

Porphobilinogen (PBG) Test Kit

SYMBOLS IN PRODUCT LABELLING

	Authorized Representative		Temperature Limitation
	For in vitro diagnostic use		Use by/Expiration Date
	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
	Catalogue number		Manufactured by
	Consult instructions for use		C - Corrosive
	Xn - Harmful		

INTENDED USE

This kit is intended for the in vitro qualitative determination of porphobilinogen (PBG) in urine to aid in the differential diagnosis of acute porphyria.

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 neurological symptoms.

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.

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.

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 colored 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 color 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 color develops. By comparing the color developed with the color 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 specializing in the investigations of porphyria.

KIT COMPOSITION

ACTIVE INGREDIENTS

ACTIVE INGREDIENTS	QUANTITY
Resin filled syringes	20
Filters (5 micron)	20
Dimethyl aminobenzaldehyde Powder(DMAB)	2x10 Tests (15mL)
DMAB Diluent (6.1 Molar HCl)	2x10 Tests (15mL)
Reaction tubes	20
Elution Reagent (1 Molar Acetic Acid)	20 Tests (20mL)
Color 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 2-8°C.

SPECIMEN COLLECTION AND HANDLING⁴

Urine: A random urine sample is suitable for use with this method. Urine should be collected without the use of a preservative or stabilizer.

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 deionized water.
- Timer.
- Ammonia solution, approximately 8% (Concentrated ammonia solution is approx. 33%. Dilute this 1:4 with distilled water to approx. 8%).

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 1 mL of distilled or deionized 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 color of the solution to the Color 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 specializing 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 color reaction by compounds present in the urine.
4. The method is sensitive to 25 µmol/L (6 mg/L) of PBG, independent of the color 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 color chart which represents the following PBG concentrations A: 25 µmol/L, B: 50 µmol/L and C: 100 µmol/L.

The Color 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
2. This kit should not be used to diagnose latent phase porphyria disorders with PBG levels <25 µmol/L (<6 mg/L).⁵

EXPECTED VALUES⁵

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 Porphyrins" 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. "Porphyrins 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.



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REF

Reorder Information

Catalogue No.	Configuration
TR52001	20 Test