



HKSH Introduces Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Biotyper

Bacterial Identification Speed Doubled with 90% Accuracy

(13 September, 2012 – Hong Kong) Hong Kong Sanatorium & Hospital (HKSH) is the first private hospital in Hong Kong to introduce Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometer for clinical service. With the innovative methodology of analyzing protein components of microorganisms and comparing them to reference patterns in a database, MALDI-TOF reduces the time needed for bacterial identification by over a half, allowing doctors to pinpoint the cause of illness and provide pertinent treatment at the earliest opportunity and increase patient survival. This state-of-the-art technology provides greater efficiency and accuracy than conventional bacterial identification processes, thereby facilitating earlier and more precise treatment plans. Conventionally, bacterial identification takes a minimum of 2 days from culture of bacteria to testing. Now with MALDI-TOF, the testing takes less than 1 hour after 1-day culture of bacteria.

Research has shown that patients given correct antibiotic treatment within one hour of onset of septicemia due to microbial infection has a survival rate of 80%. However, if treatment is delayed, survival rate drops by 8% every hour starting from 6 hours after onset.¹

Conventional Biochemical Bacterial Identification Processes Require At Least One Day For Each Test

Previously, most conventional means of microorganism identification are based on biochemical tests, which are applied in fields such as medicine and food quality control. Disease-causing microorganisms may include bacteria, fungi and mycobacteria. After a sample is taken, cultures are grown on an agar plate (figure 1), where some of the bacteria will develop into various single colonies after a day.

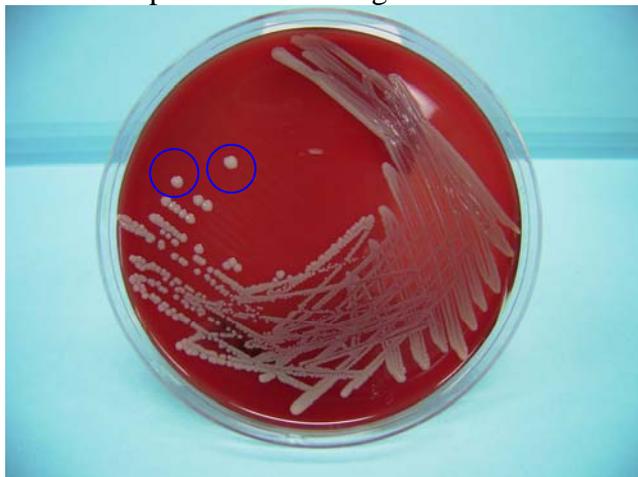


Figure 1: Single colonies (as shown by blue circles)

From there, a sample is extracted from any single colony formed. Reagents are selected to be used on that sample based on the experience and knowledge of the technologist, who infers the possibilities for the identity of the microorganism in question based on the visual characteristics of the sample culture, thereby gauging its usual metabolism pattern and picking out relevant tests to pinpoint its species (figure 2). For instance, Bacteria A, B and C all grow into cultures of a similar appearance, but each metabolises different reagents.

Hence, all these possible reagents would be applied to samples from the culture and left for at least a day to await the completion of metabolism, with certain microorganisms requiring more time. After that, any colour change or other chemical changes would be analysed by the computer against known characteristics in its database, with a 90-odd percent certainty deemed as acceptable. Alternative tests are performed when results are less than conclusive, which would further push back the availability of results. Currently, 50% of cases can be identified within 2 days, with the remaining half requiring 3 days or more. In one recent case where a Hospital patient was infected with the *Burkholderia pseudomallei*, confirmation of the species required 11 days – a less than ideal situation for both doctor and patient.

Dr. Bone Tang, HKSH Specialist in Clinical Microbiology and Infection has recently published a paper which details the successful use of MALDI-TOF to promptly identify *Burkholderia pseudomallei*, with results ready in 3 days. “Prompt identification of highly-contagious infectious agents like *Burkholderia pseudomallei* can aid both in early treatment and infection control,” remarks Dr. Tang.



Figure 2: Conventional bacterial identification processes apply various reagents respectively to the bacteria in question, and determine the species according to its biochemical reactions. Under 1,000 species of microorganisms can be identified with this method. About 20% unidentifiable cases using the conventional method would be further investigated using DNA sequencing.

State-of-the-Art Process Identifies Bacteria with Mass Spectrometry Within One Hour In One Go

The MALDI-TOF employs a completely different approach, namely mass spectrometry. Each species of microorganism has a unique set of proteins. After a sample is taken from a single colony formed on an agar plate grown from the bacteria in question, a matrix solution is added to it, which breaks down the bacterial proteins in preparation for analysis. This matrix/sample mixture is then applied onto a steel plate and fed into the MALDI-TOF Biotyper (figure 3), where a laser beam irradiates and evaporates the matrix solution, releasing positive protein ions from the steel plate.



Figure 3: MALDI-TOF

1. Matrix solution breaks down bacterial protein and triggers ionisation. The laser vapourises the ionised protein molecules.
2. These ions are now suspended in vacuum and attracted by an electrostatic field, travelling at a speed directly proportional to their mass – lighter protein ions travel faster, heavier protein ions slower – until they reach a detector.
3. With the difference in their arrival time recorded, the speed pattern of the ions can be precisely measured, which in turn yields an accurate molecular mass. This is referred to as the “Molecular Fingerprint”, which is a species-specific characteristic mass for the majority of microorganisms.
4. Results are cross referenced with database, yielding identification of up to 4,600 entries of Molecular Fingerprints, which covers 95% of microorganisms which cause diseases in humans.

90% overall accuracy Direct Blood Culture Detection Possible

Should there be any unknown patterns recorded, the sample would be sent for sequencing, and cross referenced against any known academic findings, in order to identify the exact species and strain of the sample. Coupled with updates twice a year with the newest findings of the rest of the region, MALDI-TOF’s current identification success rate of over 90% is on a continuous rise.

“The Hospital introduces MALDI-TOF with the aim of reducing the time needed for bacterial identification while increasing its accuracy, allowing doctors and patients to formulate precise treatment plans at the earliest opportunity, which in turn minimises the patient’s stay and maximises survival,” states Dr. Edmond Ma, Director of Clinical Pathology Molecular Pathology of HKSH.



In addition, MALDI-TOF can speed up analysis of the cause of blood stream infections, which may cause fever and septicemia in patients. Once a blood sample is obtained, it is added to a liquid culture solution and placed into an analysis machine. If bacteria are present in the sample, the machine would sound in alarm. With the conventional methods, the positive blood culture would be further cultured in a plate and tested, where a minimum of 2 days is required. Using MALDI-TOF, the positive blood culture can be tested directly within one hour, expediting treatment.

HKSH Specialist in Clinical Microbiology and Infection Dr. Bone Tang comments, “MALDI-TOF can go as far as to calculate the possible cultures in the bacterial mixture, and a re-test can immediately be arranged without the days’ wait for metabolism of reagents which plagues conventional biochemical tests. Reduced turnaround time means more than efficiency; it translates into faster treatment for patients by minimising the need for multiple antibiotics while awaiting conclusive test results, and maximises the window of opportunity for contingency management, should a potentially contagious sample be identified, thereby protecting both the patient as an individual and the safety of the staff and visitors to the Hospital as a whole.”

References

- ¹ KUMAR, A. et al. Crit Care Med 2006, Vol. 34, No. 6 “Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock.”
- ² LAU, S.K.P. et al., Journal of Clinical Microbiology, Sep 2012, Vo. 50, No. 9 “Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry for Rapid Identification of Burkholderia pseudomallei: Importance of Expanding Databases with Pathogens endemic to Different Localities.”

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About Hong Kong Sanatorium & Hospital

Hong Kong Sanatorium & Hospital is one of the leading private hospitals in Hong Kong. With the motto “Quality in Service Excellence in Care”, the Hospital is committed to serving the public as well as promoting medical education and research.

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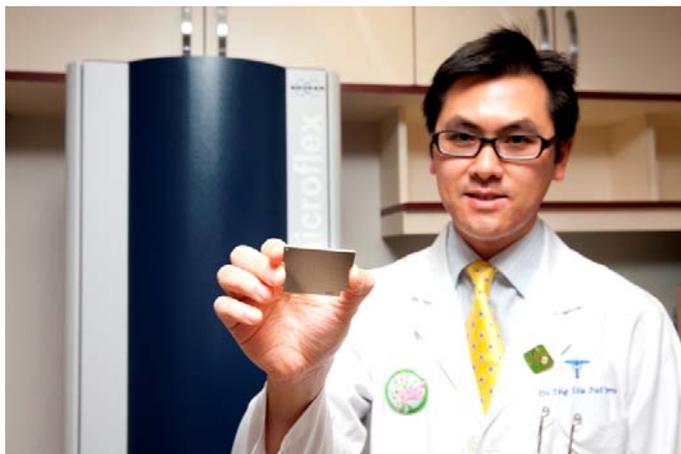
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Photos:

1. Dr. Edmond Ma, Director of Clinical Pathology / Molecular Pathology of HKSH remarked that HKSH is introducing MALDI-TOF to elevate the accuracy of identification of pathogenic microorganisms to 90%. With the 10% unidentifiable cases, DNA sequencing would be used for further analysis.

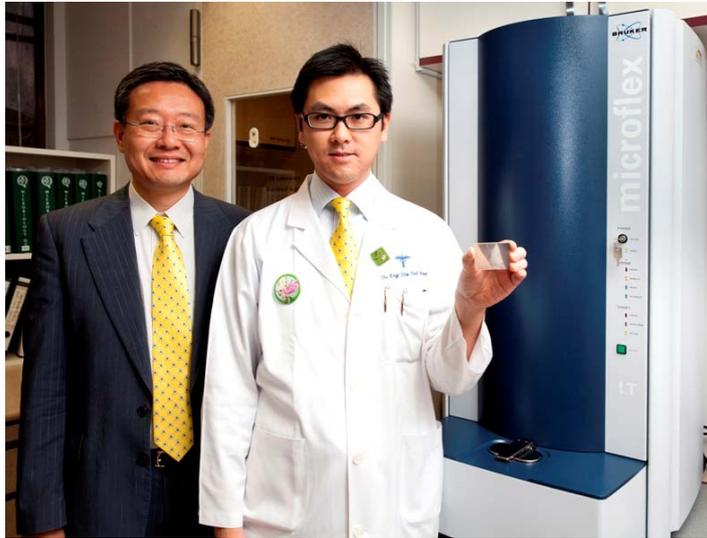


2. Dr Bone Tang, Specialist in Clinical Microbiology and Infection explained that the MALDI-TOF employs a completely different approach, namely mass spectrometry. Each species of microorganism has a unique set of proteins for analysis.





3. Dr. Edmond Ma, Director of Clinical Pathology / Molecular Pathology of HKSH (Left) and Dr Bone Tang, Specialist in Clinical Microbiology and Infection (right)



4. Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Biotyper



5. Technician demonstrating the operation of MALDI-TOF Biotyper



6. The Sample Plate of Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Biotyper



7. By generating and recording a characteristic mass and intensity distribution of the mainly proteins, it illustrates a 'molecular fingerprint', which is species-specific for a large number of microorganisms.

