The impact of preanalytical factors on glucose concentration measurement
Glucose concentration measurement

- Samples without glucose: 40%
- Samples with glucose: 60%

- Other biochemistry tests: 15%
- Glucose: 85%

Samples:
- Ascites
- Urine
- CSF
- Blood
- Serum
- Plasma
- Capillary
- Venous
Total testing cycle

Preanalytical errors!
Sources of preanalytical errors

**Variability**
- Patient preparation
- Sample type
- Type of container
- Time of measurement
- Interferences

**Diagnostic errors**
- Delayed diagnosis
- Misdiagnosis
- Wrong diagnosis
# 1. Patient preparation

<table>
<thead>
<tr>
<th>Fasting glucose</th>
<th>Postprandial glucose</th>
<th>Oral glucose tolerance test</th>
</tr>
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<tr>
<td>• Fasting time?</td>
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<td>• Adherence to instructions</td>
</tr>
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<td>• Definition of fasting?</td>
<td>• Therapy (medications)</td>
<td>• Gestational DM</td>
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- Fasting glucose
  - Fasting time?
  - Definition of fasting?

- Postprandial glucose
  - Type of meal?
  - Therapy (medications)

- Oral glucose tolerance test
  - Adherence to instructions
  - Gestational DM
Croatian survey

- **CSM BLM** – WG for patient preparation
- March 2014
- Online survey on practices for patient preparation
- Heads of the laboratories
- Response rate: 118/206 = 57%
**Question 13:** According to your instructions for patient preparation, what is the required fasting time for glucose concentration measurement:

- A) at least 8 hours
- B) at least 10 hours
- C) at least 12 hours
- D) number of hours not specified
Definition of fasting?

Original papers

Are patients well informed about the fasting requirements for laboratory blood testing?

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- Survey on outpatients in the primary care laboratory
- N = 150

What does the fasting state mean?

- A) 12 hours since the last meal
- B) 10 hours since the last meal
- C) 8 hours since the last meal
- D) tea or coffee can be consumed in the morning
- E) light breakfast can be consumed in the morning
- F) last meal is the day before, exact time is not relevant
Harmonization and education

- Patients are not informed
- Heterogeneity of instructions
- Revision of existing guidelines
- Education of patients and laboratory staff

National recommendation on patient preparation

2015

CSM/BLM
WG for patient preparation
2. Sample type

- **Plasma** sample
  - Lower water content
  - Consumption of glucose during clotting

- **Serum** sample

Higher in plasma
2. Sample type

- Venous sample
- Capillary sample

- Fasting state
- Postprandial state

- Rate of the glucose consumption in the tissues
Quality of the capillary sample?

Venous plasma sample

Capillary plasma samples

Glucose concentration

$N = 20$ volunteers
$x 3$ (sample types)
$x 3$ (manufacturers)

Heated Cold

Simundic AM, Nikolac N, et al. Capillary sample quality: verification of three different lancets for capillary blood sampling; *Publication in process.*
Simundic AM, Nikolac N, et al. Capillary sample quality: verification of three different lancets for capillary blood sampling; Publication in process.

![Box plot showing bias vs. venous glucose](image)

- **P = 0.024**

**Bias vs. venous glucose**

- **Sarstedt**
- **BD**
- **Greiner**

- **Venous**
- **Capillary_warm**
- **Capillary_cold**
2. Sample type

- Serum/plasma and venous/capillary samples cannot be used interchangeably.
- Glucose measurement should always be performed in the same sample type.
3. Type of container / Time of measurement

- Metabolic processes continue in vitro (glucose, ammonia, lactate)

- Icy water slurry

- Rapid centrifugation (30 min)

- Separation from the cells

- Special additives to block the process
Glycolysis

Mannose

Glucose

Hexokinase

ATP

ADP

Phosphoglucoisomerase

Fructose 6-phosphate

ATP

ADP

Phosphofructokinase

Fructose 1,6-biphosphate

Aldolase

Dihydroxyacetone phosphate

Glyceraldehyde 3-phosphate

Glyceraldehyde 3-phosphate dehydrogenase

NAD$^+$ + Pi

NADH + H$^+$

1,3-Bisphosphoglycerate

Phosphoglycerate kinase

Glyceraldehyde 3-phosphate

Phosphoglycerate mutase

2-Phosphoglycerate

Enolase

Water

Phosphoenolpyruvate

Pyruvate kinase

F + inorganic phosphates $\rightarrow$ bound to Mg

F + inorganic phosphates $\rightarrow$ bound to Mg
Is NaF efficient?


Immediate centrifugation and separation from cells is superior to NaF!

NaF has up to 3 hours delay in glycolysis inhibition!
Problem with NaF?

Fluoride waiting for 30-90 minutes?
NaF blocks production of lactate immediately!
Glycolysis

Phosphorilation of sugars occurs until ATP is exhausted (60-90 minutes)

↓ 5-7% per hour

NaF
(Not so) New inhibitors?

Patent in 1986 by Terumo corporation:
- Citric acid, trisodium citrate pH 5.9
- Disodium EDTA (chelate Mg)
- NaF (prolonged inhibition)
Glucose concentration in the new tubes?

<table>
<thead>
<tr>
<th></th>
<th>Loss of glucose 2 h</th>
<th>Loss of glucose 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate buffer</td>
<td>↓ 0.3%</td>
<td>↓ 1.2%</td>
</tr>
<tr>
<td>NaF tube</td>
<td>↓ 4.6%</td>
<td>↓ 7.0%</td>
</tr>
</tbody>
</table>

Minimal loss of glucose in the first 2 hours, stable up to 24 hours.
Implementing new citrate buffer tubes:

- Higher mean glucose value for 0.8 mmol/L
  
  (Norman M, Jones I. Clin Biochem 2014;47:683-5.)

- Higher prevalence of gestational diabetes
  

- Higher prevalence of diabetes and lower prevalence of normal results
  
  (Juricic G, et al. Publication in process.)
Guidelines and recommendations

- Existing guidelines use cut-off values based on the old tubes
- Revision of cut-off values is required using the new tubes
- Notify clinicians about the change
4. Hemolysis

- Fluoride tubes have increased hemolysis rate
- NaF disrupts RBC membrane
- Catalase is released from RBC
- RBC glucose lower than serum

Hb > 0.15 g/L: 86.2% NaF vs. 2.2% SST
Generally, glucose is not sensitive to hemolysis

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Hb conc. (g/L)</th>
<th>Glucose conc. (mmol/L)</th>
<th>Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abbott</strong> (Abbott Park, IL, USA)</td>
<td>10</td>
<td>4.3</td>
<td>4.4% (10 g/L), 8.3% (20 g/L)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>6.6</td>
<td>1.7% (10 g/L), 4.0% (20 g/L)</td>
</tr>
<tr>
<td><strong>Beckman Coulter</strong> (Brea, California, USA)</td>
<td>5</td>
<td>?</td>
<td>&lt;3%</td>
</tr>
<tr>
<td><strong>Roche</strong> (Penzberg, Germany)</td>
<td>10</td>
<td>3.9</td>
<td>&lt;10%</td>
</tr>
<tr>
<td><strong>AMS Diagnostics</strong> (Weston, FL, USA)</td>
<td>1</td>
<td>?</td>
<td>&lt;10%</td>
</tr>
<tr>
<td><strong>Pointe Scientific</strong> (Canton, MI, USA)</td>
<td>?</td>
<td>?</td>
<td>? Do not analyse hemolysed samples.</td>
</tr>
<tr>
<td><strong>Teco Diagnostics</strong> (Anaheim, CA, USA)</td>
<td>4</td>
<td>?</td>
<td>? Negligible interference.</td>
</tr>
<tr>
<td><strong>Thermo Scientific</strong> (Waltham, MA, USA)</td>
<td>10</td>
<td>?</td>
<td>? No interference.</td>
</tr>
</tbody>
</table>

Acceptance criteria based on biological variation (Ricos et al. 2014):

\[ I = 2.3\%, \quad B = 1.8\%, \quad TE = 5.5\% \]
To conclude...

Preanalytical phase is the major source of variability for glucose measurement!

- Verify patient preparation
- Standardise sample type
- Follow recommendations on sample handling
- Type of container
- Verify interferences