CE

BabySafe™

17-OHP Rapid Diagnostic Test

Rapid Test for detection of 17 Alpha-hydroxyprogestrone (17-OHP) in Human Whole blood

DEVICE

INTENDED USE

The BabySafe 17-OHP Rapid test kit is an *in-vitro* diagnostic immunochromatographic assay for the semi-quantitative detection of 17α-hydroxyprogestrone (17-OHP) in New Born whole blood samples. This is a screening test.

TEST PRINCIPLE

The BabySafe 17-OHP Rapid Test Cassette (Whole Blood) is Semi-Quantitative membrane-based immunoassay for the detection of 17α-OH Progesterone(17-OHP) in new-born whole blood samples. This test is based on competitive inhibition assay. There are two components, one is conjugate pad containing Anti-17-OHP antibody conjugated to colloidal gold and other is 17-OHP immobilized on to the membrane at test zone "T". Cases in which, 17-OHP is very high in specimen, specimen will flow through the membrane assembly of the device, 17-OHP in the specimen binds to the highly specific antibody for 17-OHP colloidal gold conjugate and travels on the membrane due to capillary action. Since conjugate is already saturated with 17-OHP, it will not bind to Capture test line and move further. Samples in which 17-OHP is in normal levels or lesser, the conjugate will have enough binding sites to bind to capture as it travels across test region "T". Based on the concentration of 17-OHP present in the specimen, colour intensity of the Test "T" will vary. The colloidal gold conjugate and unbound complex moves further to the reference region (R) that contains pre-calibrated for rabbit globulin corresponding to 50ng/ml,17-OHP immobilized on the membrane. The intensity of the coloured band formed at the reference region (R) corresponds to a17-OHP concentration of 50ng/ml. The unreacted conjugate and unbound complex, if any, move further on the membrane and are subsequently immobilized by the agglutinating sera for rabbit globulin coated on the membrane at the control region (C), forming a pink to pink-purple coloured band. The intensity of the coloured band formed at the control region(C) corresponds to a 17-OHP concentration of 15ng/ml.

INTENDED USER

The test is intended to be performed by a trained user.

SPECIMEN REQUIRED

Capillary Whole Blood Sampling by Heel Prick:

- Blood samples should ideally be collected between the third and fifth day of baby's life (48 to 120 hrs after birth) by Heel prick. However, in some screening programs the sampling timing may vary. Consult local regulations for appropriate timing for specimen collection.
- Blood from the new-born's Heel should be collected **ONLY** from the medial (closest to the body center-line) or lateral portion (furthest from the body center-line) of the planter surface (walking surface).
- Clean the skin with an alcohol swab and allow to air-dry.
- Puncture the infant's Heel with a sterile lancet to the depth of approximately 1.0-2.0 mm. Puncturing deeper than 2.0 mm on small infants may cause bone damage. (Reference: WHO Guidelines for Paediatric and Neonatal patients sampling).
- Puncture infant's heel with one quick, continuous and deliberate stroke, to achieve a good flow of blood and to prevent the need to repeat the puncture.
- Wipe away the first drop of blood because it may be contaminated with tissue fluid or debris (sloughing skin). Collect 10 µl of blood sample with the help of a Micropipette (10 µl) from a large well-formed drop of blood.
- Avoid squeezing the heel too tightly because this dilutes the specimen with tissue fluid (plasma) and increases the probability of haemolysis.
- When the blood collection procedure is complete, apply firm pressure to the site to stop the bleeding.

Venous blood:

- Fresh whole blood collected in EDTA, Heparin or ACD. Freezing is not recommended. With the help of micropipette transfer 10 µl whole blood in to the test cassette 'A' region.

WARNINGS AND PRECAUTIONS:

- Read the instructions carefully before performing the test
- For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
- Do not use the kit beyond expiry date and do not re-use the test device.
- Do not intermix reagents from different lots.
- Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS#7646-79-9) should be kept to minimum. Inhalation / swallowing may cause harm.

- Handle all specimens as if potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infectious material.

MATERIALS

Materials Provided:

A. Individual pouches, each containing:
   1. DEVICE : Membrane assembly pre-dispensed with 17-OHP (indicator) colloidal gold conjugate, 17-OHP (capture) and Agglutinating sera for rabbit globulin coated at the respective regions.
   2. Desiccant pouch.

B. BUF : Assay buffer vials.

C. Package insert.

PRESENTATION

REF	526020010	526020025
	10 T	25 T

OPTIONAL MATERIAL REQUIRED BUT NOT PROVIDED

i) NBS-RDT Single Channel Pipette (10 µl) (Cat. No.: 10SCPRDT016)
ii) NBS-RDT Micropipette Tips-Tip Box Set (Cat. No.: 10MPTMTB019)

Materials required but not provided:

Alcohol swab, Sterile blood lancet (0.85 mm- 2 mm max depth) and stopwatch.

** For premature neonates, a lancet depth of 0.85 mm is recommended.

TEST KIT STORAGE AND STABILITY

- Store the sealed pouches at 4°C to 30°C .
- Do not use beyond the expiration date .
- The test kit is stable until the expiration date marked on the RDT box and /or the packaging of the individual components when stored as specified.
- DO NOT FREEZE.

TESTING PROCEDURE

Before testing:

1. Bring the kit components of Baby Safe 17-OHP device to ambient temperature before testing.
2. Check that the cassette packaging is not damaged. (If damaged, discard the cassette packaging and use another test).
3. Open the cassette packaging by tearing along the notch indicated and check the desiccant. (If it shows saturation i.e. colour changed from blue to pink or colourless), discard the cassette and take another cassette packaging. If the colour of the desiccant does not show any colour change the cassette can be used for the test.
4. Throw the desiccant in the non-sharp disposal container.
5. Take the cassette and place it on the horizontal surface.
6. The cassette will have:
   - A result window (marked as C, R & T).
   - A sample port marked "A".
   - A circle well marked "B".
7. Write the patient name or patient identification on the cassette.
8. Put the gloves. Use new pair of gloves for each patient.

Test Procedure

Capillary whole blood from Heel prick

1. Wear gloves.
2. Open the packaging of the alcohol swab. Take out the alcohol swab. Do not throw away the empty packaging (wrapper) but keep it aside.
3. Wipe the complete area to be pricked with the alcohol swab.
4. Wait until the area has completely dried (minimum 30 seconds).
5. Place the alcohol swab in the wrapper and set it aside (you will use it again to stop the bleeding after you collected the blood).
6. Take the safety-seal lancet.
7. Detach the cap of the lancet. Puncture the infant's Heel (from medial or lateral portion of plantar surface) with a sterile Lancet (0.85 mm-2 mm) . Dispose the lancet immediately into the sharps box.
8. Make sure a well-formed drop of blood is present.

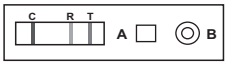
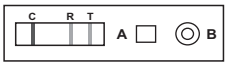
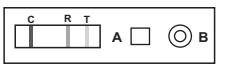
9. If there is no well-formed drop of blood, repeat the prick at some other area.
10. Use a new lancet and choose a different puncture site.
11. Collect 10 µl of blood sample with the help of a Micropipette (10ul) from a large well-formed drop of blood. Take the alcohol swab you put aside (step 5). Press it to the site of pricking to stop the bleeding. After use, put the alcohol swab into the non-sharps disposal container.
12. Dispense 10 µl blood sample in the sample port 'A', followed by the addition of 4 drops of the Assay buffer in port 'B' (**Do not move the device after addition of Assay buffer**).
13. Do not read and interpret after 25 minutes.
14. Use a separate micropipette tip for each patient to avoid any contamination.

Venous whole blood

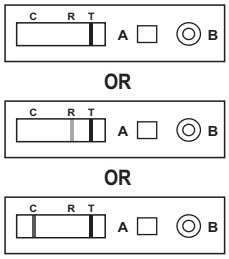
1. Wear Gloves.
2. Collect fresh whole blood by standard venipuncture procedure into tube containing anticoagulant (EDTA or heparin).
3. Mix the tube gently.
4. Perform steps 11 -13 of the previous section ("Capillary whole blood from heel prick").
5. **Storage duration**: If assays are not completed immediately, Whole blood samples should be stored at +2°C to +8°C no longer than 2-3 days.

INTERPRETATIONS OF THE RESULTS

1. At the end of 25 minutes record the test result as noted in the table below.
2. Where possible have the results confirmed by a second reader within this time frame.
3. Line intensities may vary from faint to strong intensity. Consider the faint lines also as a visible band.
4. Record the test result as noted in the table below:

	**17-OHP concentration below 15ng/ml: T>C** Presence of three coloured band at, Test '**T**', Reference '**R**' and Control '**C**', provided that colour intensity observed at Test '**T**' is higher to Control '**C**' it should be inferred as the concentration of 17-OHP in the specimen is below 15ng/ml	**Interpretation:** Presumptive Negative
	**17-OHP concentration @15ng/ml: T=C** Presence of three coloured band at, Test '**T**', Reference '**R**' and Control '**C**', provided that colour intensity observed at Test '**T**' is equal to Control '**C**' it should be inferred as the concentration of 17-OHP in the specimen is @15ng/ml.	**Interpretation:** Presumptive Negative
	**17-OHP concentration between 15- 50ng/ml: C>T>R** Presence of three coloured band at, Reference '**R**', Control '**C**' and Test '**T**', provided that colour intensity observed at Test '**T**' is more than Reference '**R**' and less than Control '**C**' it should be inferred as the concentration of 17-OHP in the specimen is between 15 to 50ng/ml.	**Interpretation:** Borderline Range
	**17-OHP concentration @50ng/ml: T=R** Presence of three coloured band at, Test '**T**', Reference '**R**' and Control '**C**', provided that colour intensity observed at Test "**T**" is equal to Reference "**R**" it should be inferred as the concentration of 17-OHP in the specimen is @50ng/ml.	**Interpretation:** Presumptive Positive
	**17-OHP concentration > 50ng/ml: T<R** Presence of two or three coloured band at Reference **'R'**, Control **'C'** and/or Test **"T"**, provided that colour intensity observed at Test **"T"** is lesser to Reference **"R"** it should be inferred as the concentration of 17-OHP in the specimen is more than 50ng/ml.	**Interpretation:** Presumptive Positive

Note: All samples tested as Borderline or Presumptive positive; Necessitate Confirmation by Serum Analysis, 2nd Tier Testing & Correlation with other Clinical Findings (e.g. Sampling Age, Prematurity, Birth Weight, sick infants etc.)

	**Invalid Result:** Absence of band in control '**C**' and/ or Reference '**R**' region irrespective of presence or absence of band at Reference '**R**' and '**T**' regions.	

LIMITATIONS OF THE PRODUCT

(1). The test procedure, precautions and interpretation of results for this test must be followed while testing. (2). The BabySafe 17-OHP Rapid Test Cassette (Whole Blood) is for in vitro diagnostic use only. The test should be used for the detection of 17-OHP in whole blood specimens only. (3). Test kit directly exposed to sunlight and heat can lead to wrong results. (4). As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician. (5). BabySafe Rapid 17-OHP is a Screening test; which is a process of Filtration that separates the newborn infants into two groups; one who may have the given disorder or condition from the other group of infants who do not have it. If the screening 17-OHP rapid test results are borderline or presumptive positive, it should be followed by further 2nd Tier testing by LC-MS/MS & Confirmatory diagnostics tests according to the Screening algorithm of CAH (stratified by Birth weight and Collection time). (6). Results must be confirmed using a Serum ELISA test. Presumptive Positive samples needs to be confirmed by 2nd Tier testing (e.g. Steroid Profiling) and caution must be exercised in correlating test results to clinical status of specimens from new born less than 48 hrs after birth, premature, low birth weight and hospitalized sick new born. (7). Values expressed is in ng/ml of whole blood. (8). This test does not determine the absolute value of 17-OHP in specimen. (9). This test is designed for full-term, normal weight babies. Hence, interpretation should be done very carefully and needed to be confirmed with confirmatory assay. (10). Elevated concentrations of 17-OHP are not per se diagnostic of CAH the circulating 17-OHP concentration may be elevated in infants who are pre-term, under stress, have respiratory disorders or other severe illness.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1). WHO Guidelines for Paediatric and Neonatal patients sampling. (2). Nimkarn S, Gangishetti PK, Yau M, et al. 21-Hydroxylase-Deficient Congenital Adrenal Hyperplasia. 2002 Feb 26 [Updated 2016 Feb 4]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. Gene Reviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1171/. (3). Krone N, Roscher AA, Schwarz HP, Braun A. (1998) Comprehensive analytical strategy for mutation screening in 21- hydroxylase deficiency. Clin Chem . 44:2075–2082. (4). Speiser, P. W., Azziz, R., Baskin, L. S., et.al. (2010) Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 95(9), 4133-60. (5).Krone, N., & Arlt, W. (2009). Genetics of congenital adrenal hyperplasia. Best practice & research. Clinical endocrinology & metabolism, 23(2), 181-92. (6).Data on file: Zephyr Biomedicals.

SYMBOL KEYS

	Temperature Limitation		Manufacturer	LOT	Batch Number / Lot Number	DEVICE	Device		Do not reuse
	Use by		Consult Instructions for use	Σ	Contains sufficient for <n> tests	EC REP	Authorised Representative in the European Community		Do not use if package is damaged
	Date of Manufacture	REF	Catalogue Number	IVD	*In vitro* Diagnostic Medical Device	BUF	Assay Buffer		This side up

Manufactured by:

Zephyr Biomedicals

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EC REP

CMC Medical Devices & Drugs S.L., Spain.

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