiens assembly 38) with specified capture regions (NeoGenomics panel) and using a germline resource and a panel of normals for filtering. Orientation bias filtering and contamination estimation were applied during variant calling. The resulting variant call format (VCF) files were further filtered using GATK's FilterMutectCalls tool. The filtered VCF files were annotated using VEP (v98) and converted to mutation annotation format (MAF) using vcf2maf (v1.6.17).^{38,39} Variants were filtered using in-house scripts with the following criteria: (1) depth >100, (2) variant allele frequency (VAF) >5% to filter sequencing errors (3) VAF not in 40-60% or >90% to filter germline mutations (4) filter for 'Missense_Mutation', 'Nonsense_Mutation', 'Frame_Shift_Ins', 'Frame_Shift_Del', 'Splice_Site', 'In_Frame_Ins', 'In_Frame_Del' (5) allele frequency <1% in the GNOMAD v.3.0 database.⁴⁰ For investigation of top variants, filtering was performed for known driver genes listed in the COSMIC Cancer Gene Census (v100).³⁴ Oncoplots were generated with maftools (v2.18.0)³⁵ and RColorBrewer (v1.1-3) in R (v4.3.2).

ctDNA Sequencing Analysis:

Analysis of ctDNA was performed in accordance with the RaDaR assay protocol as previously described.^{26,36} NeoGenomics was blinded to all clinical data and participant outcomes at the time of sample analysis. Briefly, FFPE tumor was sent for WES. Tumor DNA was extracted using the Maxwell RSC DNA FFPE kit (Promega) and quantified with the Quanti-IT dsDNA Broad Range (BR) assay (Invitrogen). The KAPA Hyper Prep kit (KAPA Biosystems) and unique dual-indexed (UDI) adapters were used for library preparation as previously described.^{26,37} The IDT xGen Exome Research panel v1.0 was used for exome enrichment of the resulting pre-capture libraries followed by fragment analysis and quantification of enriched libraries prior to pooling, normalization, and sequencing on the Illumina HiSeq4000 platform.

Tumor-only exome sequencing data analysis was executed using a custom-designed workflow including fastq file processing, alignment to the human genome (hg38), duplicate marking, and variant calling. Germline variants were filtered by applying tailored filters that considered prior information sourced from public single nucleotide polymorphism (SNP) databases. Somatic variants initially detected

in the tumor tissue through WES underwent prioritization via a proprietary algorithm to formulate an individualized primer panel, encompassing up to 48 primer pairs, each capturing at least one somatic variant (Sup. Fig. 1B).

Following panel preparation, tumor DNA and baseline buffy coat were subjected to targeted sequencing to validate selected somatic variants. Plasma cfDNA was evaluated on personalized panels alongside a baseline buffy coat DNA control sample. This control sample played a dual role: identification and elimination of germline variants and the removal of variants associated with CHIP from ctDNA calls, while also serving as a positive amplification control. Additional details of the entire RaDaR assay workflow have been described previously (Sup. Fig. 1B).^{26,36,37}

Supplementary Tables:

Supplementary Table 1. Baseline Whole Exome Sequencing Characteristics for Assay Generation.

Supplementary Table 2. Summary Characteristics of the Assay Panel Variant Selection and Validation.

Supplementary Table 3. Individual Participant Time Point Assessment with the Assay and Summary Characteristics.

Supplementary Table 4. Supplementary Table Legend.

Supplementary Table 5. Sensitivity, Specificity, and Predictive Value of ctDNA Detection and Recurrence.

Sup. Table 1

Patient ID	Panel ID	Reads (million)	Aligned %	Duplication %	On target %	Usable %	Mean Coverage	Tumor	Total Variants	Panel	Tumor passing variants	Mean Tumour VAF	QC Passing Variants	Mean Tumour VAF	WES Passing Variants
LIB-02-0001	30008241P01	616.2600098	99.48200226	58.83800125	91.45700073	37.5320015	155	48	27			0.121308209	0.1624375		
LIB-02-0005	30007748P01	297.8999939	99.91699982	12.99600029	94.0530014	81.86100006	190	49				0.168442383	0.168204082		
LIB-02-0006	30004560P01	396.3739929	99.93899976	21.13299942	94.82600226	74.57700348	218	48	37			0.257294719	0.244907315		
LIB-02-0010	30007477P01	307.019989	99.94100189	13.72999973	94.46900177	81.95899963	191	49	39			0.250214662	0.179510204		
LIB-02-0011	30008310P01	363.589963	97.32800293	29.77000046	89.44699986	61.1230011	141	49				0.177473679	0.165775511		
LIB-02-0012	30008262P01	702.6199951	99.6309967	31.3029995	92.71299744	63.56999969	298	28	24			0.199289107	0.148877553		
LIB-02-0013	30008239P02	521.9299927	99.74700165	52.60400009	90.77000092	42.96899951	154	48				0.221293113	0.212604167		
LIB-02-0014	30004560P01	401.7599978	99.94700047	93.7559997	80.80199738	61.7559997	251	25	17			0.235209251	0.239520001		
LIB-02-0015	30008324P01	299.3599854	99.47100067	75.5500305	85.93299866	22.6000031	39	49	18			0.191081784	0.15022449		
LIB-02-0016	30008242P01	646.6989929	99.81500244	30.83200073	93.52700043	64.68299866	254	49	33			0.377682009	0.269285715		
LIB-02-0018	30004560P01	579.9400024	99.90299988	18.84939986	92.89499664	75.43000031	342	40	19			0.290425498	0.158775		
LIB-02-0019	30004571P01	484.9580078	99.92700195	27.36499977	95.16899872	69.17900085	239	46	44			0.332862845	0.267608696		
LIB-02-0020	30004558P01	447.1799927	99.9280036	14.93800022	92.8259964	79.02300262	272	35	24			0.340285749	0.090971428		
LIB-02-0021	30008309P01	660.1199951	99.66799927	52.51300049	92.34100342	43.80099869	200	48	45			0.28721701	0.282000001		
LIB-02-0022	30008362P01	1004.530029	99.40699768	43.68000031	92.16999817	51.69300079	356	50	21			0.209407732	0.12762		
LIB-02-0023	30004560P01	480.5499878	99.93499756	15.95600033	92.6760025	77.97000122	295	49	39			0.206572601	0.149857143		
LIB-02-0025	30008314P01	657.8599854	99.76899719	31.42699814	92.32500153	61.55199814	268	48				0.308603551	0.310645834		
LIB-02-0026	30008351P01	547.8900146	99.83200073	31.7899993	93.94900711	64.23000336	222	49	27			0.235594849	0.191244986		
LIB-02-0027	30004582P01	562.3729858	99.85099702	26.52700043	92.15899658	67.73100171	306	32	42			0.210368635	0.208781249		
LIB-02-0028	30008361P01	718.1900024	99.6780014	28.16300011	92.9980011	66.72299957	294	49	46			0.163498309	0.164897959		
LIB-02-0029	30004581P01	379.3800049	99.89600372	13.38799953	93.30699921	80.86699677	242	49	43			0.344708956	0.304999998		
LIB-02-0030	30007748P01	322.2900085	99.9260025	14.4299984	94.5490036	80.91100311	208	47	43			0.116249773	0.131801383		
LIB-02-0031	30004580P01	402.6700234	99.9289986	9.47299957	94.34000269	76.06999969	235	48	41			0.270925887	0.2453125		
LIB-02-0032	30004577P01	487.0400085	99.89399719	26.84499931	93.72799683	68.619003	253	49	0.2			0.192081634	0.158520833		
LIB-02-0033	30008383P01	453.7099915	95.94300079	36.57300186	89.73000336	54.60300064	164	48			NA		0.195833334		
LIB-02-0035	30008408P01	731.5100098	99.72000122	27.76000023	92.93000031	67.07499965	307	48	46			0.161419018	0.190583334		
LIB-02-0036	30004579P01	477.4599915	99.94899975	26.28600006	94.0599976	69.45700073	256	48			0.2		0.192166667		
LIB-02-0037	30008431P01	1027.500044	99.70400238	38.82399979	93.30999884	56.95100021	373	49	41			0.099772694	0.116081633		
LIB-02-0038	30008426P01	609.1099854	99.64399719	51.6230011	93.09100342	44.9469986	170	24	8			0.225163003	0.145208333		
LIB-02-0039	30007474P01	308.2600098	99.94599915	25.37199974	95.22399902	71.09700012	161	49	43			0.431342464	0.326408162		
LIB-02-0041	30008390P01	530.5800171	99.4509964	33.67699814	93.68699646	61.86299896	213	48	42			0.461638865	0.423770836		
LIB-02-0042	30008414P01	107.649015	99.6669982	34.31200027	92.38500214	60.60300174	422	51	29			0.313697138	0.27921567		
LIB-02-0044	30004580P01	448.950015	99.91799127	16.6299916	93.35600281	77.88400269	271	49	44			0.10689937	0.221		
LIB-02-0045	30004743P01	262.5400085	99.94200134	14.01399994	94.84400177	81.58300018	171	45	49			0.292253997	0.219044442		
LIB-02-0046	30004557P01	522.789978	99.92099762	16.99600029	92.59799957	76.93599701	316	49	30			0.163362199	0.125204082		
LIB-02-0048	30015759P01	681.9699707	99.69300079	57.68999863	92.10199738	38.94300079	182	43	43			NA	0.192860465		
LIB-02-0051	30004559P01	408.1099854	99.89499664	16.83799934	93.31600189	77.64000208	243	48				0.225562499	0.225562499		
LIB-02-0053	30008430P01	781.3099976	99.6229998	58.56601089	92.9199962	38.19200134	204	48	46			0.325687601	0.295687601		
LIB-02-0054	30008464P02	676.3900146	99.68499756	55.84400177	92.06099701	40.64099954	184	48	47			0.152711898	0.166833332		
LIB-02-0055	30007473P01	270.3599854	99.93599701	12.69400024	94.17500305	82.25800323	168	48				0.20083047	0.182062498		
LIB-02-0056	30008477P01	639.289978	99.33899689	54.81900024	92.10999884	41.40399976	166	48	40			0.328666016	0.319275003		
LIB-02-0058	30007476P01	275.5	99.80399919	92.3030014	80.35999792	62.3030014	204	48	41			0.307541896	0.147858073		
LIB-02-0059	30015752P01	1326.500024	99.58200073	39.58700118	93.08100128	56.13999399	172	18	17			0.144900229	0.11612		
LIB-02-0060	30007748P01	314.589963	99.93599701	25.40500069	94.48600006	70.52700043	473	50	47			0.248639459	0.211666668		
LIB-02-0061	30008489P01	620.2999878	99.8089811	32.02600098	92.92299652	63.13899994	260	48	19			0.175355358	0.166187498		
LIB-02-0062	30004561P01	478.1099854	99.92299652	15.14599991	92.76699929	78.77000256	295	48				0.178811755	0.172958333		
LIB-02-0064	30004560P01	489.9599976	99.92600073	17.55999958	94.02699969	77.48200226	294	47	41			0.182766981	0.153467141		
LIB-02-0065	30004563P01	421.1600037	99.90100098	17.67099953	93.60800171	71.3309967	244	49	42			0.18742872	0.141387755		
LIB-02-0066	30008521P01	515.7199707	99.68599701	30.20000076	92.48100281	64.489975	232	49	30			0.30623403	0.236755101		
LIB-02-0067	30007748P01	325.8699951	99.93699646	12.88000011	93.94499969	81.90299988	202	46	33			0.112350801	0.104608696		
LIB-02-0068	30007748P01	299.3800049	99.94200134	13.34200001	95.10500336	82.47000122	159	50	48			0.505566113	0.4336		
LIB-02-0069	30004560P01	489.959977	99.92600073	16.9829994	92.30200348	77.45200348	234	48	41			0.267497345	0.267497345		
LIB-02-0071	30004586P01	444.5799866	99.9240036	16.27199936	93.51699829	78.34000177	273	48				0.151418487	0.1401875		
LIB-02-0072	30008547P01	1760.280029	99.58799744	45.57600021	92.9489975	50.4799954	596	49	42			0.203449254	0.207102042		
LIB-02-0073	30008569P01	899.4899902	99.46499634	30.9439814	92.7480011	42.25299835	246	48	36			0.158996469	0.148625001		
LIB-02-0074	30008575P01	390.8300171	99.7440033	30.37700081	92.08399963	64.05899811	371	49	24			0.34414895	0.19795102		
LIB-02-0076	30008560P01	461.9500122	99.91900122	9.31780001	92.93399811	61.33399811	249	48	41			0.33895133	0.180204082		
LIB-02-0077	30008519P01	928.539978	99.74199634	37.80400085	93.54199982	56.80099884	377	48	31			0.307559404	0.219500001		
LIB-02-0078	30004588P01	497.8410034	99.9260025	18.45400047	93.83000183	76.55699921	293	48	48			0.296476075	0.273354167		
LIB-02-0079	30008506P01	915.1300049	99.75900269	31.9899963	92.05899811	62.50400177	403	48				NA	0.140229167		
LIB-02-0081	30008314P01	483.9400024	99.69799805	27.83300073	90.61299896	62.57700043	220	48	23			0.207813114	0.146208333		
LIB-02-0083	30008547P01	1023.73999	99.93099618	35.6460104	92.9599967	60.01900103	403	48	40			0.329065837	0.234175		
LIB-02-0084	30004578P01	499.7799988	99.93399811	24.45400047	93.1289978	70.42500305	278	48	47			0.291303416	0.26385834		
LIB-02-0085	30008554P01	899.6599731	99.76699829	44.4599915	92.09500122	51.14500046	327	47	41			0.426683969	0.372404258		
LIB-02-0087	30008520P01	1032.150024	99.6302001	30.02899933	91.97699738	55.99000168	407	48				0.299838633	0.2820625		
LIB-02-0088	30007748P01	304.480011	99.94300079	13.78000045	94.0410037	81.72299957	188	42	24			0.174341496	0.102404762		
LIB-02-0089	30008312P01	489.8300007	99.5899802	40.86799895	92.7399798	64.54989920	279	48	42			0.274225184	0.158897027		
LIB-02-0090	30004578P01	526.3200073	99.91400146	16.63100087	92.61299896	77.33400299	322	48				0.182202917	0.164333333		
LIB-02-0091	30008337P01	1024.699951	99.70200348	38.09400177	91.6269989	56.66999882	404	49	38			0.30942855	0.241959183		
LIB-02-0092	30008613P01	636.3200073	99.66200256	60.2820015	91.85399628	36.41400189	147	47	22			0.239352177	0.156829787		
LIB-02-0093	30007748P01	403.1499939	99.93699976	1											

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Sup. Table 3

Year	Region	Baseline	Date of Collection	Specimen Type	Sample ID	Panel ID	Returned	Total Examined	Total Collected	Examined	QC	QC%	QC Status	All Pass Specimens	Mean Score	QC Pass Rate
18-00-0001	C1	Aug-17	Plasma	300071903	300071903	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	C1	Jul-17	Plasma	300071901	300071901	4.056	44.216	22.000	3.214	3.214	3.214	100.00	27	28336.284	548.629	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071902	300071902	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071903	300071903	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071904	300071904	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071905	300071905	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071906	300071906	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071907	300071907	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071908	300071908	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071909	300071909	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071910	300071910	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071911	300071911	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071912	300071912	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071913	300071913	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071914	300071914	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071915	300071915	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071916	300071916	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071917	300071917	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071918	300071918	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071919	300071919	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071920	300071920	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071921	300071921	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071922	300071922	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071923	300071923	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071924	300071924	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071925	300071925	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071926	300071926	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071927	300071927	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071928	300071928	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071929	300071929	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071930	300071930	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071931	300071931	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071932	300071932	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071933	300071933	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071934	300071934	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071935	300071935	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071936	300071936	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071937	300071937	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071938	300071938	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071939	300071939	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071940	300071940	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071941	300071941	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071942	300071942	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071943	300071943	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071944	300071944	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071945	300071945	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071946	300071946	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071947	300071947	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071948	300071948	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071949	300071949	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071950	300071950	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071951	300071951	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071952	300071952	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071953	300071953	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071954	300071954	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071955	300071955	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071956	300071956	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071957	300071957	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071958	300071958	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071959	300071959	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071960	300071960	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071961	300071961	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071962	300071962	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071963	300071963	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071964	300071964	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071965	300071965	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071966	300071966	2.801	7.066	6.120	9.818	9.818	9.818	100.00	27	28336.984	425.004	TRUE
18-00-0001	PCST-OP	Jul-17	Plasma	300071967	300071967											

UR-02-0025	Baseline	Jun-18	Plasma	300040408	30004040802	30004040901	4.507	9,612	7,240	1,320.04	1,320.02	48	15,361,237	298,984	TRUE	
UR-02-0025	CS	Jun-18	Plasma	300040408	30004040802	30004040901	4.511	9,610	7,240	1,320.03	1,320.01	48	15,361,236	298,983	TRUE	
UR-02-0025	FU.1	Jan-19	Plasma	300040408	30004040801	30004040901	4.396	10,006	10,020	1,241.13	1,241.11	48	12,762,778	266,475	FALSE	
UR-02-0025	FU.2	May-19	Plasma	300040408	30004040801	30004040901	3.873	13,780	10,600	1,420.13	1,420.11	48	11,382,156	237,121	FALSE	
UR-02-0025	FU.3	Nov-19	Plasma	300040408	30004040801	30004040901	3.873	13,780	10,600	1,320.13	1,320.11	48	11,382,156	237,121	FALSE	
UR-02-0025	FU.4	Mar-21	Plasma	300040408	30004040801	30004040901	4.134	12,277	9,440	1,116.14	1,116.12	48	8,950,180	207,399	FALSE	
UR-02-0025	FU.5	Nov-22	Plasma	300040408	30004040801	30004040901	4.003	9,400	7,800	1,060.03	1,060.01	48	8,900,180	207,399	FALSE	
UR-02-0025	FU.6	Nov-22	Plasma	300040408	30004040801	30004040901	3.847	13,624	10,480	1,768.14	1,768.12	48	10,137,474	894,427	FALSE	
UR-02-0026	Baseline	Aug-18	Plasma	300040479	30004047901	30004047901	3.671	10,800	8,400	1,460.13	1,460.11	48	8,932,176	185,807	FALSE	
UR-02-0026	CS	Aug-18	Plasma	300040479	30004047901	30004047901	3.671	10,800	8,400	1,768.17	1,768.15	48	10,682,150	137,125	FALSE	
UR-02-0026	FU.1	Jan-19	Plasma	300040479	30004047901	30004047901	3.206	12,440	9,200	1,208.14	1,208.12	48	12,122,150	238,120	FALSE	
UR-02-0026	POST-OP	Jan-19	Plasma	300040479	30004047901	30004047901	3.769	7,488	5,760	8,780.17	8,780.17	48	13,759,056	370,730	FALSE	
UR-02-0026	POST-OP	Jul-19	Plasma	300040479	30004047901	30004047901	3.228	9,440	7,440	1,111.13	1,111.12	48	12,996,048	418,848	FALSE	
UR-02-0026	FU.1	Jul-19	Plasma	300040479	30004047901	30004047901	4.001	5,112	3,120	5,112.28	5,112.28	48	12,127,127	441,117	FALSE	
UR-02-0026	FU.3	Mar-21	Plasma	300040479	30004047901	30004047901	3.557	8,008	6,160	4,790.20	4,790.20	48	12,824,159	645,678	FALSE	
UR-02-0026	FU.4	Mar-21	Plasma	300040479	30004047901	30004047901	3.553	14,456	11,320	1,375.17	1,375.15	48	10,681,002	531,079	FALSE	
UR-02-0027	Baseline	Aug-18	Plasma	300040481	30004048102	30004048101	3.447	14,820	11,520	2,806.04	2,806.02	48	8,481,984	283,515	TRUE	
UR-02-0027	CS	Aug-18	Plasma	300040481	30004048102	30004048101	4.524	10,800	8,400	2,326.10	2,326.08	48	8,837,947	301,767	FALSE	
UR-02-0027	POST-OP	Jan-19	Plasma	300040481	30004048102	30004048101	4.410	14,002	10,840	2,026.11	2,026.09	48	8,684,052	240,401	FALSE	
UR-02-0027	POST-OP	Feb-19	Plasma	300040481	30004048101	30004048101	4.474	12,700	9,040	2,006.12	2,006.10	48	8,251,942	220,648	FALSE	
UR-02-0027	FU.2	Sep-20	Plasma	300040481	30004048101	30004048101	3.925	10,764	8,280	1,778.14	1,778.12	49	8,038,978	185,734	FALSE	
UR-02-0027	FU.3	Mar-21	Plasma	300040481	30004048101	30004048101	4.863	10,348	7,960	2,778.16	2,778.14	49	7,940,577	160,612	FALSE	
UR-02-0027	FU.4	Sep-21	Plasma	300040481	30004048101	30004048101	3.599	7,312	5,680	2,002.12	2,002.10	49	7,962,717	162,504	FALSE	
UR-02-0028	Baseline	Sep-18	Plasma	300040482	30004048202	30004048201	2.658	6,012	4,640	2,994.12	2,994.10	24	5,167,001	238,120	FALSE	
UR-02-0028	POST-OP	Oct-18	Plasma	300040482	30004048201	30004048201	1.761	8,802	6,840	1,101.07	1,101.05	24	4,741,013	377,628	FALSE	
UR-02-0029	Baseline	Sep-18	Plasma	300071741	3000717403	3000717403	3.808	5,804	4,480	2,866.07	2,866.05	49	10,181,391	1,431,912	TRUE	
UR-02-0029	CS	Sep-18	Plasma	300071741	3000717403	3000717403	3.798	8,800	6,800	2,866.07	2,866.05	49	10,181,391	1,431,912	TRUE	
UR-02-0029	CS	Nov-18	Plasma	300071790	3000717903	3000717403	4.2	16,192	10,000	5,006.11	5,006.09	49	15,877,911	638,149	FALSE	
UR-02-0029	POST-OP	Feb-19	Plasma	300071790	3000717903	3000717403	3.795	15,964	9,960	2,346.14	2,346.12	49	14,684,124	703,774	FALSE	
UR-02-0029	FU.1	Jul-19	Plasma	300071792	3000717923	3000717403	4.37	12,844	9,880	1,778.13	1,778.11	49	15,164,798	778,547	FALSE	
UR-02-0029	FU.2	Oct-19	Plasma	300071793	3000717933	3000717403	4.403	13,068	10,000	1,046.11	1,046.09	49	15,266,169	791,473	FALSE	
UR-02-0029	FU.3	Mar-21	Plasma	300071793	3000717933	3000717403	4.37	11,676	10,574	7,028.12	7,028.10	49	15,712,028	804,450	FALSE	
UR-02-0029	FU.4	Nov-21	Plasma	300071774	3000717742	3000717403	4.405	11,760	8,760	1,106.13	1,106.11	49	12,761,193	778,861	FALSE	
UR-02-0029	FU.5	May-22	Plasma	300071776	3000717762	3000717403	4.239	16,328	11,560	1,206.12	1,206.10	49	12,338,136	251,799	FALSE	
UR-02-0031	Baseline	Oct-18	Plasma	300080902	30008090202	30008090901	3.25	5,668	4,440	9,946.05	9,946.05	48	18	27,071,757	544,976	TRUE
UR-02-0031	CS	Dec-18	Plasma	300080902	30008090202	30008090901	3.84	5,360	4,140	5,550.06	5,550.04	48	18	27,071,757	544,976	TRUE
UR-02-0031	CS	Jan-19	Plasma	300080902	30008090202	30008090901	4.13	23,296	17,020	9,946.05	9,946.03	48	18	27,071,757	544,976	TRUE
UR-02-0031	CS	Jan-19	Plasma	300080902	30008090202	30008090901	4.27	23,296	17,020	2,376.07	2,376.05	48	18	27,071,757	544,976	TRUE
UR-02-0031	PRE-OP	Apr-19	Plasma	300080902	30008090201	30008090901	4.383	8,580	6,600	1,246.23	1,246.21	48	17,734,011	579,875	FALSE	
UR-02-0031	POST-OP	Apr-19	Plasma	300080902	30008090201	30008090901	4.252	8,480	6,500	1,246.23	1,246.21	48	17,734,011	579,875	FALSE	
UR-02-0031	FU.1	Aug-19	Plasma	300080902	30008090201	30008090901	5.076	10,848	8,360	1,076.21	1,076.19	48	18,117,227	482,776	FALSE	
UR-02-0031	FU.2	Aug-19	Plasma	300080902	30008090201	30008090901	5.076	10,848	8,360	1,076.21	1,076.19	48	18,117,227	482,776	FALSE	
UR-02-0031	FU.3	Sep-21	Plasma	300080902	30008090201	30008090901	3.821	7,764	5,840	1,646.24	1,646.22	48	16,318,840	544,976	FALSE	
UR-02-0032	Baseline	Oct-18	Plasma	300080904	30008090402	30008090901	4.387	12,120	9,160	2,008.14	2,008.12	51	22	18,966,166	361,645	FALSE
UR-02-0032	POST-OP	Nov-18	Plasma	300080904	30008090401	30008090901	4.13	11,116	8,156	1,546.03	1,546.01	51	22	18,966,166	361,645	FALSE
UR-02-0032	CS	Dec-18	Plasma	300080904	30008090401	30008090901	3.880	10,840	8,280	9,426.14	9,426.12	51	22	18,966,166	361,645	FALSE
UR-02-0032	POST-OP	Apr-19	Plasma	300080904	30008090401	30008090901	3.886	11,120	8,160	2,040.14	2,040.12	51	22	18,966,166	361,645	FALSE
UR-02-0032	FU.1	Jul-19	Plasma	300080904	30008090401	30008090901	4.008	10,760	8,200	4,666.14	4,666.12	51	22	18,966,166	361,645	FALSE
UR-02-0032	FU.2	Aug-19	Plasma	300080904	30008090401	30008090901	4.809	12,760	9,800	2,146.14	2,146.12	51	22	18,966,166	361,645	FALSE
UR-02-0032	FU.3	Aug-21	Plasma	300080904	30008090401	30008090901	4.908	12,220	9,260	4,056.14	4,056.12	51	22	18,966,166	361,645	FALSE
UR-02-0034	Baseline	Oct-18	Plasma	300040483	30004048303	30004048303	3.203	10,400	7,920	1,341.10	1,341.08	48	11,626,127	41,264	FALSE	
UR-02-0034	CS	Dec-18	Plasma	300040483	30004048303	30004048303	4.5	3,880	2,800	2,086.14	2,086.12	22	18	2,712,464	124,323	FALSE
UR-02-0034	CS	Apr-19	Plasma	300040483	30004048303	30004048303	4.409	8,076	6,096	1,406.12	1,406.10	22	18	2,712,464	124,323	FALSE
UR-02-0034	CS	Apr-19	Plasma	300040483	30004048303	30004048303	4.357	18,200	14,000	1,476.19	1,476.17	22	18	2,712,464	124,323	FALSE
UR-02-0034	PRE-OP	May-19	Plasma	300040483	30004048303	30004048303	4.595	6,012	4,640	1,628.12	1,628.10	22	18	2,712,464	124,323	FALSE
UR-02-0034	POST-OP	Jul-19	Plasma	300040483	30004048303	30004048303	3.2	2,600	1,920	1,346.13	1,346.11	22	18	2,712,464	124,323	FALSE
UR-02-0034	FU.1	Jan-20	Plasma	300040483	30004048303	30004048303	3.598	5,588	4,240	4,706.13	4,706.11	22	18	2,712,464	124,323	FALSE
UR-02-0034	FU.2	Mar-21	Plasma	300040483	30004048303	30004048303	4.580	4,840	3,680	4,326.11	4,326.09	22	18	2,712,464	124,323	FALSE
UR-02-0034	FU.3	Aug-21	Plasma	300071799	3000717993	30004048303	3.899	8,000	6,020	2,886.10	2,886.08	22	18	2,712,464	124,323	FALSE
UR-02-0034	FU.4	Oct-21	Plasma	300071799	3000717993	30004048303	3.706	8,020	6,040	3,716.08	3,716.06	22	18	2,712,464	124,323	FALSE
UR-02-0034	FU.5	Mar-22	Plasma	300071777	3000717772	30004048303	4.2	9,308	7,136	4,216.09	4,216.07	22	18	2,712,464	124,323	FALSE
UR-02-0034	FU.6	Nov-22	Plasma	300071778	3000717782	30004048303	3.613	12,440	9,480	2,713.12	2,713.10	22	18	2,712,464	124,323	FALSE
UR-02-0035	Baseline	Nov-18	Plasma	300071793	3000717933	3000717403	3.559	7,956	6,036	6,036.05	6,036.03	45	33	80,966,126	1,799,256	TRUE
UR-02-0035	CS	Nov-18	Plasma	300071793	3000717933	3000717403	3.559	7,956	6,036	6,036.05	6,036.03	45	33	80,966,126	1,799,256	TRUE
UR-02-0035	CS	Feb-19	Plasma	300071793	3000717933	3000717403	4.827	22,724	17,480	8,016.06	8,016.04	45	34	46,768,194	1,099,032	TRUE
UR-02-0035	PRE-OP	Apr-19	Plasma	300071793	3000717933	3000717403	4.121	13,012	10,240	1,308.19	1,308.17	45	34	46,768,194	1,099,032	TRUE
UR-02-0035	FU.1	Oct-19	Plasma	300071793	3000717933	3000717403										

US-02-0009	PRE-OP	Oct-19	Plasma	30003008	3000040881	3000050402	4.435	13.302	10.080	3.651-12	3.651-10	48	37	12,009,795	250,203	FALSE
US-02-0009	FU 1	Mar-20	Plasma	30004617	3000040872	3000050403	4.592	12.754	9.840	2.802-12	2.802-10	48	37	29,747,249	412,421	FALSE
US-02-0009	FU 2	Aug-21	Plasma	30004618	3000040873	3000050404	4.592	12.754	9.840	2.774-12	2.774-10	48	37	29,747,249	412,421	FALSE
US-02-0071	Baseline	Jul-19	Plasma	30004603	3000040869	3000050405	4.039	13.960	10.800	1.781-11	1.781-09	48	42	21,848,503	317,147	FALSE
US-02-0071	PRE-OP	Jul-19	Plasma	30004602	3000040868	3000050406	4.039	13.960	10.800	1.809-11	1.809-09	48	42	21,848,503	317,147	FALSE
US-02-0071	CS	Aug-19	Plasma	30004624	3000040870	3000050407	4.039	13.912	20.000	4.700-11	4.700-09	48	42	113,110,009	1,513,314	FALSE
US-02-0071	FU 1	Jan-20	Plasma	30004604	3000040869	3000050408	4.039	13.912	20.000	4.688-11	4.688-09	48	42	113,110,009	1,513,314	FALSE
US-02-0071	FU 2	Nov-21	Plasma	30004649	3000040870	3000050409	4.039	13.944	20.000	4.131-11	4.131-10	48	42	138,666,889	2,081,511	FALSE
US-02-0071	PRE-OP	Nov-21	Plasma	30007749	3000040869	3000050410	4.252	13.798	20.000	3.360-14	3.360-12	48	42	138,666,889	1,544,729	FALSE
US-02-0071	Baseline	Jul-19	Plasma	30004647	3000040870	3000050411	4.252	13.798	20.000	2.212-12	2.212-10	48	42	138,666,889	1,544,729	FALSE
US-02-0072	CS	Jul-19	Plasma	30004604	3000040868	3000050412	3.546	13.523	16.640	1.150-10	1.150-09	48	42	111,417,475	208,130	FALSE
US-02-0072	PRE-OP	Jul-19	Plasma	30004605	3000040869	3000050413	3.546	13.523	16.640	1.240-10	1.240-09	48	42	111,417,475	208,130	FALSE
US-02-0072	FU 1	Aug-19	Plasma	30004655	3000050502	3000060401	4.461	29.210	20.000	2.400-10	2.400-08	48	42	5,928,875	104,026	FALSE
US-02-0072	FU 2	Aug-19	Plasma	30004656	3000050503	3000060402	4.461	29.210	20.000	4.258-12	4.258-10	48	42	5,928,875	104,026	FALSE
US-02-0073	Baseline	Aug-19	Plasma	30004659	3000050602	3000060500	3.852	13.586	12.000	2.000-12	2.000-12	48	34	8,760,785	129,545	TRUE
US-02-0073	PRE-OP	Aug-19	Plasma	30004660	3000050701	3000060501	4.2	13.586	12.000	1.175-12	1.175-12	48	34	17,770,242	270,681	TRUE
US-02-0073	CS	Aug-19	Plasma	30004671	3000050702	3000060502	4.644	14.872	11.440	7.000-12	7.000-12	48	34	9,578,140	152,566	FALSE
US-02-0073	PRE-OP	Aug-19	Plasma	30004672	3000050703	3000060503	4.644	14.872	11.440	2.000-12	2.000-12	48	34	9,578,140	152,566	FALSE
US-02-0074	Baseline	Aug-19	Plasma	30004675	3000050702	3000060504	4.039	13.960	20.000	2.502-10	2.502-08	48	42	18,611,009	279,809	TRUE
US-02-0074	PRE-OP	Aug-19	Plasma	30004676	3000050801	3000060505	4.039	13.960	20.000	4.176-10	4.176-08	48	42	18,611,009	279,809	TRUE
US-02-0074	CS	Aug-19	Plasma	30004687	3000050802	3000060506	4.059	11.328	8.560	1.431-13	1.431-11	48	42	4,865,909	173,253	FALSE
US-02-0074	PRE-OP	Aug-19	Plasma	30004688	3000050803	3000060507	4.059	11.328	8.560							

Patient ID: Internal study patient identifier

Mutation code: Variant code summarizing location and nature of the variant included in the panel

Chromosome: Chromosome of the variant

Locus: Genomic locus of the variant (hg38 coordinates)

Present in buffy coat: Boolean (TRUE/FALSE), indicating presence/absence in the buffy coat control (if FALSE the variant is retained for ctDNA assessment)

Present in tumor: Boolean (TRUE/FALSE), indicating presence/absence in the tumor DNA control (if TRUE the variant is retained for ctDNA assessment)

VAF in WES: VAF (Variant Allele Frequency) in the Whole Exome Sequencing data

Sup. Table 5

Timepoint	Patient Population	Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
Baseline ctDNA Detection	All Patients	95%	15%	21%	92%
	HER2-positive	100%	25%	3%	100%
	HER2-negative	94%	28%	32%	93%
Mid-NAT ctDNA Detection	All Patients	43%	81%	35%	86%
	HER2-positive	0%	69%	0%	95%
	HER2-negative	46%	91%	67%	81%
Postoperative ctDNA detection	All Patients	75%	100%	100%	96%
	HER2-positive	0%	100%	NaN	97%
	HER2-negative	82%	100%	100%	94%