BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024, Tamil Nadu, India

Programme: M.Sc., Biomedical Science

Course Title : Drug Discovery and Assay Development

Course Code : 18BMS48ES

Unit-IV

Antimicrobial Assay

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- To determine susceptibility to the antimicrobial agents
- To detect possible drug resistance in clinical microbiology laboratories.
- No yet Any reported resistance of *Streptococcus pyogenes* to penicillin
- In the case of routine methods for AST, it usually takes at least 24 h to obtain growth of bacterial colonies and an additional 24 h to obtain isolate characterization, namely biochemical identification and phenotypic AST

Minimum Inhibitory Concentration (MIC)

- The methods generally used afford qualitative results, using the susceptible, intermediate or resistant categories, quantitative results

- The MIC is defined as the lowest drug concentration that inhibits visible growth of a micro-organism after a certain incubation.

Agar disk-diffusion method

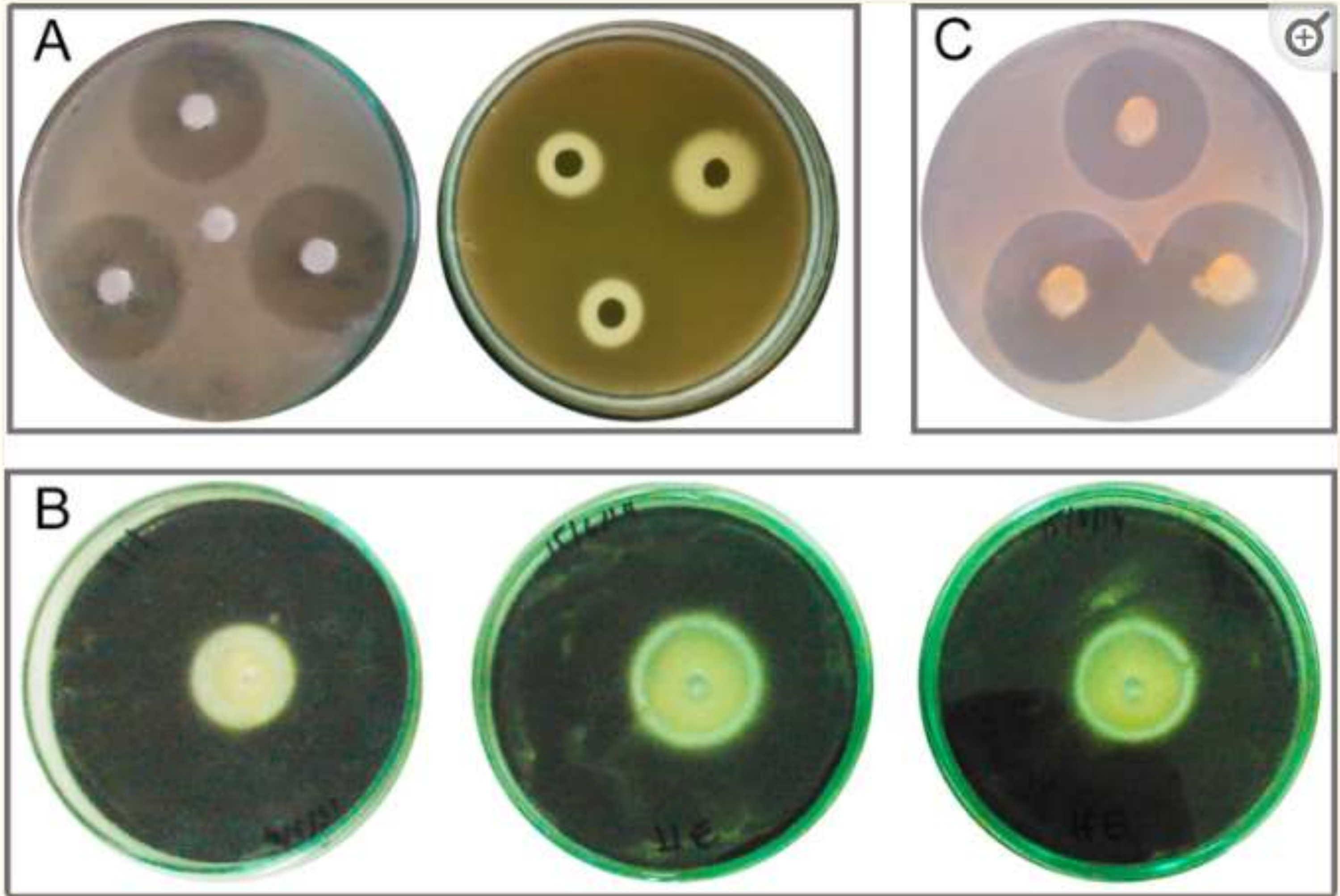
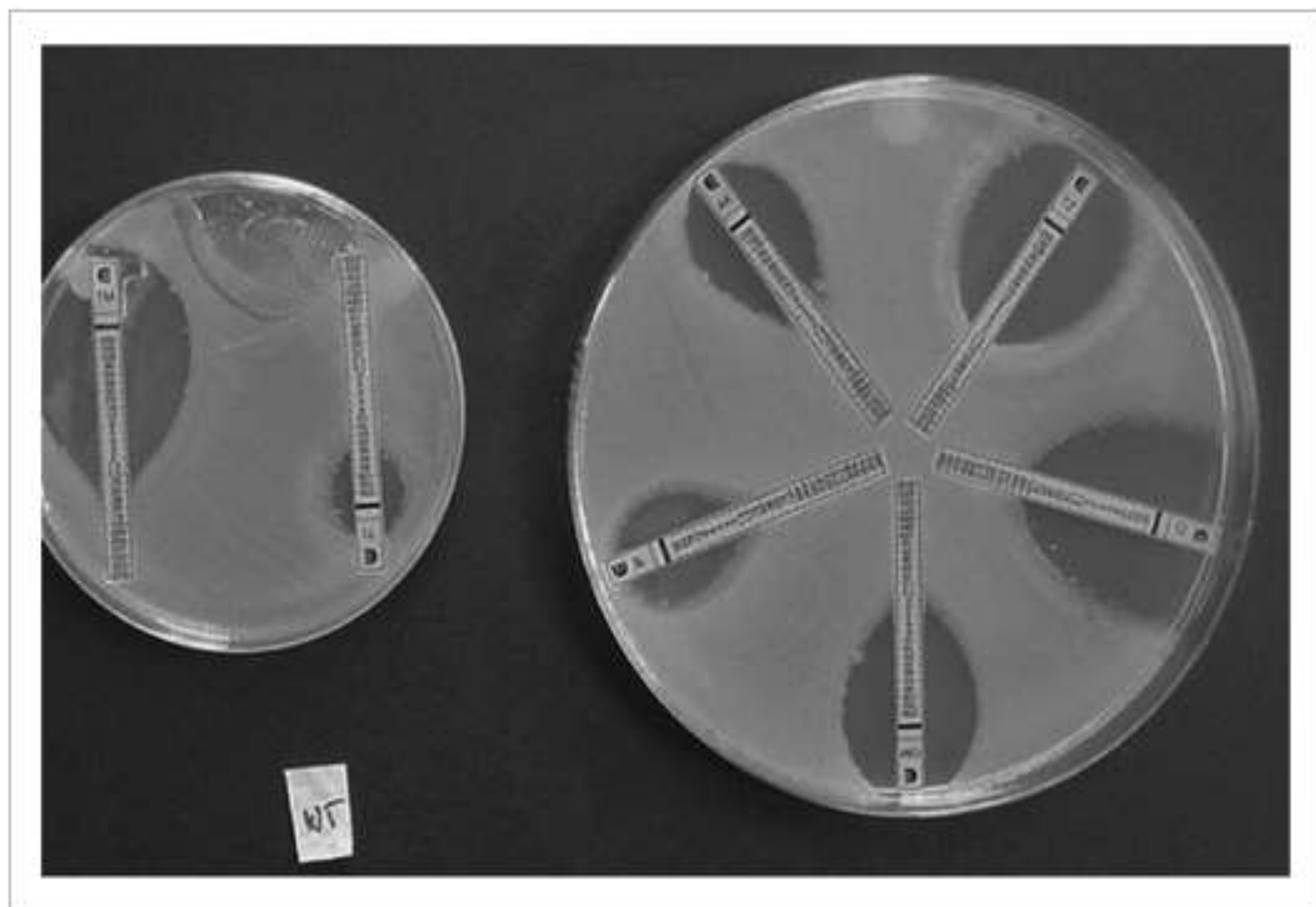


Fig. 1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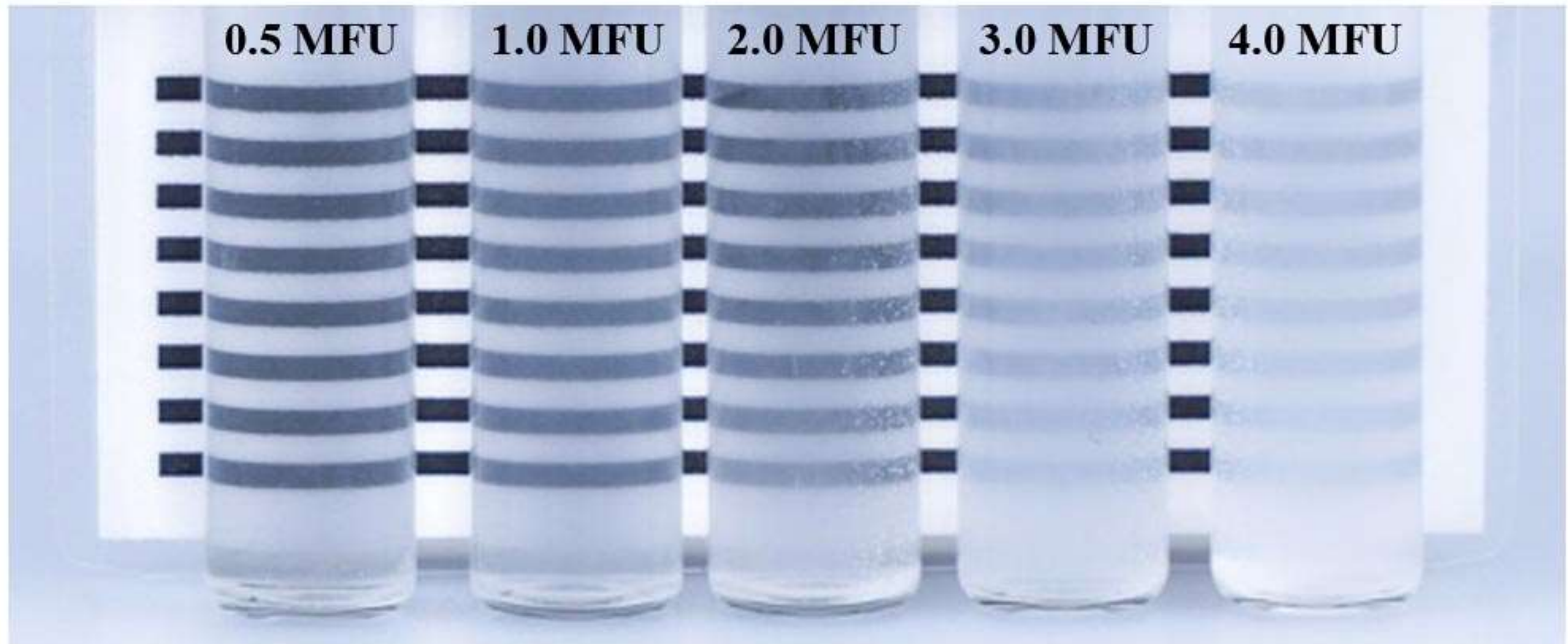
- A McFarland Standard is a chemical solution of barium chloride and sulfuric acid. The chemical reaction between these two chemicals results in the production of a fine precipitate of barium sulfate. After shaking well, the turbidity of a McFarland Standard is visually comparable to a bacterial suspension of known concentration.

McFarland Standards



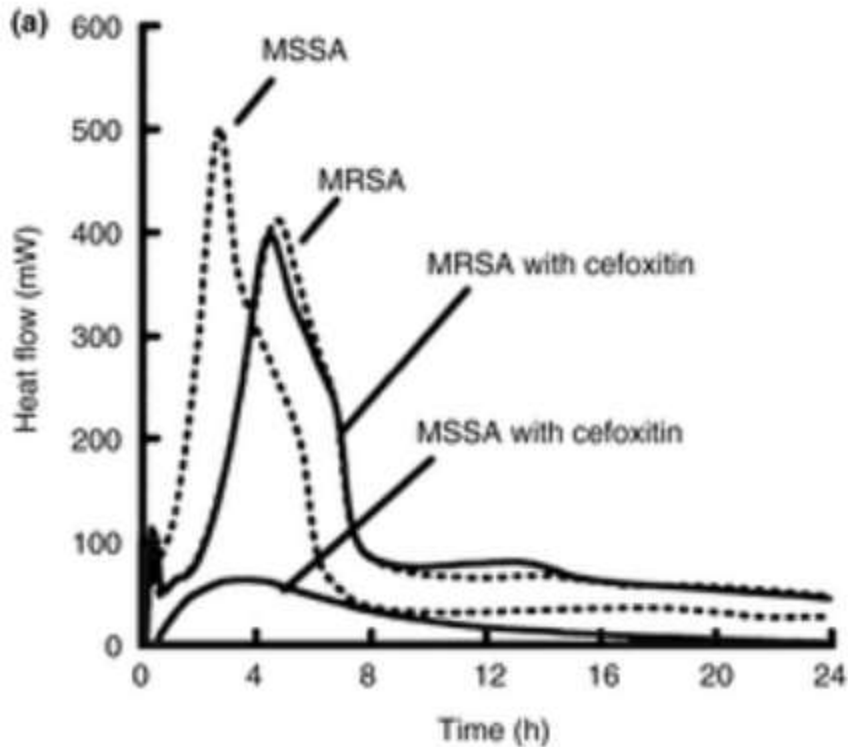
- McFarland Standard 0.5
- 1% $BaCl_2$(ml) 0.05
- 1% H_2SO_4 (ml) 9.95
- Approximate Cell Count Density ($\times 10^8$ cells)
- 1.5×10^8

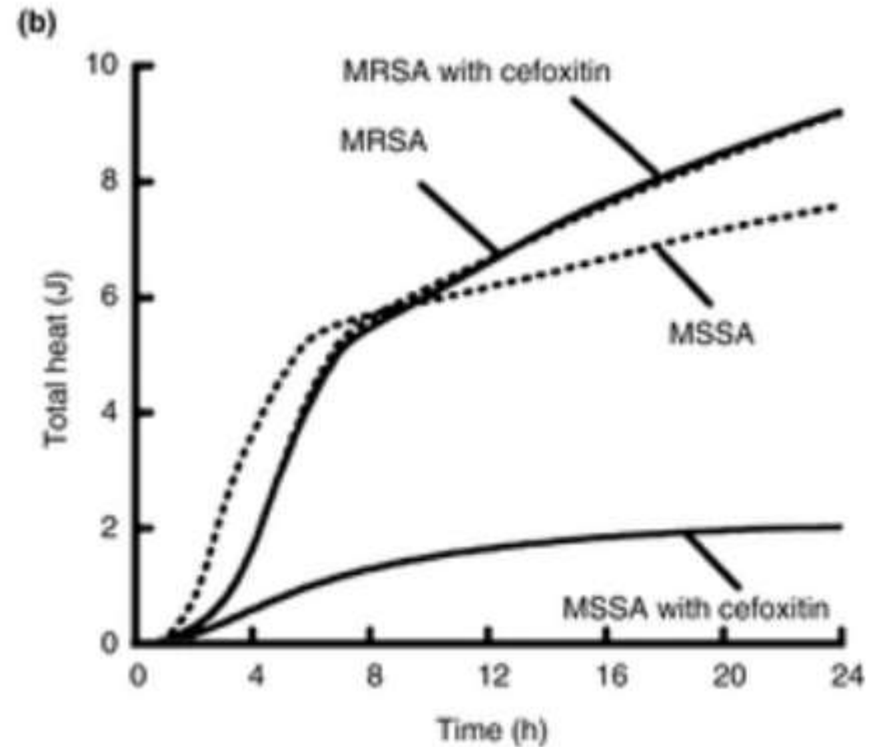
- The procedure is performed by preparing twofold dilutions of the antimicrobial agent (expressed in μg ml^{-1}) in a liquid growth medium dispensed in test tubes containing a minimum volume (2 ml) of the standardized microbial suspensions adjusted to 0·5 McFarland turbidity scale
- Following overnight incubation at 37°C for 24 h (bacterial strains) or at 25°C for 4–10 days (fungal strains), the tubes are examined for the presence of visible microbial growth by turbidity.
- The lowest concentration of the antimicrobial agent where the growth was completely inhibited (no turbidity) represents the MIC .

Isothermal microcalorimetry

- Using real-time (IMC), the heat generated or consumed by chemical or physical processes in micro-organisms can be measured.
- Cumulative heat production increases or decreases in parallel with growth curves, and correspondingly with lag, log and stationary phases.
- The total number of cells, that is, **maximum bacterial growth rate, is represented by maximum heat production.**
- The antibiotic dose, at first, increases the rate of heat production due to the activity of resistant mechanisms that are energy-dependent, and afterwards **heat production decays due to the loss of activity and death of the bacteria**

- *S. aureus*, *E. coli,* methicillin-resistant *S. aureus* (MRSA) , *Mycobacterium* sp. and *Aspergillus* sp.
- The major advantages of the method include testing in sealed vials that moderates safety
- The possibility to differentiate quickly bacterial species susceptible or resistant to antibiotics





Graphs show (a) heat flow and (b) total heat generated by growth of methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA)

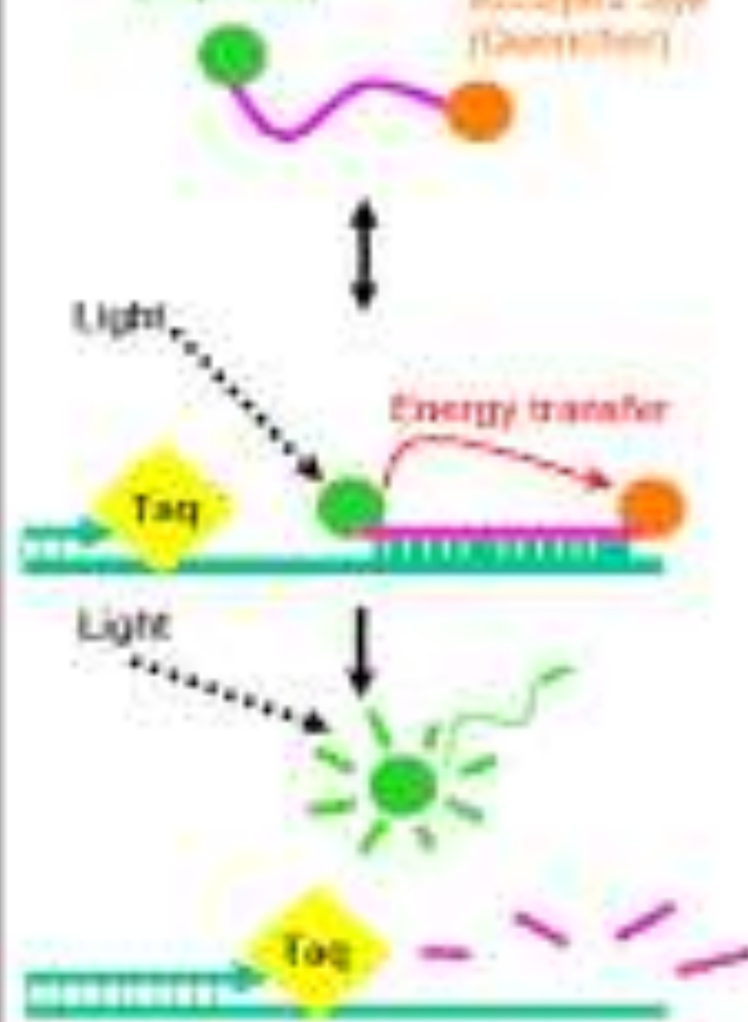
Polymerase chain reaction-based methods

- PCR relies on the amplification of DNA sequences that are specific for a particular pathogen and its drug susceptibility or resistance.
- PCR was initially used for the rapid identification and quantification of the pathogen
- Developed to detect the presence of genetic determinants of resistance to different antimicrobial agents

Real-time PCR,

- using hydrolysis probes, hybridization probes, or double-stranded DNA-binding fluorescent dyes
- uses the ability to quantify the number of specific nucleic acid copies in a clinical sample for measuring the growth of bacteria in the presence of the antibiotic being tested, and indirectly measures phenotypic resistance.
- Moreover, the sample does not always need to be purified, can be nonsterile, and may contain bacterial mixtures.
- In general, PCR-based methods are efficient, reliable and fast screening tools with high specificity and sensitivity, and with the test results available within 2 h

- *mecA* gene, commonly found in MRSA, encoding a modified penicillin-binding protein PBP2a with reduced affinity to beta-lactam antibiotics
- This method can also detect the presence of resistance genes in gram-negative bacteria (such as carbapenemase- and cephalosporinase-encoding genes)
- vancomycin resistance associated with the *vanA* and *vanB* genes primarily for resistance in *Enterococcus* species
- The gene is amplified using *Bst* DNA polymerase instead of *Taq* polymerase at a constant temperature of 60–65°C



Unbound probe free in solution

Probe and primer bind target, FRET occurs

Taq polymerase extends and hydrolyzes probe, donor dye free to emit fluorescence → accumulation of signal

MALDI-TOF MS

- Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
- Determination of mass/charge (*m*/*z*) ratio and strain differentiation using their ribosomal proteins
- MALDI-TOF MS does not depend on metabolic activity and can directly identify within minutes colonies of Gram-positive and Gram-negative bacteria and yeasts grown on agar plates

- MALDI-TOF MS is cocrystallization, where the crystal is created by mixing the microbial sample (for bacteria 10^4–10^6 CFU) and a matrix solution (a benzoic acid or cinnamic acid derivative)
- Placed on the steel surface of the target plate to dry at room temperature.
- The target plate can hold up to 384 samples.
- The spotted target is then inserted into the machine and transported to the measuring chamber in the vacuum environment.
- The laser beams desorb the ribosomal proteins of the bacteria or yeasts, contributing to ionization of the proteins.
- Using an electromagnetic field, the ions are accelerated in a pulsed fashion intjo a linear flight tube.
- The molecular mass (m) and the charge (z) determine the time of flight (TOF) of the desorbed particles.
- The TOF is measured using a detector at the end of the vacuum flight tube. The generated mass spectrum is compared to reference spectra in the database by the use of algorithms, and the resulting fingerprint is unique for an individual species

Acknowledgement

- The Presentation is being used for educational and non commercial Purposes
- Thanks are due to all the original contributors and entities whose pictures were used in the creation of this presentation.