Theoretical bases for standardizing pancreatic lipase measurements

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

Measurement of lipase activity

- Titrimetry
- Turbidimetry
- Colorimetry (glyceride or analogue substrates)
Measurement of lipase in titrimetry (pH-stat)

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.

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

Routine methods for lipase

- **Turbidimetry**
  - triolein \(\rightarrow\) 2,3-diolein \(\rightarrow\) 2-monoolein
  - Has been widely used
  - Narrow measuring range
  - Is subjected to unexpected absorbance increases (“negative” lipase activity)

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

- **Reflectometry**
  - \(1\)-oleyl \(2,3\)-diacetylglycerol \(\rightarrow\) \(2,3\)-diacetylglycerol \(\rightarrow\) glycerol
  - Low specificity
  - Substrate also hydrolysed by nonpancreatic esterases

  (Mauck et al., Clin. Chem., 1984)
Routine methods for lipase

- **Spectrophotometry (550 nm)**
  
  1,2-diglyceride $\xrightarrow{\text{pancr. lipase}}$ 2-monoglyceride $\xrightarrow{\text{bact. lipase}}$ glycerol

  than, auxiliary enzymes: glycerokinase, glycerolphosphate oxydase, peroxydase (quinone monooimine dye)


  - 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)

- **Spectrophotometry (580 nm)**

  1,2-O-dilauryl-rac-glycerol - 3-glutaric acid - (6'-methylresorufin) ester (DGGR) $\rightarrow$ pancreatic lipase

  1,2-O-dilauryl-rac-glycerol + glutaric acid - (6'-methylresorufin) ester $\rightarrow$ spontaneously, OH$^-$

  glutaric acid + methylresorufin

  (Prinzeng 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, ...)


Colorimetric method for lipase using DGGR

1,2-O-dilauryl-rac-glycerol-3-glutamic acid-resorufin ester

Lipase

1,2-O-dilauryl-rac-glycerol

+ H$_2$O

$\text{glutamic acid-resorufin ester}$

$\text{glutamic acid}$

+$\text{resorufin}$ $\lambda_{\text{max}}=572\text{ nm}$

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

A new procedure for lipase using a thioester substrate

\[
\begin{align*}
\text{Dibutyrylthiopropyl olate} & \xrightarrow{\text{Lipase (pH 9.2), Collapsed/Mg^+}} \text{Dibutyrylthiopropyl alcohol} + \text{Oleate anion} \\
\text{Dibutyrylthiopropyl alcohol} & \xrightarrow{\text{Spontaneous, pH 9.2}} \text{BAL} + 2 \text{Butyrate anion} \\
\text{BAL} & \xrightarrow{2 \text{DTNB, very fast}} [\text{OCOC}_6\text{H}_3(\text{NO}_2)_2\text{SS}]_2\text{C}_2\text{H}_5\text{OH} + 2\text{TNB dianion} \\
\end{align*}
\]

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


- 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

Exemple of optimisation of conditions

1. Purified human pancreatic lipase
2. Lipase from human plasma
3. Calcium, mmol/L
4. pH

(Hermoso et al., 1995)

Results of lipase activity according to routine method

Example of the following of a patient with acute pancreatitis


Commutability of three materials

Patients’ results (mean ± 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)
**Effect of different calibration conditions**

<table>
<thead>
<tr>
<th>Conditions of calibration</th>
<th>Between-method ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>manufacturer’s instructions</td>
<td>2.39</td>
</tr>
<tr>
<td>same titration procedure of calibrators</td>
<td>1.25</td>
</tr>
<tr>
<td>same titration procedure + common calibrator</td>
<td>1.71, 1.17, 1.01</td>
</tr>
</tbody>
</table>

- Between-method coherency of patients’ results was dramatically improved by using cRM as calibrator
  
  \[(\text{Lessinger et al., Clin. Chem., 1996})\]
- Calibration with commutable materials permits a correction of original intermethod differences
  
  \[(\text{Cattozzo et al., Clin. Chem., 2001})\]

**Lipase reference materials**

(BCR, IRMM)

- Two lyophilised materials
  - Human pancreatic juice: BCR 693
  - Recombinant: BCR 694

- Catalytic properties
  - Identical to those exhibited by plasma enzyme
  - \(\text{DOC, colipase, calcium, optimal pH, apparent Km}\)

- Catalytic concentrations
  - Standardized titrimetric procedure, optimized (\(\text{DOC, colipase, calcium, pH}\), 37 °C)
Commutability of lipase RMs

![Graph showing commutability of lipase RMs](graph.png)


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
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

(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
**Lipase reference methodology (3)**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Titrimetry, Triolein</th>
<th>Spectrophotometry, DGGR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- Well documented</td>
<td>- Same instrumentation, procedures and expertise than for the other enzymes</td>
</tr>
<tr>
<td></td>
<td>- Proposed for many years</td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td>- Need of specific instrumentation, procedures and expertise</td>
<td>- Patent - Need of further studies</td>
</tr>
<tr>
<td></td>
<td>- Reproducibility and stability of the micellar substrate</td>
<td></td>
</tr>
</tbody>
</table>

**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