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ACE kinetic

Angiotensin Converting Enzyme

KK-ACK	100 tests
KK-ACK2	2 x 50 tests
KK-ACK4	400 tests
KK-ACKX	1200 tests

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ENGLISH

INTENDED USE

The BÜHLMANN ACE kinetic test is intended for the direct and quantitative *in vitro* diagnostic determination of angiotensin converting enzyme (ACE) activity in serum by an enzymatic assay.

PRINCIPLE OF THE ASSAY

ACE catalyses the conversion of angiotensin I to angiotensin II. The enzyme also mediates the cleavage of the synthetic substrate (FAPGG) into an amino acid derivative and a dipeptide. The kinetic of this cleavage reaction is measured by recording the decrease in absorbance at 340 nm (1,2). The ACE kinetic method is standardized with the BÜHLMANN ACE colorimetric kit according to the reference method described in 3 and 4.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity			Code	Reconsti-tution
	KK-ACK/ KK-ACK4	KK-ACK2	KK-ACKX		
Substrate	1 vial/ 4 vials 26 ml	2 vials 13 ml	3 vials 100 ml	B-ACK-SUB B-ACK2-SUB[1] B-ACKX-SUB[2]	Ready to use
Calibrator[3]	1 vial/ 2 vials	2 vials	3 vials	B-ACK-CA	add 2 ml of sterile water
Controls[4] Normal and High	1x2 vials/ 2x2 vials	2x2 vials	3x2 vials	B-ACK-CONSET	add 2 ml of sterile water

Table 1

1 Order Codes for KK-ACK2.
2 Order Codes for KK-ACKX.
3 Lyophilized ACE Calibrator in a protein serum matrix with lot specific activity. After reconstitution leave for 15 minutes at 18-28°C and mix well before use. The Calibrator has been standardized with the BÜHLMANN ACE colorimetric kit (*cf.* above).
4 Lyophilized ACE Normal and High Controls in a protein serum matrix with lot specific activity. Reconstitute for 15 minutes at 18-28°C and mix well before use.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
Stable at 2-8°C until expiration date printed on the label	
Opened / Reconstituted Reagents	
Substrate	Stable until exp. date at 2-8°C
Calibrator	Stable for 6 months at 2-8°C
Controls	

Table 2

WARNINGS AND PRECAUTIONS

All reagents of this kit contain components of human origin. Each serum used to prepare the kit components was tested by an FDA approved method and found negative for HBV surface antigen, as well as for HCV and HIV1/2 antibodies. Although these methods are highly accurate, there is no guarantee that this material cannot transmit Hepatitis or AIDS. *Therefore, all patient specimens and kit components should be handled as potentially infectious.* All products containing human source material should be handled according to good laboratory practice using appropriate precautions.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipetes: 25, 100, 150, 250 µl and 2 ml
- Plastic test tubes for sample or calibrator incubation
- Vortex mixer
- Waterbath for incubation set at 37°C
- Spectrophotometer with temperature controlled cuvette holders for incubation at 37°C and for measurement of absorbance at 340 nm
- Clinical chemistry analyser with 340 nm filter (optional)

SPECIMEN COLLECTION AND STORAGE

Since EDTA inhibits ACE activity, serum specimen should be used for the determination of ACE activity.
Collect sufficient blood (at least 0.5 ml) by venipuncture into an appropriate tube without anticoagulant. coagulate for 2-3 hours at room temperature, centrifuge at 4°C and 1000 x g and collect the serum. Freeze the serum specimen at –20°C if not assayed within 5 days. ACE activity in sterile serum is stable for up to 30 days at 2-8°C and 6 months at –20°C.
To avoid lipemic sera, blood samples should be taken from fasting patients. Due to interference with the photometric determination, lipemic sera must be pretreated either with an Ultracentrifuge or with LipoClear from StatSpin Inc. (www.statspin.com). Icteric or hemolytic sera can not be used for ACE activity determination.

ASSAY PROCEDURE

Assay Protocol (For automatic procedure *cf.* next chapter below)
Allow the substrate to reach room temperature. Avoid heating of the substrate. Pretreat all lipemic sera according to the procedure described above.

1. Label two plastic tubes for calibrator and controls and samples.
2. Pipet 25 µl of Calibrator, Control serum and patient sample, respectively, into the corresponding tubes.

NOTE: The measurement of the enzyme activity as described below has to be started according to the capacity of the respective photometer.

3. Add 250 µl of substrate to each sample and vortex thoroughly.
4. Incubate for 5 minutes at 37°C in a water bath.
5. Blank your photometer with distilled water.
6. Transfer Calibrators, Controls and samples into a microcuvette, incubate them at 37°C and measure the samples twice at an interval of exactly 10 minutes at 340 nm.

RESULTS AND AUTOMATION

Standardization
The results obtained using this assay is identical to the values obtained with the standard BÜHLMANN ACE colorimetric assay. The ACE kinetic (KK-ACK) is standardized against the reference method established by Lieberman (3).
Automation with Clinical Analysers
ACE kinetic can be performed on every open chemistry analyser. Bühlmann will assist you to adapt your instrument settings. In order to guarantee equivalent kinetic characteristics between manual and automated version we recommend using the volumes and/or ratios established for the manual version. For instructions on programming and operation please refer to the “Operators Manual” of the

Instrument. We also advise that each laboratory should evaluate the assay performance on their instrument.

PARAMETER SETTINGS FOR SEVERAL CLINICAL CHEMISTRY ANALYSERS ARE AVAILABLE UPON REQUEST.

CALCULATION

Calculate the corresponding enzyme activity (E_x) of each unknown sample by dividing the difference in absorbance of the individual sample (ΔA_x) through the mean of the absorbance difference of the calibrator vial (ΔA_c) and multiply the results by the enzyme activity (E_c) indicated on the data sheet (for an example see Table 11):

$$E_x = \frac{\Delta A_x}{\Delta A_c} \times E_c$$

Automated procedure: For calculation of results use the RATE mode. Refer to the instrument manual for further details.

Definition: One unit of ACE activity is defined as the amount of enzyme required to release one μmol of Hippuric Acid per minute and per liter of serum at 37°C as determined by the colorimetric assay:

$$1\,\text{ACE unit} = \frac{1\,\mu\text{mol hippuric acid}}{\text{min} \times \text{L}} = 1\,\text{U/l}$$

QUALITY CONTROL

The values of the Normal and High Controls provided with the kit must be within the lot specific range indicated on the corresponding data sheet. Otherwise, the assay has to be repeated.

It is good laboratory practice to record the following data for each assay: kit lot number, reconstitution dates of kit components, concentration value of calibrator and controls, concentration values of internal serum pool.

PERFORMANCE CHARACTERISTICS

Intra-Assay Precision: 2.7%. The intra-assay precision has been determined by measuring three different serum samples 20 times in a single test run (*cf.* Table 12).

Inter-Assay Precision: 8.1%. The inter-assay precision has been determined by measuring three control serum samples in 20 consecutive runs (*cf.* Table 13).

Dilution Linearity: 108.9%. 14 serum samples with elevated ACE activity (range: 100-172 U/l) have been diluted with physiological NaCl solution from 1:1 up to 1:32 in various runs. In total 140 values has been analysed. The median value of dilution linearity (observed vs. expected) is 108.9% and the 5-95th percentile is 90 to 137% (*cf.* Table 14 and Figure 1). The kinetic method is linear up to at least 150 U/l.

Spiking Recovery: 99.8%. Two different serum samples have been spiked with increasing amounts of ACE and analyzed according to the assay procedure (*cf.* Table 15).

Analytical Sensitivity: <5 U/l. The analytical sensitivity is dependent on the precision of the clinical chemistry analyser used. By repeated measurements with water (blank reagent) the imprecision of the analyser was determined by calculating the mean +3 SD value and converted it into ACE U/l.

For Cobas Mira the instrument detection limit is calculated as 2.5 U/l (n=100) and for the Kone T30 analyser as 3.6 U/l (n=33).

Functional Sensitivity: ~12 U/l. The functional sensitivity was determined by repeated measurements (n=356) of a series of sera (n=45) with medium to low activity (range: 1.5-35.5 U/l). The ACE activity at 20 %CV was determined to be 12 U/l.

Specificity: Inhibition by its natural substrate Ang I and by EDTA and H-Val-Trp-OH. A serum sample of defined ACE activity has been inhibited and measured again (*cf.* Table 16).

EXPECTED VALUES

The serum ACE activity strongly depends on the genotype of the patients investigated (5). Therefore, reference values depend on the genetic pattern of the donors and differences can be explained by the frequency of the three genotypes within group of donors on investigation.

From a group of **80 adult** (age 20-70 years) normal blood donors from Switzerland, a reference range (2.5-97.5th Percentile) has been determined to be (cf. Table 17):

20 – 70 U/l

In a study 159 Caucasian volunteers, classified as apparently healthy by a medical examination, were tested for ACE activity. No correlation between serum ACE activity and age could be detected. A median value of 45.1 U/l and a normal range (2.5th -97.5th Percentile) of 16 – 85 U/l was described (5).

In a study with 50 sarcoidosis patients, serum ACE levels were within a range of 45-135 U/l (6).

Serum ACE levels in children are substantially higher and more variable than in adults (5). Elmlinger et al. (Children's Hospital, Tübingen, Germany), examined **84 children** from 6 months to 18 years to estimate serum ACE reference range. A normal serum range (2.5-97.5th Percentile) has been determined to be (cf. Table 17):

29 – 112 U/l

No significant differences have been observed by splitting into gender or age categories. In Newborns (0 to 6 months) very low ACE activity has been detected (Data not shown).

METHOD COMPARISON

80 sera from apparently healthy donors (see above) were analyzed with the three different assays from Bühlmann AG. The results from ACE kinetic assay, ACE colorimetric and ACE direct radioenzymatic were correlated:

ACE kinetic = 1.065 * ACE colorimetric – 6.05 U/l;
r = 0.94; R^2 = 0.88

ACE kinetic = 1.368 * ACE direct – 13.96 U/l;
r = 0.94; R^2 = 0.89

APPENDIX I
TABLES/ TABELLEN/ TABLES/ TABELLE/ TABLAS

Table 11 **Example of results**

Sample	Abs. t=0min	Abs. t=10min	ΔAbs. t=10min	ACE U/l
Calibrator	1.4737	1.3805	0.093	82.1
Normal Contr	1.5083	1.4663	0.042	37.1
High Control	1.5499	1.4689	0.081	71.6
Unknown	1.4884	1.4174	0.071	62.7

$$\mathbf{E_x = \frac{0.071}{0.093} \times 82.1 = 62.7\ ACE\ U/l}$$

Table 12 **Intra-assay precision**

Mean [ACE U/l]	SD [ACE U/l]	n	CV [%]
38.6	1.3	20	3.4
63.6	1.7	20	2.7
85.3	1.8	20	2.1
Mean			2.7

Table 13 **Inter-assay precision**

Mean [ACE U/l]	SD [ACE U/l]	n	CV [%]
20.2	2.6	20	12.8
48.5	3.6	20	7.4
78.1	3.1	20	4.0
Mean			8.1

Table 14 **Dilution linearity (observed/expected)**

n	140
mean	110.7 %
95% CI	108.0 – 113.5 %
SD	16.4 %
Median	108.9 %
96.5% CI	105.7 – 111.7 %
IQR	17.7 %

Figure 1 **Dilution Linearity**

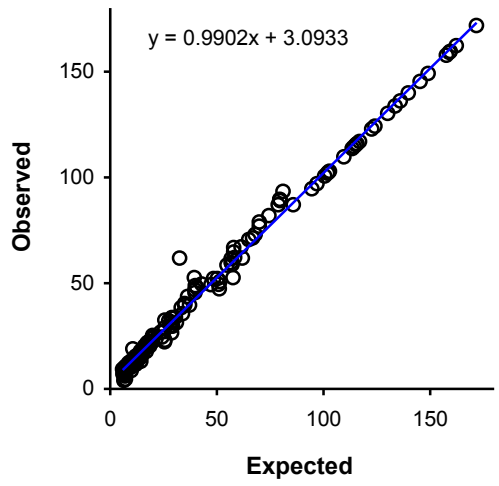

Table 15 **Spiking recovery**

	Basic value	Spiked with	Calculated	Observed	Recovery[%]
Sample 1	18.5	12.5	31.0	29.8	96
		25	43.5	46.1	106
		50	68.5	70.4	102
Sample 2	29.5	12.5	42.5	40.8	96
		25	54.8	53.1	97
		50	79.8	81.3	102
Mean recovery					99.8

Table 16 **Specificity**

	Initial ACE value [U/l]	Conc. of inhibitor [M]	Inhibited ACE value [U/l]	Inhibition [%]
Angiotensin 1	49.0	$1 * 10^{-5}$	46.5	5.1
		$1 * 10^{-4}$	32.5	33.7
		$1 * 10^{-3}$	6.5	86.7
EDTA	49.0	$3.9 * 10^{-5}$	46.9	4.3
		$3.9 * 10^{-4}$	20.3	58.6
		$3.9 * 10^{-3}$	ND	100.0
H-Val-Trp-OH * 2 H_2O	49.0	$1.3 * 10^{-6}$	46.1	5.9
		$1.3 * 10^{-5}$	36.2	26.1
		$1.3 * 10^{-4}$	10.3	79.0
		$1.3 * 10^{-3}$	3.6	92.7

Table 17 **Normal values**

	Adults Data Bühlmann AG	**Adults** Biller et al. 2006 (5)	**Children** Elmlinger et al.
n	80	159	84
Age (years)	20-70	18 - 64	0.5-18
Mean (U/l)	42.2	47.0	70.3
SD (U/l)	14.5	17.3	21.5
Mean ± 2SD	13.2 – 71.2	12.4 – 81.6	27.3 – 113.3
Median (U/l)	40.7	45.1	66.9
IQR (U/l)	21.5	22.5	32.0
2.5-97.5th Percentile (U/l)	**19.8 – 70.2**	**16.1 – 85.3**	**29.3 – 112.2**

Table description: cf. “Calculation” (page 2) and corresponding “Performance Characteristics” (page 3)

Tabellenbeschreibung: siehe “Berechnung der Resultate” (Seite 4) und “Leistungsmerkmale” (Seite 5)

Explications relatives aux tableaux: voir “Calcul des Résultats“ (page 6) et “Caractéristiques de Performance” (page 7)

Descrizione tavola: *cf.* “Elaborationen dei Resultati” (pagina 8) e “Caratteristiche di Prestazione” (pagina 9).

Explicaciones relativas a las Tablas: ver “Cálculos” (página 9) y “Características de Eficiencia” (página 11)

APPENDIX II

REFERENCES/ LITERATURREFERENZEN/ RÉFÉRENCES/ RIFERIMENTI/ REFERENCIAS

1. Ronca-Testoni S.: *Direct spectrophotometric assay for angiotensin-converting enzyme in serum.* Clin Chem **29**, 1093-1096 (1983).
2. Bénéteau B. and Baudin B. et al.: Automated *kinetic assay of angiotensin-converting enzyme in serum*. Clin Chem **32**, 884-886 (1986).
3. Lieberman J.: *Elevation of serum angiotensin converting enzyme (ACE) level in Sarcoidosis.* Am J Med **59**, 36-72 (1975)
4. Hurst P.L. and Lovell-Smith C.J.: *Optimized assay for serum angiotensin converting enzyme activity.* Clin Chem **27**, 2048-2052 (1981).
5. Biller H, Zissel G, Müller-Quernheim J et al.: Genotype-corrected reference values for serum angiotensin-converting enzyme. Eur Respir J 28, 1085-90 (2006).
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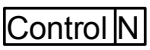
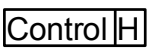
APPENDIX III

SYMBOLS/ SYMBOLE/ SYMBOLES/ SIMBOLI/ SIMBOLOS

Symbol	Explanation
	Use By Verwendbar bis Utiliser jusqu'au Utilizzare entro Fecha de caducidad
	Catalogue number Bestellnummer Référence du catalogue Numero di catalogo Número de catálogo
	Batch code Chargenbezeichnung Code du lot Codice del lotto Codigo de lote
	*In Vitro* Diagnostic Medical Device *In Vitro* Diagnostikum Dispositif médical de diagnostic *in vitro* Dispositivo medico-diagnostico *in vitro* Producto sanitario para diagnóstico *in vitro*
	Temperature limitation Zulässiger Temperaturbereich Limites de température Limiti di temperatura Límites de temperatura
	Consult Instructions for Use- Gebrauchsanweisung beachten Consulter le mode d'emploi Consultare le istruzioni per l'uso Consulte las instrucciones de uso

Symbol	Explanation
	Contains sufficient for <n> tests Ausreichend für „n" Ansätze Contenu suffisant pour „n" tests Contenuto sufficiente per „n" saggi Contenido suficiente para <n> ensayos
	Normal Control Normalkontrolle Contrôle Normal Controllo normale Control Normal
	High Control Kontrolle hoch Contrôle Elevé Controllo alto Control Alto
	Calibrator Kalibrator Calibrateur Calibratore Calibrador
	Substrate Substrat Substrat Substrato Substrato

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