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Inherited metabolic disorders: Quality management for laboratory diagnosis

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ABSTRACT

Background: The advancements in laboratory technology and knowledge of the mechanisms behind metabolic disorders have facilitated accurate and reliable laboratory testing in screening, diagnosis and treatment of inherited metabolic disorders. Therefore, quality assurance and improvement in diagnostic proficiency have become essential in this area. In most developing countries, standard practices for quality assurance in testing of enzymes, hormones and metabolites involved in these genetic disorders have not been fully implemented. We highlight the benefits of quality assurance and aim to create awareness for greater compliance with the criteria established for quality control to ensure accuracy in biochemical genetic testing.
Methods: Establishing the limit of detection and testing range for each analyte and enzyme are useful as a reference while setting up new assays. To minimize error, %CV should be monitored regularly. Evaluation of proficiency testing performance provides scope to the laboratory for improving testing quality.
Results: Low precision seen in lysosomal enzyme assays does not undermine their diagnostic efficacy as differentiation between patients and normal subjects is possible by setting % coefficient of variation cutoffs.
Conclusions: The study will facilitate the collaboration with other screening and diagnostic systems and help in development of new laboratory standards.

1. Introduction

1.1. Need of quality assurance in biochemical genetics testing

Biochemical genetic testing is critical for proper diagnosis and management of patients with inborn errors of metabolism. There is a wide spectrum of genetic disorders that are diagnosed by highly specialized biochemical techniques which require complex equipments, software and interpretation of results by experienced personnel. In most emerging countries, experienced personnel are not always available. Patients frequently travel between countries for these specialized tests thereby increasing the need for satisfactory quality assurance, including external quality control, to guarantee the comparability of results between different centers. Therefore, it is necessary to raise the level of accuracy, precision, reproducibility and harmonization of biochemical genetics testing [1]. Compliance to the internal quality control criteria and participation in external quality assessment schemes increase the diagnostic proficiency of the laboratory by helping to reduce analytical deficiencies, improving the testing procedures and taking necessary actions for prevention of errors in future [2].

1.2. Availability of quality assurance schemes for inherited metabolic disorders

Newborn Screening Quality Assurance Program (NSQAP), Centers for Disease Control and Prevention (CDC), Atlanta provides an essential public health service for various metabolic and genetic disorders free of cost and helps to ensure the quality and accuracy of screening tests for >4 million infants born each year (http://wwwn.cdc.gov/nsqap/public/default.aspx) [3]. The Preventive Medicine Foundation (PMF), Taiwan with the government support operates an external quality assurance program to monitor the performance of screening and diagnostic services for the qualitative and quantitative analysis of glucose-6-phosphate dehydrogenase (G6PD) deficiency free of cost (http://g6pd.qap.tw) [4]. European Research Network for evaluation and improvement of screening, Diagnosis and Treatment of Inherited Disorders of Metabolism (ERNDIM), UK also operates a quality

Abbreviations: G6PD, glucose 6 phosphate deficiency; 17OHP, 17 hydroxy progesterone; GALT, galactose 1-phosphate uridyl transferase; SUAC, succinyl acetone; DBS, dried blood spots; EQAS, External Quality Assurance Scheme.

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assurance program for enzyme assays for the diagnosis of inborn errors of metabolism but due to the high cost for participation, the number of laboratories that can afford to participate in their schemes is limited. However, it is heartening to note that they give discounts to developing countries for participation (https://www.erndim.org).

1.3. Study aim and objectives

We describe the internal quality control methods set up for newborn screening assays, lysosomal enzymes and other metabolite assays to minimize testing errors and produce high quality results. Proficiency testing results obtained from CDC, Atlanta for thyroid stimulating hormones (TSH), 17 hydroxy progesterone (17OHP), biotinidase deficiency, galactose-1-phosphate uridyl transferase (GALT), galactose and succinyl acetone (SUAC), ERNDIM, UK for lysosomal enzymes, and PMF, Taiwan for neonatal G6PD were used to evaluate laboratory performance in terms of false positive and false negative for these assays. Though new generation techniques like LC-MS/MS, GC-MS and tandem mass spectrometry with high precision rates are in use for screening assays these days; their setup is not easy for the laboratories in developing countries with limited resources. Further, the standardization and validation status are still in question for some of the disorders diagnosed using these expensive equipments. Our study demonstrates the diagnostic efficacy of manual enzyme assays to discriminate between patients and normal cases by establishing the acceptable %CV cutoffs. The study is intended for continued monitoring of % coefficient of variation and evaluation of error rate to ensure and improve testing quality.

2. Materials and methods

2.1. Assay methods, samples, calibration and techniques used

The dried blood spot (DBS) specimens obtained for proficiency testing were tested by several biochemical methods. The assays of TSH, 17OHP, and G6PD were performed by time resolved fluoroimmunometric method (Perkin Elmer kits, manufactured by Wallac Oy) using the Victor 2D instrument (Multi label Counter, Perkin Elmer) as a reader. Biotinidase (EC.3.5.1.12) was estimated in nmol/h/punch by a quantitative spectrophotometric method [5,6] and the results were reported qualitatively as normal or deficient activity on CDC website. GALT (EC.2.7.7.12) (U/gm Hb) and galactose (mg/dl) assays were done by fluorometric methods [7,8]. Standard curves of 4 methylumbelliferone, 4 nitrocatechol, galactose and hemoglobin [9] were prepared monthly to check instrument performance and reagent quality for use in enzyme assays. One standard was always run per assay. Succinyl acetone (μmol/l) was estimated by a spectrophotometric method. It can be measured in either urine/plasma samples [10] or in DBS samples [11]. The assay is based on the conversion of δ-aminolevulinic acid to porphobilinogen by δ-aminolevulinic acid dehydratase (δ-ALAD) (E.C.4.2.1.24), which is inhibited by succinyl acetone. For the measurement of succinyl acetone in urine and plasma samples, the enzyme δ-ALAD source is heparin blood of a normal individual which varies every time and causes variation in the values of calibrators while in dried blood spot, enzyme δ-ALAD is provided by the patient sample itself. The problem of variation in calibrators can be avoided by using DBS sample of a same person (stored at 4 °C for 6 months with desiccant) for setting up calibration curves. Lysosomal enzymes (www.chem.qmul.ac.uk/iubmb/enzyme/ for enzyme EC numbers): Aryl sulfatase B (E.C.3.1.6.12), α-galactosidase (E.C.3.2.1.22), α-glucosidase (E.C.3.2.1.20), β-glucosidase (E.C.3.2.1.21), chitotriosidase (EC.3.2.1.14), β-galactosidase (E.C. 3.2.1.23), β-hexosaminidase (E.C. 3.2.1.52), total hexosaminidase (EC.3.2.1.52), sphingomyelinase (E.C. 3.1.4.12), β-galactocerebrosidase (EC.3.2.1.46), palmitoyl protein thioesterase (EC.3.1.2.22), tripeptidyl peptidases (EC.3.4.14.9), α-fucosidase (EC:3.2.1.51), α-mannosidase (EC.3.2.1.24), iduronate 2-sulfatase (E.C. 3.1.6.13), α-iduronidase (E.C.3.2.1.76), heparan sulfamidase (E.C. 3.2.1.76), acetyl Co A: glucosamine N-acetyl transferase (EC.2.3.1.4), n-acetyl glucosamine 6 sulfatase (EC.3.1.6.14), galactose 6 sulfatase (EC.2.5.1.5), β-glucuronidase (EC.3.2.1.31) and acid lipase (EC.3.1.1.3) were assayed by the fluorometric method using artificial 4 methylumbelliferone conjugated substrates and enzyme activity was measured in nmol/h/mg or nmol/h/ml except for aryl sulfatase A (E.C.3.1.6.8) which was estimated by a spectrophotometric technique using 4-nitrocatechol sulfate as a standard [12–23].

2.2. Control reference materials and day to day validation

Control reference materials were either prepared in-house or purchased commercially (Table 1). Levy–Jennings charts were drawn routinely for the assays of biotinidase, TSH, 17OHP, G6PD, GALT, galactose and succinyl acetone to detect the outliers immediately using quality control rules. The %CV was monitored routinely for each parameter.

2.3. Quality control rules and acceptance/ rejection criteria

For internal quality control, quality control (QC) rules were prepared on the basis of Westgard's multi control QC rules for all quantitative assays as referred in NABL document no. 112 (www.nabl-india.org).

2.4. Proficiency testing results and reporting

After analysis, the results of proficiency testing samples were reported on-line as per external quality assessment schemes schedule given on their respective websites. An evaluation criterion was based on the final clinical assessment as positive/abnormal and negative/normal as per the respective cutoffs set by laboratory for different parameters. Results were compiled in terms of % error.

2.5. Informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients/normal subjects for being included in the study.

3. Results

3.1. Internal quality control

Various assays, sample types, control reference materials, preparation of in-house controls, calibration procedures, frequency of internal quality control checks and compliance criteria are summarized in Table 1. To control errors in testing, assay technique, limit of detection, testing range, laboratory %CV, acceptance criteria, storage time, temperature conditions for standards, control samples and substrates utilized for all assays are given in Table 2. The % variation was also kept within permissible limit (%CV: 15–20) in repeat testing, replicates and retained samples for each analyte and monitored routinely. Data of the samples tested for the same during 2013–2015 for different parameters with sample retention time and temperature are shown in Table 3. These are alternative ways to keep testing errors in control and are generally important for those assays where external quality assessment schemes are not available.

For the manual (enzymes and metabolites) assays, the %CV was <9.2 and a threshold of <15% was acceptable (Table 2). The %CV was slightly higher (10–14) in all assays done on dried blood spots in comparison to assays done on other samples (urine/plasma/whole blood leukocytes/whole blood RBCs) except succinyl acetone (SUAC) assay. The %CV was less (8.65) in dried blood assay of SUAC in comparison to liquid