SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Multi-analyte test system with algorithmic analysis for detection of prostate cancer

Device Trade Name: 4Kscore Test

Device Procode: QRF

Applicant's Name and Address: OPKO Health, Inc.
4400 Biscayne Boulevard
Miami, FL 33137

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P190022

Date of FDA Notice of Approval: December 7, 2021

II. INDICATIONS FOR USE

The 4Kscore Test is an in vitro serum or plasma test that combines the results of four immunoassays (Roche Elecsys total PSA (prostate specific antigen), Roche Elecsys free PSA, intact PSA, and human kallikrein 2) into a single numerical score that also incorporates the following information: a patient's age, previous biopsy, and digital rectal exam (DRE). The 4Kscore Test is indicated for use with other patient information as an aid in the decision for prostate biopsy in men 45 years of age and older who have an abnormal age-specific total PSA and/or abnormal DRE. The 4Kscore Test is intended to aid in detection of aggressive prostate cancer (Gleason score $\geq$ 7/Gleason Grade Group $\geq$ 2) for whom a biopsy would be recommended by a urologist, based on current standards of care before consideration of the 4Kscore Test.

A 4Kscore < 5.0 is associated with decreased likelihood of a Gleason score $\geq$ 7 on biopsy. Prostate biopsy is required for the diagnosis of cancer. The test is not recommended more than once every 6 months.

The test is intended for professional use only, and is performed at a single-site BioReference Laboratories, Inc.

III. CONTRAINDICATIONS

The 4Kscore Test is not indicated for use in men with:

- A previous diagnosis of prostate cancer

- Digital rectal exam (DRE) performed within 96 hours before blood draw
- Use of 5-alpha reductase inhibitors within the previous 6 months
- Prostate procedures within the previous 6 months

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the 4Kscore Test Instructions for Use.

V. DEVICE DESCRIPTION

4Kscore Test uses software to incorporate the values for four analytes from separately run immunoassays (described below) from patients' sample (serum or K_2EDTA plasma), the patient's prior prostate biopsy status, digital rectal exam (DRE) finding and age, into a numerical value based on logistic regression. The test is performed at a single-site: BioReference Laboratories, Inc., Elwood Park, New Jersey.

A. Device Components – Immunoassays

The four analytes and corresponding immunoassays used in the 4Kscore Test are summarized in Table 1:

Table 1: Constituent immunoassays of the 4Kscore Test

Analyte	Reagent(s)	Instrument
Total PSA (tPSA)	Roche Elecsys Total PSA	Roche Cobas 8000, e602
Free PSA (fPSA)	Roche Elecsys Free PSA	Roche Cobas 8000, e602
Intact PSA (iPSA)	iPSA Assay Reagent Master Lot[1], assay ancillary materials, iPSA Controls	Perkin Elmer AutoDELFIA 1235
Human kallikrein 2 (hK2)	hK2 Assay Reagent Master Lot[2], assay ancillary materials, hK2 Controls	Perkin Elmer AutoDELFIA 1235

[1] iPSA Master Lot (200 plates) contains: iPSA Capture, iPSA Tracer, streptavidin-coated plates, iPSA Standards, and assay buffer

[2] hK2 Master Lot (200 plate) contains: hK2 Capture, hK2 Tracer, streptavidin-coated plates, hK2 Blocker, hK2 Standards, and assay buffer

Among the four constituent assays, Elecsys total PSA (tPSA) and Elecsys free PSA (fPSA) were commercialized by Roche Diagnostics and approved in P990056 and P000027, respectively. These tests are performed following the manufacturer's instructions for use on the Roche cobas e602 immunochemistry analyzer module, cleared in K060373.

Tests for intact PSA (iPSA) and kallikrein 2 (hK2), are proprietary sandwich fluoroimmunoassays, performed on the Perkin-Elmer AutoDELFIA fluorescence immunoassay analyzer, cleared in K935047. Proprietary reagents, calibrators, and controls for iPSA and hK2 assays are manufactured by OPKO Diagnostics (Woburn,

MA) and shipped to the BioReference Laboratories (Elmwood Park, NJ) facility. A description of required equipment, software, reagents, vendors, and storage conditions were provided and are described in the device labeling and/or assay protocols/procedures. The iPSA and hK2 assays are run by following OPKO's internal documents: "iPSA and hK2 Assay Using AutoDELFIA - Patient Samples (rev 008)" and "EP-SOP-1150 hK2 and iPSA Autodelfia (Rev 11)". OPKO assumes responsibility for the device.

i. **iPSA**: The iPSA assay is a non-competitive sandwich time-resolved fluoroimmunoassay in 96-well plate format, using the AutoDELFIA analyzer (K935047). Sample analyte is bound to capture monoclonal $F(ab')_2$ antibody fragments immobilized to the solid phase plate surface by streptavidin, followed by washing. Tracer monoclonal antibody binds a distinct epitope of the analyte, labeled with europium chelate to enable time-resolved fluorescence detection of the antigen:antibody complex at 615 nm. The assay uses a seven-point calibration, traceable to master lot. Assay controls are run at three levels in duplicate for each control.

ii. **hK2**: The hK2 assay is a non-competitive sandwich time-resolved fluoroimmunoassay in 96-well plate format, using the AutoDELFIA analyzer (K935047). Sample analyte is bound to capture monoclonal $F(ab')_2$ antibody fragments immobilized to the solid phase plate surface by streptavidin, followed by washing. The hK2 assay incorporates monoclonal blocker antibodies to minimize interference from immunochemically similar PSA epitopes. Tracer monoclonal antibody binds a distinct epitope of the analyte, labeled with europium chelate to enable time-resolved fluorescence detection of the antigen:antibody complex at 615 nm. The assay uses a seven-point calibration, traceable to master lot. Assay controls are run at three levels in duplicate for each control.

B. Test Procedure

The 4Kscore Test should be prescribed and ordered by physician. For sample submission for 4Kscore Test, the 4Kscore shipper, provided by BioReference Laboratory (BRL) includes the required materials: foam insulation, serum separator tube (SST) Vacutainer, K_2EDTA Vacutainer, sample bag, gel pack, protective bubble wrap envelope and FedEx shipping envelope.

Patient specimens should be collected aseptically by venipuncture. Centrifuging specimens and separating serum from the clot or plasma from the cells need to be performed within one hour and shipped with cold pack overnight to Bioreference Lab. If a sample is received within 72 hours from the time of blood draw, and before 4 PM Eastern time of a business day, at BioReference Lab, Elmwood Park, NJ, the sample is processed the same day, if not, the sample is stored in -20ºC freezer until the next available run.

The 4Kscore Test is implemented as an internal web service system for the measurements. The patient's file (sample type, previous biopsy, DRE status and age) is uploaded to the Specimen Processing Module (SPM). Sample is tested in accordance with the procedures, "EP-SOP-1150 version 17 for iPSA and hK2", "EP-SOP-0241 version 11" for tPSA and EP-SOP-0233 version 10 for fPSA. The 4Kscore Test results are determined automatically by the validated laboratory information system algorithm

calculation software. All eight essential inputs: four immunoassay results based on selected sample type, patient's age, biopsy status, and DRE result are exported automatically to the laboratory information system (B2 LIS). The system automatically triggers the internal web-based service 4Kscore Algorithm software to calculate the 4Kscore value for each sample.

C. Result Interpretation

The valid numerical value interval for 4Kscore is from 0.1 – 100.0.

A 4Kscore < 5.0 is associated with decreased likelihood of a Gleason score ≥ 7 on biopsy. Prostate biopsy is required for the diagnosis of cancer. Patient management should be based on clinical judgement. Other clinical information, along with the 4Kscore Test results, should be considered in the shared decision making process.

The test is not recommended more than once every 6 months.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Prostate biopsy with an appropriate number of cores is required for a diagnosis of prostate cancer. There are several other alternatives and procedures that aid in the detection of prostate cancer, including one or more of the following: digital rectal examination (DRE), transrectal ultrasound (TRUS), histological examination such as needle biopsy or transurethral resection, and MRI. Other IVD devices for measuring total PSA, %free PSA, and *phi* with venous serum or plasma samples are currently available to aid in the detection of prostate cancer in conjunction with DRE in men aged 50 and older. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

The 4Kscore Test has been marketed in the United States as a single-site laboratory developed test (LDT), subject to enforcement discretion by the FDA.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

For the detection of prostate cancer, measurements of circulating biomarkers (e.g., tPSA, fPSA) are used along with DRE and other signs and symptoms to aid in the decision to biopsy; prostate biopsy confirms the presence of prostate cancer and later management and treatment strategies. The 4Kscore Test is intended to provide additional information for patients who are indicated for prostate biopsy by current standard of care: elevated tPSA and/or abnormal DRE findings – to aid in the detection of aggressive prostate cancer and aid in the decision to proceed to biopsy.

Potential adverse effects include:

- A falsely elevated 4Kscore value could lead to a medical decision causing unnecessary biopsy.
- A falsely low 4Kscore value may not receive a necessary biopsy and therefore, could delay recognition of the presence of prostate cancer by the physician, and could adversely delay the initiation of treatment.

4Kscore results should be used in conjunction with symptoms, clinical evaluation, DRE, and other laboratory tests or imaging techniques.

IX. SUMMARY OF NONCLINICAL STUDIES

The analytical performances of Roche Elecsys total PSA (tPSA) and Roche Elecsys free PSA (fPSA) were established in P990056 and P000027, respectively. Analytical performance of intact PSA (iPSA) and human kallikrein 2 (hK2) were summarized below. In addition, analytical performance for the composite the 4Kscore value, based on analytical performance of the individual analytes is described below:

A. Precision Studies

Precision performance was determined for the 4Kscore Test using native samples. In addition, precision for 4Kcore was computationally simulated. Precision was also evaluated individually for the iPSA and hK2 assays. The design of precision studies was in accordance with CLSI EP05-A3, "Evaluation of Precision of Quantitative Measurement Procedures", and FDA "Guidance for Industry and Food and Drug Administration Staff, Class II Special Controls Guidance Document: Ovarian Adnexal Mass Assessment Score Test System" issued on March 23, 2011, for assessment of the score precision.

i. Precision of the 4Kscore Test:

a. A 20-day study was conducted by testing five serum samples in four replicates per run, one run per day for 20 days using one lot of reagents on one set of instruments, for a total of 80 measurements per sample. The results are summarized in Table 2:

Table 2: 20-day 4Kscore Within-Laboratory Precision

			Repeatability (Within-Run)		Between-Day/Run		Within-Lab Precision	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV
1	75	2.8	0.31	11.0%	0.29	10.4%	0.42	15.3%
2	80	6.6	0.57	8.6%	0.58	8.8%	0.81	12.3%
3	78	15.0	1.00	6.7%	1.59	10.6%	1.88	12.6%
4	80	15.0	1.57	10.5%	2.73	18.2%	3.15	21.0%
5	76	48.2	1.59	3.3%	2.00	4.1%	2.55	5.3%

b. Another 6-day study was conducted to evaluate the precision of the 4Kscore incorporating sources of variability as different operator and different instrument. Five serum samples were tested in four replicates per run, one run per day for six days on three instruments (three operators, one operator per instrument) using one reagent lot, for a total of 72 measurements per sample. Operators were cycled through instruments over the course of this 6-day study. The results are summarized in Table 3:

Table 3: 6-day 4Kscore Within-Laboratory Precision

			Repeatability (Within-Run)		Between-Day/Run		Between-Instrument		Between-Operator		Within-Lab	
ID	Mean	N	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	3.00	71	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.0	0.0%
2	7.63	71	0.45	5.9%	0.05	0.7%	0.21	2.7%	0.00	0.0%	0.50	6.5%
3	11.38	72	1.55	13.6%	1.03	9.1%	0.19	1.7%	0.30	2.7%	1.90	16.7%
4	17.03	70	0.77	4.5%	0.30	1.8%	0.30	1.8%	0.09	0.5%	0.89	5.2%
5	82.00	72	1.08	1.3%	0.50	0.6%	0.81	1.0%	0.14	0.2%	1.44	1.8%

c. Lot-to-lot imprecision: Five serum samples with 4Kscore values at 3.0, 6.5, 12.3, 18.0, and 63.6 were analyzed using three reagent lots. Each sample was tested in five replicates per run, one run per day, for five days, for a total of 75 measurements per sample. For each of the five samples, the mean, repeatability, between-day/run between-lot component of variance were calculated. The %CV for the between-lot imprecision was ≤ 5.4% for all five samples.

ii. Simulated precision for 4Kscore numerical values:

The precision of 4Kscore was evaluated at five different 4Kscore value levels. For a multivariate index assay, the precision performance can be different at the same score value when the underlying combinations for tPSA, fPSA iPSA, hK2, age, previous biopsy, and DRE result are different. In order to evaluate the precision characteristics of 4Kscore numerical values under different combinations of underlying variables, precision was computationally simulated from empirically obtained precision of the component immunoassays: iPSA and hK2 below; and tPSA, fPSA from manufacturer's labeling. Random measurement error was considered normally distributed and a generator of random normally distributed numbers was used. Mean values of of the analytes were considered as values of the corresponding analyte 937 subjects from the clinical validation study (see §X below). The standard deviation (SD) for each individual subject for each of the four individual analytes was calculated based on the precision profile of the analyte by linear interpolation. Each subject random measurement error was simulated with 1,000 iterations. There were simulated repeatability of the 4Kscore based on repeatability of the individual analytes and within-laboratory precision of the 4Kscore based on the within-laboratory precision of the individual analytes. There were considered six ranges of the 4Kscore values and for each range, there were calculated: mean of 4Kscore, maximum of SD and maximum %CV. The results are summarized in Table 4.

Table 4: Simulated Precision for 4Kscore								
			Repeatability (Within-Run)			Within-Laboratory Precision		
		N	Mean	SD_{max}	$\%CV_{max}$	Mean	SD_{max}	$\%CV_{max}$
4Kscore interval	< 5	194	2.66	0.42	11.0%	2.67	0.72	17.9%
	5–10	146	7.46	0.95	10.3%	7.48	1.67	16.8%
	11–20	198	14.48	1.33	9.0%	14.51	2.19	14.8%
	21–40	188	28.34	2.48	8.1%	28.36	3.63	13.8%
	41–60	95	49.17	3.36	7.9%	49.17	5.31	12.4%
	> 60	116	80.52	6.90	8.4%	80.47	8.03	9.9%

%CV of the repeatability of the 4Kscore values was ≤ 11% and the %CV of the within-laboratory precision of the 4Kscore values was ≤ 18%.

iii. Precision of Intact PSA (iPSA) and kallikrein 2 (hK2):

Five individual sera spanning the measuring intervals for iPSA and hK2 were tested in four replicates per run, one run per day for 20 days using one lot of reagent, for a total of 80 measurements per sample. The results are summarized in Table 5:

Table 5: 20-day Within-Laboratory Precision for iPSA and hK2									
			Mean	Repeatability		Between-Day/Run		Within-Laboratory	
	Sample	N	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
iPSA	1	80	0.037	0.0040	10.7%	0.0044	11.8%	0.0060	15.9%
	2	80	0.264	0.0068	2.6%	0.0084	3.2%	0.0108	4.1%
	3	80	0.462	0.0092	2.0%	0.0094	2.0%	0.0131	2.8%
	4	80	1.446	0.0157	1.1%	0.0286	2.0%	0.0326	2.3%
	5	80	5.231	0.0474	0.9%	0.0888	1.7%	0.1007	1.9%
hK2	6	80	0.041	0.0017	4.1%	0.0017	4.1%	0.0024	5.8%
	7	80	0.082	0.0024	3.0%	0.0021	2.5%	0.0032	3.9%
	8	80	0.121	0.0017	1.4%	0.0020	1.7%	0.0027	2.2%
	9	80	0.269	0.0053	2.0%	0.0044	1.6%	0.0069	2.6%
	10	80	2.282	0.0385	1.7%	0.0478	2.1%	0.0613	2.7%

Five sera spanning the measuring intervals for iPSA and hK2 were tested in four replicates per run, one run per day for nine days by three operators on three instruments using three lots of reagents, for a total of 108 measurements per sample. The results are summarized in Table 6:

Table 6: 9-day Within-Laboratory Precision for iPSA and hK2

			Mean	Repeatability		Between-Day/Run		Between-Operator		Between-Lot		Between-Instrument		Total	
	Sample	N	(ng/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
iPSA	1	108	0.043	0.0051	11.9%	0.0010	2.3%	0.0009	2.1%	0.0017	3.9%	0.0033	7.7%	0.0064	14.9%
	2	108	0.296	0.0110	3.7%	0.0000	0.0%	0.0007	0.3%	0.0000	0.0%	0.0040	1.3%	0.0117	4.0%
	3	108	0.459	0.0123	2.7%	0.0038	0.8%	0.0000	0.0%	0.0079	1.7%	0.0040	0.9%	0.0156	3.4%
	4	108	1.404	0.0316	2.2%	0.0176	1.3%	0.0073	0.5%	0.0159	1.1%	0.0067	0.5%	0.0407	2.9%
	5	108	5.330	0.1198	2.2%	0.0321	0.6%	0.0325	0.6%	0.0316	0.6%	0.0297	0.6%	0.1353	2.5%
hK2	6	108	0.040	0.0016	4.0%	0.0006	1.6%	0.0003	0.7%	0.0000	0.0%	0.0000	0.0%	0.0017	4.3%
	7	108	0.082	0.0026	3.1%	0.0007	0.9%	0.0002	0.2%	0.0020	2.5%	0.0016	2.0%	0.0037	4.5%
	8	108	0.120	0.0030	2.5%	0.0015	1.2%	0.0000	0.0%	0.0015	1.3%	0.0016	1.3%	0.0040	3.3%
	9	108	0.239	0.0056	2.3%	0.0010	0.4%	0.0000	0.0%	0.0045	1.9%	0.0042	1.8%	0.0084	3.5%
	10	108	1.792	0.0291	1.6%	0.0085	0.5%	0.0000	0.0%	0.0121	0.7%	0.0031	0.2%	0.0327	1.8%

B. Linearity

Linearity of tPSA and fPSA were previously demonstrated in P990056 and P000027 for the measurement intervals incorporated into the 4Kscore Test.

Linearity studies for iPSA and hK2 were conducted and analyzed in accordance with CLSI EP06, 2nd Ed. "Evaluation of Linearity of Quantitative Measurement Procedures". For each of the iPSA and hK2 assays, one high serum sample from an individual donor with analyte concentration above the intended assay upper limit was diluted into a low serum sample from a donor with an undetectable level of analyte to generate eight concentration levels across the anticipated linear range of the assay. Each dilution sample was tested with four replicates. Based on dilution scheme, the Expected values were calculated. For Predicted values, the weighted least squares linear regression was performed and the best fitted linear model was used for calculation of Predicted values. Deviation from linearity for each sample was calculated as a difference between the Mean value and Predicted value. %Deviation was calculated as Deviation/Predicted value. Data from these analyses for iPSA and hK2 are summarized in Table 7 and Table 8:

Table 7: Linearity of iPSA

		[Conc] (ng/mL)		(%)	(ng/mL)	(ng/mL)	(ng/mL)	(%)
Sample	Relative Concentration	Mean Observed	SD	%CV	Expected [Conc]	Predicted [Conc][1]	Deviation from Linearity	
1	1.0000	6.264	0.064	1.0%	6.264	6.076	0.188	3.1%
2	0.7986	4.912	0.061	1.2%	5.003	4.853	0.060	1.2%
3	0.3850	2.384	0.018	0.8%	2.412	2.340	0.044	1.9%
4	0.1566	0.916	0.016	1.7%	0.981	0.952	−0.036	−3.8
5	0.0626	0.360	0.008	2.2%	0.392	0.380	−0.021	−5.4%
6	0.0250	0.134	0.003	2.5%	0.157	0.152	−0.018	
7	0.0100	0.050	0.002	4.2%	0.063	0.061	−0.0112	
8	0.0040	0.016	0.003	21.3%	0.025	0.024	−0.009	

[1] Predicted concentration values derived as Y=0.97×Expected [Conc]

For the iPSA assay, acceptable deviations from linearity are:
±10% for values ≥ 0.18 ng/mL and
±0.018 ng/mL for values < 0.18 ng/mL.

The linearity data support that the linear interval for the iPSA assay is 0.016–6.0 ng/mL.

Table 8: Linearity of hK2

		[Conc] (ng/mL)		(%)	(ng/mL)	(ng/mL)	(ng/mL)	(%)
Sample	Relative Concentration	Mean Observed	SD	%CV	Expected [Conc]	Predicted [Conc][1]	Deviation from Linearity	
1	1.0000	4.416	0.078	1.8%	4.416	4.546	−0.130	−2.9%
2	0.5000.	2.237	0.019	0.8%	2.208	2.273	−0.036	−1.6%
3	0.1993	0.912	0.009	1.0%	0.880	0.906	0.006	0.6%
4	0.0793	0.355	0.004	1.0%	0.350	0.360	−0.006	−1.6%
5	0.0318	0.151	0.002	1.2%	0.141	0.145	0.006	4.3%
6	0.0129	0.060	0.001	1.9%	0.057	0.058	0.002	3.3%
7	0.0051	0.024	0.001	3.6%	0.023	0.023	0.001	
8	0.0020	0.011	0.0007	6.5%	0.009	0.009	0.002	

[1] Predicted concentration values derived as Y=0.97×Expected [Conc]

For the hK2 assay, acceptable deviations from linearity are:
±10% for values ≥ 0.03 ng/mL and
±0.003 ng/mL for values < 0.03 ng/mL.

The linearity data support that the linear interval for hK2 assay is 0.012–2.5 ng/mL.

C. Detection Capability

The detection capabilities for the iPSA and hK2 assays were evaluated by Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies in accordance with CLSI EP17-A2, "Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures".

i. Limit of Blank: Four serum samples from female donors were tested for each of iPSA and hK2 using two reagent lots over four days with four replicates per day for a total of 64 datapoints per lot. Non-parametric calculation interpolated the 61.3rd ordered value, corresponding to target type I error rate $\alpha = 0.05$, from the ranked distribution of datapoints per lot. The greatest value of LoBs from the two lots was selected as the LoB for the assay.

ii. Limit of Detection: Seven low-level serum samples from male donors were tested for each of iPSA and hK2 using two reagent lots over five days with eight replicates per day for a total of 40 datapoints per sample for each lot. Analysis of the data was performed according to the CLSI EP17-A2 for type II error rate $\beta = 0.05$, from the pooled standard deviation for each lot. The greatest estimate from the LoDs of two lots was selected as the LoD for the assay.

iii. Limit of Quantitation: Six low-level serum samples from male donors were tested for each of iPSA and hK2 using two reagent lots over five days with eight replicates per day for a total of 40 datapoints per sample for each lot. These samples were identical

to those used above for evaluation of LoD. A precision profile method for %CV calculated an interpolated value for a target precision value of %CV of 15% from a best-fit curve for each lot. The greatest estimate of the LoQs from two lots was selected as the LoQ for the assay.

Claims for detection capability for the iPSA and hK2 assays are summarized in Table 9:

Table 9: Detection capability for iPSA and hK2

	iPSA	hK2
Limit of Blank (LoB)	0.0069 ng/mL	0.0003 ng/mL
Limit of Detection (LoD)	0.015 ng/mL	0.0020 ng/mL
Limit of Quantitation (LoQ)	0.035 ng/mL	0.0050 ng/mL

D. Analytical Measuring Interval

Taking into consideration precision, linearity, LoB, LoD, and LoQ study results, the analytical measuring intervals for the iPSA assay is 0.035–6.0 ng/mL and for the hK2 assay, 0.012–2.5 ng/mL.

E. Matrix Comparison

To validate the comparability of K_2EDTA plasma and serum sample types for the 4Kscore Test, a matrix comparison study was performed in a subset of 380 subjects from the clinical studies (§X below), spanning the measuring intervals for each assay, tested for each of the four assays. Comparison was performed for each assay separately and subjects with assay values below and above the analytical measuring interval were excluded from the regression analysis. The Passing-Bablok regression analysis (X-axis is serum and Y-axis is plasma) was performed for each of the four individual assays. The results are summarized in Table 10:

Table 10: Matrix comparison: K_2EDTA Plasma vs Serum for individual analytes

		$[Conc]_{Serum}$ (ng/mL)		Slope		Intercept	
Analyte	N	Min	Max	Estimate	(95% CI)	Estimate	(95% CI)
tPSA	348	0.59	84.45	0.979	(0.972; 0.984)	0.014	(-0.0165; 0.0482)
fPSA	349	0.19	10.52	1.023	(1.016; 1.030)	-0.001	(-0.006; 0.006)
iPSA	346	0.084	3.755	1.097	(1.073; 1.128)	0.042	(0.029; 0.053)
hK2	337	0.015	1.768	1.007	(1.000; 1.022)	0.001	(0.000; 0.002)

Passing-Bablok regression analysis (X-axis is serum and Y-axis is plasma) was also performed for the 4Kscore values and the scatter plot, slope and the intercept along with 95% confidence intervals are presented in Figure 1 and Table 11:

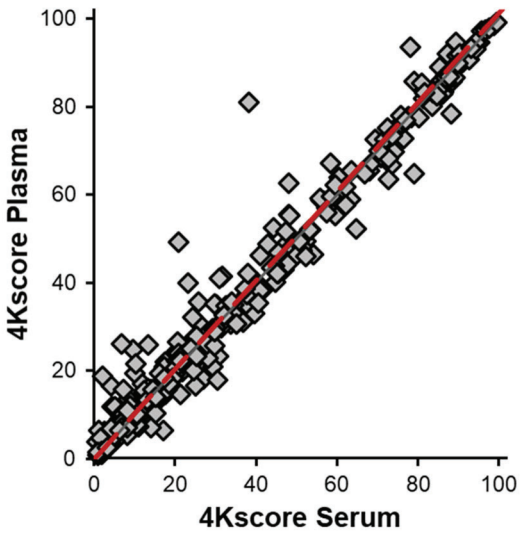

Figure 1: Matrix comparison of 4Kscore values from paired K_2EDTA Plasma versus Serum samples

Table 11: Matrix comparison of K_2EDTA Plasma vs Serum for 4Kscore values

		$4Kscore_{Serum}$		Slope		Intercept	
	N	Min	Max	Estimate	(95% CI)	Estimate	(95% CI)
4Kscore	349	0.6	99.5	0.989	(0.980; 0.996)	0.001	(-0.000; 0.004)

F. Analytical Specificity

The effect of potential endogenous, exogenous substances and cross reactivity to the 4Kscore Test and its component assays, iPSA and hK2, was evaluated in interference studies in accordance with CLSI EP07, 3rd Ed, "Interference Testing in Clinical Chemistry".

For the iPSA and hK2 assays, two levels of iPSA (0.13, 5.12 ng/mL) and hK2 (0.018, 2.490 ng/mL) were tested in the presence of the maximum concentrations of interferent listed in Table 12 and Table 13, and compared to an unspiked control condition. Interference testing for 4Kscore numerical values was performed on three sample pools, contrived to represent 4Kscore values < 5.0 with 34 replicates, 6.0–8.0 with 20 replicates, and > 20.0 with 10 replicates. Interference was determined as systematic difference between the mean of the results for the spiked sample (test sample), X_{test}, and the mean of the results of the unspiked sample (control sample), $X_{control}$: Interference = $X_{test} - X_{control}$. %Interference was determined as Interference/$X_{control}$. No significant interference was determined: %Interference is within ±10% for 4Kscore values ≥ 10 and Interference is within ±1 for 4Kscore < 10.

For endogenous substances, no significant interferences were observed in the study up to the concentrations listed in Table 12:

Table 12: Endogenous Interferences, Cross-reactivities			
	No Significant Interference up to Interferent Concentration		
Interfering Substance	iPSA	hK2	4Kscore
bilirubin	50 mg/dL	50 mg/dL	50 mg/dL
hemoglobin	500 mg/dL	500 mg/dL	500 mg/dL
human serum albumin	7 g/dL	7 g/dL	7 g/dL
human IgG	3.52 g/dL	5 g/dL	3.4 g/dL
triglycerides	3 g/dL	3 g/dL	3 g/dL
rheumatoid factor	161 U/mL	161 U/mL	161 U/mL
HAMA	52.5 ng/mL	52.5 ng/mL	52.5 ng/mL
prostatic acid phosphatase (PAP)	1000 ng/mL	1000 ng/mL	1000 ng/mL
alpha 1-antichymotrypsin (α_1ACT)	10 µg/mL	10 µg/mL	10 µg/mL
biotin[i]	3600 ng/mL	30 ng/mL	25.2 ng/mL
human kallikrein-2 (hK2)	15 ng/mL	n/a	n/a
free PSA (fPSA)	n/a	15.7 ng/mL	n/a
PSA-ACT	100 ng/mL	31.6 ng/mL	100 ng/mL[ii]

i. Biotin Interference: The effect of presence of biotin in the patient samples on the iPSA, hK2 and 4Kscore Test was evaluated by testing both serum and plasma samples each at two levels of iPSA (low and high), two levels of hK2 (low and high), and four levels of 4Kscore (2, 7.5, 15.2, and 45.6). Biotin concentration was tested at multiple levels up to 3600 ng/mL. The %Deviation of results from the samples with biotin comparing to the control sample without biotin (%Interference) was calculated.

   For iPSA: No interference (%Deviation within ±10%) was identified for iPSA assay up to 3600 ng/mL of biotin concentration.

   For hK2: Specimens with biotin concentrations up to 30 ng/mL demonstrated a less than or equal to 10% change in results. Biotin concentrations greater than this falsely decrease hK2 assay results for patient samples.

   For 4Kscore Test: Specimens with biotin concentrations up to 25.2 ng/mL demonstrated a less than or equal to 10% change in results. Biotin concentrations greater than this falsely decrease the 4Kscore Test results for patient samples.

ii. PSA-ACT Interference for 4Kscore: the effect of presence of PSA-ACT of 100 ng/mL in the patient samples on the 4Kscore values was evaluated by simulation of 4Kscore values at tPSA of 100 ng/mL and different combinations of other analytes (fPSA, iPSA, hK2) and clinical patient covariates (age, biopsy, DRE). The %Deviation of the 4Kscores was within 10%.

For exogenous substances, no significant interferences were observed in the study up to the concentrations listed in Table 13:

Table 13: Exogenous Interferences			
	No Significant Interference up to Concentration		
Interfering Substance	iPSA	hK2	4Kscore
tamsulosin	124.8 ng/mL	124.8 ng/mL	124.8 ng/mL
silodosin	184.8 ng/mL	184.8 ng/mL	184.8 ng/mL
doxazosin mesylate	1.33 µmol/L	1.33 µmol/L	1.33 µmol/L
ciprofloxacin[1]	30.2 µmol/L	30.2 µmol/L	30.2 µmol/L
nitrofurantoin	16.8 µmol/L	16.8 µmol/L	16.8 µmol/L
sulfamethoxazole	1.58 mmol/L	1.58 mmol/L	1.58 m mol/L
trimethoprim	138 µmol/L	138 µmol/L	138 µmol/L
sildenafil citrate	12.9 pmol/L	12.9 pmol/L	12.9 pmol/L
tadalafil	975 ng/mL	975 ng/mL	975 ng/mL
acetaminophen	1325 µmol/L	1325 µmol/L	1325 µmol/L
acetylsalicylic acid	3.62 mmol/L	3.62 mmol/L	3.62 mmol/L
ibuprofen	2425 µmol/L	2425 µmol/L	2425 µmol/L
lisinopril	0.74 µmol/L	0.74 µmol/L	0.74 µmol/L
atorvastatin	600 µg/mL	600 µg/mL	600 µg/L
amlodipine	245 nmol/L	245 nmol/L	245 nmol/L
hydrochlorothiazide	20.2 µmol/L	20.2 µmol/L	20.2 µmol/L
metformin	310 µmol/L	310 µmol/L	310 µmol/L
omeprazole	17.4 µmol/L	17.4 µmol/L	17.4 µmol/L
gadobutrol	0.9 mmol/L	0.9 mmol/L	0.9 mmol/L

[1] see clinical study for further evaluation of medications (§X)

G. Stability

Stability for the 4Kscore Test is based on reagent stability for the individual component assays. The 4Kscore Test only uses the reagents which are stored under the claimed storage condition and within the claimed expiration date for each assay.

Stability for tPSA and fPSA was established in P990056 and P000027, respectively. Stability for iPSA, hK2, and 4Kscore were evaluated based on the following studies:

i. Reagent Stability

Reagent stability (shelf-life, short-term, freeze-thaw, and on-board) studies for the iPSA and hK2 assays were performed in accordance with CLSI EP25-A, "Evaluation of Stability of In Vitro Diagnostic Reagents". Five plasma samples, one set of 3-level Assay Controls and one set of 7-level Standards, were tested in four replicates with three reagent lots at five timepoints (0, 3, 6, 12, 18 months) at reagent-specific storage conditions in shelf-life and short-term stability studies. Only the frozen reagents (Assay Controls and Standards for each of iPSA and hK2 assays) were assessed for freeze-thaw stability. One additional freeze-thaw cycle was tested beyond the claimed freeze-thaw stability. The acceptable degradation was 10% from the baseline values. The reagent stability was summarized in Table 14 and Table 15:

Table 14: iPSA Assay Reagent Stability			
	Storage Time @ Condition		Number of Cycles
Description	Shelf-Life	Short-Term	Freeze-Thaw
iPSA Antigen	1.5 year @ -90 – -70°C	n/a	n/a
fPSA Antigen	3.0 year @ -90 – -70°C	n/a	n/a
iPSA Capture mAb	2.5 year @ 2–8°C	n/a	n/a
iPSA Tracer mAb	2.0 year @ -20 ±5°C	n/a	n/a
fPSA Solution	1.0 year @ -90 – -70°C	n/a	n/a
iPSA Solution	1.0 year @ -90 – -70°C	n/a	n/a
iPSA Capture	12 months @ 2–8°C	2 weeks @ 25 ±2°C	n/a
iPSA Tracer	12 months @ 2–8°C	2 weeks @ 25 ±2°C	n/a
iPSA Assay Buffer	12 months @ 2–8°C	1 week @ 25 ±2°C	n/a
iPSA Standards	12 months @ -90 – -70°C	21 hours @ 25 ±2°C	1
iPSA Assay Controls	12 months @ -20 ±5°C	21 hours @ 25 ±2°C	1

Table 15: hK2 Assay Reagent Stability			
	Storage Time @ Condition		Number of Cycles
Description	Shelf-Life	Short-Term	Freeze-Thaw
hK2 Antigen	1.5 year @ -90 – -70°C	n/a	n/a
hK2 Capture mAb	1.0 year @ -20 ±5°C	n/a	4
hK2 Capture mAb, bulk	1.5 year @ -20 ±5°C	n/a	n/a
hK2 Blocking mAbs	1.5 year @ -20 ±5°C	n/a	n/a
	1.0 year @ -20 ±5°C	n/a	4
	1.5 year @ -20 ±5°C	n/a	4
hK2 Tracer mAb	1.5 year @ -20 ±5°C	n/a	4
hK2 Solution	6 months @ -90 – -70°C	n/a	n/a
hK2 Capture	12 months @ 2–8°C	2 weeks @ 25 ±2°C	n/a
hK2 Blocker	12 months @ 2–8°C	2 weeks @ 25 ±2°C	n/a
hK2 Tracer	12 months @ 2–8°C	2 weeks @ 25 ±2°C	n/a
hK2 Assay Buffer	12 months @ 2–8°C	3 weeks @ 25 ±2°C	n/a
hK2 Standards	12 months @ -20 ±5°C	21 hours @ 25 ±2°C	1
hK2 Assay Controls	12 months @ -20 ±5°C	21 hours @ 25 ±2°C	1

On-board stability studies were performed by placing the reagents on-board of instrument at the conditions used for the iPSA or hK2 assays. An additional timepoint exceeding the normal reagent on-board time was tested. The results demonstrated stability of iPSA and hK2 reagents on-board the AutoDELFIA instrument at 15–25ºC:

- Over 24 hours for multiple runs
- Over 22 hours with 10 hours kept refrigerated between runs.

The iPSA, hK2 immunoassays and 4Kscore were tested and there was no impact on the results of analytes and the 4Kscore.

ii. Sample Stability

a. Sample storage stability:

Stability of samples used for the 4Kscore Test is based on the stability of samples used with each individual assay, i.e., tPSA, fPSA, iPSA and hK2. Stability studies for all four constituent measurement procedures (i.e., tPSA, fPSA, iPSA, hK2) were performed in accordance with CLSI EP25-A, "Evaluation of Stability of In Vitro Diagnostic Reagents". A panel of 20 samples from 20 donors were collected and prepared for serum (on-gel) and plasma. Stability of serum samples were evaluated by keeping the serum samples on-gel at room temperature for (24, 48, and 72 hours) and then transferred and stored at 2–8ºC for additional 24, 48, 72, and 96 hours. Stability of K_2EDTA plasma samples were evaluated at 2–8ºC for 24, 48, 72, 96, 120, and 144 hours. At each timepoint, samples were tested in four replicates for each of the tPSA, fPSA, iPSA and hK2 assays. 4Kscore value was also evaluated by comparing value obtained at 96 hours to the value from the initial day. The results support the following initial stability for four assays and 4Kscore when no more than 10% deviation of the value of samples was observed:

- K_2EDTA plasma samples are stable up to 120 hours (5 days) at 2–8ºC
- Serum samples are stable on-gel up to 72 hours at room temperature followed with up to 72 hours stored at 2–8ºC.

In addition, long-term sample stability was evaluated real-time for iPSA and hK2 using six serum and six plasma sample stored at -80ºC (±10ºC). The data support sample stability up to one year for these two assays.

The sample storage stability are summarized in Table 16:

Table 16: Sample Stability for the 4Kscore Test and individual assays

		Ambient (RT)	Refrigerated	Frozen
Assay	Sample Type	20–25°C	2–8°C	-20°C, unless specified
tPSA (P990056)	Serum and plasma (K_2EDTA, Li-Heparin)	24 hours	5 days (120 hours)	6 months
fPSA (P000027)	Serum and plasma (K_2EDTA, Li-Heparin)	8 hours	5 days (120 hours)	3 months
iPSA	Serum	72 hours	3 days (72 hours)	12 months (-90 – -70°C)
	Plasma (K_2EDTA)	not tested	5 days (120 hours)	12 months (-90 – -70°C)
hK2	Serum	72 hours	3 days (72 hours)	12 months (-90 – -70°C)
	Plasma (K_2EDTA)	not tested	5 days (120 hours)	12 months (-90 – -70°C)
4Kscore	Serum	8 hours	3 days (72 hours)	3 months
	Plasma (K_2EDTA)	not tested	5 days (120 hours)	

Serum samples used for the 4Kscore Test are stable up to 8 hours when stored at 20–25°C, 3 days when stored at 2–8°C; K_2EDTA plasma samples are stable up to 5 days when stored at 2–8°C. Both serum and K_2EDTA plasma samples are stable up to 3 months when kept at -25 – -15°C.

b. Sample Shipping Stablity:

The samples used for the 4Kscore Test requires preparation of serum or plasma samples within one hour after sample collection, which are then shipped with a cold pack to the Bioreference Laboratory, Inc.

To demonstrate the effect of 4Kscore Test results during transportation of patient samples shipped overnight in the shipping kit provided by BioReference Laboratory, Inc., sample stability was evaluated by testing six native serum and six native K_2EDTA plasma samples stored under shipping conditions modeled as temperature flux in ambient winter conditions (sequential steps with 4 hours (18°C), 2 hours (-10°C), 12 hours (2–8°C), 8 hours (18°C), 6 hours (-10°C), for a total of 32 hours); and summer conditions (sequential steps with 4 hours (22°C), 2 hours (35°C), 12 hours (30°C), 8 hours (22°C), 6 hours (35°C), for a total of 32 hours). At the end of each simulated shipping condition, all samples were tested with the 4Kscore Test, using the same parameters for biopsy and DRE result. The %Deviation of 4Kscore of each sample from the control sample (stored at 2–8°C without temperature modification) was evaluated. The results demonstrated all samples are within 10% deviation on 4Kscore value up to 32 hours post sample collection and under simulated winter and summer shipping conditions.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The clinical performance and reference population studies were conducted under approved IRB protocols to establish the performance of the 4Kscore Test for use as an aid in the detection of aggressive prostate cancer (Gleason score ≥ 7) and aid in the decision for prostate biopsy in men 45 years of age and older who are found to have abnormal age-specific tPSA and/or abnormal DRE.

CLINICAL PERFORMANCE STUDY

A. Study Design

To determine the performance of the 4Kscore Test as an aid in the detection of aggressive prostate cancer (Gleason score ≥ 7) and aid in the decision for prostate biopsy in men 45 years of age and older who have abnormal age-specific tPSA and/or abnormal DRE, the study consists of two multi-center double blinded clinical studies – Study 1 and Study 2. The studies included patients enrolled between 2013 through 2016 from 23 urology centers (a total of 27 sites) (Study 1, "US Validation Study") and eight hospitals (Study 2, "Veteran Administration Validation Study") in the U.S. All subjects had prior assessment including a tPSA test and a digital rectal exam (DRE). All subjects participating in the study met the inclusion/exclusion criteria described below and underwent their previously scheduled prostate biopsy. The goals for the clinical studies were to evaluate clinical performance of the 4Kscore Test to support the proposed intended uses: aid in detecting of Gleason score ≥ 7 of prostate cancer in men ≥ 45 years of age with elevated age-specific tPSA and/or suspicious DRE, thus, aid in assisting the biopsy decision for these patients.

1. Inclusion and Exclusion Criteria

   The study subjects from Study 1 and Study 2 were comprised by patients who met the following inclusion criteria and exclusion criteria.

   Inclusion criteria:

   - Men aged 45–80 years old who have scheduled to undergo prostate biopsy
   - Prior total PSA and DRE performed by a urologist
   - Have abnormal age-specific tPSA and/or abnormal DRE; elevated age-specific total PSA values are defined as follows:
     - 45–54 years old: ≥ 2.0 ng/mL total PSA
     - 55–75 years old: ≥ 3.0 ng/mL total PSA
     - ≥ 76 years old: ≥ 4.0 ng/mL total PSA
   - Informed consent
   - All patients must receive a conventional TRUS-guided biopsy, minimum of 10 cores, evaluated by "pathology service routinely utilized by the center"
   - All patients must be able to undergo phlebotomy for the collection of 20–30 mL of blood.

   Exclusion criteria:

   - A previous diagnosis of prostate cancer
   - DRE within 96 hours, prior to sample collection. DRE performed after the phlebotomy is acceptable
   - Treatment with a 5-alpha reductase inhibitor (5ARI), e.g., dutasteride, finasteride within 6 months prior to sample collection
   - Undergoing any procedures to treat symptoms of benign prostatic hyperplasia (BPH)
   - Undergoing any invasive urologic procedures associated with elevated PSA levels within the previous six months prior to phlebotomy. The procedures include but not limited to:
     - Thermotherapy
     - Microwave therapy
     - Laser therapy
     - Transurethral resection of the prostate (TURP)
     - Urethral catheterization
     - Lower genitourinary tract endoscopy

2. Follow-up Schedule

   Not applicable

3. Clinical Endpoints

   The objective of the clinical performance study was to evaluate the 4Kscore in detection of aggressive prostate cancer with Gleason Score ≥ 7 upon biopsy. Clinical performance of the 4Kscore was described by likelihood (probability) of Gleason Score ≥ 7 for four intervals of the 4Kscore results. In addition, performance of the 4Kscore was described by sensitivity, specificity, positive predictive value (PPV) and

negative predictive value (NPV) for the 4Kscore cut-points of 5.0, 10.0, and 20.0. These calculations are based on the following definitions:

True Positive (TP): Men with prostate cancer biopsy findings (Gleason Score ≥ 7), for whom 4Kscore numerical value is reported at or above the cut-points of 5.0, 10.0, and 20.0;

True Negative (TN): Men with negative biopsy findings (no prostate cancer) or low-grade prostate cancer (Gleason Score = 6), for whom 4Kscore numerical values fall below the cut-points of 5.0, 10.0, and 20.0;

False Positive (FP): men with negative or low-grade biopsy findings, for whom 4Kscore numerical value is reported at or above the cut-points of 5.0, 10.0, and 20.0;

False Negative (FN): men with prostate cancer biopsy findings (Gleason Score ≥ 7), for whom 4Kscore numerical values fall below the cut-points of 5.0, 10.0, and 20.0.

B. Accountability of PMA Cohort

A total of 937 patients including 574 patients from Study 1 and 363 patients from Study 2 met the finalized eligibility criteria and included for the 4Kscore Test clinical performance analysis.

C. Study Population Demographics and Baseline Parameters

From a total of 937 subjects in the study, the distributions of demographic and clinical variables are described in Table 17:

Table 17: Distribution of demographic, clinical variables in Clinical Performance Study

	Demographic or Clinical Variable	N	% Total
Race, Ethnicity	White, Caucasian	631	67.3%
	African American	254	27.1%
	Hispanic or Latino	24	2.6%
	Asian or Asian American	5	0.5%
	American Indian or Alaska Native	3	0.3%
	Native Hawaiian or other Pacific Islander	1	0.1%
	Other	12	1.3%
	Unknown	7	0.8%
Age (years)	45–54	106	11.3%
	55–75	785	83.8%
	76–80	46	4.9%
Prior Biopsy	Prior negative biopsy	184	19.6%
	No prior biopsy	753	80.4%

Table 17: Distribution of demographic, clinical variables in Clinical Performance Study			
	Demographic or Clinical Variable	N	% Total
DRE and tPSA	Normal DRE, elevated tPSA	714	76.2%
	Abnormal DRE, not elevated tPSA	49	5.2%
	Abnormal DRE, elevated tPSA	166	18.6%
Biopsy Result	Negative	444	47.4%
	Gleason Score 6	235	25.1%
	Gleason Score 7 (3+4)	109	11.6%
	Gleason Score 7 (4+3)	61	6.5%
	Gleason Score 8	57	6.1%
	Gleason Score 9	26	2.8%
	Gleason Score 10	5	0.5%
	Total:	937	

D. Safety and Effectiveness Results

1. Safety Results

   The 4Kscore Test involves testing blood samples using venipuncture, which has potential risk of hematoma. However, these specimens are routinely taken as part of the practice of medicine, and therefore, sample collection presents no additional safety hazard to the patient being tested.

   In this study, all enrolled subjects were men presenting to a practicing urologist with symptoms that would lead to an evaluation for prostate cancer and who were scheduled to receive a prostate needle biopsy. Although the most significant safety concern with respect to biopsy is often associated with infectious complications following the procedure, the 4Kscore Test result for these study subjects did not alter the medical decision for these patients, therefore, present no additional safety hazard to the subjects being tested.

2. Effectiveness Results

   The clinical performance evaluation was based on a total of 937 evaluable patients of the clinical performance study (Study 1 and Study 2). Each patient in the study had 4Kscore Test result and biopsy findings. Results of the 4Kscore were divided into four intervals: < 5.0, $5.0 - < 10.0$, $10.0 - < 20$ and ≥ 20. Biopsy findings were divided into two groups: Gleason Score ≥ 7, and Gleason Score < 7. The data of the clinical performance study were presented in Table 18–Table 20:

Table 18: 4-by-2 table for 4Kscore clinical performance study				
		Biopsy findings		
		Gleason Score ≥ 7	Gleason Score < 7	
4Kscore	< 5.0	8	186	194
	5.0 – < 10.0	14	132	146
	10.0 – < 20.0	39	159	198
	≥ 20.0	197	202	399
	Total:	258	679	937

Performance of the 4Kscore Test is described by likelihood (probability) of Gleason Score ≥ 7 for each interval of 4Kscore along with prevalence and summarized in Table 19:

Table 19: Likelihood of Gleason Score 7 or higher, by 4Kscore values, total study population				
	N		Likelihood of Gleason Score ≥ 7	
4Kscore	Total	Gleason Score ≥ 7	Estimate	(95% CI)
< 5.0	194	8	4.1%	(2.1%; 7.9%)
5.0 – < 10.0	146	14	9.6%	(5.8%; 15.5%)
10.0 – < 20.0	198	39	19.7%	(14.8%; 25.8%)
≥ 20.0	399	197	49.4%	(44.5%; 54.3%)
Total:	937	258	27.5%	

Confidence intervals for binomial proportions were calculated by the Wilson score method (CLSI EP12-A2 "User Protocol for Evaluation of Qualitative Test Performance"). Likelihood (probability) of Gleason Score ≥ 7 for the three 4Kscore intervals (< 5.0, 5.0 – < 10.0, and 10.0 – < 20.0) were lower (statistically significantly) than the prevalence of 27.5% and the likelihood (probability) of Gleason Score ≥ 7 for the 4Kscore interval (≥ 20) was higher (statitically significantly) than the prevalence of 27.5%. Using 937 samples from subjects who had an age-specific elevated total PSA value and/or abnormal DRE, the results indicated that 4Kscore Test is statistically informative as aid in detection of aggressive prostate cancer (Gleason Score ≥ 7).

In addition, clinical performance was described by metrics: sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with corresponding 95%CI for three 4Kscore cut-points: 5.0, 10.0, and 20.0. The results are summarized in Table 20:

Table 20: Clinical performance summary for the 4Kscore Test (Prevalence: 27.5% (258/937))				
4Kscore cut-point	Sensitivity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)
5.0	96.9% (250/258) (94.0%; 98.4%)	27.4% (186/679) (24.2%; 30.9%)	33.6% (250/743) (30.3%; 37.1%)	95.9% (186/194) (92.1%; 97.9%)

Table 20: Clinical performance summary for the 4Kscore Test (Prevalence: 27.5% (258/937))				
4Kscore cut-point	Sensitivity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)
10.0	91.5% (236/258) (87.4%; 94.3%)	46.8% (318/679) (43.1%; 50.6%)	39.5% (236/597) (35.7%; 43.5%)	93.5% (318/340) (90.4%; 95.7%)
20.0	76.4% (197/258) (70.8%; 81.1%)	70.3% (477/679) (66.7%; 73.6%)	49.4% (197/399) (44.5%; 54.3%)	88.7% (477/538) (85.7%; 91.1%)

The performance of the 4Kscore test compares to tPSA alone are shown by Receiver Operating Characteristic (ROC) plot and the Area Under the Curve (AUC) of the ROC statistics in Figure 2 and Table 21Error! Reference source not found.:

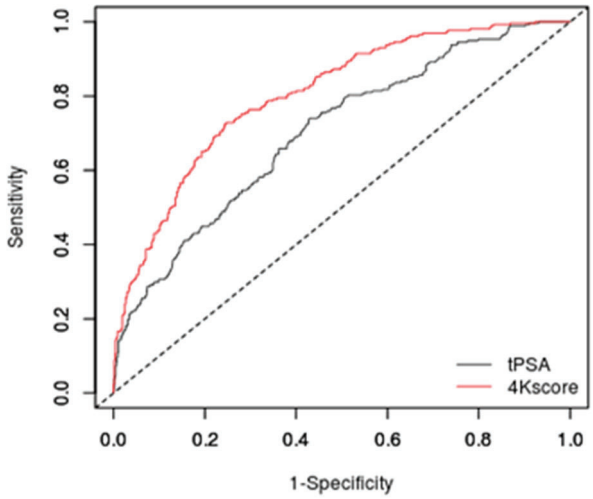

Figure 2: ROC plot of 4Kscore Test in comparison to total PSA

Table 21: ROC Analysis Results			
	N	AUC	(95% CI)
tPSA	937	0.7021	(0.6651; 0.7391)
4Kscore	937	0.8039	(0.7734; 0.8344)

Performance of the 4Kscore Test for detection of Gleason Score ≥ 7 is better than performance of the total PSA test alone.

3. Subgroup Analyses

Clinical performance of the 4Kscore Test based on 937 subjects of the clinical performance study was presented stratified by different demographic and other clinical characteristics.

a. Age

Prevalence of prostate cancer is well-known to increase with advancing age. The definition of "abnormal age-specific total PSA", as stated in the Intended Use reflects this age-related increase in clinical and laboratory parameters associated with prostate cancer:

- 45–54 years old and total PSA ≥ 2 ng/mL
- 55–75 years old and total PSA ≥ 3 ng/mL
- 76–80 years old and total PSA ≥ 4 ng/mL

The performance of the 4Kscore Test was analyzed for the different age groups: 45–54 years old, 55–75 years old, and 76–80 years old. The results are summarized in Table 22 and Table 23:

Table 22: Likelihood of Gleason Score 7 or higher, by 4Kscore values, stratified by age

Age (years)	4Kscore	N		Likelihood of Gleason Score ≥ 7	
		Total	Gleason Score ≥ 7	Estimate	(95% CI)
45–54	< 5.0	39	1	2.6%	(0.5%; 13.2%)
	5.0 – < 10.0	18	2	11.1%	(3.1%; 32.8%)
	10.0 – < 20.0	23	4	17.4%	(7.0%; 37.1%)
	≥ 20.0	26	10	38.5%	(22.4%; 57.5%)
	Total:	106	17	16.0%	
55–75	< 5.0	149	7	4.7%	(2.3%; 9.4%)
	5.0 – < 10.0	124	12	9.7%	(5.6%; 16.2%)
	10.0 – < 20.0	168	33	19.6%	(14.3%; 26.3%)
	≥ 20.0	344	171	49.7%	(44.5%; 55.0%)
	Total:	785	223	28.4%	
76–80	< 5.0	6	0	0.0%	(0.0%; 39.0%)
	5.0 – < 10.0	4	0	0.0%	(0.0%; 49.0%)
	10.0 – < 20.0	7	2	28.6%	(8.2%; 64.1%)
	≥ 20.0	29	16	55.2%	(37.5%; 71.6%)
	Total:	46	18	39.1%	

In addition, sensitivity and specificity with 4Kscore cut-points of 5.0, 10.0, and 20.0 were stratified by age groups and presented in Table 23:

Table 23: Performance of the 4Kscore Test, stratified by Age Groups

		4Kscore cut-points					
		5.0		10.0		20.0	
Age (years)	Prevalence of Gleason Score ≥ 7	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)
45–54	16.0% (17/106)	94.1% (16/17) (73.0%; 99.0%)	42.7% (38/89) (32.9%; 53.1%)	82.4% (14/17) (59.0%; 93.8%)	60.7% (54/89) (50.3%; 70.2%)	58.8% (10/17) (36.0%; 78.4%)	82.0% (73/89) (72.8%; 88.6%)
55–75	28.4% (223/785)	96.9% (216/223) (93.7%; 98.5%)	25.3% (142/562) (21.9%; 29.0%)	91.5% (204/223) (87.1%; 94.5%)	45.2% (254/562) (41.1%; 49.3%)	76.7% (171/223) (70.7%; 81.8%)	69.2% (389/562) (65.3%; 72.9%)
76–80	39.1% (18/46)	100% (18/18) (82.4%; 100%)	21.4% (6/28) (10.2%; 39.5%)	100% (18/18) (82.4%; 100%)	35.7% (10/28) (20.7%; 54.2%)	88.9% (16/18) (67.2%; 96.9%)	53.6% (15/28) (35.8%; 70.5%)

b. Race and Ethnicity

Increased prevalence and severity are known and well-documented for African American populations in the United States. The performance of the 4Kscore Test was evaluated by stratifying into two subgroups: African American (N=254) and non-African American (N =676) subpopulations, as in the table below. Study subjects who did not self-report any race/ethnicity identifiers ("unknown", N=7) were excluded from this analysis; whereas subjects who reported as "other" were incorporated into the non-African American group. The results are summarized in Table 24.

Table 24: Likelihood of Gleason Score 7 or higher, by 4Kscore values, stratified by Race and Ethnicity

Race/Ethnicity	4Kscore	N		Likelihood of Gleason Score ≥ 7	
		Total	Gleason Score ≥ 7	Estimate	(95% CI)
Non-African American	< 5.0	150	6	4.0%	(1.8%; 8.5%)
	5.0 – < 10.0	118	10	8.5%	(4.7%; 14.9%)
	10.0 – < 20.0	144	24	16.7%	(11.5%; 23.6%)
	≥ 20.0	264	114	43.2%	(37.3%; 49.2%)
	Total:	676	154	22.8%	
African American	< 5.0	42	2	4.8%	(1.3%; 15.8%)
	5.0 – < 10.0	26	4	15.4%	(6.1%; 33.5%)
	10.0 – < 20.0	53	14	26.4%	(16.4%; 39.6%)
	≥ 20.0	133	81	60.9%	(52.4%; 68.8%)
	Total:	254	101	39.8%	

In addition, sensitivity and specificity with 4Kscore cut-points of 5.0, 10.0, and 20.0 were stratified by race and ethnicity and presented in Table 25:

Table 25: Performance of the 4Kscore Test, stratified by Race and Ethnicity

	4Kscore cut-points					
	5.0		**10.0**		**20.0**	
	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)
Non-African American	96.1% (148/154) (91.8%; 98.2%)	27.6% (144/522) (23.9%; 31.6%)	89.6% (138/154) (83.8%; 93.5%)	48.3% (252/522) (44.0%; 52.6%)	74.0% (114/154) (66.6%; 80.3%)	71.3% (372/522) (67.2%; 75.0%)
African American	98.0% (99/101) (93.1%; 99.5%)	26.1% (40/153) (19.8%; 33.6%)	94.1% (95/101) (87.6%; 97.2%)	40.5% (62/153) (33.1%; 48.4%)	80.2% (81/101) (71.4%; 86.8%)	66.0% (101/153) (58.2%; 73.0%)

Benefit and risk per 10,000 patients from the intended use population were evaluated for detection of Gleason Score ≥ 7 and presented in Table 26 and Table 27 below.

Table 26: Benefit-Risk Analysis for 10,000 non-African American Patients

		Benefit		Risk			
		TP	TN	FN	FP		
Standard of Care:		2,278	0	0	7,722	10,000	
4Kscore	< 5.0	0	2,130	89	0	2,219	10,000
	5.0 – < 10.0	0	1,598	148	0	1,746	
	10.0 – < 20.0	0	1,775	355	0	2,130	
	≥ 20.0	1,686	0	0	2,219	3,905	
	Total:		5,503	592			

For non-African American patients, the ratio of TN: FN was 9.3 true negative to 1 false negative (5,503/592 = 9.3). It means that in average, for every 9 patients who avoided unnecessary biopsy, there is 1 patient with potentially missed prostate cancer.

Table 27: Benefit-Risk Analysis for 10,000 African American Patients

		Benefit		Risk			
		TP	TN	FN	FP		
Standard of Care:		3,976	0	0	6,024	10,000	
4Kscore	< 5.0	0	1,575	79	0	1,654	10,000
	5.0 – < 10.0	0	867	157	0	1,024	
	10.0 – < 20.0	551	0	0	1,536	2,087	
	≥ 20.0	3,189	0	0	2,046	5,235	
	Total:		2,442	236			

For African American patients, the ratio of TN: FN was 10.3 true negative to 1 false negative (2,442/236 =10.3). It means that in average, for every 10 patients who avoided unnecessary biopsy, there is 1 patient with potentially missed prostate cancer.

c. DRE status

The performance of the 4Kscore Test was analyzed based on the DRE status: DRE (−) refers to normal DRE finding, DRE (+) refers to abrnomal DRE finding. The results are summarized in Table 28:

Table 28: Performance of the 4Kscore Test stratified by DRE Status

	4Kscore cut-points					
	5.0		**10.0**		**20.0**	
	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)
DRE(−)	95.9% (186/194) (92.1%; 97.9%)	30.3% (160/528) (26.5%; 34.4%)	89.2% (173/194) (84.0%; 92.8%)	49.4% (261/528) (45.2%; 53.7%)	72.7% (141/194) (66.0%; 78.5%)	71.6% (378/528) (67.6%; 75.3%)
DRE(+)	100% (64/64) (94.3%; 100%)	17.2% (26/151) (12.0%; 24.0%)	98.4% (63/64) (91.7%; 99.7%)	37.7% (57/151) (30.4%; 45.7%)	87.5% (56/64) (77.2%; 93.5%)	65.6% (99/151) (57.7%; 72.7%)

d. Prior Biopsy Status

The performance of the 4Kscore Test was analyzed based on the prior biopsy history: no previous biopsy and prior biopsy(−) (refers to no cancer identified at prior biopsy). The results are summarized in Table 29:

Table 29: Performance of the 4Kscore Test stratified by Prior Biopsy history

	4Kscore cut-points					
	5.0		**10.0**		**20.0**	
	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)
No prior biopsy	98.3% (232/236) (95.7%; 99.3%)	14.9% (77/517) (12.1%; 18.2%)	92.8% (219/236) (88.8%; 95.5%)	36.0% (186/517) (32.0%; 40.2%)	78.8% (186/236) (73.2%; 83.5%)	62.9% (325/517) (58.6%; 66.9%)
Prior biopsy (−)	81.8% (18/22) (61.5%; 92.7%)	67.3% (109/162) (59.7%; 74.0%)	77.3% (17/22) (56.6%; 89.9%)	81.5% (132/162) (74.8%; 86.7%)	50.0% (11/22) (30.7%; 69.3%)	93.8% (152/162) (89.0%; 96.6%)

e. Comorbidities

From the total number of 937 subjects, concurrent comorbid diagnoses were evaluated to assess the impact of other disease conditions upon the clinical performance of the 4Kscore test. From 29 disease categorizations, the top six most frequent are represented in the tables below, in descending order of prevalence. In addition, 13 cancer types were reported and summarized as “all

cancer" to account for the low prevalence of several solid tumor types. The results are summarized in Table 30:

Table 30: Performance of the 4Kscore Test, stratified by comorbid diagnostic conditions

	4Kscore cut-points					
	5.0		10.0		20.0	
	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)
cardiovascular disease, hypertension	97.0% (162/167) (93.2%; 98.7%)	23.9% (88/368) (19.8%; 28.5%)	92.8% (155/167) (87.9%; 95.8%)	43.8% (161/368) (38.8%; 48.9%)	79.0% (132/167) (72.3%; 84.5%)	69.3% (255/368) (64.4%; 73.8%)
hyperlipidemia	97.6% (124/127) (93.3%; 99.2%)	30.3% (79/261) (25.0%; 36.1%)	92.1% (117/127) (86.1%; 95.7%)	46.4% (121/261) (40.4%; 52.4%)	79.5% (101/127) (71.7%; 85.6%)	70.5% (184/261) (64.7%; 75.7%)
benign prostatic hyperplasia	96.2% (50/52) (87.0%; 98.9%)	33.3% (60/180) (26.9%; 40.5%)	86.5% (45/52) (74.7%; 93.3%)	53.3% (96/180) (46.1%; 60.5%)	73.1% (38/52) (59.7%; 83.2%)	76.7% (138/180) (70.0%; 82.2%)
gastrointestinal disease, benign	97.9% (46/47) (88.9%; 99.6%)	29.8% (42/141) (22.9%; 37.8%)	91.5% (43/47) (80.1%; 96.6%)	44.0% (62/141) (36.0%; 52.2%)	78.7% (37/47) (65.1%; 88.0%)	68.8% (97/141) (60.7%; 75.9%)
diabetes, any	94.4% (51/54) (84.9%; 98.1%)	23.1% (25/108) (16.2%; 31.9%)	88.9% (48/54) (77.8%; 94.8%)	46.3% (50/108) (37.2%; 55.7%)	75.9% (41/54) (63.1%; 85.4%)	73.1% (79/108) (64.1%; 80.6%)
arthritis	96.9% (31/32) (84.3%; 99.4%)	25.9% (21/81) (17.6%; 36.4%)	90.6% (29/32) (75.8%; 96.8%)	49.4% (40/81) (38.8%; 60.0%)	71.9% (23/32) (54.6%; 84.4%)	69.1% (56/81) (58.4%; 78.1%)
cancer, all[1]	100% (12/12) (75.8%; 100%)	16.3% (7/43) (8.1%; 30.0%)	83.3% (10/12) (55.2%; 95.3%)	41.9% (18/43) (28.4%; 56.7%)	75.0% (9/12) (46.8%; 91.1%)	67.4% (29/43) (52.5%; 79.5%)

[1] sum of skin (N=33), leukemia (N =4), colorectal (N=3), kidney (N=3), lung/liver (N=2), adrenal (N=2), penile (N=2), and (N=1) for each of GI, muscle, bladder, bone, lymphoma, and testicular cancers.

Additional comorbid diagnoses were in limited number (n<50 each) to derive conclusions, including allergy/asthma, chronic obstructive pulmonary disorder (COPD), renal disease, hypogonadism, atrial fibrillation, and vitamin D deficiency, as well as individual cancer types, not shown here.

Altogether, the performance estimates for these comorbid conditions did not appear to impact the performance of the 4Kscore Test results.

f. Medications

From the total number of 937 subjects, concurrent medications taken within a week prior to testing with the 4Kscore Test were captured from patient health records to assess the impact of medication usage upon the clinical performance of the 4Kscore Test. From 28 listed medications, the 18 most frequent are represented in Table 31, in descending order of frequency:

Table 31: Performance of the 4Kscore Test stratified by medication usage

	4Kscore cut-points					
	5.0		10.0		20.0	
	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)
Lisinopril	97.0% (64/66) (89.6%; 99.2%)	22.9% (36/157) (17.0%; 30.1%)	92.4% (61/66) (83.5%; 96.7%)	37.6% (59/157) (30.4%; 45.4%)	81.8% (54/66) (70.9%; 89.3%)	63.7% (100/157) (55.9%; 70.8%)
Ciprofloxacin[1]	91.1% (41/45) (79.3%; 96.5%)	30.3% (47/155) (23.6%; 38.0%)	75.6% (34/45) (61.3%; 85.8%)	52.3% (81/155) (44.4%; 60.0%)	64.4% (29/45) (49.8%; 76.8%)	71.0% (110/155) (63.4%; 77.5%)
Acetylsalicylic acid	95.9% (47/49) (86.3%; 98.9%)	25.0% (30/120) (18.1%; 33.4%)	91.8% (45/49) (80.8%; 96.8%)	46.7% (56/120) (38.0%; 55.6%)	79.6% (39/49) (66.4%; 88.5%)	70.0% (84/120) (61.3%; 77.5%)
Atorvastatin	100% (50/50) (92.9%; 100%)	21.2% (25/118) (14.8%; 29.4%)	94.0% (47/50) (83.8%; 97.9%)	36.4% (43/118) (28.3%; 45.4%)	88.0% (44/50) (76.2%; 94.4%)	61.9% (73/118) (52.9%; 70.1%)
Amlodipine	100% (61/61) (94.1%; 100%)	25.7% (26/101) (18.2%; 35.0%)	93.4% (57/61) (84.3%; 97.4%)	42.6% (43/101) (33.4%; 52.3%)	80.3% (49/61) (68.7%; 88.4%)	64.4% (65/101) (54.6%; 73.0%)
Omeprazole	100% (40/40) (91.2%; 100%)	22.6% (21/93) (15.3%; 32.1%)	95.0% (38/40) (83.5%; 98.6%)	38.7% (36/93) (29.4%; 48.9%)	82.5% (33/40) (68.1%; 91.3%)	71.0% (66/93) (61.1%; 79.2%)
Hydrochloro-thiazide	97.7% (42/43) (87.9%; 99.6%)	22.5% (20/89) (15.0%; 32.2%)	93.0% (40/43) (81.4%; 97.6%)	47.2% (42/89) (37.2%; 57.5%)	72.1% (31/43) (57.3%; 83.3%)	71.9% (64/89) (61.8%; 80.2%)
Multivitamin	100% (32/32) (89.3%; 100%)	27.6% (27/98) (19.7%; 37.1%)	96.9% (31/32) (84.3%; 99.4%)	44.9% (44/98) (35.4%; 54.8%)	87.5% (28/32) (71.9%; 95.0%)	74.5% (73/98) (65.0%; 82.1%)
Aimvastatin	97.5% (39/40) (87.1%; 99.6%)	25.0% (22/88) (17.1%; 35.0%)	92.5% (37/40) (80.1%; 97.4%)	48.9% (43/88) (38.7%; 59.1%)	77.5% (31/40) (62.5%; 87.7%)	71.6% (63/88) (61.4%; 80.0%)
Tamsulosin	94.1% (16/17) (73.0%; 99.0%)	29.4% (30/102) (21.4%; 38.9%)	82.4% (14/17) (59.0%; 93.8%)	52.0% (53/102) (42.4%; 61.4%)	70.6% (12/17) (46.9%; 86.7%)	71.6% (73/102) (62.2%; 79.4%)
Metformin	92.6% (25/27) (76.6%; 97.9%)	20.9% (19/91) (13.8%; 30.3%)	92.6% (25/27) (76.6%; 97.9%)	49.5% (45/91) (39.4%; 59.5%)	77.8% (21/27) (59.2%; 89.4%)	69.2% (63/91) (59.1%; 77.8%)
Sildenafil	100% (45/45) (92.1%; 100%)	28.4% (19/67) (19.0%; 40.1%)	97.8% (44/45) (88.4%; 99.6%)	44.8% (30/67) (33.5%; 56.6%)	84.4% (38/45) (71.2%; 92.3%)	64.2% (43/67) (52.2%; 74.6%)
Metoprolol	97.4% (37/38) (86.5%; 99.5%)	27.7% (18/65) (18.3%; 39.6%)	89.5% (34/38) (75.9%; 95.8%)	50.8% (33/65) (38.9%; 62.5%)	73.7% (28/38) (58.0%; 85.0%)	70.8% (46/65) (58.8%; 80.4%)
Levofloxacin	95.7% (22/23) (79.0%; 99.2%)	26.8% (19/71) (17.9%; 38.1%)	91.3% (21/23) (73.2%; 97.6%)	46.5% (33/71) (35.4%; 58.0%)	73.9% (17/23) (53.5%; 87.5%)	80.3% (57/71) (69.6%; 87.9%)

Table 31: Performance of the 4Kscore Test stratified by medication usage

	4Kscore cut-points					
	5.0		10.0		20.0	
	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)	Sensitivity (n/N) (95% CI)	Specificity (n/N) (95% CI)
Vitamin D	100% (30/30) (88.7%; 100%)	22.4% (13/58) (13.6%; 34.7%)	93.3% (28/30) (78.7%; 98.2%)	39.7% (23/58) (28.1%; 52.5%)	80.0% (24/30) (62.7%; 90.5%)	55.2% (32/58) (42.5%; 67.3%)
Pravastatin	94.1% (16/17) (73.0%; 99.0%)	34.0% (16/47) (22.2%; 48.3%)	94.1% (16/17) (73.0%; 99.0%)	55.3% (26/47) (41.2%; 68.6%)	82.4% (14/17) (59.0%; 93.8%)	83.0% (39/47) (69.9%; 91.1%)
Acetaminophen	100% (22/22) (85.1%; 100%)	19.4% (7/36) (9.8%; 35.0%)	100% (22/22) (85.1%; 100%)	50.0% (18/36) (34.5%; 65.5%)	72.7% (16/22) (51.8%; 86.9%)	80.6% (29/36) (65.0%; 90.2%)
Allopurinol	100% (20/20) (83.9%; 100%)	12.1% (4/33) (4.8%; 27.3%)	100% (20/20) (83.9%; 100%)	39.4% (13/33) (24.7%; 56.3%)	65.0% (13/20) (43.3%; 81.9%)	66.7% (22/33) (49.6%; 80.3%)

[1] See discussion of ciprofloxacin usage below

Additional medications were in limited number (N < 50 each) to derive conclusions, including fish oil, ibuprofen, losartan, levothyroxine, tadalafil, sulfametoxazole, trimethoprim, doxazosin, silodosin, and biotin, not shown here.

Altogether, the performance estimates for usage of these medications, with the possible exception of ciprofloxacin (see below), did not appear to impact the performance of 4Kscore Test results.

In this clinical performance study, patients recorded as receiving ciprofloxacin (N=200) collecting samples for 4Kscore testing at or around the time of TRUS-guided biopsy, for which prophylactic antibiotics are indicated appeared to be different from other patients.**Error! Reference source not found.** The interpretation in Table 32 below present likelihood (probability) of Gleason Score ≥ 7 findings from biopsy, split by patients reported as receiving ciprofloxacin versus those who did not:

Table 32: Likelihood of Gleason Score 7 or higher, by 4Kscore values, stratified by ciprofloxacin use

		N		Likelihood of Gleason Score ≥ 7	
	4Kscore	Total	Gleason Score ≥ 7	Estimate	(95% CI)
cipro(+)	< 5.0	51	4	7.8%	(3.1%; 18.5%)
	5.0 – < 10.0	41	7	17.1%	(8.5%; 31.3%)
	10.0 – < 20.0	34	5	14.7%	(6.4%; 30.1%)
	≥ 20.0	74	29	39.2%	(28.9%; 50.6%)
	Total:	200	45	22.5%	

Table 32: Likelihood of Gleason Score 7 or higher, by 4Kscore values, stratified by ciprofloxacin use

		N		Likelihood of Gleason Score ≥ 7	
	4Kscore	Total	Gleason Score ≥ 7	Estimate	(95% CI)
cipro(−)	< 5.0	143	4	2.8%	(1.1%; 7.0%)
	5.0 – < 10.0	105	7	6.7%	(3.3%; 13.1%)
	10.0 – < 20.0	164	34	20.7%	(15.2%; 27.6%)
	≥ 20.0	325	168	51.7%	(46.3%; 57.1%)
	Total:	737	213	28.9	

Because of the uncertainty contained in these performance estimates, in addition to the finding that analytical interference was not detected (see §IX.F above), and an unclear biologic or clinical mechanism, the increased false negative risk at low-to-intermediate 4Kscore numerical values was included in the limitation section of the instructions for use.

Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 35 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

REFERENCE INTERVAL STUDY

A. Study Design

To establish the reference interval (i.e., "reference range") for the four individual analytes (tPSA, fPSA, iPSA, hK2) as well as 4Kscore numerical values, a cohort of 547 "apparently healthy men", originally assembled for the approval of P170037 and further supplemented here, were tested.

1. Inclusion and Exclusion Criteria

Enrollment in the reference interval study was limited to donor subjects who met the following criteria:

- Men age ≥ 45 years old
- "Apparently healthy", all race/ethnicities
- No known history of prostate disease
- Informed consent

- Within 30 days of physician visit for non-urological or prostate-related purposes
- Blood draws for standing physician orders (e.g., glucose, PT/INR, other non-urological conditions)
- All medications not listed among the exclusion criteria

Subjects were not permitted to enroll in the reference interval study if they met any of the following exclusion criteria:

- Men age < 45 years old
- Men age ≥ 81 years old
- Any known history of prostate disease including:
  - Prostate cancer (PCa)
  - Benign prostatic hyperplasia (BPH)
  - Prior negative prostate biopsy
  - Prostatitis
- Prostate-related medications, including:
  - 5-alpha reductase inhibitors (5ARI) (e.g., dutasteride, finasteride)
  - Any BPH-related alpha-blocker (e.g., tamsulosin, silodosin)
- Total PSA findings classified as "abnormal" or "elevated", by age:
  - 45–54 years old: total PSA ≥ 2 ng/mL
  - 55–75 years old: total PSA ≥ 3 ng/mL
  - ≥ 76 years old: total PSA ≥ 4 ng/mL

2. Follow-up Schedule

   Not applicable

3. Endpoints

   The purpose of this reference interval study was to establish the reference intervals for the 4Kscore numerical value, as well as its constituent analytes in an apparently healthy population of men, 45 years and older.

B. Accountability of Study Population

This study was conducted by enrolling a total of 547 subjects from 14 collection sites across the U.S. These subjects aged ≥ 50 years old provided samples used in the reference interval study for P170037. In addition, samples from men between 45–50 years of age, N=46 from two sites, were included. From these 547 subjects, 136 were excluded for not conforming to the above criteria. Subtracting these excluded subjects resulted in a final, evaluable reference interval population cohort of 411 subjects.

C. Study Population Demographics and Baseline Parameters

The median age of 411 study subjects was 61.4 years. The demographics and baseline characteristics of the reference population subjects are shown in Table 33:

Table 33: Distribution of demographic variables in reference interval study			
		Subgroup	
	Demographic Variable	N	% Total
Race, Ethnicity	White, Caucasian	187	45.5%
	Hispanic or Latino	131	31.9%
	African American	30	7.3%
	Asian or Asian American	24	5.8%
	American Indian or Alaska Native	4	1.0%
	Other	21	5.1%
	Unknown	14	3.4%
Age (years)	45–54	113	27.5%
	55–64	160	38.9%
	65–80	138	33.6%
	Total:	411	

D. Study Results

The 411 evaluable subjects were stratified into three age-groups. The distributions of 4Kscore values and the constituent analytes from reference population subjects, stratified by age, are shown in Table 34:

Table 34: Descriptive statistics for reference interval population, stratified by Age					
	Age (years)	N	95th percentile	Median	Mean
4Kscore	45–54	113	4.1	2.4	2.4
	55–64	160	9.4	4.2	5.3
	65–80	138	15.6	7.5	8.7
tPSA	45–54	113	1.74	0.66	0.79
	55–64	160	2.35	0.85	1.04
	65–80	138	2.59	1.06	1.21
fPSA	45–54	113	0.50	0.21	0.23
	55–64	160	0.58	0.24	0.27
	65–80	138	0.80	0.30	0.34
iPSA	45–54	113	0.342	0.137	0.150
	55–64	160	0.421	0.148	0.191
	65–80	138	0.555	0.219	0.233
hK2	45–54	113	0.077	0.028	0.032
	55–64	160	0.085	0.033	0.040
	65–80	138	0.124	0.044	0.053

Individual analyte values are represented as units of ng/mL.

The percent of subjects from the reference interval study with 4Kscore values above the cut-points of 5.0, 10.0, and 20.0 were calculated along with 95% confidence intervals, and reported in Table 35:

Table 35: Percent of reference interval study subjects with 4Kscore values above cut-points of 5.0, 10.0, and 20.0 stratified by Age

Age (years)	N	4Kscore ≥ 5.0		4Kscore ≥ 10.0		4Kscore ≥ 20.0	
		Percent (n/N)	(95% CI)	Percent (n/N)	(95% CI)	Percent (n/N)	(95% CI)
45–54	113	1.8% (2/113)	(0.5%; 6.2%)	0.0% (0/113)	(0.0%; 3.3%)	0.0% (0/113)	(0.0%; 3.3%)
55–64	160	28.8% (46/160)	(22.3%; 36.2%)	5.0% (8/160)	(2.6%; 9.6%)	1.3% (2/160)	(0.3%; 4.4%)
65–80	138	83.3% (115/138)	(76.2%; 88.6%)	30.4% (42/138)	(23.4%; 38.6%)	0.7% (1/138)	(0.1%; 4.0%)
Total:	411	39.7% (163/411)	(35.0%; 44.5%)	12.2% (50/411)	(9.4%; 15.7%)	0.7% (3/411)	(0.2%; 2.1%)

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Analytical studies demonstrate acceptable analytical performance of iPSA and hK2 assays from 0.035–6.0 ng/mL and 0.012–2.5 ng/mL, respectively.

The effectiveness of the 4Kscore Test which combines the results of four immunoassays (Roche Elecsys total PSA, Roche Elecsys free PSA, iPSA, and hK2), a patient's age, previous biopsy, and digital rectal exam (DRE) result has been demonstrated for use with other patient information as an aid in the decision for prostate biopsy in men 45 years of age and older who have an abnormal age-specific total PSA and/or abnormal DRE, and an aid in detection of aggressive prostate cancer (Gleason Score ≥ 7/Gleason Grade Group ≥ 2) for whom a biopsy would be recommended by a urologist, based on current standards of care before consideration of the 4Kscore Test.

B. Safety Conclusions

As an *in vitro* diagnostic (IVD) test, the 4Kscore Test requires routine collection of venous blood for testing, to be performed by trained healthcare professionals in healthcare settings. Sample collection presents no more safety hazard than other blood-derived IVD sample collection by venipuncture. The risk from complications from biopsy (e.g., infection, fever, rectal bleeding, hematuria and hematospermia (blood in the urine

and semen), urinary retention, and hospitalization in some cases) if the 4Kscore indicated biopsy.

The results of the analytical performance, clinical performance, and reference interval studies demonstrated the acceptable safety of the 4Kscore Test when used in accordance with its instructions for use, warnings and precautions, and limitations specified in the product labeling, together with the urologist's assessment of test result, other risk factors, and professional guidelines.

C. Benefit-Risk Determination

The Clinical Study only included men who were recommended by urologists for biopsy. Therefore, the performance of the 4Kscore Test has not been established in men for whom a biopsy was not already recommended.

1. Summary of Benefits

   The goal of prostate biopsy in men of the age group specified in the IU is to identify potentially aggressive prostate cancers and to avoid biopsy in men with prostate cancers that are expected to be indolent and would likely not significantly affect longevity and quality of life. The decision to perform a prostate biopsy is complex and depends on multiple factors including patient age, family history, results of genetic tests, PSA level, results of digital rectal examination, results of other laboratory tests, and importantly, patient preferences. These factors are taken into account in the discussion between the urologist and patient in which the likelihood of various outcomes is estimated and accordingly the risks and benefits of doing the biopsy are openly discussed.

   The benefits of performance of a prostate biopsy in the specified intended use population include identification of an aggressive prostate cancer for which the patient would benefit from immediate treatment (i.e., for which the expected benefit of treatment is increased longevity and/or improvement of quality of life). The benefits of not performing a prostate biopsy include not identifying a cancer that is expected to be indolent, and therefore not subjecting the patient to a diagnosis of cancer, as well as surgery, radiation and/or other therapies that would have significant morbidity without providing a concomitant benefit with respect to longevity.

   Demonstration of the 4Kscore Test performance in the three age groups (based on tPSA cut-points) and the two sets of subjects from the two prospective clinical validation studies was provided in this submission. A total of 937 subjects, of which 574 subjects with K_2EDTA plasma samples and 363 subjects with serum samples, qualified for the clinical performance analyses. A single cut-point was not intended to be used, but rather a numeric 4Kscore value is provided. The probability (likelihood) of Gleason Score ≥ 7 biopsy findings are provided in four intervals of 4Kscore numeric values.

   Demographics of each of two studies (Study 1 and Study 2) are similar, except the proportion of African American, which are 8.9% and 56% in the Study 1 (US study) and Study 2 (VA study), respectively. Consistent with published data, comparison of the outcome of these two studies showed that African Americans are more likely to

present high abnormal PSA at younger ages and more advanced prostate cancer upon first detection.

The clinical study, based on a total of 937 patients, each of which had a 4Kscore and biopsy findings, demonstrated that the clinical performance characteristics of the 4Kscore Test at the three relevant cut-points, with results reflecting the proportion of patients with prostate cancer of Gleason Score ≥ 7, are summarized above in Table 19.

Using the 5.0 cut-point as cited in the Intended Use statement, the 96.9% sensitivity will provide significant benefit in the identification of aggressive prostate cancers. The specificity, PPV and NPV at this cut-point are in the appropriate range to contribute to an overall beneficial clinical decision as to whether a prostate biopsy should be performed and accordingly minimize unnecessary biopsies without excessive risk.

With the receiver-operating characteristic curves of the entire clinical performance study IU population the AUCs for the total PSA alone and 4Kscore were calculated and the 4Kscore is better than total PSA alone for detecting prostate cancer of Gleason Score ≥ 7. Data based on analyses of all three cut-points support the intended use. The sensitivity and NPV of the 4Kscore Test at 5.0 and 10.0 are above 90% with no significant difference. At the higher cut-point of 20.0, the sensitivity is lower (76.4%); however, this is mitigated by the higher PPV and the high likelihood of performing a prostate biopsy when the probability of a Gleason Score ≥ 7 cancer is in the high range indicated by this high score.

With respect to race differences, the data suggest a different level of likelihood of prostate cancer of Gleason Score ≥ 7 for African American men as compared to non-African American men. The 95% CIs of these likelihoods overlap in the 4Kscore < 5.0, 5.0 – < 10.0, and 10.0 – < 20.0 groups, and only reach statistical significance for the ≥ 20.0 group. The true negative to false negative ratio at the 5.0 cut-point further supports the value of the test in minimizing unnecessary biopsies. For non-African American men, this ratio was 9.3, and for African American men, 10.3. This information will be provided in the labeling to assist in the interpretation of the 4Kscore results in light of the race of the patient.

In summary, the results provided by the 4Kscore Test in the IU population, when used in conjunction with other clinical factors and patient preferences, can contribute to a properly informed decision as to whether or not to proceed with a prostate biopsy, with an expected outcome that is probabilistically more favorable for the individual patient.

2. Summary of Risks

Conversely to the above discussion of benefits, the risks of performance of a prostate biopsy in the specified intended use population include identification of an indolent prostate cancer for which the patient would not likely benefit from immediate treatment (i.e., for which treatment is not expected to increased longevity and/or improve quality of life). This risk is mitigated by the current practice of active surveillance of such expected indolent tumors. The risks of not performing a prostate

biopsy include failure to identify a cancer that is expected to be aggressive, and therefore not treating the patient with surgery, radiation, and/or other therapies that could have provided a benefit with respect to longevity and/or quality of life.

Based on the 4Kscore Test performance in the two pivotal clinical trials discussed above, the test provides probabilistic information that can inform the decision as to whether to perform a biopsy. As this information provides only probabilities, it is imperfect and there is a risk that this information could mislead the patient and physician to make a decision that ultimately does not have a favorable risk/benefit balance for the particular patient. This is mitigated by the standard practice of not using this information on its own, but rather, in conjunction with the other clinical information which include patient age, family history, results of genetic tests, PSA level, results of digital rectal examination, results of other laboratory tests, and importantly, patient preferences.

3. Benefit-Risk Conclusion

The data provided demonstrate that, in the context of this population, there is reasonable assurance that the use of the 4Kscore Test in the specified intended use population is safe and effective. More specifically, there is reasonable assurance that the use of this device, shown by the provision of probabilities of the occurrence of aggressive prostate cancer (i.e., Gleason Score $\geq$ 7) in the population of men in the specified age groups with elevated age-specific total PSA levels and/or abnormal DRE, can provide important information with acceptable uncertainty about the risk of such prostate cancer and accordingly assist in the clinical assessment of whether performance of a prostate biopsy is in the best interests of the patient considering the benefits and risks of such a biopsy. This is provided that the test is not used alone but rather is used in conjunction with other available clinical information as per the standard of care for such patients who are considered at risk of prostate cancer.

In conclusion, given the available information above, the 4Kscore Test is safe and effective for the indications contained in the Intended Use Statement. The probable benefits outweigh the probable risks.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from nonclinical and clinical studies support the use of the 4Kscore Test as an aid in the decision for prostate biopsy in men 45 years of age and older who have an abnormal age-specific total PSA and/or abnormal DRE, and as an aid in detection of aggressive prostate cancer (Gleason score $\geq$ 7/Gleason Grade Group $\geq$ 2) for whom a biopsy would be recommended by a urologist, based on current standards of care before consideration of the 4Kscore Test.

XIII. CDRH DECISION

CDRH issued an approval order on December 7, 2021.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.