# Genotype MTBDR Plus (Hain) Test in Suspected MDR-TB Patients†

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***Background:*** *Incidence of MDR-TB in Thailand is higher. Diagnosis of multi-drug resistance (MDR-TB) is often delayed because of insufficient investigations. Therefore, there is a need to introduce an efficient testing method. Genotype MTBDR plus (Hain test) is a new technology of line probe assay (LPAs) In general practice, it had questionable benefit on negative and positive direct smear specimens.*

***Objective:*** *Evaluate the accuracy of the Hain test as compared to the conventional culture and drug susceptibility test (DST), and evaluate the judgment of the physician on starting MDR-TB treatment after receiving the result of the Hain test.*

***Material and Method:*** *An observational prospective study was done of 100 suspected MDR-TB patients who visited the Central Chest Institute of Thailand between September and December 2012. Ninety-four patients were included in the present study. They were assessed by direct sputum smear test, Hain test, sputum conventional culture, and drug-susceptibility test (DST) in the first visit. Followed-up treatment until cure/definite treatment were observed for definite diagnosis.*

***Results:*** *Hain tests identified 65 (69%) MTB positives, 25 (26%) MTB negatives, and four (4%) Non-tuberculous Mycobacteria (NTM), respectively. Fourteen of 17 negative direct smear but positive Hain test samples were culture negative. We followed these 14 patients. Four patients with TB treatment were cured. Six patients received MDR treatment and improved. Two patients were misdiagnosed CA. Two patients had old scar TB lesion. Sensitivity, specificity, and accuracy of Hain test for MDR-TB detection compared to standard conventional culture were 95%, 86%, and 88%, respectively. Twelve patients were NTM culture positive. Most of them (75%) were M. abscessus. Sixty-three percent of chest physicians used the result of the Hain test for extended treatment of standard regimen in non-IR resistance or changing of TB regimen. There was correlation between judgments of starting MDR-TB treatment and results of Hain test ($p = 0.001$), no correlation between judgments of physician and pattern (default/relapse/failure) of previous TB treatment history ($p = 0.6$), and periods of time from first diagnosis of TB to develop suspected MDR-TB ($p = 0.09$) were demonstrated.*

***Conclusion:*** *We recommend Hain test to diagnose MDR-TB in highly suspected cases, and to wait for conventional culture results to confirm NTM. Hain test has benefit in negative direct smear.*

***Keywords:*** *MDR-TB, Genotype MTBDR and MTBDR plus (Hain) test*

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Tuberculosis (TB) population in Thailand is large [137 in 100,000 population]. Incidence of MDR-TB in Thailand is as high 2,000 patients per year. Diagnosis of multi-drug resistance (MDR-TB) is often delayed because of insufficient investigations. Therefore, there is a need to introduce an efficient testing method. Genotype MTBDR plus (Hain test [2,3,5]) is a new technology of line probe assay (LPAs) that has become popular technology in recent years because it makes it easy and rapid to identify mycobacterium species and to characterize drug susceptibilities of isoniazid (INH) and rifampicin (RIF) in two to three days. The WHO[1] analysis showed that LPAs are highly sensitive (≥97%) and specific (≥99%) for the detection of RIF resistance, alone or in combination with INH (sensitivity ≥90%; specificity ≥99%), on isolates of *M. tuberculosis*, and on smear-positive sputum specimens. Hain test in general practice had questionable benefit for specimens including negative and positive direct smear specimens. The present study evaluated the accuracy of the Hain test compared to conventional culture and drug susceptibility test (DST), and to evaluate the judgment of chest physician for starting MDR-TB treatment after receiving result of Hain test.

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## Material and Method

It was an observational prospective study in conjunction with an evaluation of molecular diagnostic method. Inform consent was received in all volunteers by local EC of chest disease institute (27/2555). One hundred MDR-TB suspects in TB clinic, Central Chest Institute of Thailand between September and December 2012 were included in the present study. Suspected MDR-TB patients were identified by failure of standard treatment, suspected relapse, and default treatment. Failed standard treatment mean that the patients were treated with TB standard treatment of two HERZ/4HR with no improvement in clinical test, Chest X-ray, and/or no sputum conversion. Suspected relapse means a previous cure TB treatment and new episode of suspected recurrence. Default means loss of treatment. Positive and negative sputum smear specimens were included. The patients were assessed by direct sputum smear test, Hain test, sputum conventional culture, and drug-susceptibility test (DST) in the first visit. Hain test is a new technique by extraction of DNA from sample material, specifically amplified via PCR and detected on a membrane strio using reversed hybridization and an enzymatic color reaction (Fig. 1). physicians were observed for changing treatments or continuing standard treatment of TB after received Hain test. Eight chest physicians had more than five years TB experience. Followed-up treatment until cure/definite treatment were observed for definite diagnosis was done. Hain test was performed as recommended by the Hain Lifescience Gmb H manufacturer, Germany. It was performed under control of trained technician.

### *Statistical analysis*

All medical records were evaluated and analyzed using SPSS (version 13) program. The data were presented as descriptive statistics. Chi-square test was used in determine association between judgments of starting MDR-TB treatment and results of Hain test.

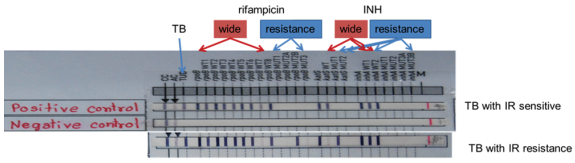


**Fig. 1** Strip of Hain test.

## Results

One hundred MDR-TB suspected patients were included in the present study. Six patients were excluded because sputum was not collected properly. The remaining 94 patients were included in the study. Patients consisted of 53 failure sputum conversion, 23 suspected relapse, and 24 default cases. There were 53 males and 47 females, age between 16 and 91 years old (mean 46 years old). It took 4 to 240 months (median time 12 months) from first TB diagnosis to develop suspected MDR-TB condition. Hain tests identified 65 (69%) MTB positives, 25 (26%) MTB negatives, and four (4%) non-tuberculous mycobacteria (NTM), respectively. Comparison between Hain tests and standard culture was shown in Table 1. Forty-three of 94 patients (45%) were positive for both Hain tests and standard culture of TB, 14 (14%) patients were negative for both, 18 (14%) patients were negative for standard culture but positive for Hain test, and five (5%) patients had positive culture of TB but negative/MOTT pattern in Hain tests as shown as Table 1 and Fig. 2.

Hain tests detected MTB in 17 (43%) samples of 39 negative direct smears. Fourteen samples of 17 positive Hain test and negatives direct smear

**Table 1.** Hain test and standard culture of TB comparison

| Hain test | Standard culture of TB | | | | Total (n) |
|---|---|---|---|---|---|
| | MTB | | Negative C/S (n) | Nontuberculous-mycobacterium (n) | |
| | IR resistance (n) | IR sensitive (n) | | | |
| MTB | | | | | |
| IR resistance (n) | 19 | 3 | 6 | 1 | 29 |
| IR sensitive (n) | 0 | 21 | 12 | 3 | 36 |
| Negative Hain (n) | 1 | 3 | 14 | 7 | 25 |
| Nontuberculous-mycobacterium (n) | 0 | 1 | 2 | 1 | 4 |
| Total (n) | 20 | 28 | 34 | 12 | 94 |

TB = tuberculosis; MTB = Mycobacterium tuberculosis; IR = isoniazid and rifampin; C/S = culture/smear

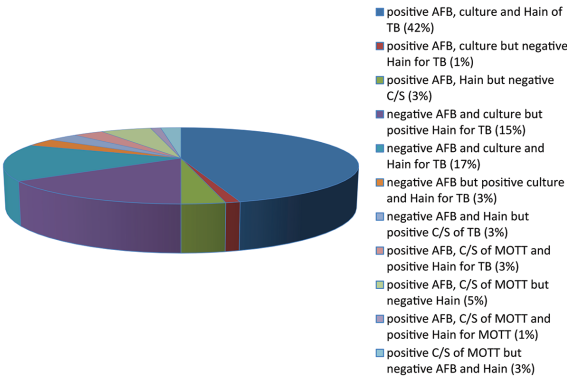


**Fig. 2** Chart of direct smear, culture and Hain tests.

were negative culture (Fig. 2). On the other hand, three of them were positive in culture.

We followed these 14 patients with positive Hain tests but negative for both direct smear and culture. Four patients were treated with antituberculous medication, and were cured, six MDR treated patients improved, two CA lung patients were under diagnosis, and the last two patients had old scar-TB lesion (Table 2).

Drug sensitivity of Hain tests showed that 29 (30%) samples were INH and Rifampicin (RIF) resistant (MDR-TB), while INH and RIF mono-resistant were 11% and 2%, respectively. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of Hain test for MDR-TB detection compared to standard conventional culture were 95% (95% CI 85.45 to 104.55%), 86% (95% CI 78.70 to 94.28%), 65% (95% CI 48.22 to 82.82%), 98% (95% CI 95.47 to 101.45%), and 88% (95% CI 81.79 to 94.55%), respectively. In NTM detection, only one patient was positive in both Hain test and conventional culture for NTM. Twelve patients had positive NTM culture; most of them (75%) were *M. abscessus*. Others were *M. siminae*, *M. avium*, and *M. kansasii*. The sensitivity, specificity, PPV, NPV, and accuracy for NTM diagnosis of Hain test were 8% (95% CI -7.3 to 23.95%), 96% (95% CI 92.28 to 100.4%), 25% (95% CI -17.43 to 67.43%), 87% (95% CI 81.01 to 94.55%), and 89% (95% CI 77.91 to 92.31%). Sixty-three percent of chest physicians used the result of Hain tests for treatments, such as extended treatment of standard regimen or changing regimen. Physicians decided to start MDR-TB treatment regimens when they recognized IR resistance from Hain tests reports. There was correlation between judgment of starting MDR-TB treatment and results of Hain tests ($p$ = 0.001). No correlation between judgments of Physician and pattern (default/relapse/failure) of previous TB treatment history ($p$ = 0.6), and period of time from first diagnosis of TB to develop suspected MDR-TB ($p$ = 0.09) were demonstrated.

## Discussion

The present study was an observational review. The authors did not want to interfere in the routine treatment. Limitation of our study was that there was no liquid culture media in all specimen. We used long-term follow-up to confirm definite diagnosis. The study showed that judgments of chest physician depended on Hain tests more than pattern of previous TB treatment or time period from first diagnosis of TB to develop suspicious of MDR-TB. This is because the physicians believed in the new technology to help them confirm MDR-TB earlier.

The mutations of rifampicin[1,5] are predominate in rpoB gene. In contrast, the mutations causing INH resistance are located in several genes and regions, such as mutations of KatG gene and the inhA regular regions. The purpose of the author was to identify MDR-TB, meaning resistance in both INH and RIF. Line probe assay such as Hain test[2,3] was the best method because it can identify both INH and Rifampicin resistance. The result shows 41% of positive Hain tests and culture and 16% negative. Sensitivity, Specificity, PPV, NPV, and Accuracy of Hain tests for MDR-TB detection compared to standard

**Table 2.** Result of positive Hain tests but negative direct smear

| | | Positive Hain tests for TB, n (%) | Final diagnosis after follow-up | |
|---|---|---|---|---|
| | | | Disease | Number of patients (n) |
| Negative direct smear (AFB) | Positive standard C/S | 3 (3%) | TB | 3 |
| | Negative standard C/S | 14 (15%) | TB | 4 |
| | | | MDR-TB | 6 |
| | | | CA lung | 2 |
| | | | Old scar TB | 2 |

MDR-TB = multi-drug resistant tuberculosis; CA lung = lung canner

conventional culture in positive and negative sputum were 95%, 86%, 65%, 98%, and 88%, respectively. Other studies[3,4] showed sensitivities of the Genotype MTBDR were reported as 99% a 96.4% in RIF-resistant strains and 88.4% and 84.4% in INH-resistant strains, respectively. Accuracy of Hain test for MDR-TB in the present study was high, but false positive was high too. In the present study, 18 patients had positive Hain test but negative conventional culture. This is because identification of TB needs more organisms in culture than Hain tests. The present study included suspected MDR-TB specimens who had previous treatments of TB or ongoing standard TB treatments. Sometimes, the standard culture of TB (2HRZE/4HR)[1] can kill small amount of MDR-TB bacilli. Therefore, culture specimens were negative. The present study demonstrated benefit of Hain test in negative direct smear. It found three positive in Hain tests and cultures but negative direct smears and 14 negative in direct smears and cultures but positive in Hain tests. On follow-up, 10 of 14 patients were definitely diagnosed of TB/MDR-TB. Only four patients were misdiagnosed (two old scar and two CA lung patients). In Thailand, *M. abscessus* will become difficult problem in the future. The present study showed that the most common MOTT in suspected MDR-TB patients was *M. abscessus*. Hain test is not a proper investigation for MOTT because it is designed to identify MTB. Technician sometimes reported mycobacterium other than TB when probe shows negative in condition of positive sputum smear. The physicians were confused by the reports. The present study showed low accuracy for using Hain test for detecting MOTT. Waiting for conventional culture is suggested.

**Conclusion**

The authors recommend Hain test to diagnose MDR-TB in highly suspected cases, but should wait for conventional culture results to confirm NTM. Hain test will be a benefit in negative direct smear because gene detection used only few organisms. Most physicians in Thailand decided to start MDR-TB regimens after received Hain tests reports.

**What is already known on this topic?**

High sensitivity, specificity, and accuracy of Hain test in INH and RIF resistance individual.

**What this study adds?**

Knowledge of Hain test in both INH and RIF resistance together. Based on the judgment of the physicians, they believe in Laboratory test. No benefit of Hain test in MOTT.

**Potential conflicts of interest**

None.

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## การตรวจโดยวิธี genotype MTBDR and MTBDR plus (Hain) ในผู้ป่วยที่สงสัยวัณโรคดื้อยา

เปี่ยมลาภ แสงสายัณห์, เจริญ ชูโชติถาวร

**ภูมิหลัง:** อุบัติการณ์ของวัณโรคดื้อยามีแนวโน้มสูงขึ้น การวินิจฉัยวัณโรคมักจะใช้เวลานานและมีความต้องการการสืบค้นใหม่ ๆ มาใช้ช่วยการวินิจฉัย การตรวจวิธีใหม่ Hain เป็นการตรวจโดยวิธี line probe assay ยังมีข้อกังขาในการตรวจทั่วไปในห้องปฏิบัติการ โดยเฉพาะในกลุ่มผู้ป่วยที่เสมหะไม่พบเชื้อ

**วัตถุประสงค์:** เพื่อหาความแม่นยำของการตรวจ Hain เมื่อเปรียบเทียบกับการเพาะเชื้อวัณโรค และเพื่อประเมินการตัดสินใจในการรักษาของอายุรแพทย์โรคปอดหลังจากทราบผลการตรวจ Hain

**วัสดุและวิธีการ:** การศึกษาผู้ป่วยที่สงสัยภาวะวัณโรคดื้อยาที่เข้ารับการรักษาที่สถาบันโรคทรวงอก ระหว่างเดือนกันยายน ถึง ธันวาคม พ.ศ. 2555 จำนวน 94 ราย ที่เข้าร่วมตลอดการศึกษา ทำการตรวจเสมหะเพื่อหาเชื้อวัณโรค ส่งเพาะเชื้อ และตรวจโดยวิธี Hain หลังจากนั้น ติดตามการรักษาจนทราบผู้ป่วยหายขาดและทราบผลการวินิจฉัยโรค

**ผลการศึกษา:** พบผู้ป่วยจำนวน 65 ราย (69%) มีการตรวจโดยวิธี Hain แสดงผลเป็นวัณโรค 25 ราย (26%) ไม่พบลักษณะของวัณโรค และ 4 ราย (4%) แสดงเชื้อมัยโคแบคทีเรียที่ไม่ใช่วัณโรค จากการศึกษาพบว่า 14 ราย ใน 17 ราย มีผลเสมหะ direct smear เป็นลบ แต่มีผลการตรวจ Hain เป็นบวก ไม่พบเชื้อในการเพาะเสมหะหาเชื้อวัณโรค เมื่อติดตามผู้ป่วยกลุ่มนี้ พบว่าผู้ป่วย 4 ราย หายจากการรักษาวัณโรค ผู้ป่วย 6 ราย หายจากการรักษาวัณโรคดื้อยา ผู้ป่วยมะเร็ง 2 ราย วินิจฉัยผิด ผู้ป่วย 2 ราย เป็นแผลเป็นเก่าในปอด การตรวจความไว ความจำเพาะ และความแม่นยำของการตรวจโดยวิธี Hain ในการวินิจฉัยวัณโรคดื้อยา พบค่า 95%, 86% และ 88% ตามลำดับ จากการเพาะเชื้อ มี 12 ราย พบเชื้อมัยโคแบคทีเรียที่ไม่ใช่วัณโรค 75% ของผู้ป่วยกลุ่มนี้เป็นเชื้อ M. abscessus การตัดสินในการรักษาแพทย์อายุรกรรมปอดพบการตัดสินใจรักษาตามผลการตรวจโดยวิธี Hain เช่น ยืดระยะเวลาของการรักษา หรือ เปลี่ยนการรักษา 63% มีความสัมพันธ์ระหว่างการตัดสินใจรักษาและผลการตรวจโดยวิธี Hain อย่างมีนัยสำคัญทางสถิติ ($p = 0.001$) แต่ไม่มีความสัมพันธ์ระหว่างการตัดสินใจรักษาและระยะเวลาตั้งแต่เริ่มรักษาวัณโรคจนสงสัยวัณโรคดื้อยา ($p = 0.09$) และประวัติการรักษาในอดีต ได้แก่ ประวัติการขาดยา การเป็นซ้ำ ($p = 0.6$)

**สรุป:** สามารถใช้การตรวจโดยวิธี Hain ในผู้ป่วยสงสัยวัณโรคดื้อยา แต่แนะนำให้รอผลเพาะในผู้ป่วยที่ผลการตรวจโดยวิธี Hain แสดงผลเป็นมัยโคแบคทีเรียที่ไม่ใช่วัณโรค การตรวจโดยวิธี hain ยังมีประโยชน์ในการวินิจฉัยในผู้ป่วยที่มีผลเสมหะ direct smear เป็นลบ