I. The TB Diagnosis Challenge

My presentation will focus on the new diagnostic methods of tuberculosis, developed by USTAR Biotechnologies. Today, we witness an extremely rapid accumulation of biotechnologies that can be used to develop more advanced diagnosis instruments for tuberculosis and other infectious diseases. Unfortunately, most of these technologies require the use of large, complex, expensive machines. Such machines are typically found merely at central laboratories and hospitals. The mass population, particularly in developing countries or in rural areas, does not enjoy access to such facilities, and is typically served by local clinics. Since such clinics are resource-limited, they cannot afford to purchase these complex machines, and their staff does not possess the necessary skills to operate them.

Thus, there is a significant gap between the available technology and the services supplied to the mass population in poor countries. The main goal of USTAR Biotechnologies is to bridge this gap by providing local clinics in poor countries with simple, user-friendly, affordable diagnostic tools, which require as few instruments as possible.

It is important to remember that today, modern transportation and globalisation help spread infectious diseases more rapidly. We therefore require effective products for the rapid detection of infections. Currently, most products do not meet the needs of developing countries at the local level. However, we strongly believe that unless widespread testing will become available in every area, infectious diseases will reach every world region, either poor or wealthy.

Thus, in order to serve the populations in the countryside and in poor countries, the available technology should be adapted to the specific needs and resources of these populations. To achieve this goal, the World Health Organisation has formulated the ASSURED guidelines, which define the characteristics of the ideal diagnostic technology. Such a technology should be Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free and Deliverable. These characteristics should apply to all the three stages of Nucleic Acid Testing: Sample preparation, Amplification, and Detection.

It is noteworthy that simplifying the diagnostic process required extensive technology development. I believe a useful analogy is the photography sector, and the shift from complex, manual cameras, which few people could afford and operate, to simpler, lower-cost, digital cameras. This shift was made possible through advancements in electronics.
II. USTER TB Diagnosis Technology Platform

I would now like to describe the TB diagnosis technology platform developed by USTER Biotechnologies.

1. Sample Preparation

Today, most sample preparation methods for tuberculosis diagnosis require at least a centrifuge, and usually more complex equipment. In order to simplify the preparation process, we developed a method that requires merely a syringe and a membrane.

The process is fairly simple. First, the sputum collected from the patient is mixed with a liquefying buffer, which is already included in the kit. Then, the liquefied sample is pulled through a membrane. At that stage, all the particles in the mixed solution, including the TB cells, remain on the membrane. Then, the liquid is discarded, and the membrane unit is placed back. At this stage, it is possible to wash the particles with a washing buffer by pushing the syringe up and down three times. Again, the bacteria cells would remain on the membrane. The washing buffer is then discarded.

During the next step, the TB bacteria is washed down into the lysing buffer. An incubation of five minutes at 95 degrees Celsius is performed, in order to lyse and kill the bacteria. Then, the same membrane is used to pull the lysing buffer through the membrane. At this stage, the bacteria DNA would be lyzed in the solution. Thus, the DNA would remain in the syringe, and the solid waste would be filtered. Finally, the DNA extract is collected in a fresh tube, and the sample is ready for the amplification stage.

2. Amplification

The second stage of the diagnosis process is amplification. Today, most hospitals use complex, expansive amplification instruments such as PCR. Unfortunately, most small clinics in developing countries cannot afford to purchase such a machine, and their employees lack the skills required to operate it. We have therefore attempted to develop an amplification method that is simple, rapid, and requires either portable or no instruments.

One of the main reasons PCR is so complex and expansive is that it requires up to 35 thermal cycles in order to perform the amplification process. Our strategy was to simplify the process by using isothermal amplification, which requires merely a single temperature. The only instrument required for the process is a thus water bath or a thermal cup with a thermometer.

One of the simplified amplification technologies we developed is called NEMA - Nicking Enzyme Mediated Amplification. While the forward primer of the NEMA process is linear, rather than exponential, any strand produced during the forward amplification could be later used as a template for the next round of amplification, which utilises a reverse primer. Thus, the final amplification process is exponential, and is actually significantly faster than PCR.

The operation of the amplification process is rather simple, and requires merely a 30-45 incubation in a single temperature of 65 °C.
3. Detection

The final stage of the process is detection. Real-time PCR machines provide a ratio that reveals whether the result is positive of negative. However, since our goal is to provide an instrument-free test, we were obliged to devise a simple, more visual, detection tool.

We decided that it would be most convenient to use the strip technology. Most people are already familiar with this technology, since it has been used for urine tests for the past 30 years. Naturally, several modifications were necessary in order to adapt the technology to the detection of nucleic acids.

The strip employs coloured nano-particles, which are coated with an antibody, such as Anti-A, which would bind to the analyte in question. Then, during the amplification stage of the nucleic acid, one of the primers is labelled with an Anti-B antibody, while one of the detection probes is labelled with an Anti-A antibody. The strip includes two lines, one for control, and one for the test itself.

When the specific amplicon is present, the labelled probe and the amplicon would form an hybridisation complex that contains both A and B antigens. When this complex flows through the test membrane, it would be captured by the antibody on the test line of the strip, and detected by the coloured nano-particles, indicating a positive result. On the other hand, when the specific amplicon is not present, the complex would not form, and the coloured nano-particles would go through the test line straight to the absorbent paper. No colour would be marked at the Anti-B line, indicating a negative result.

Developing the strip technology for detecting nucleic acids took several years. The first strip was introduced in 2004, and since then, it has been significantly improved. I believe this strip is a revolutionary tool, since it can be easily used in local clinics in the countryside. In the future, we aim at developing a kit that would combine the three processes.

4. Cross-Contamination Proof

One the significant challenges of diagnostic tests is the problem of cross-contamination in the laboratory, and the false-positive results that it might incur. For that purpose, we have developed an amplification and detection process that can take place in an enclosed environment. The basic idea is to detect the amplicon without ever opening the amplification reaction tube.

5. Transportation and Storage of Reagents

Another significant problem is that most of the existing biological reagents technology utilises the liquid form, which requires freezing conditions for transportation and storage. However, developing countries and the countryside, which often need to transfer the test solutions of diagnostic reagents to hospitals or more sophisticated laboratories, do not possess the required refrigeration devices.

However, there are three other states of bio-reagents – rubber, glass, and freeze-dry. By freeze-drying the reagents, we can ensure that they are safe for transportation for short periods of time, without further requirements. In the future, we plan to explore the glass state, which would enable the storage of reagents at room temperature for a couple of years.
III. Conclusions

1. Satisfaction of the ASSURED Guidelines

Returning to the ASSURED programme, I believe we have managed to develop a testing device that answers most of the requirements.

- It is affordable, since it requires no setup costs, and almost no instrument costs. The R&D and manufacturing costs in China and Indonesia are also low.
- It is highly sensitive, as the device can detect less than 5 bacteria or virus.
- It is highly specific, since it detects merely the target pathogen. In fact, our data indicates that its specificity is almost 100%.
- It is user-friendly, which was one of the most difficult challenges. It is simple, easy to operate, and does not require highly-trained personnel.
- It is rapid and robust. The sample-to-result process takes approximately one hour. The amplification stage takes around 45 minutes, and sometimes as little as 30. The preparation stage takes around 15 minutes, and the detection stage takes around 10 minutes. Furthermore, the results are repeatable, indicating that the process is stable.
- It is almost equipment-free, since water bath or a thermal cup are sufficient for conducting the test.
- With respect to delivery, today the products are already shipped at an ambient temperature. We hope that in the future they could also be stored at such a temperature.

2. Clinical Trials

USTAR has already registered several patents for its technology. Several products are already ready for large-scale clinical trials, not merely in the field of Mycobacterium tuberculosis, but also in the area of Hepatitis B, Hepatitis C, and Chlamydia.

USTAR has collaborated with the Shanghai CDC on the laboratory testing of M. tuberculosis. According to the data obtained, the product’s sensitivity is rather high. When we compared the USTAR technology to the 3D liquid culture, we found out that both provide a specificity of 100%. USTAR’s technology has a sensitivity of 93%, compared to the 100% recorded by the 3D liquid culture. We have also conducted an HBV clinical trial with the Zheijian University School of Medicine. The results indicated a 99% sensitivity, and a 100% specificity.

I thus firmly believe that this device is ready for large-scale testing, and I would like to cooperate with additional hospitals in this aspect, both in China and in other countries. Finally, I would like to note that the applications of this platform are not limited to the infectious diseases, but that we are also examining other areas, such as SNP detection technology.
IV. Question and Answer Session

Participant

What is the sensitivity of other clinical samples, such as CSF?

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So far, we have focused merely on sputum. However, we will test all the other samples in the future.