METABOLISME GLUKOSA, UREA, DAN TRIGLISERIDA (TEKNIK SPEKTROFOTOMETRI)

Tujuan: i) Mengerti prinsip–prinsip dasar mengenai teknik spektofotometri (yaitu prinsip dasar alatnya, kuvet, standard, blanko, serta Hukum Beer-Lambert dll).
ii) Latihan pengenceran/pembuatan dan penggunaan larutan stok
iii) Kumpulkan data kadar glukosa, trigliserida dan urea darah
iv) Latihan pembuatan dan interpretasi grafik
v) Persiapan untuk praktikum Metabolisme II" di mana Anda akan mendesain dan melakukan percobaan yang berdasarkan teknik-teknik pratikum ini

**Kegiatan praktikum ini diadaptasi dari bahan:*
Renato M. Passos, R.M., Se´, A.B., Wolff, V.L., Nobrega, Y.K.M. & Hermes-Lima, M. 2006. Pizza and pasta help students learn metabolism. *Adv Physiol Educ* 30: *89–93*.

Pendahuluan*:* Spektrofotometri merupakan salah satu dari beberapa teknik yang sering dipakai secara rutin di laboratorium biokimia. Pada dasarnya, dengan teknik spektrofotometri kita dapat mengukur jumlah cahaya yang melewati sampel larutan. Jumlah cahaya yang diserap oleh larutan sampel berkaitan dengan konsentrasi unsur di dalam larutan sampel tersebut. Teknik ini dapat digunakan untuk memonitor perubahan warna (yaitu perubahan pada jumlah cahaya yang diserap) yang kualitatif dan mengukur konsentrasi bahan secara kuantitatif.
Ingatlah dari bahan kuliah spektrofotometri:

$$\mathbf{A} = \boldsymbol{\varepsilon}\mathbf{dc}$$

dimana **c** = konsentrasi larutan itu (satuan adalah **M**),
$\boldsymbol{\varepsilon}$ = koefisien absorpsi molar ($\mathbf{M^{-1}cm^{-1}}$),
d = panjang larutan yang dilalui cahaya (**cm**)
A = serapan

Ingatlah pula Hukum Beer-Lambert, untuk larutan standard (LS) $\mathbf{A_{LS}} = \boldsymbol{\varepsilon}\mathbf{dc_{LS}}$
menyusun kembali: $\mathbf{A_{LS}} / \mathbf{c_{LS}} = \boldsymbol{\varepsilon}\mathbf{d}$ *(persamaan 1)*

Sama juga dengan LX $\mathbf{A_{LX}} = \boldsymbol{\varepsilon}\mathbf{dc_{LX}}$ dan $\mathbf{A_{LX}} / \mathbf{c_{LX}} = \boldsymbol{\varepsilon}\mathbf{d}$ *(persamaan 2)*

Dari persamaan 1 dan 2 kita bisa menulis

$$\mathbf{A_{LS}} / \mathbf{c_{LS}} = \mathbf{A_{LX}} / \mathbf{c_{LX}}$$

menyusun kembali: $\mathbf{c_{LX}} = \mathbf{A_{LX}} \cdot \mathbf{c_{LS}} / \mathbf{A_{LS}}$ *(persamaan 3)*

Akibatnya, Anda bisa menghitung $\mathbf{c_{LX}}$ ketika Anda sudah mengetahui nilai $\mathbf{A_{LX}}$, $\mathbf{c_{LS}}$ and $\mathbf{A_{LS}}$.

Cara Kerja*:*

Alat dan Bahan:

pipet Mohr: (1ml & 5ml)	Kit pemeriksaan urea	urea
sentrifus	Kit pemeriksaan glukosa	glukosa
alat spektrofotometer	Kit pemeriksaan trigliserida	pipet otomatik 10µl - 100µl
Waterbath 37°C	tabung reaksi dan rak	kuvet

Larutan stok yang perlu disiapkan

Meja 1, 3, 5: **Larutan stok urea:** siapkan 10mL larutan urea pada kadar 50g/L (atau 500mg/dL)

∴jumlah bubuk urea yang dibutuhkan: _____________g

Meja 2, 4: **Larutan stok glukosa:** siapkan 10mL larutan 50mM glukosa (ingatlah rumus kimiawi glukosa adalah: $C_6O_6H_{12}$)

∴jumlah bubuk glukosa yang dibutuhkan: _____________g

Pengenceran untuk kalibrasi dari larutan stok tersebut:

Siapkan pengenceran logarithmic berlipatgandaan (*doubling dilution*) serta pengenceran logarithimik berdasar 10 (*decimal dilution*) dari larutan stok urea atau larutan stok glukosa (sesuai dengan larutan stok yang Anda siapkan tadi).

Caranya untuk *doubling dilution*

1) Sediakan 8 tabung reaksi dalam rak tabung beri tanda 1 s/d 8. Tambahkan 1 ml akudes masing-masing pada tabung reaksi 2 s/d 8.
2) Pada tabung reaski 1 tambah 2 ml larutan stok.
3) Pada tabung reaksi 2 tambah 1 ml larutan yang diambil dari tabung reaksi 1. Campur dengan baik.
4) Pada tabung reaksi 3 tambah 1 ml larutan yang diambil dari tabung reaksi 2. Campur dengan baik.

   Buat pengenceran larutan stok selanjutnya dengan cara yang sama (yaitu 1 ml larutan dari tabung reaski sebelumnya ditambahkan ke tabung reaksi yang selanjutnya). Lihat tabel yang dibawa sebagai ringkasaan *doubling dilution*.

nomor tabung	1	2	3	4	5	6	7	8
pengenceran urea/glukosa	stok	1:1	1:3	1:7	1:15	1:31	1:63	1:127
faktor	-	2	4	8	16	32	64	128

Caranya untuk *decimal dilution*

1) Sediakan 6 tabung reaksi dalam rak tabung beri tanda 1 s/d 6.
2) Siapakan pengenceran sesuai dengan tabel yang dibawah:

nomor tabung	1	2	3	4	5	6
faktor	stok	3	10	30	100	300

Pemeriksaan Glukosa, Trigliserida dan Urea

Kita akan menggunakan kit DisSys untuk pemeriksaan glukosa, trigliserida dan urea. Prosedur kerjanya dilampirkan tapi cara kerja secara singkat seperti berikutnya:

1. ~ 1 ml darah diambil ke dalam wadah yang berisi EDTA. Menggunakan alat sentrifugasi klinik untuk memisahkan sel-sel darah dari plasma. Diharap dapat ± 500µl plasma tapi hanya 10µl dibutuhkan untuk pemeriksaan glukosa, trigliserida dan urea masing-masing.
2. Pemeriksaan terhadap glukosa, trigliserida serta urea berdasar reaksi enzim (lihat lampiran-lampiran). <u>Perhatikanlah bahwa waktu pada reaksi enzim harus dicatat dan diikuti dengan benar</u> – kalau sampel-sampel tidak diperlakukan secara sama, hasilnya pasti kurang bagus.
3. Oleh karena kepentingan periode reaksi diatur dengan baik, kerjakan bagian inkubasi untuk sampel-sampel pengenceran *doubling* dan *decimal* maupun pemeriksaan glukosa, trigliserida dan urea, satu per satu.
4. Alat spektrofotometer yang akan kita pakai berada di Lab Sitogenetika. Supaya tidak jadi antrian yang sangat lama untuk menggunakan alat tersebut, diharap grup meja masing-masing membagi sampel-sampel yang mau diperiksa dalam dua atau tiga bagian dan membawa bagian-bagian tersebut ke Lab Sitogenetika seketika siap untuk diperiksa (daripada kumpulkan semua sampel dulu dan dibawa sekaligus).
5. Bahan (reagensia kit serta standar) untuk pemeriksaan glukosa, trigliserida dan urea disediakan di tempat masing-masing di Lab Terpadu. Bawalah tabung reaksi dengan rak

tabungnya ke tempat pemeriksaan untuk mengambil reagensia yang dibutuhkan (daripada reagensia dipindahkan ke tempat meja kerja Anda).

6. Cara persiapan sampel plasma untuk pemeriksaan glukosa, trigliserida dan urea, atau sampel pengenceran *doubling* dan *decimal* (glukosa atau urea) dicatat di bawah ini:

	GLUKOSA	**TRIGLISERIDA**	**UREA**
volume reagensia kit	1000μl reagensia glukosa	1000μl reagensia **R2**	1000μl reagensia **A**, kemudian periode inkubasi pertama; tambah 1000μl reagensia **B**
volume sampel plasma atau standard atau sampel dari kegiatan pengenceran	10μl	10μl	10μl
konsentrasi standard kit	100mg/dl	200mg/dl	40mg/dl
periode dan temperature inkubasi	10 min @ 37°C	5 min @ 37°C	5 min @ 25°C ** **2X****
periksa pada λ =	500nm	530nm	600nm

7. Catat hasil serapan (*absorbance*) yang diperoleh dengan alat spektrofotometer pada tabel-tabel berikutnya. Kumpulkan data dari grup meja yang lain supaya data lengkap, yaitu
   - ☐ hasil pengenceran *doubling* dan *decimal* urea dan glukosa (Tabel 1a, 1b, 2a, 2b)
   - ☐ hasil periksaan glukosa, trigliserida dan glukosa dari 5-9 mahasiswa (Tabel 4)

LaporanPraktikum Spektrofotometri: * satu laporan/ grup meja

1-2 halaman selain Tabel 3 dan 5 grafik yang dibuat dari data yang di Tabel 1a, 1b, 2a, 2b serta Table 4.

Buat laporan praktikum dengan kata-kata sendiri. Kalau ada perubahan dari yang ditulis di bahan penuntun praktikum ini, catatlah dalam laporan.

Sebutkan 3 kesimpulan dari setiap grafik yang kalian buat – bagi grafik-grafik yang bertautan dengan pengenceran stok glukosa dan urea, berikan komentar atas kebuktian (atau tidak) Hukum Beer-Lambert)

Berilah komentar atas hasil yang kaliam peroleh pada Tabel 3.

Berikanlah saran atas praktikum spektrofotometri ini sehingga praktikum selanjutnya akan lebih baik lagi.

Proposal untuk Praktikum Metabolisme II (dibuat masing-masing)

Buatlah proposal untuk percobaan lanjut mengenai metabolisme glukosa, trigliserida dan/atau urea. Dari data dan pengalaman Anda pada praktikum ini, pikirkan suatu hipotesis dan mendesain suatu percobaan yang bisa membuktikan hipotesis Anda itu benar atau tidak **dan** yang bisa diuji dalam konteks praktikum (ingatlah keterbatasan waktu dan alat!!)

Siapkan cara kerja/proposal yang lengkap dan jelas untuk percobaan yang Anda rencanakan (termasuk tujuan, pendahuluan singkat, alat dan bahan, langkah-langkah cara kerja dan bagaimana hasilnya akan dianalisa).

Proposal ini dikumpulkan (secara hardcopy) pada saat Anda ikut UTS (tgl 10 Nopember).

Proposal Anda akan dikomentar oleh staf pengajar praktikum biomedis dan akan dikembalikan kepada Anda sebelum akhir bulan Nopember 2011.

Anda boleh bergabung dengan seorang mhs lain (terserah Anda) sebagai grup meja untuk praktikum Metabolisme II dan boleh memilih satu proposal di antara kalian berdua untuk mengerjakan bersama-sama pada jam praktikum pada tgl 15 Desember 2011.

Tabel 1a : UREA – data untuk kalibrasi *doubling dilution*

Konsentrasi stok urea =500mg/dl

faktor	konsentrasi	grup meja__	grup meja__
1			
2			
4			
8			
16			
32			
64			
128			
blanko			

Buatlah grafik dengan konsentrasi sebagai sumbu X dan serapan (A) sebagai sumbu Y.

Tabel 1b : UREA – data untuk kalibrasi *decimal dilution*

Konsentrasi stok urea =500mg/dl

faktor	konsentrasi	grup meja__	grup meja__
1			
3			
10			
30			
100			
300			
blanko			

Buatlah grafik dengan konsentrasi sebagai sumbu X dan serapan (A) sebagai sumbu Y.

Tabel 2a : GLUKOSA – data untuk kalibrasi *doubling dilution*

Konsentrasi stok glukosa =50mM

faktor	konsentrasi	grup meja__	grup meja__
1			
2			
4			
8			
16			
32			
64			
128			
blanko			

Buatlah grafik dengan konsentrasi sebagai sumbu X dan serapan (A) sebagai sumbu Y.

Tabel 2b : GLUKOSA data untuk kalibrasi *decimal dilution*

Konsentrasi stok glukosa =50mM

faktor	konsentrasi	grup meja__	grup meja__
1			
3			
10			
30			
100			
300			
blanko			

Buatlah grafik dengan konsentrasi sebagai sumbu X dan serapan (A) sebagai sumbu Y.

Tabel 3 Konsentrasi glukosa dan urea dalam plasma yang dibaca pada grafik 1a s/d 2b, serta yang dihitung melalui rumus kit

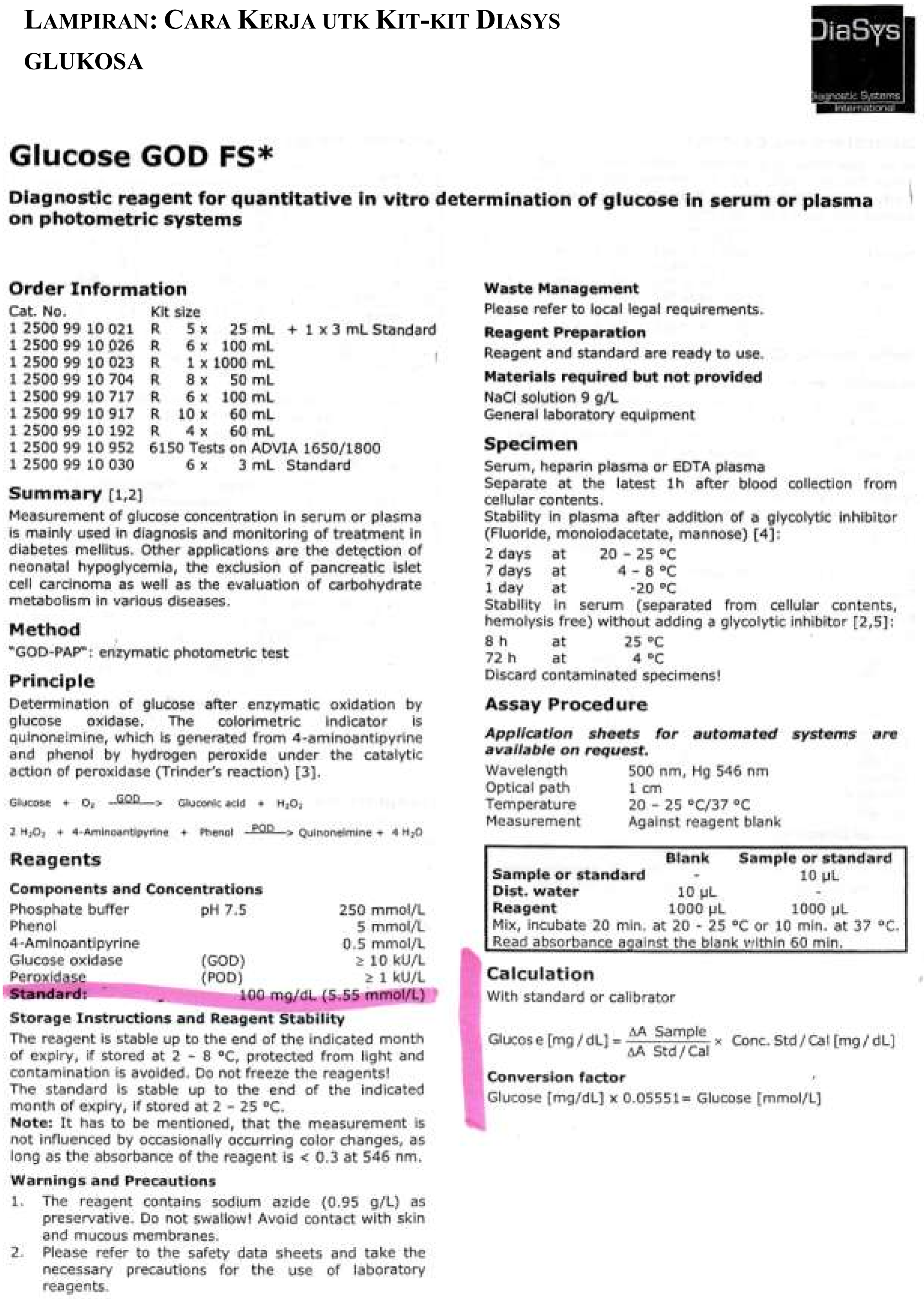
	GLUKOSA		UREA	
	mhs: ______	**mhs: ______**	**mhs: ______**	**mhs: ______**
Serapan sampel				
dari grafik 1a/2a				
dari grafik 1b/2b				
dari rumus kit				

Tabel 4 Hasil pemeriksaan glukosa, trigliserida dan urea plasma mahasiswa

detil^2 mhs (berapa lama sejak makan; rata-rata apa yg dimakan; jenis kelaminan; umur)	GLUKOSA		TRIGLISERIDA		UREA	
	A	**kadar**	**A**	**kadar**	**A**	**kadar**
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
10.						

LAMPIRAN: CARA KERJA UTK KIT-KIT DIASYS

GLUKOSA



Glucose GOD FS*

Diagnostic reagent for quantitative in vitro determination of glucose in serum or plasma on photometric systems

Order Information

Cat. No.	Kit size		
1 2500 99 10 021	R	5 x 25 mL	+ 1 x 3 mL Standard
1 2500 99 10 026	R	6 x 100 mL	
1 2500 99 10 023	R	1 x 1000 mL	
1 2500 99 10 704	R	8 x 50 mL	
1 2500 99 10 717	R	6 x 100 mL	
1 2500 99 10 917	R	10 x 60 mL	
1 2500 99 10 192	R	4 x 60 mL	
1 2500 99 10 952	6150 Tests on ADVIA 1650/1800		
1 2500 99 10 030		6 x 3 mL	Standard

Summary [1,2]

Measurement of glucose concentration in serum or plasma is mainly used in diagnosis and monitoring of treatment in diabetes mellitus. Other applications are the detection of neonatal hypoglycemia, the exclusion of pancreatic islet cell carcinoma as well as the evaluation of carbohydrate metabolism in various diseases.

Method

"GOD-PAP": enzymatic photometric test

Principle

Determination of glucose after enzymatic oxidation by glucose oxidase. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder's reaction) [3].

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4\ H_2O$$

Reagents

Components and Concentrations

Phosphate buffer	pH 7.5	250 mmol/L
Phenol		5 mmol/L
4-Aminoantipyrine		0.5 mmol/L
Glucose oxidase	(GOD)	≥ 10 kU/L
Peroxidase	(POD)	≥ 1 kU/L
Standard:		100 mg/dL (5.55 mmol/L)

Storage Instructions and Reagent Stability

The reagent is stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C, protected from light and contamination is avoided. Do not freeze the reagents!

The standard is stable up to the end of the indicated month of expiry, if stored at 2 – 25 °C.

Note: It has to be mentioned, that the measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the reagent is < 0.3 at 546 nm.

Warnings and Precautions

1. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Reagent and standard are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma

Separate at the latest 1h after blood collection from cellular contents.

Stability in plasma after addition of a glycolytic inhibitor (Fluoride, monoiodacetate, mannose) [4]:

2 days	at	20 – 25 °C
7 days	at	4 – 8 °C
1 day	at	-20 °C

Stability in serum (separated from cellular contents, hemolysis free) without adding a glycolytic inhibitor [2,5]:

8 h	at	25 °C
72 h	at	4 °C

Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	500 nm, Hg 546 nm
Optical path	1 cm
Temperature	20 – 25 °C/37 °C
Measurement	Against reagent blank

	Blank	**Sample or standard**
Sample or standard	-	10 µL
Dist. water	10 µL	-
Reagent	1000 µL	1000 µL
Mix, incubate 20 min. at 20 - 25 °C or 10 min. at 37 °C.		
Read absorbance against the blank within 60 min.		

Calculation

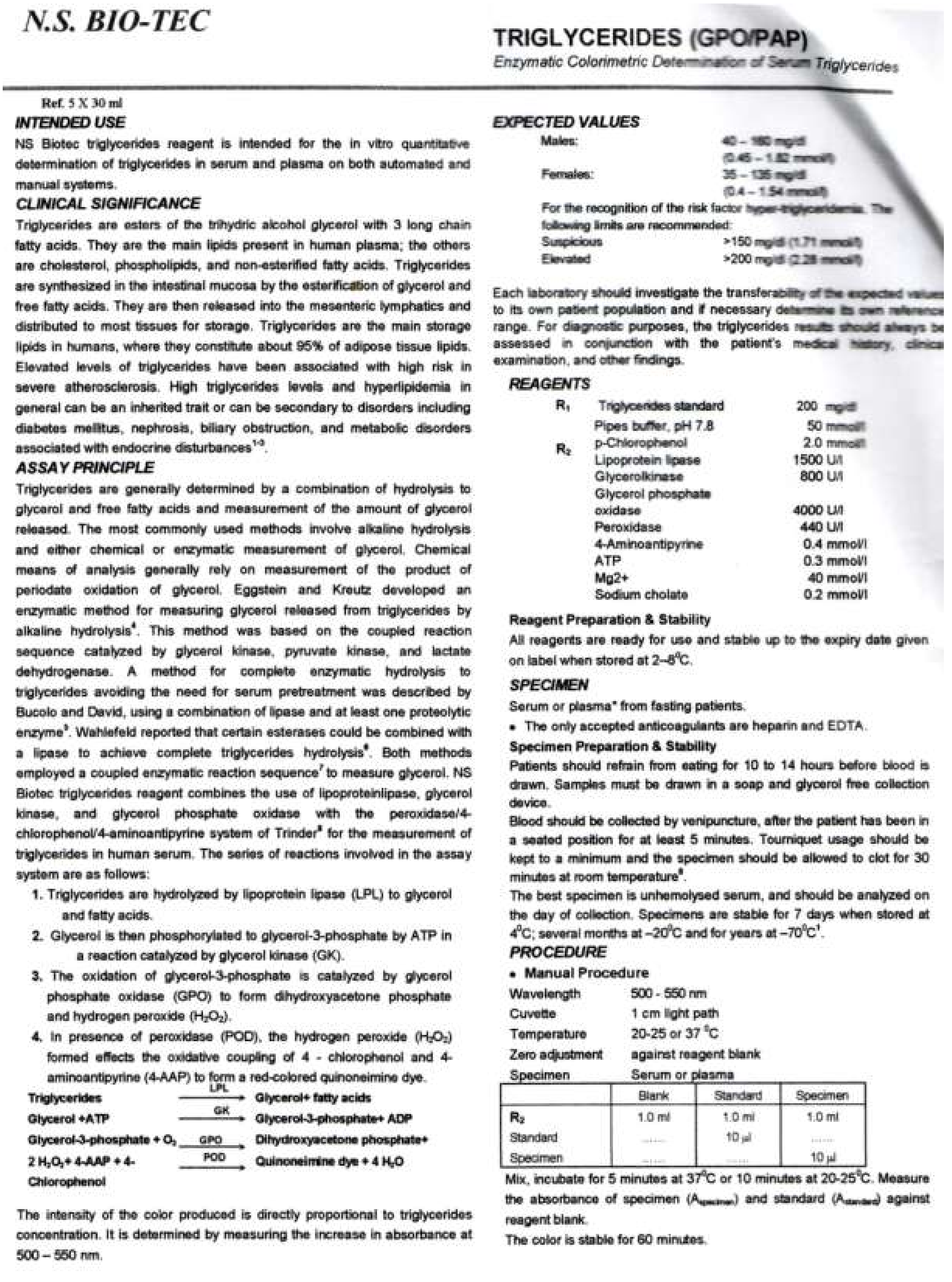
With standard or calibrator

$$\text{Glucose}\ [\text{mg/dL}] = \frac{\Delta A\ \text{Sample}}{\Delta A\ \text{Std/Cal}} \times \text{Conc. Std/Cal}\ [\text{mg/dL}]$$

Conversion factor

Glucose [mg/dL] x 0.05551 = Glucose [mmol/L]

TRIGLISERIDA



N.S. BIO-TEC

TRIGLYCERIDES (GPO/PAP)

Enzymatic Colorimetric Determination of Serum Triglycerides

Ref. 5 X 30 ml

INTENDED USE

NS Biotec triglycerides reagent is intended for the in vitro quantitative determination of triglycerides in serum and plasma on both automated and manual systems.

CLINICAL SIGNIFICANCE

Triglycerides are esters of the trihydric alcohol glycerol with 3 long chain fatty acids. They are the main lipids present in human plasma; the others are cholesterol, phospholipids, and non-esterified fatty acids. Triglycerides are synthesized in the intestinal mucosa by the esterification of glycerol and free fatty acids. They are then released into the mesenteric lymphatics and distributed to most tissues for storage. Triglycerides are the main storage lipids in humans, where they constitute about 95% of adipose tissue lipids. Elevated levels of triglycerides have been associated with high risk in severe atherosclerosis. High triglycerides levels and hyperlipidemia in general can be an inherited trait or can be secondary to disorders including diabetes mellitus, nephrosis, biliary obstruction, and metabolic disorders associated with endocrine disturbances[1-3].

ASSAY PRINCIPLE

Triglycerides are generally determined by a combination of hydrolysis to glycarol and free fatty acids and measurement of the amount of glycerol released. The most commonly used methods involve alkaline hydrolysis and either chemical or enzymatic measurement of glycerol. Chemical means of analysis generally rely on measurement of the product of periodate oxidation of glycerol. Eggstein and Kreutz developed an enzymatic method for measuring glycerol released from triglycerides by alkaline hydrolysis[4]. This method was based on the coupled reaction sequence catalyzed by glycerol kinase, pyruvate kinase, and lactate dehydrogenase. A method for complete enzymatic hydrolysis to triglycerides avoiding the need for serum pretreatment was described by Bucolo and David, using a combination of lipase and at least one proteolytic enzyme[5]. Wahlefeld reported that certain esterases could be combined with a lipase to achieve complete triglycerides hydrolysis[6]. Both methods employed a coupled enzymatic reaction sequence[7] to measure glycerol. NS Biotec triglycerides reagent combines the use of lipoproteinlipase, glycerol kinase, and glycerol phosphate oxidase with the peroxidase/4-chlorophenol/4-aminoantipyrine system of Trinder[8] for the measurement of triglycerides in human serum. The series of reactions involved in the assay system are as follows:

1. Triglycerides are hydrolyzed by lipoprotein lipase (LPL) to glycerol and fatty acids.
2. Glycerol is then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase (GK).
3. The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidase (GPO) to form dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2).
4. In presence of peroxidase (POD), the hydrogen peroxide (H_2O_2) formed effects the oxidative coupling of 4 - chlorophenol and 4-aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye.

$$\textbf{Triglycerides} \xrightarrow{LPL} \textbf{Glycerol + fatty acids}$$

$$\textbf{Glycerol + ATP} \xrightarrow{GK} \textbf{Glycerol-3-phosphate + ADP}$$

$$\textbf{Glycerol-3-phosphate} + O_2 \xrightarrow{GPO} \textbf{Dihydroxyacetone phosphate+}$$

$$2\,H_2O_2 + \textbf{4-AAP + 4-Chlorophenol} \xrightarrow{POD} \textbf{Quinoneimine dye} + 4\,H_2O$$

The intensity of the color produced is directly proportional to triglycerides concentration. It is determined by measuring the increase in absorbance at 500 – 550 nm.

EXPECTED VALUES

Males:	40 – 160 mg/dl (0.45 – 1.82 mmol/l)
Females:	35 – 135 mg/dl (0.4 – 1.54 mmol/l)

For the recognition of the risk factor hyper-triglyceridemia. The following limits are recommended:

Suspicious	>150 mg/dl (1.71 mmol/l)
Elevated	>200 mg/dl (2.28 mmol/l)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the triglycerides results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R_1	Triglycerides standard	200 mg/dl
	Pipes buffer, pH 7.8	50 mmol/l
R_2	p-Chlorophenol	2.0 mmol/l
	Lipoprotein lipase	1500 U/l
	Glycerolkinase	800 U/l
	Glycerol phosphate oxidase	4000 U/l
	Peroxidase	440 U/l
	4-Aminoantipyrine	0.4 mmol/l
	ATP	0.3 mmol/l
	Mg2+	40 mmol/l
	Sodium cholate	0.2 mmol/l

Reagent Preparation & Stability

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8°C.

SPECIMEN

Serum or plasma* from fasting patients.

- The only accepted anticoagulants are heparin and EDTA.

Specimen Preparation & Stability

Patients should refrain from eating for 10 to 14 hours before blood is drawn. Samples must be drawn in a soap and glycerol free collection device.

Blood should be collected by venipuncture, after the patient has been in a seated position for at least 5 minutes. Tourniquet usage should be kept to a minimum and the specimen should be allowed to clot for 30 minutes at room temperature[8].

The best specimen is unhemolysed serum, and should be analyzed on the day of collection. Specimens are stable for 7 days when stored at 4°C; several months at –20°C and for years at –70°C[1].

PROCEDURE

- **Manual Procedure**

Wavelength	500 - 550 nm
Cuvette	1 cm light path
Temperature	20-25 or 37 °C
Zero adjustment	against reagent blank
Specimen	Serum or plasma

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	……	10 µl	……
Specimen	……	……	10 µl

Mix, incubate for 5 minutes at 37°C or 10 minutes at 20-25°C. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.

The color is stable for 60 minutes.

- **Automated Procedure**

  User defined parameters for different auto analyzers are available upon request.

CALCULATION

Calculate the triglycerides concentration by using the following formulae:

Triglycerides Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \text{ X Standard}$$

- **Unit conversion**

  mg/dl x 0.0114 = mmol/l

LINEARITY

When run as recommended, the assay is linear up to 900 mg/dl (20.7 mmol/l).

If result exceeds 900 mg/dl (10.26 mmol/l), specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

SENSTIVITY

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.

When run as recommended the sensitivity of this assay is 3.0 mg/dl (0.034 mmol/l).

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established triglycerides values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact NS Biotec Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**

  The only acceptable anticoagulants are heparin and EDTA.
- **Bilirubin:**

  No interference from free bilirubin up to level of 10 mg/dl and from conjugated bilirubin up to a level of 12 mg/dl.
- **Drugs:**

  Methyldopa and noramidopyrine causes artificially low triglycerides values at the tested drug level. For a more comprehensive review of drugs affecting triglycerides assays refer to the publication by Young[9].
- **Haemoglobin:**

  No interference from haemoglobin up to a level of 600 mg/dl.
- **Lipemia:**

  Extremely lipemic samples can produce a normal triglycerides result (triglycerides grater than 3000 mg/dl).
- **Others:**

  Ascorbic acid levels higher than 2.0 mg/dl decrease the apparent triglycerides concentration significantly.

WARNING & PRECAUTIONS

- NS Biotec triglycerides reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- Warm up working solution to the corresponding temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- The reagent blank will not exceed an absorbance of 0.06 but don't use the reagent if it is turbid or if the absorbance is greater than 0.2 at 500 nm.
- Extremely lipemic specimens can produce a normal result. Dilute specimens' 1+4 with saline and reassayed. Multiply the result by 5.

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📖	Consult Instruction for Use
⚠	Caution Consult Accompanying Documents
IVD	In Vitro Diagnostic Medical Device
🌡	Temperature Limitation
🏭	Manufacturer
EC REP	Authorized Representative In The European Community
REF	Catalogue Number
LOT	Batch Code
⌛	Use By

N.S BIOTEC **MEDICAL EQUIPMENTS** 7 Sad Zaghloul sq. Mehatet Elraml, Alexandria – Egypt Tele: 002 03 486 5140 Fax: 002 03 485 7597 Website : www.nsbiotec.com E- mail : info@nsbiotec.com	CE EC REP **Wellkang Ltd** **suite B , 29 Harley** **street LONDON ,** **W 1 G 9 QR , U.K.** **www.CE-marking .EU**

UREA

Principle

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

$$2NH_4^+ + \text{Salicylate} + \text{Hypochlorite} \longrightarrow \text{Indophenol derivative}$$

Reagents

Kit 5 x 100 ml (Ref.99 36 48). Contents:

A. 5 x 100 ml. Urease / Salicylate.	(Ref. 99 21 04)
B. 1 x 15 ml Alkaline hypochlorite.	(Ref. 99 14 75)
C. 1 x 5 ml Standard.	(Ref. 99 02 41)

Aqueous solution of Urea equivalent to 40 mg / dl. (6.6 mmol/L). Ready-to-use.

Working reagent

A. Dissolve the contents of the Urease/Salicylate vial with the volume of deionized water stated on the label.

The concentrations in the working reagent are:

Phosphate buffer pH 6.8	20 mM
Sodium salicylate	61 mM
Sodium nitroprusiate	3.4 mM
EDTA-Na_2	1.34 mM
Urease	≥ 23 U/ml
Stabilizers	

B. Dilute the contents of the Alkaline hypochlorite vial up to 500 ml. of deionized water.

Concentrations of reagent solution are:

Alkaline hypochlorite	7.5 mM
NaOH	160 mM

Storage and stability

The components of the kit, stored at 2-8ºC, will remain stable until the expiration date stated on the label. Once the Urease/Salicylate vial has been dissolved, will remain stable for 3 weeks, if stored in amber bottle at 2-8º C. The Alkaline hypochlorite solution will remain stable for 8 months, if stored in the same way.

Sample

Serum,plasma and urine. Urea will remain stable in serum for at least 1 day at room temperature (≤ 25ºC), 5 days at 2-8ºC and 6 months when frozen (-20º C). In urine, urea will remain stable,when kept at 2-8ºC, for 5 days, provided that the pH value be lower than 4.

If a urine sample is to be assayed, it should be previously diluted 1/100 with deionized water.

Multiply the final result by 100.

Caution

Reagent **B**: In case of contact with the skin, mucose or eyes, wash thoroughly with water and ask the physician.

The reagent **A** contains Sodium azide at 0.09%. Handle with care. The disposal of the residues has to be made according to legal local regulations.

Procedure	**BL** ml	**SA** ml	**ST** ml
Standard	—	—	0.01
Sample	—	0.01	—
Reagent A	1.00	1.00	1.00

Mix and incubate 3 min. at 37ºC. or 5 min. at room temperature (≤ 25ºC).

Reagent B	1.00	1.00	1.00

Mix and incubate again 3 min. at 37ºC. or 5 min. at room temperature (≤ 25ºC).

Reading

Wavelength: Hg 578 nm; 600 nm.
Blank: The contents of the BL tube.
Colour stability: 4 hours.

Calculations

$$\frac{\text{SA O.D.}}{\text{ST O.D.}} \times 40 = \text{mg urea / dl}$$

S.I. Units

(mg/dl) x 0.1665 = mmol/L

Normal values

Serum, plasma: 15 - 45 mg/dl.
Urine: 20 - 35 g / L.

Performance Characteristics

Linearity: Up to 400 mg/dl of Urea. For higher values, dilute the sample 1/2 in deionized water and assay once again. Multiply the final result by 2.

The analytical performance characteristics of the product depend both of the reagent and the reading system used, manual or automatic. The following data have been obtained manually.

Intraseries Variation Coefficient: 1.66%
Interseries Variation Coefficient: 2.05%
Recovery: 97.9 %.

Any glassware contamination by ammonium salts or ammonia should be avoided. Serum samples should be free from hemolysis and turbidity. Fluoride as well as ammonium heparinate inhibit the reaction.

Quality control

Seriscann Normal (Normal Control Serum) (Ref. 99 41 48) and Seriscann Anormal (Abnormal Control Serum) (Ref. 99 46 85).

References

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