

STANDARDIZATION IN CLINICAL ENZYMOLOGY:
A CHALLENGE FOR THE THEORY OF METROLOGICAL TRACEABILITY
(CIRME)

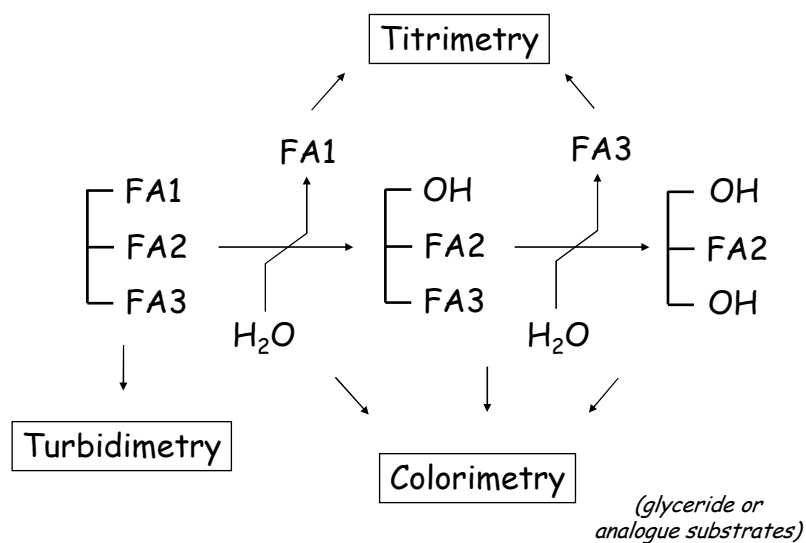
Jean-Marc Lessinger (Milano, November 2008)

Theoretical bases for standardizing pancreatic lipase measurements

- Methods for lipase
- Lipase calibrators and lipase reference materials
- Possibilities for lipase reference procedure

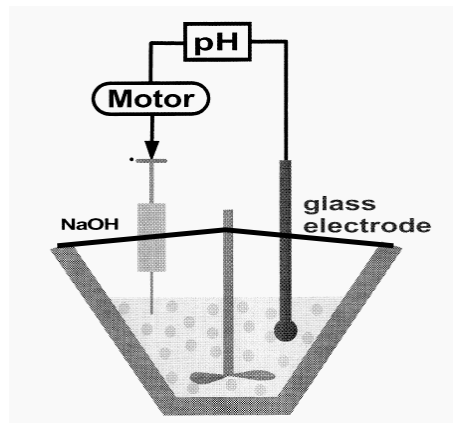
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Measurement of lipase activity



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Measurement of lipase in titrimetry (pH-stat)



(Beisson *et al.*, Eur. J. Lipid Technol., 2000)

Lipase is injected in a closed reaction vessel containing the emulsified substrate

The lipase activity is measured by recording the amount of titrant (NaOH) added to maintain the pH at a constant value during the reaction

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Routine methods for lipase

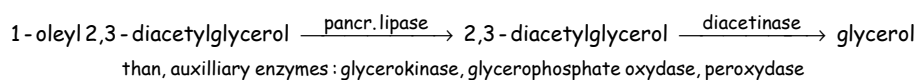
• Turbidimetry



(Ziegenhorn *et al.*, Clin. Chem., 1979)

- Has been widely used
- Narrow measuring range
- Is subjected to unexpected absorbance increases ("negative" lipase activity)

• Reflectometry



(Mauck *et al.*, Clin. Chem., 1984)

- Low specificity
- Substrate also hydrolysed by nonpancreatic esterases

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Routine methods for lipase

- Spectrophotometry (550 nm)

1,2-diglyceride $\xrightarrow{\text{pancr. lipase}}$ 2-monoglyceride $\xrightarrow{\text{bact. lipase}}$ glycerol
 than, auxilliary enzymes : glycerokinase, glycerophosphate oxydase, peroxydase (quinone monoimine dye)

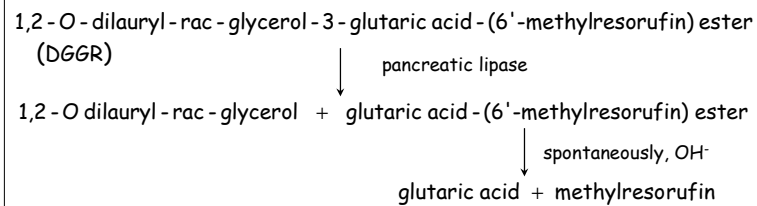
(Imamura *et al.*, Clin. Chem., 1989)

- 1-position : predominantly palmitic and oleic acids
- Board range of linearity
- Has replaced turbidimetric assays (good correlation) - Often used during the past decade
- Subject to interference from glycerol ?, esterases ?
- Reagent-to-reagent carryover : cannot conveniently be used on instruments on which triglyceride (cholesterol) assays are performed (washing procedures)

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Routine methods for lipase

- Spectrophotometry (580 nm)

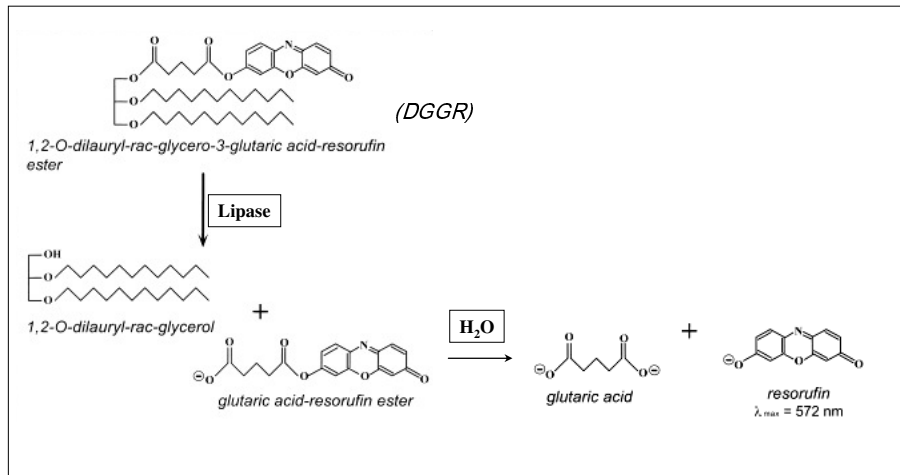


(Prinzing *et al.*, Clin. Chem., 1998)

- Compared to methods based on the use of diglyceride as substrate :
 - Simpler reaction scheme (2 reactions as opposed to 5)
 - Less interferences
 - Seems to have increased specificity
- Replaces progressively diglyceride-based assays (*e.g.* Roche, Beckman-Coulter, Siemens, ...)

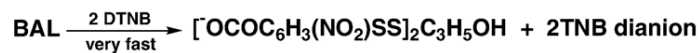
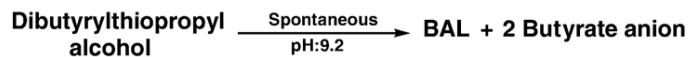
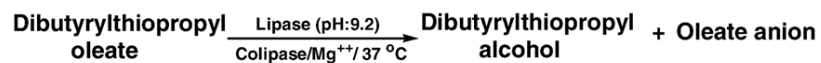
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Colorimetric method for lipase using DGGR



(Beisson *et al.*, Eur. J. Lipid Technol., 2000) 7

A new procedure for lipase using a thioester substrate



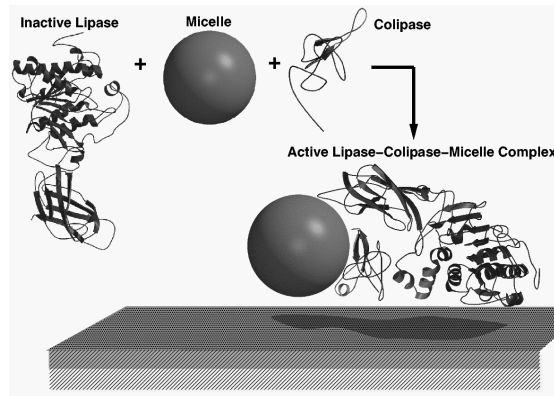
BAL: 2,3-Dimercapto-1-propyl alcohol
 DTNB: 5,5'-Dithio-bis-(2-nitrobenzoic acid)
 TNB: 5-Thio-2-nitrobenzoic acid

(Yamada *et al.*, Clin. Chim. Acta, 2007)

- Spectrophotometry (TNB, 412 nm)
- Not yet commercially available method
- Seems to have similar performance than DGGR method

Effectors for specificity and optimal reactivity

Bile salts, colipase, calcium chloride

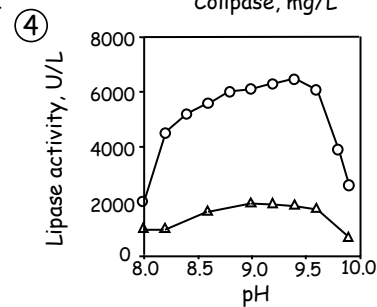
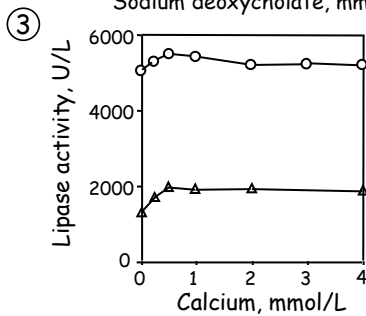
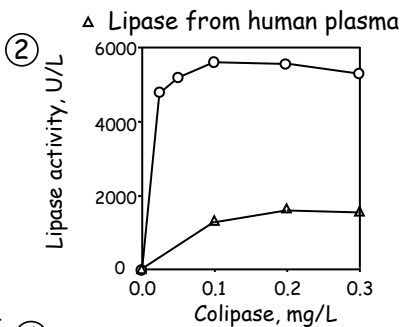
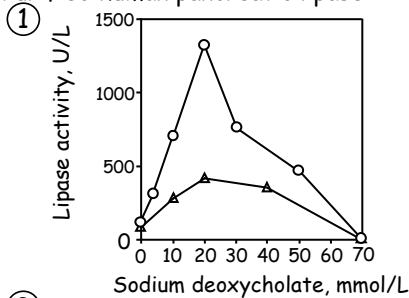


(Hermoso *et al.*, 1995)

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Exemple of optimisation of conditions

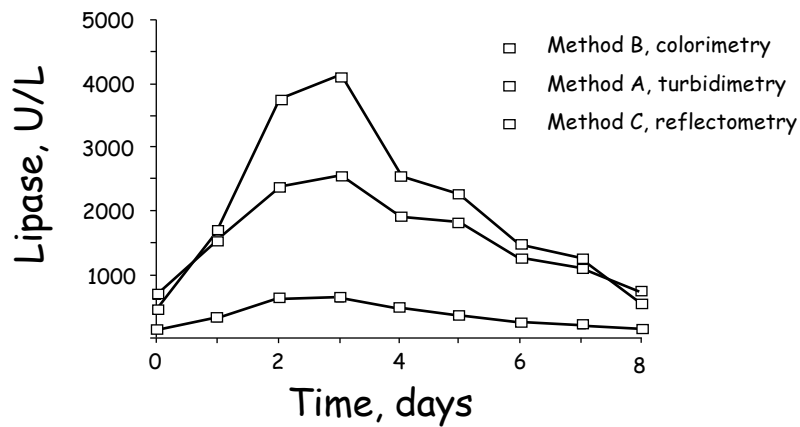
○ Purified human pancreatic lipase



(Lessinger *et al.*, Clin. Chim. Acta, 1996) 10

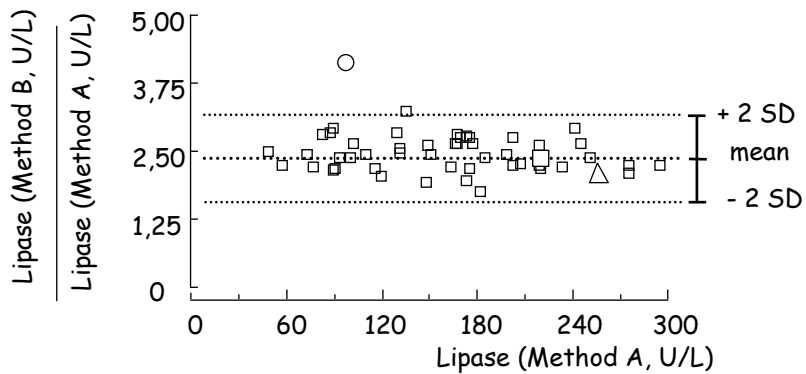
Results of lipase activity according to routine method

Example of the following of a patient with acute pancreatitis



(Lessinger *et al.*, Eur. J. Clin. Chem. Clin. Biochem., 1994) 11

Commutability of three materials



- Patients' results (mean \pm 1 SD) : 2.39 ± 0.31
- Calibrator C_B : at mean + 4.36 SD
- △ Calibrator C_A : at mean - 1.75 SD
- Candidate RM : at mean - 0.17 SD

(Lessinger *et al.*, Clin. Chem., 1996) 12

Effect of different calibration conditions

Conditions of calibration	Between-method ratio
• Manufacturer's instructions	2.39
• Same titration procedure of calibrators	1.25
• Same titration procedure + common calibrator	1.71
	C_B
	C_A
	1.17
	cRM
	1.01



- Between-method coherency of patients' results was dramatically improved by using cRM as calibrator
(Lessinger et al., Clin. Chem., 1996)

- Calibration with commutable materials permits a correction of original intermethod differences
(Cattozzo et al., Clin. Chem., 2001) 13

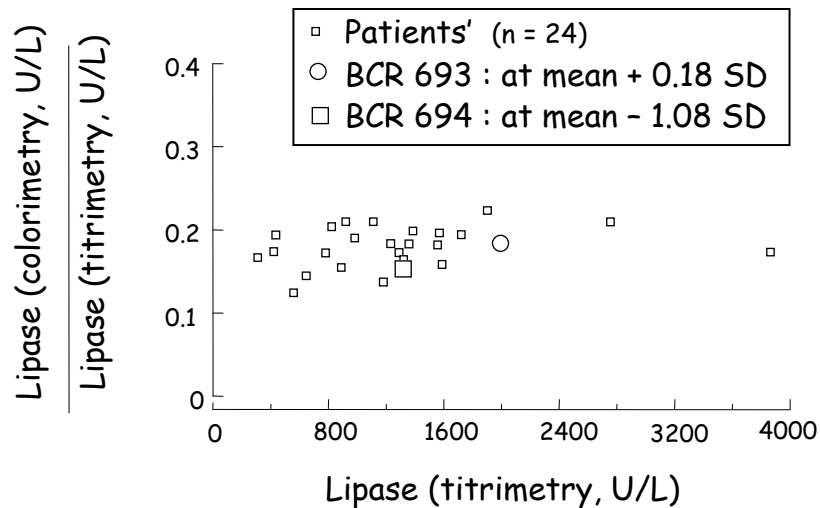
Lipase reference materials

(BCR, IRMM)

(Lessinger et al., Clin. Chem. Lab. Med., 2003 & 2004)

- Two lyophilised materials
 - Human pancreatic juice : BCR 693
 - Recombinant : BCR 694
- Catalytic properties
 - Identical to those exhibited by plasma enzyme
 - DOC, colipase, calcium, optimal pH, apparent Km
- Catalytic concentrations
 - Standardized titrimetric procedure, optimized (DOC, colipase, calcium, pH), 37 °C

Commutability of lipase RMs



(Lessinger *et al.*, Clin. Chem. Lab. Med., 2003)

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Possibilities for lipase reference procedure

- Progress in standardization for lipase depends on an international agreement on :
 - The substrate
 - Triglyceride (or analogue) with long-chain alkyl groups
 - Micelles of stable and reproducible size
 - The measurement procedure
- Possible candidates ? ...
 - Titrimetry, triolein
 - Spectrophotometry, DGGR
 - ...

37 °C, optimized conditions :
bile salt(s), colipase, calcium, pH

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Lipase reference methodology : (1) Titrimetry

- Is the proposed reference method for lipase for many years (Tietz et al., Clin. Chem., 1989 & 1993 - reviews)
- Optimized conditions have already been published (triolein, 100 g/L, 50 g/L, 5 g/L)
- Transferability of a standardized procedure has been described
- Need of specific instrumentation and procedures (different than for other enzymes)
- Problem of reproducibility and stability of the emulsion
→ preparation of "reference substrate" :
 - stabilized in a dry form by nebulization (feasibility has been done)
 - ex : stock emulsion in powder → [triolein] = 5 g/L (20 apparent Km) after reconstitution by the solution of effectors in concentration corresponding to optimized conditions

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Lipase reference methodology : (2) Spectrophotometry

- Same instrumentation than for other enzymes
- DGGR is the most commonly used substrate in routine
- Patent : substrate (DGGR), emulsification and stabilization process
- The micellar-solubilized substrate must not absorb or scatter incident light (clear solution), making it suitable for direct colorimetric reading
- Need to study the release and the absorption properties of methylresofurin in the reaction medium
- Need of information on the optimization of the reaction conditions
- Need of complementary studies on interferences

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Lipase reference methodology (3)

	Titrimetry, Triolein	Spectrophotometry, DGGR
Advantages	- Well documented - Proposed for many years	- Same instrumentation, procedures and expertise than for the other enzymes
Disadvantages	- Need of specific instrumentation, procedures and expertise	- Patent - Need of further studies
	- Reproducibility and stability of the micellar substrate	

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Conclusion

- Need for a reference system for pancreatic lipase measurement
- Feasibility studies have been done
- Interest of intermethod calibration with commutable materials has been demonstrated
- Necessity of consensus on the choice of the reference method

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