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IVD

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NEFA-HR(2)

For the quantitative determination of Non-Esterified Fatty Acid (NEFA) in serum

Date of issue: 2025/02/07 BX003

[Intended purpose]

NEFA-HR(2) is an *in vitro* assay for the quantitative determination of Non-esterified fatty acid (NEFA) in serum and is designed to be applied to general clinical chemistry analyzers. NEFA measurement aids to diagnosis of patients with diabetes and hepatic diseases. The usage and application of this reagent is reserved for professional use only.

[Summary and explanation of the test]

NEFA in serum binding albumin, is used as an important energy source of peripheral tissues. The amount of NEFA in serum depends on a balance between intake in liver and peripheral tissues, and the release from adipose tissues. Amount of NEFA decreases by physical exercise, increases by starvation, cold,fear or smoking. And then increase or decrease of NEFA is observed in diabetes, hepatic diseases or endocrine diseases.
NEFA had been assayed by organic solvent extraction method, which was complicated to operate. Enzymatic method using acyl-CoA-oxidase (ACOD) has become widespread due to excellent specificity and concise procedure. NEFA-HR(2) is the reagent kit for NEFA assay based on enzymatic method using 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) as a violet color agent.

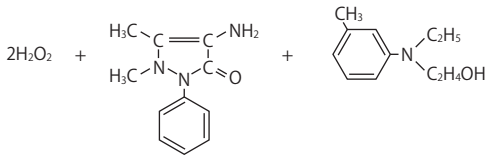
[Principle of the method]

NEFA in sample is converted to acyl-CoA, AMP and pyrophosphoric acid (PPi) by the action of acyl-CoA synthetase (ACS), under coexistence with coenzyme A (CoA) and adenosine 5-triphosphate disodium salt (ATP). Obtained acyl-CoA is oxidized and yields 2,3-trans-enoyl-CoA and hydrogen peroxide by the action of ACOD. In the presence of peroxidase (POD), the hydrogen peroxide formed yields a blue purple pigment by quantitative oxidation condensation with MEHA and 4-aminoantipyrine (4-AA).
NEFA concentration is obtained by measuring absorbance of the blue purple color.

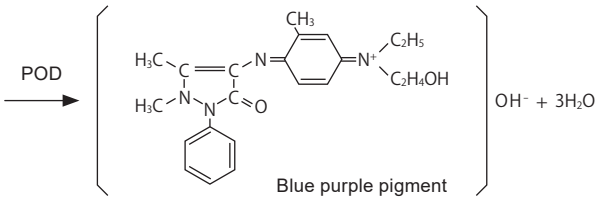
Reactions

$$\text{RCOOH + ATP + CoA} \xrightarrow{\text{ACS}} \text{Acyl-CoA + AMP + PPi}$$

$$\text{Acyl-CoA + } O_2 \xrightarrow{\text{ACOD}} \text{2,3-trans-Enoyl-CoA + } H_2O_2$$



4-aminoantipyrine MEHA



Blue purple pigment

[Reagents and Contents]

Contents

R1 Set:	(R1a) + (R1b)	
	(R1a) Color Reagent A	4 × for 50 mL
	(R1b) Solvent A	4 × 50 mL
R2 Set:	(R2a) + (R2b)	
	(R2a) Color Reagent B	4 × for 25 mL
	(R2b) Solvent B	4 × 25 mL

Ingredients

R1a:	(when reconstituted) Buffer 4-Aminoantipyrine (4-AA)	 1.42 mmol/L
R1b:	Buffer	
R2a:	(when reconstituted) Buffer Acyl-CoA- oxidase (ACOD)	 12.7 U/mL
R2b:	3-Methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA)	2.34 mmol/L

[Reagent preparation]

R1 Set: Prepare R1 by dissolving one vial of R1a with one bottle of R1b. After reconstituted R1, the reagent should be store at 2 – 10°C and use within one month.
R2 Set: Prepare R2 by dissolving one vial of R2a with one bottle of R2b. After reconstituted R2, the reagent should be store at 2 – 10°C and use within one month.

[Specimen collection and preparation]

Serum can be used as specimen.
It is recommended to measure NEFA immediately after collection, because the enzymes such as lipoprotein lipase, phospholipase, etc., hydrolyze lipids and form fatty acids. If analysis is not immediately possible, store specimen in freeze.
Stability of NEFA in the specimen is summarized below. All results come from in-house evaluation. Stability of NEFA in the specimen should be different due to specimen characteristics.
Heparin administration promotes *in vitro* lipolysis by the release of lipoprotein lipase from the endothelium. This can cause the NEFA in the specimen to be falsely high[1].
It is recommended that specimen collection is carried out in accordance with local and national regulations. Since all specimens are potentially infectious, they should be handled in accordance with any other local or national regulation relating to the safe handling of such materials.

Storage temperature	Stability of NEFA in the specimen*
-30°C	98 – 103% in 2 months
8°C	100 – 110% in 1 day

* Rate of change relative to the measured value at the start.

[Physical or chemical indications of instability]

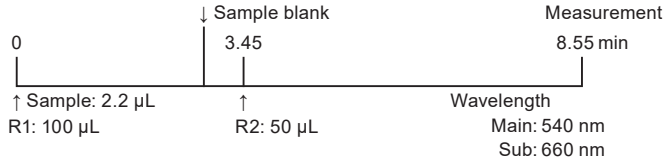
The presence of precipitates in the reagents or values of control sera outside the manufacturer’s acceptable range may be an indication of reagent instability.

[Instruments]

The reagent is designed to be used on commercially available automatic analyzers. Refer to the instruction manual for a description of instrument operation and specifications.
A validation by the user in practice at the customer’s site in the form of measurements of adequate control or patient sera in sufficient number is indispensable.

[Standard procedure]

Temperature: 37°C (BECKMAN COULTER DxC 700 AU)



Calibrator: NEFA Standard (Available separately)

Calculation of NEFA concentration

Calculate NEFA concentration from the calibration curve which was created from absorbance of calibrator.

Application to the various automatic analyzers

Input the parameters according to the instruction manual to perform the measurement. Instrument applications are available upon request.

Limitations of the procedure

When NEFA concentration in a specimen exceeds the upper limit of the measurable range, dilute the specimen with saline solution, repeat assay and multiply the result by the dilution factor.

[Results]

The final results are automatically calculated and printed in concentration units (mEq/L).

[Expected values[2)]]

Men: 0.1 – 0.60 mmol/L
Women: 0.1 – 0.45 mmol/L

As the expected values depend on the population of the test, each laboratory is required to set their own expected values.

[Performance characteristics]

1. Analytical sensitivity

a) When saline is assayed, the absorbance is not more than 0.140.
b) When sample with assigned value (oleic acid 1 mEq/L) is assayed, the absorbance is 0.100 – 0.380.

2. Analytical specificity

Specificity was studied by adding substances that were thought to endogenously interfere with NEFA test result, into serum.

1) Hemoglobin

Hemoglobin	(mg/dL)	None	100	200	300	400	500
NEFA	(mEq/L)	0.79	0.78	0.76	0.76	0.76	0.75
	Recovery (%)	100.0	98.7	96.2	96.2	96.2	94.9

2) Bilirubin

Bilirubin	(mg/dL)	None	10	20	30	40	50
NEFA	(mEq/L)	0.79	0.76	0.74	0.76	0.76	0.74
	Recovery (%)	100.0	96.2	93.7	96.2	96.2	93.7

3) Conjugate bilirubin

Conjugate bilirubin	(mg/dL)	None	4	8	12	16	20
NEFA	(mEq/L)	0.78	0.76	0.74	0.72	0.70	0.68
	Recovery (%)	100.0	97.4	94.9	92.3	89.7	87.2

4) Intralipos

Intralipos	(%)	None	0.4	0.8	1.2	1.6	2.0
NEFA	(mEq/L)	0.80	0.80	0.80	0.80	0.80	0.81
	Recovery (%)	100.0	100.0	100.0	100.0	100.0	101.3

5) Ascorbic acid

Ascorbic acid	(mg/dL)	None	10	20	30	40	50
NEFA	(mEq/L)	0.80	0.80	0.80	0.80	0.80	0.80
	Recovery (%)	100.0	100.0	100.0	100.0	100.0	100.0

3. Accuracy

3-1. Trueness (bias)

The trueness of this method was determined by a recovery study.

Serum sample 1

Added (mEq/L)	0.00	0.23	0.39	0.83
Measured (mEq/L)	0.22	0.48	0.64	1.07
Obtained (mEq/L)	–	0.26	0.42	0.85
Recovery (%)	–	113.0	107.7	102.4

Serum sample 2

Added (mEq/L)	0.00	0.23	0.39	0.83
Measured (mEq/L)	2.20	2.45	2.61	3.01
Obtained (mEq/L)	–	0.25	0.41	0.81
Recovery (%)	–	108.7	105.1	97.6

Serum sample 3

Added (mEq/L)	0.00	0.23	0.39	0.83
Measured (mEq/L)	2.96	3.21	3.35	3.79
Obtained (mEq/L)	–	0.25	0.39	0.83
Recovery (%)	–	108.7	100.0	100.0

The recovery rate of NEFA is 97.6% – 113.0% in the concentration ranges shown in the above table.

3-2. Precision (1) Repeatability

Below are representative data of the repeatability. The CV of NEFA obtained by measuring each sample 21 times was not more than 2.2% at 0.23 mEq/L or higher NEFA concentrations.

Sample No.	Sample 1	Sample 2	Sample 3
Mean (mEq/L)	0.23	2.94	3.73
SD (mEq/L)	0.005	0.012	0.016
CV (%)	2.2	0.4	0.4

3-3. Precision (2) Reproducibility

Below are representative data of the reproducibility. All data were collected in accordance with CLSI EP05-A3.

Sample No.	Mean Value (mEq/L)	N	Repeatability		Between-Day		Between-Site		Reproducibility	
			SD (mEq/L)	CV (%)	SD (mEq/L)	CV (%)	SD (mEq/L)	CV (%)	SD (mEq/L)	CV (%)
1	0.22	75	0.003	1.4	0.003	1.4	0.004	1.8	0.006	2.7
2	0.39	75	0.000	0.0	0.003	0.8	0.007	1.8	0.008	2.1
3	0.94	75	0.006	0.6	0.005	0.5	0.016	1.7	0.018	1.9
4	2.34	75	0.011	0.5	0.016	0.7	0.018	0.8	0.027	1.2
5	3.64	75	0.020	0.5	0.018	0.5	0.028	0.8	0.039	1.1

3-4. Precision (3) Total precision

Below are representative data of the total precision. All data were collected in accordance with CLSI EP05-A3.

Sample No.	Sample 1	Sample 2	Sample 3
Total mean (mEq/L)	0.227	2.97	3.69
Total precision SD (mEq/L)	0.0026	0.025	0.027
Total precision CV (%)	1.1	0.8	0.7

4. Limit of detection and quantitation

LoB, LoD and LoQ were determined by calculating in accordance with CLSI EP17-A2.

Limit of blank (LoB): 0.00 mEq/L
Limit of detection (LoD): 0.01 mEq/L
Limit of quantitation (LoQ): 0.43 mEq/L

5. Measuring range (Linearity)

The assay was verified to be linear for the reportable ranges of 0.01 – 4.00 mEq/L.

[Metrological traceability of calibrator]

The concentration of NEFA is determined by measuring a standerd with the manufacturer's standing measurement procedure and the manufacturer's internal standard. The concentration of NEFA of the manufacturer's internal standard was determined by dissolving the weighed oleic acid in the buffer.

[Warnings and precautions]

- For *in vitro* diagnostic use.
- The usage and application of this reagent is reserved for professional use only. Please refer to respective national and local regulations and legislation.
- Not to be used internally in humans or animals.
- Do not use the reagents described above in any procedures other than those described herein. Performance cannot be guaranteed if the reagents are used in other procedures.
- Do not use the containers and other materials in the package for any purposes other than those described herein.
- Operate the instruments according to instruction manuals under appropriate conditions. Consult the instrument manufacture for details.
- The results of this assay should be used in conjunction with physician judgment and clinical symptoms.
- If the reagents come in contact with mouth, eyes or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
- All devices including reagents and reagent containers that come in contact with specimen should be considered potentially infectious.
- Store the reagents under the specified conditions. Do not use reagents past the expiration date stated on each container label.
- After reconstituted the reagents, it is recommended to use them immediately. When the opened reagents are stored, cap the bottles and keep them under the specified conditions.
- Do not use the reagents that were frozen in error. Such reagents may give false results.
- Be careful not to cut yourself with the aluminum cap when removing it from the vial.
- The vial is stoppered at reduced pressure. Slowly remove the stopper in order not to blow the powder in the vial.
- Do not mix the reagents, which have different lot numbers.
- When the reagent for NEFA is used at the same time as reagent for cholesterol and triglyceride, the cholesterol esterase and lipoprotein lipase in the reagent are adsorbed to cuvettes and may interfere the measured value of NEFA.
- When discarding the reagents, dispose of them according to local or national regulations.
- R1b contains cyano complex (1 mg/L as cyan).
- R1a contains 0.75% and R1b contains 0.05% sodium azide as a preservative. When R1a is dissolved in R1b, the sodium azide concentration is 0.055%. Sodium azide may react with lead or copper plumbing to form explosive compounds. Even though the reagents contain minute quantities of sodium azide, drains should be flushed well with a large amount of water, when discarding the reagents.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

NEFA-HR(2) R1 Set (R1a) Color Reagent A contains components as follows according to Regulation (EC) No.1272/2008. Please note the safety data sheet!

- **Hazard statements**
  H412 Harmful to aquatic life with long lasting effects.
- **Precautionary statements**
  P273 Avoid release to the environment.
  P501 Dispose of contents/container in accordance with local/regional/national/international regulations.
- **Additional information:**
  EUH208 Contains Ascorbate Oxidase. May produce an allergic reaction.

NEFA-HR(2) R2 Set (R2a) Color Reagent B contains components as follows according to Regulation (EC) No.1272/2008. Please note the safety data sheet!

- **Hazard pictograms**

GHS08

- **Signal word** Danger
- **Hazard-determining components of labelling:**
  Acyl Coenzyme A Oxidase
  Peroxidase
- **Hazard statements**
  H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
- **Precautionary statements**
  P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
  P284 [In case of inadequate ventilation] wear respiratory protection.
  P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
  P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.

NEFA-HR(2) R2 Set (R2b) Solvent B contains components as follows according to Regulation (EC) No.1272/2008. Please note the safety data sheet!

- **Additional information:**
  EUH208 Contains Mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one, maleimide. May produce an allergic reaction.
  EUH210 Safety data sheet available on request.

[Quality control]

A quality control program is recommended for all clinical laboratories.

[Storage and shelf life]

Product	Storage condition	Shelf life
NEFA-HR(2) R1 Set	2 – 10°C	12 months
NEFA-HR(2) R2 Set	2 – 10°C	12 months

Check the expiration date on the label before use.

[References]

1) Krebs M., et al.: Clin. Chem., 2000; 46 (7): 950 - 954.
2) Greiling H., et al.: Lehrbuch der Klinischen Chemie und Pathobiochemie (3rd Edition)., 1995; 319 - 320.

[Information]

Catalogue number	Product	Contents	
994-91801	NEFA-HR(2) R1 Set	(R1a) Color Reagent A (R1b) Solvent A	4 × for 50 mL 4 × 50 mL
990-91901	NEFA-HR(2) R2 Set	(R2a) Color Reagent B (R2b) Solvent B	4 × for 25 mL 4 × 25 mL
991-91791	NEFA Standard	NEFA Standard	2 × 10 mL

[Symbols]

IVD	In vitro diagnostic medical device
UDI	Unique Device Identifier
CONT.	Contents of the kit
	Consult instructions for use or consult electronic instructions for use
	Temperature limit
LOT	Batch code
	Use-by date (last day of the month)
	Manufacturer
	Importer
EC REP	Authorized representative in the European Community/ European Union
REF	Catalogue number

 https://diagnostic-wako.fujifilm.com/cemarkingproducts/index.html

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