มหาวิทยาลัยมหิดล
คณะแพทยศาสตร์ศิริราชพยาบาล

Laboratory tests in endocrine pancreatic diseases

Busadee Pratumvinit, MD
Department of Clinical Pathology
Faculty of Medicine Siriraj Hospital

“เอกสารและสื่อนี้ ใช้เพื่อ
ประกอบการเรียนการสอน ใน
หลักสูตรแพทยศาสตรบัณฑิต
คณะแพทยศาสตร์ศิริราชพยาบาล
ห้ามผู้ใดนำไปใช้ในวัตถุประสงค์อื่น
หากฝ่าฝืนอาจมีความรับผิดตาม
พระราชบัญญัติลิขสิทธิ์”

Outline

Laboratory tests in

- Diagnosis & monitor treatment of DM
- Complications of DM
- Hypoglycemia

Diagnosis and monitor treatment of DM

Glucose

Hemoglobin A1c

Glycated protein

Diagnosis & monitor treatment

Glucose

Diagnosis

1. FPG ≥ 126 mg/dL (7 mmol/L); no caloric intake 8 hr
2. RPG ≥ 200 mg/dL + symptom

Establish diagnosis of DM
Screening of high-risk individuals

→ Venous plasma

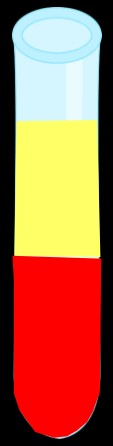
Fasting

→ Overnight fast (≥8 h)
Drawn in the morning

Water as desired

Sacks et al. Clin Chem. 2023 Aug 2;69(8):808-868

Glucose: specimen collection



Plasma contact with cells

➡ Glycolysis

5-7% (10 mg/dL) per hour

Varies with the glucose concentration, temperature, WBC count, other factors

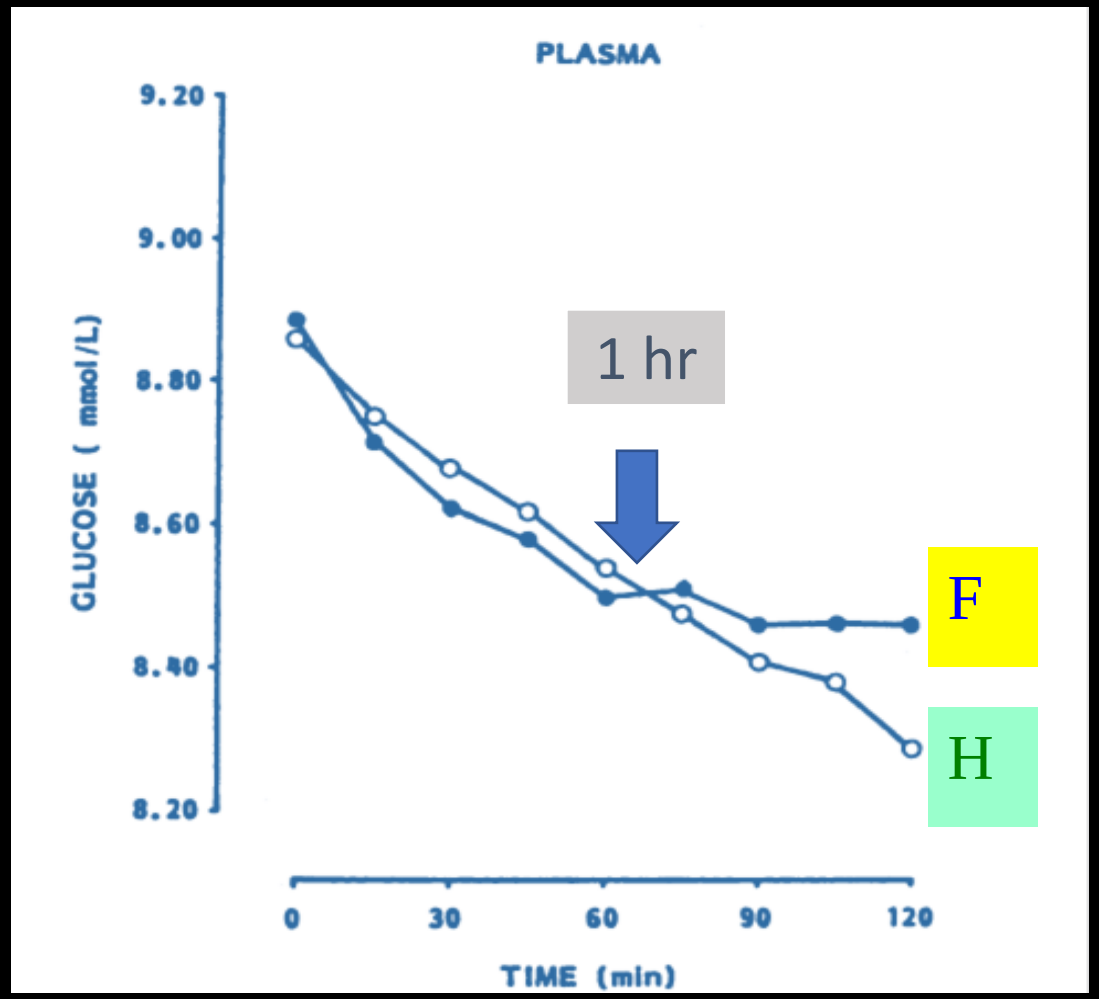
ถ้าไม่สามารถแยก plasma จากเซลล์ใน 30 นาที

-kept on ice

-sodium fluoride: prevent in vitro glycolysis: ineffective

Henry's Clinical diagnosis and management by laboratory methods, 24th ed, 2022

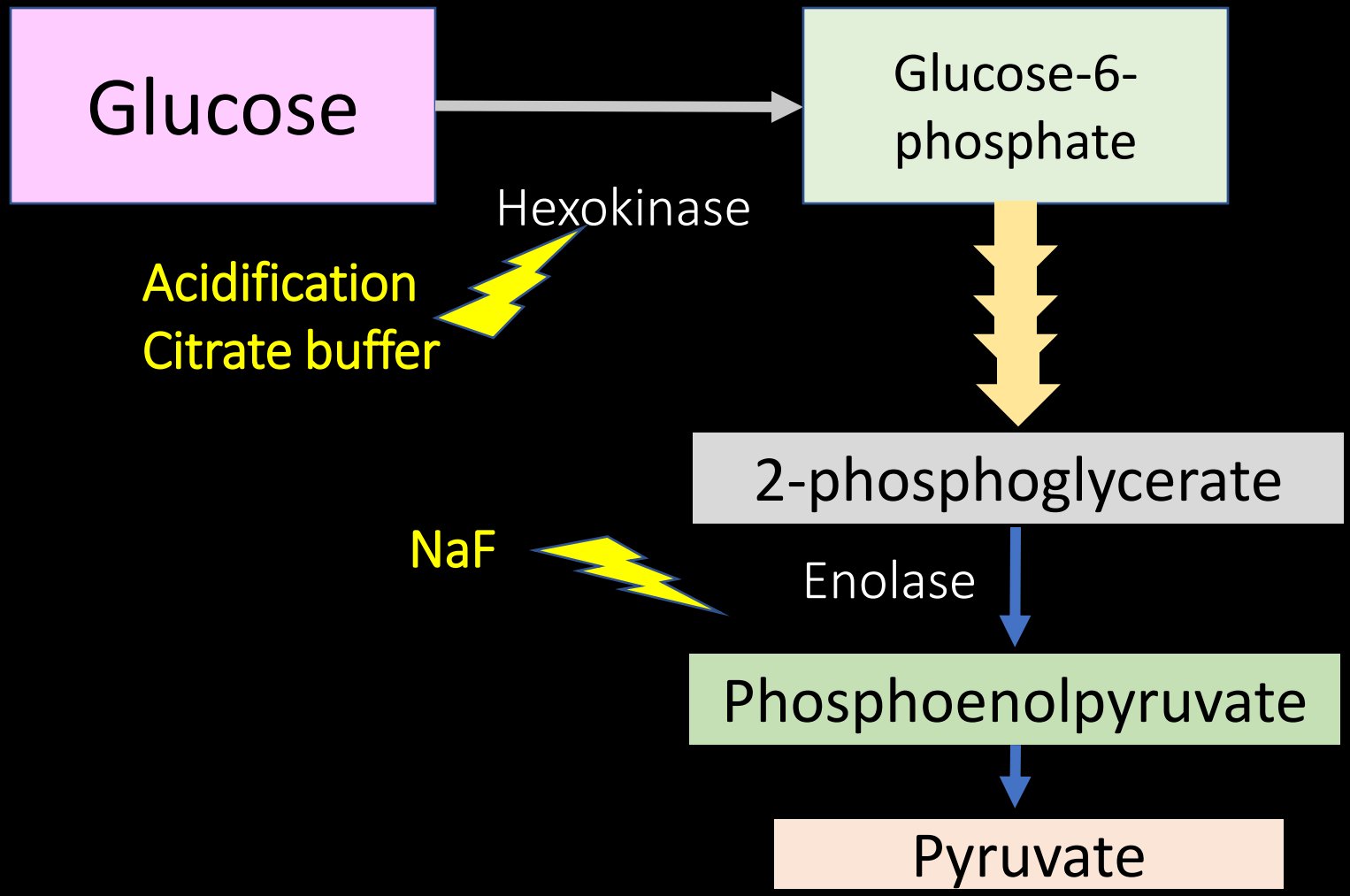
Plasma glucose between NaF- and heparinized tube



Time, h	Plasma glucose (mmol/L) (NaF)
0	9.19
1	8.83
2	8.75
4	8.66
24	8.65
48	8.61
72	8.62

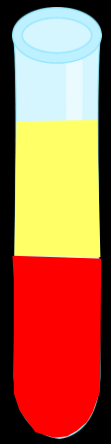
Chan,Clin Chem 1989; 35:315-7.

Glycolytic pathway



Montagnana, Ann Transl Med. 2017; 5(12):257.

Glucose: specimen collection



Plasma contact with cells

Glycolysis

ถ้าไม่สามารถแยก plasma จากเซลล์ใน 30 นาที ใส่ Glycolysis inhibitor เช่น citrate buffer

แต่ยังใช้ไม่แพร่หลาย

Tietz textbook of laboratory medicine 2023

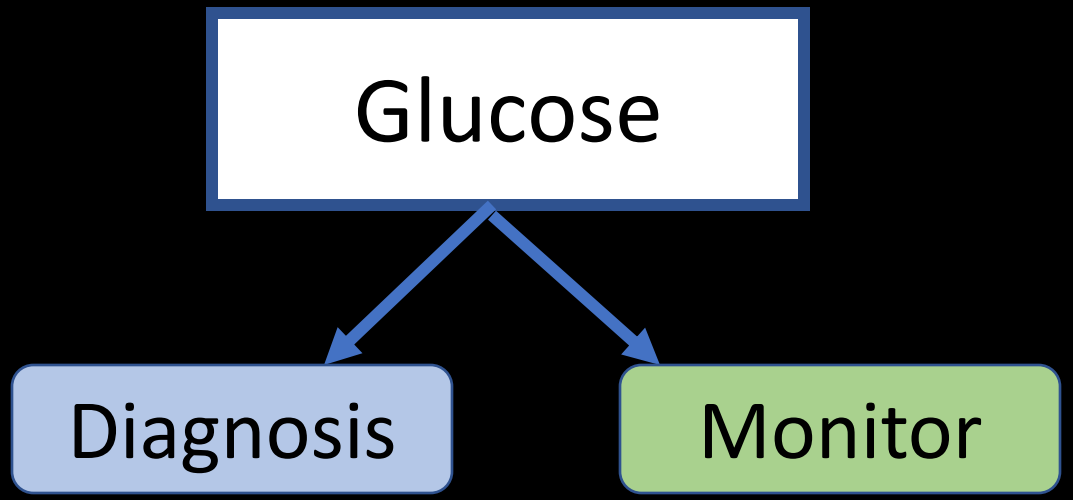
Glucose: factors affecting results

Fasting

Stress, severe illness

Stability

Diagnosis & monitor treatment



1. FPG ≥ 126 mg/dL; no caloric intake 8 hr
2. RPG ≥ 200 mg/dL + symptom
3. OGTT (2-hr PG ≥ 200 mg/dL)

Oral glucose tolerance test

75 g glucose
+ 250-300 mL H_2O
within 5 min

Morning

3 days

Unrestricted diet
>150 g/day of CHO

Usual physical activity

WHO 1999

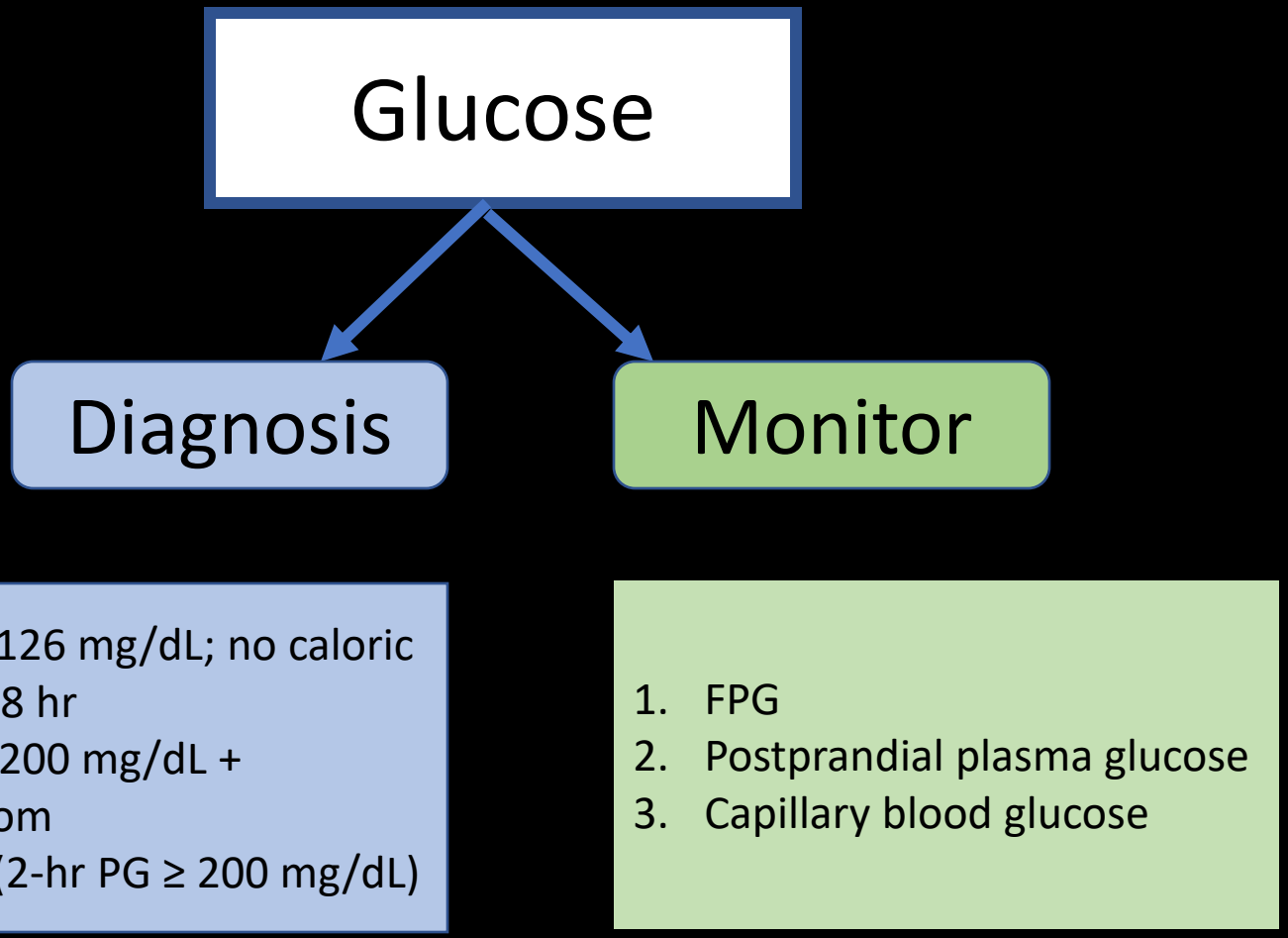
Oral glucose tolerance test

Fasting

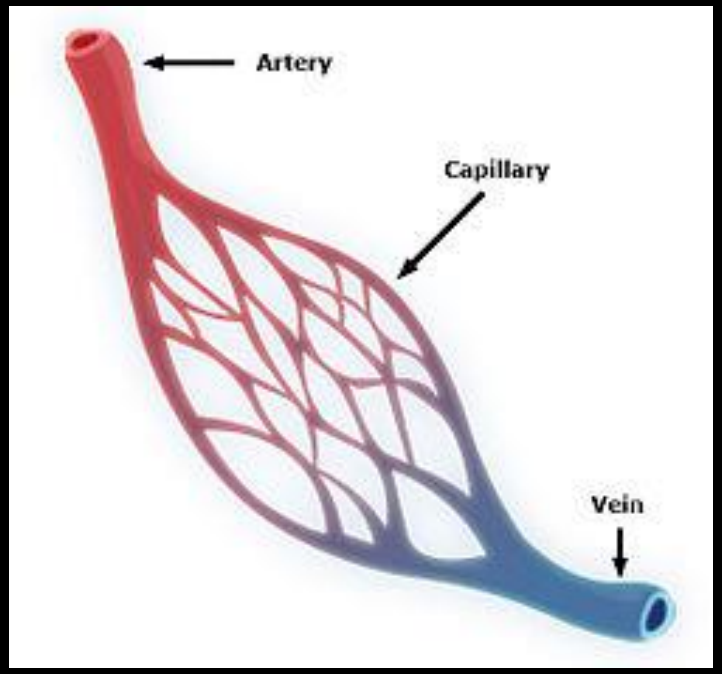
2 hr

WHO 1999

Diagnosis & monitor treatment



Capillary blood glucose



Capillary glucose ≈ arterial glucose, but can vary from venous samples depend on timing of food ingestion

Henry's Clinical diagnosis and management by laboratory methods, 24th ed, 2022

Capillary blood glucose

Whole blood gives 10-15% lower than plasma glucose

Henry's Clinical diagnosis and management by laboratory methods, 24th ed, 2022

	Abbott	Arkray	Nova Biomedical	Roche Diagnostics
How results are displayed	Plasma equivalent values	True values	Plasma equivalent values	Plasma equivalent values

CAP today Mar 2022

Capillary blood glucose

Not use for diagnosis of DM & hypoglycemia

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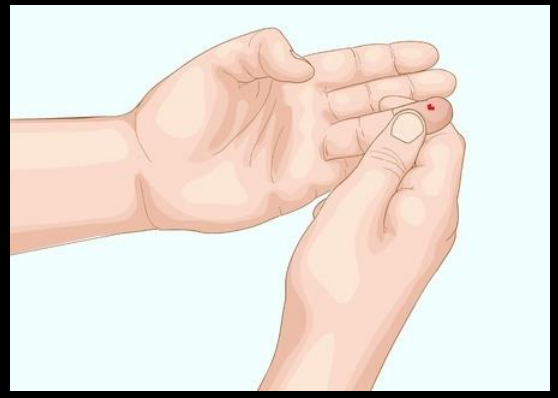
Causes of errors in using glucose meters



-Insufficient blood
-Milking the finger
-Hypotension

-Outside Hct range
-Environmental factors (humidity, heat, altitude)

-lipid, bilirubin
-Nonglucose sugar (icodextrin, maltose)
-ยาบางชนิด

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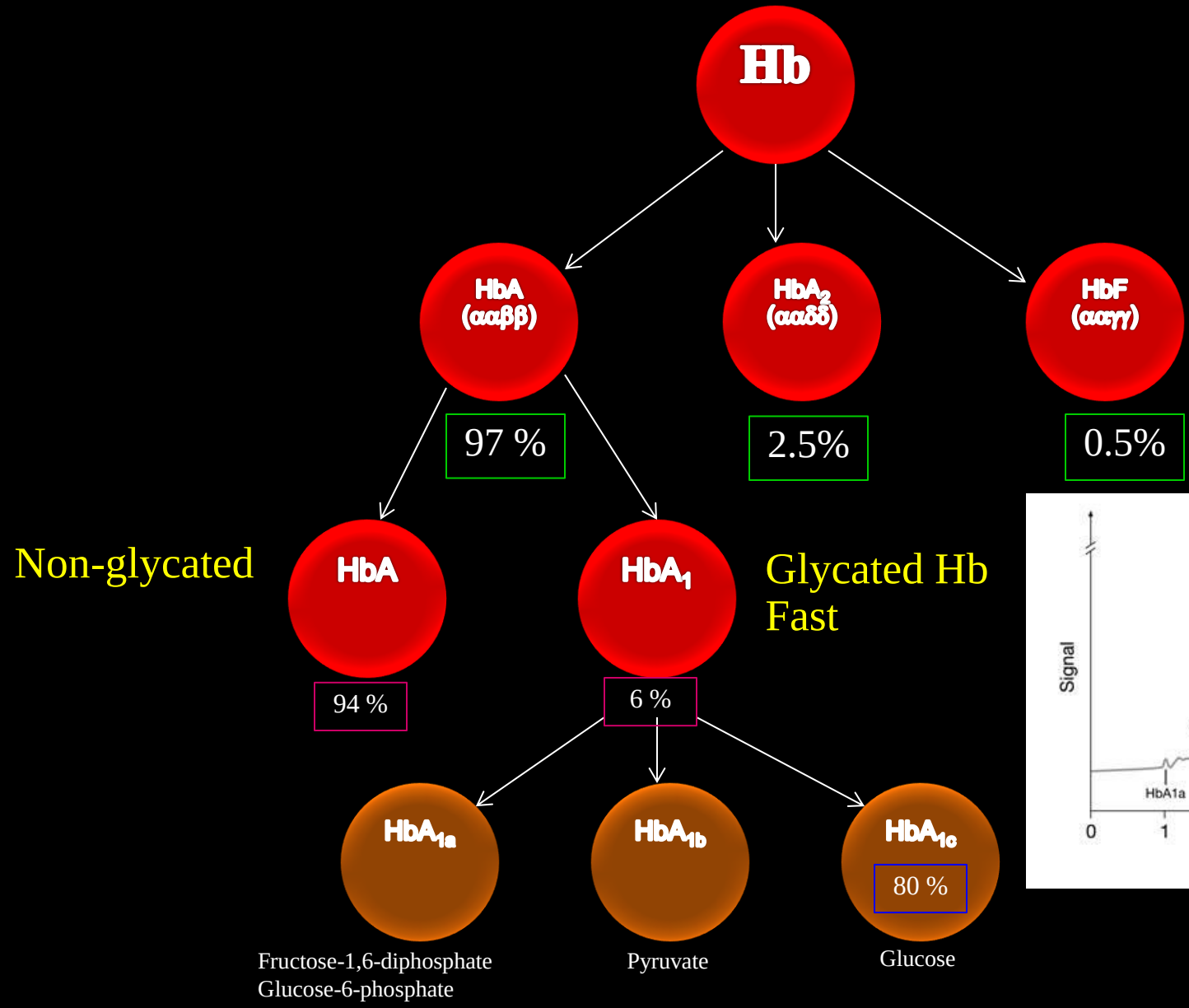
Diagnosis and monitor treatment of DM

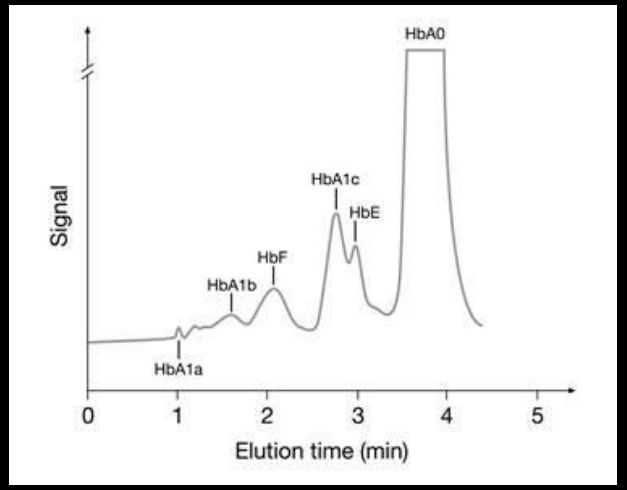
Glucose

Hemoglobin A1c

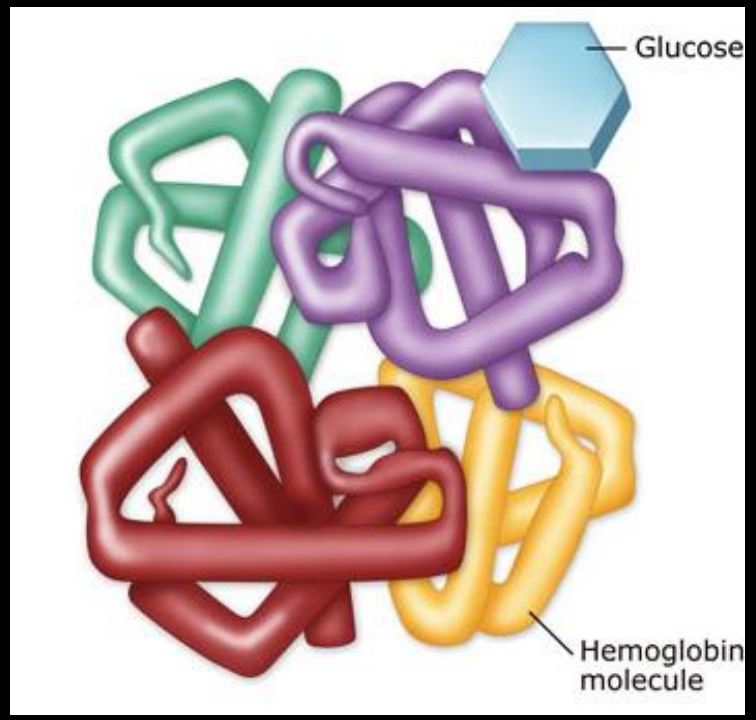
Glycated protein

Adult hemoglobin



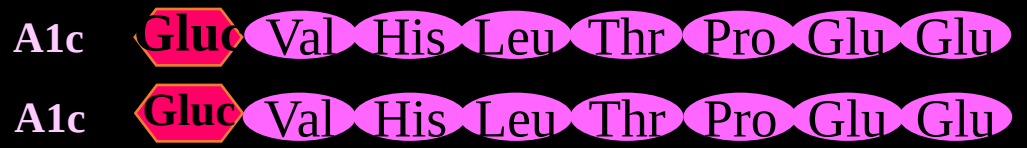


HbA_{1c}, A1C



Nonenzymatic attachment of a glucose molecule to the *N*-terminal amino acid (valine) of the **β-globin** chain molecule of hemoglobin

N-terminal of β-globin



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HbA_{1c}

Reflect average glycemia over approximately 2-3 months

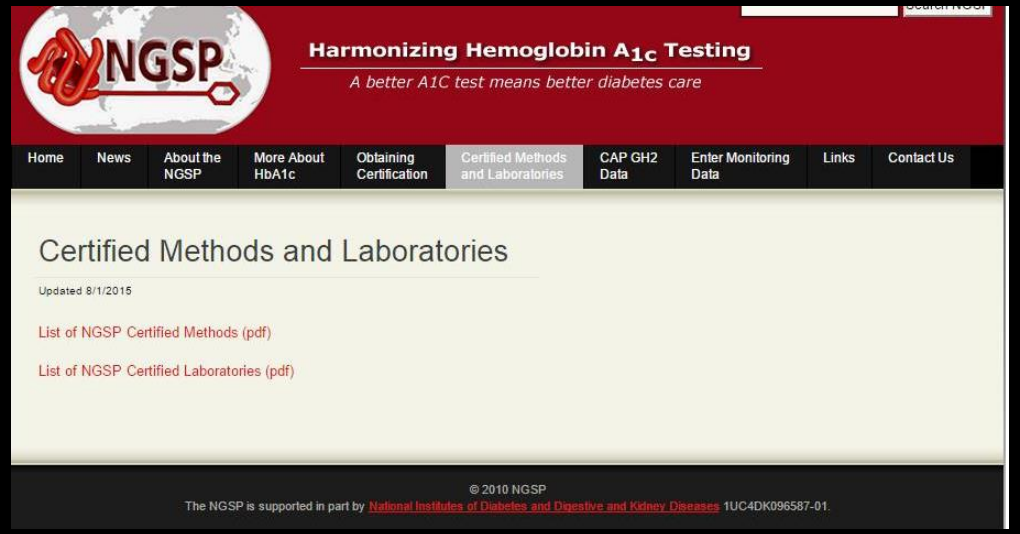
Strongly linked to diabetes complications

American Diabetes Association Professional Practice Committee. 6. Glycemic goals and hypoglycemia: Standards of Care in Diabetes—2025. Diabetes Care 2025;48(Suppl. 1): S128–S145

Hemoglobin A1c

- Diagnosis
- Monitor

A1C ≥6.5%.: The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay



National Glycohemoglobin Standardization Program

American Diabetes Association Professional Practice Committee. 2. Diagnosis and classification of diabetes: Standards of Care in Diabetes—2025. Diabetes Care 2025;48(Suppl. 1): S27–S49

Factors affecting HbA1c results

Factors affecting HbA1c interpretation

Factors affecting HbA1c measurement

Factors affecting HbA1c interpretation

- Physiological e.g. age, race
- Chronic kidney disease
- Erythrocyte lifespan
- Glycation phenotype

Welsh 2016, Diabetes Care; 39:1299-306

Factors affecting HbA1c measurement

Hemoglobin variants

Uremia (carbamylated Hb)

www.ngsp.org

Welsh 2016, Diabetes Care; 39:1299-306

Use and interpretation of laboratory measurements of glucose and A1C (1/2)

	Glucose	A1C
Cost	Inexpensive, available in most laboratories	More expensive, not widely availabe
Time frame of hyperglycemia	Acute	Chronic
Pranalytic stability	Poor	Good
Sample	Measurement can vary depending on sample type (plasma, serum, whole blood) and source (capillary, venous, arterial)	Requires whole-blood sample

American Diabetes Association Professional Practice Committee. 2. Diagnosis and classification of diabetes: Standards of Care in Diabetes—2025. Diabetes Care 2025;48(Suppl. 1): S27–S49

Use and interpretation of laboratory measurements of glucose and A1C (2/2)

	Glucose	A1C
Assay standardization	Not standardized	Well standardized
Fasting	Fasting or timed samples required	Nonfasting test; no participant preparation is needed
Acute factors that can affect levels	Food intake, stress, recent illness, activity	Unaffected by recent food intake, stress, illness, activity
Other individual factors that can affect test results	Diurnal variation, medications, alcohol, smoking, bilirubin	Altered erythrocyte turnover, HIV, cirrhosis, renal failure, dialysis, pregnancy
Test interferences	sample handling/processing time, hemolysis, severe hypertriglyceridemia, severe hyperbilirubinemia	Hemoglobin variants, severe hypertriglyceridemia, severe hyperbilirubinemia

Diabetes Care 2025;48(Suppl. 1): S27–S49

RATIONAL LAB USE

HbA1c testing frequency?: stable glycemic control

ADA:

- Assess glycemic status (A1C, continuous glucose monitoring) at least two times a year, and more frequently (e.g.,every 3 months) for individuals not meeting glycemic goals or with recent treatment changes

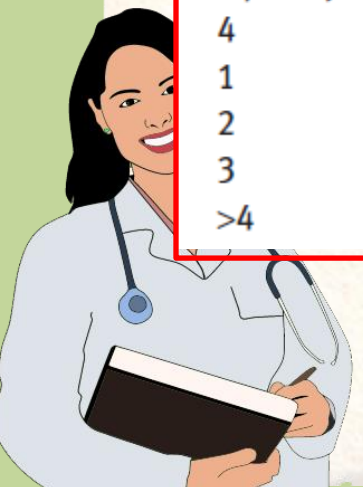
Guideline: describe minimum amount but no mention of a maximal limit

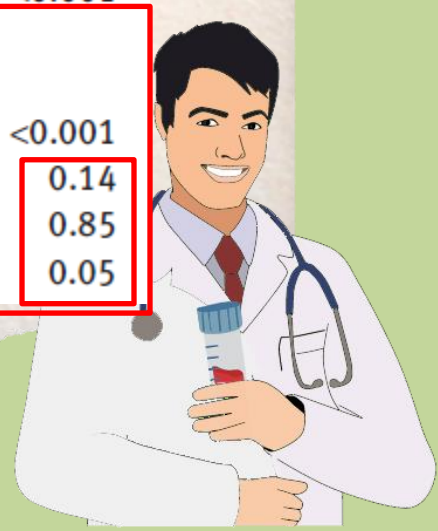
Diabetes Care 2025;48(Suppl. 1): S128–S145

RATIONAL LAB USE

The frequency of testing for glycated haemoglobin, HbA_{1c}, is linked to the probability of achieving target levels in patients with suboptimally controlled diabetes mellitus

Explanatory variable	Mean difference in HbA_{1c} levels (95% CI)		p-Value
	(mmol/mol)	(%)	
Sex			
Female	Reference	Reference	
Male	−0.11 (−0.55, 0.34)	−0.010 (−0.050, 0.032)	0.63
Age			
Linear term	−0.27 (−0.29, −0.25)	−0.025 (−0.026, −0.023)	<0.001
Quadratic term	0.003 (0.002, 0.004)	0.0003 (0.0002, 0.0003)	<0.001
Frequency of tests			
4	Reference	Reference	
1	2.64 (2.00, 3.28)	0.242 (0.183, 0.300)	<0.001
2	0.39 (−0.12, 0.90)	0.036 (−0.011, 0.083)	0.14
3	0.05 (−0.47, 0.57)	0.005 (−0.043, 0.052)	0.85
>4	0.52 (−0.001, 1.03)	0.047 (−0.0001, 0.094)	0.05





RATIONAL LAB USE

Diagnosis and monitor treatment of DM

Glucose

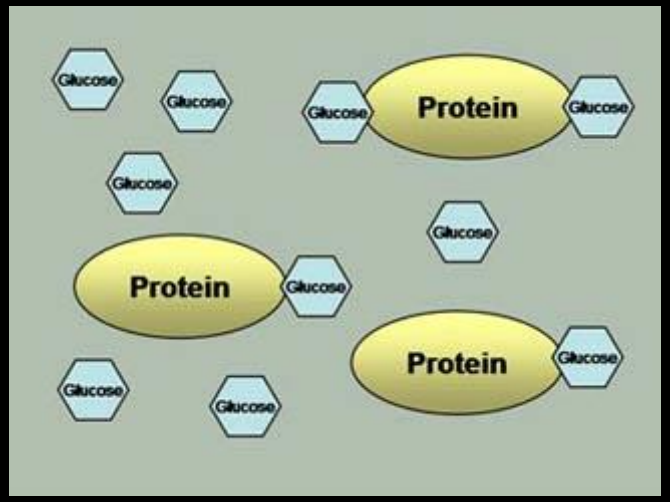
Hemoglobin A1c

Glycated protein

Glycated Protein

Reflect mean glucose over a period of 2-3 weeks

Use in conditions in which HbA1c may be unreliable



Fructosamine

Glycated albumin

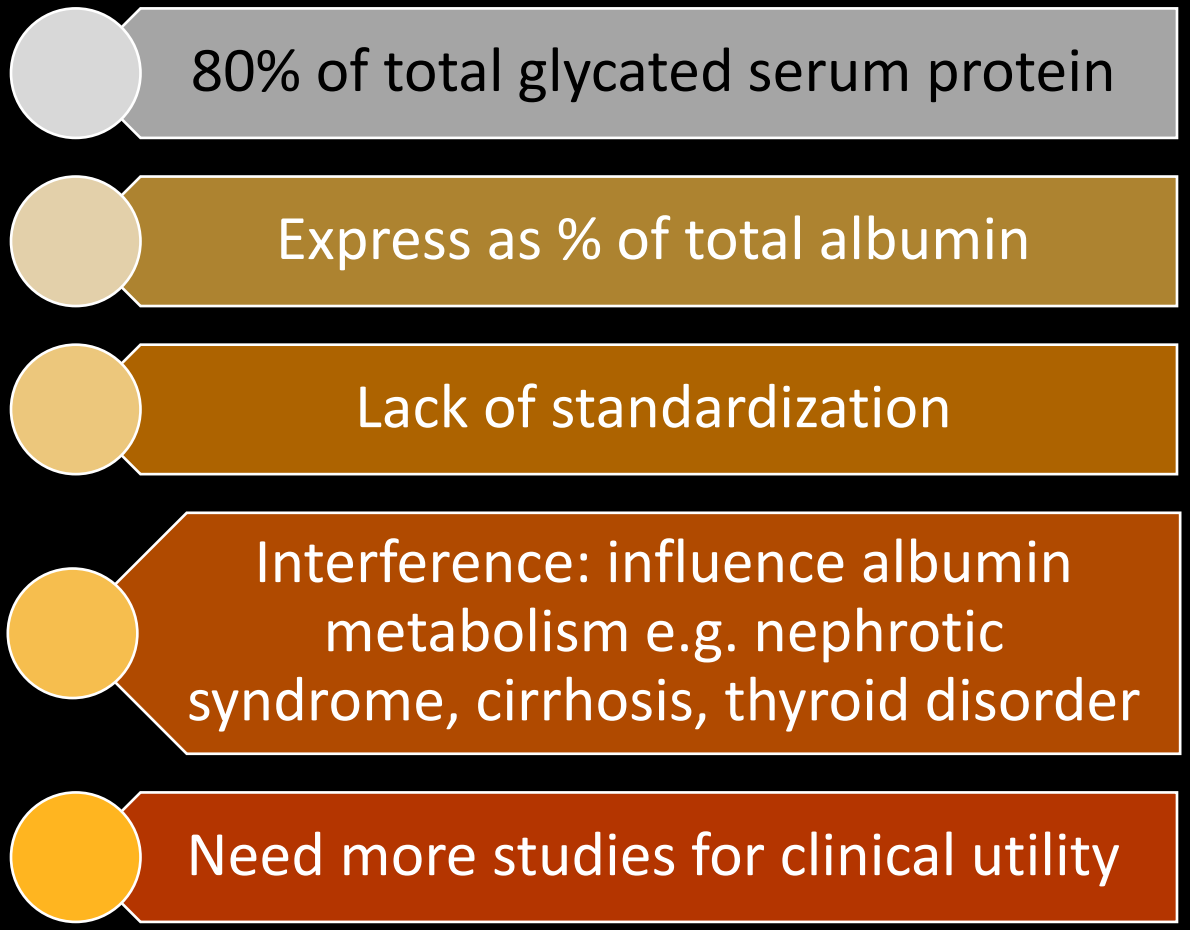
Welsh, Diabetes Care 2016;39:1299-1306.

Fructosamine

- All glycated serum protein mainly glycated albumin
- Not valid when albumin < 3 g/dL
- Interference: reducing substance e.g. uric, vitamin C
- Lack of evidence linking to long-term complication

Welsh, Diabetes Care 2016;39:1299-1306.

Glycated albumin



Welsh, Diabetes Care 2016;39:1299-1306.

Diagnosis and monitor treatment of DM

Glucose

Hemoglobin A1c

Glycated protein

Outline

Laboratory tests in

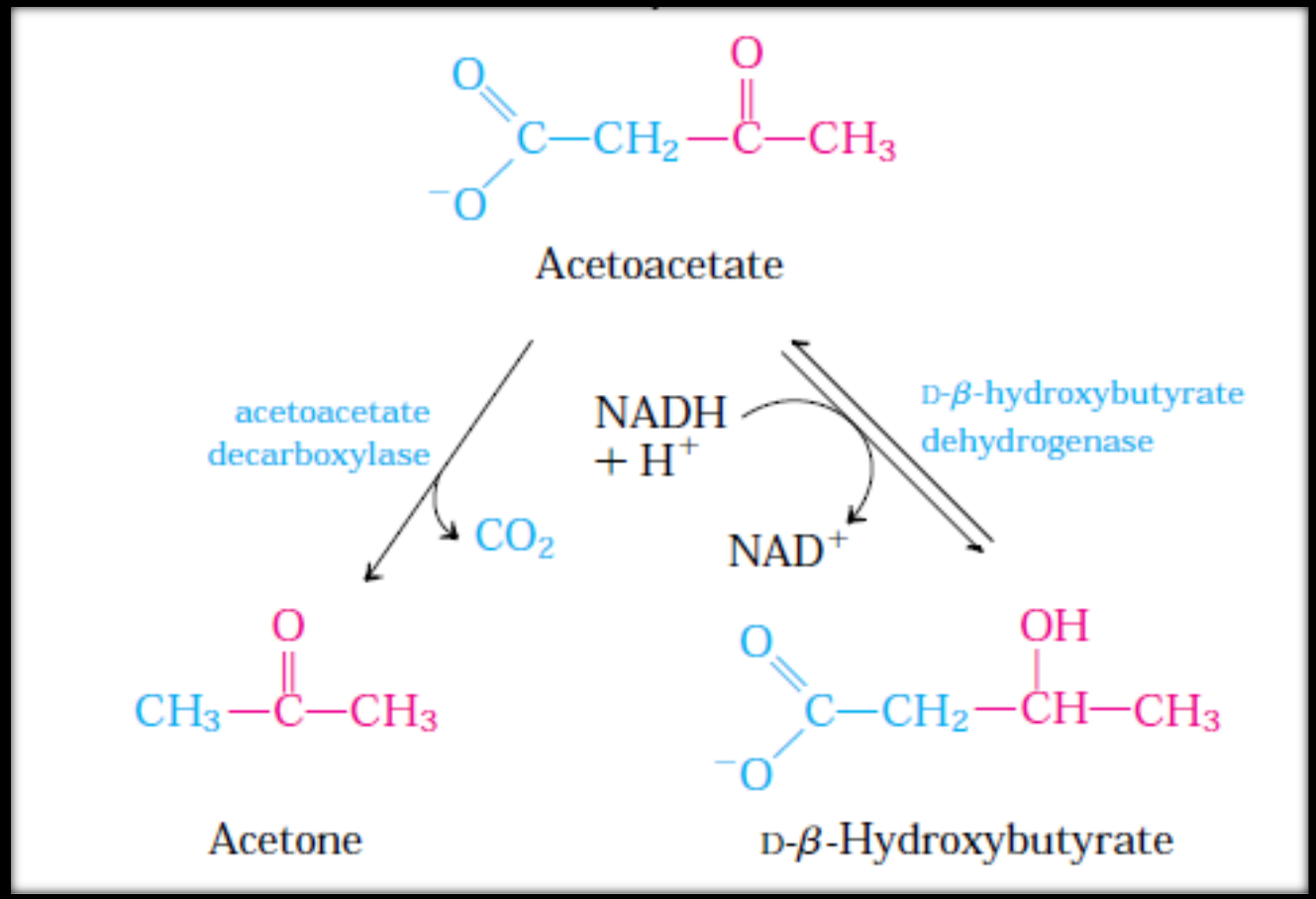
- Diagnosis & monitor treatment of DM
- Complication of DM
- Hypoglycemia

Tests in complication of DM

Ketone

Albuminuria

Ketone bodies: DKA



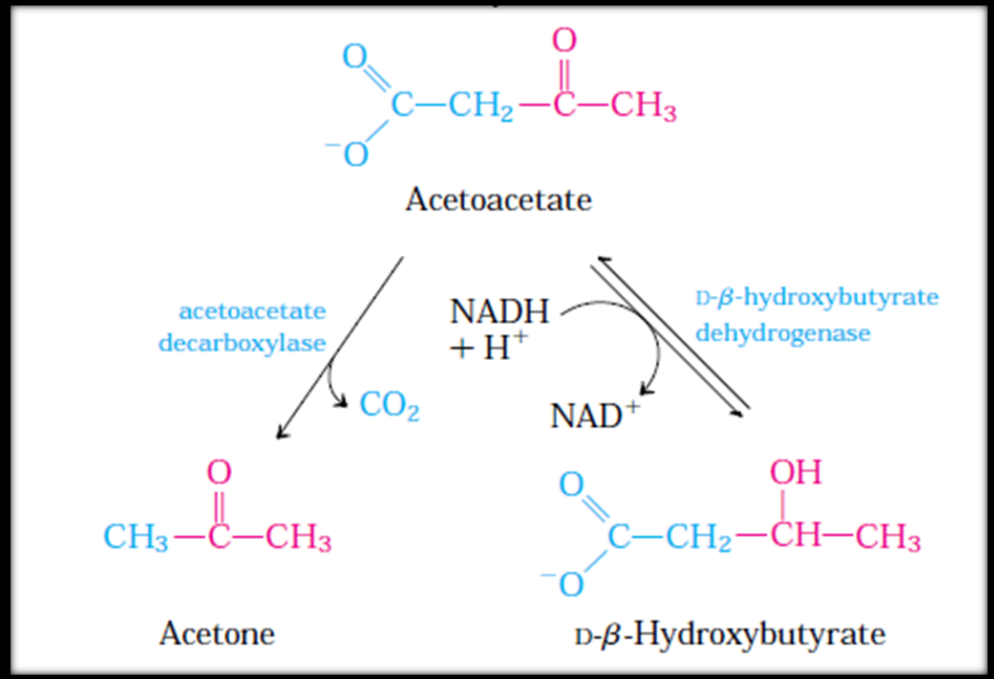
Lehninger Principles of Biochemistry. 4th ed: W. H. Freeman 2004. p. 631-55.

Ketone testing

Sodium nitroprusside

- Detect: acetoacetic acid, acetone; not B-OHB
- False positive: captopril, acetylcysteine, penicillamine, mesna
- False negative: large intake of vitamin C
- Should not use for monitoring

β-hydroxybutyric acid quantitation
: can use for monitoring



Tests in complication of DM

Ketone

Albuminuria

Albuminuria

- diabetic kidney disease
- Urine albumin 1/yr
  - timed or 24 h: burdensome
- Urine albumin/creatinine: immunoassay or by using a sensitive dipstick test specific for albuminuria

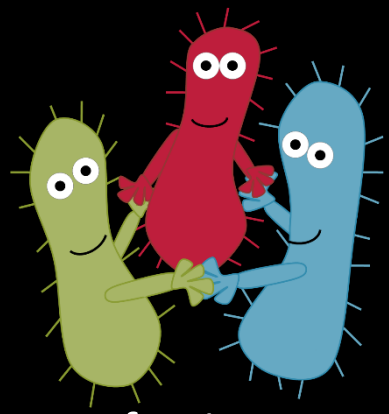
Category	Spot collection (mg/g creatinine)
Normal	<30
Moderately increased	30-299
Severely increased	≥300

Diabetes Care 2021;44(Suppl. 1):S151–S167

Albuminuria

Fever

Exercise within 24h



Infection

CHF

Marked hyperglycemia, marked HT

↓

↑ Albuminuria

- High biological variation: 2 in 3 specimens collected in 3-6 months

Diabetes Care 2023;46(Suppl. 1):S191–S202

Outline

Laboratory tests in

- Diagnosis & monitor treatment of DM
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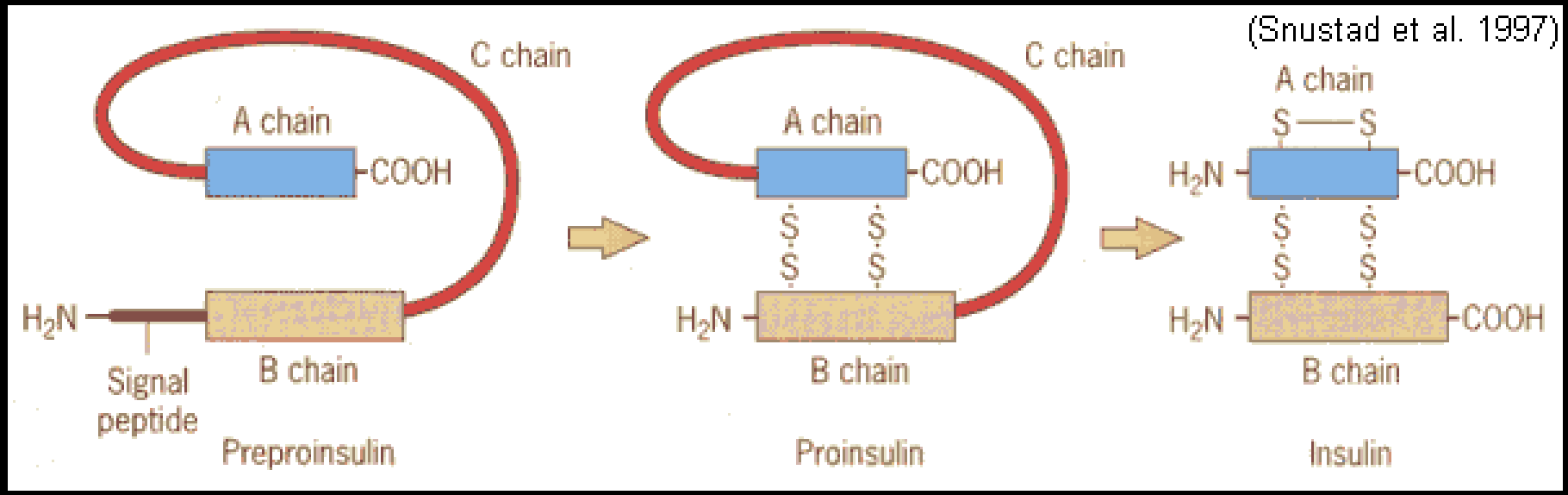
- Hypoglycemia

Test in hypoglycemia

Insulin

C-peptide

Insulin & C-peptide



Connecting peptide (C-peptide) and insulin are secreted in equimolar amounts into portal vein

http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2005/Dresser/My%20favorite%20Protein.html

Insulin & C-peptide

- Evaluation of hypoglycemia

Glucose	Insulin	C-peptide	Diagnosis
Low	High	Low	Exogenous insulin
Low	High	High	Endogenous insulin

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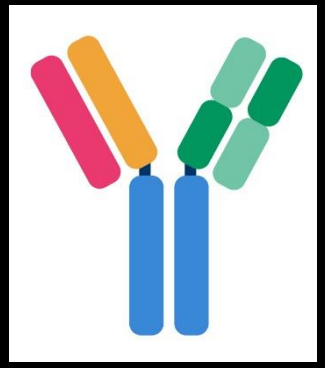
Insulin

Glucose	Insulin

measure concomitantly

Falsely low in hemolysis
(insulin-degrading enzyme in RBC)



Insulin antibodies:

falsely elevated or suppressed

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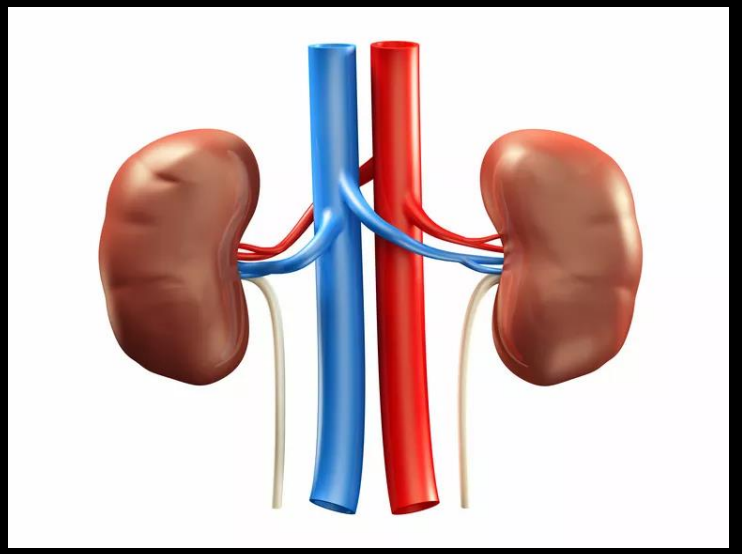
C-peptide

- less stable than insulin
- Separate quickly & frozen



- Degrade in kidney
  - Elevated level in renal failure

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Conclusions

Laboratory tests in

Diagnosis & monitor treatment of DM

Complication of DM

Hypoglycemia

นึกถึง Rational Lab use

นึกถึง พยาธิวิทยาคลินิก ศิริราช