Explanation and Interpretation of *OneTest*™

Basis of this Test

***OneTest*™ is a blood test and machine learning algorithm that was developed to aid in the detection of multiple cancers *before* symptoms occur when treatments are usually more effective.** It is based on a cancer screening paradigm that has been very common in the Far East (Japan, Korea, China) for nearly two decades. In that region, tens of millions of individuals receive what are often day-long check-ups that include blood tests that measure the levels of the same biomarkers that are part of *OneTest*™**.**

The machine learning algorithm was co-developed by 20/20 GeneSystems, Inc. (www.2020gene.com) together with Chang Gung Memorial Hospital, the largest and most reputable medical center in Taiwan, which enjoys a level of healthcare comparable to that in the U.S. Biomarker and clinical data from over 28,000 individuals tested at Chang Gung over a 15-year period, including whether they were diagnosed with cancer within one year of their test, were the foundation of the machine learning algorithm. A key element of the algorithm is the inclusion of the individual's personal health information (i.e. clinical factors) which has been demonstrated to be superior to just measuring biomarker levels.

Beginning in mid-2018, data from an American population who use *OneTest*™ will be integrated into the algorithm for continuous improvement and refinement. Until a statistically significant quantity of data from a U.S. population (e.g. data from more than 7,500 individuals) is integrated the algorithm, scores should be deemed exploratory not definitive.

The results of this test should be used by physicians in conjunction with other tests and clinical information and should not be the only tool leading to a diagnosis of cancer. Various non-malignant conditions may also cause abnormal tumor marker values leading to high biomarker values and/or an increased *OneTest*™ risk score. Please contact your healthcare professional for a clinical interpretation of *OneTest*™ results and recommendations for next steps for additional testing. The results of *OneTest*™ are adjunctive to the ordering physician's workup.

Regulatory Status

OneTest™ is a Laboratory Developed Test (LDT) that is not currently regulated by the U.S. Food & Drug Administration (FDA) but is instead regulated by the Center for Medicare & Medicaid Services (CMS) and the Maryland Department of Health under the Clinical Laboratory Improvement Amendments (CLIA). In general, CLIA approval is directed at the technical performance and analytical validity of the test (i.e. whether the test delivers consistent results) rather than the impact of the test on disease outcomes.

The laboratory equipment and kits used to analyze the biomarkers in *OneTest*™ are approved by the FDA for certain uses. Except for PSA, which is approved by the FDA for use in screening for prostate cancer, most of the other biomarker test kits used here are approved by the FDA generally for assessments of those suspected of having cancer or detecting recurrence of cancer among those previously diagnosed. However, several foreign counterparts to the FDA have approved these biomarker test kits for use in screening and early detection resulting in these tests being employed in those countries for the yearly screening of tens of millions of individuals.

It is presently the intent of the test developer, 20/20 GeneSystems, Inc., to seek FDA approval after the real-world outcome data (i.e. the numbers of true cancers detected early with the aid of this test vs. false alarms) from a

statistically significant number of Americans can be obtained. We therefore seek the assistance of the consumers of this test and their healthcare providers to assist us in collecting reliable outcome data. Until then, the results of the algorithms, which were derived mainly from an overseas population, should be deemed for evaluation purposes only.

Accuracy & Performance

The accuracy of tests of this nature is generally characterized by two separate metrics:
"Sensitivity" (the percentage of true cancers properly classified) and,
"Specificity" (the percentage of true non-cancers properly classified).
Thus, the higher the test sensitivity, the few cancers missed. The higher the test specificity, the fewer false positive results.

Accuracy of Biomarkers Alone

There are hundreds of reports in the scientific and medical literature assessing the performance of the biomarkers in *OneTest™* for various cancers, alone and in combination. (See www.OneTestforCancer.com for representative citations).

In what is perhaps the most comprehensive study of these biomarkers from a real-world screening setting (i.e. testing of individuals prior to diagnosis of cancer) involving over 41,516 study participants over 15 years (published in 2015*) concluded that using the panel of biomarkers in *OneTest™* (based on any one marker in the panel above its threshold; no algorithm) at a specificity of 88.7% the sensitivities of detecting liver cancer, lung cancer, prostate cancer, and colorectal cancer was 90.9%, 75.0%, 100% and 76.9% respectively. By biomarker the following sensitivities were found (at 88.7% specificity):

Table 1*. Sensitivities of the individual tumor markers for each malignancy.

Type of Malignancy	PSA	AFP	CEA	CA19-9	CYFRA 21-1	CA 125	SCC	CA15-3	Panel
Prostate Cancer	100	0	0	4.8	5.9	-	5.6	-	100
Hepatocellular carcinoma	13.3	63.3	5.6	31.6	10	0	0	0	92.3
Pancreatic cancer	0	0	55.6	62.5	33.3	66.7	0	0	88.9
Colorectal cancer	7.1	5.9	53.8	25	38.9	22.2	5.9	12.5	76.9
Lung cancer	9.1	5.7	72.2	12.9	40.9	20.0	8.7	20.0	75.0
Bladder cancer	25	0	33.3	69.2	57.1	50.0	60.0	0	64.3
Cervical cancer	-	7.1	20.8	5	11.1	30.4	20.8	0	44.4
Gastric cancer	0	6.3	25	6.7	41.7	0	9.1	0	38.9
Breast cancer	-	5.4	8.1	9.7	11.1	20.5	3.1	5.4	37.5
Ovarian cancer	-	0	0	50	0	0	0	0	33.3
Oral cancer [a]	0	0	0	0	0	0	0	0	0

Data are given as percentages unless otherwise indicated.

Abbreviations: PSA, prostate specific antigen; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; CA, cancer antigen; CYFRA, cytokeratin fragment; SCC, squamous cell-specific antigen

[a] Oral cancer included malignancies arising in the tongue, oral cavity and oropharynx

*Source: Wen YH, Chang PY, Hsu CM, Wang HY, Chiu CT, Lu JJ. (2015) Cancer screening through a multi-analyte serum biomarker panel during health check-up examinations: Results from a 12-year experience. Clinica chimica acta; international journal of clinical chemistry. 2015; 450:273–6.

As pointed out elsewhere in this document, the comparison of sequential testing with single time point measurements is the best way to maximize the value of these biomarkers. That is, the change in levels of the various biomarkers over time is expected to give the most valuable information for predicting the appearance and progression of cancer.

Accuracy of Algorithms

Together with our Taiwanese collaborators, we have generated compelling evidence that machine learning algorithms that integrate clinical factors (e.g. age, gender, etc.) with biomarker levels substantially improve accuracy over biomarker levels alone.

- The performance for all cancers (including those like skin cancer for which good biomarkers are lacking) using only biomarker measurements yielded a sensitivity of 57% at a specificity of 89%. The algorithms in *OneTest* improved those results to **82% sensitivity and 80% specificity in men** (excluding Stage 4).
- Based on an independent study from individuals in a second East Asian country, for men with any of the following cancer types (excluding stage 4): Liver, Lung, Colorectal, or Prostate, the test had a **sensitivity of about 88% at a specificity of about 80%**. For women with Lung, Liver, or Colorectal Cancer (excluding stage 4) the **sensitivity was about 80% at a specificity of about 80%**. (The test is substantially less accurate for breast cancer detection.)

Cautionary Note: These algorithms were built using data from a Taiwanese population. Until sufficient data generated from the US population is obtained, their accuracy for the US population cannot be predicted.

The machine learning algorithms are expected to substantially improve accuracy over time as additional data (including many clinical factors) from both American and Asian populations is incorporated, especially from individuals who take this test on a yearly basis.

Summary of Strengths & Limitations of Test

Strengths

- Data was generated from a real-world screening center with cancer patients tested up to one year before diagnosis.
- The population studied for the development of *OneTest™* was a generally healthy population who visited a health screening center. This real-world population may offer better accuracy than "case controlled" studies that look at cohorts after they were already diagnosed with cancer.
- Large data sets (over 40,000) were the basis of *OneTest™*.
- The Biomarker test kits and analyzers employed by *OneTest™* are used routinely in thousands of clinical labs worldwide to screen millions of individuals each year
- The *OneTest™* algorithm uses machine learning, a form of artificial intelligence (AI), in which a computer system has the ability to continually process and incorporate new data and thus improve accuracy over time.
- Because the algorithm used to combine and interpret patient biomarker levels with relevant clinical factor data is derived from machine learning, this algorithm is amenable to periodic review and redefinition.

- While the current algorithm is fixed on the basis of rigorous studies performed to date, 20/20 GeneSystems, Inc. is committed to the performance of regular review of the algorithm as the existing patient dataset grows.
- By providing us with outcome data from patient follow-up subsequent to the *OneTest™*, real-world experiences can inform further development and fine-tuning of the *OneTest™* algorithm and continuously improve the accuracy of the test.
- Our database will grow in volume and geographic /ethnic diversity as the test is used throughout the U.S. and elsewhere as reliable outcome data is collected. Thus, our algorithms are expected to continuously learn and improve.

Limitations

- **This is NOT a definitive test for cancer. The only definitive test for any cancer is analysis of biopsied tissue by a qualified pathologist.**

- *OneTest™* is intended as an initial screen to indicate the potential need for further follow-up testing to confirm the presence of cancer. It is possible to have a high probability of cancer on the *OneTest™* but not have cancer. Low *OneTest™* score result should not be used to rule out the presence of cancer.
- Algorithms derived from data from East Asia may not exactly translate into an American population.
- A high score (false positive) for an individual tumor marker may result from benign conditions which are not cancer. See chart below.
- False negative results when a person receives a low score but may have cancer.
- Accuracy in women is currently not as high as for men, probably due to (at least in part) failure to control for menstrual periods which contribute to elevation of one or more biomarkers in *OneTest™*.

Interpretation of Part I—Biomarker Values

Part I of this report simply provides the raw biomarker levels and the test kit manufacturer's cut-offs. It is comparable to information provided to tens of millions of individuals worldwide. The accuracy of this approach by cancer type is set forth in the Accuracy & Performance section (table above).

Association between Biomarkers and Disease (Cancer & Benign Conditions)

The biomarkers in *OneTest™* are each associated with one or more cancers and several benign conditions which can result in high tumor marker level when cancer is not actually present.

This table summarizes information gleaned from publications authored by reputable biomarker experts worldwide. See www.OneTestforCancer.com for citations.

Biomarker	Normal Range	Benign Diseases (i.e. potential sources of false positives)	Cancers

PSA	< 4ng/ml	Prostatitis; Benign Prostatic Hyperplasia (BPH); Ejaculation within the previous 48 hours; (Older men are more likely to have false positives than younger men)	Prostate cancer
AFP	< 8.30 ng/ml	Cirrhosis of the liver; Hepatitis and other forms of liver disease; Inflammatory bowel disease; Hereditary tyrosinosis; Pregnancy; Ataxia Telangiectasia; Hepatobiliary disorders	Liver cancer, testicular and ovarian cancers
CA 19-9	< 35 U/ml	Endometriosis; Gallstones; Acute cholangitis; Jaundice; Biliary disease; Ulcerative colitis; Inflammatory Bowel Disease; Cirrhosis of the liver; Renal failure (<400 U/ml); Cholecystitis; Pancreatitis (<1,000 U/ml); Mucinous cysts or bronchiectasis (<500 U/ml)	Pancreatic, liver, and gastrointestinal cancers
CEA	< 4.7 ng/mL (nonsmokers) <5.6 ng/ml (smokers)	Inflammatory Bowel Disease; Pancreatitis; Liver disease; Diverticulitis; Hepatitis; Peptic ulcers; Crohn's disease; Hypothyroidism; Cirrhosis of the liver; COPD; Lung infection; Pleural effusions; Biliary obstruction	Lung, pancreatic, and gastrointestinal cancers
CYFRA 21-1	< 2.37 ng/ml	Systemic skin disorders (pemphigus, psoriasis); Liver disease (<15ng/ml); Cirrhosis of the liver; Renal failure (<20ng/ml)	Lung, head and neck cancer, breast, ovarian and bladder cancers
CA 125	< 38.10 U/ml	Ovulatory peak; Menstruation; Pulmonary infections; COPD (<100U/ml); Nephrotic syndrome; Gynecological disorders: cysts, myomas, Liver disease; Renal failure (<300 U/ml); Pregnancy (amniotic fluid); Fluid retention: serous effusion (<1000U/ml)	Ovarian and lung cancers
CA15-3	< 25 U/ml	Liver diseases (<100U/ml); Benign breast disorders; Renal failure; Megaloblastic anemia	Breast cancer

Source: R. Molina, 2013, *Clinical Value of Tumor Markers*, Roche Diagnostics

Benefits of Repeat Testing and Biomarker "Trends"

Physicians in the clinics and health check centers that routinely test these biomarkers tend to repeat tests of biomarkers that are above the cut-off or otherwise ambiguous as part of the overall patient examination. One of the world's most widely recognized experts in tumor markers, Rafael Molina, M.D. who is the longstanding Chairman of both the International Society of Oncology and Biomarkers and the European Group on Tumor Markers offers the following "rule of thumb" for sequential testing:

> "An isolated finding of high levels of any tumor marker is of limited value. When there are doubts regarding a result, two or three sequential measurements should be carried out at intervals of more than its plasma half-life (15-20 days for the majority of tumor markers). If the tumor marker values show a continuous increase over the period (above the normal level), it can be concluded with a high level of probability that it is of malignant origin, as it reflects the growth of tumor. Conversely, if the serum level does not change or show a downward trend, the origin should be sought in another non-malignant condition." (R. Molina, 2013, *Clinical Value of Tumor Markers*, Roche Diagnostics)

Repeat testing of high biomarkers may be covered by some insurance plans depending on the manner in which it is ordered by the physician.

Interpretation of Part II—*OneTest* Multi-Tumor Score (1st algorithm)

As explained in the tables above, some of the biomarkers tested here are associated with more than one tumor type. We have therefore developed a software program that assesses the biomarkers collectively while integrating them with various clinical factors (e.g. age, gender, etc.) to provide an overall cancer risk score. The algorithm does not employ the pre-determined manufacturer's cutoff for any given marker but rather assesses each biomarker value in the context of a given patient's demographic profile and values of the other tumor biomarkers. As such, it is possible to have a high score even when all tumor marker values fall below the standard manufacturer's cutoff values.

While the *OneTest*™ score generally correlates with a cancer likelihood index, **it is important to note that most individuals with even a High OneTest score will not have cancer.** For these reasons, we translate the score into the number of people out of 100 found to have cancer. The goal is to permit each customer to assess the risk and benefits of follow-up testing such as repeat biomarkers or non-invasive imaging.

What should follow OneTest?

We are not positioned to recommend specific follow-up testing based on individual test results. This task is reserved for qualified medical professionals selected by the consumer.

We will provide on our website www.OneTestforCancer.com published information from trusted sources about using biomarkers in connection with various cancers. This is expected to also include a clinical case study section where American clinicians can query overseas experts based on the results of *OneTest*™ for their patients (anonymized).

In general, these tests tend to be followed up with either repeat tests of those biomarkers that are high or borderline and then imaging test such as ultrasound, colonoscopy, CT, PET, and bone scans, etc.

At a minimum, yearly testing is highly recommended based on studies with ovarian, pancreatic, and colon cancers.