AUTOMATED DETERMINATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) ON A SPOTCHECK® MICROFLOW ANALYZER

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Abstract. Glucose-6-phosphate dehydrogenase (G6PD) is the initial enzyme in the hexose monophosphate pathway of glucose metabolism. Deficiency of G6PD has been linked to increased sensitivity of red cells to hemolytic anemia due to certain oxidant drugs, infectious agents or fava beans. It is an inherited error in metabolism and has a high incidence in certain ethnic groups. Astoria-Pacific has developed an automated assay for use on the SPOTCHECK Microflow Analyzer for the semi-quantitative determination of G6PD activity in erythrocytes. After sample extraction, all assay steps are automated including reagent addition, incubation and data collection. Use of on-line dialysis removes interferences. The assay is intended primarily as a screening tool in the diagnosis and treatment of disease states associated with G6PD deficiency in newborns. G6PD in the dried blood spot is extracted and placed on the instrument. Samples are then aspirated into the system at a rate of 90 samples/hour. All other reagents are added by the SPOTCHECK Analyzer on-line during sample processing. Incubation of each sample occurs on-line at 37°C, and after dialysis the NADPH reaction product is excited at 365 nm. Fluorescence is measured at 500 nm. A lack of fluorescence indicates a probable G6PD deficiency. Data reduction occurs real time through a FASPac software thus individual results are available during a run as soon as each sample analysis is complete. The Astoria-Pacific International G6PD reagent kit paired with the SPOTCHECK Microflow Analyzer provides an effective and easy to use screening tool for determining G6PD deficiency in newborns.

INTRODUCTION

The Astoria-Pacific International SPOTCHECK Analyzer was evaluated as a tool for use in screening glucose-6-phosphate dehydrogenase (G6PD) deficiency in newborn specimens. After sample extraction, all assay steps are automated in the SPOTCHECK analyzer including reagent addition, incubation, data collection and data analysis. Use of on-line dialysis removes interferences.

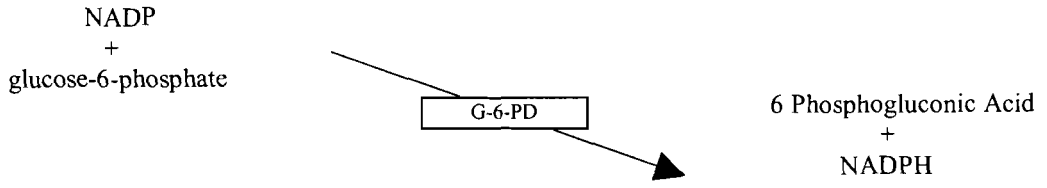
G6PD in the dried blood spots is extracted and the extract placed on the instrument. Samples are then introduced into the system at a rate of 90 samples/hour. This method is a modification of the established fluorometric methods (Fig 1). Glucose-6-phosphate dehydrogenase catalyzes the oxidation of glucose-6-phosphate (G6P) to 6-phosphogluconate (6-PG) and simultaneously reduces one mole of NADP+ to NADPH. Maleimide, an inhibitor of 6-phosphogluconate dehydrogenase (6-PGD) activity, is added to inhibit the production of NADPH from 6-phosphogluconate. All reagents are automatically added by the SPOTCHECK Analyzer during sample processing. Incubation of each sample occurs automatically at 37°C. After dialysis to remove interferences, the NADPH reaction product is excited at 365 nm and the fluorescence is measured at 500 nm. A lack of fluorescence indicates a probable G6PD deficiency. Data reduction occurs real time through a FASPac software making individual results available during a run as soon as each sample reaches the detector.

Fig 2 below graphically depicts the results of 901 specimens including some partial and deficient samples, analyzed by Astoria-Pacific using the G6PD 50 Hour reagent kit on the SPOTCHECK analyzer.

Twenty-four newborn specimens classified as deficient by the manual fluorometric method were run on the SPOTCHECK analyzer. The results are shown in Fig 3 which also shows the cut-off levels used for the semi-quantitative determination of Deficient and Intermediate specimens.

REACTION

This method is a modification of the established fluorometric methods. Glucose-6-phosphate dehydrogenase catalyzes the oxidation of glucose-6-phosphate (G6P) to 6-phosphogluconate (6-PG) and simultaneously reduces one mole of $NADP^+$ to NADPH.



Maleimide, an inhibitor of 6-phosphogluconate dehydrogenase activity, is added to inhibit the production of NADPH from 6-phosphogluconate.

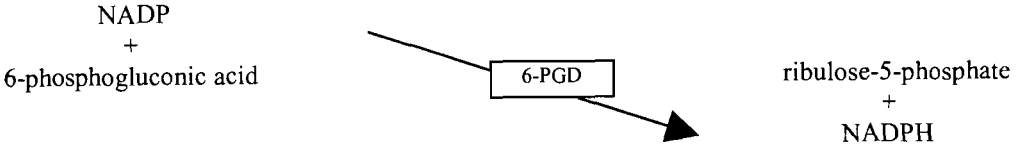


Fig 1. Reaction mechanism of G6PD assay.

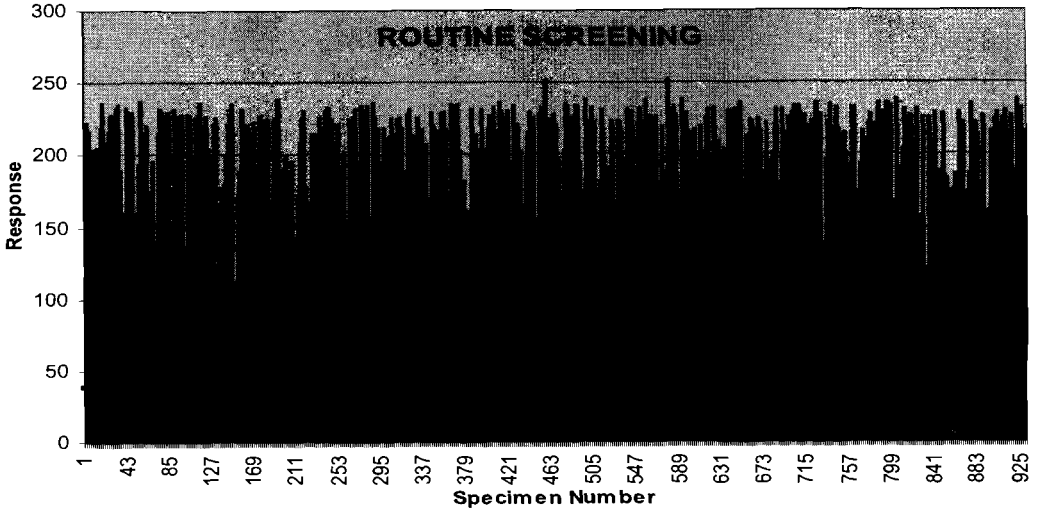


Fig 2. Performance of the SPOTCHECK Analyzer during routine screening.

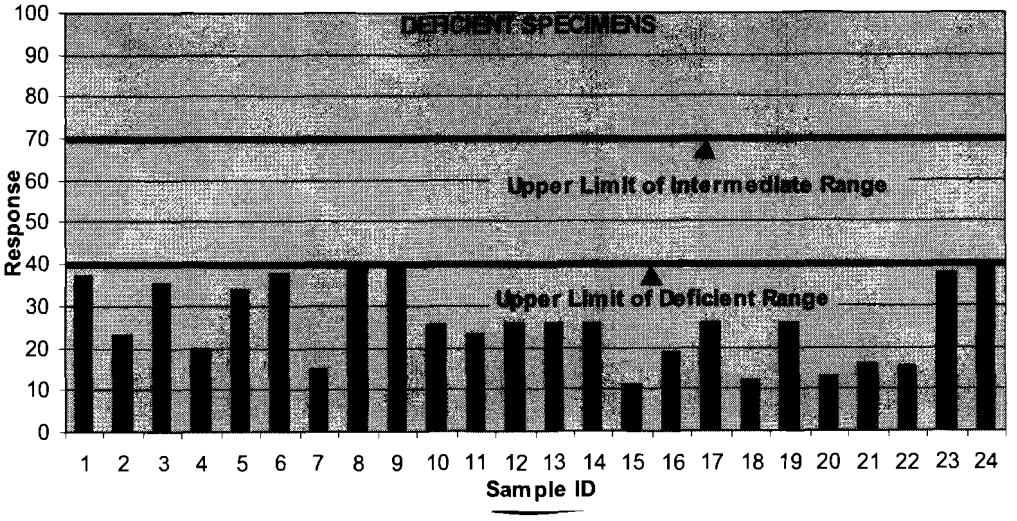


Fig 3. Performance of the SPOTCHECK Analyzer on G6PD deficient specimens.

RESPONSE LINEARITY

Linearity of the substrate reaction process

A specimen with normal G6PD activity and a G6PD deficient specimen were mixed in the following ratios:

- 100% Normal (NNNN)
- 75% / 25% (NNND)
- 50% / 50% (NNDD)
- 25% / 75% (NDDD)
- 100% Deficient (DDDD)

This simulates the majority of the range of activities encountered in routine screening. See Response Linearity Graph to the right.

Fig 4. Linearity of the substrate reaction process.

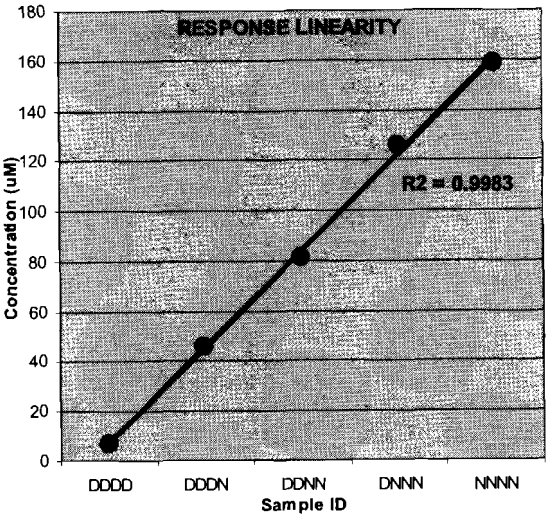


Fig 5. Graphical representation of Fig 4.

The response linearity of the substrate reaction process is shown in Figs 4 and 5. Two samples, one with normal activity and one categorized as deficient were mixed in varying ratios listed below and run as samples on the SPOTCHECK system.

PRECISION

Within-run and total precision were evaluated for this method. Samples with three levels of activities were assayed in duplicate in 2 runs per day over 4 days to estimate the within-run and total precision. The data is summarized below.

WITHIN-RUN PRECISION, SWR

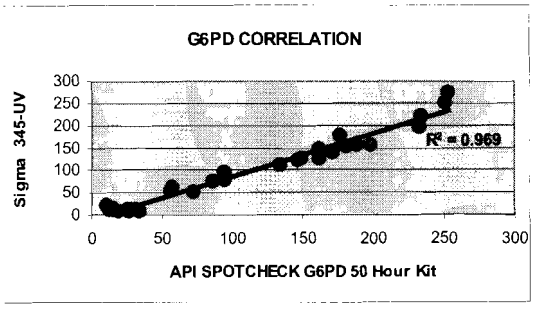
	G6PD Deficient	Near Cut off	Normal
Average	7.8 mM	43.0 mM	158 mM
S.D.	0.25 mM	1.9 mM	4.9 mM
C.V.	3.2%	4.4%	3.1%

TOTAL PRECISION, ST

	G6PD Deficient	Near Cut off	Normal
Average	7.8 mM	43.0 mM	158 mM
S.D.	0.42 mM	2.6 mM	13.7 mM
C.V.	5.4%	6.0%	8.7%

COMPARISON METHOD CORRELATION

The performance of the SPOTCHECK Analyzer G6PD system and a Sigma Diagnostics Method 345-UV (on COBAS BIO) were compared by analyzing samples known to be normal (16), intermediate (4) and G6PD deficient (10). Based upon the evaluation of these 30 samples, it was determined that the two methods produced equivalent results. The results are represented in Fig 6.



G6PD USING THE SPOTCHECK ANALYZER

Fig 6. SPOTCHECK Analyzer G6PD System vs. Sigma Diagnostics 345-UV Reagent System run on the COBAS BIO.

CONCLUSIONS

Analysis of G6PD using the SPOTCHECK Analyzer and Reagent System is a viable alternative to other methods used today. It has good discriminatory properties which when applied with cut-off values allow for semi-quantitative determination of each specimen as Normal, Intermediate, or Deficient. The system offers the advantage of providing results on the same day it was taken. Other advantages include:

- the ability to screen simultaneously other assays including Biotinidase, Galactose-1-Phosphate Uridyltransferase, Phenylalanine, Tyrosine, and Total Galactose
- automated sampling, incubation and analysis
- reduced labor as compared to current methods
- high sample throughput (systems operate at 90 samples per hour)
- low cost per test

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