OnSite HBsAg Combo Rapid Test

A lateral flow chromatographic immunoassay for the qualitative detection of Hepatitis B surface antigen (HBsAg) in human serum, plasma, or whole blood.

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Section A) PRODUCT DESCRIPTION

1.) Product Description

The OnSite HBsAg Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a colored conjugate pad containing murine anti-HBsAg antibodies conjugated with colloidal gold (HBsAg Ab conjugates) and a control antibody conjugated with colloidal gold; and 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The test line is pre-coated with a different, unconjugated murine anti-HBsAg antibody, and the control line is pre-coated with a control antibody.

2.) Intended Use

The OnSite HBsAg Combo Rapid Test is a lateral flow chromatographic immunoassay for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum, plasma or whole blood. It is intended to be used by healthcare professionals as an aid in the diagnosis of infection with hepatitis B virus (HBV).

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be used to confirm the test result obtained by this device.

3.) Device Classification

- USA: Class III device
- Europe: Class IIA device
- India: Critical test device

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: 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	5-6 mm x 3.5 ± 0.3 mm Containing HBsAg antibody conjugates and Chicken IgY-gold conjugates
	c. Nitrocellulose membrane	20 ± 0.5 mm x 3.5 ± 0.3 mm, Flow rate: 4cm / 110 - 165 seconds backing spotted with HBsAg antibody (T band), and goat anti-Chicken IgY (C band).
	d. Absorbent pad	22 ± 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
Mouse Anti-HBsAg Antibody No1		Purified via affinity chromatograph ≥ 95% purity by SDS-PAGE ≥ 1.5 mg/mL by Bradford Protein Assay Pass HBsAg Specificity and Positive Detection panel
Mouse Anti-HBsAg Antibody No2		Purified via affinity chromatograph ≥ 95% purity by SDS-PAGE ≥ 1.5 mg/mL by Bradford Protein Assay Pass HBsAg Specificity and Positive Detection panel
Goat anti-chicken IgY		Purified via affinity chromatograph > =95% purity by SDS-PAGE >= 5.0 mg/mL Pass activity test (control line appear in 2 minutes)
Chicken IgY		Purified via ion-exchange chromatograph > =95% purity by SDS-PAGE >= 5.0 mg/mL Pass activity test (control line appear in 2 minutes)
Gold Chloride		Chemical formula: $HAuCl_4.3\,H_2O$
Conjugate pad fabric		Absorb water within 1 second.
Plastic Cassette		72 ± 4.1 mm x 20± 1 mm The position of the T and C lines are marked on the cassette Strip groove fits 3.5 mm x 64 mm strips Pass flow rate test with specimens
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, with no dirty spots. Pass 3 day integrity of seal test
Desiccant		1 ± 0.1 g
Instructions for Use		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
Sample Diluent	Colorless Free of particulate matter
Sample Diluent Bottle	7 mL dropper bottle, 30 µL-40 µL /drop Pass vacuum leakage test (0.2 PSI x 2 minutes

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 mouse anti-HBsAg Antibody No1, a C line pre-coated with goat-anti chicken IgY, and a conjugate pad containing colloid gold conjugated mouse anti-HBsAg antibody No2 and chicken IgY conjugate assembled into a strip.

5.3.2) Kit composition and specifications

Kit Composition

Composition	Specification
1. Device	30 single use devices in each kit. Each is individually sealed, and contains two items inside: 1. One cassette device 2. One desiccant
2. Plastic dropper	30 single use plastic dropper
3.Sample Diluent	1 bottle, 5 mL
4. Instructions for Use	One IFU in each kit

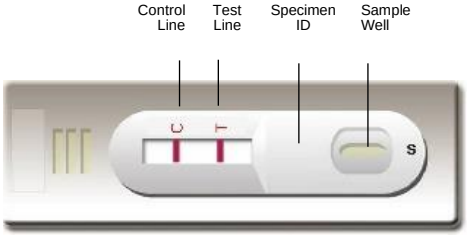
Kit Box

Specification	Dimension	Capacity
CTK Generic (PM-R0001)	12.4 cm (W) x 22.2 cm (L) x 7.05 cm (H)	30 test devices

5.3.3) Test Appearance

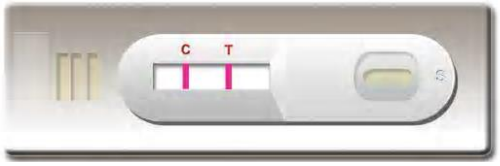
The *OnSite* HBsAg Combo Rapid Test is a cassette device. The device has the following letters on the surface of the cassette:

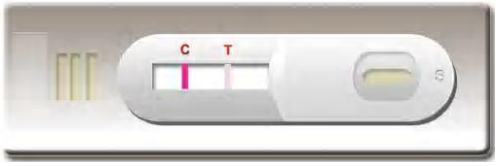
- T: Test Band position
- C: Control Band position
- S: Sample receiving well



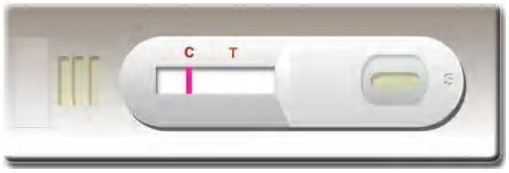
Prior to use, the device appears as follows. Both the Test bands and the Control band are not visible before applying any samples.

If both C and T bands are developed, the test indicates that the specimen contains HBsAg. The result is positive.





If only the C band develops, the test indicates that the level of HBsAg is not detected in the specimen. The result is negative.



6.) CLINICAL STUDIES & PERFORMANCE EVALUATIONS

See Attachment 4: Product Performance

7.) STABILITY STUDIES

See Attachment 5: Stability Study

8.) MANUFACTURING

8.1) Manufacturing Process

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

Step #1 **Conjugate mouse anti-HBsAg antibody No1 and chicken IgY 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 mouse-anti HBsAg antibody No1 and chicken IgY 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 positive detection 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 positive detection and specificity panels and reference reagents if necessary

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

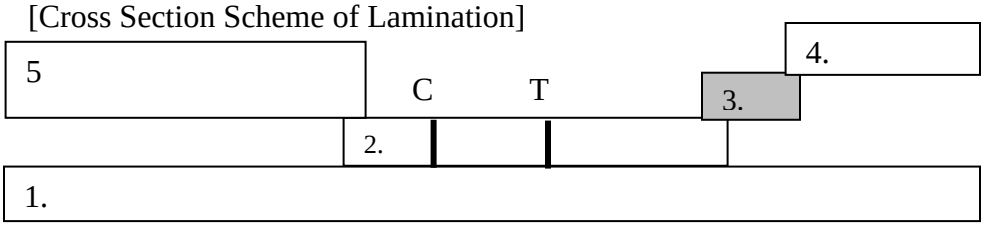
- Preparation of membrane lamination: Fix a 20 mm x 300 mm of nitrocellulose membrane onto the 300 mm x 66 mm of vinyl matte adhesive.
- According to the incoming material QC inspection result, prepare the T band (mouse anti-HBsAg antibody No2) and C band (goat anti-chicken IgY) coating solutions.
- 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 positive detection, specificity, reproducibility, interference and stability panels.

[Cross Section Scheme of Lamination]



1. Vinyl matte adhesive
2. NC membrane coated with T and C band.
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 **Cassette Assembling and Sealing**

- Pouch is labeled with name, mfg date, exp. date, and catalog, according to the documentation
- Assemble the cut strip into the plastic housing cassette and press to close the cassette
- Pack one cassette, one dropper, 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 pouches assembled, and the quantity of unused components.

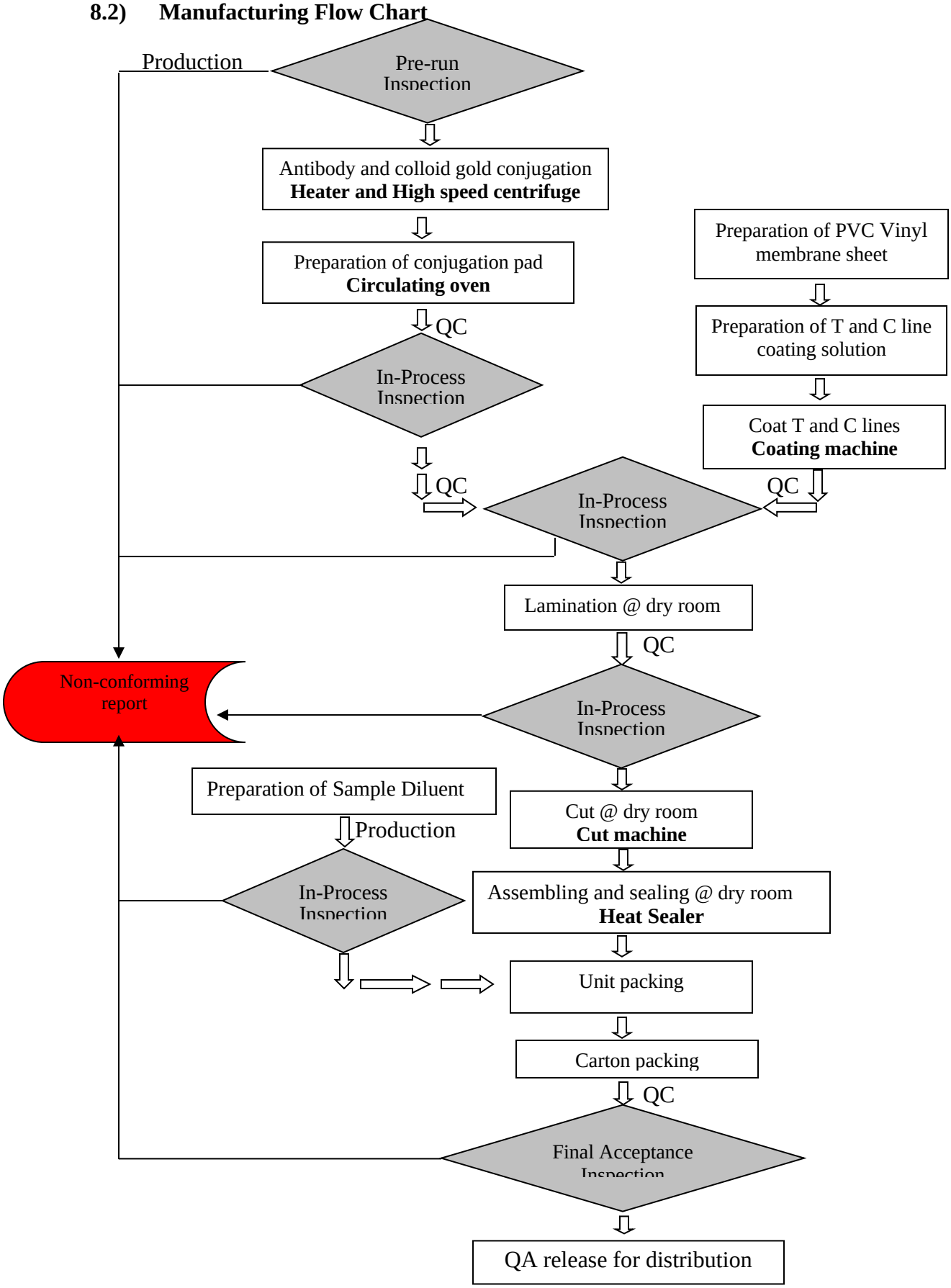
Step #6 **Preparation of Sample Diluent**

- Prepare Sample Diluent according to correct procedure
- Aliquot 5 mL to each bottle
- Inspect all bottles for leakage at 2 Psi for 2 minutes

Step #7 **Unit Packing**

- Pick up all required components (droppers, device, diluent) and labels including package inserts, zip lock bag (bulk package) and kit box
- Pack to unit
- 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 bands 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.
Cassette assembling and pouching	All the strips are properly assembled into the cassettes 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.
Preparation of Sample Diluent	Solution is made with the correct formulation Fill in volume 5 mL + 0.5 mL based on the sampling plan No leaking on 100% test inspection
Unit Packing	All the components are packed correctly by reconciliation of the quantity of the components 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* HBsAg 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: At least 90 tests submitted by the Production group are inspected with four *Onsite* HBsAg Combo QC control panels. The panels consist of 11 members of a positive detection panel, 20 members of a specificity panel, 1 member of a precision panel, 3 members of limit of detection, 2 members of stability panel and 3 members of background panel for testing positive detection, specificity, precision, limit of detection, stability and background.
- 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 90 tests for future analysis.

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

Introduction

Each kit of *Onsite* HBsAg Combo Rapid Test (R0042C-CE) contains the following components:

a. 30 test devices, each sealed in a foil pouch with two items inside:
   - One Cassette device
   - One desiccant
b. 30 single use plastic dropper
c. Sample Diluent (1 bottle, 5 mL)
d. Instructions 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* HBsAg Combo Rapid Test is inspected for its positive detection, specificity, precision, limit of detection, stability and background.

   - Positive Detection Inspection
     - Inspection is carried with the Onsite HBsAg Combo Rapid Sensitivity QC panel. The panel consists of 11 members, numbered SM-R0042-P1~P11 and contains one whole blood positive.

- The assay is performed and interpreted using the procedure described in the Instructions for Use.
- SM-R0042-P1~P10 is assayed in singlet and SM-R0042-P11 is assayed in duplicate.

▪ Specificity Inspection
- Inspection is carried with the HBsAg Combo Rapid Test Specificity QC panel. The panel consists of 20 members which numbered SM-R0042-N1~N20 and 100 clinical negative specimens.
- The assay is performed and interpreted using the procedure described in the Instructions for Use

▪ Precision Inspection
- Inspection is carried out with a precision panel consisting of one HBsAg positive specimen
- The assay is performed and interpreted using the procedure described in the Instructions for Use.
- Each sample is assayed in 10 replicates. 10 Devices are used for the inspection.

▪ Limit of Detection
- Inspection is carried out with 3 members of Limit of Detection Panel.
- The assay is performed and interpreted using the procedure described in the Instructions for Use.
- Each member is assayed in triplicate.

▪ Stability Inspection
- The stability Inspection is carried out with a 45°C accelerated stability protocol. Test devices are incubated at the 45°C accelerated stability chamber for two weeks. Inspection is carried out with the Stability Panel.

▪ Background inspection
- Inspection is carried out with the Background Panel consisting of 2 members.
- The assay is performed and interpreted using the procedure described in the Instructions for Use.
- Each member is assayed 5 times.

▪ Flow rate inspection
- Inspection is carried out with the sample diluent.
- The assay is performed in duplicate.
- The flow rate of each device is recorded during the inspection.

c. Acceptance Criteria

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

HBsAg Combo Positive Detection Panel	Sample (N)	Result
SM-R0042-P1~P10	11	100% positive
Whole blood positive	1	positive

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

HBsAg Combo Specificity Panel	Sample (N)	Result
SM-R0042-N1~N20	20	100% negative
Clinical negative specimen	100	100% Negative

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

HBsAg Combo Precision Panel	Replicates	Result
SM-R0042-C	10	Equivalent test line intensity

- The Limit of Detection Inspection result must meet the specification indicated in the following table.

HBsAg Combo Limit of Detection Panel	Result
SM-R0042-L1	Positive
SM-R0042-L2	Positive
SM-R0042-L3	Positive or negative

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

HBsAg Combo Stability Panel	Result
1 weak positive	Positive after two weeks at 45°C
1 negative	Negative after two weeks at 45°C

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

HBsAg Combo Background Panel	Runs	Result
Whole blood, male	5	background clear, no red blood cell on membrane
Whole blood, female	5	background clear, no red blood cell on membrane

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

HBsAg Combo Flow Rate Panel	Runs	Flow Rate	Result
SB-R0042-CE	2	Migration: ≤120s	negative

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* HBsAg Combo Rapid Test is developed, manufactured, and marketed according to the ISO13485 quality standard. The test is deemed acceptable for marketing and sale wherever the regulatory requirement is completed.