New diagnostic tools in China

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Outline:

- Introduction of National Clinical Laboratory (NCL) on tuberculosis
- New diagnostic tools in China
National Clinical Laboratory (NCL)

Functions of NCL

- Clinical specimen examination service
- Technical support for NTP
- Research
Space

Equipment

Strain bank

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Isolate</td>
<td>~ 20000</td>
</tr>
<tr>
<td>Standard strain</td>
<td>~ 60 species</td>
</tr>
<tr>
<td>Clinical sample</td>
<td>~ 40000</td>
</tr>
</tbody>
</table>

Biggest in China in TB area
## Diagnostics performed in NCL and the working load

<table>
<thead>
<tr>
<th>Diagnostics</th>
<th>technique</th>
<th>Annual working load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear</td>
<td>LED microscopy</td>
<td>60000</td>
</tr>
<tr>
<td>Solid Culture</td>
<td>L-J medium</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>Liquid Culture</td>
<td>MGIT960</td>
<td>8000</td>
</tr>
<tr>
<td>Blood culture</td>
<td>BACTEC 9120</td>
<td>400</td>
</tr>
<tr>
<td>Phenotypic DST</td>
<td>Micro-well plate (16 drugs)</td>
<td>3000</td>
</tr>
<tr>
<td>NAAT</td>
<td>SAT (RNA detection test)</td>
<td>3000</td>
</tr>
<tr>
<td>Genotypic DST for RIF</td>
<td>GeneXpert</td>
<td>15000</td>
</tr>
<tr>
<td>Genotypic DST for RIF &amp; INH&amp;FQs&amp;EMB</td>
<td>High resolution melting (HRM)</td>
<td>3000</td>
</tr>
<tr>
<td>Mycobacteria species identification</td>
<td>Gene Sequencing</td>
<td>400</td>
</tr>
</tbody>
</table>
Domestic products for TB diagnosis in China
Automatic equipment for smear testing

Sample Collecting
- Sputum processing

Smear preparing

Smear Staining
- ZN staining, Fluoresce staining, Kinyoun staining

Auto Smear Preparing:
1. 50 smear/hour
2. Well distributed
3. Different specimen types

Auto Staining:
1. 240 smear/hour
2. Closed system, no water sink needed

Auto Reading:
1. 3 min for ZN, 1 min for fluorescence smear
2. Higher positivity

Data managing

Reporting
Mycobacteria Susceptibility Test kit with microplate

Characteristics:

• No specific equipment needed.
• Easy: Inoculation with multiple-channel pipet
• Faster: 7~10 days
• 16 drugs, 1st and 2nd line
• Mycobacteria identification in the same plate
48-well microplates diagram

1/10 and 1/100

Positive control

TCH

PNB

Negative control

SIRE

Second-line antibiotics
Two concentration

Test1

Test2

Negative control

Positive control

1/10 and 1/100

TCH

PNB

Negative control

SIRE

Second-line antibiotics
Two concentration

Test1

Test2

48-Well Microplates M. Tuberculosis Drug Diagram

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>E</td>
<td>1/10/Ref</td>
<td>GC</td>
<td>S</td>
<td>1</td>
<td>0.2</td>
<td>R</td>
<td>1</td>
<td>E</td>
<td>2.5</td>
<td>Rft</td>
<td>Lft</td>
</tr>
<tr>
<td>B</td>
<td>F</td>
<td>1/100/Ref</td>
<td>GC</td>
<td>2</td>
<td>0.4</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
<td>NC</td>
<td>TCH</td>
<td>0.25</td>
<td>4</td>
<td>0.8</td>
<td>4</td>
<td>10</td>
<td>Mfx</td>
<td>0.5</td>
<td>Pas</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>H</td>
<td>NC</td>
<td>PNB</td>
<td>400</td>
<td>8</td>
<td>1.6</td>
<td>8</td>
<td>20</td>
<td>2</td>
<td>8</td>
<td>16</td>
<td>3</td>
</tr>
</tbody>
</table>
Procedure of a microplate assay

48/96 wells Microplate Test procedure

Step 1:
Add sterile diluent into antibacterial agent and shake.

Step 2:
Add 100ul (For 96wells:200ul) step 1 mixture liquid into culture medium and shake.

Step 3:
Transfer 180ul of culture medium into A1/E1 (For 96wells:E1) well as 1/10 reference control; And Transfer 180ul into B1/F1 (For 96wells:F1) well as 1/100 reference control. Transfer 200ul of culture medium into C1 and D1 well or G1 and H1 (For 96wells:A1 and B1) well as negative control.

Step 4:
Isolated strains should be prepared to 1mg/ml by grinding turbidity.

Step 5:
Inoculate 100ul (For 96wells:200ul) of Step 4 suspension into the liquid culture medium and shake up.

Step 6:
Add the 200ul of step 5 inoculated culture medium in the rest of well, then transfer 20ul into A1/E1 (For 96wells:E1) well as 1/10 reference control, and transfer 20ul liquid from A1/E1 (For 96wells:E1) to B1/F1 (For 96wells:F1) as 1/100 reference control.

Step 7:
Cover and seal incubate at 35-37°C.

Step 8:
Note down the result on the form provided in the kit or report it by YK-909 Reader.
Comparison between Microplate DST and L-J medium in Beijing Chest Hospital

<table>
<thead>
<tr>
<th></th>
<th>SM</th>
<th>INH</th>
<th>EMB</th>
<th>RFP</th>
<th>EMB</th>
<th>RFT</th>
<th>LFX</th>
<th>AM</th>
<th>CM</th>
<th>PAS</th>
<th>RFB</th>
<th>PTO</th>
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<tbody>
<tr>
<td>Total number</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
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<tr>
<td>Conforming number</td>
<td>68</td>
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<td>69</td>
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<td>70</td>
<td>57</td>
<td>67</td>
<td>62</td>
<td>68</td>
<td>59</td>
<td>66</td>
</tr>
<tr>
<td>Coincidence rate</td>
<td>97.10%</td>
<td>98.60%</td>
<td>97.10%</td>
<td>98.60%</td>
<td>100%</td>
<td>81.40%</td>
<td>96%</td>
<td>88.60%</td>
<td>97.10%</td>
<td>84.30%</td>
<td>94.30%</td>
<td></td>
</tr>
</tbody>
</table>

ps. All strains were isolated from L-J medium with age more than 30 days.
同一温度下，首先通过M-MLV反转录酶产生靶标核酸（RNA）的一个双链DNA拷贝。

然后利用T7 RNA多聚酶从该DNA拷贝上产生多个（100～1000个）RNA拷贝；

带有荧光标记的探针和这些RNA拷贝特异结合，产生荧光。该荧光信号可由荧光检测仪器实时捕获，直观反映扩增循环情况；

同时，每一个RNA拷贝再从反转录开始进入下一个扩增循环。
Simultaneous amplification and testing method (SAT-TB assay)

SAT and PCR

- Differentiate “dead” or “live” bacilli
- Higher positivity
- Less chance of contamination

Amplification to $10^{10}$ in 30 mins
Site evaluation with sputa of 387 PTB patients

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Method</th>
<th>Diagnosis</th>
<th>Sen. (%)</th>
<th>Spe. (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TB-SAT</strong></td>
<td></td>
<td>Total</td>
<td>171</td>
<td>0</td>
<td>67.6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>82</td>
<td>134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S+</td>
<td></td>
<td>+</td>
<td>120</td>
<td>0</td>
<td>97.6</td>
<td>100</td>
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<td></td>
<td>-</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-</td>
<td></td>
<td>+</td>
<td>51</td>
<td>0</td>
<td><strong>39.2</strong></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>79</td>
<td>132</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cui Z. Journal of Clinical. 2012, 50 (3) :646
MMCA®

**M**ulticolor: multiple probes in one tube

**M**elting: melting curve analysis

**C**urve: each mutation has its Tm

**A**nalysis: single step like real-time PCR
MeltPro® TB diagnosis system

Step 1
Automatic sample extraction

Step 2
DNA loading

Step 3
Amplification and results output

Lyophilized reagent

Hands on time ~0.5h, turn around time ~4.0h
# MeltPro® DR TB assays: Mutation sites

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Mutation Sites</th>
<th>Coverage rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampin</td>
<td><em>rpoB</em> 507~533 (81bp)</td>
<td>~95</td>
</tr>
<tr>
<td>Isoniazid</td>
<td><em>ahpC</em> promoter (-44<del>30, -15</del>3), <em>inh94</em>, <em>inhA</em> promoter (-17~8), and <em>katG</em> 315</td>
<td>70-90</td>
</tr>
<tr>
<td>Ethambutol</td>
<td><em>embB</em> 306, 406, 497</td>
<td>75-90</td>
</tr>
<tr>
<td>Streptomycin</td>
<td><em>rpsL</em>43, <em>rpsL</em>88, <em>rrs</em> 513<del>517 and <em>rrs</em> 905</del>908</td>
<td>70-90</td>
</tr>
<tr>
<td>Fluoroquinolone</td>
<td><em>gyrA</em> 88~94</td>
<td>70-90</td>
</tr>
<tr>
<td>Injectable 2nd line</td>
<td><em>rrs</em>1401, 1402, 1484 and <em>eis</em> promoter -37, -14, -13, -10</td>
<td>AMK &gt; 80, KAN &gt; 85, CAP: 70~80</td>
</tr>
</tbody>
</table>
MeltPro® TB MDx Approval Status

CFDA & CE approved

RIF, INH, EMB, STR, FQ and MTBC

CE approved

Injectable 2nd Line
**Isoniazid** is metabolized primarily in the liver by arylamine N-acetyltransferase 2 (NAT2).

**Hepatitis** is a common adverse effect of isoniazid administration. The risk of drug-induced hepatotoxicity varies depending on acetylation status determined by NAT2 gene alleles.

![Diagram](chart.png)

- **INH** → **AcINH**
  - 50% ~ 90%

- **Hz** → **AcHz**

**Hepatitis**
MeltPro® NAT2 Test

The test is used for **NAT2 genotyping** based on polymerase chain reaction and melting curve analysis.

It covers 4 SNPs of NAT2 gene, including c.341T>C, c.481C>T, c.590G>A and c.857G>A.

According to the genotypes of NAT2, individuals can be classified as rapid acetylators (**RAs**), intermediate acetylators (**IAs**) and slow acetylators (**SAs**), respectively. It is well established that SAs are at greater risk to develop hepatotoxicity compared to IAs and RAs.
The genotypes and acetylation metabolism types can be automatically read out.
NAT2 genotype among Chinese

1082 PTB patients:

- RA+SA accounted for 50.28%
- HRM had 100% consistency with gene sequencing
The working principle of 2D label

Liao, Y., Application of Probe-based Fluorescence Melting Curve Analysis in Virus Diagnosis, 2013
Identify 19 mycobacteria species in a single reaction.
MeltPro® McSpoligotyping

Typical results of H37Rv and BCG
More is coming !!
Thank you!

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Email: huanghairong@tb123.org