

### INTENDED USE

**Alere™ TestPack Plus hCG Combo** with On Board Controls (OBC) (**Alere™ TestPack hCG Combo**) is a rapid immunoassay for the qualitative detection of human Chorionic Gonadotropin (hCG) in serum and urine for early detection of pregnancy. For professional *in vitro* diagnostic use only.

### INTRODUCTION

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone that is produced by the blastocyst.<sup>1</sup>

The background concentration of hCG in urine and serum increases with age, but is normally <5mIU/ml in women of childbearing age<sup>2</sup>. This rapidly increases after conception, reaching 50-250mIU/ml by the day of the expected period and peaks at approximately 100,000 to 200,000mIU/ml during the first trimester<sup>3,4</sup>. The sudden rapid rise in concentration of hCG in urine and serum following conception makes it an excellent marker for pregnancy.

### TEST PRINCIPLE

Serum or urine is added to the Sample Well of the reaction disc with the aid of a transfer pipette, and allowed to migrate through the membrane. As the serum or urine proceeds through the membrane, it mobilises the anti-hCG monoclonal antibody-colloid. If hCG is present in the specimen, it will form a complex with the antibody-colloid. The antibody-colloid complex migrates through the membrane and is captured by the immobilised anti-hCG polyclonal antibody in the Result Window, providing a visual indication of the presence of hCG.

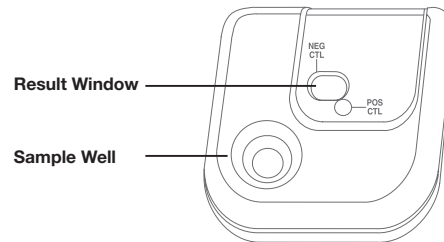
The test should be read at 5 minutes. If hCG is present in the urine or serum at levels of 25mIU/ml or greater, a Plus Sign “+” appears in the result window. A Minus Sign “-” indicates no hCG was detected.

### KIT CONTENTS AND STORAGE

- 20 reaction discs
- Pack of 20 transfer pipettes
- One package insert

Store at 2-30°C. Do not use after the expiry date.

Safety data sheet available for professional user on request.



Result Window

Sample Well

### PRECAUTIONS

Standard guidelines for handling infectious agents should be observed throughout all procedures.

1. Do not use reaction discs that have become wet or if the foil pouch has been opened or damaged.
2. Properly dispose of all contaminated waste such as reaction discs and transfer pipettes.

### SPECIMEN COLLECTION & STORAGE

**Urine Specimens:** A urine specimen collected at any time of the day is suitable, but a first morning specimen is recommended for early detection of pregnancy, as it should contain the highest concentration of hCG<sup>5</sup>.

Urine specimens must be collected in **clean**, dry, plastic or glass containers. Specimens may be stored in the refrigerator (2-8°C) for up to 48 hours, or frozen **once** (-20°C) for up to 3 months. Specimens should not be repeatedly frozen and thawed.

**Alere™ TestPack hCG Combo** has not been validated for use with urine specimens containing preservatives other than sodium azide (0.1%).

No centrifugation or filtration of specimens is required prior to testing. However, particulate matter in specimens should be allowed to settle before removing a sediment-free aliquot for testing.

**Serum Specimens:** No special preparation of serum is required. Serum specimens not tested immediately must be stored refrigerated (2-8°C) for up to 48 hours or frozen **once** (-20°C) for up to 3 months. Specimens should not be repeatedly frozen and thawed.

### TEST PROCEDURE

Allow the reaction disc and patient specimen to equilibrate to room temperature (18-30°C) for a minimum of 30 minutes before beginning the assay. Do not open foil pouches until ready to perform the assay.

1. Remove the reaction disc from its foil pouch. Label with patient or control identifications. Place on a clean, flat, dry surface.
2. Draw specimen to the line marked on the transfer pipette. Dispense the entire contents drop wise into the Sample Well on the reaction disc.
3. Read results 5 minutes after addition of sample to the Sample Well. **Ignore any results that appear after this time.**

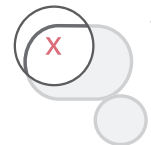
## INTERPRETATION OF RESULTS

### On Board Controls

**Alere™ TestPack hCG Combo** utilises three On Board Controls to ensure the assay is functioning properly.

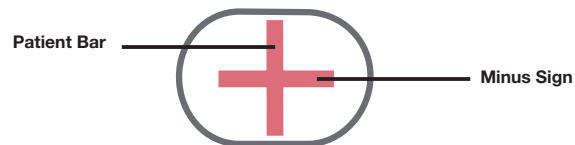


- The **Positive On Board Control** (POS CTL “√”) indicates that both the antibody-colloid complex and capture antibody systems are functional. The POS CTL “√” must appear for the result to be valid.



- The **Negative On Board Control** (NEG CTL “X”) indicates that the test specimen may contain a non-specific entity that could cause a false positive result. If the NEG CTL “X” appears in the Result Window, the result is invalid.

- The **Minus Sign** “-” indicates that migration of the specimen has occurred. The Minus Sign “-” must appear for the result to be valid.



### Positive Result

A positive result consists of one vertical line (Patient Bar) and one horizontal line (Minus Sign) in the Result Window forming a Plus Sign “+”. Pink/red colour (darker than the background) on the Patient Bar is interpreted as a positive result even if it has less colour than the Minus Sign. Randomly occurring red dots should not be evaluated in the interpretation of results.



### Negative Result

A negative result is indicated by a horizontal line (Minus Sign “-”) in the Result Window. A negative result means that no hCG was detected, or that the levels of hCG in the specimen were below the detection limit of the assay.

Weak positive results may occur with hCG levels below 25mIU/ml. It is good laboratory practice to resample and retest these weak positive specimens after an additional 48-72 hours. The use of controls at hCG levels near the assay sensitivity may guide in the interpretation of weak positive results.

The reaction area may, on occasion, exhibit outlines. An outline can be described as a colourless area which surrounds all or part of the Patient Bar. If outlines are present, an impression of the Patient Bar may be seen. However, in the absence of hCG (negative specimens) this impression is comparable to the background and should be interpreted as a negative result.

Specimens with high levels of hCG may yield colour on the Patient Bar as quickly as 1 minute after specimen addition. Specimens with levels of hCG at or above the level of sensitivity of the assay remain positive with time. Specimens with levels of hCG below the sensitivity of the assay may yield some colour on the Patient Bar with time however the test should be read 5 minutes after addition of sample.

## EXTERNAL QUALITY CONTROL

Good laboratory practice recommends the use of control materials to ensure proper kit performance. Each laboratory should refer to guidelines established internally and by local, national or other accrediting organisations.

Due to variation in analyte composition and/or matrices, external quality control materials and proficiency survey samples may not elicit identical results across all hCG assays. Each laboratory needs to determine the suitability of each control material for specific immunoassays and validate the material prior to use.

## LIMITATIONS OF THE TEST

- Positive results from very early pregnancy may later prove negative due to natural termination of the pregnancy. This is estimated to occur in 31% of all conceptions<sup>9</sup>. It is recommended when using urine specimens with a sensitive pregnancy test such as **Alere™ TestPack hCG Combo** that weak positive results be retested with a first morning urine specimen taken 48-72 hours later.
- A negative result may be obtained if the urine specimen tested is too dilute.
- If a negative result is obtained and pregnancy is still suspected, the patient should be retested 48-72 hours later.
- Abnormal pregnancies (e.g. ectopic) may produce lower concentrations of hCG than expected for a given gestational age. Abnormal pregnancy cannot be distinguished from normal pregnancy by hCG levels alone<sup>8</sup>.
- hCG remains elevated for a time after pregnancy<sup>9</sup>. Pregnancy tests carried out fewer than 3 weeks after giving birth, or 9 weeks after natural loss or termination, may need further evaluation.
- A number of conditions other than pregnancy can cause elevated levels of urinary or serum hCG, e.g. menopause, ovarian cysts, trophoblastic disease, and certain non-trophoblastic neoplasms<sup>10</sup>.
- Occasionally, specimens containing <25mIU/ml hCG may test positive.
- Drugs containing hCG may interfere with **Alere™ TestPack hCG Combo** and produce misleading results.
- False positive and false negative pregnancy tests have been observed in patients with abnormal bladder or kidney function e.g. enterocystoplasties and renal failure.

10. Inconsistent results may be obtained if the urine specimen contains an excessive amount of bacteria.
11. False results can occur with **Alere™ TestPack hCG Combo** due to non-specific protein binding<sup>11, 12, 13</sup>.
12. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays<sup>14, 15</sup>.
13. Grossly haemolysed, plasma, lipaemic or icteric specimens are not suitable for testing with **Alere™ TestPack hCG Combo**, since they may give inaccurate and/or erratic results.
14. If the test result is not consistent with clinical evidence, further evaluation may be required.

#### EXPECTED RESULTS

Urine and serum specimens from pre-menopausal females generally contain <5mIU/ml hCG; levels are generally <10mIU/ml in healthy males and post menopausal females<sup>2</sup>. On the first day of the first missed period, the levels of maternal hCG are normally 50-250mIU/ml<sup>6</sup>.

#### PERFORMANCE CHARACTERISTICS

##### Sensitivity

**Alere™ TestPack hCG Combo** can detect hCG in urine and serum at concentrations of 25mIU/ml or greater. This sensitivity has been determined against the 4th International hCG Standard (WHO)<sup>1</sup>. Specimens containing less than 5mIU/ml should give negative results.

##### Prozone effect

**Alere™ TestPack hCG Combo** has been shown to produce positive results with specimens containing up to and including 1,000,000mIU/ml hCG, which is higher than the maximum level expected during typical pregnancy.

##### Specificity

**Alere™ TestPack hCG Combo** has been evaluated for its cross-reactivity with a variety of substances, including other hormones found in urine and serum. No cross-reactivity was detected when the following substances were added to both “positive” (containing 25mIU/ml hCG) and “negative” urine and serum specimens: LH (1000mIU/ml), FSH (1000mIU/ml), TSH (1000µIU/ml).

#### INTERFERING SUBSTANCES

The following potentially interfering substances were added to hCG negative and hCG positive (containing 25mIU/ml hCG) specimens. None of the substances interfered with the assay at the concentrations tested.

##### Interfering Substances (in Urine)

Acetaminophen	(20 mg/dL)
Acetoacetic acid	(2000 mg/dL)
Acetone	(1000 mg/dL)
Acetylsalicylic acid	(20 mg/dL)
Albumin (human serum)	(1200 mg/dL)
Ampicillin	(20 mg/dL)
Ascorbic acid	(200 mg/dL)
Atrophine	(20 mg/dL)
Biotin	(25 µg/dL)
Bilirubin	(1 mg/dL)
Caffeine	(20 mg/dL)
Creatinine	(200 mg/dL)
Dextromethorphan	(20 mg/dL)
Diphenhydramine	(20 mg/dL)
EDTA	(40 mg/dL)
Estrone β-D glucuronide	(100 µg/dL)
Ethanol	(1%)
Glucose	(10000 mg/dL)
Haemoglobin	(360 mg/dL)
Human serum proteins	(2000 mg/dL)
Hydroxybutyric acid	(100 mg/dL)
Ephedrine	(20 mg/dL)
Gentisic acid	(20 mg/dL)
Ibuprofen	(40 mg/dL)
Nicotine	(20 µg/dL)
Oxalic acid	(60 mg/dL)
Oxytetracycline	(30 mg/dL)
Phenylpropanolamine	(4000 mg/dL)

5β-pregnane-3α, 20α-diol glucuronide	(100 µg/dL)
Riboflavin	(2 mg/dL)
Salicylic acid	(20 mg/dL)
Sodium Carbonate	(800 mg/dL)
Sodium Chloride	(6800 mg/dL)
Tetracycline	(30 mg/dL)
Urea	(2000 mg/dL)
Uric acid	(100 mg/dL)

##### Interfering Substances (in Serum)

Bilirubin	(40 mg/dL)
Estrone β-D glucuronide	(1 µg/mL)
Haemoglobin	(1000 mg/dL)
5β-pregnane-3α, 20α-diol glucuronide	(1 µg/mL)
Triglycerides	(1395 mg/dL)

In addition there was no pH effect in urine in the range pH 4.5 to pH 8.5.

### ACCURACY

In a study, 295 urine and 186 serum specimens collected from women for the purpose of pregnancy testing, were evaluated with **Alere™ TestPack hCG Combo** and with the Quidel QuickVue®\* One Step hCG Combo test (QuickVue® hCG Combo).

Of the 295 urine specimens evaluated, 126 specimens tested positive by both methods and 168 specimens tested negative by both methods. One specimen, which tested less than 5mIU/ml hCG on a quantitative test, tested negative on the **Alere™ TestPack hCG Combo** test but positive on the QuickVue® hCG Combo.

An agreement of over 99% was determined for these urine specimens. The relative sensitivity and relative specificity were found to be greater than 99% for these specimens.

Of the 186 serum specimens evaluated, 110 specimens tested positive by both methods and 76 specimens tested negative by both methods. An agreement of over 99% was determined for these serum specimens. The relative sensitivity and relative specificity were found to be greater than 99% for these specimens.

The study results are summarised below:

#### Urine

TestPack hCG Combo	QuickVue® hCG Combo	
	+	-
+	126	0
-	1	168

#### Serum

TestPack hCG Combo	QuickVue® hCG Combo	
	+	-
+	110	0
-	0	76

\*QuickVue® is a registered trademark of Quidel Corporation.

#### ADVICE LINE

For further information, please contact your distributor, or call Alere Technical Specialists:

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