510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K202852

B Applicant

Biovica International AB

C Proprietary and Established Names

DiviTumTKa

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QTE	Class II	21 CFR 866.6010 - Tumor-Associated Antigen Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Thymidine Kinase Activity (TKa)

C Type of Test:

Semi-quantitative, enzyme immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

DiviTumTKa is an in vitro diagnostic device intended for the semi-quantitative measurement of thymidine kinase activity (TKa) in human serum. The assay is to be used as an aid in monitoring disease progression in previously diagnosed hormone receptor positive, metastatic postmenopausal female breast cancer patients. A TKa value of < 250 DuA is associated with the decreased likelihood of disease progression within 30 days or 60 days post testing. DiviTumTKa results should be used in conjunction with other clinical methods for monitoring breast cancer.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

WARNINGS:

- DiviTumTKa should not be used for cancer screening or diagnosis.
- DiviTumTKa is not for serial testing because the test result at given timepoint does not compare to the test result at previous timepoint, but to a fixed cut-off value
- DiviTumTKa is not indicated as a standalone test to determine the outcome of disease, nor to suggest or infer an individual patient's likely benefit from therapy.

D Special Instrument Requirements:

ELISA microplate reader capable of measuring optical density (OD) at 405 nm and 630 nm

IV Device/System Characteristics:

A Device Description:

Reagents

DiviTumTKa kit contains reagents to test 37 patient samples in duplicates. Each kit is stored at 4–8 °C and contains the following:

- Reaction Plate DiviTumTKa: one 96 well plate in sealed aluminum foil, contains immobilized DNA strands, acts as reaction chamber
- Reagent A: one vial, lyophilized, contains 0.04% sodium azide as preservative and necessary enzymes for the enzyme reaction
- Reagent B: one vial, lyophilized, contains the substrate bromodeoxyuridine (BrdU)
- Antibody-Enzyme Conjugate: one vial, lyophilized, contains 0.05% sodium azide as preservative
- Substrate: one tablet for reaction with the enzyme in Antibody-Enzyme Conjugate
- Buffer: four vials [one vial each of Conjugate Buffer (12.5 mL liquid), Substrate Buffer (13.5 mL liquid), Reaction Buffer (46.5 mL liquid) and Wash Buffer (13.0 mL liquid)], contains sodium azide as preservative
- Calibrator: seven vials (level 0 to 6), lyophilized, with pre-determined value for generating calibration curve, contains human serum

- Control: three vials (Low, Medium and High), lyophilized, contains human serum, with pre-determined value, for assay run quality control

B Principle of Operation:

The DiviTumTKa is a multi-step end-point ELISA assay involving a cascade of enzymatic reactions and one antibody binding reaction. In the DiviTumTKa test, a serum sample is combined with a reaction mixture containing the substrate bromodeoxyuridine (BrdU). Since BrdU is a substrate analog to thymidine, TK from the serum sample phosphorylates the BrdU to its monophosphate, BrdUMP. The BrdUMP is then further phosphorylated to its triphosphate, BrdUTP and incorporated into a DNA/RNA hybrid, bound to the 96-well microplate solid surface using a reverse transcriptase DNA polymerase. An alkaline phosphatase-conjugated anti-BrdU antibody is added to the reaction product after washing. The amount of phosphatase conjugate bound to the DNA is determined by a colorimetric reaction, turning the substrate color from colorless to yellow, and the absorbance reading at 405 nm and 630 nm are used to calculate the TK-activity level in the sample. Calibrators with pre-determined nominal TKa values are included in the kit. These are used to generate a standard curve by which the optical density (OD) readings from the patient samples are converted to TK activity expressed as DiviTum Units of Activity (DuA).

V Substantial Equivalence Information:

A Predicate Device Name(s):

CYFRA 21-1 EIA Kit

B Predicate 510(k) Number(s):

K100831

C Comparison with Predicate(s):

Device & Predicate:	K202852	K100831
Device Trade Name	DiviTumTKa	CYFRA 21-1 EIA Kit
General Device Characteristic Similarities		
Classification	Class II	Same
Regulation	21 CFR § 866.6010, Tumor-associated antigen immunological test	Same
Principles of operation	Enzyme-linked immunosorbent assay (ELISA)	Same
Sample Type	Serum	Same
Traceability	Traceable to in-house reference	Same
Instrument	ELISA Plate Reader	Same
General Device Characteristic Differences		

Device & Predicate:	K202852	K100831
Analyte	Thymidine Kinase activity	CYFRA 21-1 (soluble cytokeratin 19 fragments)
Intended Use/ Indications For Use	DiviTumTKa is an in vitro diagnostic device intended for the semi-quantitative measurement of thymidine kinase activity (TKa) in human serum. The assay is to be used as an aid in monitoring disease progression in previously diagnosed hormone receptor positive, metastatic postmenopausal female breast cancer patients. A TKa value of < 250 DuA is associated with the decreased likelihood of disease progression within 30 days or 60 days post testing. DiviTumTKa results should be used in conjunction with other clinical methods for monitoring breast cancer.	The CYFRA 21-1 EIA kit is intended for the quantitative determination of soluble cytokeratin 19 fragments in human serum. The assay is to be used as an aid in monitoring disease progression during the course of disease and treatment in lung cancer patients. Serial testing for patient CYFRA 21-1 assay values should be used in conjunction with other clinical methods used for monitoring lung cancer.
Product Code	QTE	OVK
Units	DiviTum Units Activity (DuA)	ng/mL
Reportable Range	100 DuA to 2,000 DuA	0.5 to 50 ng/mL
Testing and Evaluation Method	Not for serial testing (TKa test result value is compared to a fixed Cut off value of 250 DuA)	Serial testing (CYFRA 21-1 test result value is compared to the immediate previous sample test result value)

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06, 2nd Edition, Evaluation of Linearity of Quantitative Measurement Procedures
- CLSI EP07, 3rd Edition, Interference Testing in Clinical Chemistry
- CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition
- CLSI EP17-A2, 2nd Edition, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline
- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition

- CLSI EP34, 1st Edition, Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking
- CLSI EP37, 1st Edition, Supplemental Tables for Interference Testing in Clinical Chemistry
- ISO 15223-1, 4th Edition (2021-07), Medical devices, Symbols to be used with information to be supplied by the manufacturer, Part 1: General requirements
- ISO 17511, 2nd Edition (2020-04) In vitro diagnostic medical devices—Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

All results presented below met the manufacturer's pre-determined acceptance criteria.

1. Precision/Reproducibility:

Precision testing was performed in accordance with CLSI guideline EP05-A3.

a) Within-laboratory Precision:

The studies were performed at a single site using one lot of DiviTumTKa kit reagents. Six levels of native serum sample were run in two replicates per run, two runs daily over the course of 20 days (2 x 2 x 20, n =80 for each sample). The data were analyzed for repeatability (within-run), between-run, between-day, and within-laboratory precision. The mean DuA and percent coefficient of variation (%CV) are summarized in table below.

Sample	Mean activity (DuA)	N	Within-Run (Repeatability)		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	172	80	9	5.4%	20	11.4%	0	0.0%	22	12.6%
2	255	79*	12	4.8%	20	8.0%	6	2.3%	25	9.6%
3	552	80	28	5.0%	45	8.2%	0	0.0%	53	9.6%
4	990	80	46	4.7%	81	8.1%	0	0.0%	93	9.4%
5	1698	80	106	6.2%	153	9.0%	0	0.0%	186	11.0%

*One outlier was removed. The 'within-laboratory' results (%CV) including the outlier in the dataset for sample 2 was 16.2%.

b) Lot-to-Lot Precision:

The study was performed at a single site using three different lots of DiviTumTKa kit reagents. Seven levels of native serum sample were run in five replicates per run, one run per day for five days (3 x 5 x 5, n=75 for each sample). The data were analyzed for the

repeatability (within-run), between-day, between-lot and total precision. The results are summarized in table below.

Sample	Mean activity (DuA)	N	Within-Run (Repeatability)		Between-Day		Between-Lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	121	75	8	6.7%	0	0.0%	16	13.6%	18	15.2%
2	201	75	16	7.9%	0	0.0%	21	10.6%	27	13.2%
3	345	75	22	6.4%	12	3.3%	24	6.9%	34	9.9%
4	887	75	47	5.3%	51	5.8%	47	5.3%	84	9.4%
5	1243	75	68	5.4%	0	0.0%	118	9.5%	136	10.9%
6	1685	75	107	6.4%	70	4.2%	167	9.9%	211	12.5%
7	1892	75	94	5.0%	149	7.9%	184	9.7%	255	13.5%

c) *Site-to-Site Reproducibility:*

The study was performed at three sites (including one internal site and two external sites) using single lot of DiviTumTKa kit reagents. Five levels of native serum sample were run in five replicates per run, one run daily over the course of five days (3 x 1 x 5 x 5, n=75 for each sample). The combined results with the factors of site and run used to calculate the repeatability (within-run), between-run, between-site and reproducibility are summarized in table below:

Sample	Mean activity (DuA)	N	Within-Run (Repeatability)		Between-Run		Between-Site		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	156	75	9	5.4%	17	10.6%	0	0%	19	11.9%
2	243	75	12	5.1%	19	7.9%	18	7.5%	29	12.0%
3	590	75	28	4.7%	31	5.3%	84	14.2%	94	15.9%
4	703	75	30	4.3%	33	4.7%	103	14.6%	112	16.0%
5	1710	74*	107	6.2%	104	6.1%	194	11.3%	244	14.3%

*One outlier was removed. The 'reproducibility' results (%CV) including the outlier found in the dataset for sample 5 was 14.5%.

2. Linearity:

a) *Linearity studies:*

Linearity studies were conducted in accordance with CLSI guideline EP06, 2nd Edition. A 'high sample pool' and a 'low sample pool' were prepared using native serum samples. The 'high sample pool' had a DuA value (as measured by DiviTumTKa) higher than the 'upper limit of the linearity interval' (ULLI). The 'low sample pool' had a DuA value lower than the 'lower limit of the linearity interval' (LLLI). The two sample pools were mixed to obtain 10 intermediate concentrations with TK activity distributed throughout the 'analytical measuring interval' (AMI) and near the assay clinical decision point, 250 DuA. The final linearity sample panel consisted of 12 samples. The samples were tested with DiviTumTKa in replicates of four and all members of a sample set were run together. Measured values were plotted on the vertical (y-) axis and the expected values

on the horizontal (x-) axis and weighted least squares linear regression analysis with an intercept was applied on the dataset. Predicted values obtained from the fitted line equation were calculated and the deviation between measured and predicted values were calculated. This deviation was then compared to the allowable deviation from linearity (ADL) as shown in table below.

Sample*	Measured Value	Expected Value (E)	Predicted Value (Y)**	% Deviation***
High	2249	2249	2163	4%
2	1868	2030	1953	-4%
3	1782	1811	1743	2%
4	1522	1592	1533	-1%
5	1243	1374	1323	-6%
6	1126	1155	1113	1%
7	916	936	903	1%
8	700	717	693	1%
10	281	280	273	3%
11	179	171	168	7%
Low	61	61	63	-3%

*One outlier sample (number 9) was removed from the data set due to large deviation (19.3%) between actual test value and the expected value.
**Predicted Value, Y = kE + m (k=0.96, m=4.157)
***% Deviation = 100 [(Measured –Predicted)/Predicted]

The weighted least squares linear regression analysis results are provided in table below.

Range (DuA)	Slope (95% CI)	Intercept (95% CI)	R^2
61–2249	0.96 (0.9123 to 1.008)	4.157 (-59.70 to 68.01)	0.996

The results support the linearity of the claimed DiviTumTKa 'analytical measuring interval' (AMI) of 100 DuA to 2,000 DuA.

b) *Spiking and Dilutional Recovery Studies:*

Dilutional recovery studies were conducted using two independent patient serum sample panels (Sample panel A and B) which were generated by pooling sera from cancer patients with high levels of endogenous TK activity, and one sample panel (Sample panel C) which was generated by diluting a known amounts of recombinant TK1 protein in pooled human serum with very low endogenous TK activity. The initial TK activity in all three sample panels were above the ULOQ of the assay (~2000 DuA). Five two-fold serial-dilutions were prepared right before assaying each sample panel using DiviTumTKa kit component reaction buffer as diluent. The TK activity in three dilution panels were independently determined in duplicates by two operators on individual days using DiviTumTKa assays. The dilution adjusted TK activity calculated for each sample and the % recovery of measured TK activity and target (expected) TK activity is presented in the table below.

Target (DuA)	Measured (DuA)	% Recovery
Sample Panel A		
2,653	2,653	100%
1,327	1,181	89%
663	599	90%
332	301	91%
166	154	93%
Sample Panel B		
2,321	2,321	100%
1,161	1,151	99%
580	607	105%
290	313	108%
145	161	111%
Sample Panel C		
3,014	3,014	100%
1,507	1,405	93%
754	690	92%
377	373	99%
188	178	95%

c) *High Dose Hook Effect:*

Hook effect of the DiviTumTKa was evaluated in accordance with CLSI guideline EP34. Human serum samples with low TK activity were pooled and then spiked with high concentrations of recombinant TK to approximately 100,000 DuA (about 50 times higher than the ULOQ). The sample was serially diluted seven times to create samples with TK activity of approximately 50000, 25000, 12500, 6250, 3125, 1563 and 781 DuA and tested in six replicates using a single reagent lot DiviTumTKa. No high dose hook effect was observed when samples were up to approximately 100,000 DuA of DiviTumTKa.

3. Analytical Specificity/Interference:

Interference study was performed according to CLSI EP07 (3rd Edition) and EP37 (1st Edition) guidelines to determine the effect of various endogenous and exogenous substances on the DiviTumTKa assay. Two native human serum pools with a target concentration of 100–174 DuA (Low pool) and 328–435 DuA (High pool) were supplemented with potentially interfering compounds, and the percent bias was determined by comparing the result of sample with interferent to a control sample without the interferent. An interference within ± 15% bias between the mean spiked sample value and the mean control value was considered non-significant.

a) *Endogenous Substance Interference:*

The following endogenous substances were tested using DiviTumTKa assay. No significant interference was found for each substance at the concentrations listed below.

Endogenous Substance	Concentration
Free Bilirubin (unconjugated)	40 mg/dL
Hemoglobin	10 g/L
Albumin and y-globulin	60 g/L
Human anti-mouse antibodies (HAMA)	68 ng/mL
Rheumatoid factor (RF)	400 IU/mL
Alkaline phosphatase	980 U/L

Triglyceride-rich lipoproteins (at ≥427 mg/dL) and conjugated bilirubin (at ≥18 mg/dL) interfere with DiviTumTKa. Results from a dose response study are presented below.

Substance	Substance Concentration	% Bias
Triglyceride-rich lipoproteins (low pool)	375 mg/dL	6.2%
	750 mg/dL	30.5%
	1125 mg/dL	69.0%
	1500 mg/dL	119.6%
Bilirubin, conjugated (high pool)	10 mg/dL	-14.2%
	20 mg/dL	-18.9%
	30 mg/dL	-23.9%
	40 mg/dL	-24.4%

The package insert of the DiviTumTKa includes the following limitations: patients who have high levels of triglycerides may show falsely elevated values when tested, and who have high levels of bilirubin (conjugated) may show falsely depressed values when tested with DiviTumTKa.

b) *Exogenous Substance Interference:*

The potential interference of 47 commonly used drugs, including those used for cancer treatment, was evaluated using DiviTumTKa assay. No significant interference was found for each substance at the concentrations listed below.

Exogenous Substance	Concentration	Exogenous Substance	Concentration
Acetylcysteine	5.1 mmol/L	Heparin	3,000 U/L
Ampicillin-Na	152 µmol/L	Ibuprofen	510 µmol/L
Anastrozole	0.6 µg/mL	Levodopa	2000 ng/ml
Ascorbic acid	342 µmol/L	Methotrexate	45 µg/mL
Capecitabine	4 mg/L	Metoklopramid	1.5 µmol/mL
Carboplatin	500 µg/mL	Metronidazole	701 µmol/L
Cyclophosphamide	500 µg/mL	Mitomycin C	17.2 µg/mL
Cyclosporine	1200 ng/mL	Morphine	1.75 µmol/L
Docetaxel	103.8 µg/mL	Omeprazole	17.4 µmol/L
Doxorubicin	10 µg/mL	Paclitaxel	222 µmol/L
Epirubicin	103.8 µg/mL	Palbociclib	75 µg/mL
Everolimus	6 µg/mL	Paracetamol	240 µg/mL

Exogenous Substance	Concentration	Exogenous Substance	Concentration
Exemestane	15 ug/mL	Theophylline	60 mg/L
Foscarnet	0.5 µg/mL	Toremifene	3.6 µg/ml
Fulvestrant	0.3 mg/mL	Zidovudine	12 µmol/L
Goserelin	25.8 ng/ml		

The test pools, shown in the table below, that obtained differences higher than 15% of the mean control pool or were statistically significant for at least one serum pool were investigated further through 'dose response' testing. The interference from these substances in a 'dose response' testing did not exceed the allowable bias criterion of 15%.

Exogenous Substance	Concentration	Exogenous Substance	Concentration
5-fluorouracil	280 µg/mL	Letrozole	1.5 ug/mL
Abemaciclib	240 µg/mL	Leuoprorelin	15.6 ng/ml
Acetylsalycilic acid	3.62 mmol/L	Loperamide	2.67 mg/L
Aminoglutethimidine	34.5 µg/ml	Megestrol Acetate	270 ng/ml
Brivudine	75 µg/mL	Ribociclib	360 µg/mL
Doxycycline	67.5µmol/L	Tamoxifen	5.0 µg/mL
Eribulin	1.3 µg/mL	Trastuzumab	360 µg/mL
Lapatinib	2,107 ng/mL		

Among exogenous substances, Cisplatin was the only exogenous substance that resulted in a TK activity that exceeded the acceptance criteria of 15% difference from control. Results from a dose response study are presented below.

Substance	Substance Concentration	% Bias
Cisplatin	0.138 mmol/L	-28.5%
	0.275 mmol/L	-30.0%
	0.413 mmol/L	-39.7%
	0.550 mmol/L	-53.5%

The package insert of the DiviTumTKa includes the following limitation: patients who are taking Cisplatin may show falsely depressed TKa values when tested with DiviTumTKa.

4. Assay Reportable Range:

The 'Analytical Measuring Interval' (AMI) for the DiviTumTKa is 100 DuA to 2,000 DuA. Any value below 100 DuA is reported as <100 DuA and any sample with a value above 2,000 DuA is reported as >2,000 DuA.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) *Traceability:*

There is no recognized standard for Thymidine Kinase Activity (TKa). Calibration of the DiviTumTKa is traceable to in-house reference calibrators (Master calibrators), whose TKa values have been assigned, in accordance with ISO 17511 (2nd Edition), according to the protein concentration used in each calibrator: 1 DuA (DiviTum unit of Activity) is the TKa of 1 pg/mL of standard recombinant TK1.

b) *Kit Stability:*

The shelf-life of the DiviTumTKa reagent kit was determined according to CLSI guideline EP25-A.

i) Reagent kit stability: A real-time stability study at –24 ±6ºC and +2 to +8°C was performed using three DiviTumTKa kit lots. Three samples with TKa levels within the AMI (near LLOQ, medical decision point and high) were tested in six replicates per sample, in three assay runs. Testing was performed at multiple time points throughout the claimed stability period and at least one month past the expiration date. Results support the 12-month shelf-life stability claim for the DiviTumTKa reagent kit at –24 ±6ºC and +2 to +8°C.

ii) Transport simulation: To evaluate reagent transport stability, three DiviTumTKa kit lot reagents were stored at -24 ±6°C until transport simulation started. When initiating the transport simulation, the kits were stored at room temperature (RT) for 24 hours. Thereafter, the kits were placed in the freezer until all liquid had become frozen (3–4 hours). The kits were then placed in RT again for 96 hours. Thereafter, the kits were placed in 2°C to 8°C and stored there until analysis was performed at respective testing occasion. The results from the combined 'transport simulation' and stability study at 2°C to 8°C show that DiviTumTKa is stable at least for 12 months in 2°C to 8°C after transport.

c) *Sample Stability:*

To demonstrate that serum samples stored at -70°C (with no freeze/thaw cycles) and those used in the clinical study have acceptable levels of Thymidine kinase activity (TKa), an accelerated stability study was conducted for 21 days at four elevated temperatures. Three pools of serum from breast cancer patients were aliquoted and stored at -70°C until moved to four different elevated temperatures (4°C, 25°C, 30°C, and 37°C) for up to 3 weeks. The degradation and estimated shelf-life were calculated for both storage temperature -20°C and -70°C using the Arrhenius equation. The results obtained with the Arrhenius calculation indicated that no relevant degradation would occur for samples stored at -70°C for 15 years and for samples stored at -20°C for 6 years.

6. Detection Capability:

   CLSI guideline EP17-A2 were followed to determine the Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the DiviTumTKa.

   LoB: Four blank samples were obtained by affinity purification of healthy blood donor serum. The samples were tested in replicates of six over the course of five days using two DiviTum TKa reagent lots (Lot A and Lot B; 120 replicates for each lot). The LoB was calculated using the non-parametric option. This gave the LoB value 32.5 DuA for Lot A and 33.3 DuA for Lot B. The claimed LoB for the DiviTumTKa is 33.3 DuA.

   LoD: Four low-level samples derived from breast cancer patient serum were run in replicates of six over the course of five days using two DiviTum TKa reagent lots (Lot A and Lot B; 120 replicates for each lot). The parametric analysis was used as 120 measurement results from four LoD samples followed normal distribution in each group with no outliers being observed. LoD was defined as the lowest TK-activity that can be determined with a 95% confidence of being higher than LoB. This gave the LoD value 47.0 DuA for Lot A and 46.8 DuA for Lot B. The claimed LoD for the DiviTumTka is 47.0 DuA.

   LoQ: A precision profile was created using nine native low TK activity serum samples using one lot of DiviTumTKa kit reagents. A within-laboratory precision study of 80 measurements (20 days x 2 runs x 2 replicates) was performed as described in CLSI EP05-A3. Within-laboratory %CV was plotted against DuA and a power function was fitted to the data. The estimated LoQ of DiviTumTKa was 80 DuA using the precision profile power function, with a CV criterion of 15%. The lowest measurand concentration that can be measured with respect to predefined accuracy goals was set to 100 DuA. The claimed LoQ for the DiviTumTKa is 100.0 DuA.

7. Assay Cut-Off:

   See Clinical Cut-off below.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable. The predicate measures a different analyte.

2. Matrix Comparison:

   Not applicable. The assay is for serum samples only.

C Clinical Studies:

The effectiveness of the DiviTumTKa as an aid in monitoring disease progression in postmenopausal female patients previously diagnosed with HR+ metastatic breast cancer and undergoing treatment, was determined through a clinical validation study using banked serum samples from metastatic breast cancer patients undergoing treatment. The primary objective was

to evaluate whether Thymidine Kinase activity (TKa) above a preset value (clinical cut-off) at TKa ≥ 250 DuA in patient's serum sample during treatment at cycles 2, 3, 4, 7 is prognostic for progression of metastatic breast cancer.

Inclusion and exclusion criteria

- Disease characteristics
  - Histologically confirmed breast cancer meeting one of the following criteria:
    - Metastatic disease (M1)
    - Multiple sites of new disease that is clinically obvious metastatic disease (e.g., multiple sites of new osseous disease)
- Metastasis: No known brain or CNS metastases
- Hormone receptor status
  - Estrogen-receptor positive* and/or
  - Progesterone-receptor positive*

*=Positivity defined as estrogen binding of > 10 fmol/mg cytosol protein by ligand binding assay or positive by immunohistochemistry

Patient characteristics

- Female
- Postmenopausal
- Zubrod scale 0–2
- Hematopoietic: No bleeding diathesis
- Hepatic 'international normalized ratio' (INR) ≤ 1.6
- Other: HIV negative; No other malignancy within the past 5 years except adequately treated basal cell or squamous cell skin cancer, carcinoma in situ of the cervix, or adequately treated stage I or II cancer currently in complete remission

Non-progression was assumed for all sample collections (with TKa measurements) unless progression was confirmed by RECIST criteria. (RECIST v.1.0). Death was counted as progression.

Serum samples from 454 patients were collected by several different clinical facilities in the U.S. and Canada. The serum samples were shipped on dry ice to a biobank where they were stored at -80°C until analysis. The samples were de-identified prior to being shipped to Biovica. A total of 1,726 serum samples were analyzed in replicates. Out of these, 118 observations (samples) were excluded due to missing values for "actual time of visit", 10 TKa results (samples) were excluded due to insufficient serum for analyses or invalid (due to imprecise on of the replicates in the test results) and 52 samples were obtained after the patient had progressed. The 52 serum samples were distributed over the 454 patients. The final analysis contained 1546 test results (samples representing 454 patients), of which 382 samples were collected at baseline (actual time baseline) and 1164 samples were collected during cycles 2, 3, 4, and 7. The 454 patients included in the study showed age distribution (years) of 65±10.3 (Mean), 64 (Median), 58 (Q1), 72 (Q3), 27 (minimum) and 92 (maximum).

Variable	Statistic	Category	Subjects (N=454)
Race	N (%)	White	408 (89.9%)
		Black	30 (6.6%)
		Other	16 (3.5%)
Metastatic sites: Visceral	N (%)	Yes	231 (50.9%)
		No	223 (49.1%)
Metastatic sites: Other	N (%)	Yes	131 (28.9%)
		No	323 (71.1%)
Metastatic sites: Bone only	N (%)	Yes	92 (20.3%)
		No	362 (79.7%)
Measurable disease	N (%)	Yes	260 (57.3%)
		No	194 (42.7%)

The multiple time-point test results were divided into time intervals based on the original sampling day (phlebotomy) – i.e., actual visit (sampling) day at physician after registration. Patient treatment was initiated according to patient information – i.e., after baseline (>21 days) sampling. The number of the DiviTumTKa measurements per patient (N=454) and TKa mean, median, minimum, and maximum values per time interval are listed in the table below.

Variable		Patients (N) (%)
Number of TKa measurements per patient (after baseline)	1	55 (12.1%)
	2	50 (11.0%)
	3	100 (22.0%)
	4	102 (22.4%)
	5	147 (32.3%)
Interval (# of Day) (Treatment Cycle #)	**TKa Measurement (DuA)**	
baseline (<21 day)	Mean (SD)	585 (1395)
	Median	227
	Min; Max	33; 15,288
21–70 days; cycle 2	Mean (SD)	414 (1227)
	Median	160
	Min; Max	30; 14,439
71–98 days; cycle 3	Mean (SD)	239 (408)
	Median	153
	Min; Max	32; 3,961
99–154 days; cycle 4	Mean (SD)	241 (410)
	Median	144
	Min; Max	30; 3,699
>154 days; cycle 7	Mean (SD)	243 (966)
	Median	142
	Min; Max	25; 14,674

Elevated serum TKa was defined as ≥250 DuA (clinical reference value).

The visits were classified by whether or not patients showed progression, and whether or not the 'level of TKa' exceeded cut-off. From the 1164 DiviTumTKa monitoring tests (during

treatment), disease progression within 30 days from testing was seen in 63 cases. Thirty-three (52.4%) of the 63 samples with disease progression had TKa above the clinical reference value (≥ 250 DuA). Eight hundred and eighty-eight (80.7%) of the 1101 samples with TKa < 250 DuA were not associated with disease progression within the next 30 days. Table below presents the data in a 2 x 2 format and also includes data on progression within 60 days of testing.

		30 Days Post TKa Measurement			60 Days Post TKa Measurement		
		Progression	**No Progression**	**Total**	**Progression**	**No Progression**	**Total**
TKa (DuA)	**≥250**	33	213	246	57	189	246
	<250	30	888	918	60	858	918
Total		63	1101	1164	117	1047	1164

Table below shows the performance characteristics of the DiviTumTKa relative to progression outcome within 30 days and within 60 days at a TKa cut-off value of 250 DuA.

TKa measurement: 21-154 days; cycles 2-7 included

Performance measurement	**Progression within 30 Days from TK Measurement**	**Progression within 60 days from TKa Measurement**
N of samples	1164	1164
Cases	63	117
Sensitivity (95% CI)*	52.4% (33/63) (42.6% – 61.9%)	48.7% (57/117) (40.5% – 57.1%)
Specificity (95% CI)	80.7% (888/1101) (78.1% – 83.1%)	81.9% (858/1047) (79.3% – 84.6%)
Concordance (95% CI)	79.1% (921/1164) (76.6% – 81.8%)	78.6% (915/1164) (74.0% – 81.1%)
Risk of Progression	5.4% (63/1164)	10.1% (117/1164)
Risk of Progression for TKa ≥250 DuA	13.4% (33/246)	23.2% (57/246)
Risk of progression for TKa<250 DuA	3.3% (30/918)	6.5% (60/918)
Probability of "No progression"	96.7% (888/918)	93.5% (858/918)
Positive Likelihood Ratio	2.71	2.70
Negative Likelihood Ratio	0.59	0.63

* CI=Confidence Intervals

To account for dependent observations, confidence intervals for sensitivity and specificity have been calculated using bootstrap confidence intervals using the Boot package (version 1.3-28) in R (version 4.1.1).

Interval (days)	n	Cases	Sensitivity (95% CI)	Specificity (95% CI)
Progression within 30 days				
21-70	494	42	50.0% (38.5% – 61.5%)[1]	76.8% (73.3% – 80.2%)[1]
71-98	294	7	42.9% (15.8% –75.0%)[0]	81.5% (78.1% – 84.9%)[1]
99-154	146	6	66.7% (25.0% – 84.2%)[0]	85.0% (80.3% – 89.4%)[1]
>154	230	8	62.5% (30.6% – 86.3%)[0]	84.7% (79.4% – 88.8%)[0]
Total	1164	63	52.4% (42.6% – 61.9%)[1]	80.7% (78.1% – 83.1%)[1]
Progression within 60 days				
21-70	494	71	50.7% (40.3% – 60.6%)[1]	78.7% (75.2% – 82.3%)[1]
71-98	294	17	47.1% (26.2% – 69.0%)[0]	82.7% (79.2% – 85.9%)[1]
99-154	146	13	46.2% (23.2% – 70.9%)[0]	85.7% (81.1% – 90.2%)[1]
>154	230	16	43.8% (23.1% – 66.8%)[0]	85.0% (79.7% – 89.2%)[0]
Total	1164	117	48.7% (40.5% – 57.1%)[1]	81.9% (79.3% – 84.6%)[1]

'0' denotes Score Confidence Intervals, and '1' denotes Bootstrap Confidence Intervals.
When there are no dependent observations, the score confidence intervals have been used.

Sensitivities were 52.4% and 48.7% for progression within 30 and 60 days, respectively, and specificities were 80.7% and 81.9% for progression within 30 and 60 days, respectively.

Positive predictive values (PPV) and negative predictive values (NPV) along with 95% confidence intervals shown in table below.

Interval	PPV (95% CI)	NPV (95% CI)
Progression within 30 days		
21-70	16.7% (11.6% – 21.7%)	94.3% (92.5% – 96.1%)
71-98	5.4% (1.1% – 14.9%)	98.3% (97.3% – 99.4%)
99-154	16.0% (4.5% – 36.1%)	98.3% (96.7% – 99.5%)
>154	12.8% (4.3% – 27.4%)	98.4% (95.5% – 99.7%)
Total	13.4% (10.2% – 16.7%)	96.7% (95.8% – 97.6%)
Progression within 60 days		
21-70	28.6% (22.0% – 35.3%)	90.5% (87.8% – 93.2%)
71-98	14.3% (6.4% – 26.2%)	96.2% (94.4% – 97.9%)
99-154	24.0% (9.4% – 45.1%)	94.2% (91.0% – 97.3%)
>154	17.9% (7.5% – 33.5%)	95.3% (91.2% – 97.8%)
Total	23.2% (18.3% – 27.9%)	93.5% (92.0% – 94.9%)

In summary, the clinical study results support the intended use of DiviTumTKa as "an aid in monitoring disease progression in previously diagnosed postmenopausal HR+ metastatic breast cancer patients undergoing treatment." NPV for progression within 30 days or 60 days exceeded 90% for all time points analyzed, thereby supporting intended use statement "a TKa value of < 250 DuA is associated with the decreased likelihood of disease progression within 30 days or 60 days post testing."

D Clinical Cut-Off:

The TKa clinical cut-off value was determined by analyzing serum samples from 123 apparently healthy post-menopausal female and based on the 95th percentile of that distribution in the 'Reference Range study.' The test is used for monitoring but would not be characterized as serial testing because the test result at given timepoint does not compare to the test result at previous timepoint, but to a fixed cut-off value.

E Expected Values/Reference Range:

a) Reference range:
The reference range of the DiviTum TKa was established per CLSI EP28-A3c. Serum specimens from 123 apparently healthy post-menopausal female subjects (age ≥ 55 and < 85, collected in the U.S.) were assessed using the DiviTum TKa. The results are presented in the table below:

N = 123	
	TKa (DuA)
Mean	139
Standard Deviation	56
Median	125
Max	301
95th percentile	254
97.5th percentile	276

b) Expected value:
In addition to the normal cohort, serum specimens obtained from postmenopausal female subjects with benign conditions and postmenopausal female subjects with malignant diseases were tested using the DiviTum TKa. The observed ranges are summarized in the following tables:

Value of DiviTum TKa in Apparently Healthy and Benign Subjects

	Number of Subjects				
	N	**DiviTum TKa Assay Values (DuA)**			
Subject Category	**Subjects**	0–254	255–302	303–1,000	>1,000
Apparently Healthy Subjects					
All Normal	123	118 (96%)	5 (4%)	0 (0%)	0 (0%)
Non-malignant Disease or Condition					
Parkinson's Disease	43	41 (95%)	1 (2%)	1 (2%)	0 (0%)
Type 2 Diabetes	41	37 (90%)	0 (0%)	4 (10%)	0 (0%)
Rheumatoid Arthritis	46	31 (67%)	0 (0%)	15 (33%)	0 (0%)
NASH	41	32 (78%)	5 (12%)	4 (10%)	0 (0%)
CKD	40	37 (93%)	2 (5%)	0 (0%)	1 (3%)
COPD	45	34 (76%)	4 (9%)	7 (16%)	0 (0%)
EBV & HSV	41	30 (73%)	2 (5%)	5 (12%)	4 (10%)

Note: EBV Epstein-Barr Virus; HSV Herpes Simplex Virus; NASH Nonalcoholic Steatohepatitis; CKD Chronic Kidney Disease; COPD Chronic Obstructive Pulmonary Disease

Value of the DiviTum TKa in Subjects with Cancer

Subject Category	N	Number of Subjects DiviTum TKa Assay Values (DuA)			
		0–254	255–302	303–1,000	>1,000
Breast Cancer Stage IV	60	26 (43%)	4 (7%)	23 (38%)	7 (12%)
Breast Cancer Stage III	48	38 (79%)	0 (0%)	7 (15%)	3 (6%)
Breast Cancer Stage II	43	37 (86%)	2 (5%)	3 (7%)	1 (2%)
Breast Cancer Stage I	42	39 (93%)	1 (2%)	2 (5%)	0 (0%)
Liver Cancer (all stages)	26	12 (46%)	1 (4%)	10 (38%)	3 (12%)
Lung Cancer (all stages)	43	31 (72%)	1 (2%)	9 (21%)	2 (5%)
Bone Cancer (all stages)	59	29 (49%)	2 (3%)	16 (27%)	12 (20%)
Brain Cancer (all stages)	42	17 (40%)	4 (10%)	16 (38%)	5 (12%)

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.