आई सी एम आर – राष्ट्रीय विषाणु विज्ञान संस्थान
भारतीय आयुर्विज्ञान अनुसंधान परिषद
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय, भारत सरकार
I C M R - NATIONAL INSTITUTE OF VIROLOGY
Indian Council of Medical Research
Department of Health Research
Ministry of Health & Family Welfare, Govt. of India
20 - ए. डा. आंबेडकर मार्ग, पोस्ट बॉक्स संख्या 11, पुणे - 411 001, भारत. 20-A. Dr. Ambedkar Road, Post Box No. 11, Pune 411 001, India.
Tel. : NIV Camp +91-020-26127301, 26006290, Fax : 26122669, 26126643 / NIV Pashan +91-020-26006390 Fax : No. 25871895 / 25870640
E-mail : director.niv@icmr.gov.in Website : www.niv.co.in

No: Inf/COVID-19/Evaluation/2020- 1606

1 Oct 2021

To,
Molbio Diagnostics Private Limited
Plot No. L-46, Phase II D,
Verna Industrial Estate, Verna,
Goa - 403 722, India

Sub: Performance evaluation report of Truenat Influenza H1N1 kit Version-03

Sir,
We have evaluated Truenat Influenza H1N1 kit Version-03 (REF 601070020) RT PCR kit manufactured by Molbio Diagnostics Private Limited. Detailed report is attached for your information.

Thank you.

Prof. Priya Abraham
Director

ICMR- National Institute of Virology, Pune

PERFORMANCE EVALUATION REPORT FOR RT-PCR DIAGNOSTIC KIT

• Name of the Kit	Truenat Influenza H1N1 kit Version-03
• Name of the manufacture	Molbio Diagnostics Pvt. Ltd
• Catalog and batch no	REF 601070020, batch no HN035, HN036
• Date of Expiry	HN035: 2022-09, HN036: 2022-10
• Application	Truenat H1N1 is a chip-based real time RT-PCR assay intended for the semi-quantitative detection of H1N1 virus in human throat and nasal swab specimen and aids in the diagnosis of infection with H1N1. RT-PCR test is based on Taqman chemistry.
• Kit components	Truenat H1N1 is a disposable, chip based RT-PCR test with dried MgCl2 in reaction well and freeze dried RT-PCR reagents in micro-tube. Individually sealed pouches containing a Truenat H1N1 micro PCR chip, Microtube with freeze dried RT-PCR reagents and DNase & RNase free pipette tip.
• Method	RNA was extracted using MagMax RNA extraction kit as per kit protocol. The samples were tested simultaneously with gold standard (WHO/CDC protocol for Influenza A/H1N1pdm09) and Truenat Influenza H1N1 kit. The gold standard assay was performed on Applied Biosystems 7500 Fast Real Time PCR System. Truenat Influenza H1N1 assay was performed as per kit protocol on Truelab Quattro real time quantitative micro PCR analyzer. Two different lots of Truenat Influenza H1N1 kit (REF 601070020, batch no HN035, HN036) were evaluated for sensitivity and specificity against the gold standard. Respiratory clinical samples well stored at -80 °C were used for evaluation.
• Sample Panel o Positive samples o Negative samples	64 Influenza A/H1N1, 86* Influenza negatives and 8 Influenza A/H3N2 and 4 Influenza B positives. *A total 11 samples showed invalid results hence removed from the final analysis.
• Results interpretation	Result interpretation of Truenat Influenza H1N1 kit was as per kit protocol. At the end of the test run, result is displayed on machine screen as H1N1 'DETECTED' for positive result or 'NOT DETECTED' for negative result. For test validity, internal positive control (IPC) is also checked and depending on IPC detection validity of test run is counted. The validity of the test run is also displayed as 'VALID' or 'INVALID'. IPC validates the test run from samples to result. The result screen also display the viral load as 'HIGH', 'MEDIUM' or 'LOW' with cT values.

Sensitivity and specificity of the Truenat Influenza H1N1 kit

		Gold standard (WHO/CDC Protocol)		
		Positive	Negative	Total
Truenat Influenza H1N1 kit	Positive	64	1	65
	Negative	0	86	86
	Total	64	87	151

	Estimate (%)	CI 95%
Sensitivity	100	94.40 to 100
Specificity	98.85%	93.76 to 99.97

Lot to lot variation: table showed ct values of samples tested with two different lots (HN035, HN036)

Sample	Lot No	H1N1	IPC	Lot No	H1N1	IPC
Sample 1	HN035	18	26	HN036	18	26
Sample 2	HN035	25	27	HN036	24	27
Sample 3	HN035	22	27	HN036	20	28
Sample 4	HN035	24	26	HN036	21	26
Sample 5	HN035	25	33	HN036	23	32
Sample 6	HN035	25	27	HN036	25	27
Sample 7	HN035	26	29	HN036	24	28
Sample 8	HN035	25	24	HN036	23	25
Sample 9	HN035	26	24	HN036	24	24
Sample 10	HN035	26	26	HN036	26	26

Standard deviation (SD) and coefficient of variation (CV) of two lots (HN035, HN036)

Samples	H1N1			Control (IPC)		
	Mean ct value	SD	CV (%)	Mean ct value	SD	CV (%)
Sample 1	18	0	0.0%	26	0	0.0%
Sample 2	24.5	0.71	2.9%	27	0	0.0%
Sample 3	21	1.41	6.7%	27.5	0.71	2.6%
Sample 4	22.5	2.12	9.4%	26	0	0.0%
Sample 5	24	1.41	5.9%	32.5	0.71	2.2%
Sample 6	25	0	0.0%	27	0	0.0%
Sample 7	25	1.41	5.7%	28.5	0.71	2.5%
Sample 8	24	1.41	5.9%	24.5	0.71	2.9%
Sample 9	25	1.41	5.7%	24	0	0.0%
Sample 10	26	0	0.0%	26	0	0.0%

Standard deviation (SD) and coefficient of variation (CV) values were in acceptable range.

• Conclusions: **SATISFACTORY**

ICMR guideline was followed for kit performance evaluation.

Disclaimers

1. ICMR's validation process does not approve/disapprove the kit design

2. ICMR's validation process does not certify user friendliness of the kit/assay

3. Validation of a kit by ICMR is not an assurance that the kit specifications would be included in the tendering process.

This evaluation report is exclusively for **Truenat Influenza H1N1** kit REF 601070020 version 3 manufactured by Molbio Diagnostics Pvt Ltd.

(Sensitivity and specificity have been assessed in controlled lab setting using the kits of the batch no. (HN035, HN036) provided by the manufacturer)

Dr. ML Choudhary
Scientist D, Influenza Group

Dr. VA Potdar
Scientist D, Influenza Group Leader

Dr. Priya Abraham
Director