

ORIGINAL ARTICLE

Performance Evaluation of MolecuTech REBA Myco-ID Using HybREAD480 for Identification of Nontuberculous Mycobacteria

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SUMMARY

Background: Rapid and accurate identification of nontuberculous mycobacteria (NTM) species is essential for the diagnosis and treatment of NTM disease. MolecuTech REBA Myco-ID (YD Diagnostics, Yongin, Korea) is a line probe assay for identification of NTM species and can be performed using HybREAD480, an instrument for automating the post-PCR steps. In this study, we assessed the performance of MolecuTech REBA Myco-ID using HybREAD480.

Methods: Seventy-four reference strains, including 65 *Mycobacterium* strains and nine non-*Mycobacterium* strains within the order *Mycobacteriales*, were used to determine the analytical specificity of MolecuTech REBA Myco-ID. The clinical performance of this assay was evaluated with 192 clinical *Mycobacterium* strains, and the assay results were compared to those of multigene sequencing-based typing.

Results: The accuracy of MolecuTech REBA Myco-ID for the 74 reference strains and 192 clinical strains was 77.0% (57/74; 95% confidence interval [CI], 65.8 - 86.0%) and 94.3% (181/192; 95% CI, 90.0 - 97.1%), respectively. Although some rarely isolated NTM species are misidentified, the most commonly isolated NTM species, including *M. avium* complex, *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. fortuitum* complex, were all correctly identified. Of note, all *M. lentiflavum* strains tested (reference strain, n = 1; clinical strain, n = 10) were misidentified as *M. gordonae*.

Conclusions: MolecuTech REBA Myco-ID using HybREAD480 was accurate for identifying commonly isolated NTM species and for discriminating between *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense*. However, the main limitations of this assay, including misidentification of some rarely isolated NTM species and cross-reactivity between *M. lentiflavum* and *M. gordonae*, should be considered.

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Supplementary Data

Table S1. Analytical specificity test results of MolecuTech REBA Myco-ID.

Species	Strain	MolecuTech REBA Myco-ID Identification results	Interpretation
<i>Mycobacterium</i> spp. (n = 65)			
<i>M. abscessus</i> subsp. <i>abscessus</i>	KCTC 19621	<i>M. abscessus</i>	Correct
<i>M. abscessus</i> subsp. <i>bolletii</i>	KCTC 19281	<i>M. abscessus</i>	Correct
<i>M. abscessus</i> subsp. <i>massiliense</i>	KCTC 19086	<i>M. massiliense</i>	Correct
<i>M. acapulcensis</i>	KCTC 9501	<i>Mycobacterium</i> spp.	Correct
<i>M. agri</i>	KCTC 9502	<i>Mycobacterium</i> spp.	Correct
<i>M. alvei</i> ^a	KCTC 19709	<i>M. fortuitum</i> complex	Correct
<i>M. anyangense</i>	KCTC 29443	<i>M. genavense</i> / <i>M. simiae</i>	MisID
<i>M. asiaticum</i>	KCTC 9503	<i>Mycobacterium</i> spp.	Correct
<i>M. aubagnense</i>	KCTC 19639	<i>M. aubagnense</i>	Correct
<i>M. austroafricanum</i>	KCTC 9504	<i>Mycobacterium</i> spp.	Correct
<i>M. avium</i>	KMRC 00136-41011	<i>M. avium</i>	Correct
<i>M. brisbanense</i> ^a	KCTC 19641	<i>M. fortuitum</i> complex	Correct
<i>M. brumae</i>	KCTC 19711	<i>Mycobacterium</i> spp.	Correct
<i>M. canariensis</i>	KCTC 19644	<i>Mycobacterium</i> spp.	Correct
<i>M. celatum</i>	KCTC 19714	<i>M. celatum</i>	Correct
<i>M. chelonae</i>	KCTC 9505	<i>M. chelonae</i>	Correct
<i>M. chlorophenicum</i>	KCTC 19089	<i>Mycobacterium</i> spp.	Correct
<i>M. chubuense</i>	KCTC 19712	<i>Mycobacterium</i> spp.	Correct
<i>M. colombiense</i>	KMRC 00136-96001	<i>Mycobacterium</i> spp.	Correct
<i>M. conceptionense</i> ^a	KCTC 19640	<i>M. fortuitum</i> complex	Correct
<i>M. cookie</i>	KCTC 19715	Negative for <i>Mycobacterium</i> spp.	MisID
<i>M. cosmeticum</i>	KCTC 19713	Negative for <i>Mycobacterium</i> spp.	MisID
<i>M. diernhoferi</i>	KCTC 9506	<i>Mycobacterium</i> spp.	Correct
<i>M. fallax</i>	KCTC 9508	<i>M. fortuitum</i> complex	MisID
<i>M. farcinogenes</i> ^a	KCTC 19647	<i>M. fortuitum</i> complex	Correct
<i>M. fortuitum</i> ^a	KMRC 00136-60004	<i>M. fortuitum</i> complex	Correct
<i>M. frederiksbergense</i>	KCTC 19100	<i>M. fortuitum</i> complex	MisID
<i>M. gilvum</i>	KCTC 19423	<i>Mycobacterium</i> spp.	Correct
<i>M. gordonae</i>	KCTC 9513	<i>M. gordonae</i>	Correct
<i>M. heckeshornense</i>	KCTC 19648	<i>Mycobacterium</i> spp.	Correct
<i>M. holsaticum</i>	KCTC 19650	<i>M. fortuitum</i> complex	MisID
<i>M. interjectum</i>	KCTC 19649	<i>Mycobacterium</i> spp.	Correct
<i>M. intracellulare</i>	KCTC 9514	<i>M. intracellulare</i>	Correct
<i>M. intracellulare</i> subsp. <i>yongonense</i>	KCTC 19555	Negative for <i>Mycobacterium</i> spp.	MisID
<i>M. kansasii</i>	KCTC 9515	<i>M. kansasii</i>	Correct
<i>M. koreense</i>	KCTC 19819	<i>Mycobacterium</i> spp.	Correct
<i>M. lentiflavum</i>	ATCC 51985	<i>M. gordonae</i>	MisID
<i>M. manitobense</i>	KCTC 9977	<i>Mycobacterium</i> spp.	Correct
<i>M. marinum</i>	KMRC 00136-21108	<i>M. ulcerans</i> / <i>M. marinum</i>	Correct
<i>M. marseillense</i>	KMRC 00136-83001	<i>M. genavense</i> / <i>M. simiae</i>	MisID
<i>M. moriokaense</i>	KCTC 9516	<i>M. fortuitum</i> complex	MisID

Table S1. Analytical specificity test results of MolecuTech REBA Myco-ID (continued).

Species	Strain	MolecuTech REBA Myco-ID Identification results	Interpretation
<i>M. mucogenicum</i>	KCTC 19088	<i>M. mucogenicum</i>	Correct
<i>M. neoaurum</i>	KCTC 19096	<i>Mycobacterium</i> spp.	Correct
<i>M. nonchromogenicum</i>	KMRC 00136-46003	<i>M. terrae/M. nonchromogenicum</i>	Correct
<i>M. obuense</i>	KCTC 19097	<i>M. fortuitum</i> complex	MisID
<i>M. oryzae</i>	KCTC 39560	<i>M. fortuitum</i> complex	MisID
<i>M. paraintracellulare</i>	KCTC 29084	<i>M. intracellulare</i>	Correct ^b
<i>M. parakoreense</i>	KCTC 19818	<i>Mycobacterium</i> spp.	Correct
<i>M. parascrofulaceum</i>	KCTC 9979	Negative for <i>Mycobacterium</i> spp.	MisID
<i>M. paraseoulense</i>	KCTC 19145	<i>M. scrofulaceum</i>	MisID
<i>M. paraterrae</i>	KCTC 19556	<i>Mycobacterium</i> spp.	Correct
<i>M. peregrinum</i> ^a	KMRC 00136-75003	<i>M. fortuitum</i> complex	Correct
<i>M. phlei</i>	KCTC 9087	<i>Mycobacterium</i> spp.	Correct
<i>M. porcinum</i> ^a	KCTC 9517	<i>M. fortuitum</i> complex	Correct
<i>M. pulveris</i>	KCTC 9518	<i>Mycobacterium</i> spp.	Correct
<i>M. saskatchewanense</i>	KCTC 9978	<i>Mycobacterium</i> spp.	Correct
<i>M. sediminis</i>	KCTC 19999	Negative for <i>Mycobacterium</i> spp.	MisID
<i>M. senuense</i>	KCTC 19147	<i>Mycobacterium</i> spp.	Correct
<i>M. smegmatis</i>	KCTC 9108	<i>Mycobacterium</i> spp.	Correct
<i>M. stephanolepidis</i>	KCTC 39843	<i>M. chelonae</i>	Correct ^b
<i>M. szulgai</i>	KCTC 9520	<i>M. szulgai</i>	Correct
<i>M. terrae</i>	KCTC 9614	<i>M. terrae/M. nonchromogenicum</i>	Correct
<i>M. tuberculosis</i>	ATCC 27294	<i>M. tuberculosis</i> complex	Correct
<i>M. vaccae</i>	KCTC 19087	<i>Mycobacterium</i> spp.	Correct
<i>M. vanbaalenii</i>	KCTC 9966	<i>Mycobacterium</i> spp.	Correct
Non- <i>Mycobacterium</i> spp. within the order <i>Mycobacteriales</i> (n = 9)			
<i>Gordonia araii</i>	KCTC 19354	Negative for <i>Mycobacterium</i> spp.	Correct
<i>Gordonia polyisoprenivorans</i>	KCTC 9953	Negative for <i>Mycobacterium</i> spp.	Correct
<i>Gordonia terrae</i>	KCTC 9807	Negative for <i>Mycobacterium</i> spp.	Correct
<i>Nocardia brasiliensis</i>	ATCC 19296	Negative for <i>Mycobacterium</i> spp.	Correct
<i>Rhodococcus corynebacterioides</i>	KCTC 9613	<i>Mycobacterium</i> spp.	MisID
<i>Rhodococcus ruber</i>	KCTC 9806	<i>Mycobacterium</i> spp.	MisID
<i>Tsukamurella inchonensis</i>	KCTC 9866	Negative for <i>Mycobacterium</i> spp.	Correct
<i>Tsukamurella pulmonis</i>	KCTC 9963	Negative for <i>Mycobacterium</i> spp.	Correct
<i>Tsukamurella strandjordii</i>	KCTC 19178	Negative for <i>Mycobacterium</i> spp.	Correct

KCTC - Korean Collection for Type Cultures, KMRC - Korea Mycobacterium Resource Center, ATCC - American Type Culture Collection, MisID - misidentification.

^a These species are included in *M. fortuitum* complex.

^b Identification results of these strains were considered as correct because they were very closely related to the species of these strains.