Original Article

External Proficiency Testing Programmes in Laboratory Diagnoses of Inherited Metabolic Disorders

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Abstract

Introduction: This paper shows the importance and value of external proficiency testing programmes in monitoring and improving a laboratory's diagnostic skills. It reviews and documents the wide variety of inherited metabolic disorders (IMDs) encountered in the programmes organised by the Human Genetics Society of Australasia and the College of American Pathologists. Materials and Methods: The programmes used actual patient specimens to assess a laboratory's ability to provide diagnoses based on laboratory tests results and brief clinical information. Participating laboratory was also required to suggest additional test(s) to confirm diagnoses. Results: The results of diagnoses on 116 samples were reviewed. Altogether 49 IMDs were encountered, including 26 organic acidurias, 16 aminoacidurias, 3 urea cycle defects, 5 mucopolysaccharidoses, and 1 each of mucolipidosis and purine disorder. Our report for 21 of the 116 samples (18.1%) deviated from the actual diagnoses. Deviations from the final diagnoses were recorded along with the reasons for them. The main reasons for the deviations were: the lack of standards for recognising metabolites of pathognomonic significance, absence of characteristic metabolites in samples collected during treatment, the presence of misleading unusual metabolites, inadequate clinical information, and inability to perform additional tests due to insufficient specimens. Conclusions: The programmes provided a wide variety of IMDs, some of which we have yet to encounter in our patients. They also enabled us to learn about the varied biochemical manifestations at different stages of disease and the identity of previously unidentified metabolites. They enhanced our knowledge and experience and improved our diagnostic skills.

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Key words: External proficiency testing, Inherited metabolic disorders, Laboratory diagnosis, Quality assessment

Introduction

In order to monitor and ensure the quality of our laboratory diagnoses for inherited metabolic disorders (IMDs), our laboratory participated in the proficiency testing programmes organised by the Human Genetics Society of Australasia (HGSA) from Australia,[1] and the College of American Pathologists (CAP) from USA.[2] As the prevalence of IMDs in the population is low, we were quite certain that it would be most unlikely for us ever to come across the wide range of disorders, with equally varied clinical manifestations, in patients presented to our laboratory. Without the experience of investigating and diagnosing known positive cases, we would have no idea as to our ability to arrive at a correct diagnosis should we encounter them. Participation in well-recognised programmes which employ specimens obtained from actual patients, both with and without IMDs, would provide us with an excellent opportunity to learn about our strengths and weaknesses as well as the opportunity to improve our diagnostic skills through sharing of experience and knowledge disseminated by the organisers of the programmes and how other laboratories performed. Patient specimens for proficiency testing programmes are contributed by various national medical centres known for their expertise and experience in specific areas in the field. The aim of this paper was to show the importance and value of proficiency testing programmes in improving our laboratory's diagnostic skills as well as to review and document the wide variety of IMDs encountered in these programmes.

Materials and Methods

The HGSA programme had 11 participating laboratories, of which all except one were specialised genetic laboratories serving major cities in Australia and New Zealand. It

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provided 10 or 11 urine samples per year from previously diagnosed patients and participating laboratories were required to perform appropriate analyses for each specimen. Brief clinical information was provided for each sample. Based on the results of analyses and clinical information, laboratories were required to arrive at a diagnosis. In addition, they had to suggest other tests that would be helpful in confirming the diagnosis. The CAP programme had 68 participating laboratories worldwide. It provided 8 plasma and urine samples per year and participating laboratories were required to perform analyses as instructed. The specimens were either from actual patients or from non-IMD patients spiked with abnormal metabolite(s).

Laboratory investigations included the detection and quantitative analyses of plasma and urine amino acids, urine organic acids and urine mucopolysaccharides. Urine and plasma amino acids were separated by ion-exchange chromatography, followed by reaction with ninhydrin and then quantitated by spectrophotometry on the dedicated Beckman 6300 Amino Acid Analyzer (High-Performance Amino Acid Analyzer and System Gold: The Personal Chromatograph, Spinco Division, Beckman Instruments Inc. Palo Alto, California 94304). Urine organic acids were extracted with ethyl acetate/diethyl ether and derivatised with bistrimethylsilyltrifluoroacetamide (BSTFA) to form stable, volatile trimethylsilylated (TMS) derivatives. Separation and identification of the organic acid derivatives were achieved using the Hewlett Packard 5890A Series II chromatograph and 5971A mass spectrometer (GC-MS System by Hewlett-Packard, Analytical Product Group, Delaware, USA).[3-6] Urine mucopolysaccharides were measured using the dimethylmethylene blue (DMB) dye-binding method.[7,8] Separation of the various mucopolysaccharides was achieved by high-resolution electrophoresis (HRE) on cellulose acetate plates.[9] Specimens received from proficiency testing programmes were not given any special treatment and were analysed with other specimens received from patients.

For each specimen, the HGSA programme organiser provided 2 reports, initially by post and subsequently by email, to each participating laboratory. The "Participant Summary Report" provided an overall summary of the results submitted by all participants. A second "Case Summary Report" gave the detailed history, clinical manifestation, results of significant confirmatory tests such as enzyme analyses, and actual diagnosis of the patient. Information on treatment and progress of patient was also provided when available. The CAP organiser also provided 2 reports for each participating laboratory. The "Evaluation Report" evaluated results using comparable peer groups. The "Participant Summary" gave a summary of the performance of all available instrument and reagent combinations. A discussion of the clinical history, clinical manifestation and management, actual diagnosis of the patient, and significant analytical findings was also provided.

Results

From 1996 when the programme was first established, to 2004, a total of 93 urine samples were analysed for the HGSA programme. Participation in the CAP programme commenced some years later, when our department sought CAP accreditation and participation in its proficiency programme was a requirement. A total of 23 plasma and urine samples were analysed for the CAP programme between 2002 and 2004. Both programmes did not provide an actual score or qualitative grading (e.g., excellent, good, satisfactory, poor) for participants' performance. However, we are able to calculate the rate of correct diagnosis obtained by our laboratory and by all participating laboratories, by reviewing the reports on every specimen, counting the total number of specimens received for proficiency testing and the number of correct diagnosis made by our own laboratory, and by all laboratories. Calculations are made as follows:

Rate of correct diagnosis obtained by our laboratory =
(No. of samples correctly diagnosed by our laboratory)/
(total no. of samples analysed) x 100

Average rate of correct diagnosis obtained by all participating laboratories =
(No. of specimens correctly diagnosed by all laboratories)/
(total no. of specimens analysed) x 100

Our laboratory provided correct diagnoses for about 82% of cases for both programmes. This is well above the average rate of 72.4% obtained by all participating laboratories. CAP considered our performance sufficiently well to deserve accreditation. CAP will not hesitate to inform a laboratory, seeking its inspection, of any deficiency and area of improvement required and will not grant accreditation unless any unsatisfactory performance is rectified.

Altogether 49 IMDs were encountered in these programmes, consisting of 26 organic acidurias, 13 amino-acidurias, 3 urea cycle defects, 5 mucopolysaccharidoses (MPS), 1 mucolipidosis, and 1 purine disorder.[10] Table 1 provides a detailed list of the IMDs presented by these programmes and the clinical presentations of each case. Our report for 21 of the 116 samples (18.1%) deviated from the actual diagnoses. Table 2 lists the IMDs, which could not be diagnosed, and the reasons for misdiagnoses or an inability to provide a diagnosis.

Discussions

External proficiency testing programmes for IMD investigations, such as the HGSA and CAP programmes,

Table 1. IMDs presented by the HGSA and CAP Proficiency Testing Programmes

IMD		Clinical information provided
(1)	2-hydroxyglutaric aciduria (D-2-hydroxyglutaric acid dehydrogenase deficiency)	Macrocephaly, ataxia
(2)	2-methylacetoacetyl CoA thiolase deficiency (beta-ketothiolase deficiency)	Profound acidosis
(3)	3-methylglutaconic acidaemia (3-methylglutaconyl-CoA hydratase deficiency)	Encephalopathy
(4)	3-methylcrotonyl CoA carboxylase deficiency	Vomiting
(5)	5-oxoprolinuria (pyroglutamic aciduria, glutathione synthetase deficiency)	Hepatosplenomegaly
(6)	Alkaptonuria (homogentisic oxidase deficiency)	Encephalopathy
(7)	Aminoadipic aciduria (ketoadipic aciduria, 2-ketoadipic acid dehydrogenase deficiency)	Intellectual handicap
(8)	Argininosuccinic acid lyase deficiency	Developmental delay
(9)	Argininosuccinic acid synthetase deficiency (citrullinaemia)	Seizure, hyperammonaemia
(10)	Aromatic amino acid decarboxylase deficiency	Feeding problems
(11)	Barth Syndrome (3-methyl glucatonic aciduria)	Cardiomyopathy
(12)	Biotinidase deficiency	Seizures, developmental delay
(13)	Cystathionine beta-synthase deficiency	Glaucoma, dislocated lens, tall stature
(14)	Fumarase deficiency	Seizures, developmental delay
(15)	Fumarylacetoacetase deficiency (tyrosinaemia type I or hepatorenal tyrosinaemia)	Abnormal liver function tests
(16)	Gamma glutamyltransferase deficiency	Petit mal
(17)	Glutaryl CoA dehydrogenase deficiency (GAI)	Macrocephaly, dystonia, convulsions
(18)	Glycerol kinase deficiency	Developmental delay, acidosis, vomiting
(19)	Hartnup disease (neutral amino acid transport defect)	No information provided
(20)	Hawkinsinuria (4-hydroxyphenylpyruvate dehydrogenase deficiency)	Asthma
(21)	Hereditary orotic aciduria [uridine monophosphate synthase (UMPS) deficiency]	FTT, anaemia, developmental delay
(22)	Hyperlysinaemia (lysine alpha glutarate reductase and saccharopine dehydrogenase deficiency)	Epilepsy with drop attacks, difficulty walking
(23)	Hyperoxaluria Type I (alanine:glyoxylate aminotransferase deficiency)	Nephrocalcinosis
(24)	Hyperoxaluria Type II (D-glycerate dehydrogenase deficiency)	Recurrent renal calculi
(25)	Hypophosphatasia (alkaline phosphatase deficiency)	Exfoliation of desiduous teeth, premature teeth loss
(26)	I-cell disease (mucolipidosis II) (N-acetylglucosamine-1-phosphotransferase deficiency)	Dysmorphic features, mild joint contracture of hands/elbows, cardiac anomalies
(27)	isovaleryl CoA dehydrogenase deficiency (isovaleric acidaemia)	Severe acidosis
(28)	Long chain 3-hydroxy acyl CoA dehydrogenase deficiency (LCHAD)	Fits, hypoglycaemia
(29)	Lysinuria protein intolerance (deficient carrier involved in the membrane transport of dibasic amino acids in the small intestine and renal tubules)	Altered conscious state

Table 1. Contd.

IMD		Clinical information provided
(30)	Malonyl CoA decarboxylase deficiency (malonic aciduria)	Vomiting, seizures, UTI
(31)	Maple syrup urine disease (MSUD, branched-chain ketoacid dehydrogenase deficiency)	Vomiting, extensor spasms, developmental delay
(32)	Medium chain acyl CoA dehydrogenase deficiency (MCAD)	Hypoglycaemia, seizures
(33)	Methylmalonic CoA mutase deficiency (methylmalonic aciduria)	No information provided
(34)	Molybdenum cofactor deficiency (combined sulphite oxidase and xanthine oxidase deficiency, with urinary excretion of S-sulphocysteine)	Seizures
(35)	Mucopolysaccharidosis I (MPS IH - Hurler syndrome) (alpha-L-iduronidase deficiency)	Umbilical hernia, unusual facies, macrocephaly, hepatomegaly
(36)	MPS II - Hunter syndrome (Iduronate sulphatase deficiency)	Speech delay, deafness, coarse facies
(37)	MPS III - San Filippo syndrome	Macrocepahaly, hepatosplenomegaly
(38)	MPS IV - Morquio syndrome (galatcose-6-sulfatase or beta-galactosidase deficiency)	Bone dysplasia, skeletal abnormalities
(39)	MPS VI - Maroteaux-Lamy syndrome (N-acetylglucosamine-4-sulphatase deficiency)	Joint contractures, airway obstruction, and dysmorphic features
(40)	Multiple acyl CoA dehydrogenase deficiency (Glutaric aciduria type II or MADD)	Hypoglycaemia, hyperammonaemia, and acidosis
(41)	Multiple carboxylase deficiency	Seizures, dermatitis
(42)	Non-ketotic hyperglycinaemia (glycine cleavage system defect)	Hiccups, hypotonia
(43)	Ornithine carbamoyl transferase deficiency	Episodic vomiting, behavioural changes
(44)	Phenylalanine hydroxylase deficiency (classical PKU)	Intellectual disability, strong urine smell
(45)	Propionyl CoA carboxylase deficiency (propionic aciduria)	Acidosis
(46)	Succinic semialdehyde dehydrogenase deficiency	Developmental delay in speech
(47)	Tyrosine aminotransferase deficiency (tyrosinaemia type II, occulocutaneous tyrosinaemia)	Corneal ulcers, plantar/palmer keratosis, and photophobia
(48)	Uridine monophosphate synthase deficiency [UMPS deficiency (hereditary orotic aciduria)]	Failure to thrive, developmental delay and anaemia
(49)	Xanthine oxidase deficiency (xanthinuria)	Renal calculi

CAP: College of American Pathologists; FTT: failure to thrive; HGSA: Human Genetics Society of Australasia; IMD: inherited metabolic disorder; PKU: phenylketonuria; UTI: urinary tract infection

are very different from all other external quality assessment programmes for analyses performed in routine biochemical, immunological or microbiological laboratories. While the former required laboratories to provide an actual diagnosis and suggestions for further investigations in addition to test results, the latter required laboratories to report only results of quantitative or qualitative analyses which were then compared against the target values for the control specimens or the mean values obtained by all participants using the same test procedures and instruments. The programmes provide a real challenge to participating laboratories as they present the opportunity for studying a wide variety of IMDs, some of which have yet to be encountered in diagnostic work on patients referred to the laboratory. The samples taken include those collected during different phases of the various IMDs (i.e., acute phase, remission and during treatment) as well as from patients with no apparent IMDs. This mimics the true range of specimens received by the laboratory. The Participant Summary Reports enabled us to assess our performance, provided information on

Table 2. Specimens on Which Diagnoses Could Not be Offered or Incorrect Diagnoses Were Given

Year	No. of samples		Final diagnoses (reason for incorrect diagnoses or inability to diagnose)
	Total	(Problem Cases)	
1996	7 (2)	(1)	SSADH deficiency (unable to recognise significance of 4-hydroxybutyric acid)
		(2)	MPS III (did not perform high-resolution electrophoresis because urine MPS level was within reference range; this taught us not to rely on quantitative assay alone for interpretation.)
1997	14 (3)	(1)	OTC deficiency carrier (unable to detect orotic acid and uracil in first urine extract)
		(2)	Hypophosphatasia (unable to recognise significance of elevated phosphoethanolamine)
		(3)	Argininosuccinic acid lyase deficiency (unable to identify argininosuccinic acid and its anhydrides; this later helped us to identify the "unknown peaks" in the amino acid chromatogram.)
1998	8 (1)	(1)	Molybdenum cofactor deficiency (unable to recognise S-sulphocysteine)
1999	16 (3)	(1)	LCHAD deficiency (unable to recognise significance of 3-hydroxydicarboxylic acids)
		(2)	Barth's syndrome (unable to associate cardiomyopathy with 3-methylglutaconic aciduria)
		(3)	Patient with no mucopolysaccharidoses (misled by clinical presentation)
2000	12 (2)	(1)	GGT deficiency (unable to identify reduced glutathione peak)
		(2)	Mucolipidosis type II (unable to perform thin-layer chromatography because of insufficient urine specimen provided)
2002	20 (3)	(1)	MCAD deficiency (unable to distinguish between MCAD deficiency and GAII based on organic acids profile)
		(2)	Aromatic L-amino acid decarboxylase deficiency (unable to identify the significance of vanillactic acid)
		(3)	Succinic semialdehyde dehydrogenase deficiency (unable to recognise significance of 4-hydroxybutyric acid, insufficient experience of new lab staff)
2003	20 (3)	(1)	MPS III (unable to distinguish MPS from that of a healthy child)
		(2)	MCAD (missed hexanoylglycine)
		(3)	Biotinidase deficiency (unable to distinguish between another 2 disorders with isovaleric acidemia)
2004	19 (4)	(1)	Ketoacidosis (unable to correlate absence of glycine conjugates excludes fatty acid oxidation defects)
		(2)	Hepatic failure due to paracetamol toxicity (did not recognise significance of paracetamol)
		(3)	Alkaptonuria (homogentisic acid was not detected)
		(4)	MPS I (dermatan sulphate band was not detected in MPS HRE)

Total 116 (21)

GGT: gamma-glutamyl transferase; HRE: high-resolution electrophoresis; LCHAD: long chain 3-hydroxy acyl CoA dehydrogenase; MCAD: medium chain acyl CoA dehydrogenase; MPS: mucopolysaccharidosis; OTC: ornithine transcarbamylase (or ornithine carbamoyl transferase); SSADH: succinic semialdehyde dehydrogenase

alternative methods of analysis and helped to improve our approach to patient investigations and diagnoses.

Communications with HGSA programme organisers on misdiagnosed cases provided us with the opportunity to identify previously "unknown" metabolites, which are now stored in our "library of standards" on abnormal metabolites. This will enable us to arrive at a correct diagnosis for future patients with similar presentations and laboratory findings. For example, we learnt in the case of one of the specimens that some unknown peaks labelled by us were actually argininosuccunic acid and its anhydrides, which are present in the urea cycle defect, argininosuccinic acid lyase deficiency. This helped us to identify these metabolites in one patient. We also learnt from the programme that alloisoleucine is an excellent marker for maple syrup urine disease (MSUD), as the branched chain amino acids and their keto-acid metabolites may not be markedly elevated in some patients. The inclusion of patients without an IMD has alerted us to the need to interpret laboratory findings with an open mind. For example, abnormal metabolites could arise from disturbed metabolism due to bacterial infestation of the gut, poisoning or other metabolic derangement not associated with an IMD. We found a high level of propionic acid in one urine specimen. This acid should not be present in the urine of normal subjects. The clinical information provided was that of a gastrointestinal problem, which was not characteristic of the IMD propionic aciduria. We concluded that the "propionic aciduria" was probably due to bacterial contamination. It turned out to be a patient with short bowel syndrome, with bacterial growth producing propionic acid.

A deficiency of the CAP programme was the way participating laboratories were asked to provide a single specific diagnosis when several were possible. When crucial clinical information or characteristic laboratory test results are not available, making a specific diagnosis is neither possible nor desirable. Doing so without clear supportive evidence may lead to incorrect, inappropriate or unnecessary patient treatment. It is like giving a diagnosis of myocardial infarction based solely on an elevated serum creatine kinase (CK) test result, without the relevant clinical information and/or a concurrent increase in serum CKMB

isoenzyme and troponin T concentrations. Clearly, elevated serum CK level can be caused by a variety of pathological and physiological states, such as trauma or injury to the muscle, diseases affecting the muscle, insect bites, and malignancy. When markedly elevated tyrosine was found in a plasma specimen, a general diagnosis of tyrosinaemia could be made with certainty. However, it was not possible to arrive at a definitive diagnosis, whether it is a case of tyrosinaemia type I (hepatorenal tyrosinaemia) with prominent hepatic pathology, or a tyrosinaemia type II (occulocutaneous tyrosinaemia) with obvious ocular symptoms. A characteristic feature of the former is the presence of succinylacetone in urine. When urine specimen was not provided for testing of succinylacetone and clinical information on the presence of hepatic or ocular symptoms was not available, it was not possible to distinguish between type I and II tyrosinaemia, which require different management. Another example of CAP programme's deficiency is its requirement to provide a definitive diagnosis for a MPS subtype when urine mucopolysaccharide analysis alone was not adequate for such a decision. Contrary to CAP's inflexibility, the organiser of the Australasian programme advised all participating laboratories that differentiation of MPS types I, II and VI based on the pattern of mucopolysaccharide excretion in urine is unreliable. Enzyme analyses need to be performed. Therefore, HGSA programme advised laboratories to report a diagnosis of "MPS types I, II or VI". Had our general diagnoses of tyrosinaemia and MPS for 2 specimens been considered acceptable in the absence of crucial information, our rate of correct diagnosis in the CAP programme would have been 91.3% instead of 82.6%.

An annual meeting was held for HGSA programme participants to share experience and to learn more from the experts invited by the programme organisers. The proceedings of these meetings are sent to all participating laboratories. We are not aware of similar meeting organised by CAP. We were able to communicate with experts and other participants of the HGSA programme directly should we need to seek information and assistance. This was not the case with the CAP programme.

Conclusion

We performed well in both the Australasian HGSA and the American CAP proficiency testing programmes for IMD laboratories, having consistently diagnosed about 82% of cases correctly for each. These programmes were helpful in evaluating not only our analytical performance but also in improving our ability in interpreting results. They greatly increased our knowledge and experience, and enhanced our confidence in the competency and quality of our diagnostic service. The experience also shows that it is important for physicians referring patients for laboratory investigations to indicate the time of specimen collection, patient history, presenting symptoms, and medication to enable the proper interpretation of tests results.

(Note: The HGSA programme was revised in mid-2005 to take into consideration the specific national accreditation requirements of Australia. In its new format, participants must become a member of the HGSA before they can subscribe to the programme. Participating laboratories were no longer listed by name. Although all reported results were still included in the summary report distributed by the programme organiser, they could not be traced to specific laboratories to maintain anonymity. We continue to participate in the new programme.)

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