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Laboratory Guide to the Methods in Biochemical Genetics

Foreword by C. R. Scriver

With 176 Figures and 113 Tables

Springer

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Foreword

This book, which is authored by numerous authorities, presents as a stand-alone handbook for those working in the field of human biochemical genetics. It is a far cry from the day when there were rather simple descriptions of "side room tests", as Garrod called them.

Garrod recognized patients in whom there were disruptions of dynamic biochemistry, that is of pathways, as we now know them. He also recognized that the disruption could be inherited; hence his descriptive term "inborn errors of metabolism". Garrod used his tests to reveal "chemical individuality", a theme that dominated his writing and thinking throughout his lifetime. Garrod described chemical phenotypes as they were revealed in his patients with inborn errors of metabolism. Garrod's observations introduced a new paradigm in medical thinking.

The range and sophistication of the methods used to describe the chemical phenotype at the beginning of the 21st century are very different from those that were available to Garrod at the beginning of the 20th century. This new book describes a spectrum of tests, from simple screening methods that we could all do, to analytical methods that are dependent on technologies that very few of us will ever use.

Behind the chemical phenotype is a "biochemical phenotype". The latter describes disorder in a dynamic function; any function that modifies a molecule or moves it from here to there. How to measure a function that can be disrupted by a mutant gene is an important part of this laboratory guide.

The present laboratory guide goes beyond the chemical phenotype, beyond the biochemical phenotype, to examine the genotype, where instructions to maintain normal biochemical function and the normal metrical trait values begin. In my own experience, for example, I have witnessed the power of methods that can identify a novel chemical phenotype by chromatography, then confirmed the corresponding biochemical phenotype by using the appropriate functional analysis, and finally revealed the actual mutant allele in the gene in question.

Authors of this book, and certainly many of its readers, will recall the series called "Methods in Enzymology"; this laboratory guide could become a counterpart. In the post-genome era, when one can talk about the metabolome and the phenome, or refer to networks, nodes, hubs, edges and exemplars, it is likely that this laboratory guide only begins to describe the methods we will soon be using in human biochemical genetics. And surely we will need to have colleagues who can create the mathematical models and algorithms to describe the complexity of the phenotype.

Last, but not least, there is always the need for interpretation of results and assessment of outcomes, particularly now that tandem mass spectrometry is increasingly embedded in newborn screening. This laboratory guide serves the expertise upon which interpretation depends. Accordingly, the correct use of a laboratory guide is certain to be multidisciplinary, and at the end of the inquiry and analysis, one will know better why this person has this disease, now.

Charles R. Scriver

Preface

Experienced chefs may harbor several recipes for delicious entrees in their memory banks. The rest of us, who cook either for enjoyment or sustenance, often follow recipes, where lists of ingredients are meticulously described, accompanied by details for mixing and baking that are key to a successful outcome. Which of us hasn't realized that the omission of one small item such as baking powder can lead to a very "flat" cake? We learn that adherence to the recipe is the key to good results, and this is the basis of the usefulness of "cookbooks."

So follows the current tome entitled "Laboratory Guide to the Methods in Biochemical Genetics". The last methodology-driven book on this subject was edited by Dr. Frits Hommes in 1991, a comprehensive and well-referenced manual that is found in the laboratories of most biochemical geneticists and clinical biochemists. While a stalwart in the laboratory, Hommes' tome has become dated due to methodological advances and the addition of high-throughput, high-sensitivity equipment. The current endeavor is geared to fill the "methodological" gap in laboratory techniques that has developed over the last several years. Together with two previous books "Physician's Guide to the Diagnosis of Metabolic Diseases" and "Physician's Guide to the Treatment and Follow-Up of Metabolic Diseases", this book completes the trilogy.

In recent years, biochemical genetics has witnessed enhanced exposure as a laboratory discipline because of the advent of expanded newborn screening around the world. Sample numbers in most biochemical genetics laboratories are growing because of the required evaluation of positive newborn screening results. Accordingly, it is in everyone's interest (physician, patient, metabolic specialist, and other healthcare providers) that biochemical genetics laboratories have the most up-to-date methods available on their test menu.

The editors of this new book have thereby attempted a compilation of laboratory tests that will be useful in most laboratories. Each chapter has been developed by experts, with the goal of making each chapter "self-sufficient." The utility of such a structure will prevent the need to reference other technique papers, enabling a more rapid implementation of techniques in the laboratory. The majority of methods in biochemical genetics are labor-intensive and complex, especially when compared to the automated testing laboratory, and therefore a laboratory-based compendium should be a useful and valuable adjuvant. This has been our goal in compiling this edition.

To those of our readers and colleagues who are well-versed in the biochemical genetics laboratory, we hope that this book will serve to augment already established protocols or to fill gaps in the test menu. To others just starting a laboratory, we wish you happy biochemical cooking!

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Abbreviations

^{1}H NMR	proton nuclear magnetic resonance
2-AB	2-aminobenzamide
3α5β	3α-hydroxy-5βH
3-MD	3-methyldopa
3OHB	3-hydroxybutyrate
4-MU	4-methylumbelliferone
5α-RD-2	5α-reductase-2
5αTHF	5α-tetrahydrocortisol
5HIAA	5-hydroxyindoleacetic acid
5-OH-Me-ura	5-hydroxymethyluracil
5PD	5-pregnene-3β,20α-diol
5PT	5-pregnene-3β,17α,20α-triol
7DHC	7-dehydrocholesterol
7DHPT	5β-pregn-7-ene-3α,17α,20α-triol
8DHPT	5β-pregn-8-ene-3α,17α,20α-triol
18-oxo-THF	18-oxo-tetrahydrocortisol
6-MPH$_4$	6-methyltetrahydropterin
17HP	17α-hydroxypregnenolone
17-OHP	17-hydroxyprogesterone
18-OHF	18-hydroxycortisol
18-OH-THA	18-hydroxytetrahydro-11-dehydrocorticosterone
AA	arachidonic acid
AAA	amino acid analyzer
AADC	aromatic amino acid decarboxylase
AASA	α-aminoadipic semialdehyde
AA-TLC	amino acid thin layer chromatography
ABCA1	ATP-binding cassette transporter A
ABL	abetalipoproteinemia
ACAC	acetoacetate
ACRS	amplification-created restriction site
ACTH	adrenocorticotrophic hormone
AdoHcy	S-adenosylhomocysteine
AdoMet	S-adenosylmethionine
AGAT	L-arginine:glycine amidinotransferase
AIP	acute-intermittent pophyria
ALAD	δ-aminolevulinate dehydratase

ALAS	δ-aminolevulinic acid synthese
ALD	adrenoleucodystrophy
ALD	aldehyde dehydrogenase
AME	apparent mineralocorticoid excess syndrome
AMACR	2-methyl acyl-CoA racemase
AMN	adrenomyeloneuropathy
An	androsterone
APCI	atmospheric pressure chemical ionization
apoA-1	apolipoprotein A1
apoA-IV	apolipoprotein AIV
apoB100	apolipoprotein B-100
apoC	apoliprotein C
apoCII	apolipoprotein CII
ApoC-III	apolipoprotein C III
ApoE	apolipoprotein E
ApoE2	apolipoprotein E-2
APPI	atmospheric pressure photoionization
AR	aldose reductase
ASA	argininosuccinate
BAs	bile acids
BE	Bond-Elut
BH_2	7,8-dihydrobioperin
BH_4	tetrahydrobiopterin
BME	2-mercaptoethanol
bp	base pairs
B-PABA	biotinyl-p-aminobenzoic acid
Br-AMN	2-bromoacetyl-6-methoxynaphthalene
BSA	bovine serum albumin
BSTFA	N,O,-bis-(trimethylsilyl)trifluoroacetamide
CA	cholic acid
CAH	congenital adrenal hyperplasia
CACT	carnitine acylcarnitine translocase
CDCA	chenodeoxycholic acid
CDG	congenital disorders of glycosylation
CDPX2	Conradi-Hunermann syndrome
CE	capillary electrophoresis
CEP	congenial erythropoitic prophyria
CER	cholesterol esterification rate
CETP	cholesteryl ester transfer protein
CFD	cerebral folate deficiency
CMP	cytidine-5'-monophosphate
CNS	central nervous system
COMT	catechol-O-methyltransferase
CPA UK	UK Clinical Pathology Accreditations
CPC	cetylpyridinium chloride
CPT-I	carnitine palmitoyltransferase type I
CPT-II	carnitine palmitoyltransferase type II
Cr	creatine
CR	carbonyl reductase

CSF	cerebrospinal fluid
CTX	cerebrotendinous xanthomatosis
CV	coefficient of variance
d_2CDCA	[11,12-d_2] chenodeoxycholic acid
D_2O	deuterium oxide
DABCO	1,4-diazabicyclo(2,2,2)octane
DCA	deoxycholic acid
DEAE	diethylaminoethyl
DGGE	denaturing gradient gel elecrophoresis
DHA	docosahexaenoic acid
DHA-P	dihydroxyacetone phosphate
DHB	2,5-dihydroxybenzoic acid
DHCA	dihydroxycoprostanic acid
DHCR7	7-dehydrocholesterol reductase
DHEA	dehydroepiandrosterone
DHPLC	denaturing high-pressure liquid chromatography
DHPR	dihydropteridine reductase
DHT	dihydrotestosterone
DMAB	dimethyl-aminobenzaldehyde
DMB	1,9-dimethylene blue
DMEM	Dulbecco`s Modified Eagle Medium
DMG	N,H-dimethylglycine
DMSO	dimethyl sulfoxide
DNPH	dinitrophenylhydrazine
DOC	deoxycorticosterone
DRD	dopa-responsive dystonia
DTE	dithioerythriol
DTNB	dithionitrobenzoic acid
DTPA	diethylenetriaminepentaacetic acid
DTT	dithiothreitol
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EIC	extracted-ion chromatogram
ELISA	enzyme-linked immunosorbent assay
ELSD	evaporative light scattering detector
EPA	eicosapentaenoic acid
EQA	external quality assurance
ERNDIM	European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism
ES	electrospray
ESI	electrospray ionization
Et	etiocholanolones
F-1,6-P	D-fructose 1,6-6 bisphosphate
F-1-P	D-fructose-1-phosphate
F-6-P	fructose-6-phosphate
FAO	fatty acid β-oxidation
Fb	fibroblasts
FCS	fetal calf serum

FDB	familial defective apolipoprotein B-100
FH	familial hypercholesterolemia
FHBL	familial hypobetalipoproteinemia
FIGLU	formiminoglutamate
FRET	fluorescence resonance energy transfer
G-3-P	glycerol-3-phosphate
G-6-P	glucose-6-phosphate
G6-PDH	glucose-6-phosphate dehydrogenase
GABA	gamma-aminobutryc acid; 4-aminobutyric acid
GABA-T	GABA-transaminase
Gal-DH	galactose dehydrogenase
GALT	galctose-1-phosphate uridyltransferase
GAMT	guanidinoacetate methyltransferase
GC	gas chromatography
GCA	glycocholic acid
GC-EI-MS	gas chromatography coupled with electron impact ionization mass spectrometry
GC/FID	gas chromatography with flame ionization detection
GCDCA	glycochenodeoxycholic acid
GC-MS	gas chromatography-mass spectrometry
GDCA	glycodeoxycholic acid
GDH	α-glycerophosphate dehydrogenase
GEMO	glycerol-ether-monooxygenase
GK	glycerol kinase
GKD	glycerol kinase deficiency
GLCA	glycolithocholic acid
GRA	glucocorticoid remediable aldosteronism
GSD	glycogen storage disease
GTP	guanosine triphosphate
GTPCH	GTP cyclohydrolase I
GUDCA	glycoursodeoxycholic acid
H_2NTP	7,8-dihydroneopterin triphosphate
H6PDH	hexose-6-phosphate dehydrogenase
Hb	hemoglobin
HBDH	hydroxybutyrate dehydrogenase
HC	hereditary coproporphyria
HCA	hyocholic acid
HCS	holocarboxylase synthetase
Hcy	homocysteine
HDCA	hyodeoxycholic acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HDL	high-density lipoproteins
HFB	heptafluorobutyric acid
HFI	hereditary fructose intolerance
HL	hepatic lipase
HMP	2-hydrazino-1-methylpyridine
HMBS	hydroxymethylbilane-synthase
HPA	hyperphenylalaninemia
HPLC	high performance liquid chromatography

HPIC	ion-exchange chromatography
HRP	horseradish peroxidase
HSDH2	3α-hydroxysteroid dehydrogenase type 2
HTLC	high-turbulence liquid chromatography
HTP	hexamethyl disilazane/chlorotrimethyl silane/pyridine
HVA	homovanillic acid
IDL	intermediate-density lipoproteins
IEF	isoelectric focusing
ILBP	ileal lipid-binding protein
INF-γ	interferon-γ
IQC	internal quality control
IS	internal standard
ISSD	infantile sialic acid storage disease
IVPM	in vitro probe medium
KB	ketone body
LAH	lipoid adrenal hyperplasia
LC-MS/MS	liquid chromatography mass spectrometry/mass spectrometry
LCA	lithocholic acid
LCAT	lecithin:cholesterol acyltransferase
LCHAD	long-chain 3-hydroxyacyl-coenzyme A dehydrogenase
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
LDLR	low-density-lipoprotein receptor
L-dopa	3,4-dihydroxy- L-phenylalanine
LLO	lipid-linked oligosaccharides
LOD	lower detection limit
LOQ	lower reporting levels
LPDS	lipoprotein-deficient medium
LPL	lipoprotein lipase
Lp(X)	lipoprotein X
MALDI-TOF	matrix assisted laser desorption ionization – time of flight mass spectrometry
ManNAc	N-acetylmannosamine
MAO	monoamine oxidase
MAT	methionine adenosyltransferase
MCA	muricholic acid
MCAD	medium-chain acyl-CoA dehydrogenase
MEM	minimum essential medium
MLPA	multiplex ligation-dependent probe amplification
MnO_2	manganese dioxide
MoM	multiples of the median
MO-TMS	methoximetrimethylsilyl
MPH	male pseudohermaphroditism
MPS	mucopolysaccharidosis
MPS I	mucopolysaccharidosis type I, Hurler syndrome
MPS II	mucopolysaccharidosis type II, Hunter syndrome
MPS III	mucopolysaccharidosis type III, Sanfilippo syndrome
MPS IV	mucopolysaccharidosis type IV, Morquio syndrome
MPS V	mucopolysaccharidosis type V, Scheie syndrome

MPS VI	mucopolysaccharidosis type VI, Maroteaux-Lamy syndrome
MPS VII	mucopolysaccharidosis type VII, Sly syndrome
MPS-EP	mucopolysaccharide electrophoresis
MRC	mitochondrial respiratory chain
MRI	magnetic resonance imaging
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MSUD	maple syrup urine disease
MTBSTFA	N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide
MtDNA	mitochondrial DNA
MTHF	5,10-methylene-tetrahydrofolate
MTHFR	5,10-methylene-tetrahydrofolate reductase
MTP	microsomal triglyceride transfer protein
MU-Gal	4-methylumbelliferyl-β-D-galactopyranoside
MU-NeuAc	4-methylumbelliferyl-α-D-N-acetylneuraminic acid
MW	molecular weight
NAD	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
NCI	negative chemical ionisation
NEQAS	National External Quality Assessment Service
NeuAC	N-acetylneuraminic acid
NeuGc	N-glycolylneuraminic acid
NKH	nonketotic hyperglycinemia
NMR	nuclear magnetic resonance
NPC	Niemann-Pick disease type C
OD	optical density
ODS	octadecylsilyl
OGS	oligosaccharide
OMIM	Online Mendelian Inheritance in Man
OPA	orthophthalaldehyde
ORD	oxidoreductase deficiency
OSS	octane sulphonic acid
PA	pipecolic acid
PAGE	polyacrylamide gel electrophoresis
PABA	4-aminobenzoic acid
PAH	phenylalanine-4-hydroxylase
PBC	primary biliary cirrhosis
PBG	porphobilinogen
PBS	phosphate-buffered saline
PC	pyruvate carboxylase
PCA	perchloric acid
PCD	pterin-4a-carbinolamine dehydratase
PCR	polymerase chain reaction
PCSK9	proprotein convertase subtilisin/kexin 9
PCT	porphyria cutanea tarda
PDA	pentadecanoic acid

p-DABA	p-dimethylaminobenzaldehyde
PDH	pyruvate dehydrogenase
PFB	pentafluorobenzyl bromide
PFBBr	pentafluorobenzylbromide
PGDH	3-phosphoglycerate dehydrogenase
PGI	phosphoglucose isomerase
PH-I	primary hyperoxaluria type I
PH-II	primary hyperoxaluria type II
PKU	phenylketonuria
PLP	pyridoxal phosphate
PMI	phosphomannose isomerase
PMM	phosphomannomutase
PNPO	pyridox(am)ine-5'-phosphate oxidase
PT	pregnanetriol
PTONE	pregnanetriolone
PTP	6-pyruvoyltetrahydrobiopterin
PTPS	6-pyruvoyltetrahydrobiopterin synthase
PUFA	polyunsaturated fatty acids
PV	porphyria variegata
PVDF	polyvinylidene fluoride
QC	quality control
RBC	red blood cells
RCDP	rhizomelic chondrodysplasia punctata
RIA	radioimmunoassay
RO	reverse osmosis
RPA	relative peak area
RPI	ribose-5-phosphate isomerase
rpm	revolutions per minute
RXLI	recessive X-linked ichthyosis
SASD	free sialic acid storage disease
SBDF	ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate
SCAD	short-chain acyl-CoA dehydrogenase
SD	Salla disease
SDS	sodium dodecylsulfate
SIM	single ion monitoring
SLC6A8	Na^+-Cl^--dependent creatine transporter
SLOS	Smith-Lemli-Opitz syndrome
SPE	solid-phase extraction
SR	sepiapterin reductase
SR-BI	scavenger receptor B1
SSA	sulfosalicylic acid
SSADH	succinate semialdehyde dehydrogenase
SSCP	single-stranded conformational polymorphism
StAR	steroidogenic acute regulatory
STSD	steroid sulfatase deficiency
TAE	Tris-acetate-EDTA buffer
TALDO	transaldolase
TBA	thiobarbituric acid

TBE	Tris-borate-EDTA buffer
TCA	taurocholic acid
TCA	trichloroacetic acid
TCDCA	taurochenodeoxycholic acid
TDCA	taurodeoxycholic acid
TDeABr	Tetrakis (decyl) ammonium bromide
TEa	total error allowable
TEA	triethanolamine
TFA	trifluoroacetic acid
TH	tyrsosine-3-hydroxylase
THA	tetrahydro-11-dehydrocorticosterone
THAldo	tetrahydroaldosterone
THB	tetrahydrocorticosterone
THE	tetrahydrocortisone
THF	tetrahydrocortisol
THCA	trihydroxycoprostanic acid
THcy	total homocysteine
THS	tetrahydro-11-deoxycortisol
TIC	total ion current
TIM	triosephosphate
TLC	thin-layer chromatography
TLCA	taurolithocholic acid
TMA	trimethylamine
TMCA	tauromuricholic acid
TMCS	trimethylchlorosilane
TMR	tetramethylrhodamine
TMS	trimethylsilyl
TMSI	trimethylsilylimidazole
TNF-α	tumor necrosis factor-α
TOCSY	total correlated spectroscopy
TOF	time of flight
TPH	tryptophan-5-hydroxylase
Tris	tris(hydroxymethyl)-amino-methane
TSP	trimethylsilyl-2,2,3,3-tetradeuteropropionic acid
TUDCA	tauroursodeoxycholic acid
UDCA	ursodeoxycholic acid
UDP	uridine diphosphate
UDP-GlcNAc	UDP-N-acetyl-D-glucosamine
uE_3	unconjugated estriol
UEFA	unesterified fatty acids
UFE	urinary free cortisone
UFF	urinary free cortisol
VLCFA	very-long-chain fatty acids
VLDL	very-low-density lipoproteins
X-ALD	X-linked adrenoleukodystrophy
ZSDs	Zellweger spectrum disorders

1.1 Laboratory Strategies in Biochemical Genetics

Rodney Pollitt

1.1.1 Introduction

For the first 50 years after Archibald Garrod outlined the concept of an inborn error of metabolism in 1904 the number of such conditions identified grew only slowly. Thereafter, the pace of discovery quickened, powered by the successive emergence of new laboratory techniques such as paper chromatography, gas chromatography and gas chromatography-mass spectrometry (GC-MS), techniques that were all rapidly taken up by routine diagnostic laboratories. Simultaneously, laboratory automation and the commercial availability of a wider variety of biochemicals were bringing enzyme assays, for lysosomal disorders in particular, within the range of a routine service. In the last decade the application of electrospray ionisation tandem mass spectrometry (MS/MS) has opened up additional prospects. Even more recently, the automation of DNA analysis has developed to the stage where it is practicable to use gene sequencing for primary diagnosis. A quick glance at the contents page of this book indicates the scope of what is now possible. The problem is how to incorporate these new capabilities into routine healthcare provision in a rational and cost-effective manner. This entails both an informed approach to test requesting and a laboratory service organised appropriately for the range and volume of work thus generated.

1.1.2 Selection for Investigation

Unless there is a population-wide screening programme in place, the first steps in the diagnosis of an inherited metabolic disease are clinical suspicion and putting in train the appropriate laboratory investigations. The number of samples submitted and the range of tests being requested are both increasing.

In some disorders there is a clearly recognisable phenotype, often a named syndrome that has later been linked to a specific biochemical cause. However, once the biochemical basis of such a syndrome has been recognised it often becomes apparent that milder variants, *formes frustes* showing only some of the features of the classical syndrome, are relatively common. The diagnostic assay then changes from being a rarely used confirmatory test to one that is requested relatively frequently for a variety of less specific indications. The same phenomenon is seen where the abnormal phenotype develops progressively, as in most of the lysosomal disorders, and a bat-

tery of specific enzyme assays may be requested for any child showing possible early signs.

At the other end of the scale, many of the acutely presenting metabolic disorders give few clinical indicators of their underlying biochemical cause, and a rather non-selective approach to laboratory investigation is appropriate. Where time is of the essence, there is a natural tendency to order broad-spectrum "screening" tests and more specific investigations simultaneously, with little attempt to follow a logical diagnostic sequence. There is particular pressure with investigations such as organic acid or amino acid chromatography where results may be required "immediately", but most laboratories find that the proportion of samples giving a diagnostic result is extremely low. However, suggestions that such investigations should be performed in an acutely ill child only if other biochemical indications such as hypoglycaemia or hyperammonaemia are present have been strongly opposed [1]. Another approach to limiting demand is to proscribe common clinical presentations where metabolic investigations are usually unproductive. In the opening page of their "Vademecum Metabolicum" [2], Zschocke and Hoffmannn list six categories of patient where special metabolic investigations are not required. The trouble with either of these proposals is that they will result in a small proportion of cases being diagnosed late or not at all. This may not only compromise the effective treatment of the patient concerned, but also result in further affected children being unwittingly born into the family.

A more structured approach has been devised by Saudubray and Charpentier [3] that is based on the careful consideration of clinical detail and uses an extensive series of diagnostic algorithms. However, the editors' note that accompanies that article warns that the chapter is "an experiment", that patients have derangements in complex, overlapping, "non-categorical biological systems", and that we are "currently faced with the classical dilemma of balancing the practicality of categorical thinking against the reality of biologic variation". Perhaps clinical judgement is still as much an art as a science, and therefore any attempt on the laboratory's part to manage work-load by systematically restricting access would be highly problematical. A better solution is to increase laboratory capacity, although, depending on the financial structures in place, this may not always be a practical proposition.

1.1.3 Choosing the Biological Level

In classical inborn errors of metabolism there is a causal thread linking the defect in the gene with defective function or absence of an enzyme or transporter protein, the consequent excess or lack of one or more metabolites, and the clinical phenotype. Correspondingly, laboratory investigation can be at the nucleotide, protein or metabolite levels. Selecting the appropriate level at which to begin an investigation is an important component of the dialogue that should exist between laboratory and clinical services. In general (the lysosomal disorders being a notable exception), the metabolite level is to be preferred because a single analysis of urinary organic acids or plasma steroids, for example, has the potential to uncover many different disorders. Measurement of overall metabolite flux through a pathway using cultured cells can also be a useful group test, as in fatty acid oxidation disorders. Assays of enzymic activity are specific to individual disorders and are often most useful as con-

firmatory tests. Analysis at the DNA/RNA level is even more specific. Most disorders are heterogeneous with many disease-causing mutations, only a small proportion of which are covered by commercially available test kits. One can readily confirm a diagnosis by analysing for such mutations, but total exclusion is seldom possible. However, techniques that can quickly check for mutations in long stretches of DNA are passing into routine diagnostic use and will overcome this problem. In some of the glycogenoses, where the metabolic indicators are rather non-specific and enzyme assays are difficult and require a liver sample, gene sequencing is proving to be a practicable alternative, even though there are sometimes several candidate genes to be investigated.

The apparent simplicity of the one gene, one protein, one disease paradigm has long been discounted, but we are only now beginning to appreciate fully the complexity of the biological systems involved. Interpretational problems abound. A priori prediction of the clinical effect of a new mutation may be difficult, as apparently benign mutations can affect gene splicing or exert other effects. Even with well-known mutations there are limitations. Databases linking genotype to phenotype are available for several disorders, but there is often a considerable range of clinical expression. Siblings concordant for the same mutant genes may be affected to very different degrees and we have only slight inklings as to what the modifying factors might be. Newborn screening for cystic fibrosis or for medium-chain acyl-CoA dehydrogenase deficiency both suffer from the resulting uncertainty. In both disorders "mild" and "severe" genotypes have been identified, correlating with phenotype in cystic fibrosis and with varying degrees of residual enzyme activity in vitro in medium-chain acyl-CoA dehydrogenase deficiency, respectively. In theory, this additional information should help to refine the screening process. In practice, defining appropriate management schemes for presymptomatic cases with "mild" genotypes presents a major challenge. Similar problems arise in some disorders that are normally diagnosed and characterised only at the metabolite level, phenylketonuria being a prime example.

It is important that molecular genetic tests are integrated properly with other investigations and it is unfortunate that in some health systems molecular genetics has evolved in isolation from other diagnostic services. Some molecular genetics laboratories offer highly specific tests, for the common 985A>G mutation of the medium-chain acyl-CoA dehydrogenase gene in cases of unexplained infant death, for example, without any assurance that appropriate preliminary investigations have been performed and they are at least examining the right gene. In unselected cases of sudden infant death, for every definitive diagnosis (two copies of the 985A>G mutation) there will be approximately two cases where only one copy of the mutant gene is found. In only a minority of the latter will a second disease-causing mutation be present; most will be unaffected carriers – a situation that must be explained to the parents and requires very delicate handling.

The undoubted successes of molecular genetics have lead to exaggerated perceptions of its predictive powers.[1] The resulting sensitivity that legislators and the general public now attach to "genetic information" is an additional, extraneous complication when investigating metabolic disease. Genetic testing has been variously

1 "A smudge of spit on a beer glass leaves a person as vulnerable as if they had left their medical dossier, business card and personal journal lying on the bar top." Science correspondent – The Guardian (UK), February 2002

defined, from the very general "tests that provide information used for diagnosing an inherited disorder" to the more specific "a test to detect the presence or absence of, or change in, a particular gene or chromosome". Thus, even amino acid chromatography could be classed as a genetic test and, according to some official guidelines, be the subject of a formal informed consent process before the sample is collected, seemingly out of place in the context of emergency investigation of an acutely ill child. In practice, attention is generally focussed on analysis of the gene itself; equally specific information at the protein level, in sickle cell anaemia for example, is seen as less threatening – a good example of "genetic exceptionalism". Legal requirements vary from country to country, but in most it would be unwise to proceed to DNA analysis without explicit patient or parental permission.

1.1.4 Laboratory Provision

There is huge diversity in the way that specialist laboratory services in biochemical genetics are provided. Configurations often owe as much to historical chance as to rational planning. At one time, most laboratories serving the larger hospital centres would offer a selection of test-tube and chromatographic tests for the classical inborn errors of metabolism. With the growing sophistication and complexity of the field, the introduction of external quality-assessment schemes, and an ever-increasing workload in other areas, such investigations have tended to migrate progressively to more specialised biochemical genetics centres. However, clinical chemistry departments serving acute paediatric units still need to perform at least a limited range of tests, such as ammonia or lactate in plasma, on a 24 h/day emergency basis. A rapid turn-around service for plasma amino acids and urinary organic acids chromatography would also be advantageous. However, adequate local provision is not always feasible and external quality assessment has shown that laboratories carrying out such work on an occasional basis often perform poorly. It is usually better to have a more comprehensive service organised on a regional basis with rapid courier transport of samples.

Regional centres need to be of a certain size with sufficient sample throughput to justify the capital investment required, the effort needed to ensure technical reliability of the assays offered, and to give staff sufficient on-going experience of specialist interpretation. As a minimum, each centre requires a quantitative amino acid analyser, GC-MS for organic acids analysis and, increasingly, a tandem mass spectrometer with an electrospray ion source. Mixed usage, combining paediatric work with a toxicology service, for example, has in general not proved a satisfactory solution. The larger biochemical genetics laboratories will cover a wider range of activities including newborn screening, tissue culture, enzymatic assays, and molecular genetics, although few, if any, will offer the full range of investigations described in this "Laboratory Guide". Thus, at all levels, a collaborative networking approach is required.

Many of the larger biochemical genetics laboratories are in academic medical centres and make a significant contribution to research. They have collectively been responsible for much of the progress made in recent years. However, there is always a tension between research, where data need to be accumulated into publishable packets and a degree of secrecy prevails, and clinical care, where results need to reported immediately, perhaps to distant clinicians with their own publication needs.

The belief in some quarters that patients have proprietary rights over their samples and any information generated therefrom, and that even anonymised data should not be divulged without permission may cause difficulties even for routine services with no overt research brief, putting interlaboratory audits and external quality assessment schemes at risk.

Particular problems arise when a laboratory attached to some other academic discipline such as paediatrics or pure biochemistry has become the world centre for investigating some extremely rare or difficult disease. Such laboratories are unlikely to satisfy the increasingly arduous demands of accreditation and are by nature evanescent, depending on the research interests of the academic head and supported by time-limited research funds. They tend to work at a different tempo to the routine service and new information, perhaps obtained months or years after the sample was first received, does not always find its way back into the patient's clinical notes. At some stage in the life cycle of such projects the balance will have shifted from new, cutting-edge research to routine service provision, calling for some form of technology-transfer partnership with a larger diagnostic centre.

1.1.5 Impact of New Technologies

The workload is increasing in both volume and scope in all branches of laboratory medicine. In mainstream clinical chemistry, the past 25 years have seen astonishing developments in assay technology and instrumentation; assays that were performed manually with a few dozen assays a day can now be accomplished automatically by the thousands. In biochemical genetics (molecular genetics aside), automation has had a less dramatic impact.

Instrumental developments in GC-MS have brought this technique into routine diagnostic service. It is no longer necessary, as in the early 1970s, to sit by the machine and press a button when a mass scan is required, but sample preparation and interpretation of the output remain labour-intensive. For urinary organic acids, for example, the maximum daily throughput is still only a dozen or so samples per instrument, and attempts to automate interpretation of the resulting profiles by pattern recognition [4] or deviation of prescribed metabolites from a defined range [5] have so far have not resulted in general use. Other more traditional investigations, qualitative and quantitative amino acid chromatography, for example, also remain low-volume and labour-intensive investigations.

More recently, MS/MS and related ion-trap techniques have opened up groups of metabolites that have previously been difficult to analyse, acylcarnitines being the prime example. High throughput (40 or more samples per hour) is achievable and very little sample preparation is required. However, this will do little to reduce the workload elsewhere in the laboratory, as plasma acylcarnitine analysis is complementary to urinary organic acid analysis rather than replacing it. Amino acids are also amenable to MS/MS, but the technique has significant limitations for this group of metabolites and can not completely replace the more time-consuming traditional methods.

High-resolution nuclear magnetic resonance spectroscopy also has the potential for rapid sample throughput and could expand the range of metabolites readily detectable. The instrumentation is expensive and interpretation requires considerable

experience, restricting the technique at present to a few academic centres. If this technique does become affordable for routine diagnostic laboratories it, like GC-MS and MS/MS, is likely to increase the overall workload rather than reduce it.

Newborn screening is a special case where automation has considerable potential. In several countries screening is being concentrated in laboratories screening between 50,000 and 100,000 births per year, this being regarded as the optimum range for economical operation of a single-tandem mass spectrometer. Such a throughput has only become achievable through automatic data handling; specimens where a metabolite concentration or a particular concentration ratio falls outside the specified range are flagged for individual attention. Microarray technology is also being introduced into newborn screening, with a commercial laboratory in the USA screening over 300,000 babies per year, making extensive use of microarray mutation analysis as a second-tier screen.

Centralisation on this scale is out of the question for most biochemical genetics investigations, as the cost of developing the systems required can not be supported by the overall level of demand. There may be some scope for laboratory networks to rationalise test repertoires and invest in a more limited degree of automation, an approach most suited to assays giving simple, readily interpretable, numerical results. More complex tests, particularly related group tests such as organic acids, acylcarnitines and amino acids, are best performed and evaluated together in the same centre. The range of options available will vary with the healthcare environment in which laboratories operate; only free-standing commercial laboratories are able to adopt an outright business-lead approach. Many existing laboratories are embedded within hospitals that provide tertiary paediatric services and, as discussed in the previous section, would need to maintain a substantial core of investigations for urgent use. The quest for micro-economic "efficiency" should not be allowed to destroy the essentially interactive nature of biochemical genetics, where relationships between clinic and laboratory at different levels provide not just a more effective service, but also the intellectual and emotional stimulation that has made this such a rewarding field in which to work.

References

1. Holton JB. Diagnosis of inherited metabolic disease in acutely ill children. Ann Clin Biochem 1982; 19:389–395
2. Zschocke J, Hoffmann GF. Vademecum Metabolicum. A Manual of Metabolic Paediatrics. Stuttgart and New York: Milupa-Schattauer, 1999
3. Saudubray JM, Charpentier C. Clinical phenotypes: diagnosis/algorithms. In: Scriver CR, Beaudet AL, Sly WS, et al. (eds) The Metabolic and Molecular Bases of Inherited Disease, 8th edn, New York: McGraw Hill, 2001, pp 1327–1403
4. Karjalinen EJ, Karjalinen UP. Speeding up the identification of anomalies in urinary organic acids. J Inherit Metab Dis 2003; 26 Suppl 2:49
5. Kimura M, Yamamoto T, Yamaguchi S. A personal computer-based system for interpretation of gas chromatography mass spectrometry in the diagnosis of organic acidaemias. Ann Clin Biochem 1999; 36:671–672

1.2 Quality Control and Quality Assurance in the Biochemical Genetic Laboratory

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1.2.1 Introduction

When using medical laboratories, physicians frequently assume that the results provided are accurate and precise. Those working in the laboratory environment know that this is true only within the constraints of the methods, technology and processes available. In this chapter we will explore how internal quality control (IQC) and external quality assurance (EQA) procedures can inform both the analyst and the physician about the limitations of laboratory testing and permit a more intelligent use of laboratory data, while at the same time indicating areas where improvement is required.

1.2.2 Laboratory Accreditation

While the specialist physician may develop an in-depth knowledge of the strengths and limitations of the laboratory that they use regularly, the occasional user and the patient require the reassurance offered by accreditation systems. In general, accreditation systems follow the principles of healthcare assessment described by Donabedian [5]:

1. Providing guidance concerning the appropriate structures to be in place, for example staffing, equipment and buildings.
2. Ensuring that suitable processes are employed to guarantee reliable testing, for example the use of standard operating procedures, the conduct of IQC and participation in EQA.
3. Requiring examination of outcomes, for example audit and user survey.

The way in which accreditation is organised varies in different countries with organisations such as the Clinical Pathology Accreditation (CPA UK) in the UK and CCKL Test in The Netherlands. Accreditation may be optional or may be mandatory, as in the USA, and mediated by "deemed authorities" such as the College of American Pathologists. However, the standards to which these accreditation bodies operate are usually cross referenced to internationally agreed formulae such as:

1. ISO 9001:2000, which covers: the design of quality management systems; the role and practice of management; equipment, infrastructure and staffing; product realisation; monitoring, including audit and incident reporting. It is worth noting