July 29, 2022

Biovica International AB
Joakim Arwidson
Regulatory and QA Director
Dag Hammarskjölds väg 54B
Uppsala, 75237
Sweden

Re: K202852
Trade/Device Name: DiviTumTKa
Regulation Number: 21 CFR 866.6010
Regulation Name: Tumor-Associated Antigen Immunological Test System
Regulatory Class: Class II
Product Code: QTE
Dated: April 28, 2022
Received: May 2, 2022

Dear Joakim Arwidson:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ying Mao, Ph.D.
Branch Chief
Division of Immunology
and Hematology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K202852

Device Name
DiviTum®TKa

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(K) OWNER: Biovica International AB
ADDRESS: Dag Hammarskjölds Väg 54B, 752 37 Uppsala, Sweden
PHONE NUMBER: + 46 (0) 18 44 44 830
EMAIL: info@biovica.com
NAME OF CONTACT PERSON: Joakim Arwidson
DATE THE SUMMARY WAS PREPARED: April 7th, 2021

A. 510(K) NUMBER

K202852

B. PURPOSE OF SUBMISSION

New Device

C. MEASURAND

Thymidine Kinase Activity (TKa)

D. TYPE OF TEST

Semiquantitative, enzyme immunoassay

E. APPLICANT

Biovica International AB

F. PROPRIETARY AND ESTABLISHED NAMES

DiviTum®TKa
DiviTum®

G. REGULATORY INFORMATION

Regulation Section
21 CFR §866.6010, Tumor – Associated antigen immunological test system

Classification
Class II

Product Code
QTE

Panel
Immunology (82) (Assay)
Clinical Chemistry (75) (Calibrators and Controls)

H. INTENDED USE

1. Intended Use(s)
   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.

Warnings:

- DiviTum®TKa should not be used for cancer screening or diagnosis.
- DiviTum®TKa 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.

2. Indication(s) for Use
   Same as above.

3. Special Conditions for Use Statement(s)
   For prescription use only.

4. Special Instrument Requirements
   ELISA Plate Reader at 405 and 630 nm.

I. DEVICE DESCRIPTION

Each DiviTum®TKa kit contains all necessary reagents, including calibrators and assay controls for analyses of 37 patient samples in duplicates.

Table 1 - Components of DiviTum®TKa kit

Component	Quantity	Description
Reagent A	1 vial, lyophilized	Forms Reaction buffer A after reconstitution, containing necessary enzymes for the enzyme reaction.
Reagent B	1 vial, lyophilized	Forms Reaction buffer B after reconstitution, containing the substrate BrdU.
Conjugate DiviTum®TKa	1 vial, lyophilized	Forms antibody-enzyme conjugate solution. after reconstitution with Conjugate Buffer
Control Low DiviTum®TKa	1 vial, lyophilized	With pre-determined value, for assay run quality control. To be reconstituted with Reaction Buffer before use
Control Medium DiviTum®TKa	1 vial, lyophilized	
Control High DiviTum®TKa	1 vial, lyophilized	
Calibrator 0 DiviTum®TKa	1 vial, lyophilized	With pre-determined value, for generating calibration curve. To be reconstituted with Reaction Buffer before use
Calibrator 1 DiviTum®TKa	1 vial, lyophilized	

Calibrator 2 DiviTum®TKa	1 vial, lyophilized	
Calibrator 3 DiviTum®TKa	1 vial, lyophilized	
Calibrator 4 DiviTum®TKa	1 vial, lyophilized	
Calibrator 5 DiviTum®TKa	1 vial, lyophilized	
Calibrator 6 DiviTum®TKa	1 vial, lyophilized	
Reaction Plate DiviTum®TKa	One 96 well plate in sealed aluminum foil	Contains immobilized DNA strands, acts as reaction chamber

J. SUBSTANTIAL EQUIVALENCE INFORMATION:

1. Predicate Device Name(s):
   CYFRA 21-1 (soluble cytokeratin 19 fragments)

2. Predicate 510(k) Number(s):
   K100831

3. Comparison with Predicate:

Table 2- Summary Predicate Comparison – Similarities

Device Name	DiviTum® TKa	CYFRA 21-1 EIA Kit
Device Type	*In vitro* diagnostic	Same
Classification	Class II	Same
Regulation	21 CFR § 866.6010, Tumor – Associated antigen immunological test system	Same
Clinical function	Aid in monitoring disease progression in cancer patients	Same
Type of specimen	Serum samples	Same
Intended clinical environment	Clinical and hospital laboratory	Same
Type of test	Enzyme-linked immunosorbent assay (ELISA)	Same

Interpretation of results	Calibration curve	Same
Supply of calibrators and controls	Supplied with kit	Same
Special condition for use statement	For prescription use only	Same
Special instrument requirements	ELISA Plate Reader	Same

Table 3 - Summary Predicate Comparison – Differences

Device Name	DiviTum®TKa	CYFRA 21-1 EIA Kit
Intended 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.
Reference value	250 DuA	Relative percentage change
Target analyte	TK activity (TKa)	CYFRA 21-1 (soluble Cytokeratin 19 fragments)

K. STANDARD/GUIDANCE DOCUMENT REFERENCED:

CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline -Third Edition
CLSI EP06-2nd edition: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
CLSI EP07-3rd edition: Interference Testing in Clinical Chemistry
CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline –Second Edition
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 Guidelines – 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 Fourth edition 2021-07. Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements.

ISO 17511: 2020: In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples

L. TEST PRINCIPLE:

The DiviTum®TKa is a multi-step end-point ELISA assay involving a cascade of enzymatic reactions and one antibody binding reaction. During the DiviTum®TKa 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 phosphorylated to BrdUTP and incorporated into a DNA/RNA hybrid, bound to the 96 well microplate solid surface, using a reverse transcriptase DNA polymerase. After washing, an alkaline phosphatase conjugated anti-BrdU antibody is added to the reaction well. 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 optical density readings (OD) hence indicate the TKa in the sample. Calibrators with predetermined nominal values are included in the kit. These are used to generate a standard curve by which the OD readings from the patient samples are converted to TKa expressed as DiviTum®TKa Units (DuA).

M. PERFORMANCE CHARACTERISTICS:

1. ANALYTICAL PERFORMANCE

a. Precision/Reproducibility

The precision evaluation study for DiviTum®TKa was designed in accordance with CLSI guideline EP05-A3 to test reproducibility, repeatability, and other possible sources of variation for the assay: Operator, reagent lot and laboratory site. The study was divided into four parts: Two single site studies, a multi-site study and a lot-to-lot study.

The 1st single site precision evaluation study was performed at one site with five samples in five replicates per assay run, by two operators for 10 days per operator, and with three kit lots. The 2nd single site precision evaluation study was performed with six samples in two replicates per assay run for 20 days with two assay runs per day. The study was conducted by two operators at one site. The multisite precision evaluation study was conducted at three individual study sites (of which two are external CLIA certified laboratories) with five samples in five replicates per assay run for five days per site for a total of fifteen days. The lot-to-lot precision evaluation study was performed at one site by two operators using 3 unique (regarding critical raw materials) kit lots. Three kit lots were assayed per day for 5 days. The study was conducted with 5 replicates per run and per kit lot.

The results from the 1st single site precision evaluation study, the multisite precision evaluation study, the 2nd single site precision evaluation study, and the lot-to-lot precision evaluation study fulfilled the pre-defined acceptance criteria for acceptable variation between measurements using DiviTum®TKa. The results of site-to-site reproducibility and within laboratory precision are presented below in Tables 4 (multisite precision evaluation study) and 5 (2nd single site precision evaluation study).

Table 4 - Results for the multisite precision evaluation study. Samples consisted of pooled breast cancer serum and healthy blood donor serum respectively.

Sample	Mean activity (DuA)	N	Within-assay (Repeatability)		Between-assay		Between-Site		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
VS5	156	75	9	5.4%	17	10.6%	0	0%	19	11.9%
VS7	243	75	12	5.1%	19	7.9%	18	7.5%	29	12.0%
VS9	590	75	28	4.7%	31	5.3%	84	14.2%	94	15.9%
VS10	703	75	30	4.3%	33	4.7%	103	14.6%	112	16.0%
VS12	1710	74*	107	6.2%	104	6.1%	194	11.3%	244	14.3%

*One outlier was found; one replicate was therefore removed.

Table 5 - Results for the 2nd single site precision evaluation. Samples consisted of pooled breast cancer and healthy blood donor serum.

Sample	Mean activity (DuA)	N	Within-Assay (Repeatability)		Between-Assay		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.

b. Linearity/Assay Reportable Range

i. Linearity.

The assay linearity interval was defined as 100 – 2000 DuA. Sample pools "HIGH" and "LOW" were prepared using native serum samples. The HIGH sample pool had a DuA value (as measured by DiviTum®TKa) 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 (HIGH and LOW) were mixed to obtain 10 intermediate concentrations with TK activity distributed throughout the whole AMI and near the assay clinical decision point at 250 DuA.

Four replicates per linearity panel member were analysed using DiviTum®TKa. Expected values were calculated using the measured value of sample HIGH and LOW together with proportional data obtained from the mixing of samples.

Measured values were plotted on the vertical (y-) axis and the expected values on the horizontal (x-) axis. Weighted least squares (WLS) linear regression analysis with an intercept was applied on the dataset. The results from WLS linear regression is presented in Table 6. The curve is described by the following equation (curve-equation): Measured value=4.157+0.96 x Expected value

Table 6. WLS linear regression results. Curve-equation: y = 4.157 + 0.96x.

Range (DuA)	Slope (95% CI)	Intercept (95% CI)	R2
61 – 2249	0.96 (0.9123 to 1.008)	4.157 (-59.70 to 68.01)	0.996

ii. *High Dose Hook Effect*

A dilution linearity test was performed in accordance with CLSI guideline EP34 1st edition to prove the absence of a Hook effect within the expected range of

DiviTum®TKa in patient samples with high TKa. No Hook effect was observed within a TK-concentration range that reaches up to approximately 100,000 DuA, which is almost 50 times higher than the ULOQ in DiviTum®TKa.

c. Traceability, Stability, Expected values (controls, calibrators, or methods)

i. *Traceability and Calibrators*

DiviTum®TKa is a complex multi-step end-point assay involving five enzymatic reactions and one antibody binding reaction. It is not possible to characterize the measured TK activity in this assay using SI units. According to ISO 17511:2020, in IVD assays using units that are non-SI traceable, the calibration hierarchy shall be defined in a way that enables consistent realization of the corresponding (non-SI) units of measure. Biovica therefore established in-house measurement procedures and calibrators to support values assigned to the product calibrators.
The definition of 1 DuA (DiviTum unit of Activity) is the TK activity of 1 pg/mL of recombinant TK1, MyBiosource. It equals the reaction rate catalyzed by 1 pg/mL of recombinant TK under the standard DiviTum®TKa assay conditions.

ii. *Reagent Stability*

DiviTum®TKa is stable for 12 months in storage at +2 to +8°C. The expiry date stated on the labeling will be 12 months in storage at +2 to +8°C. The FDA recognized consensus standard CLSI EP25A was used as guideline for stability studies.

iii. *Stability during assay run*

To verify in use stability, assay runs were performed at different temperatures using a minishaker chamber to control/maintain the temperature during thawing (30min), mixing of reagents (10min), and incubation (1.5h). The temperatures tested were +18˚C, +23˚C, and +28˚C. The %CV between the three assays run at each temperature was <15% for all temperatures, and hence the stability of the kit is not significantly affected by variations in lab temperature in the range of normal room temperature (+18-28˚C.)

d. Detection Limit

A detection capability study has been performed in accordance with CLSI guideline EP17-A2 to characterize measurement accuracy in the low-end region of the assay.

i. *Limit of Blank (LOB):*

LOB was calculated to 33 DuA, using the non-parametric option.

ii. *Limit of Detection (LOD):*

LOD was calculated to 47 DuA.

iii. *Limit of Quantitation (LOQ):*

The estimated LoQ of DiviTum®TKa is 80 DuA.

iv. *Analytical Measuring Interval (AMI)*

The Analytical Measuring Interval (AMI) for DiviTum®TKa is set to 100 DuA to 2000 DuA. The AMI is established from the LoQ, Linearity and Precision studies. Samples below 100 DuA will be reported as <100 DuA and samples above 2000 DuA will be reported as >2000 DuA.

e. Analytical Specificity

The effects of 55 potential interfering substances (Table 7) were tested according to the CLSI guidelines EP07 3rd edition and EP37 1st edition. When tested within normal reference values, none of the 55 tested substances showed any significant interference with DiviTum®TKa. However, interference was observed for serum samples with high lipemia (>427 mg/dl) or with abnormally elevated Bilirubin (at ≥18 mg/dL) or using Cisplatin or with abnormally elevated ALP levels (>980 U/l), the DiviTum®TKa results shall be interpreted with caution.

Table 7. List of the 52 tested substances not interfering with DiviTum®TKa

Exogenous substances
5-fluorouracil
Abemaciclib
Acetylcysteine
Acetylsalycilic acid
Aminoglutethimidine
Ampicillin-Na
Anastrozole
Ascorbic acid
Brivudine
Capecitabine
Carboplatin
Cyclophosphamide
Cyclosporine
Docetaxel
Doxorubicin
Doxycycline
Epirubicin
Eribulin
Everolimus
Exemestane
Foscarnet
Fulvestrant
Goserelin
Heparin
Ibuprofen
Lapatinib
Letrozole
Leuoprorelin
Levodopa
Loperamid
Megestrol Acetate
Methotrexate
Metoklopramid
Metronidazole
Mitomycin C
Morphine
Omeprazole
Paclitaxel
Palbociclib
Paracetamol
Ribociclib

Tamoxifen
Theophylline
Toremifene
Trastuzumab
Zidovudine
Endogenous substances
Hemoglobin
Bilirubin, unconjugated
Albumin and y-globulin
Alkaline phosphatase
HAMA
RF

f. Clinical reference value ("cut-off")

See clinical reference value below.

2. COMPARISON STUDIES

a. *Method comparison with predicate device*

Not applicable. The predicate measures a different analyte. Extensive analytical and clinical studies are presented to demonstrate a high safety and efficacy profile.

b. Matrix comparison

Not applicable. Serum samples are the only matrix used for analysis.

3. CLINICAL STUDIES

The effectiveness of the DiviTum®TKa as an aid in monitoring disease progression in previously diagnosed postmenopausal HR+ metastatic breast cancer patients undergoing treatment, was determined through a clinical validation study using left-over banked serum samples from metastatic breast cancer patients undergoing treatment. Multiple time-point measurements of TKa in serum samples from patients were linked to clinical data on disease progression. The clinical validation study included 454 patients (subjects) with a total of 1598 DiviTum®TKa serum samples/test results. 52 serum samples/test results obtained after the patient had progressed were excluded before the statistical analysis. These 52 serum samples were distributed over the 454 patients. The number of serum samples/test results – included in all the statistical analyses, and still representing 454 patients, hence constituted 1546 test results of which 1164 test results (samples) were captured during treatment, but still representing all 454 patients. The remaining 382 test results (samples) were from baseline (i.e., pre-treatment). 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 DiviTum®TKa measurements per patient (N=454) and TKa mean, median, minimum, and maximum values per time interval are listed in Table 6.

Table 6 - Number of DiviTum®TKa measurements per patient (N=454) and TKa mean, median, minimum, and maximum values per time interval

Variable		**Statistic**	**Patients (%)**
Number of TKa measurements per patient	1	n_1 (%)	55 (12.1%)
	2	n_2 (%)	50 (11.0%)
	3	n_3 (%)	100 (22.0%)
	4	n_4 (%)	102 (22.4%)
	5	n_5 (%)	147 (32.3%)

		Measurement (DuA)
TKa baseline (<21 day)	Mean (SD)	585 (1395)
	Median	227
	Min; Max	33; 15 288
TKa (21-70 days; cycle 2)	Mean (SD)	414 (1226)
	Median	160
	Min; Max	30; 14 439
TKa (71-98 days; cycle 3)	Mean (SD)	239 (408)
	Median	153
	Min; Max	32; 3961
TKa (99-154 days; cycle 4)	Mean (SD)	241 (410)
	Median	144
	Min; Max	30; 3699
TKa (>154 days; cycle 7)	Mean (SD)	243 (966)
	Median	142
	Min; Max	25; 14 674

Elevated serum TKa was defined as above or equal to 250 DuA (clinical reference value).

From the 1164 DiviTum®TKa 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 9 presents the data in a 2 x 2 format and also includes data on progression within 60 days of testing.

Table 9 - Number of patient samples showing progression vs no-progression within 30- and 60 days after blood sampling

Time interval from TKa measurement (days)	30 days		60 days	
	Progression (samples)	No progression (samples)	Progression (samples)	No progression (samples)
≥250 DuA	33	213	57	189
<250 DuA	30	888	60	858
Total	63	1101	117	1047

a. *Clinical Sensitivity and Specificity:*
The diagnostical performance is presented in Table 710. At a clinical reference value at 250 DuA, a DiviTum®TKa value ≥ 250 DuA is 52.4% sensitive for disease progression within the next 30 days (48.7% for disease progression within 60 days). A value below the clinical reference value (250 DuA) is 80.7% specific that disease will not progress within the next 30 days (81.9% specific for no-progression within the next 60 days). Sensitivity is represented as the proportion of patients with disease progression that had elevated DiviTum®TKa. Specificity is represented as the proportion of patients without disease progression that did not have elevated DiviTum®TKa. Positive Likelihood Ratio (PLR) is represented as the fold increase of a patient's likelihood of disease progression when having elevated DiviTum®TKa. Negative Likelihood Ratio (NPR) is represented as the fold decrease of a patient's likelihood of disease progression without elevated DiviTum®TKa.

Table 7 - Overview Diagnostical Performance

Time interval	Progression within 30 days post testing		Progression within 60 days post testing	
TKa measurement: 21-154 days; cycles 2-7 incl.	**Value**	**95% CI**	**Value**	**95% CI**
n (samples)	1164	NA	1164	NA
Cases (patients)	63	NA	117	NA
Sensitivity	52.4 % (33/63)	42.6 – 61.9	48.7% (57/117)	40.5 – 57.1
Specificity	80.7% (888/1101)	78.1 - 83.1	81.9% (858/1047)	79.3 – 84.6
Concordance	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 LR	2.71	NA	2.70	NA
Negative LR	0.59	NA	0.63	NA

4. Clinical reference value ("cut off")

To determine the clinical reference value, the intended patient population, and the intended purpose of DiviTum®TKa was considered. As TK activity is an indicator for cell proliferation, it was important to determine which values can be expected in a healthy population and a population with other clinical conditions leading to cell proliferation. This was done to get a first indication for a suitable clinical reference value. The clinical reference value was determined by analyzing 123 serum samples from apparently healthy post-menopausal females in a reference study. The clinical reference value was based on the 95th percentile of the distribution in this study. The sample size and statistical method evaluation were done according to the CLSI guideline EP28-A3. Based on the study results the assay clinical reference value for DiviTum®TKa was set to 250 DuA. This clinical reference value/cut-off was verified by published data (Larsson et.al. "Serial evaluation of serum thymidine kinase activity is prognostic in women with newly diagnosed metastatic breast cancer" vol 10, no. 4484, 2020). To assess sensitivity and specificity at various clinical reference values, an ROC analysis was also performed. Results are shown in Figure 1.

A B

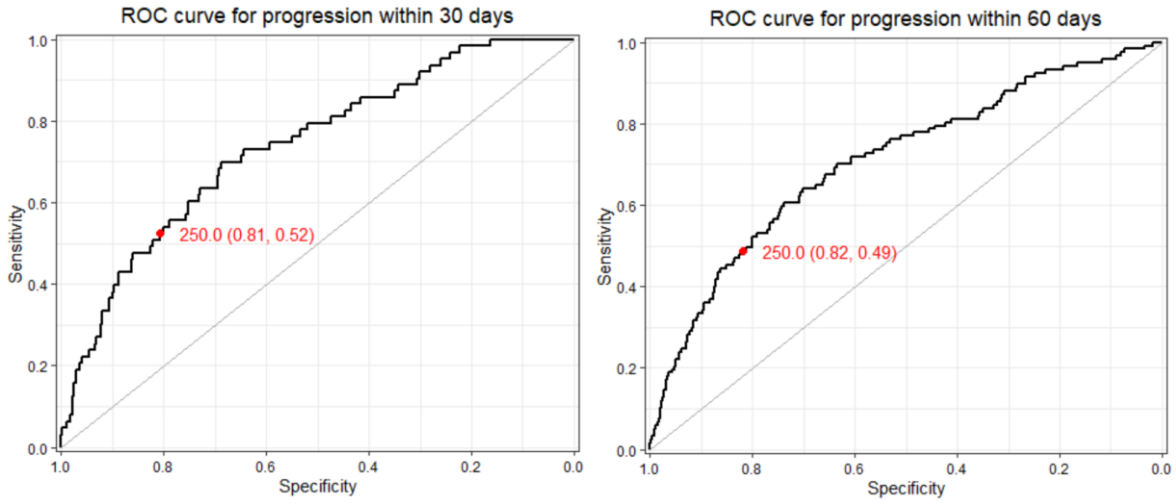

Figure 1 – A and B: ROC curves for progression within 30 days (A) and 60 days (B), with the clinical reference value/cut-off at 250 DuA noted.

5. Expected values/Reference range:

The reference range was established by measuring the TKa in 123 healthy blood donors. The reference interval for healthy serum donors is 55 to 254 DuA. The 95th percentile for healthy serum donors is 254 DuA.

Groups with non-malignant conditions were analyzed to evaluate possible effects on the TKa value, see Table 811. The groups were chosen based on the possibility that the disorder affects the cell cycle and/or cell proliferation or being common in the study population.

Groups with malignant conditions were also analyzed, see Table 12. They were chosen based on the association with breast cancer.

Table 8 - Non-malignant diseases or conditions and rationales for inclusion of chosen groups

Group	Sample size	Rationale for inclusion
Viral Infection (EBV & HSV)	41	Enhanced cellular proliferation
Parkinson's Disease	43	Common in intended population
Non-alcoholic Steatohepatitis (NASH)	41	Enhanced cellular proliferation
Type 2 Diabetes	41	Enhanced cellular proliferation
Rheumatoid Arthritis	46	Enhanced cellular proliferation
Chronic Kidney Disease (CKD)	40	Common in intended population
Chronic Obstructive Pulmonary Disease (COPD)	45	Common in intended population

Table 9 - Malignant diseases and rationales for inclusion of chosen groups. BC: Breast Cancer

Group	Sample size	Rationale for inclusion
Breast cancer stage IV	60	Patient population; intended use
Breast cancer stage III	48	Can develop into BC stage IV

Breast cancer stage II	43	Can develop into BC stage IV
Breast cancer stage I	42	Can develop into BC stage IV
Lung cancer (all stages)	43	Common breast cancer metastatic site
Bone cancer (all stages)	59	Common breast cancer metastatic site
Brain cancer (all stages)	42	Common breast cancer metastatic site
Liver cancer (all stages)	26	Common breast cancer metastatic site

To calculate the reference interval the Harrel-David non-parametric bootstrap method was chosen. The method generated a 95th percentile of 254 DuA. The results are summarized in Table 10.

Table 10 - Results for upper reference limit estimation using non-parametric Harrell-Davis bootstrap method. Mean, SD, Median and Max values are presented.

Apparently Healthy Post-Menopausal Female Specimens, Age ≥ 55 < 85; U.S. Collected	
N	**123**
Mean (DuA)	139
SD (DuA)	56
Median (DuA)	125
Max (DuA)	301
95th percentile (DuA)	254
97.5th percentile (DuA)	276

Based on these results, it can be concluded that values below 254 DuA are within the normal range for a healthy population of post-menopausal women (U.S. collected). The TKa was found to be slightly increased in patients with cell cycle affecting viral infections (acute Herpes Simplex and Epstein-Barr viral infections) as well as in certain autoimmune diseases (Rheumatoid Arthritis). Elevated levels of TKa are seen in patients with any type of late stage or aggressive malignant disease (cancer) as TKa is a biomarker for cell proliferation.

N. PROPOSED LABELING:

Labeling has been done in accordance with the requirements of 21 CFR Part 809.10**.**

O. CONCLUSION:

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