Quality approved by SD BIOSENSOR / For *in vitro* use only

STANDARD™ G6PD Test

REF 02GS10

PLEASE READ THE INSTRUCTIONS CAREFULLY BEFORE YOU PERFORM THE TEST

EXPLANATION AND SUMMARY

[Introduction]

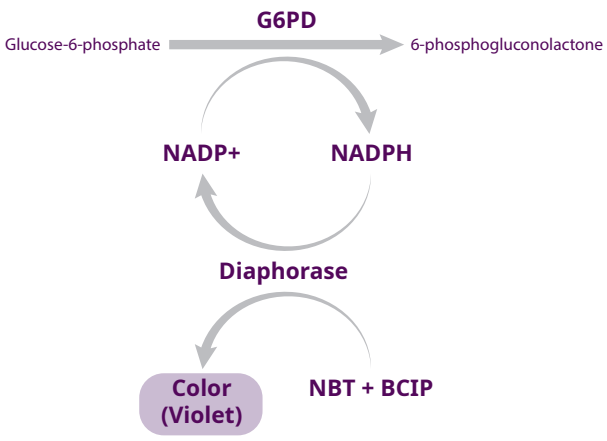
G6PD (glucose-6-phosphate dehydrogenase) deficiency is one of the most common enzymopathological diseases, described as a widespread, heritable, X-chromosome linked abnormality. It is estimated that it affects approximately 400 million people worldwide and the prevalence can be as high as 25 percent in ethnic populations originating from Africa, the Middle East, Asia, and the Mediterranean.[1,2] The G6PD enzyme plays an important role in survival of erythrocytes. The G6PD enzyme is involved in the PPP (pentose phosphate pathway) and provides the NADPH (reduced nicotinamide adenine dinucleotide phosphate) and GSH (reduced glutathione). GSH produced by PPP can react with H202 and reduce H_20_2 to H_20. This helps protect erythrocytes from certain generation of oxidative stress.[3,4] The most common medical problem associated with G6PD deficiency is hemolytic anemia, which can lead to paleness, yellowing of the skin and whites of the eyes, dark urine, fatigue, shortness of breath, and a rapid heart rate. The STANDARD G6PD Test is a fast, simple and reliable test system that provides results in 2 minutes and point-of-care diagnosis of G6PD deficiency.

[Intended use]

The STANDARD G6PD System is an enzymatic colorimetric assay intended for the semi-quantitative measurement of G6PD activity and total hemoglobin (T-Hb) concentration in non-anticoagulated capillary (finger-stick) whole blood or venous whole blood (K2-EDTA, sodium heparin, or acid citrate dextrose [ACD]). The STANDARD G6PD Test System is indicated for differentiating normal, intermediate, and deficient G6PD activity levels to aid in the detection of G6PD deficiency in individuals and in the determination of hemoglobin level. The test will provide results expressed as the ratio of units of G6PD activity per gram of hemoglobin (G6PD U/g Hb) which can be used to determine G6PD status. Samples which generate a G6PD deficient or intermediate result should be assayed using a quantitative G6PD test to verify a deficiency. T-Hb results expressed in grams per deciliter (g/dL) are used to determine G6PD status and not state of anemia. T-Hb results should be confirmed by a quantitative test when monitoring disease progression or making treatment decisions. The STANDARD G6PD Test System is intended for use by health care professionals in a laboratory or point-of-care environment. The system is for in vitro diagnostic use only. The STANDARD G6PD Test has not been validated for use with neonatal samples. The STANDARD G6PD Analyzer must be used exclusively with the STANDARD G6PD Test Device, STANDARD G6PD Control, and STANDARD G6PD Check strip manufactured by SD BIOSENSOR.

[Test principle]

The STANDARD G6PD Test contains a flow through STANDARD G6PD Test Device with treated membrane and mesh. The test is based on a colorimetric detection system for the automatic calculation of G6PD activity on the codechip for each test device. G6PD catalyzes the first step in the PPP, oxidizing G6P (glucose-6-phosphate) to 6-phosphogluconolactone and reducing NADP (nicotinamide adenine dinucleotide phosphate) to NADPH. When NADPH is generated by G6PD, the BCIP (5-bromo-4-chloro-3-indolyl-phosphate) and NBT (nitro blue tetrazolium) are reduced by the diaphorase reaction to yield a violet color. The rate of the color production is directly proportional to the concentration of G6PD present in the specimen (see schematic below). The color intensity can be measured through reflectance photometry of the reduced BCIP and NBT. The T-Hb concentration is also measured by reflectance on a separate location on the test device. The STANDARD G6PD Analyzer's screen displays the G6PD enzyme activity in U/g Hb and the concentration of T-Hb in g/dL. The Analyzer has a temperature monitor and a pre-programmed algorithm corrects the values for temperature as long as the test was run within the operating temperature range of the test (15°C to 40°C / 59°F to 104°F).



MATERIALS PROVIDED

Materials	Ea/kit
STANDARD G6PD Test Device	25 ea
Extraction buffer	25 ea
STANDARD Ezi tube+(10µl)	50 ea
Instructions for Use	1 ea
Codechip	1 ea

MATERIALS REQUIRED BUT NOT PROVIDED

1. STANDARD G6PD Analyzer
2. STANDARD G6PD Control (Level 1: 1 x 10ea, Level 2: 1 x 10ea)
3. Standard blood drawing equipment or lancet, lancing device, alcohol swab

KIT STORAGE AND STABILITY

1. The sealed pouch containing the test device may be stored at 2°C to 30°C / 36°F to 86°F out of direct sunlight for the duration of its shelf life.
2. The test device must remain in the sealed pouch until use and should be used immediately after removal from the pouch.
3. DO NOT FREEZE, but test device may be stored in a refrigerator at 2°C to 8°C / 36°F to 46°F.
4. Do not use beyond the expiration date.
5. Keep the codechip either in the analyzer or the test device package.
6. Allow the test device to be at room temperature at least 1 hour before starting the test.

SPECIMEN COLLECTION AND PREPARATION

[Whole blood]

Capillary whole blood

1. Capillary whole blood should be collected aseptically by fingertip.
2. Clean the area to be lanced with an alcohol swab.
3. Squeeze the end of the fingertip and pierce with a sterile lancet.
4. Collect the capillary whole blood; it must be tested immediately after collection.

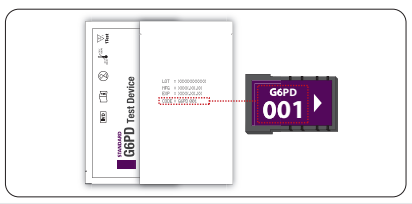
Venous whole blood

1. Collect the venous whole blood into a commercially available anticoagulant tube, such as K2-EDTA, sodium heparin, or ACD by venipuncture.
2. The venous blood treated by K2-EDTA, sodium heparin, or ACD should be tested within 12 hours at room temperature.
3. If stored venous blood treated by K2-EDTA, sodium heparin, or ACD is kept in a refrigerator, the specimen blood can be used for testing within 5 day (120 hours) after collection.

TEST PROCEDURE

[Preparation]

1. Check the expiration date printed on a test device pouch.
2. Check the code number on the codechip matches the code number printed on the test device pouch.



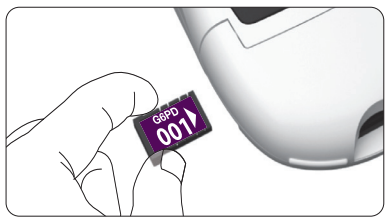
NOTE

- Make sure the code number printed on the test device pouch and the codechip match.

[Components]

Test device | Sample collector (STANDARD™ Ezi Tube+(10µl)) | Extraction buffer | Codechip | Instructions for Use

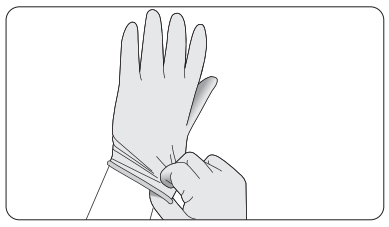
3. Insert a new codechip until it snaps into place.
4. Turn on the analyzer; the codechip number will appear on the screen.
5. Make sure the codechip number matches with the number on the screen.



NOTE

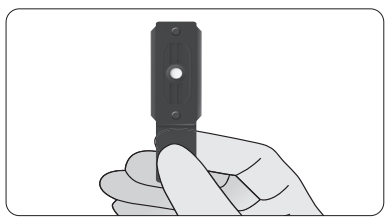
- Make sure the analyzer is turned off.
- Remove an old codechip if one is installed.
- Refer to the analyzer manual for detailed display information.

6. Put on gloves. Use new gloves for each patient.



7. Open the test device pouch and take out a test device.

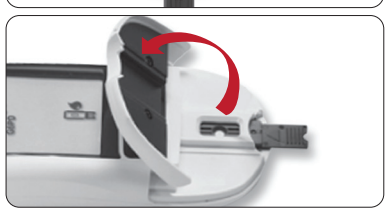
8. Hold the test device with thumb and index finger so that the upper test device is facing upward.



9. Insert the test device into the test device slot of a STANDARD G6PD Analyzer until it will go no further.

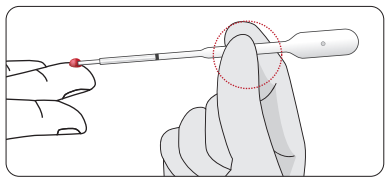
10. The screen will display 'OPE.

11. Open the measurement chamber flap.

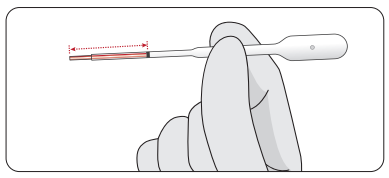


[Specimen Collection]

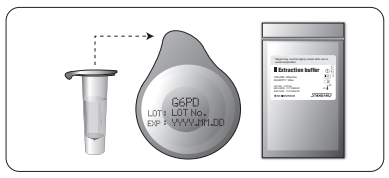
1. Hold a STANDARD Ezi tube+(10µl) horizontally, and touch the tip of STANDARD Ezi tube+(10µl) to the blood specimen (10 µl).



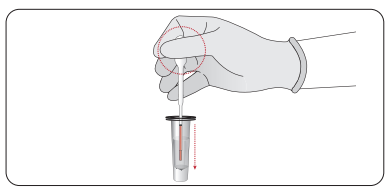
2. Capillary action will automatically draw the specimen to the black line and stop.
   ※**Insufficient or excessive volume of specimen causes an inaccurate result.**



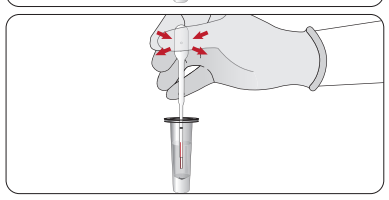
3. Open an extraction buffer pouch and take an extraction buffer out of the pouch.
   ※**An extraction buffer should not be used beyond the printed expiration date, EXP.**



4. Place the STANDARD Ezi tube+(10µl) with the specimen into the extraction buffer.



5. Mix the collected specimen with the extraction buffer, pressing and releasing the STANDARD Ezi tube+(10µl) 8 to 10 times with the hole closed.



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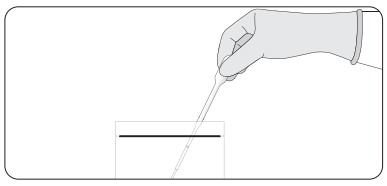
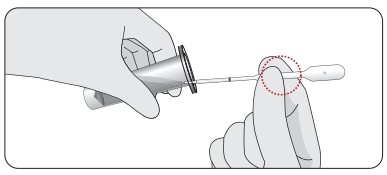
6. Discard the STANDARD Ezi tube+(10µl) in a biosafety box.

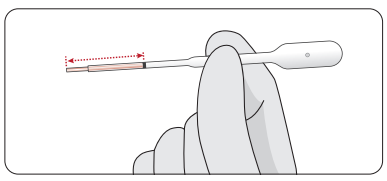
7. Open the STANDARD Ezi tube+(10µl) pouch and take out an unused STANDARD Ezi tube+(10µl).



8. Hold the STANDARD Ezi tube+(10µl) horizontally, and touch the tip of STANDARD Ezi tube+(10µl) to the mixed specimen.
   ※**Do not collect mixed specimen until the bubbles are completely gone.**

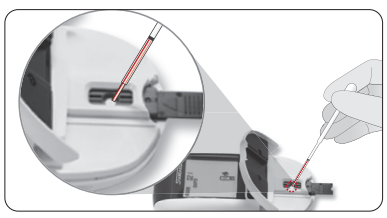


9. Capillary action will automatically draw the mixed specimen to the black line and stop.
   ※**Insufficient or excessive volume of specimen causes an inaccurate result.**
   ※**Apply the sample mixture within 1 minute after mixing the blood sample and extraction buffer solution. Failure to apply the sample-extraction buffer within one minute will lead to an inaccurate result.**



[Operation]

1. Apply the mixed specimen to the specimen application hole of the test device.



2. The screen will display 'CLo'.

3. Close the measurement chamber flap immediately after applying.
   ※**Exposing the test device to direct and strong light may cause false results.**

4. The analyzer will automatically count down from 2 minutes once the flap is closed.
   ※**Do not open the chamber flap during this time.**

5. Discard the used STANDARD Ezi tube+(10µl) and extraction buffer in a biosafety box.

6. After 2 minutes of reaction time, the test result will be appear on the screen.

NOTE

- An E-7 error occurs if the cover is open for 20 seconds after applying the control material (specimen), or the cover is opened during measurement.

MEASURING RANGES

STANDARD G6PD Test measures T-Hb and G6PD activity of numerical values as the following ranges.

Total Hemoglobin	4-25 g/dL (40-250 g/L)
G6PD	0-20 U/g Hb

If the result is outside of the measuring range, the STANDARD G6PD Analyzer will display a "Lo" or "HI" message.
- When the T-Hb is "Lo" or "HI", the test result of the G6PD enzyme activity will be displayed as "N-A".
- When G6PD activity is higher than 20, the test result of the G6PD enzyme activity will be displayed as "HI".
- When G6PD activity is lower than 0, the test result of the G6PD enzyme activity will be displayed as "Lo".

Lo	Below the measuring range (Low)
HI	Above the measuring range (High)

CAUTION

- Interpretation of the G6PD Test results should only be performed when Hb level is greater than 7g/dL.
- We recommend performing a T-Hb confirmatory test if the measured value of the T-Hb test is less than 7g/dL.

INTERPRETATION OF TEST RESULT

Male		Female	
G6PD Deficient*	≤ 4.0 U/g Hb	G6PD Deficient*	≤ 4.0 U/g Hb
G6PD Normal	≥ 4.1 U/g Hb	G6PD Intermediate**	4.1-6.0 U/g Hb
		G6PD Normal	≥ 6.1 U/g Hb

*Deficient was determined during clinical evaluation as approximately 30% of the adjusted male median of specimens tested.
**Intermediate was determined during clinical evaluation as females with activity greater than 30% and less than or equal to 70% of the adjusted male median.

PERFORMANCE CHARACTERISTICS

1. Method comparison
   To demonstrate the accuracy of the STANDARD G6PD Test System, fresh prospective venous and capillary whole blood was collected from participants at four (4) clinical sites and then tested on the STANDARD G6PD Test System in a point-of-care setting. Performance of the G6PD result is shown in the tables below.

Venous G6PD Performance

		Reference			Total
		Deficient	Intermediate	Normal	
SD Biosensor	Deficient	79	17	20	116
	Intermediate*	0	23	52	75
	Normal	0	2	1892	1894
Total		79	42	1964	2085

*Intermediate only includes female participants

Capillary G6PD Performance

		Reference			Total
		Deficient	Intermediate	Normal	
SD Biosensor	Deficient	81	19	22	122
	Intermediate*	0	21	71	92
	Normal	0	5	1906	1911
Total		81	45	1999	2125

*Intermediate only includes female participants
Performance of the T-Hb results is shown in the tables below.

Venous T-Hb Performance

		Reference			Total
		Non/Mild Anemia	Moderate Anemia	Severe Anemia	
SD Biosensor	Non/Mild Anemia	1,124	28	0	1,152
	Moderate Anemia	34	69	2	105
	Severe Anemia	2	5	9	16
Total		1,160	102	11	1,273

Capillary T-Hb Performance

		Reference			Total
		Non/Mild Anemia	Moderate Anemia	Severe Anemia	
SD Biosensor	Non/Mild Anemia	1,133	30	0	1,163
	Moderate Anemia	83	66	3	152
	Severe Anemia	2	7	8	17
Total		1,218	103	11	1,332

2. Sensitivity
   Limit of Blank (LoB) and Limit of Detection (LoD) studies were performed to determine the sensitivity of the assay. The sensitivity was determined as shown below.

G6PD (U/g Hb)		T-Hb (g/dL)	
LoB	**LoD**	**LoB**	**LoD**
0.06	0.44	0.9	2.2

3. Precision
   Precision study was performed using 9 venous sample spanning the analytical ranges for both G6PD and T-Hb.

Sample	N	Mean	Within-Run (SD, %CV)	Between-Run (SD, %CV)	Between-Day (SD, %CV)	Total (SD, %CV)
G6PD – L1	80	1.12	0.21, 18.7%	0.08, 6.9%		0.22, 19.9%
G6PD – L2	80	3.24	0.30, 9.4%	0.09, 2.6%		0.32, 9.8%
G6PD – L3	80	6.23	0.45, 7.2%		0.16, 2.6%	0.48, 7.6%
G6PD – L4	80	8.94	0.34, 3.9%		0.17, 1.9%	0.39, 4.3%
T-Hb – L1	80	5.91	0.37, 6.2%	0.14, 2.4%		0.39, 6.7%
T-Hb – L2	80	7.48	0.31, 4.1%		0.07, 0.9%	0.31, 4.2%
T-Hb – L3	80	10.39	0.41, 4.0%		0.09, 0.8%	0.42, 4.1%
T-Hb – L4	80	14.23	0.33, 2.4%	0.15, 1.1%		0.37, 2.6%
T-Hb – L5	80	18.78	0.43, 2.3%		0.12, 0.6%	0.45, 2.4%

Capillary whole blood precision was determined by testing multiple capillary whole blood samples from individuals with varied G6PD concentrations. A total of 120 samples were tested from 15 participants resulting in a standard deviation of 0.64 U/g Hb G6PD and 0.79 g/dL T-Hb.

4. Linearity
   Linearity was determined for G6PD activity, as well as T-Hb concentration.

G6PD	T-Hb
0 - 20 U/g Hb	4 - 25 g/dL

5. Interference
   Interference studies have been conducted to determine the level of interference. Samples spiked with potentially interfering materials at the following concentrations were shown to have no effect on assay performance.

No.	Interfering Substance	Interfering Level	No.	Interfering Substance	Interfering Level
1	Total cholesterol	250 mg/dL addition	10	Ascorbic acid	3.0 mg/dL addition
2	Elevated lipid-TG	500 mg/dL addition	11	Dopamine	0.9 mg/dL addition
3	Elevated lipid-HDL	70 mg/dL addition	12	Uric acid	9.0 mg/dL addition
4	Bilirubin	1.0 mg/dL addition	13	Aspirin	4.34 mM/L addition
5	Protein	8.0 g/dL addition	14	Paracetamol	3.0 mg/dL addition
6	Lactic acid	23.4 mg/dL addition	15	Ibuprophen	50 mg/dL addition
7	Copper chloride	0.1 mM/dL addition	16	Ethanol	86.8 mM/L addition
8	Copper sulfate	0.1 mM/dL addition	17	Caffeine	5.15 mM/L addition
9	Abnormally hemocrit	66%			

WARNINGS

1. The STANDARD G6PD Test device should only be used with the STANDARD G6PD Analyzer.
2. The test device should not be used beyond the printed expiration date.
3. Make sure the codechip and the code number printed on the test device pouch match.
4. The test should be performed at 15°C to 40°C / 59°F to 104°F (10-93% RH, Relative Humidity).
5. A test device is for single use only. Do not reuse.
6. Insert a test device and codechip into the test device slot and the codechip slot of the analyzer, respectively.
   - Insert a test device into the test device slot with blood application chamber facing up and toward the analyzer.
   - Insert a codechip into the codechip slot with the surface printed with the code number facing up and toward the analyzer.
   - Insert a test device into the analyzer gently until it will go no further
7. Severe hemolysis or patients who have hemolytic anemia can produce inaccurate results.
8. In cases of extreme anemia, extreme elevated white blood cell counts, or very low levels of red cell G6PD activity, the contribution of white blood cell G6PD activity to the total may be significant, and may result in false normal. White blood cell depletion may correct for this.
9. Ensure the proper specimen volume for the test device is used. The specimen volume should be at least 10µl.
10. Do not apply mixed specimen on another site except blood application area of a test device.
11. Do not ingest.
12. Discard the used test devices according to local guidelines.
13. Extraction buffer contains Triton X-100 which can cause serious eye irritation.

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Information about sponsor

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REF Reference number | IVD *In vitro* Diagnostics | Consult Instructions for Use | Contains Sufficient for <n> Tests | Caution | Do not re-use. | Use by | To indicate the temperature limitations in which the transport package has to be kept and handled. | LOT Batch code | Manufacturer | Date of manufacture | CE Fulfill the requirements of Directive 98/79/EC on *in vitro* diagnostic medical devices