

## Intended use

The HR Series NEFA-HR (2) is an in vitro enzymatic colorimetric method assay for the quantitative determination of non-esterified fatty acids (NEFA) in serum.

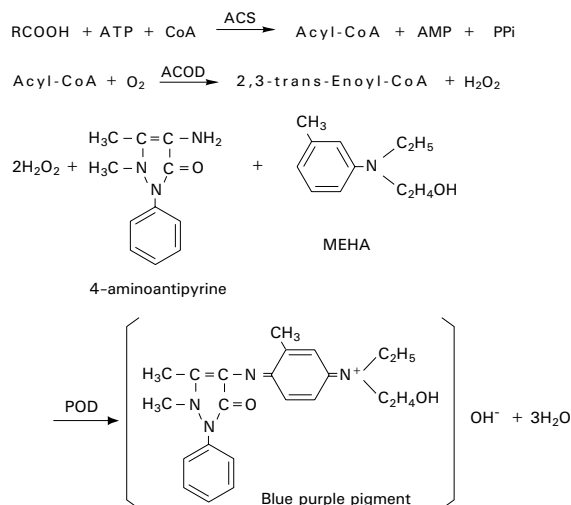
## Summary and explanation of the test

Extraction methods are widely used for the colorimetric determination of non-esterified fatty acids (NEFA) in serum. NEFA are converted to their copper salts that are extracted into an organic solvent. The salts are then complexed with a dye for the purpose of colorimetric measurement<sup>(1,2,3,4)</sup>. Alternatively, extracted NEFA are titrated with standard alkali to an acid-base indicator endpoint<sup>(5,6)</sup>. These approaches are time consuming, hazardous and not easily automated. Wako has made extensive studies of NEFA quantitation and has succeeded in developing an original enzymatic method which is accurate, precise, simple and fast. The need for an extraction step has been eliminated and the method can be automated.

The Wako enzymatic method relies upon the acylation of coenzyme A (CoA) by the fatty acids in the presence of added acyl-CoA synthetase (ACS). The acyl-CoA thus produced is oxidized by added acyl-CoA oxidase (ACOD) with generation of hydrogen peroxide. Hydrogen peroxide, in the presence of peroxidase (POD), permits the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple colored product which can be measured colorimetrically at 550 nm.

## Principle of the method

Non-esterified fatty acids (NEFA) in serum, when treated with acyl-CoA synthetase (ACS) in the presence of adenosine triphosphate (ATP) and CoA, form the thiol esters of CoA known as acyl-CoA along with the byproducts adenosine monophosphate (AMP) and pyro-phosphate (PPI). In the second portion of the procedure, the acyl-CoA is oxidized by added acyl-CoA oxidase (ACOD) to produce hydrogen peroxide which in the presence of added peroxidase (POD) allows for the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple colored end product with an absorption maximum at 550 nm. Hence the amount of NEFA in the sample can be determined from the optical density measured at 550 nm.



## Reagents

- Color Reagent A  
When reconstituted  
0.53 U/mL Acyl-coenzyme A synthetase (ACS) (*Pseudomonas* sp.)  
0.31 mmol/L Coenzyme A (CoA, *Candida*)  
4.3 mmol/L Adenosine triphosphate (ATP) (*Bacterium* sp.)  
1.5 mmol/L 4-aminoantipyrine  
2.6 U/mL Ascorbate oxidase (pumpkin)  
0.062% Sodium azide (as Color Reagent A Solution)  
Store at 2-10°C.
- Solvent A  
50 mmol/L phosphate buffer, pH 7.0  
0.05% Sodium azide  
Store at 2-10°C.
- Color Reagent B  
When reconstituted  
12 U/mL Acyl-coenzyme A oxidase (ACOD) (*Arthrobacter* sp.)  
14 U/mL Peroxidase (POD) (horseradish)  
Store at 2-10°C.

- Solvent B  
2.4 mmol/L 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA)  
Store at 2-10°C.

## Warnings and precautions

- For in vitro diagnostic use.
- Not to be used internally in humans or animals.
- Do not use reagents past the expiration date stated on each reagent container label.
- Do not use the reagents described above for any purpose other than described herein.
- Do not use reagents which were frozen in error. Such reagents may give false results.
- If the reagents come in contact with the mouth, eyes or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
- Color Reagent A and Solvent A contain sodium azide as a preservative (0.062% as reconstituted solution). Sodium azide may react with copper or lead plumbing to form explosive compounds. Even though the reagents contain minute quantities of sodium azide, drains should be well flushed with a large amount of water when discarding the reagents.
- The vials are sealed under vacuum. Slowly remove the stopper in order not to release the powder in the vial.
- Be careful not to cut yourself with the aluminium cap when removing it from the vial.

## 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 automated analyzers such as Beckman SYNCHRON CX5<sup>®</sup> analyzer.

Refer to the operating manual for a description of instrument operation, specifications and calibration.

## Specimen collection and preparation

Use serum as a specimen. Blood should be collected in the early morning after the patient has fasted for at least 12 hours. Collection of only a few mL of blood from the antecubital vein into a plain evacuated tube will be quite satisfactory.

After the blood has been allowed to clot, the serum should be separated by centrifugation as soon as possible. Please note that serum is the specimen of choice, but alternate collections may be acceptable.

Any specimen containing heparin is unsuitable for this analysis. Hence, any patient receiving heparin therapy, or any specimen collected in a heparinized collection vessel is unsuitable for this analysis.

Specimens that are noticeably icteric, hemolyzed or lipemic may yield inaccurate results unless a specimen blank is also analyzed.

## Warning/Biohazard

It is recommended that specimen collection be carried out in accordance with NCCLS Document M29-T2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

## Procedure for Beckman SYNCHRON CX5<sup>®</sup>

### Materials supplied

Refer to the section entitled "Reagents."

### Materials required but not supplied

Beckman SYNCHRON CX5<sup>®</sup> analyzer

NEFA Standard Solution

Quality control material

All analyzer applications should be validated in accordance with NCEP and CLIA recommendations. For further assistance contact Wako Diagnostics Technical Service Department at 1-877-714-1924 or e-mail [diagnostics@wakousa.com](mailto:diagnostics@wakousa.com).

### Reagent preparation

Color Reagent A Solution :

Add one bottle of Solvent A to one vial of Color Reagent A. Mix gently by inverting the vial until the contents are completely dissolved. Reconstituted solution is stable for one month at 2-10°C.

Color Reagent B Solution :

Add one bottle of Solvent B to one vial of Color Reagent B. Mix gently by inverting the vial until the contents are completely dissolved. Reconstituted solution is stable for one month at 2-10°C.

## Test procedure

### Parameter setting (Beckman SYNCHRON CX5®)

Reagent	HR Series NEFA-HR (2)
Test name	NEFA
Reaction Type	ENDPOINT2
Reaction Direction	Positive
Unit	mEq/L
Decimal Position	X.XX
Calculation Factor	0
Math Model	LINEAR
Cal. Time Limit	9999hr
No. of Calibrator	2
Cal#1	saline/0.00/*2
Cal#2	Calibrator/*1/*2
Cal#3	
Cal#4	
Cal#5	
Cal#6	
Primary Wavelength	560 nm
Secondary Wavelength	670 nm
Sample Volume	4 µL
Primary Inject R1	225 µL
Primary Inject R2	
Secondary Inject Reagent	75 µL
Add Time	592 sec.
Calibrators	
Multi point Span 1-2	0.00
2-3	
3-4	
4-5	
5-6	
6-1	
RGT Blank Start Read	528 sec.
End Read	560 sec.
Low/High Abs	- 1.5/1.5
Reaction Start Read	576 sec.
End Read	608 sec.
Low/High Abs	- 1.5/1.5
Usable Range Low/High Limit	0/9999
Substrate Depletion	
Initial Rate	99.999
Delta Abs.	1.5

\* 1 : Input the assigned value of the calibrator.

\* 2 : Input the position of the calibrator.

## Results

The final results are automatically calculated and printed in concentration. The results are given in mEq/L.

## Calibration

The HR Series NEFA-HR (2) assay must be calibrated using the NEFA Standard Solution.

## Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

## Limitations of the procedure

The linearity of HR Series NEFA-HR (2) is 0.01-4.00 mEq/L. If the NEFA value exceeds 4.00 mEq/L, dilute the sample 1 + 2 with saline, repeat the assay, and multiply the result by 3.

## Expected values

The expected normal range for serum NEFA from fasting patients is 0.1 - 0.6 mEq/L. Since expected values are affected by age, sex, diet and geographical factors, each laboratory should establish its own expected values for this procedure.

## Performance characteristics

### Accuracy

The accuracy of this method was demonstrated by a recovery study.

No.	Added value	Expected value	Measured value	Obtained value	Recovery
	(mEq/L)	(mEq/L)	(mEq/L)	(mEq/L)	(%)
1	0.15	0.42	0.42	0.15	100.0
2	0.30	0.57	0.58	0.31	103.3
3	0.59	0.86	0.89	0.62	105.1

No.	Added value	Expected value	Measured value	Obtained value	Recovery
	(mEq/L)	(mEq/L)	(mEq/L)	(mEq/L)	(%)
1	0.39	1.43	1.43	0.39	100.0
2	0.65	1.69	1.69	0.65	100.0
3	0.78	1.82	1.82	0.78	100.0

No.	Added value	Expected value	Measured value	Obtained value	Recovery
	(mEq/L)	(mEq/L)	(mEq/L)	(mEq/L)	(%)
1	0.59	2.61	2.62	0.60	101.7
2	1.18	3.20	3.16	1.14	96.6
3	1.77	3.79	3.87	1.85	104.5

### Precision

Within-run precision

Sample #	Replicates	Mean (mEq/L)	SD	CV (%)
1	20	0.51	0.0038	0.75
2	20	0.96	0.0059	0.61

Total precision

Number of assay days	Mean (mEq/L)	S <sub>WT</sub>	S <sub>T</sub>	CV (%)
20	0.548	0.0015	0.0041	0.75
20	1.082	0.0053	0.0531	4.91

### Sensitivity

The minimum detectable level of this method is estimated to be 0.0014 mEq/L.

### Correlation

A group of 97 serum samples with NEFA concentration ranging from 0.10 to 1.73 mEq/L was assayed by the described procedure and by a commercially available method. Comparison by values yielded a correlation coefficient of 0.992 and the regression equation was  $y = 1.027x + 0.041$ .

### Specificity (Beckman SYNCHRON CX5®) (Additive Study)

Hemoglobin (mg/dL)	None	100	200	300	400	500
NEFA (mEq/L)	0.48	0.47	0.45	0.44	0.42	0.40

Ascorbic acid (mg/dL)	None	10	20	30	40	50
NEFA (mEq/L)	2.16	2.15	2.14	2.13	2.14	2.11

Free Bilirubin (mg/dL)	None	10	20	30	40	50
NEFA (mEq/L)	1.74	1.69	1.65	1.60	1.57	1.56

Conjugated Bilirubin (mg/dL)	None	8	16	24	32	40
NEFA (mEq/L)	2.08	1.98	1.87	1.77	1.67	1.56

## References

- (1) Duncombe, W. G. : Clin. Chim. Acta 9. 122 (1964).
- (2) Itaya, K., and Ui, M. : J. Lipid Res. 6, 16 (1965).
- (3) Novak, M. : J. lipid Res. 6 431 (1965).
- (4) Elphick, M. D. : J. Clin. Pathol. 21, 567.
- (5) Trout, D. L., Estes, E. H. and Friedbekg, S. J. :J. Lipid Res. 1 : 199 (1960).
- (6) Dole, V.P. and Meinertz, H. : J. Biol. Chem. 235, 2595 (1960).

## Ordering information

Code No.	Product	Package
999-34691	HR Series NEFA-HR (2) Color Reagent A	4 × for 50 mL
995-34791	HR Series NEFA-HR (2) Solvent A	4 × 50 mL
991-34891	HR Series NEFA-HR (2) Color Reagent B	4 × for 25 mL
993-35191	HR Series NEFA-HR (2) Solvent B	4 × 25 mL
997-76491	Wako NEFA Linearity Set	10 mL
276-76491	NEFA Standard Solution	4 × 10 mL

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