Letter to the Editor

An improved high-throughput dried blood spot screening method for Gaucher disease

Dear Editor,

Gaucher disease is a lysosomal storage disorder caused by the deficiency of the lysosomal hydrolase glucocerebrosidase (β-glucocerebrosidase or acid β-D-glucosidase, GBA, EC 3.2.1.45). The deficiency of GBA leads to the accumulation of glucosylceramide (glucocerebroside) in the lysosomes of cells in the monocyte/macrophage system. In Gaucher disease, glycosphingolipid-engorged cells displace normal cells in the liver, spleen and bone marrow, which can lead to hepatosplenomegaly, thrombocytopenia, organ dysfunction, and skeletal deterioration [1].

The diagnosis of Gaucher disease is confirmed by measuring GBA activity in peripheral blood leukocytes; however, many patients are misdiagnosed or remain undiagnosed because Gaucher disease is rare and patients can have heterogeneous clinical symptoms [2]. The use of a simple screening method in high risk populations could increase the diagnostic rate of Gaucher disease and permit therapeutic intervention as needed to prevent serious complications. A high-throughput and reliable fluorescent assay was developed using the β-D-glucosidase substrate 4-MUG and CBE, an irreversible inhibitor of GBA that allows the distinction between GBA and other β-glucosidase isoenzymes [3].

Specimens from 20 previously diagnosed Gaucher disease patients were collected with written informed consent. Specimens from 193 healthy adults were from ProMedDx LLC (Norton, MA). Venous blood was drawn into tubes containing EDTA, shipped on cold packs overnight and held at 4 °C. Within 24 h of collection, the tubes were inverted several times to resuspend the blood cells and 75 µl aliquots were spotted onto Whatman 903® specimen collection paper with a handheld pipettor. Bloodspots were dried for at least 4 h at room temperature. The dried bloodspots (DBS) were stored in sealed plastic bags at 4 °C for up to 1 week in a desiccated cabinet and ≤−20 °C with a desiccant and humidity indicator for longer periods.

GBA activity in DBS was measured using the following modification of the method of Chamoles [4]. One 3.2 mm diameter disk was punched from each DBS sample and the enzyme was extracted in 200 µl 0.2 mol/mol/l citrate phosphate buffer, pH 5.2 containing 1% Triton® X-100 (Sigma, St. Louis, MO) and 1% sodium taurodeoxycholate (≥97% purity, Sigma) on a 96-well plate. The extraction plate was covered with an adhesive aluminum seal film (VWR, West Chester, PA), vortexed for 30 s and then incubated on a rocking platform (MaxQ Mini rocker from Barnstead International, Dubuque, IA) at 225 RPM for 1 h at RT. After incubation, the supernatant was transferred to a clean 96-well plate, which was then covered again with the aluminum seal and centrifuged at 2397 g for 30 min to remove any particulate matter.

Uninhibited substrate stock solution (12.5 mmol/l 4-MUG) was prepared by adding 100 µl 4-MUG stock solution (1 mol/l in DMSO; Sigma) to 7.9 ml HPLC grade water (Honeywell Burdick & Jacksons, Morristown, NJ). Inhibited substrate solution (12.5 mmol/l 4-MUG, 0.5 mmol/l CBE) was prepared by adding 7.5 µl CBE stock solution.

Fig. 1. Comparison of samples from 193 normal controls and 20 Gaucher disease patients. The mean GBA activity for normal control samples was 10.6 pmol punch⁻¹ h⁻¹, with minimum 5.6; maximum 23.8; and 95% CI of mean 10.2–11.1 pmol punch⁻¹ h⁻¹. Activity in the Gaucher disease samples ranged up to 4.3 pmol punch⁻¹ h⁻¹ with a mean of 2.7 pmol punch⁻¹ h⁻¹. Two Gaucher disease samples were below the LOD.

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(0.26 mol/l in DMSO, Calbiochem, San Diego, CA) to 4 ml uninhibited substrate stock solution. Uninhibited and inhibited enzyme reactions contained 80 µL of the corresponding substrates and 40 µl of DBS extract. Each was run in duplicate.

The reactions were incubated for 20 h at 37 °C in black 96-well assay plates (Corning, Corning, NY) covered with the aluminum seal film. To stop the reactions, 100 µl of 0.5 mol/l EDTA (pH 11.5) was added to each well. An eight point 4-methylumbelliferone (4-MU) standard curve (0–0.67 µmol/l) was prepared on each plate in duplicate (120 µl/well) by diluting a 4-MU stock solution (4 mmol/l in DMSO, Sigma) in water. One hundred µl 0.5 mol/l EDTA (pH 11.5) was added to each well (final standard curve is 0–0.37 µmol/l). The plate was covered with the adhesive aluminum plate seal and centrifuged at 2397 × g for 1 h at 24 °C. After centrifugation, the seal was removed and the plate was read in a fluorometer with 355 nm excitation and 460 nm emission wavelengths. Molar product quantities in the assay wells were calculated by linear regression from the standard curve. GBA activity was determined by subtracting the activities measured in the inhibited reaction from that in the uninhibited reaction. Filter paper discs from a single venous blood specimen were included as internal QC samples on all plates. A detailed protocol is available in the online Supplementary data.

As shown in Fig. 1, this assay was sensitive enough to differentiate samples from 20 previously diagnosed Gaucher disease patients and 193 normal control samples. Two Gaucher disease samples were below the LOD. These results demonstrate the capacity of this method for the detection of Type 1 Gaucher disease.

Following the procedure outlined in Clinical and Laboratory Standards Institute (CLSI) guideline EP05 [5], the estimate of assay precision was determined by testing six normal samples (high, and low GBA activity) in quadruplicates on four plates per day for 5 days by 2 operators. The CVs for samples are shown in Table 1.

The limit of detection (LOD) for GBA activity was determined to be 1.3 pmol punch⁻¹ h⁻¹ by following the CLSI EP17 [6] guidelines. The assessment was made with a proportion of false positive (α) to be <5% and false negative (β) <5% from 131 measurements (60 blanks and 71 low-activity samples). Limit of blank (LOB) was calculated to be 0.5 pmol punch⁻¹ h⁻¹.

The modified assay proved to be robust and reliable for the differentiation of normal and disease-specific samples. Transfer of the high throughput enzyme assay to newborn screening labs should be straightforward.

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cca.2008.08.024.

### References


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