आई सी एम आर – राष्ट्रीय यक्ष्मा अनुसंधान संस्थान
स्वास्थ्य अनुसंधान विभाग, स्वास्थ्य और परिवार
कल्याण मंत्रालय, भारत सरकार

ICMR - National Institute for Research in Tuberculosis
Department of Health Research, Ministry of Health and Family Welfare, Government of India

30th Sept. 2022

Ref.No.NIRT/BACT/Truenat INH/Report/2022

To,
Dr Chandrasekhar Nair
Director
Molbio Diagnostics Pvt Ltd.
Goa

Dear Dr Nair,

Enclosing herewith the evaluation report on comparison of three lots of Truenat™ MTB-INH assay manufactured by M/S Molbio Diagnostics Pvt. Ltd., at their manufacturing site Plot no. L-46, Phase II D, Verna Industrial estate, Verna, Goa – 403722.

1. Truenat™ MTB-INH – Lot no.s EIN7, EIN8, EIN9

Name of the Tests	Truenat™ MTB-INH
Manufacturer	Molbio Diagnostics Pvt Ltd, Plot no. L-46, Phase II D, Verna Industrial estate, Verna, Goa – 403722
Configuration of test Panel	A panel of 50 M.tb isolates comprising of 25 known Isoniazid resistance and 25 known Isoniazid sensitive were run in parallel, using WHO approved GenoType MTBDRplus ***line probe assay*** as comparator.
Principle of the Test	Isolation of nucleic acid based on an automated cartridge based protocol and nucleic acid amplification by real time polymerase chain reaction [PCR].
Protocol	Truenat™ tests were run on Truelab Real Time Micro PCR systems. This system comprises of the Truelab™ DuoDx, a fully automated and battery operated Real Time PCR analyser, which runs the specific Truenat™ chip based Real Time PCR test and Trueprep™ Auto, an automated, battery operated sample preparation device. Performance comparison was done using WHO approved GenoType MTBDRplus ***line probe assay*** as comparator.

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Result Summary	**Inter lot analysis of Truenat™ MTB RIF Dx assay** Truenat MTB INH is a reflux test to determine Isoniazid resistance in samples detected as positive by Truenat MTB. The 25 resistant M.tb isolates described above were run on 3 lots of Truenat MTB INH chips (Lot numbers EIN7, EIN8, EIN9 and results compared with GenoType MTBDRplus ***line probe assay*** results. All 25 samples with Isoniazid resistance were detected by GenoType MTBDRplus ***line probe assay*** also yielded similar results by Truenat MTB INH. In 24/25 samples where INH resistance was not detected by GenoType MTBDRplus ***line probe assay*** similar results were obtained by Truenat MTB INH. One M.tb isolates showed false positive and on repeat by both the test the discordant persisted. The sensitivity and specificity data has been attached as ANNEXURE - I. No variation was observed across 3 lots of chips tested. Raw data provided in Table 1.
Conclusion	All three lots of the following tests, 1. Truenat™ MTB-INH – Lot no.s EIN7, EIN8, EIN9 submitted for evaluation by the manufacturer showed 100% sensitivity and 96.15%. No significant lot to lot variation was observed as seen from the raw data.

ANNEXURE - I

Experimental data:

A panel of 50 sputum samples were analysed. Samples were run in parallel on GenoType MTBDRplus ***line probe assay*** as comparator and Truelab workstations. Three lots of test chips (for Truenat™ MTB INH) were used for evaluation. INH Resisitant samples were run to determine Isoniazid resistance and results were compared to that of GenoType MTBDRplus ***line probe assay***

2 by 2 table for sensitivity and specificity:

a) True Positive: 24	**b) False Positive: 1**
c) False Negative: 0	**d) True Negative: 25**

1. Sensitivity=[a/(a+c)]×100: **100**

2. Specificity=[d/(b+d)]×100: **96.15384615**

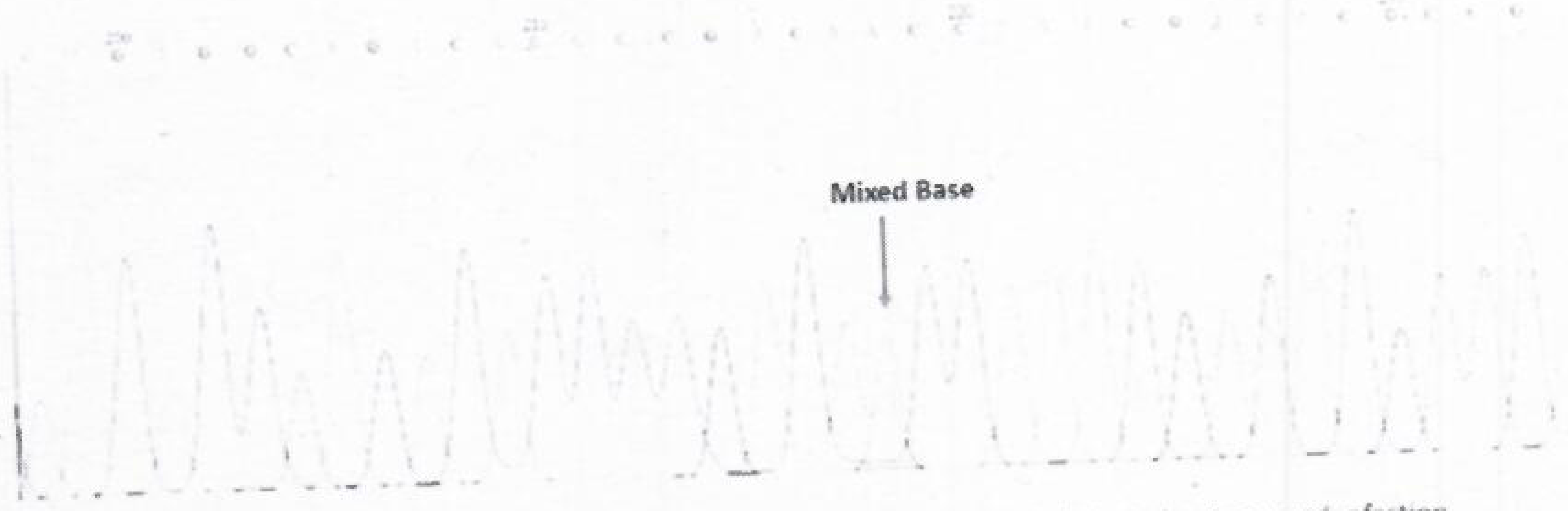
3. Positive predictive value(PPV)=[a/(a+b)]×100: **96**

4. Negative predictive value(NPV)=[d/(c+d)]×100: **100**

Discrepancy resolution:

One false positive sample was subjected to genome sequencing for discrepancy resolution and we found that there was a hetero infection, which was detected by Trunenat assay and not picked up by the comparator tests.

Electrophoretogram – NIRT Discordant Sample



DNA sequencing result showing mixed base in the probe binding region of inhA – indicating mixed infection

Dr. S Siva Kumar
Scientist D and Head
Bacteriology Department
ICMR-NIRT, Chennai

Dr. Padmapriyadarsini C
Director
ICMR-NIRT, Chennai

Table 1:

S.NO	LPA RESULT		Truenat MTB		Truenat INH detection											
					EIN07				EIN08				EIN09			
	RIF	INH	MTB	LOAD		KatG	InhA	CONTROL		KatG	InhA	CONTROL		KatG	InhA	CONTROL
1	S	R (KAT G)	DETECTED	3.2×10^{07}	DETECTED	70.3	70.48	71.58	DETECTED	69.86	70.58	71.28	DETECTED	69.97	70.94	72.58
2	S	R (INH A)	DETECTED	5.7×10^{06}	DETECTED	70.71	70.5	71.26	DETECTED	70.58	70.76	73.04	DETECTED	69.86	71.06	71.14
3	R	R (INH A)	DETECTED	4.1×10^{07}	DETECTED	70.57	70.08	72.33	DETECTED	70.69	70.58	71.65	DETECTED	69.53	70.09	73.25
4	S	S	DETECTED	4.2×10^{06}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
5	S	R (INH A)	DETECTED	2.0×10^{06}	DETECTED	74.37	64.89	71.89	DETECTED	74.18	65.94	71.61	DETECTED	73.26	66.08	72.12
6	S	R (KAT G)	DETECTED	1.0×10^{07}	DETECTED	70.78	70.5	71.86	DETECTED	70.3	70.5	71.93	DETECTED	69.46	70.45	72.33
7	R	R (KAT G)	DETECTED	1.7×10^{07}	DETECTED	70.6	70.55	72	DETECTED	69.62	70.4	72.37	DETECTED	70.11	71.06	71.77
8	S	S	DETECTED	1.9×10^{07}	DETECTED	65.29	65.39	72.67	DETECTED	66.03	65.48	73.46	DETECTED	65.4	65.4	73.14
9	S	S	DETECTED	2.9×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
10	S	S	DETECTED	7.8×10^{04}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
11	S	S	DETECTED	1.9×10^{06}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
12	S	S	DETECTED	4.6×10^{06}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
13	R	R (INH A)	DETECTED	1.1×10^{07}	DETECTED	70.55	70.46	71.49	DETECTED	70.85	71.06	71.72	DETECTED	70.2	70.51	72.6
14	S	S	DETECTED	1.1×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
15	S	S	DETECTED	4.7×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
16	R	R (KAT G)	DETECTED	2.5×10^{07}	DETECTED	70.48	70.67	72.53	DETECTED	69.74	71.01	72.56	DETECTED	70.6	70.69	74.8
17	S	S	DETECTED	1.3×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
18	S	S	DETECTED	2.5×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
19	S	S	DETECTED	2.5×10^{07}	NOT DETECTED				INDETERMINATE				NOT DETECTED			
20	S	R (KAT G)	DETECTED	4.3×10^{07}	DETECTED	70.51	70.6	72.46	DETECTED	70.13	70.2	72.3	DETECTED	69.74	70.46	72.67
21	R	R(KATG)	DETECTED	1.3×10^{07}	DETECTED	70.67	70.57	72.23	DETECTED	70.13	70.66	72.14	DETECTED	70.31	70.57	72.05
22	S	S	DETECTED	8.9×10^{06}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
23	S	S	DETECTED	5.4×10^{06}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
24	S	S	DETECTED	3.2×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
25	R	R (KAT G)	DETECTED	5.9×10^{07}	DETECTED	70.66	70.5	71.26	DETECTED	69.55	70.59	72.21	DETECTED	70.64	70.37	71.22
26	S	S	DETECTED	7.6×10^{05}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
27	S	S	DETECTED	1.4×10^{06}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
28	R	R (KAT G)	DETECTED	1.3×10^{07}	DETECTED	70.57	70.48	71.82	DETECTED	70.27	70.57	72.63	DETECTED	70.62	70.34	72.12
29	S	S	DETECTED	1.5×10^{05}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
30	S	S	DETECTED	2.5×10^{06}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
31	S	S	DETECTED	5.1×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
32	S	R(KAT G)	DETECTED	2.5×10^{07}	DETECTED	70.66	70.75	71.89	DETECTED	70.27	70.46	72.07	DETECTED	70.57	70.57	71.93
33	S	R (KAT G)	DETECTED	7.3×10^{07}	DETECTED	70.57	70.57	72.19	DETECTED	70.29	70.55	72.51	DETECTED	70.39	70.76	71.77
34	S	R (KAT G)	DETECTED	1.1×10^{06}	DETECTED	70.78	70.23	71.14	DETECTED	70.3	70.58	72.16	DETECTED	70.23	70.48	71.58
35	S	S	DETECTED	6.3×10^{06}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
36	S	R(KATG)	DETECTED	9.3×10^{06}	DETECTED	70.55	70.73	71.19	DETECTED	70.69	70.87	72.086	DETECTED	70.43	70.57	71.38
37	S	S	DETECTED	4.3×10^{04}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
38	R	R(KATG)	DETECTED	2.1×10^{07}	DETECTED	70.13	70.59	70.89	DETECTED	69.74	70.85	72.81	DETECTED	70.02	70.57	71.96
39	S	S	DETECTED	7.9×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
40	R	R(KATG)	DETECTED	7.2×10^{06}	DETECTED	70.59	70.5	70.52	DETECTED	70.46	70.83	72.21	DETECTED	70.64	70.92	70.34
41	S	R(KATG)	DETECTED	2.3×10^{02}	DETECTED	70.94	70.41	72.26	DETECTED	70.02	70.46	72.6	DETECTED	70.02	69.92	74.99
42	S	S	DETECTED	1.7×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
43	S	R (KAT G & INH A)	DETECTED	3.6×10^{06}	DETECTED	70.41	67.53	70.8	DETECTED	70.38	67.36	72.05	DETECTED	70.29	67.51	72.28
44	S	S	DETECTED	1.7×10^{06}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
45	S	R(KATG)	DETECTED	6.8×10^{07}	DETECTED	70.75	70.68	71.31	DETECTED	70.11	70.36	71.33	DETECTED	69.92	70.32	72.98
46	R	R(KATG)	DETECTED	1.3×10^{08}	DETECTED	70.85	70.64	70.91	DETECTED	70.87	70.87	71.19	DETECTED	70.78	70.85	70.26
47	S	S	DETECTED	1.1×10^{07}	NOT DETECTED				NOT DETECTED				NOT DETECTED			
48	S	R (KAT G)	DETECTED	4.7×10^{07}	DETECTED	70.38	70.66	70.33	DETECTED	70.57	70.48	72.07	DETECTED	69.73	70.94	71.96
49	S	S	DETECTED	4.2×10^{04}	INDETERMINATE				NOT DETECTED				NOT DETECTED			
50	R	R(INHA)	DETECTED	2.5×10^{07}	DETECTED	74.84	65.11	70.63	DETECTED	74.32	65.89	70.82	DETECTED	74.38	65.85	71.58

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