# REDSPOT-PE™

Urinary Congo Red Spot Test for Pre-eclampsia (PE) Screening


**SUMMARY**

Pre-eclampsia (PE), a pregnancy–induced hypertension disorders occurs in 2-8% of pregnancies and is associated with maternal and perinatal mortality. Pre-eclampsia, is characterized by onset of hypertension accompanied by significant proteinuria after 20 weeks gestation accounting for 17-24% of maternal deaths in low income settings. If left untreated PE may lead to progressive clinical deterioration resulting in seizures (eclampsia), stroke, haemorrhage, kidney damage, liver failure and death[2].

Obstetricians managing women with pre-term PE are faced with the challenge of balancing the need for achieving foetal maturation in uterus with risks to the mother and foetus from continuing the pregnancy longer. These risks include progression to eclampsia, developmental of placental abruption and HELLP syndrome[14]. On the other hand pre-term delivery is associated with higher infant mortality and increased morbidity resulting from small-for-gestational age (SGA), thrombocytopenia, bronchopulmonary dysplasia, cerebral palsy, and an increased risk of various chronic diseases in adult life, particularly type 2 diabetes, cardiovascular disease and obesity[14].

The quest to effectively predict PE is fuelled by the desire to identify women who are at high risk of developing PE. Accurate and early diagnosis of PE is still an enigma in resource limited settings. Early clinical signs of PE are frequently inconspicuous, and the effectiveness of hypertension and proteinuria as diagnostic "gold standard" is compromised when PE is superimposed on other predisposing conditions such as chronic hypertension or nephropathy[2]. PE has a large spectrum of medical signs and symptoms resulting in a range of clinical phenotypes and out-comes, making a diagnosis on available clinical and laboratory parameters challenging.

An effective combined method for prediction of pre-eclampsia is based on maternal history, mean arterial pressure (MAP), Uterine artery pulsatility index (UTPI) and biochemical markers like Pregnancy Associated Plasma Protein A (PAPP-A), Placental Growth Factor (PlGF) soluble fms-like tyrosine kinase -1 (sFlt-1), and others[16,17]. The placental biomarkers are laboratory based, require a blood sample or are employed as a part of complex algorithms making them impractical as point of care tests, especially for low resource settings[17]. The challenges associated with the affordability and availability of these biomarkers limits their usage. A simple, cost effective and non-invasive point of care method for early prediction of PE is still an unmet clinical need.

The affinity of Congo red, a synthetically formulated diazo dye, for mis-folded proteins (amyloids) is referred as Congophilia and is considered as a gold standard to identify amyloids. It has been reported that mis-folded proteins are present in the urine of pregnant women diagnosed with PE. Mis-folded proteins are known to occur in urine well before the onset of clinical symptoms of pre-eclampsia. Urinary congophilia is not affected by clinical variables like gestational age of onset, severity, super imposition by eclampsia and complication by intrauterine growth restriction and intrauterine death[15].

**REDSPOT-PE™** Congo Red Spot test, is a point-of-care test, to detect urinary congophilia useful for screening of Pre-eclampsia (PE) in pregnant females with > 20 weeks of gestation. The test has also been found to be useful to improve wait times in obstetrical triage areas and avoid unnecessary admissions in a study by K.M Rood et.al[6].


**PRESENTATION**

| | | |
|---|---|---|
| **REF** | REF | **1108230050** |
| **Pack size** | Σ | **50 Tests** |
| Congo Red Reagent | CR | 3 ml |
| Test Cards | TC | 50 Nos. |
| Sample-Reagent Mixing Vials | SRV | 50 Nos. |
| Sample Droppers | SD | 50 Nos. |
| Test Droppers | TD | 50 Nos. |
| Pack insert | | 1 No. |

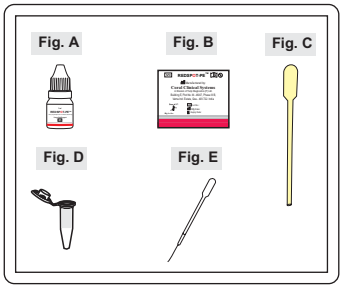
**MATERIALS AND COMPONENTS:**

**Materials provided with the test kit:**

- Congo Red Reagent (CR) - **Fig. A**
- Test Cards (TC) with cellulose test paper - **Fig. B**
- Sample Droppers (SD) - Yellow plastic droppers - **Fig. C**
- Sample- Reagent Mixing Vials (SRV) - plastic vials - **Fig. D**
- Test Droppers (TD) - White plastic fine-tip droppers - **Fig. E**

Additional Materials required but not provided:

- Disposable gloves
- Sterile Urine Collection Container
- Stopwatch.



**TEST PRINCIPLE**

The **REDSPOT-PE**™ test is based on the chemical interaction between Congo red (CR) and amyloid proteins. Congo red (CR) is a synthetic diazo dye with specific affinity for amyloid protein[10-12]. This special affinity of mis-folded protein fibres to Congo red dye is known as congophilia.

When Congo red (CR) reagent is mixed and incubated with the urine sample for few minutes and spotted on a cellulose test paper, the CR reagent will form hydrogen bonds with cellulose of the test paper, thus slowing down its flow through the porous paper surface, creating a tight circle made by aqueous CR solution on the test paper.

If the urine sample contains amyloids (PE positive urine), the CR reagent binds proportionately to the amyloid concentration in the sample during incubation. Further when spotted onto the cellulose test paper there will be little or no free CR available for cellulose bonding, hence the CR-amyloid aggregate/complex spreads on the test paper forming a wide diffused pink circle indicating positive for Pre-eclampsia. The more Congo red is bound to mis-folded proteins, the dye spreads more evenly on the paper[16].

**STORAGE AND STABILITY:**

The Kit is stable at 25°C to 30°C till the expiry date printed on the label.

**NOTE**

(1) Read the instructions carefully before performing the test. (2) For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use. (3) The test is for aiding in diagnosis of PE and results should be correlated with the patient's Blood pressure and proteinuria test results. (4) Do not use beyond expiry date. (5) The test card, sample reagent mixing tubes and droppers are for single use only.

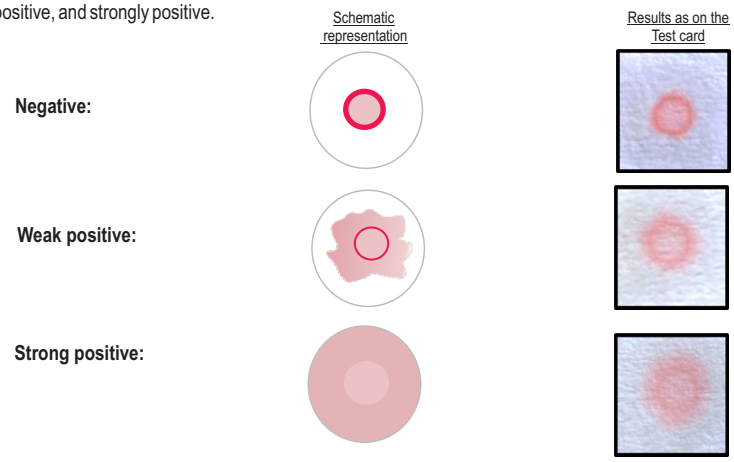
**SPECIMEN REQUIRED**

Urine sample:

- No Special preparation of the patient is necessary prior to the specimen collection.
- Midstream urine sample should be self-collected by the patient using a clean sterile urine container provided by the healthcare facility.
- If testing is not immediate, the urine specimen may be stored at 2-8$^0$C for upto 72 hours. Contaminated samples should not be used for testing. Turbid specimens should be centrifuged or allowed to settle and only the clear supernatant should be used for testing.

**TESTING PROCEDURE AND INTERPRETATION OF THE RESULTS:**

1. To reduce the risk of contamination, wear gloves while performing the test.
2. Refrigerated specimens must be brought to room temperature prior to testing.
3. Secure the desired numbers of the Test Cards (TC) and Sample-Reagent Mixing Vials (SRV) for testing and label them with the patient's identity.
4. Using **yellow** Sample Droppers (SD) held vertically add four drops of the patient's urine sample into the respective Sample-Reagent Mixing Vial (SRV).
5. Next puncture the Congo Red (CR) reagent bottle nozzle by tightening its cap in clockwise direction to pierce the bottle nozzle. Add one drop of the Congo red reagent to the collected urine sample in the Sample-Reagent Mixing Vial (SRV).
6. Mix well and incubate for **five minutes** at Room temperature (25-30$^0$C).
7. Next using a **white** Test Dropper (TD), dispense one drop of this Sample- Reagent Mix into the Test card (TC) holding the dropper vertically above the test card window. The drop should be dispensed at the centre of the test card window, without coming into direct contact with the test card surface while dispensing.
8. At the end of **three minutes** read the results and interpret using following visual representation marked as negative, weak positive, and strongly positive.




**Note**: The results so obtained are stable for upto 2 days at R.T.

**SAFETY PRECAUTIONS AND WARNINGS**

- Contact with the contents of dropper bottle containing Congo Red dye should be kept to a minimum.
- Wear protective gloves while testing. Wash off immediately with soap and plenty of water after handling the reagent and specimens.
- Inhalations / swallowing may cause harm. Immediate medical attention is required.
- Handle all specimens as potentially infectious.

**LIMITATIONS OF THE TEST**

1. As with any other in-vitro screening test, the test results themselves should not be the only reason for any therapeutic consequences. They must be corelated to other clinical observations and diagnostic tests.
2. Results obtained using **REDSPOT-PE**™ test should not be the sole source for diagnosis. Results must be interpreted in conjugation with other clinical data available to the clinician.
3. Mis-folded proteins may also be present in cases of Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis, spongiform encephalopathy and familial amyloidotic polyneuropathy and other renal diseases. Samples of such patients may yield positive results.
4. Positive results may also be obtained in a few other conditions/ disease/ disorders as follows: Kidney disease/problems, Diabetes, IgA nephropathy (Berger's disease) (kidney inflammation resulting from a build-up of the antibody immunoglobulin A), Systemic lupus erythematosus, Membranous nephropathy, Multiple myeloma, Amyloidosis (build-up of abnormal proteins in the organs), Certain drugs, such as non-steroidal anti-inflammatory drugs, Heart disease, Heart failure, Hodgkin's lymphoma (Hodgkin's disease), Orthostatic proteinuria (urine protein level rises when in an upright position) and Rheumatoid arthritis.
5. Any modification to the test procedure and / or use of other reagents will invalidate the test results.

**PERFORMANCE EVALUATION**

A) Internal Evaluation:

A total of 96 pregnant women samples derived from a hospital, were evaluated in- house with **REDSPOT-PE** ™. The results obtained with **REDSPOT-PE**™ matched with other clinical findings such as Blood pressure, proteinuria and levels of PE as indicated by the hospital. The summary of the evaluation is as follows:

| **Status of Sample** | **Result with REDSPOT- PE™** | |
|---|---|---|
| | **PE Positive** | **PE Negative** |
| **PE Positive** | 38 | 8 |
| **PE Negative** | 6 | 44 |
| **Total Samples** | 96 | |
| Sensitivity | 82.61% (95%CI: 68.58% to 92.18%) | |
| Specificity | 88.00% (95%CI: 75.69% to 95.47%) | |
| Accuracy | 85.42% (95%CI: 76.74% to 91.79%) | |

B) External Evaluation:

A study was conducted in the maternal and child health care wing of a reputed hospital in India, on 116 pregnant women who consented to be part of the study between the period June 2024 – Nov 2024. Their Blood pressure and proteinuria measurements were taken, followed by testing their urine samples with **REDSPOT-PE**™. The results of the study are summarized as follows.

| **Status of Sample** | **Result with REDSPOT- PE™** | |
|---|---|---|
| | **PE Positive** | **PE Negative** |
| **PE Positive** | 19 | 3 |
| **PE Negative** | 0 | 94 |
| **Total Samples** | 116 | |
| Sensitivity | 86.36% (95%CI: 65.09% to 97.09%) | |
| Specificity | 100.0% (95%CI: 96.15% to 100.0%) | |
| PPV | 100.0% (95%CI: 82.35% to 100.0%) | |
| NPV | 96.91% (95%CI: 91.63% to 98.90%) | |
| Accuracy | 97.41% (95%CI: 92.63% to 99.46%) | |

**WARRANTY**

This product is designed to perform as described on the label and Instructions for use. The manufacturer disclaims any implied warranty of use and sale for any other purpose. In the event of performance changes or product malfunction, please contact manufacturer.

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(1) Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. Hypertension. (2018) 72:24–43. (2) Buhimschi IA, Nayeri UA, Zhao G, et al. Protein misfolding, congophilia, oligomerization, and defective amyloid processing in preeclampsia. Sci Transl Med 2014;6:245 ra292. (3) T.T. Ashburn, H. Han, B.F. Mc Guinness et al., Amyloid probes based on Congo Red distinguish between fibrils comprising different peptides. Chem. Biol. 3(1996) 351- 358. (4) Barbara Stopa, Barbara Piekarska et al.,The structure and protein binding of amyloid-specific dye reagents, Vol. 50 No. 4/2003,1213–1227. (5) W E Klunk, J W Pettegrew and D J Abraham, Quantitative evaluation of congo red binding to amyloid-like proteins with a beta-pleated sheet conformation, J Histochem Cytochem 1989 37: 1273. (6) Kara M. Rood, Catalin S. Buhimschi et al.,Congo Red Dot Paper Test for Antenatal Triage and Rapid Identification of Preeclampsia, E Clinical Medicine 8 (2019) 47–56. (7) Aida Petca, Ruxandra Diana Sinescu, Florica Sandru et al., New approaches in predicting and diagnosing preeclampsia: Congo Red Dot Paper Test (Review), Experimental and Therapeutic Medicine, 23: 270, 2022. (8) M.P.A. Sailakshmi, M.R. Prabhu, S. Prabhakara, K. Anbazhagan ,B.M. Rupakala, Congo red dot test in the early prediction and diagnosis of pre-eclampsia in a tertiary health care centre in India, Science Direct , Pregnancy Hypertension, Volume 25, August 2021, Pages 225-229. (9) Benshuo Cai, Xiaoying Yuan, Xingmin Li, Jun Xu and Juan Du, Urinary Congophilia Confirmed With the CapCord Test Is Associated With Pregnancy Outcomes in Women With Early-Onset Pre-eclampsia.Frontiers in Medicine, www.frontiersin.org, August 2021, Volume 8 , 1-7. (10) Nancy Huynh, Congo Red Dot Test: A Groundbreaking Method to Diagnose Preeclampsia, Yale Scientific, Dec 2010. (11) Suzanne Fournier et.al., Commodity and service delivery innovations for detection and management of pre-eclampsia-Landscape report : Unitaid 2024, 1-60. (12) Homer CS, Brown MA, Mangos G, Davis GK. Non-proteinuric preeclampsia: a novel risk indicator in women with gestational hypertension. *J Hypertens*. 2008; 26:295–302. doi: 10.1097/HJH.0b013e3282 f1a953. (13) Dong, X., Gou, W., Li, C., Wu, M., Han, Z., Li, X., & Chen, Q. (2017). Proteinuria in preeclampsia: Not essential to diagnosis but related to disease severity and fetal outcomes. Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health, 8, 60–64. doi:10.1016/j. preghy. 2017.03.005. (14) The international federation of Gynaecology and obstetrics (FIGO) initiative on Preeclampsia (PE): A pragmatic guide for first trimester screening and prevention. Int. J. Gynaecol. Obstet. 2019 May; 145 (Suppl 1):1-33. (15) Urinary congophilia in preeclampsia: Experience from a rural tertiary care hospital in India. Pregnancy Hypertension. Vol. 13, July 2018, Pg 83-86. (16) A study on the role of urinary congophilia in early detection of Preeclampsia. Ann Natl Acad Med Sci (India) 2022;58:87-91. (17) Congo Red test for identification of Preeclampsia: Results of prospective diagnostic-case control study in Bangladesh and Mexico.H Bracken et.al. EClinicalMedicine 31 (2021) 100678. (18) Data on File: Coral Clinical Systems.

**SYMBOL KEYS**

| | | | |
|---|---|---|---|
| 25°C 30°C Store at 25 - 30°C | Manufacturer | This side up / Do not reuse | CR Congo Red Reagent |
| Use by | Consult Instructions for use | Contains sufficient for <n> tests | TC Test Cards |
| Date of Manufacture | REF Catalogue Number | Keep Away from Sunlight | SRV Sample-Reagent Mixing Vials |
| LOT Batch Number/ Lot Number | IVD *In vitro* Diagnostic Medical Device | TD Test Droppers | SD Sample Droppers |

Manufactured by:

**Coral Clinical Systems**

A Division of Tulip Diagnostics (P) Ltd.

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