STUDY TITLE

Limit of Detection Report

DOCUMENT NUMBER

APR-02/IM/GRA/014

Revision No. 00

STUDY ARTICLE

MERISCREEN COVID-19 Antigen Detection Test

Report Approvals:
Prepared by:
Name: Ms. Dhanya Menon Signature:  Date: 10/03/2021 Designation: Executive-QA
Reviewed by:
Name: Mr. Pradeep Kumar Signature:  Date: 10/03/2021 Designation: DGM- R&D
Approved by:
Name: Mr. Ram Kanoje Signature  Date: 11/03/2021 Designation: Head- QA

Table of Contents

1. Report Synopsis

Table 1: Report Synopsis

Name of sponsor/company: **Meril Diagnostics Pvt. Ltd.** Second Floor, D1 – D3, Meril Park, Survey No. 135/2/B & 174/2, Muktanand Marg, Chala, Vapi – 396191 Gujarat, India.	
Trade name of device: MERISCREEN COVID-19 Antigen Detection Test	
Measurand: SARS-CoV-2 Ag	
Title of study: Limit of Detection	
Study site(s) location: In-House **Meril Diagnostics Pvt. Ltd.** Second Floor, D1 – D3, Meril Park, Survey No. 135/2/B & 174/2, Muktanand Marg, Chala, Vapi – 396191 Gujarat, India.	
Name and contact information of individual responsible for the study:	
Mr. Pradeep Kumar DGM- R&D	Mr. Ram Kanoje Head QA
Study Commencement date: 10/03/2021	**Study Completion date:** 10/03/2021
Study Objectives: To determine the Limit of Detection of MERISCREEN COVID-19 Antigen Detection Test assay kit.	
Study Design: The Limit of detection of MERISCREEN COVID-19 Antigen Detection Test assay kit was determined by testing pooled real clinical matrix (e.g: Nasopharyngeal swab) spiked with inactivated virus of known concentration, which was subjected to a 2 fold dilution series and tested further with MERISCREEN COVID-19 Antigen Detection Test in replicates of 5 per concentrations. The final concentration was confirmed with 20 replicates using the Probit Model.	
Statistical methods: Probit Model shall be used to determine the LoD of MERISCREEN COVID-19 Antigen Detection Test assay kit.	

2. List of Abbreviations/Definitions

LOD is defined as the lowest amount of analyte in a sample that can be detected with probability, though it may not be quantified as an exact value. LOD is also an important performance criterion for qualitative tests even if the results is reported as "positive" or "negative" ("present" or "absent").

- COVID 19: Coronavirus-2019
- SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus
- LOD : Limit Of Detection

3. Purpose/Scope

To determine the Limit of Detection of MERISCREEN COVID-19 Antigen Detection Test kit.

Scope:

The scope of this report is applicable for MERISCREEN COVID-19 Antigen Detection Test assay kit performance only.

4. References

- EN 13612:2002 Performance evaluation of *In-Vitro* Diagnostic medical devices
- GHTF/SG1/N063:2011 Summary Technical Documentation (STED) for demonstrating conformity to the Essential Principles of Safety and Performance of *In-Vitro* Diagnostics medical devices.
- NCCLS. *Protocols for determination of limits of detection and limits of quantitation;* Approved Guideline. NCCLS document EP17-A [ISBN 1-56238-551-8]
- Clinical and Laboratory Standards Institute (CLSI) document EP17-A2. "Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline--Second edition." Wayne, PA. 2012.
- Limit of detection study protocol for MERISCREEN COVID-19 Antigen Detection Test (Doc. No.: APP-02/IM/GRA/014, Rev. 00)

- Food and Drug Administration (FDA) Guidance Document Antigen Template for Test Developers
- MERISCREEN COVID-19 Antigen Detection Test Kit Pack Insert: IFU/NCTCAG01/01, Rev.01, Nov'2020

5. Introduction

As per NCCLS guideline, EP17-A, LOD is defined as the lowest amount of analyte in a sample that can be detected with probability, though it may not be quantified as an exact value. LOD is also an important performance criterion for qualitative tests even if the results is reported as "positive" or "negative" ("present" or "absent"). LOD determination also allows monitoring the consistency of the performance of method and is an important parameter for controlling the quality of many qualitative tests.

6. Intended Use of the Device

COVID-19 Antigen Detection Test is a Lateral Flow immunochromatographic rapid assay kit for the qualitative detection of SARS-CoV-2 specific antigen (viral nucleocapsid protein) in nasopharyngeal swab from human. This test is designed for use in laboratory and Point-of-Care (POCT) environments by healthcare professional that meets the requirements specified in the Instructions for Use and local regulation. This test is intended to be used as an aid in the diagnosis of SARS-CoV-2 infection. Negative results must be combined with clinical observations, patient history, and epidemiological information

7. Device description and principle of the method

7.1 Device Description:

KIT COMPONENTS:

1. Individually packed test devices with desiccant
2. Extraction solution
3. Extraction tube
4. Extraction Tube Stand
5. Disposable dropper cap
6. Sterilized nasopharyngeal swabs for sample collection

7. Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED:

1. Medical mask and medical latex gloves
2. Micropipette and disposable pipette tips
3. Watch or timer

7.2 Principle of the method:

COVID-19 Antigen Detection Test is an immunoassay kit for rapid and qualitative determination of SARS-CoV-2 infection from swab specimens. Monoclonal anti-SARS-CoV-2 nucleocapsid antibody is coated on the test line region. Antigens of SARS-CoV-2 in the specimens react with the anti-SARS-CoV-2 monoclonal nucleocapsid antibody coupled with gold conjugate, and form an antigen-antibody complex followed by reaction with anti-SARS-CoV-2 monoclonal nucleocapsid antibodies immobilized in the test line. This complex migrates on the membrane, where it will be captured by the monoclonal anti-SARS-CoV-2 antibody. A colored test line would be visible in the result window if SARS-CoV-2 antigens are present in the specimen. The intensity of colored test line will vary depending upon the amount of SARS-CoV-2 antigen present in the specimen. If SARS- CoV-2 antigens are not present in the specimen, then no line appears in the test line. The control band is used for procedural control and should always appear if the test procedure is performed correctly.

8. Equipment and Materials

The detail of materials used in the limit of detection study is mentioned below:

Details of Kit:

- Name of the kit: MERISCREEN COVID-19 Ag Detection Test kit
- Lot No. MI032105
- Exp: 02/2022

- **Test Sample:**

  Real Negative Clinical sample (eg: Nasopharyngeal swab) spiked with inactivated virus

9. Operator of assay

Following operator details are mentioned below:

Name of the Operator: Nehali Patel

Designation: Senior Executive, R&D

10. Study design

Since this test has either “band present” or “band not present”, LOD evaluation will be done using probit approach. LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all (true positive) replicates test positive.

Real clinical matrix (i.e. Nasopharyngeal swab) samples were collected in extraction solution from healthy individuals and tested by RT PCR assay for sample status confirmation. Further these swab samples that are confirmed negative for SARS Cov-2 by RT PCR assay were pooled together to obtain a common clinical matrix. To this pooled negative nasopharyngeal swab sample, an inactivated SARS-CoV-2 virus of known concentration ("Control 2, MN908947.3, Wuhan - Hu-1" strain) procured from Twist biosciences was spiked to obtain a contrived stock specimen. This spiked stock specimen was further subjected to 2 fold dilution scheme and further tested with MERISCREEN COVID-19 Antigen Detection Test in replicates of 5 per concentration. Sample dilution was done till negative results were obtained. The final concentration was confirmed with 20 replicates.

The number of positive results observed against total number of measurements was made and hit ratio was calculated. Graph was plotted with hit rate (y-axis) Vs log10 of concentration level (copies/µl) for one lot. Only concentration level greater than zero and with hit rate >0.0 and <1.0 was used.

Linear regression model parameters was used to calculate LoD, considering target hit rate of 0.95.

11. Test samples:

As mentioned in section 10.0, Pooled stock specimen is further subjected to 2 fold serial dilution and further tested with MERISCREEN COVID-19 Antigen Detection Test in replicates of 5 per concentration. The final concentration was confirmed with 20 replicates. The dilution details with respect to viral load (copies/µl) is mentioned in the below table no. 4

Sample details such as source of procurement of virus, status of the strain and concentrations (copies/µl) are mentioned in the below table no.2:

Table 2: Sample details of SARS-CoV-2 inactivated virus used in the study

Sr. No.	SARS-CoV-2 Strain/Isolate	Source/Sample Type	Concentration (copies/µl)
1.	Control 2, MN908947.3, wuhan-Hu-1	Twist Bioscience/Synthetic RNA genome	1 x 10^6 copies /µl

SARS-CoV-2 negative specimens (i.e Nasopharyngeal swab) were collected from healthy individuals working with Meril Diagnostics Pvt Ltd, Second floor, D1-D3, Meril Park, Survey No. 135/2/B & 174/2, Muktanand Marg, Chala, Vapi-396191which were confirmed negative for sample status by RT PCR assay.

Table 3: Details of SARS-CoV-2 negative specimens used in this study

Sr. No.	Sample ID	Source/Sample Type	Physiological Condition		Sample Status
			Age	Sex	
1	MRDCON21141	Nasopharyngeal swab	32	M	Negative
2	MRDCON21142	Nasopharyngeal swab	29	F	Negative
3	MRDCON21143	Nasopharyngeal swab	24	F	Negative
4	MRDCON21144	Nasopharyngeal swab	23	F	Negative
5	MRDCON21145	Nasopharyngeal swab	39	F	Negative
6	MRDCON21146	Nasopharyngeal swab	33	M	Negative
7	MRDCON21147	Nasopharyngeal swab	26	M	Negative
8	MRDCON21148	Nasopharyngeal swab	26	M	Negative
9	MRDCON21149	Nasopharyngeal swab	28	M	Negative
10	MRDCON21150	Nasopharyngeal swab	21	F	Negative
11	MRDCON21151	Nasopharyngeal swab	39	F	Negative
12	MRDCON21152	Nasopharyngeal swab	45	M	Negative
13	MRDCON21153	Nasopharyngeal swab	49	M	Negative
14	MRDCON21154	Nasopharyngeal swab	39	M	Negative
15	MRDCON21155	Nasopharyngeal swab	38	M	Negative
16	MRDCON21156	Nasopharyngeal swab	36	F	Negative
17	MRDCON21157	Nasopharyngeal swab	26	M	Negative
18	MRDCON21158	Nasopharyngeal swab	22	F	Negative
19	MRDCON21159	Nasopharyngeal swab	37	M	Negative

20	MRDCON21160	Nasopharyngeal swab	47	M	Negative
21	MRDCON21161	Nasopharyngeal swab	37	F	Negative
22	MRDCON21162	Nasopharyngeal swab	27	F	Negative
23	MRDCON21163	Nasopharyngeal swab	35	M	Negative
24	MRDCON21164	Nasopharyngeal swab	43	F	Negative
25	MRDCON21165	Nasopharyngeal swab	48	M	Negative
26	MRDCON21166	Nasopharyngeal swab	33	M	Negative
27	MRDCON21167	Nasopharyngeal swab	56	M	Negative
28	MRDCON21168	Nasopharyngeal swab	44	F	Negative

The dilution factor and number of serial dilutions of the characterized SARS-CoV-2 that were tested to determine the LoD are mentioned in this section of the report in below table no. 4.

Table 4: Dilution Details:

Sr. No.	**Dilution**	**Viral load (**$TCID_{50}$/ml**)**
1.	Neat	250000
2.	1:2	125000
3.	1:4	62500
4.	1:8	31250
5.	1:16	15625
6.	1:32	7812.5
7.	1:64	3906.25
8.	1:128	1953.125
9.	1:256	976.5625
10.	1:512	488.28125
11.	1:1024	244.140625

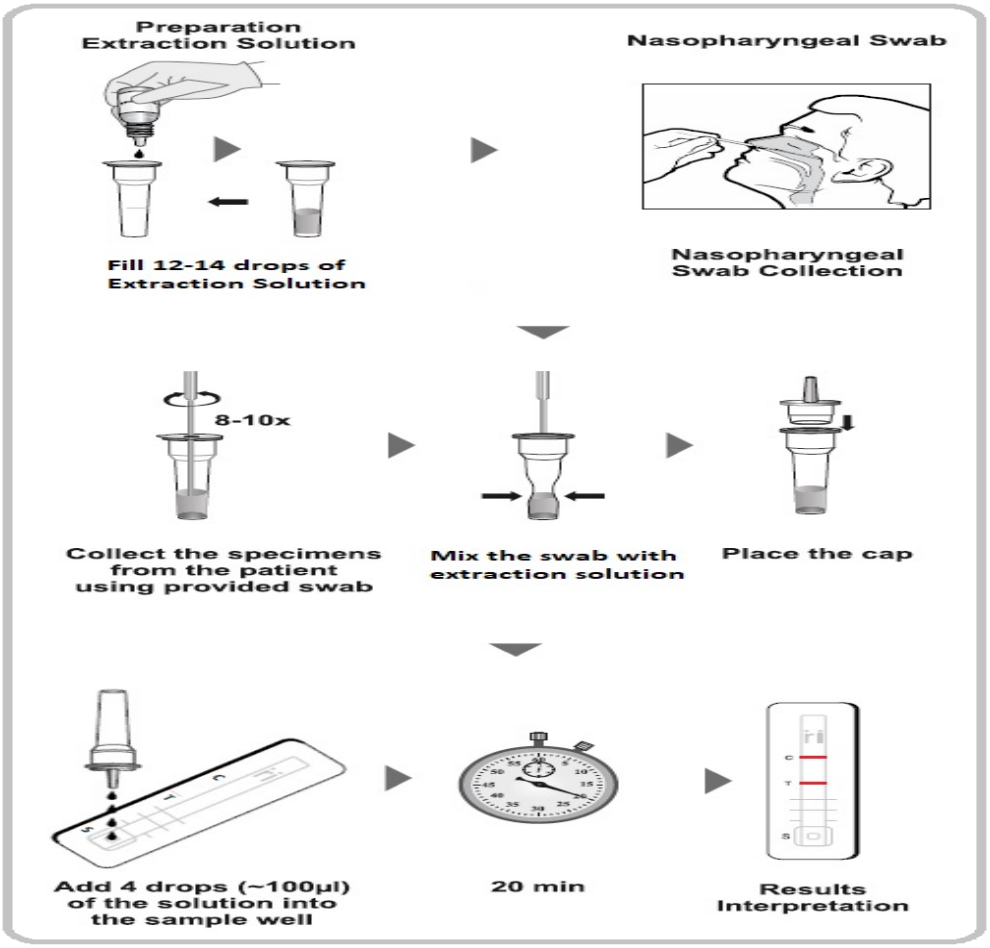
12. Test procedure

Testing was performed with MERISCREEN COVID-19 Antigen Detection Test as per the test procedure mentioned in its pack insert and as per the procedures and recommendations mentioned in the Antigen template for Test Developers.

Test Procedure:

1. Bring the specimen and test components to room temperature if refrigerated or frozen.
2. Place the device on a clean, flat surface.
3. Fill the extraction tube by adding 12-14 drops of Extraction solution.

4. Insert the nasopharyngeal swab sample into the extraction solution, then mix the swab for 8 to 10 times.
5. Remove the swab while pressing against the solution tube in order to extract most of the specimen.
6. Place the dropper cap tightly onto the tube and add 4 drops (100uL) into the sample well.
7. Interpret the test results at the end of 20 minutes. Do not read the results after 30 minutes.



INTERPRETATION OF THE RESULTS:

1. Positive result: If Control (C) and Test (T) bands are developed, the test indicates for the presence of antigens to SARS-CoV-2 in the sample. The result is positive.
2. Negative result: If only the Control (C) band is developed, the test indicates that the result is negative.

3.Invalid result: If no Control(C) band is developed, then the assay is invalid regardless of colour development on Test (T) band. Repeat the assay with a new device.

13. Acceptance criteria

The concentration at which hit rate is 0.95 should be accepted as LOD of the test.

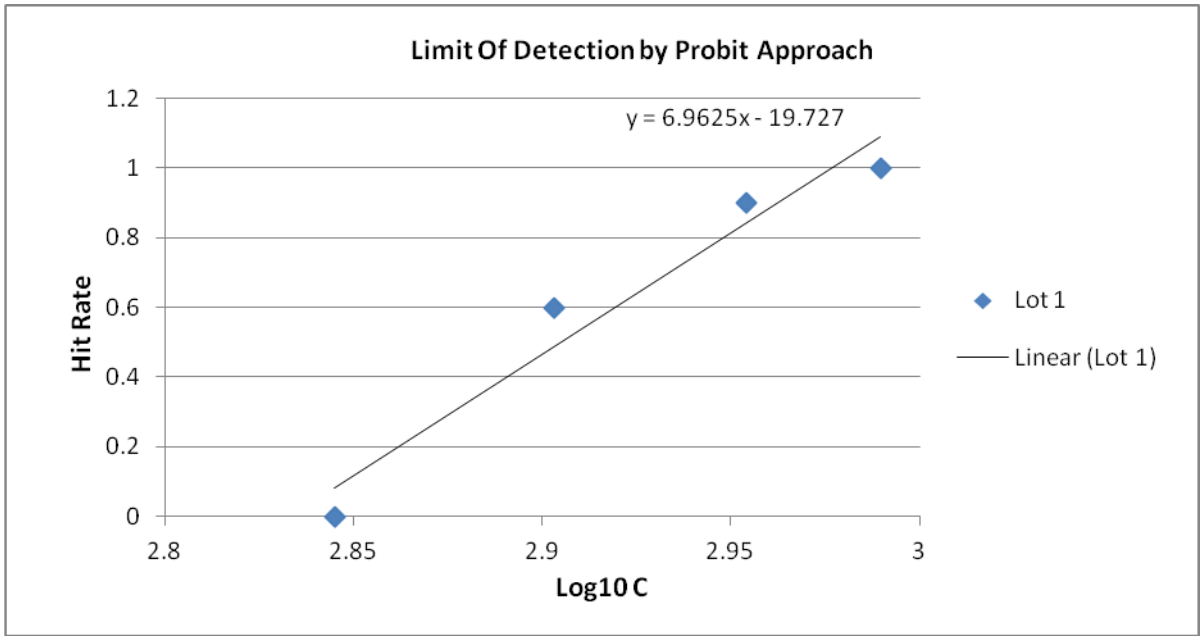
14. Results and data analysis

Test results of dilutions were evaluated to determine the limit of detection of MERISCREEN COVID-19 Antigen Detection Test using Probit Model.

Table: 5 Details of result analysis

No. of replicates tested	Concentration ($TCID_{50}$/ml)	No. of results of tested replicates	No. of replicates tested in 20 near cut off	Hit Rate	Log_{10} ($TCID_{50}$/ml)
		Lot 1	Lot 1	Lot 1	
5	250000	5/5	NA	1	5.39794
5	125000	5/5	NA	1	5.09691
5	62500	5/5	NA	1	4.79588
5	31250	5/5	NA	1	4.49485
5	15625	5/5	NA	1	4.19382
5	7812.5	5/5	NA	1	3.89279
5	3906.25	5/5	NA	1	3.59176
5	1953.125	5/5	NA	1	3.29073
5	976.5625	5/5	20/20	1	2.98970
5	488.28125	0/5	NA	0	2.68867
5	244.140625	0/5	NA	0	2.38764

On the basis of this data, Graph was plotted with hit rate (y-axis) Vs log10 of parasite density level for one lot. Only parasite density greater than zero and with hit rate >0.0 and <1.0 was used.



15. Conclusion

From results and data analysis, limit of detection for MERISCREEN COVID-19 Antigen Detection Test is ≥ 933 $TCID_{50}$/ml.

16. Enclosures / Annexure

- Enclosure 1: Raw data sheet of limit of detection study of MERISCREEN COVID-19 Antigen Detection Test (Document no.: RD-02/IM/GRA/014).
- Enclosure 2: CoA of Inactivated SARS-CoV-2 virus.

17. Amendment history

Table 6: Amendment history

Revision No.	Date	Amendment Description
00	As on approval date	Initial Issue

Meril Diagnostics	Analytical Sensitivity Study Limit Of Detection (LoD) MERISCREEN COVID-19 Antigen Detection Test	RD-02/IM/GRA/014

Results and Data Analysis

Product Name:	MERISCREEN COVID-19 Ag Detection Test		
Date of testing:	10-03-2021	**Completion:**	10-03-2021
Lot No:	MI032105		
Mfg. Date:	2021/03		
Exp. Date:	2022/02		
1 **Tested at:**	Meril Diagnostics Pvt. Ltd., Chala, Vapi, Gujarat, India.		

2	**Sample ID:**	Pooled Nasopharyngeal swab samples confirmed negative for SARS-CoV-2 by RT PCR method
		Inactivated synthetic SARS-CoV-2 procured from twist bioscience (positive control) Control 2, MN908947.3 Wuhan-Hu-1

3	**Acceptance Criteria:**
	COVID-19 Ag Rapid Test should give accurate result as per true sample status.The color development on control (C) and test (T) bands indicates for the presence of antigens to SARS-CoV-2 in the sample, whereas the development of color only on Control(C) band indicates the test result is negative. If no control band is developed, then the assay is invalid regardless of colour development on test (T) band.

4	**Introduction:**
	Analytical Sensitivity is defined as the assay's ability to detect the COVID-19 Antigen concentration, which is the minimum detectable concentration (LOD) of the COVID-19 Antigen. LOD is defined as the lowest amount of antigen in a sample that can be detected with probability, though it may not be quantified as an exact value. LOD is also an important performance criterion for qualitative tests even if the results is Reported as "positive" or "negative" ("present" or "absent"). LOD determination also allows monitoring the consistency of performance of method and is an important parameter for controlling the quality of many qualitative tests.

Table 1: Analytical Sensitivity

To determine the Limit of Detection(LoD) a study used the inactivated "Control 2, MN908947.3, Wuhan - Hu-1" strain. The titre of the cultured virus was confirmed by PCR. This strain was further spiked into the negative pooled human nasopharyngeal swab samples collected from different healthy individuals which were confirmed by RT-PCR for the sample status. The test was performed with different dilutions and each dilutions were tested in 5 replicates.The final concentration (LoD) was confirmed with 20 replicates as recommended.

Procured concentration of inactivated "Control 2, MN908947.3, Wuhan - Hu-1" strain = 1 x 10^6 copies/µl

Coverting it into copies/ml = 1x10^9 copies/ml

Further 1$TCID_{50}$/ml roughly equates to 4 x 10^3 copies/ml

Therfore converting 1x10^9 copies/ml into $TCID_{50}$/ml= 1x10^9 copies/ml/4x10^3 copies/ml= 250000 $TCID_{50}$/ml

2019-SARS CoV-2 strain tested	**Control 2, MN908947.3 Wuhan-Hu-1**					
Stock 2019-nCoV Titre	**250000 $TCID_{50}$/ml**					
Dilution	**Concentration in dilution ($TCID_{50}$/ml)**	**No. of replicates tested in 5**		**No. of replicates tested in 20 near cut off**		**Status**
		Replicates	**Results(%)**	**Replicates**	**Results(%)**	
1:02	1.25 x 10^5	5/5	100	NA	NA	Positive
1:04	6.25 x 10^4	5/5	100	NA	NA	Positive
1:08	3.125 x 10^4	5/5	100	NA	NA	Positive
1:16	1.5625 x 10^4	5/5	100	NA	NA	Positive
1:32	7.8125 x 10^3	5/5	100	NA	NA	Positive
1:64	3.90625 x 10^3	5/5	100	NA	NA	Positive
1:128	1.953125 x 10^3	5/5	100	NA	NA	Positive
1:256	9.765675 x 10^2	5/5	100	20/20	100	Positive
1:512	4.8828125x 10^2	0/5	0	0/20	0	Negative
1:1024	2.44141875 x 10	0/5	0	NA	NA	Negative

Lowest Concentration with uniform positive reactivity: 9.765675 x 10^2 $TCID_{50}$/ml

Limit of detection (LoD): 932.62304 ≈ 933 $TCID_{50}$/ml

Note : C= Control band ; T =Test Band.

Remarks :

Test interpretation time for MERISCREEN COVID-19 Ag Rapid test was 20 minutes. Background clearance time was found to be within 12-16 mins.
Band intensity criteria: 0, 1+, 2+, 3+ & 4+ = Color band intensity by using WHO color chart Prototype: A
0: Negative, 4+: Strong Positive, 3+: Positive, 2+: Weak Positive, 1+: Faint line and +: Very faint line

Conclusion :

From the above results and data analysis, Analytical sensitivity/Limit of Detection (LoD) confirmed for direct swab is **933 $TCID_{50}$/ml.**
Other testing parameters like background clearance time, Flow rate, testing time, uniform flow, release time was in acceptance range.

	Prepared By	**Reviewed By**	**Approved By**
Signature	N.S. Patel		
Date	**10-03-2021**	**10-03-2021**	**10-03-2021**
Name	**Nehali Patel**	**Kardam Dave**	**Pradeep Kumar**
Designation	**Senior Executive, R&D**	**Senior Manager, R&D**	**DGM - R&D**

Meril Diagnostics	**Analytical Sensitivity Study: Limit Of Detection (LoD) MERISCREEN COVID-19 Antigen Detection Test**	**RD-02/IM/GRA/014**

Product Name:	MERISCREEN COVID-19 Ag Detection Test		
Date of testing:	10-03-2021	Completion**:**	10-03-2021
Lot.No	MI032105		
Mfg. Date	2021/03		
Exp. Date	2022/02		

Analytical Sensitivity Study Raw Datasheet:

Sr.No.	Dilution	Number of Replicates	Start Time	End Time	Result		Background Clearance Time
					C	T	
1	1.25×10^5	1	10:21:15	10:41:18	4+	4+	12 min
		2	10:21:45	10:41:22	4+	4+	15 min
		3	10:22:05	10:42:29	4+	4+	15 min
		4	10:22:28	10:42:33	4+	4+	16 min
		5	10:22:51	10:42:40	4+	4+	16 min
2	6.25×10^4	1	10:23:16	10:43:22	4+	4+	16 min
		2	10:23:37	10:43:27	4+	4+	15 min
		3	10:24:05	10:44:35	4+	4+	15 min
		4	10:24:27	10:44:38	4+	4+	16 min
		5	10:24:39	10:44:41	4+	4+	16 min
3	3.125×10^4	1	10:25:11	10:45:09	4+	3+	16 min
		2	10:25:32	10:45:15	4+	3+	16 min
		3	10:25:53	10:45:20	4+	3+	16 min
		4	10:26:19	10:46:24	4+	3+	15 min
		5	10:26:40	10:46:43	4+	3+	16 min
4	1.5625×10^4	1	10:27:03	10:47:17	4+	3+	15 min
		2	10:27:37	10:47:31	4+	3+	16 min
		3	10:28:01	10:48:49	4+	3+	14 min
		4	10:28:17	10:48:54	4+	3+	15 min
		5	10:28:47	10:48:59	4+	3+	15 min
5	7.8125×10^3	1	10:29:12	10:49:31	4+	3+	14 min
		2	10:29:36	10:49:33	4+	3+	14 min
		3	10:29:53	10:49:41	4+	3+	16 min
		4	10:30:09	10:50:49	4+	2+	15 min
		5	10:30:29	10:50:54	4+	3+	16 min
6	3.90625×10^3	1	10:30:49	10:50:39	4+	2+	16min
		2	10:31:07	10:51:42	4+	2+	16min
		3	10:31:35	10:51:46	4+	2+	16min
		4	10:31:52	10:51:48	4+	2+	14min
		5	10:32:05	10:52:12	4+	2+	15min
7	1.953125×10^3	1	10:32:17	10:52:19	4+	1+	15min
		2	10:32:36	10:52:21	4+	1+	16min
		3	10:32:49	10:52:24	4+	1+	15min
		4	10:33:15	10:53:29	4+	1+	15min
		5	10:33:25	10:53:31	4+	1+	16min
8	9.765675×10^2	1	10:33:39	10:53:35	4+	1+	16 min
		2	10:33:56	10:53:39	4+	1+	16 min
		3	10:34:17	10:54:43	4+	1+	14 min
		4	10:34:42	10:54:47	4+	1+	16 min
		5	10:34:57	10:54:49	4+	1+	15 min
9	4.8828125×10^2	1	10:35:14	10:55:03	4+	0	16 min
		2	10:35:31	10:55:17	4+	0	16min
		3	10:35:53	10:55:27	4+	0	14min
		4	10:36:03	10:56:12	4+	0	14min
		5	10:36:21	10:56:26	4+	0	15min
10	2.44141875 x 10	1	10:36:37	10:56: 41	4+	0	16 min
		2	10:36:44	10:56:49	4+	0	16min
		3	10:36:56	10:56:56	4+	0	14min
		4	10:37:03	10:57:12	4+	0	14min
		5	10:37:16	10:57:19	4+	0	15min

Acceptance criteria :

Meriscreen COVID-19 Ag Rapid Test should give accurate result as per true sample status.The color development on control (C) and test (T) bands indicates for the presence of antigens to SARS-CoV-2 in the sample, whereas the development of color only on Control(C) band indicates the test result is negative. If no control band is developed, then the assay is invalid regardless of colour development on test (T) band.

Observation : Results were found satisfactory as per true sample status.

Result & Data Analysis

Remarks :

The test interpretation time for Meriscreen COVID-19 Ag Rapid Test was 20 minutes. The background clearance time was found within 12-16 minutes.

Band intensity criteria:

0, 1+, 2+, 3+ & 4+ = Color band intensity by using WHO color chart Prototype: A

0: Negative, 4+: Strong Positive, 3+: Positive+A25, 2+: Weak Positive, 1+: Faint line and +: Very faint line

Conclusion :

From the above results and data analysis, Analytical sensitivity/Limit of Detection (LoD) confirmed for direct swab is 933 $TCID_{50}$/ml.

	Prepared By	**Reviewed By**	**Approved By**
Signature	M.S. Patel		
Date	**10-03-2021**	**10-03-2021**	**10-03-2021**
Name	**Nehali Patel**	**Kardam Dave**	**Pradeep Kumar**
Designation	**Senior Executive, R&D**	**Senior Manager, R&D**	**DGM,R&D**

Meril Diagnostics	AnalyticalSensitivityStudy Limit Of Detection (LoD) MERISCREEN COVID-19 Antigen Detection Test	RD-02/IM/GRA/014

Product Name:	MERISCREEN COVID-19 Ag Detection Test	
Date of testing:	10-03-2021	Completion: 10-03-2021
Lot.No	MI032105	
Mfg. Date	2021/03	
Exp. Date	2022/02	

Analytical Sensitivity Study Raw Datasheet:

Sr.No.	Dilution	Number of Replicates	Start Time	End Time	Result		Background Clearance Time
					C	T	
1	9.765675×10^{2}	1	12:14:06	12:34:10	4+	1+	14min
		2	12:14:14	12:34:17	4+	1+	14min
		3	12:14:19	12:34:21	4+	1+	16min
		4	12:14:23	12:34:26	4+	1+	15min
		5	12:14:27	12:34:31	4+	1+	14min
		6	12:14:33	12:34:36	4+	1+	14min
		7	12:14:38	12:34:41	4+	1+	14min
		8	12:14:43	12:34:46	4+	1+	16min
		9	12:14:48	12:34:51	4+	1+	15min
		10	12:14:56	12:34:59	4+	1+	16min
		11	12:15:01	12:35:05	4+	1+	16min
		12	12:15:09	12:35:13	4+	1+	14min
		13	12:15:14	12:35:19	4+	1+	14min
		14	12:15:18	12:35:21	4+	1+	16min
		15	12:15:21	12:35:26	4+	1+	15min
		16	12:15:25	12:35:29	4+	1+	16min
		17	12:15:29	12:35:31	4+	1+	16min
		18	12:15:34	12:35:36	4+	1+	15min
		19	12:15:40	12:35:44	4+	1+	15min
		20	12:15:45	12:35:49	4+	1+	16min
2	9×10^{2}	1	12:16:06	12:36:10	4+	1+	14min
		2	12:16:14	12:36:17	4+	1+	14min
		3	12:16:19	12:36:21	4+	1+	16min
		4	12:16:23	12:36:26	4+	1+	15min
		5	12:16:27	12:36:31	4+	1+	14min
		6	12:16:33	12:36:36	4+	1+	14min
		7	12:16:38	12:36:41	4+	1+	14min
		8	12:16:43	12:36:46	4+	1+	16min
		9	12:16:48	12:36:51	4+	1+	15min
		10	12:16:56	12:36:59	4+	1+	16min
		11	12:17:01	12:37:05	4+	1+	16min
		12	12:17:09	12:37:13	4+	0	14min
		13	12:17:14	12:37:19	4+	1+	14min
		14	12:17:18	12:37:21	4+	1+	16min
		15	12:17:21	12:37:26	4+	1+	15min
		16	12:17:25	12:37:29	4+	1+	16min
		17	12:17:29	12:37:31	4+	0	16min
		18	12:17:34	12:37:36	4+	1+	15min
		19	12:17:40	12:37:44	4+	1+	15min
		20	12:17:45	12:37:49	4+	1+	16min
3	8×10^{2}	1	12:18:06	12:38:10	4+	0	14min
		2	12:18:14	12:38:17	4+	1+	14min
		3	12:18:19	12:38:21	4+	1+	16min
		4	12:18:23	12:38:26	4+	0	15min
		5	12:18:27	12:38:31	4+	1+	14min
		6	12:18:33	12:38:36	4+	1+	14min
		7	12:18:38	12:38:41	4+	1+	14min
		8	12:18:43	12:38:46	4+	1+	16min
		9	12:18:48	12:38:51	4+	1+	15min
		10	12:18:56	12:38:59	4+	0	16min
		11	12:19:01	12:39:05	4+	0	16min
		12	12:19:09	12:39:13	4+	0	14min
		13	12:19:14	12:39:19	4+	0	14min
		14	12:19:18	12:39:21	4+	0	16min
		15	12:19:21	12:39:26	4+	1+	15min
		16	12:19:25	12:39:29	4+	1+	16min
		17	12:19:29	12:39:31	4+	1+	16min
		18	12:19:34	12:39:36	4+	1+	15min
		19	12:19:40	12:39:44	4+	0	15min
		20	12:19:45	12:39:49	4+	1+	16min
4	7×10^{2}	1	12:20:06	12:40:10	4+	0	14min
		2	12:20:14	12:40:17	4+	0	14min
		3	12:20:19	12:40:21	4+	0	16min
		4	12:20:23	12:40:26	4+	0	15min
		5	12:20:27	12:40:31	4+	0	14min
		6	12:20:33	12:40:36	4+	0	14min
		7	12:20:38	12:40:41	4+	0	14min
		8	12:20:43	12:40:46	4+	0	16min
		9	12:20:48	12:40:51	4+	0	15min
		10	12:20:56	12:40:59	4+	0	16min
		11	12:21:01	12:41:05	4+	0	16min
		12	12:21:09	12:41:13	4+	0	14min
		13	12:21:14	12:41:19	4+	0	14min
		14	12:21:18	12:41:21	4+	0	16min
		15	12:21:21	12:41:26	4+	0	15min
		16	12:21:25	12:41:29	4+	0	16min
		17	12:21:29	12:41:31	4+	0	16min
		18	12:21:34	12:41:36	4+	0	15min
		19	12:21:40	12:41:44	4+	0	15min
		20	12:21:45	12:41:49	4+	0	16min

Acceptance criteria :

Meriscreen COVID-19 Ag Rapid Test should give accurate result as per true sample status.The color development on control (C) and test (T) bands indicates for the presence of antigens to SARS-CoV-2 in the sample, whereas the development of color only on Control(C) band indicates the test result is negative. If no control band is developed, then the assay is invalid regardless of colour development on test (T) band.

Observation : Results were found satisfactory as per true sample status.

Result & Data Analysis

Remarks :
The test interpretation time for Meriscreen COVID-19 Ag Rapid Test was 20 minutes. The background clearance time was found within 12-16 minutes.

Band intensity criteria:
0, 1+, 2+, 3+ & 4+ = Color band intensity by using WHO color chart Prototype: A
0: Negative, 4+: Strong Positive, 3+: Positive, 2+: Weak Positive, 1+: Faint line and +: Very faint line

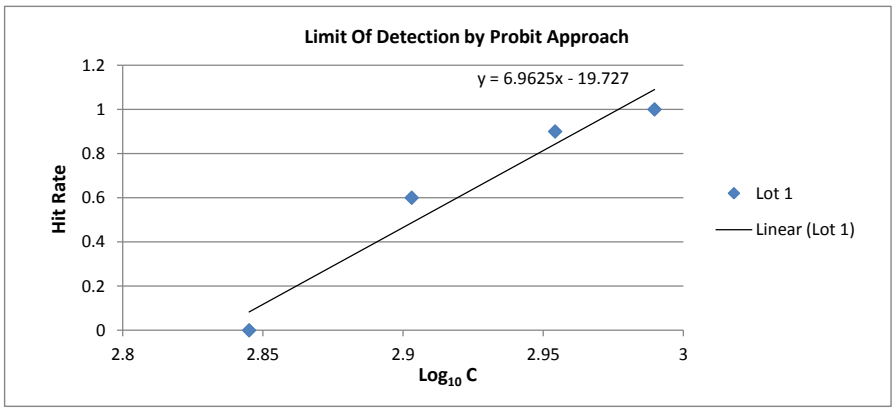
Conclusion :

From the above results and data analysis, Analytical sensitivity/Limit of Detection (LoD) confirmed for direct swab is 933 $TCID_{50}$/ml.

	Prepared By	Reviewed By	Approved By
Signature			
Date	**10-03-2021**	**10-03-2021**	**10-03-2021**
Name	**Nehali Patel**	**Kardam Dave**	**Pradeep Kumar**
Designation	**Senior Executive, R&D**	**Senior Manager, R&D**	**DGM, R&D**

Dilution	Concentration in dilution ($TCID_{50}$/ml)	No. of replicates tested in 5		No. of replicates tested in 20 near cut off		Status
		Replicates	Results(%)	Replicates	Results(%)	
1:02	1.25×10^5	5/5	100	NA	NA	Positive
1:04	6.25×10^4	5/5	100	NA	NA	Positive
1:08	3.125×10^4	5/5	100	NA	NA	Positive
1:16	1.5625×10^4	5/5	100	NA	NA	Positive
1:32	7.8125×10^3	5/5	100	NA	NA	Positive
1:64	3.90625×10^3	5/5	100	NA	NA	Positive
1:128	1.953125×10^3	5/5	100	NA	NA	Positive
1:256	9.765675×10^2	5/5	100	20//20	100	Positive
1:512	4.8828125×10^2	0/5	0	0/20	0	Negative
1:1024	2.44141875 x 10	0/5	0	NA	NA	Negative

Number of replicates	Dilution	Concentration	Observed	Log Conc.	Hit rate
			Lot 1		Lot 1
20	1:256	976.5675	20	2.9897	1
20	'1:278	900	18	2.95424	0.9
20	1:312.5 (2:625)	800	12	2.90308	0.6
20	'1:357	700	0	2.84509	0



	Lot 1
Co-relation	0.974119308
Slope (m)- C1	6.96249928
Y intercept (C) - C0	-19.72657686
LOD ($Log_{10}C$)	2.96970614
LOD ($TCID_{50}$/ml)	932.6230402

681 GATEWAY BLVD
SOUTH SAN FRANCISCO, CA 94080
TWISTBIOSCIENCE.COM

CERTIFICATE OF ANALYSIS

Product : Twist Synthetic SARS-CoV-2 RNA Control 2 (MN908947.3)
Part Number : 102024
Lot Number : 2000003176

Manufacture Date : 2020-10-29
Expiration Date : 2021-10-06

Storage Conditions : -90 °C to -70 °C
Physical State : Suspension clear solution at room temperature

SPECIFICATION	RESULT
Sequence Analysis : >99% of expected cDNA bases are present with an error rate <10% per base position	**Pass**
Physical Analysis : Capillary Electrophoresis >70% full length material	**Pass**
Quantitative Analysis : dPCR 1×10^6 copies/µL -50% to +200%	**Pass**

This document certifies that the above-mentioned product was tested by Twist Bioscience and is in conformity with all internal specifications.

RELEASE APPROVAL

Quality Andre Baxter Date 11 / 02 / 2020