Methodological accuracy and firm interpretation of enzymatic analysis: The usefulness of Bisswanger's "Practical Enzymology"

Sir,

Despite the enormous technological and scientific progress that biomedical sciences have witnessed over the last decades, the automation of the majority of laboratory techniques used for the conduction of routine assays for industrial, academic, and clinical purposes and the commercialization of a significant number of diagnostic tests into commercially-available kits, a significant percentage of the basic biomedical research output is still set up and conducted in a non-automatic, analytical bench-based biochemical manner.[1,2] Among these techniques, enzymatic analysis still plays a crucial role in pathophysiological and drug-screening basic and clinical research, as it reflects the configuration of the functionality of the studied enzymes.[3-5] Why would someone care for the overexpression of mRNA, the blotting of a monomer, or post-translational modification of a specific enzyme, if there would be no way to assess its actual activity: its ability to perform a specific reaction in relation to time? Thankfully, since the middle of the 20th century, there has been remarkable progress in the field that was (not much later) defined as "Enzymology", providing us with a very large number of specific methods that now allow us to determine with significant accuracy the activity of a plethora of enzymes in health and disease.[6] Moreover, it is also remarkable that (the majority of) these techniques are of significantly low-cost, require very basic laboratory equipment, and maintain unbeatable sensitivity dependent on the user's accuracy of setting up and execution.

Professor Hans Bisswanger of the University of Tübingen has recently published the second, completely revised edition of his book entitled *"Practical Enzymology."*[7] The book consists of five chapters, as well as an Appendix. The first chapter is a short *"Introduction"* that sets the context within which the author has approached (and served) the need for a practical manual for enzymology. The second chapter comprises a very useful and well-written piece of essential knowledge under the title *"General aspects of enzyme analysis"*; a must-read condensed version of the theoretical basis of enzymology, accompanied by amazing figures and very useful tables summarizing the basis that anyone involved in enzyme analysis should know. The third chapter is an extended assay-describing collection (entitled *"Enzyme assays"*) that provides a beautifully organized pattern of methodological approaches to a significant number of enzyme assays, overviewed in Table 1. An interesting

Table 1: Overview of the enzymes for which the activity determining assays by spectroscopic or other methods are included in Bisswanger's second edition of "*Practical Enzymology*".[7]

Enzyme's name; abbreviation (s)*	EC number	sM	oM
Alcohol dehydrogenase; ADH	1.1.1.1	[++]	-
Alcohol dehydrogenase ($NADP^+$)	1.1.1.2	[++]#	-
Homoserine dehydrogenase; AK-HDH I	1.1.1.3	[+]	-
Shikimate dehydrogenase	1.1.1.25	[+]	-
L-Lactate dehydrogenase; LDH	1.1.1.27	[+++]	-
Malate dehydrogenase; MDH	1.1.1.37	[+]	-
Malate dehydrogenase (oxaloacetate-decarboxylating) (NAD^+)	1.1.1.38	[+]	-
Malate dehydrogenase (decarboxylating)	1.1.1.39	[+]	-
Malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$)	1.1.1.40	[+]	-
Isocitrate dehydrogenase (NAD^+); IDH	1.1.1.41	[+]	-
Isocitrate dehydrogenase ($NADP^+$); ICDH	1.1.1.42	[+]	-
Glucose-6-phosphate dehydrogenase; G6P-DH	1.1.1.49	[+]	-
Glucose oxidase; GOD	1.1.3.4	[+]	-
Formate dehydrogenase; FDH	1.2.1.2	[+]	-
Glyceraldehyde-3-phosphate dehydrogenase; GAPDH	1.2.1.12	[++]	-
Pyruvate dehydrogenase (acetyl-transferring); PDH, E1p	1.2.4.1	[++]	-
Oxoglutarate dehydrogenase (succinyl-transferring); OGDH, E1o	1.2.4.2	[+]	-
Pyruvate ferredoxin oxidoreductase	1.2.7.1	[+]	-
Alanine dehydrogenase	1.4.1.1	[++]	-
Glutamate dehydrogenase	1.4.1.3	[+]	-
Leucine dehydrogenase; LeuDH	1.4.1.9	[+]	-
L-Amino acid oxidase	1.4.3.2	[+]	-
D-Amino acid oxidase	1.4.3.3	[+]	-
Monoamine oxidase	1.4.3.4	[+]	-
Primary amine oxidase	1.4.3.21	[+]	[+]
Diamine oxidase	1.4.3.22	-	[+]
Urate oxidase	1.7.3.3	[+]	-
Dihydrolipoamide dehydrogenase; E3	1.8.1.4	[++]	-
Glutathione disulfide reductase	1.8.1.7	[+]	-
Catalase	1.11.1.6	[+]	-
Peroxidase	1.11.1.7	[+++]	-

Contd...

Table 1: Contd

Enzyme's name; abbreviation (s)*	EC number	sM	oM
Luciferase	1.13.12.7	[+]	-
Dihydrolipoamide acetyltransferase; E2p	2.3.1.12	[++]	-
Fatty acid synthase	2.3.1.85	[+]	-
Phosphorylase a	2.4.1.1	[+]	-
Aspartate transaminase; AAT	2.6.1.1	[+]	-
Alanine transaminase	2.6.1.2	[+]	-
Tyrosine transaminase; TAT	2.6.1.5	[+]	-
Tryptophan transaminase; Tam 1	2.6.1.27	[+]	-
Phenylalanine transaminase	2.6.1.58	[+]	-
Hexokinase; HK	2.7.1.1	[+]	-
Pyruvate kinase; PK	2.7.1.40	[+]	-
Acetate kinase; AK	2.7.2.1	[+]	-
Phosphoglycerate kinase; PGK	2.7.2.3	[+]	-
Aspartokinase; AK	2.7.2.4	[+]	-
Lipase	3.1.1.3	[+]	[+]
Phospholipase A_2	3.1.1.4	-	[+]
Acetylcholinesterase; AChE	3.1.1.7	-	[+]
Cholinesterase; ButChE	3.1.1.8	[+]	[+]
S-Formylglutathione hydrolase	3.1.2.12	[+]	-
Alkaline phosphatase	3.1.3.1	[+]	-
Acid phosphatase	3.1.3.2	[+]	-
Ribonuclease (pancreatic); RNase I	3.1.27.5	[+]	-
α-Amylase	3.2.1.1	[+]	-
Amyloglucosidase	3.2.1.3	[+++]	-
β-1,4-Glucanase	3.2.1.4	[+]	[+]
β-Glucosidase	3.2.1.21	[+]	[+]
Lysozyme	3.2.1.17	[+]	-
Sialidase	3.2.1.18	[+]	[+]
α-Glucosidase	3.2.1.20	[++]	-
β-Galactosidase	3.2.1.23	[+]	-
β-Fructosidase	3.2.1.26	[+]	-
β-Glucuronidase	3.2.1.31	[+]	-
Proteases (general assays)	3.4	[++++]	-
Leucine aminopeptidase; LAP	3.4.11.1	[++]	-
α-Chymotrypsin	3.4.21.1	[++]	-
Trypsin	3.4.21.4	[+]	-
Pancreatic elastase	3.4.21.35	[++]	-
Pepsin	3.4.23.1	[+]	-
Asparaginase; ASNase	3.5.1.1	[+]	-
Glutaminase	3.5.1.2	[+]	[+]
Urease	3.5.1.5	[+]	[+]
Adenosinetriphosphatase; ATPase	3.6.1.3	[+]	-
Pyruvate decarboxylase; PDC	4.1.1.1	[+]	-
Glutamate decarboxylase; GAD	4.1.1.15	-	[+]
Aldolase; ALDC	4.1.2.13	[+]	-
Anthranilate synthase	4.1.3.27	[+]	-
Carbonic anhydrase; CA	4.2.1.1	[+]	[+]
Fumarase	4.2.1.2	[+]	-
Glucose/xylose isomerase	5.3.1.5	[++++]	-
Phosphoglucomutase; PGM	5.4.2.2	[+]	-
Tyrosine-tRNA ligase	6.1.1.1	[+]	[+]
Glutamine synthetase	6.3.1.2	[+]	-

Notes: *The names and abbreviations of the enzymes are presented as provided in the reviewed book, with minor modifications and additions where needed; EC, Enzyme Commission; sM, spectroscopic method described, that includes absorption (UV/Vis) photometry, fluorescence photometry, turbidity measurements, luminometry or polarimetry; oM, other method described, that includes electrochemical, radioactive or other techniques; [+], each cross in square brackets corresponds to one assay described; #identical method with the ones required for glucuronate dehydrogenase (EC 1.1.1.19), mevaldate reductase (EC 1.1.1.33) and lactaldehyde reductase (EC 1.1.1.55); Assays are also provided for the pyruvate dehydrogenase complex (EC 1.2.4.1, EC 2.3.1.12, EC 1.8.1.4) and the α-oxoglutarate dehydrogenase complex (EC 1.2.4.2, EC 2.3.1.61, EC 1.8.1.4)

Table 2: Overview of enzymes of interest, the assays of which could be included in a future edition of Bisswanger's "*Practical Enzymology*"

Enzyme name (s)*	Abbreviation (s)	EC number
NADH-dehydrogenase; cytochrome c reductase	NADH-red	1.6.99.3
Glutathione reductase	GSR; GR	1.8.1.7
Cytochrome c oxidase	CcO; COX	1.9.3.1
Glutathione peroxidase	GSH-Px, GPx	1.11.1.9
Alkylglycerol monooxygenase	AGMO	1.14.16.5
Dopamine *β*-hydroxylase	DBH	1.14.17.1
Tyrosinase	TYR	1.14.18.1
Superoxide dismutase	SOD	1.15.1.1
Ornithine carbamoyltransferase	OCT	2.1.3.3
Choline acetyltransferase	ChAT	2.3.1.6
Carnitine O-acetyltransferase	CRAT; CrAT	2.3.1.7
γ-Glutamyltransferase; *γ*-glutamyl transpeptidase	*γ*-GT; GGT	2.3.2.2
Citrate synthase	CS	2.3.3.1 #
ATP citrate lyase	ACLY; ACL	2.3.3.8 ##
Purine nucleoside phosphorylase; inosine phosphorylase	PNPase	2.4.2.1
Glutathione *S*-transferase	GST	2.5.1.18
Creatine kinase; creatine phosphokinase	CK; CPK	2.7.3.2
Protein kinase C	PKC	2.7.11.13
Glyoxalase-II; hydroxyacylglutathione hydrolase	Glo-II	3.1.2.6
Steroid sulfatase; steryl-sulfatase	STS	3.1.6.2
5'-Nucleotidase	5'NT	3.1.3.5
Glucose 6-phosphatase	G6Pase	3.1.3.9
Cyclic 3',5'-mononucleotide phosphodiesterases	PDE	3.1.4.17
α-Mannosidase; *α*-D-mannoside mannohydrolase	-	3.2.1.24
N-Acetyl-*β*-D-glucosaminidase	NAG; NAGase	3.2.1.52 ###
Alanine aminopeptidase	AAP	3.4.11.2
Angiotensin-converting enzyme	ACE	3.4.15.1
Cathepsin B; APP secretase	APPS	3.4.22.1
Guanine deaminase; guanine aminohydrolase	GAH	3.5.4.3
Magnesium adenosinetriphosphatase	Mg^{2+}-ATPase	3.6.3.2 ####
Sodium-potassium adenosinetriphosphatase	Na^+, K^+-ATPase	3.6.3.9 ####
Glyoxalase-I; lactoylglutathione lyase	Glo-I	4.4.1.5
Adenylate cyclase; adenylyl cyclase	AC	4.6.1.1
Glucose-6-phosphate isomerase	PGI	5.3.1.9
Acetyl-CoA synthetase; acetate-CoA ligase	ACS; ACAS	6.2.1.1

Notes: *The enzymes presented in this table are chosen based on the authors' subjective belief of assaying usefulness and/or frequency of appearance in the biomedical literature; #formerly listed as EC 4.1.3.7; ##formerly listed as EC 4.1.3.8; ###formerly listed as EC 3.2.1.30; ####formerly listed as EC 3.6.1.3

chapter on *"Binding measurements"* follows as the fourth chapter of Bisswanger's *"Practical Enzymology,"* providing readers with valuable data concerning the experimental design, the performance and the interpretation of binding measurements through a number of state-of-the-art and classic techniques. The final chapter focuses on *"Enzymes in*

technical applications" and elegantly categorizes and presents the methodological approaches to enzyme immobilization and analysis. Supplementary material and useful animations for the book's figures are also provided on a companion web site.[8]

The author is an experienced and accomplished enzymologist, who manages to provide the reader with an accurate, informative, and yet simple to read, understand, and use manual; the simplicity of the writing style and the avoidance of nonessential nomenclatures or information reflects the extensive knowledge of the field that forms the basis of the book's authorship as well as a focused determination to provide a practical guide for anyone who wants to set up an enzymatic assay. Moreover, the book serves in providing the reader with very useful and well-visualized data on the actual interpretation of the results that can be obtained through enzymology and the described assays; the latter is undoubtedly of high importance, as firm interpretation of any performed enzymatic assay is equally important to its methodological accuracy and *vice versa*.[9]

We strongly recommend Bisswanger's *"Practical Enzymology"* to the readers of the *Journal of Natural Sciences, Biology, and Medicine* involved in biomedical research. We believe that this manual has the potential to become a classic of its kind provided that the author will: (a) maintain the simplicity of the writing style and the practical approach to its content in future editions and (b) build up on the provided pool of enzymatic assay protocols. Table 2 provides an overview of further enzymes, the assays of which could (according to our opinion) be of benefit to readers if they were to be included in a future edition of Bisswanger's *"Practical Enzymology."*

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