13.1 Sensitivity and Range. The sensitivity of a procedure is defined as the smallest single value which can be distinguished from zero with 95% confidence. The NAG test has a sensitivity of approximately 15 μmol/h/l NAG, if the colorimeter has a minimum sensitivity of ± 0.01 OD units. The range of detection is from 15μmol/h/l to 12000μmol/h/l.

13.2 Reproducibility. Three urine samples were measured using NAG Test procedure. The results were as shown in the above table.

13.3 Correlation. Comparison of the NAG Test with two established procedures, (i) the Shionogi NAG kit procedure (6) and (ii) the fluorimetric method using 4-methylumbelliferone-GlcNAc, gave the following results:

	Number of observations	Y intercept	Slope after intercept	Correlation coefficient
(i)	20	18.1056	1.0022	0.999
(ii)	16	-2.2728	1.1087	0.999

13.4 Specificity. The kit is specific for hexosaminidase and no reaction has been detected with other glycosidase enzymes. Even with high values of haemoglobin or albumin, no interference with the assay was observed. Bilirubin was found to affect the assay only when its concentration exceed 5 mg/dl. No interference has been demonstrated by any commonly used drug, eg. aminoglycoside antibiotics or immunosuppressive agents. The effects of urinary tract analgesic dyes, such as phenazopyridine (Pyridium®)*, on the performance of the NAG Test has not been investigated. Accordingly NAG assay results in patients currently receiving such drug therapy should be interpreted with caution, given the possibility that the presence of dye(s) in the urine may interfere with the assay.

14. Precautions

- The NAG Test is intended for **in vitro diagnostic use only**.
- Although the NAG Test contains no hazardous material, normal laboratory precautions should be observed when performing this assay.
- Instructions should be strictly followed and must not be modified.

15. References

1. *Price R.G., Clinical Neprology, 1992; **38**: S14-S19*
2. *Price, R.G. & Whiting, P.H., In: Urinary Enzymes (Eds. K. Jung, H. Matteheimer & H. Burchardt), Springer-Verlag, Berlin, 1992: 203-221*
3. *Price, R.G. Toxicology, 1984; **23**; 99-134*
4. *Yuen et al., Ann. Clin. Biochem., 1984; **21**: 295-300*
5. *Yuen et al., Clin. Chem. Acta, 1982; **124**: 195-204*
6. *Noto et al., Clin. Chem.,1983; 29/10, 1713-1716*

Further Reading

*Price, R.G., Eur. J. Clin. Chem., Clin. Biochem, 1992; **30**: 693-705*

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HELIER SCIENTIFIC LIMITED

Rapichrome® CE *N*-Acetyl-ß-D-glucosaminidase (NAG) Test kit

1290050/1290100

Colourimetric Assay for Quantitation of the enzyme N-Acetyl-ß-D-glucosaminidase in Urine.

1. Name and intended use

The *N*-Acetyl-ß-**D**-glucosaminidase (NAG) Test kit is designed to detect NAG levels in urine. The kit is intended for in vitro diagnostic use only.

The determination of NAG levels in urine is recognised as a sensitive and reliable indicator of the presence of renal disease. Compared to the measurement of serum creatinine, currently the most commonly used indicator, the NAG test offers the distinct advantage of greater sensitivity in that serum creatinine levels do not become abnormally elevated until 50% of renal function has been lost. The measurement of NAG serves as an early indicator of the presence of renal disease and tubular damage (1), thus providing a means to avoid the serious consequences of untreated disease. In addition the assay has considerable potential in the monitoring of renal involvement in some major disorders including diabetes, hypertension, urogenital tract infections and rheumatoid arthritis. Elevations in urinary NAG activity provide a sensitive indicator of drug nephrotoxicity (2). The test has also been used to monitor the effects of environmental pollutants on the kidney (3).

2. Summary of the test

The NAG test is based on the hydrolysis of its substrate by the enzyme *N*-Acetyl-ß-**D**-glucosaminidase (NAG) causing the release of a phenolic compound which, in the presence of buffer salts, produces a red colour in proportion to the hydrolysis created by the level of NAG present (4,5).

3. Principle of the test

*2-Methoxy-4-(2'-nitrovinyl)-phenyl-2-acetamido-2-deoxy-β-**D**-glucopyranoside* (MNP-GlcNAc) is hyrolysed by *N*-Acetyl-ß-**D**-glucosaminidase with the release of 2-Methoxy-4-(2'-nitrovinyl)-phenol, which on the addition of alkaline buffer produces a colour which can be measured at 505 nm.

4. Reagents supplied

N *Substrate*

A yellow solution containing MNP-GlcNAc.

A *Incubation Buffer*

A mixture of citric acid monohydrate and dipotassium hydrogen phosphate.

G *Colour development Buffer*

A solution of potassium carbonate and potassium hydrogen carbonate at pH 9.5.

Calibrants

Two 1 ml freeze-dried partially purified sample of bovine kidney NAG of defined activity. Additional calibrants are available if required (1290011).

5. Storage

The NAG Test Kit should be stored at 2-8ºC.

6. Specimen Collection

6.1 Urine Samples may be preserved with 0.1-3% boric acid or with 0.02% sodium azide. Higher concentrations of these preservatives inhibit NAG activity and should not be used. Other preservatives should not be used as they may interfere with the performance of the NAG Test Kit.

6.2 NAG is stable up to 50ºC. However, due to the nature of urine, it is recommended that the NAG assay be performed as soon as possible after collection. If necessary, sample may be stored overnight at 4ºC. In the event that the NAG assay must be delayed for a longer period, samples may be frozen immediately after collection and stored at ≤-20ºC.

6.3 When it is difficult to collect 24-hour samples, the second sample of the day is preferred, although the first sample may also be used.
6.4 Serum or plasma may also be used as a sample. Modified instructions are available on request.

7. Directions for use

7.1 Preparation of Solutions:

Solution NA - reaction solution

Pour contents of bottle **N** into bottle **A** and mix well. *Store at 2-8ºC and use within 7 days after mixing.*

Solution G - colour development buffer

Bottle **G** is ready for use and stable up to the expiration date on the outer package.

7.2 Reconstituting Calibrants

Reconstitute as follows:

- Add 1.0ml of distilled water into the vial.
- Mix by gently swirling the liquid and inverting the vial twice (avoid foaming).
- Allow to stand for 5 minutes.
- Mix again by swirling and gently inverting it twice.
- Let it stand for 20 - 30 minutes.
- Mix by pipetting gently and use.

Stability of Calibrants

Lyophilised: Store at 2° to 8°C up to expiry date.

Reconstituted: Up to 10 days at 2° to 8°C. Minimise exposure to elevated temperatures.

7.3 Manual Procedure

Wavelength: 505 nm
Cuvette: 1 cm light path
Temperature: 37ºC
Measure against water

As for all enzymatic reactions, it is important that strict timing schedules are adhered to. Prewarm solution to 37ºC before commencing the assay. Add to the samples at timed intervals; *eg.* 15 or 30 seconds.

After incubation, Solution **G** is added at the same timed intervals.

Pipette into tubes in this order	Reagent Blank	Calibrant	Sample
Distilled water	50µl	---	---
NAG Calibrant	---	50µl	---
Urine sample	---	---	50µl
Reaction Solution **NA**	750µl	750µl	750µl

Incubate for 30 minutes at 37**ºC** and then add:

Colour Development Buffer (Solution **G**)	250µl	250µl	250µl

Mix and read absorbance (OD) at 505 nm after 10 minutes.
It is essential that a reagent blank be carried out each time the assay is performed.

8. Calculation of Results

$$\text{Activity} = S \times \frac{OD_{SA} - OD_{RB}}{OD_{ST} - OD_{RB}}$$

Where S = activity of NAG calibrant
OD_{SA} = sample absorbance
OD_{ST} = calibrant absorbance
OD_{RB} = reagent blank absorbance

As previously noted, timing is important. For multiple assays performed manually, the following procedure is recommended. The time between the addition of Solution **G** and the reading of the OD is 10 minutes.
eg.

- Solution **NA** (750µl) is added to tube 1, 2, 3, 4, etc., containing 50µl of sample.
- The reaction mixture is incubated at 37° C in a water bath
- After 30 minutes solution **G** is added (250µl) to each tube in the same sequence, 1, 2, 3, 4, etc.
- After 10 minutes the absorbance is read in each tube (505 nm) in the same sequence 1, 2, 3, 4, etc

Automated Method

The pack is easily automated. Instructions for discrete and centrifugal analysers and method sheets for the most widely used automated analysers are available on request.

9. Units

Activity is expressed in µmol substrate converted/hour/litre for NAG.
To convert to Enzyme units per litre (U/L) divide by 60.
To convert to nanokatal divide by 3.6.

10. Reference values

There are no apparent significant differences in excretion of NAG in males and females. There is good correlation between activities expressed as units/min and units/mmol creatinine, provided accurately timed samples are obtained. Random samples should be correlated for urine flow by factoring by creatinine concentration of the sample. The mean value of excretion of NAG is 17 ± 5.8 µmol/h/mmol of creatinine, and the lower and upper limit of normal in adults of working age is 7 and 28 µmol/h/mmol creatinine respectively. Higher values may be found in children and the elderly so activity should always be compared to age-matched controls.

11. Quality control

High, medium and low controls (129008, 129009, 129010) are available from Diagnostics Ltd. to assist the user to standardise results.

12. Limitations of the test

The NAG test covers the normal and pathological range for this enzyme in humans. Occasionally, however, levels higher than the upper limit of the spectrophotometer in use may occur. This problem is overcome by diluting the specimen 1:5 with distilled water. Full details are available on request.

13. Performance characteristics

		1	2	3
Intra-assay	**mean**	1725	127	865 µmol/h/l
	S.D.	77	6	26
	C.V.%	4.4	4.4	3.0
	N	9	9	9
Inter-assay	**mean**	1740	125	865 µmol/h/l
	S.D.	84	5	25
	C.V.%	4.8%	3.6	2.9
	N	5	5	5
Between batches	**mean**	1766	123	n.d. µmol/h/l
	S.D.	50	5	
	C.V.%	2.8	4.1	
	N	5	5	
Standards		**High**	**Medium**	**Low**
	mean	2044	642	185 µmol/h/l
	S.D.	65	20	9
	C.V.%	3.2	3.2	5.0
	N	10	10	10

NAG Test kit – Version 1.6