
بسم الله الرحمن الرحيم

Development and application of a rapid *Mycobacterium tuberculosis* detection technique using polymerase spiral reaction

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Introduction

- In 2015, 10.4 million people were infected with *M. tuberculosis*, and 1.4 million deaths were recorded due to this disease (WHO, 2016).

- China is one of the 22 countries with high prevalence of tuberculosis, and approximately one third of the Chinese population—more than 400 million (active and latent TB) are infected with *M. tuberculosis*.

Introduction

- This disease has a prolonged treatment cycle, and the drugs used have severe toxic side effects; moreover, the emergence of drug resistant and multi-drug resistant bacteria makes it very difficult to cure patients with tuberculosis.

- Therefore, a rapid, specific, and sensitive *M. tuberculosis* detection method that would benefit diagnosis and treatment of this disease should be developed urgently.

Diagnosis

- Microscopic observation and culture remain the predominant detection method for *M. tuberculosis* in several countries (WHO, 2008)
- ✓ Microscopy has a low sensitivity
- ✓ Needs multiple sampling from a patient
- ✓ Requires repeated tests.
- Detection based on sputum cultivation has 100-times higher sensitivity and high specificity → gold standard for the diagnosis of tuberculosis until date.

Diagnosis

- The growth of *M. tuberculosis* → up to 1–2 months in the traditional Löwenstein–Jensen medium
- Development of a simple, rapid, and efficient diagnostic method for this disease is a key issue.
- PCR for *M. tuberculosis* → increase in the detection sensitivity and specificity.

Diagnosis

- The complete process of PCR takes around 3–5 h
- Needs professional, well-trained personnel
- A specific experimental setting at a precisely controlled temperature.

M. tuberculosis nucleic acid detection

- A kit to extract nucleic acids from samples
- A low temperature refrigerator to store heat-unstable components
- A cold-chain transport to ensure the activity of heat-unstable components
- Well-trained personnel to perform the test operations
- And precise and expensive instruments for the test.

Cont.

There are two types of kits available for nucleic acid extraction commercially:

1) Automatic nucleic acid extractors:

- Which are expensive
- Need to be maintained by trained personnel

2) The other is more regular

- But the process involves centrifugation is tedious in operation
- And depends highly on professional skill

New Method: **GenePop**.

- Low-cost
- Rapid
- Portable nucleic acid test Platform
- Not require low temperature storage
- Not professional operation skills
- Not precise and expensive Instruments
- Making this method suitable for rapid nucleic acid testing in field settings or by staff of grass-root units and also for household use

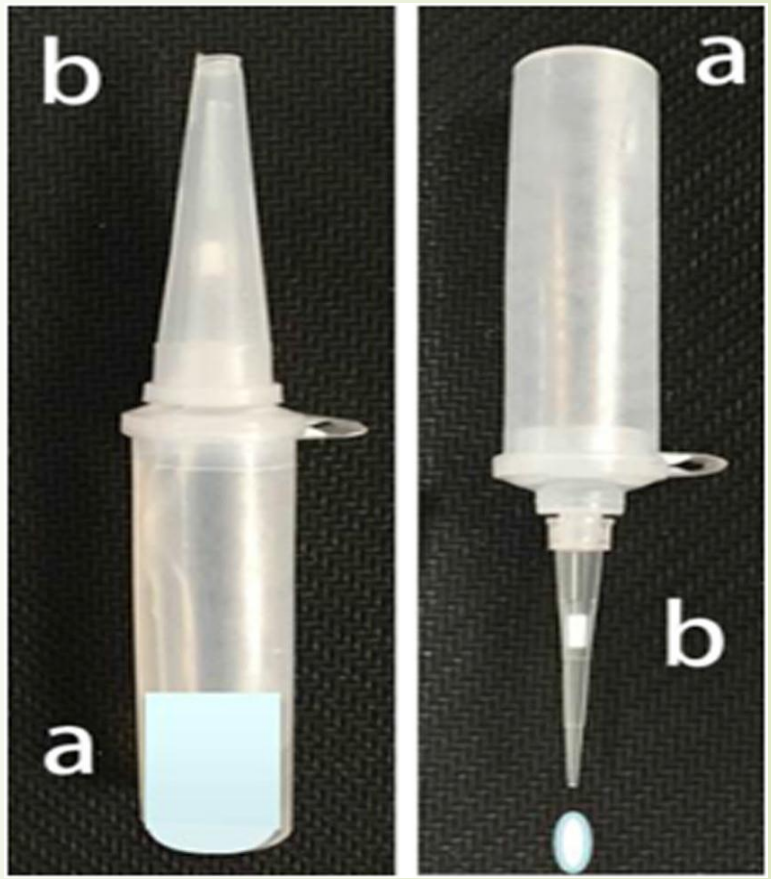
❖We **named this method GenePop**.

Methods

- The device was easy to operate with low requirement Trained personnel
- Without the need for an instrument, a pipette, or a pre-prepared solution, and could be stored at ambient temperatures
- With high extraction purity
- The complete process of DNA extraction from a sputum sample could be achieved within 15 min.

A multi-functional sample treatment device.

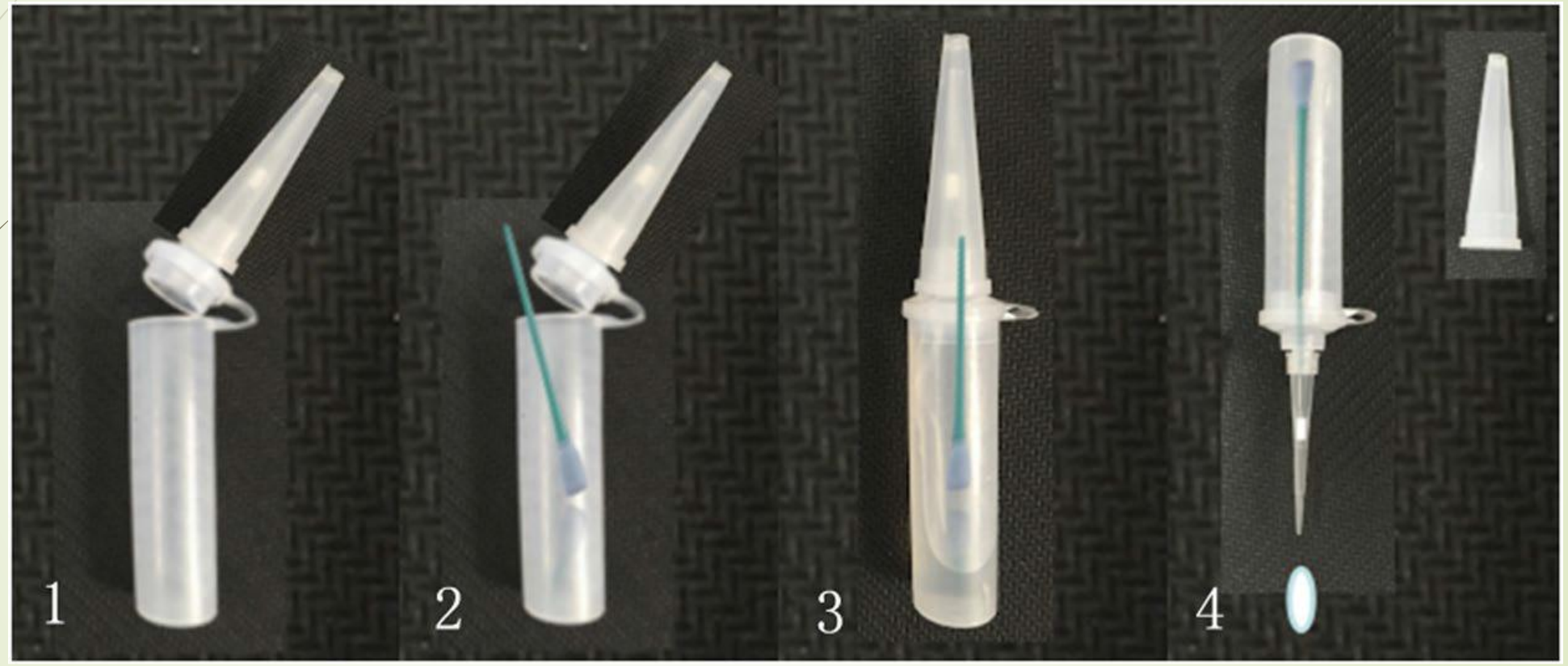
- The tube contained two ends ‘a’ and ‘b’. The end ‘a’ was the heating end where a regular lysis solution was placed
- While the end ‘b’ was a sample introduction end where a filtration membrane was fitted on the top that could filter out impurities in the sample or particulates in the lysis solution.



Lysis solution

- Optimised lysis solution (1% Triton X-100, 1% NP-40, 0.1 mM EDTA, 2% Chelex-100)
- Not inhibiting the subsequent reactions
- When used to treat complex samples (fecal and urine samples), the lysis solution could adsorb impurities from the samples and inhibit the activity of some enzymes to protect the target nucleic acids

Simple operational process



Comparison

Sample	Sample treatment method	1($C_t$value)	2($C_t$value)	3(C_t value)	4(C_t value)
Pure bacterial liquid inoculum	Takarakit	23.76	27.36	30.62	34.45
		24.14	27.54	30.89	33.57
	Multi-functional sample treatment tube	24.97	28.57	31.2	33.84
		25.04	28.67	31.18	34.45
Simulated sputum sample	Qiagen kit	25.33	27.7	30.69	N/A
		25.21	27.14	30.17	N/A
	Multi-functional	25.09	27.33	30.7	N/A
	sample treatment tube	24.84	27.24	31.46	N/A

Polymerase spiral reaction primers for the detection of *M. tuberculosis*

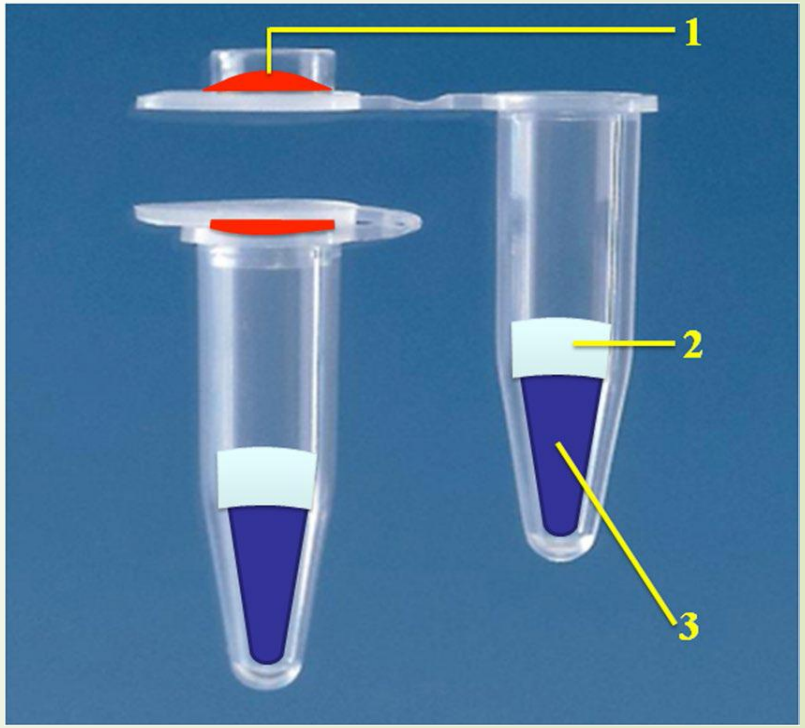
Primer	Sequence(5′ to 3′)
Ft	GTGCCCGCAAAGTGTGGCTAACTTGCGCGATGGCGAACTCA
Bt	GTGCCCGCAAAGTGTGGCTAACTTAGTTTGGTCATCAGCCGTTC
IF	ACGCGGCTGATGTGCT
IB	AGGTGGCCAGATGCACC

Vitrification

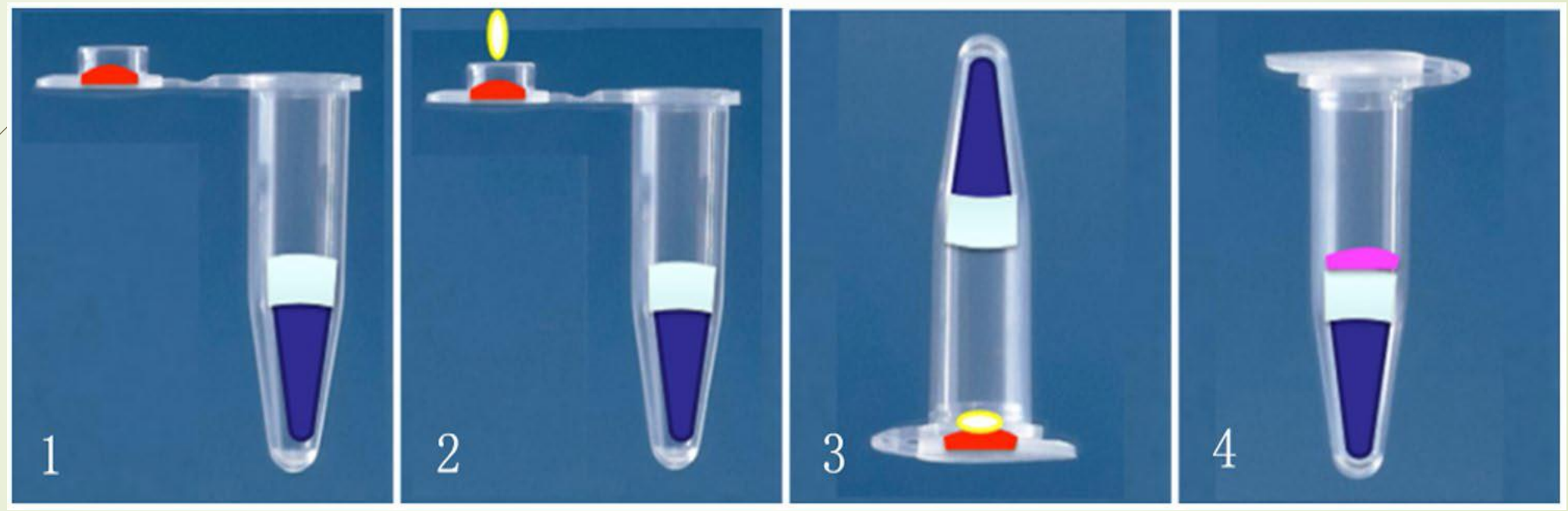
- When some substances experience a temperature equal to or lower than their glass transition temperature (Tg), they form a glass in which there are almost no molecular motions or diffusions, and therefore, the substance can be preserved for a long time at ambient temperatures
- The substances do not crystallise, but form an extremely sticky ‘super cooled’ liquid, which still shows molecular randomness a characteristic of liquids

All-in-one test kit for the detection

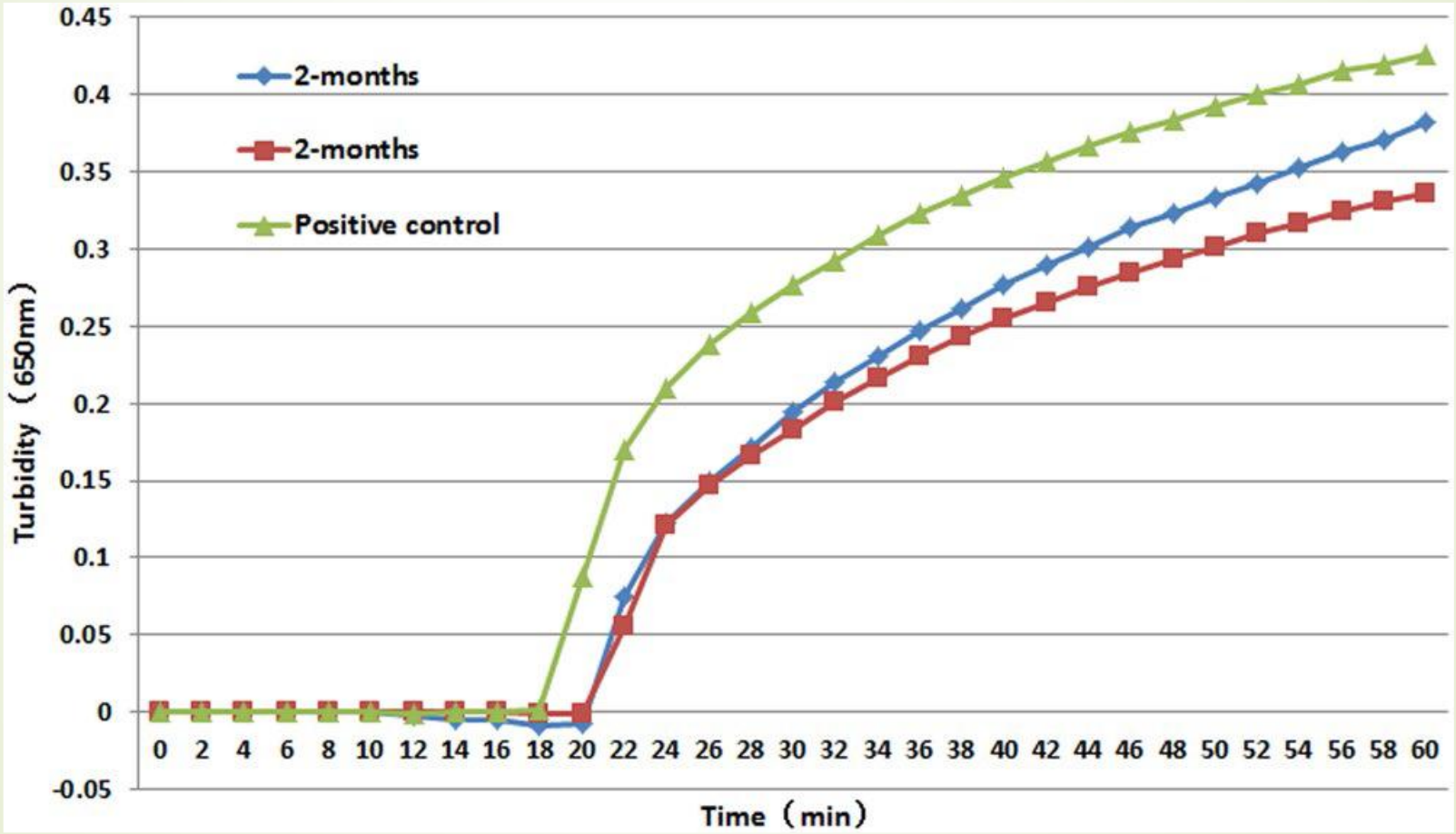
- 1. Enzymes and other heat-unstable components.
- 2. Isolating substances to prevent contamination.
- 3. A heat-stable reaction system



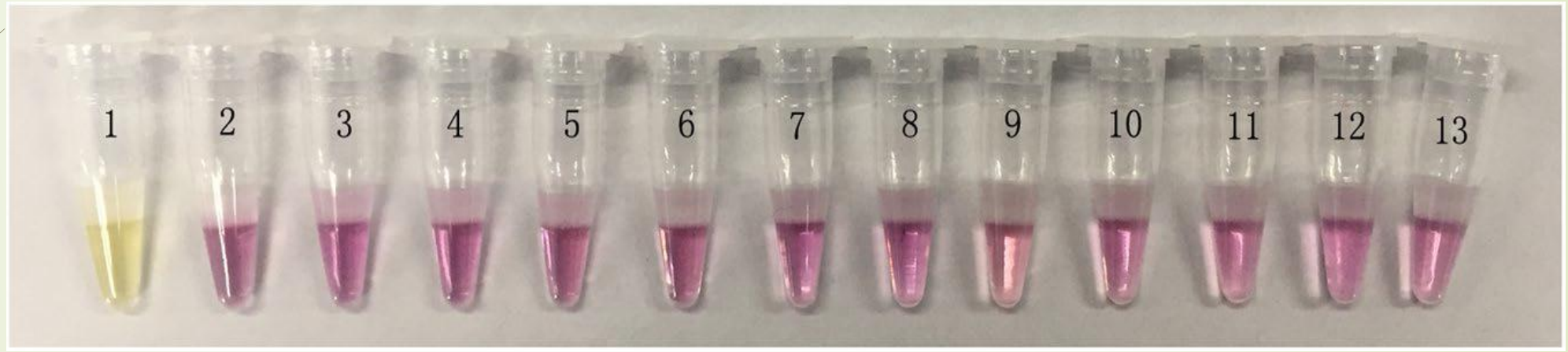
Operation System



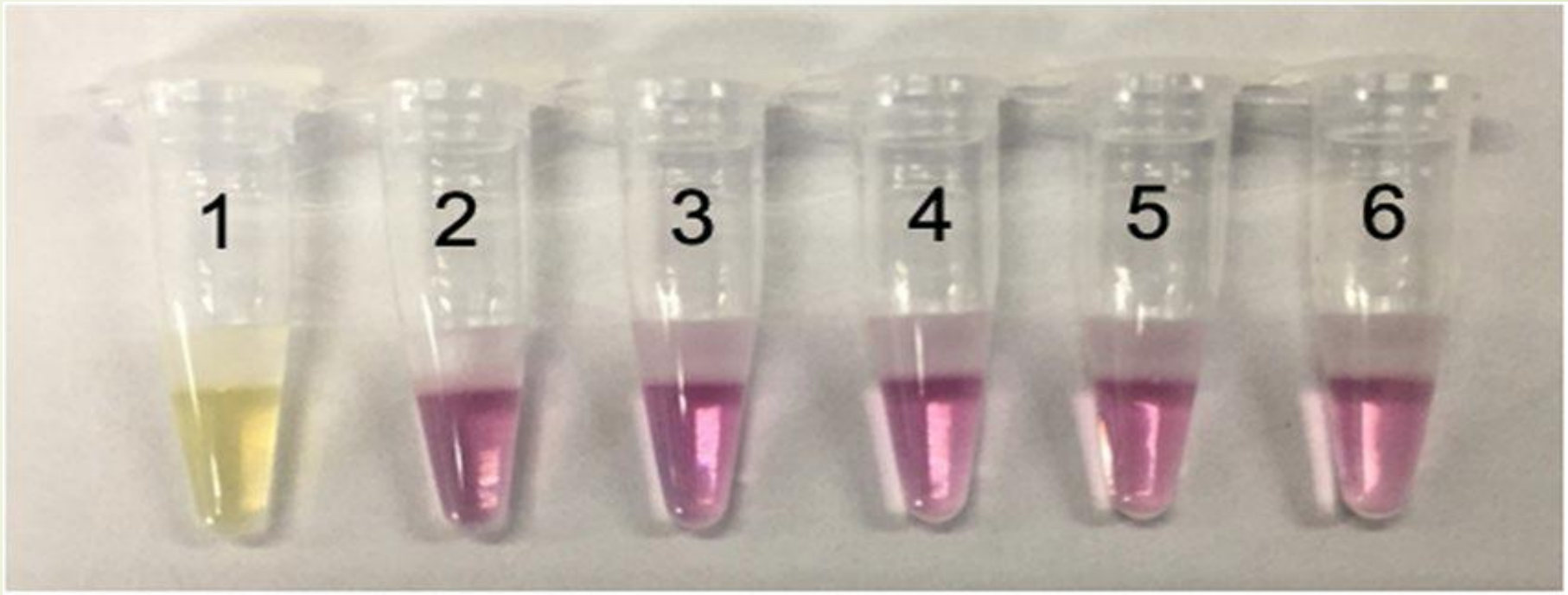
Thermal stability test



Specificity test



Specificity test



Application of the all-in-one kit for *M. tuberculosis* using sptum samples

	PSR-positive	PSR-negative	total
qPCR-positive	58	3	61
qPCR-negative	8	62	70
total	66	65	131

Discussion

1. It had good portability and was composed of only four parts.Te platform only weighed 4.5 kg, which ensured that it would be carried and transported with great ease.

2. Te operation was simple. Te whole operation process consisted of five simple steps as depicted , requiring no other device to be used or any solution to be made. An untrained person may learn the complete process within 30 min, which makes this platform very suitable for the personnel working at grass-root units.

Cont.

3. It could be stored at ambient temperatures. By employing the most cutting-edge, ambient temperature vitrifcation technology to immobilise all the heat-unstable components of the reaction solution onto the reaction tube cover, we could preserve those components for 12 months at room temperatures and transport them at temperatures of no more than 35 °C, thereby overcoming the limitations due to cold-chain transport and low temperature storage.

Cont.

4. The process could be conducted in a timely manner. The sample treatment and the amplification steps took 15 min and 50 min, respectively, and therefore the complete process would take approximately 1 h, which was 1–3 h shorter than the time required for quantitative fluorescence PCR.
5. The platform had a low cost. With the ambient temperature vitrification technique, the platform had a low consumption of electric power and thereby a low production cost, making it very suitable for wide use in developing countries

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Development and application of a rapid *Mycobacterium tuberculosis* detection technique using polymerase spiral reaction

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Mycobacterium tuberculosis is an age-old bacterium that is difficult to eliminate. A simple and rapid diagnostic method is of great importance to prevent the spread of *M. tuberculosis*. Here, we developed a low-cost rapid *M. tuberculosis* nucleic acid detection technique, named GenePop, which enabled the storage and transport of *M. tuberculosis* diagnostic reagent at ambient temperatures, without the need for professional operations or expensive instrumentation. Using a vitrification method, we vitrified heat-unstable components onto the cap of a reaction tube, and placed heat-stable components at the bottom of the reaction tube by sealing them with paraffin wax. The all-in-one detection tube, when

The End

Thank you for your attention

Your past is done, so forget it.
Your future is yet to come, so dream it.
But your present is now, so live it.