OnSite™
hCG Combo Rapid Test

A lateral flow chromatographic immunoassay for the early detection of pregnancy in human urine or serum or plasma

CTK Biotech, Inc.
13855 Stowe Dr,
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

EC REP
MDSS GmbH,
Schiffgraben 41.
30175 Hannover, Germany

Table of Contents

Section A) PRODUCT DESCRIPTION

1.) Product Description

The *OnSite* hCG Combo Rapid Test is a lateral flow chromatographic immunoassay for the early detection of pregnancy, by providing a quick direct visual test for the placental hormone, hCG in human serum, plasma and urine. The hCG detection limit of the *OnSite* hCG Combo Rapid Test is 12.5 mIU/mL in serum/plasma specimen and 25 mIU/mL in urine specimen.

The *OnSite* hCG Combo Rapid Test is not intended for quantitative results, nor for over the counter (OTC) sales. It is designed for professional use only, and provides only preliminary analytical data. For a final diagnosis of pregnancy, a more specific alternative clinical method must be used to obtain a confirmed analytical result.

2.) Intended Use

The *OnSite* hCG Combo Rapid Test is for professional use only. It provides only preliminary analytical data. For a final diagnosis of pregnancy, a more specific alternative clinical method must be used to obtain a confirmed analytical result.

.

3.) Device Classification

- USA: Class II device
- Europe: Annex III, Other

Section B) TECHNICAL REQUIREMENTS

1.) General Requirements Checklist

General requirements		Apply	Applied Standards	Demonstrated By:	Location:
1.	Safe use for Patient, User, Environments	Yes	EN ISO 14971 : 2012	Risk Management File	Attachment # 1
2.	Solutions To Ensure Safety, Including Elimination/ reduction of risk, Taking Appropriate Action when appropriate and informing users of residual risk	Yes	EN ISO 14971 : 2012	Risk Management File	Attachment # 1
3	They are suitable for the purposes referred to in Article 1(2)(b), & meet manufacturer stated Performance Expectations	Yes	EN 13612 : 2002	IFU & Product Performance	Attachment # 2 & Attachment # 4
4.	Product Safety and Performance must not be affected during product lifetime when exposed to normal stresses and conditions	Yes	EN ISO 23640 : 2013	Stability Study	Attachment #5
5.	Devices designed and manufactured so that performance is not adversely affected under storage and transport conditions.	Yes	EN ISO 23640 : 2013	Pouch Package Study	Attachment # 9
6.	In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: In vitro diagnostic reagents for professional use (ISO 18113-2:2009)	Yes	EN ISO 18113-2:2009	IFU	Attachment # 2
7.	In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions and general requirements	Yes	EN ISO 181131:2009	IFU	Attachment # 2
8.	Sampling procedures used for acceptance testing of in vitro diagnostic medical devices - Statistical aspects	Yes	EN ISO 13975: 2003	Sampling and acceptance procedure	SOP-82-04 and WI 82-04-1

2.) Design and Manufacturing Requirements

Design and Manufacturing Requirements		Apply	Standards	Demonstrated By:	Location:
1	**CHEMICAL AND PHYSICAL PROPERTIES**				
1.1	Device characteristics and performance in relation with intended use	Yes	EN 13612 : 2002	Product Performance	Attachment #4
1.2	Risks posed by device leakage, contaminants and residues	Yes	EN ISO 14971 : 2012 EN 13641: 2002	MSDS	Attachment # 8
2	**INFECTION AND MICROBIAL CONTAMINATION**				
2.1	Reduction of the risk of infection and/or contamination	Yes	EN ISO 14971 : 2012 EN 13641:2002	Risk Management, MSDS, IFU	Attachment #1, #2 & # 8
2.2	Reduce risk of biological substances	Yes	EN ISO 14971 : 2012 EN 13641:2002	Risk Management, MSDS, IFU	Attachment #1, #2 & # 8
2.3-2.6	N/A	No	N/A	N/A	N/A
2.7	Packaging	Yes	EN ISO 14971 : 2012 EN 13640:2002	Pouch Package Study	Attachment # 9
3	**MANUFACTURING AND ENVIORNMENTAL PROPERTIES**				
3.1	Tests when connected with other devices and/or accessories	No	N/A	N/A	N/A
3.2	Contact with device materials	No	N/A	N/A	N/A
3.3	Remove risk due to outside influence. (ex. Humidity, temperature)	Yes	EN ISO 14971 : 2012 EN 13640:2002	Risk Management Stability study	Attachment # 1 & Attachment #5
3.4	Flammability	No	N/A	N/A	N/A
3.5	Safe Waste Disposal	Yes	EN ISO 14971 : 2012 EN 13640:2002	IFU, MSDS	Attachment # 2 & Attachment #8
3.6	Ergonomics	No	N/A	N/A	N/A
4-7		**No**	**N/A**	**N/A**	**N/A**
8	**MANUFACTURER INFORMATION**				
8.1	Information for use	Yes	EN1041 : 2008 EN ISO 18113 : 2011 EN ISO 15223-1:2012	Labels, IFU	Section 4.2 & Attachment #2
8.2	Standard Symbol	Yes	EN ISO 15223-1 : 2012	Labels, IFU	Section 4.2
8.3	Danger Symbol	Yes	EN ISO 15223-1 : 2012	Labels	Section 4.2
8.4	Proper Label Format	Yes	EN ISO 15223-1 : 2012	Labels	Section 4.2
8.5	Intended Purpose	Yes	EN 1041 : 2008	IFU	Attachment# 2
8.6	Device and Component Identification	Yes	EN ISO 15223-1 : 2012	Labels	Section 4.2
8.7	Instructions for use	Yes	EN 1041 : 2008 EN ISO 18113:2011	IFU	Attachment # 2

3. RISK ANALYSIS

See Attachment 1: hCG Rapid Test Risk Management Report
The Risk of this product is negligible and no further action needs to be taken.

4. LABELS & INSTRUCTIONS FOR USE

4.1) Instructions for Use: See Attachment 2: I.F.U.

4.2) Direct Labeling of Product

See attached labeling

5.) MATERIALS SPECIFICATIONS

5.1) Material Specification

Materials		Specification
Test strip	Overall	64 ± 0.5 mm x 3.5 ± 0.3 mm with five subcomponents
	a. Sample pad	31 ± 1.5 mm x 3.5 ± 0.3 mm Absorb water within 1 second
	b. Conjugate pad	4-5 mm x 3.5 ± 0.3 mm containing anti-hCG Antibody and Mouse IgG -gold conjugates
	c. Nitrocellulose membrane	20 ± 0.5 mm x 3.5 ± 0.3 mm, Flow rate: 4cm / 110 - 165 seconds backing spotted with Anti-hCG Antibody (T line), and goat-anti mouse IgG (C line).
	d. Absorbent pad	15 ± 0.7 mm x 3.5 ± 0.3 mm
	e. Vinyl matte adhesive	64 ± 3.3 mm x 3.5 ± 0.3 mm Pass flow rate test at 45 °C for 7 days
Anti-hCG Antibody 1		Purified via ion-exchange chromatograph ≥ 95% purity by SDS-PAGE ≥ 1 mg/mL by OD280 nm Pass hCG Specificity and Sensitivity QC panel
Anti-hCG Antibody 2		Purified via ion-exchange chromatograph ≥ 95% purity by SDS-PAGE ≥ 1 mg/mL by OD280 nm Pass hCG Specificity and Sensitivity QC panel
Mouse IgG		Purified via ion-exchange chromatograph ≥ 95% purity by SDS-PAGE ≥ 1 mg/mL by OD280 nm Pass activity test (strong C line at 5 minutes)
Goat anti-Mouse IgG		Purified via ion-exchange chromatograph ≥ 95% purity by SDS-PAGE ≥ 1 mg/mL by OD280 nm Pass activity test (strong C line at 5 minutes)
Gold Chloride		Chemical formula: $HAuCl_4.3\,H_2O$
Conjugate pad fabric		Absorb water within 1 second.
Pouch		120 ± 0.6 mm x 65 ± 3.2 mm size with 3-5 mm sealing margin One side is printed with company information and test type. The other side is blank for labeling of production information. No dirty spots Pass 3 day integrity of seal test
Desiccant		0.5 ± 0.1 g
Package Insert		Off white or white paper, 70g A 4 paper, left and right margin: 0.5 -1.0 cm; top and bottom margin: 1.0-1.5 cm. Correct art work, color printing, no dirty spots

5.2) Product Specifications

See Attachment 3: Product Specification

5.3) Component Specifications

5.3.1) Test Characteristic

Nitrocellulose- based membrane strip with a T line pre-coated with anti-hCG antibody, a C line pre-coated with goat anti-mouse IgG, and a conjugate pad containing colloid gold conjugated anti-hCG antibodies and Mouse IgG conjugates.

5.3.2) Kit composition and specifications

Kit Composition

Composition	Specification
1. Device	50 single use devices in each kit. Each is individually sealed, and contains two items inside: 1. One dip strip device 2. One desiccant: 0.5 g
2. Package insert	One in each kit

Kit Box

Item	Specification
Dimension:	12.5 cm (W) x 22 cm (L) x 7.3 cm (H)
Capacity:	50 test devices

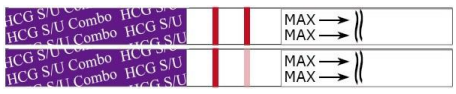
Shipping Carton Box

Item	Specification
Dimension:	54 cm (w) x 46 cm (L) x 41cm (H)
Materials:	Double side, colligated materials. Standard with 80 Kg
Capacity	40 kits of 50 tests

5.3.3) Test Appearance

The *OnSite* hCG Combo Rapid Test is a dip strip device. Both the Test line and the Control line in are not visible before applying any samples. Prior to use the device appears as follows.

In addition to the presence of the C line, if the T line develops, the test indicates for the presence of hCG in the specimen. The Result is positive.



If only the C line is developed, the test indicates that no detectable hCG is present in the specimen. The result is negative.

If no C line is developed, the assay is invalid regardless of color development of the T line. Repeat the assay with a new device.

6.) CLINICAL STUDIES & PERFORMANCE EVALUATIONS

See Attachment 4: Clinical Study and Performance Evaluation

7.) STABILITY STUDIES

See Attachment 5: Stability Study

8.) MANUFACTURING

8.1) Manufacturing Process

The entire manufacturing process has six sequential process steps and is jointly accomplished by six production groups

Step #1 **Conjugate Anti-hCG antibodies, and Mouse IgG with colloid gold and preparation of conjugation pad**

- Preparation of gold chloride solution. This solution is made by mixing 0.04% gold chloride and 0.068% sodium citrate
- Add Protein A to the above solution, mix well, incubate for 20 minutes at room temperature
- Add conjugation buffer, incubate for 15 minutes at room temperature
- Centrifuge
- Collect precipitants and wash with PB wash buffer
- Dissolve the conjugates with conjugate suspension buffer
- Use the QC specificity and sensitivity panel and reference reagents if necessary to determine the working concentration of the conjugate.
- Dilute the gold conjugates with the conjugate diluent
- Dispense the diluted conjugates to the conjugate pad material
- Dry the conjugate pad
- Cut the dried conjugate pads into strips at the size of 4 -5 mm x 30 cm.
- Seal the strips until use
- Inspect the conjugate pad with the QC sensitivity and specificity panels and reference reagents if necessary

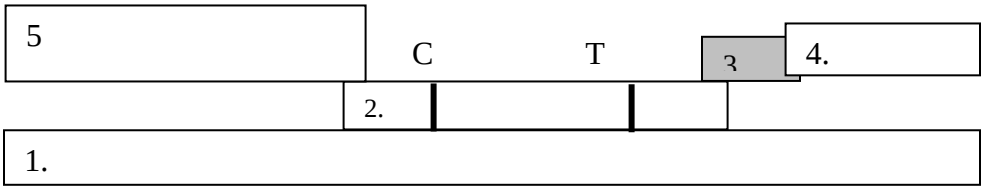
Step #2 **Coat T and C line on the NC membrane**

- Preparation of membrane lamination: Fix a 20 mm x 300 mm of nitrocellulose membrane onto the 300 mm x 66 mm of vinyl matte adhesive.
- According to the incoming material QC inspection result, prepare the T line (Anti hCG Antibodies) and C line (Goat anti-mouse IgG) coating solutions.
- Dispense the reagents to the T and C positions on the membrane with the coating machine.
- The coated membrane is then dried

Step #3 **Lamination**

- Use the QC control panel to inspect the conjugate pad strips and coated membrane prepared in Step 1 and 2 above.
- Prepare sample pad material at the size of 3.1 cm x 30 cm.
- Prepare absorbent pad material at the size of 1.5 cm x 30 cm.
- Assemble all the components to sheet according to scheme illustrated below
- Laminate the components according to scheme illustrated below
- Inspect each laminated sheet to make sure it is correctly assembled
- QC samples the uncut sheet with the QC sensitivity, specificity, reproducibility, interference and stability panels.

[Cross Section Scheme of Lamination]



1. Vinyl matte adhesive
2. NC membrane coated with T and C line.
3. Gold conjugate pad
4. Sample pad
5. Absorbent pad

Step #4 **Cutting**

- Laminated sheets are cut into the size of 3.5 mm x 68 mm.
- The size of the strip is inspected at the beginning, middle and end of the run.

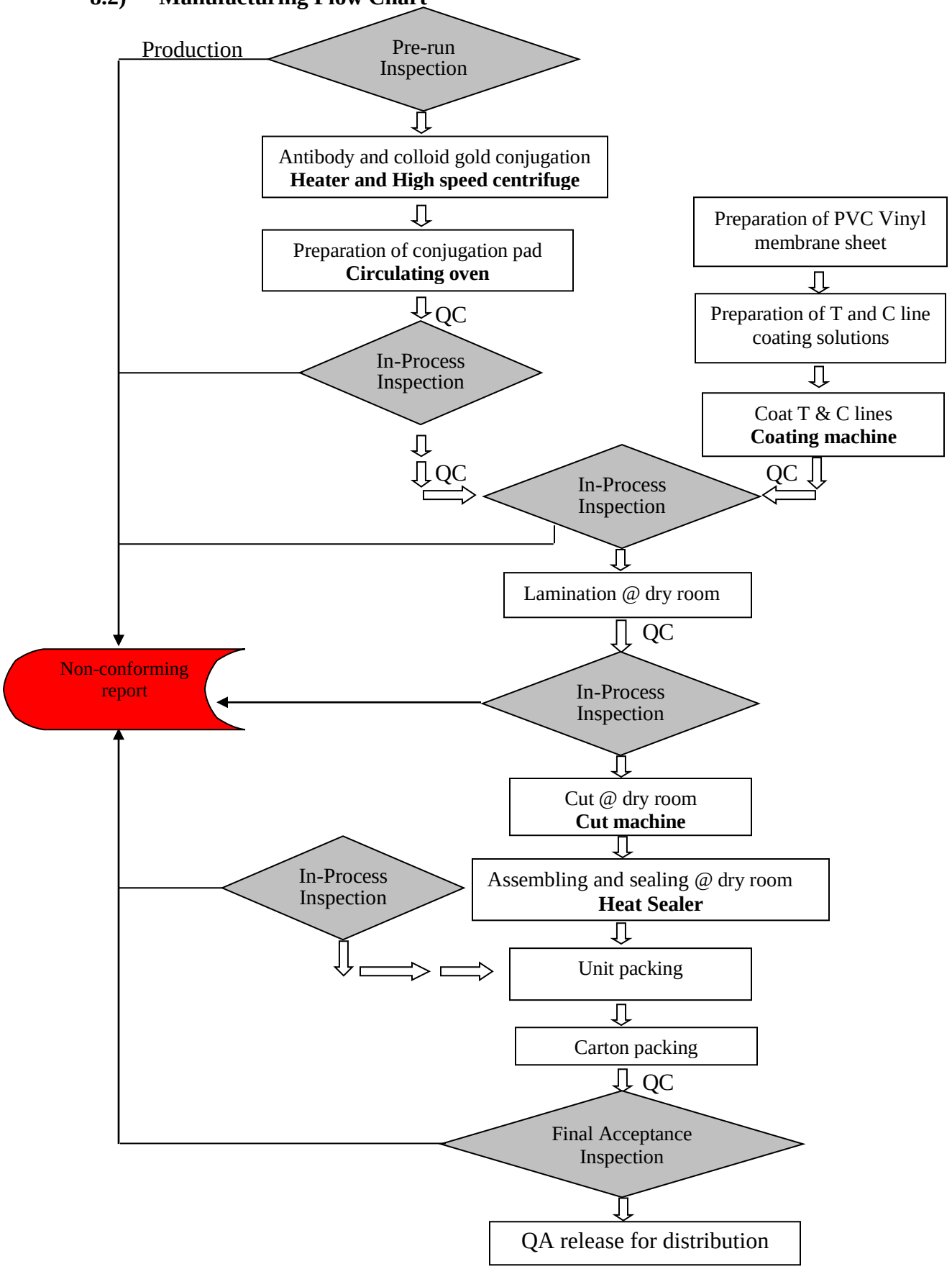
Step #5 **Assembling and Sealing**

- Pouch is labeled with name, mfg date, exp. date, and catalog, according to the documentation
- Pack one strip, and one desiccant to each pouch
- Seal the pouch with the heat sealing machine
- Inspect packing process by reconciliation of the quantity of components picked, the quantity of pouch assembled, and the quantity of unused components.

Step #6 **Unit and Carton Packing**

- Pick up all required components and labels including package inserts, zip lock bag (bulk package) and kit box
- Pack to unit
- Pack to carton
- Inspect packing process by reconciliation of the quantity of components picked, the quantity of units packed, and the quantity of unpacked components
- Packed product is moved to the quarantine area awaiting Final Acceptance Inspection

8.2) Manufacturing Flow Chart



8.3) Production Specifications

Production Process	Specification
Pre-run Inspection	Antigen or Antibody has less than 10% degradation by SDS-PAGE
Colloid Gold Solution	Validated process
Conjugation	First run inspection passes QC panel Conjugate pad is uniformly soaked with conjugates by 100% visual inspection. Dryness of the conjugate pad Conjugate pad passes test with QC panel
Coating	The dispensing volume is calibrated at the start of the process by checking the accuracy of the volumes dispensed. T and C lines are coated at the designated area and inspected at the start run, middle run, and final run. 1.0 mm coating line by 100% visual inspection. Dryness of the membrane Coated membrane passes test with QC panel
Lamination	First run inspection passes test with QC panel All the components are laminated correctly by visual inspection according to Inspection Sample Plan.
Cutting	3.5 $\pm$ 0.1 mm wide each strip verified by inspection at start of run, middle-run, and final run.
Assembling and pouching	All the dip strips are properly assembled by visual inspection during processing. All the pouches are labeled correctly by visual inspection All the components are packed correctly by reconciliation of the quantity of the components picked and used.
Unit Packing	All the components are packed correctly by reconciliation of the quantity of the components picked and packed.
Carton packing	Correct quantity of the units is packed by reconciliation of the quantity of the units picked and packed.

8.4) Documentation of Quality System

This product is manufactured in a facility certified to be in accordance with the ISO 13485 Quality System

See Attachment 6: ISO Certification

9.) BATCH RELEASE CRITERIA

Quality Control Inspection and Specifications:
Quality control inspection is performed on all raw materials and intermediate components produced, as well as the assembled kits. The inspection of the product is based on the following protocols:

9.1) Incoming Material Inspection

Incoming Material Inspections are performed on all in-coming raw materials. The

quarantined raw materials are sampled per Inspection Sample Plan by QC department for inspection per specification described at section 5.1
Upon inspection, the QC supervisor will endorse the inspection data and assign quality status to the raw materials accordingly.
Only approved raw materials are used for production of product.

9.2) In-Process Inspection

All lots of individual intermediate components produced are inspected and tested during the process. A systematic sampling of each individual lot of intermediate components is taken for In-Process Inspection per specification described at section 5.1
Function tests are performed by assaying the intermediate components with the QC control panel, and with a reference intermediate component on the *OnSite* hCG Combo Rapid Test if necessary. Physical inspections of the test components, such as the fill volume, appearance, and physical status are also carried out.
Upon analyzing the inspection results, the QC supervisor will assign the quality status of the intermediate components. The approved intermediate components are moved from the quarantined storage area to the approved storage area. Components that do not pass inspection will be rejected and not used in production.

9.3) Final Acceptance Inspection

Final acceptance inspection is carried out once all the components are assembled into the final packing unit. The inspection is to ensure only the product that meets the specification is released for distribution. The inspection includes:

- Document Inspection: Inspection of all production work records
- Physical Inspection: Inspect based on the Sampling Plan. The inspection includes checking the labels, lot number and expiration date of the individual components as well as the assembled kit. Inspection is also performed to ensure that all the components are packed.
- Performance Inspection: Around 250 tests submitted by the Production group are inspected with three *OnSite* hCG Combo QC control panels. The panels consist of 160 members of an analytical sensitivity panel, 40 members of a specificity panel, and 1 member of a hook effect panel for testing product sensitivity, specificity, and hook effect
- Kits are ready for shipment once they have passed this final stage of QC inspection.
- QA will endorse the release of the product and retain at least 250 tests for future analysis.

9.4) **Procedure of the Final Acceptance Inspection**

Introduction:
Each kit of *OnSite* hCG Combo Rapid Test (R1001S) contains the following components:

a. 50 test devices, each sealed in a foil pouch with two items inside:
   - One dip strip device
   - One desiccant

b. One package insert (instruction for use).

Quality control evaluation is performed on every lot of these components.
Inspection Procedures:
The quality control procedures used for evaluation of the finished products are:

a. Physical Inspection
   According to the Inspection Sample Plan, obtain the required quantity of tests, inspect following parameters:
   - Content in each package: Make sure each pack contains the correct quantity of components
   - Labels: Make sure all labels correspond to documentation
   - Pouch Integrity: Make sure the pouch is sealed properly
   - Pouch content: Open pouch to check contents. Make sure all the contents are included.

   Record the number of the defects observed. Refer to the Acceptance Number (Ac). Pass inspection if the number (Ac) is less than the maximum allowable defects or defectives in a sample for the lot to be accepted based on the sampling plan.

b. Performance Inspection
   Each lot of the *OnSite* hCG Combo Rapid Test is inspected for its analytical sensitivity, specificity, and hook effect.
   Analytical Sensitivity Inspection
   - Inspection is carried with the *OnSite* hCG Combo Analytical Sensitivity QC panel. The panel consists of 160 members.
   - The assay is performed and interpreted using the procedure described in the Product Insert.

   - Specificity Inspection
     - Inspection is carried with the *OnSite* hCG Combo Specificity QC panel. The panel consists of 40 members, including 20 serum specimens numbered SM-R1001-N1~N20 and 20 urine specimens numbered SM-R1001-N1~N20.
     - The assay is performed and interpreted using the procedure described in the Product Insert

   - Hook Effect Inspection
     - Inspection is carried out with the *OnSite* hCG Combo Hook Effect panel consisting of a strong hCG positive specimen at 250 IU/ml.
     - The assay is performed and interpreted using the procedure described in the Product Insert.

c. Acceptance Criteria

- The Analytical Sensitivity Inspection result must meet the specification indicated in the following table.

hCG Analytical Sensitivity Panel (SM-R1001 plasma)	Runs	Results	Flow Rate
SM-R1001-1-P50-1~20	20	100% positive	5 minutes
SM-R1001-1-P25-1~20	20	100% positive	5 minutes
SM-R1001-1-P12.5-1~20	20	≥95% positive	5 minutes
SM-R1001-1-P6.25-1~20	20	≤ 75% positive	5 minutes

hCG Analytical Sensitivity Panel (SM-R1001 urine)	Runs	T Line	Flow Rate
SM-R1001-1-U50-1~20	20	100% positive	5 minutes
SM-R1001-1-U25-1~20	20	≥95% positive	5 minutes
SM-R1001-1-U12.5-1~20	20	≤ 75% positive	5 minutes
SM-R1001-1-P6.25-1~20	20	≤ 50% positive	5 minutes

- The Specificity Inspection result must meet the specification indicated in the following table.

hCG Rapid Test Specificity Panel	Sample (N)	Result
SM-R1001-N1~N20 non-pregnant female serum samples	20	100% Negative
SM-R1001-N1~N20 non-pregnant female urine samples	20	100% Negative

- The Hook Effect Inspection result must meet the specification indicated in the following table.

hCG Rapid Test Hook Effect Panel (SM-R1001-H)	Runs	T Line	C line	Flow Rate
SM-R1001-H (hCG – 250 IU/ml)	1	positive	Strong	5 minutes

d. Reference Components Used in QC
The reference components used for inspection of individual intermediate components are approved components from previous production lots. These reference components are tested to ensure that results obtained are within the QC specification for the product.

e. See Attachment 7: Certificate of Analysis.

10.) CONCLUSION

The *OnSite* hCG Combo Rapid Test is developed, manufactured, and marketed according to the ISO13485 quality standard. In a 319 fresh urine sample comparison with an hCG ELISA reference test, the *OnSite* HCG Combo Test had a Relative Sensitivity of 100% (95% CI: 98.5% - 100%), a Relative Specificity of 100% (95% CI: 97.9% - 100%) and an Overall Agreement of 100% (95% CI: 99.1% - 100%). In a 276 fresh serum sample comparison with an hCG ELISA

reference test, the *OnSite* HCG Combo Test had a Relative Sensitivity of 100% (95% CI: 95.8% - 100%), a Relative Specificity of 99.5% (95% CI: 98.0% - 100%) and an Overall Agreement of 99.6% (95% CI: 98.4% - 100%).

The test does not require equipment and can be performed by technicians with minimal training. It can be stored for 24 months at 2-30°C. Thus, the test is deemed acceptable for marketing and sale.