PBG Test Kit

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. Consult instructions for use.
REF	Catalogue number		Manufactured by
	Consult instructions for use		
	Xn - Harmful		C - Corrosive

INTENDED USE
This kit is intended for the in vitro qualitative determination of porphobilinogen (PBG) in urine.

CLINICAL SIGNIFICANCE[1,2,3,4]
The porphyrias are a group of disorders which result from abnormalities in the biosynthesis of haem. They can be divided into two groups, the acute porphyrias and the non-acute (predominantly cutaneous) porphyrias. A number of clinically distinct disorders occur in each group. The three common acute porphyrias are:

(i)Acute Intermittent Porphyria (AIP)
(ii)Porphyria Variegata (PV); and
(iii)Hereditary Coproporphyria (HC).

AIP, PV and HC are dominantly inherited disorders. They may present in an acute phase (with neurological symptoms) or in a latent phase. PV and HC may have skin symptoms in addition to nuerological symptoms.
The presenting features of an acute attack include abdominal pain and neurological symptoms ranging from peripheral neuritis to quadriplegia. If an acute attack is not diagnosed, the patient may be subjected to surgery, with the use of anaesthetics, which may further aggravate the condition. In severe attacks weakness of the trunk muscles can cause respiratory failure and sometimes death.
Several factors, including exposure to a variety of common drugs, changes in hormonal status, diet or acute illness may precipitate an acute attack.
During an acute attack the haem precursor, porphobilinogen (PBG) accumulates in the liver, and raised levels occur in the plasma and urine. An increase in urinary PBG therefore is strongly indicative of an acute porphyria attack.

METHODOLOGY[4,5,6]
The PBG screening test kit is based on the Watson - Schwartz test. In the Watson-Schwartz test, PBG present in the urine condenses with p-dimethylaminobenzaldehyde (DMAB) in acid solution to form a magenta coloured product. In order to improve the specificity of the method it is necessary to then remove substances which may interfere. The most common one being urobilinogen which produces a magenta colour similar to PBG with DMAB/acid solution. Organic extractions with centrifugations are usually carried out to remove these interfering substances. These extractions are time consuming and a certain level of expertise is required to interpret the test.
The PBG screening method utilises an anion exchange resin which binds PBG present in the urine. Interfering compounds are then removed through a simple washing step. PBG is then eluted off the resin and added to the DMAB/acid solution. If PBG is present in the sample in abnormal amounts a magenta colour develops. By comparing the colour developed with the colour chart included in the kit, an approximate concentration of PBG can be determined.

Positive results should be confirmed by quantitative estimation. Quantitative tests require a high level of expertise and are best carried out by a reference laboratory specialising in the investigations of phorphyria.

KIT COMPOSITION

ACTIVE INGREDIENTS	QUANTITY
Resin filled syringes	20
Filters (5 micron)	20
Dimethyl aminobenzaldehyde Powder(DMAB)	2x10 Tests (10mL)
DMAB Diluent (6.1 Molar HCl)	2x10 Tests (10mL)
Reaction tubes	20
Elution Reagent (1 Molar Acetic Acid)	20 Tests (20mL)
Colour Chart	1

WARNING:
R22 Harmful if swallowed.
R34 Causes burns.
R36/38 Irritating to eyes and skin.
R37 Irritating to respiratory system.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S28 After contact with skin, wash immediately with plenty of soap and water.

For further information, consult the PBG test kit Material Safety Data Sheet.

REAGENT PREPARATION
Reconstitute the DMAB with DMAB diluent as follows:-
1. Remove cap from DMAB diluent and place pouring adapter onto the neck of the bottle.
2. Remove cap from DMAB and pour the contents of the DMAB diluent into the DMAB bottle. Mix gently.
3. Discard the Diluent bottle, cap and adapter.
4. When stored at room temperature (18-25°C) in the bottle supplied, this reagent is stable for at least 12 months. Record the expiry date (reconstitution date plus 12 months) in the place provided on the DMAB label.

STABILITY AND STORAGE
All reagents are stable until the expiry date shown on the label when stored at room temperature (18-25°C).

SPECIMEN COLLECTION AND HANDLING[4]
Urine: A random urine sample is suitable for use with this method. Urine should be collected without the use of a preservative or stabiliser.
Storage: The urine sample should be protected from light at all times. Urine should be tested for PBG within 8 hours of voiding. If this is not possible than store at -20°C or less. Samples stored at -20°C and protected from the light are stable for at least 6 months.

ADDITIONAL EQUIPMENT REQUIRED
- pH meter, pH paper or pH indicator solutions.
- Pipette.
- Distilled or deionised water.
- Timer.
- Ammonia solution, approximately 8% (Concentrated ammonia solution is approx. 33%. Dilute this 1:4 with distilled water to approx. 8%).

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PROCEDURE

1. Check urine pH is ≥ 6. If not, increase pH with dilute ammonia solution to pH 6 - 8.
2. Remove cap and fit filter securely to syringe. Expel water to waste and remove the filter. Draw up 1 mL of urine using the graduations on the side of the syringe. Introduce an air bubble into the syringe (this helps the mixing process). Replace the filter securely and mix for 10 seconds.
3. Expel the urine to waste and remove filter. Draw up 1mL of distilled or deionised water and an air bubble. Replace the filter securely and mix for 10 seconds.
4. Expel the water to waste and remove filter. Draw up 1 mL of Elution Reagent and an air bubble. Replace the filter securely and mix for 10 seconds.
5. Expel the solution in the syringe into a reaction tube containing 1 mL of DMAB reagent. Wait 3 minutes and compare the colour of the solution to the Colour chart against a white background under well lit conditions.

RESULTS

Report the results as Not Detected (ND), +, ++, +++.

where: ND : PBG < 25 μmol/L (< 6 mg/L)
+ : PBG ≈ 25 - 50 μmol/L (6 - 12 mg/L)
++ : PBG ≈ 50 - 100 μmol/L (12 - 23 mg/L)
+++ : PBG >100 μmol/L (>23 mg/L)

NOTES

1. Any positive result should be quantified using the recommended method.[5] If the confirmation assay confirms acute porphyria, it is strongly recommended that the patient be further investigated to define the type of porphyria. Follow up family studies should also be carried out. Ideally these studies should be done in a laboratory specialising in the investigation of porphyrias. Refer to the "Porphyria Reference Laboratory" section of this package insert.
2. Patients with current symptoms of acute porphyria usually have PBG levels above 100 μmol/L (+++). In the latent phase patients may have normal levels (ND) of PBG.
3. The method overcomes the major problem of the Watson-Schwartz method - interference in the colour reaction by compounds present in the urine.
4. The method is sensitive to 25 μmol/L (6 mg/L) of PBG, independent of the colour of the urine.

CALIBRATION

The PBG-Aldehyde chromophore is unstable in solution and is therefore not suitable for use as a standard.
The PBG test kit is supplied with a colour chart which represents the following PBG concentrations A: 25 μmol/L, B: 50 μmol/L and C: 100 μmol/L.
The Colour chart is provided as a visual guide only.

QUALITY CONTROL

To ensure adequate control, it is recommended that a known positive urine be run with each screening test carried out. Failure to obtain a positive result with the positive control indicates the assay may be out of control.
The following corrective actions are recommended in such situations:

- Repeat the test with the same control.
- If repeated test is still not positive, prepare a fresh control and repeat the test.
- If results on freshly prepared control still produces a negative result, then contact Technical Services or your local distributor.

LIMITATIONS

1. For a comprehensive review of factors affecting the determination of PBG in urine refer to the publication by Young et al.[7]

EXPECTED VALUES [5]

Urinary excretion of PBG is normally less than 8.8 μmol/L (2 mg/L). Values above 25 μmol/L (6 mg/L) usually indicates the presence of disease. Urine PBG is usually greater than 100 μmol/L (23 mg/L) during an attack of porphyria.

SENSITIVITY

When run as recommended the procedure is sensitive to a level of 25 μmol/L (6 mg/L).

PORPHYRIA REFERENCE LABORATORY

The PBG test kit is a screening test only. A positive result should be referred to a Porphyria Reference Laboratory where the porphyrin pattern in urine, faeces and plasma can be studied. In this way the diagnosis of acute porphyria can be confirmed and family studies carried out. For further details contact the Technical Support Group or your local distributor.

REFERENCES

1. Zilva JF and Pannall RR, "The Porphyrias" in Clinical Chemistry in Diagnosis and Treatment. Lloyd-Luke London, 1979; Chap XIX: 395-402.
2. Blake D et al, Clin. Biochem. Revs. 1985; 6: 52-61.
3. Litman D and Correia MA, Clin. Chem. 1986; 32: 1031-3.
4. Blake D, Poulos V and Rossi R, Clin. Biochem. Revs. 1992
5. Nuttall, K.L. "Porphyrias and Disorders of Porphyrin Metabolism" in Tietz Textbook of Clinical Chemistry, Second Edition. Burtis, C.A. and Ashwood, E.R. (Eds). WB Saunders Co, 1994.
6. A C Deacon and T J Peters, Ann. Clin Biochem 1998;35:726 - 732.
7. Young DS, Effects of Drugs on Clinical laboratory test. Third edition. 1990; 3 : 278-9.

Thermo Electron
189-199 Browns Road,
Noble Park, Victoria, 3174
AUSTRALIA
Phone: (03) 9790 4100
Fax: (03) 9790 4155

Thermo Electron
331 South 104th Street
Louisville, CO, 80027
U.S.A.
Phone: (800) 558 9115
Fax: (303) 581 6429

Email: sales.clinicalchemistry@thermo.com
www.thermo.com/clinicalchemistry

EC REP MediMark Europe Sarl. 11, rue Emile Zola. BP 2322
F-38033 Grenoble Cedex 2. France
Phone: +33 (0) 4 76 86 43 22
Fax: +33 (0) 4 76 17 19 82

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REF ***Reorder Information and Technical Support***

Catalogue No.	Configuration
TR52001	20 Tests

	Australia	International	U.S.A
Phone	1800 333 110	61 3 9790 4100	(800) 558 9115
Facsimile	(03) 9790 4155	61 3 9790 4155	(303) 581 6429