Automatisasi Pemeriksaan Mikrobiologi

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Pemeriksaan Mikrobiologi Klinik

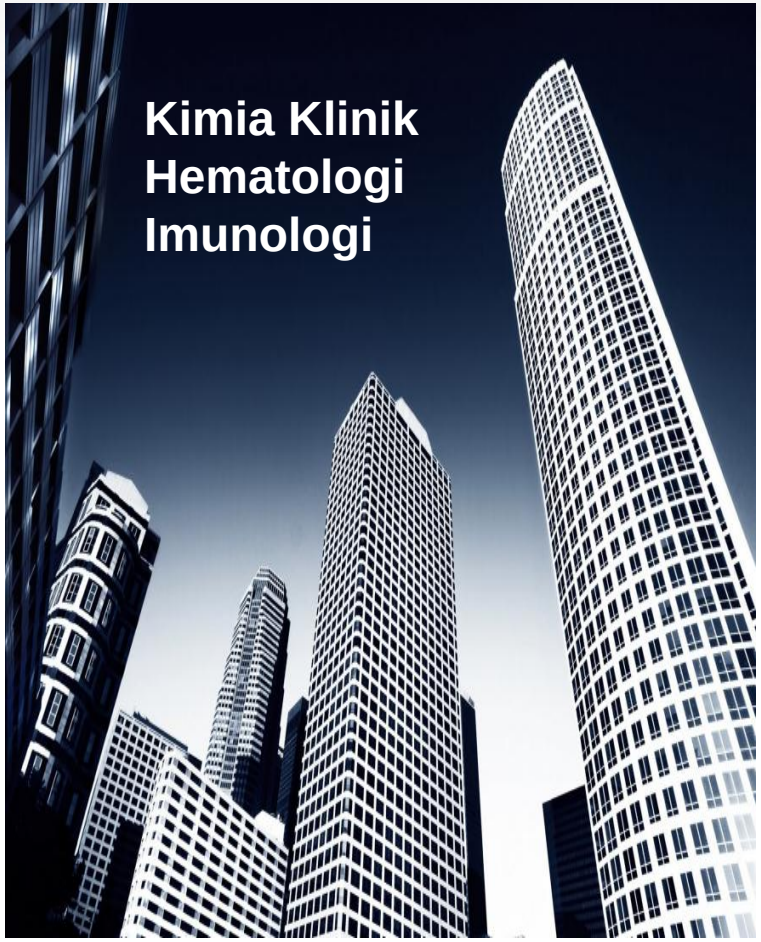
- merupakan suatu proses yang kompleks
  - ➢ Jenis mikroorganisme >>>
  - ➢ Cara Pengambilan sampel berbeda
  - ➢ Teknik pembiakan bervariasi
- membutuhkan tenaga ahli di bidangnya
- sarana prasarana yang memadai menunjang kualitas hasil pemeriksaan

Pemeriksaan Konvensional Mikrobiologi

- Proses pemeriksaan rutin meliputi
  - o pengambilan sampel
  - o pengecatan
  - o pembiakan
  - o identifikasi
  - o tes kepekaan antibiotika (TKA) bakteri.
- Tahapan yang panjang → memungkinkan terjadinya kesalahan → ketidaktepatan hasil

Automatisasi Mikrobiologi Klinik

VS



Hambatan Perkembangan automatisasi

- proses pemeriksaan yang sangat kompleks
- kemampuan mesin di bidang mikrobiologi dianggap tidak dapat menggantikan kemampuan manusia
- harga lebih mahal
- desain ruangan laboratorium yang kecil sehingga sulit dilengkapi alat automatis.

Faktor Pendukung Automatisasi

- tenaga ahli profesional di bidang mikrobiologi <<
- perkembangan inovasi teknologi yang pesat
- perubahan iklim industri kesehatan
- keinginan meningkatkan kualitas pelayanan.
- peningkatan kasus infeksi dan resistensi antibiotika
- Percepatan *turn around time*
- Perkembangan swab dengan media transpor cair.

Eswab

Perkembangan Automatisasi Mikrobiologi

- alat automatis pengecatan hapusan Gram
- alat automatis pemrosesan sampel/isolasi
- inkubator automatis
- pencitraan digital
- alat identifikasi automatis
  - reaksi biokimia
  - biologi molekuler (x)
  - MALDI-TOF Mass Spectometry
- alat TKA/AST automatis.
- *Full microbiology laboratory automation system/ Total Laboratory Automation*

Alat Automatis Pengecatan Gram

Alat Automatis Pengecatan Gram

Alat Automatis Pengecatan Gram

Prinsip Automatisasi :

1. Pencelupan slide ke wadah cat secara automatis
   - •Rawan Kontaminasi
   - •Petugas masih terpapar bahan kimia toksik
2. Penyemprotan cat ke slide dalam sistem tertutup dengan rotor berputar
   - • Reagen minimal (10 % pengecatan manual)
   - • Sistem tertutup :petugas terlindungi dari bahan kimia toksik
   - • Kualitas slide homogen, kontaminasi (-)
   - • Waktu cepat, jumlah slide >>

Microbiology : PREVI™ Color Gram

2

BIOMÉRIEUX

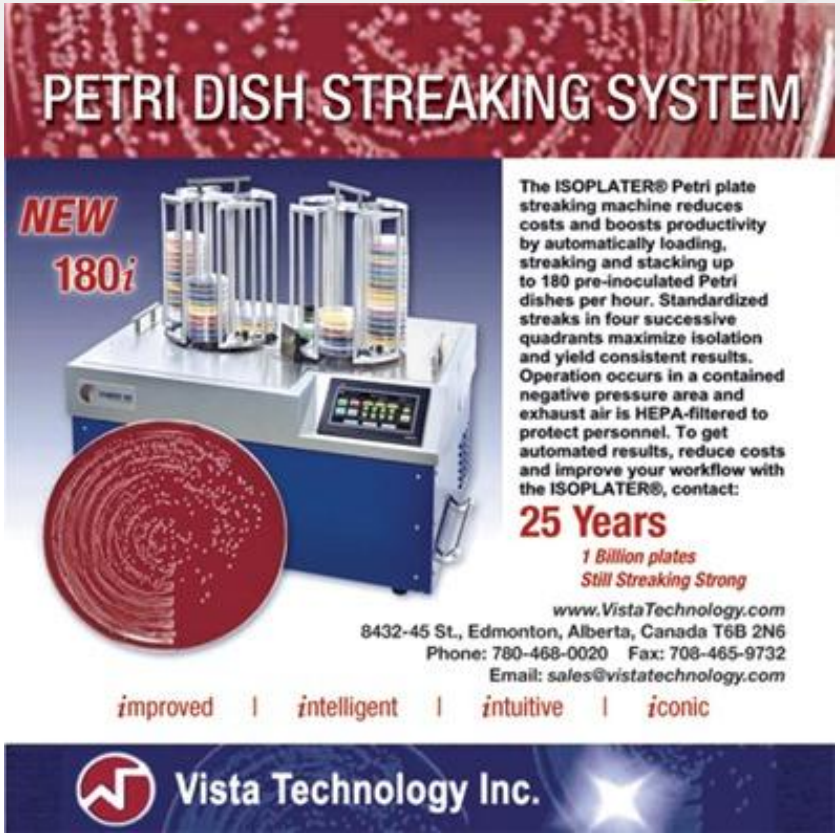
The Poly Stainer , IUL., S.A

1

Alat Automatis Pemrosesan Sampel/Isolasi

- Generasi I :
  - Isoplater (1989)
  - InoquLab
  - Semiautomatis (sampel diletakkan di *plate* → *streaking* automatis)
  - Streaking : *wire loop* dengan pola spiral

- Generasi II : *InoquLab LQH System*
- Generasi I dan II :
  - Efisiensi <
  - Troughput <
  - Akurasi <



https://www.google.com/search?q=isoplater+image&biw=1252&bih=602&tbm=isch&tbo=u&source=univ&sa=X&ved=0ahUKEwiP9J7WqqHMAhUF2KYKHZToBgsQ7AkIMA#imgrc=VbBllaL96Id5OM%3A

Alat Automatis Pemrosesan Sampel/Isolasi

Generasi III

- Lebih efisien, akurat, throughput >>>
- Dapat memproses langkah-langkah :
  1 Memilih Cawan petri yang sesuai
  2 Inokulasi sampel secara efisien
  3 Menyebarkan inokulum pada lempeng agar dan terbentuk koloni yang terpisah dengan baik setelah inkubasi
  4 Pelabelan yang akurat

Alat Automatis Pemrosesan Sampel/Isolasi

Tipe instrumen Generasi III

- *the Innova processor (BD Diagnostics, Sparks, MD),*
- *the InoqulA full automation/manual interaction (FA/MI) specimen-processing device (BD Kiestra B.V., Drachten, Netherlands),*
- *the Previ Isola automated plate streaker (bioMérieux, Inc., Hazelwood, MO)*
- *the walk-away specimen processor (WASP; Copan Diagnostics, Murrieta, CA).*

Alat Automatis Pemrosesan Sampel/Isolasi

FIG 1 Innova specimen processor.

FIG 2 InoqulA specimen processor.

FIG 3 Previ Isola specimen processor.

FIG 4 WASP.

PP Beurbou, JCM.ASM.ORG, 2016

Perbedaan alat automatis Isolasi Bakteri

	WASP	Previ-Isola	Innova	Inoqula-FLA
Company (country)	Copan (Italy)	BioMerieux (France)	Becton-Dickinson (USA)	KIESTRA (The Netherlands)
Inoculation device	Calibrated loop[a]	Combs	Calibrated loop[a]	Beads
Type of inoculation	Four quadrants, single streaking, bi-plate, etc.	Circular inoculation (semicircular for bi-plate)	Four quadrants, single streaking, bi-plate, etc.	Four quadrants, single streaking, bi-plate, etc.
Use of dispensable devices	Re-usable metal loops	Disposable combs[b] and disposable pipette tips[c]	Re-usable loops, disposable pipette tips[c]	Re-usable beads, disposable pipette tips[c]
Agar plate loading capacity	Nine silos (~350 agar plates)	Five silos (270 agar plates)	Six silos (270 agar plates)	Six buffers[d] (720 agar plates)
Sample loading capacity	72 e-swab tubes	114 samples	200 containers (on 5 drawers)	NA
Productivity (plates/h)	180 plates/h	180 plates/h	180 plates/h	400 plates/h
Decapping/recapping	Automated	Manual[e]	Automated	Automated
Agitation/centrifugation	Automated agitation and centrifugation, per specimen[e]	No automated agitation/centrifugation	Automated agitation, per rack[f]	Automated agitation, per specimen[g]

Greub and Prod'hom.Clinical Microbiology and Infection ª2011 European Society of Clinical Microbiology and Infectious Diseases, CMI, 17, 655–660

Perbedaan Bentuk Streaking Alat automatis

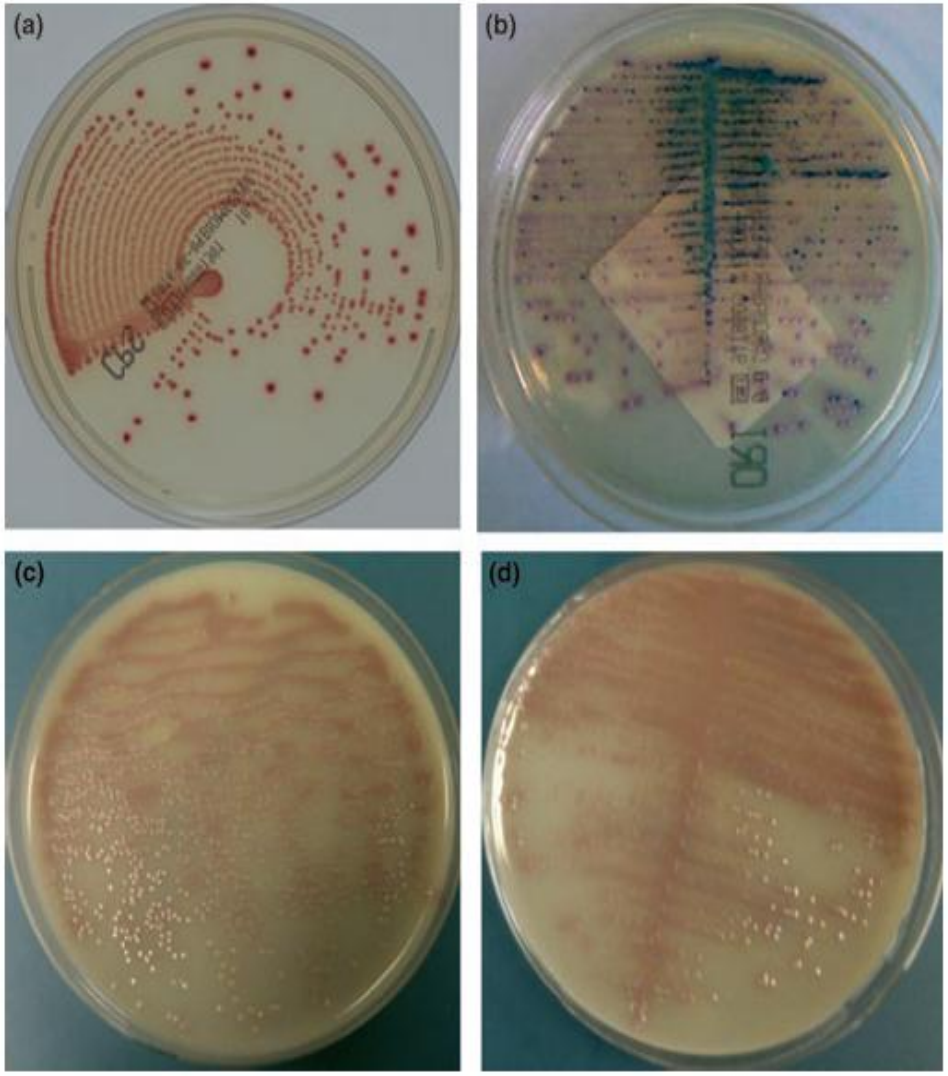


FIG. 2. Agar plates inoculated (a) with Previ-Isola, (b) with WASP, (c) withInoqula, and (d) manually. Note that isolated colonies were generally obtained, although high inocula were tested (about 10^6 bacteria/mL). The use of the Previ-Isola comb leads to circular semiquantitative inoculation (a), whereas, with WASP and Inoqula, both single-streaking (b, d) and four-quadrant inoculation (not shown) may be performed. Only 1 μL of urine was inoculated with the Inoqula bead (c), whereas 10 μL of the same urine was inoculated manually (d). Note that automated inoculation allow us to obtain more isolated colonies than manual inoculation, at least with Previ-Isola and Inoqula (Rice and Baruch, 109th ASM, 2009, Poster C064; Sturm *et al.*, 20th ECCMID, 2010, Poster 1766).

Automatic Incubator

Inkubator Konvensional

BacT/ALERT
Biomerieux

Automatic Incubator

BD Bactec

Automatic Incubator

- Kultur aerobik, anaerobik dan Mycobacteria
- Bakteri memproduksi CO^2 Larut dalam air membentuk ion H^+ pH menurun sensor *blue-green alkaline berubah menjadi* hijau-kuning terang dideteksi secara kolorimetri
- Waktu deteksi pertumbuhan lebih cepat
- Periode total inkubasi lebih cepat sampai dikatakan negatif

Deteksi Fluoresens

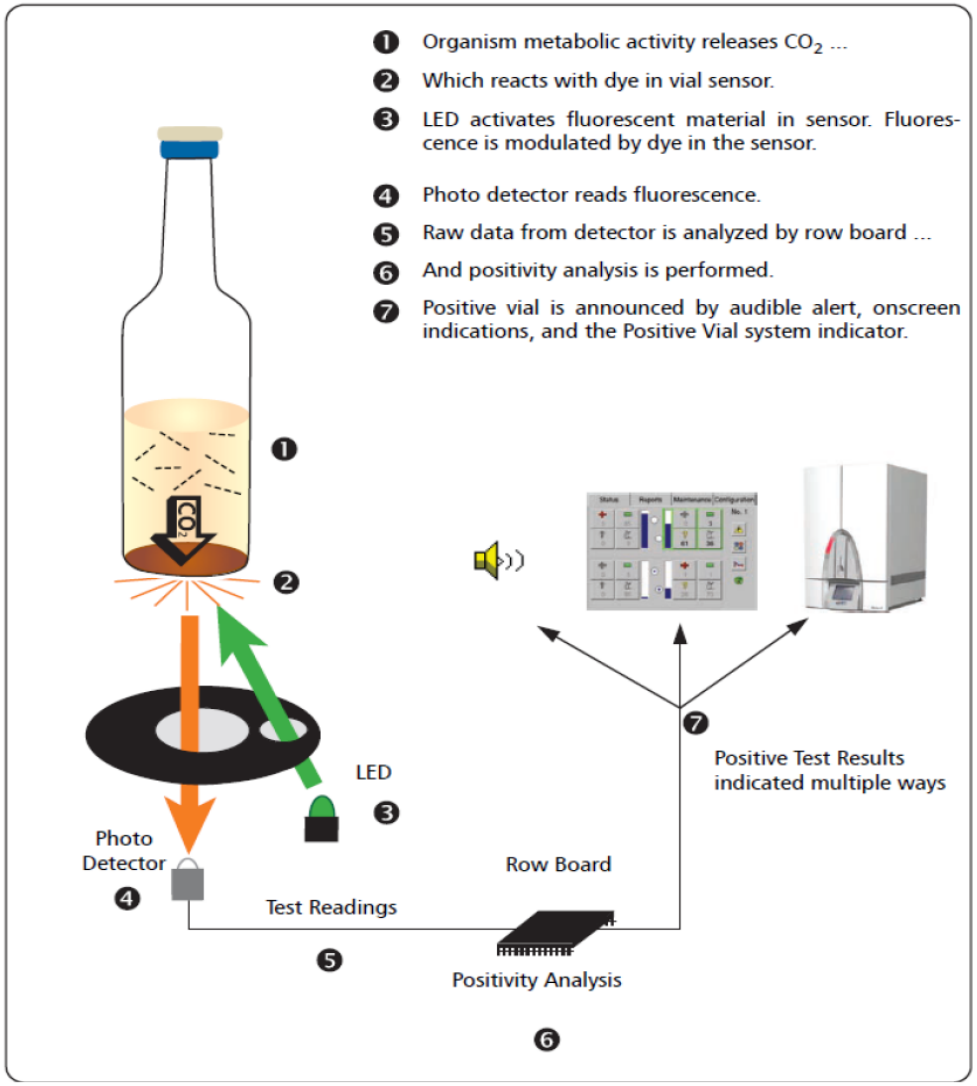


Figure 1 BACTEC Fluorescent Test Technology

BMS Diagnostics, 2014

ID/AST Konvensional

- Manual ID
  - Morfologi Koloni
  - Pengecatan Gram
  - Uji Biokimia
  - Uji Kepekaan antibiotika untuk spesies tertentu : optochin, bacitracin dll
  - Uji Serologi

- Manual AST
  - *Disk Diffusion*
  - *Agar Dilution*
  - *Broth Dilution*
  - *E test*

Alat automatis ID/AST

- *Vitek system (biomerieux Inc)*
- *Microscan WalkAway system (Dade International)*
- *Phoenix System (BD Mycrobiology system)*
- *Alfred 60/AST (Alifax)*

Vitek vs Phoenix vs Microscan

	MicroScan	Phoenix	VITEK 2
Time to perform daily maintenance	3.40 min	1.25 min	0.9 min
Time to set up one panel	3.53 min (with prompt^)	3.40 min	3.19 min
Size: Height/Width/Depth (inches)	34/39/28.5	41.7/44.3/29.5	26.3/39.4/27.7
Weight (lbs)	400	333	240
Average time to identification	2.5 h (Rapid)* 6 – 18 h (Conventional)*	4.3 h*	3 h 5.7 h (Colorimetric)
Average time to AST	20 h	12 h	9 h

*provided by company

^Prompt Inoculation System-D provides the microbiologist with a method for obtaining standardized inocula while eliminating the need for turbidity adjustment.

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St. Louis Children's Hospital[1], Missouri Department of Health and Senior Services[2] & Department of Pediatrics, Washington University School of Medicine[3]

As presented at the 105th General Meeting of the American Society for Microbiology, 2005.

ID: Vitek vs Phoenix vs Microscan

Identification Summary (Continued): Including Testing with VITEK 2 Colorimetric Card

Organism	# Tested	MicroScan Rapid					MicroScan Conventional					Phoenix				
		Match to Species	Match to Genus	Wrong ID	NO ID	Failure to fully ID	Match to Species	Match to Genus	Wrong ID	NO ID	Failure to fully ID	Match to Species	Match to Genus	Wrong ID	NO ID	Failure to fully ID
Enteric GNB	17	11	5			1	14	1			2	16	1			
Non-enteric	39	10	7		4	18	10	9	1	5	14	13	17	4	5	
Staphylococcus	7	4				3	5		2			4		2	1	
Streptococcus	11	10				1	7			1	3	8		2		1
Total	**74**	**35**	**12**		**4**	**23**	**36**	**10**	**3**	**6**	**19**	**41**	**18**	**8**	**6**	**1**
		47 (62%)					46 (62%)					59 (80%)				

Organism	# Tested	VITEK 2					VITEK 2 Colorimetric				
		Match to Species	Match to Genus	Wrong ID	NO ID	Failure to fully ID	Match to Species	Match to Genus	Wrong ID	NO ID	Failure to fully ID
Enteric GNB	17	1	6	1	1	8	7	9			1
Non-enteric	39	1	2	4	29	3	28	6	3		2
Staphylococcus	7	1		2	1	3	6		1		
Streptococcus	11				1	10	10			1	
Total	74	3	8	7	32	24	51	15	4	1	3
		11 (15%)					66 (89%)				

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AST : Vitek vs Microscan vs Phoenix

Summary of Major and Very Major Errors

Study #	Antibiotic	Organism	Reference MIC^	MicroScan	Phoenix	VITEK 2
135	Cephalothin	*K. oxytoca*	R	R	I	S
196	Clindamycin	*S. epidermidis*	S	R	R	S
63	Gentamicin	*S. aureus*	R	I	R	S
196	Levofloxacin	*S. epidermidis*	S	R	R	S
195	Levofloxacin	*S. haemolyticus*	S	R	S	S
102	Oxacillin	*S. lugdenensis*	S	R*	S	
289	Penicillin	*S. saprophyticus*	S	S		R
294	Timentin	*C. koseri*	S	S	R	S
28	Timentin	*E. coli*	R	S	R	I
66	Trimethoprim Sulfa	*S. maltophilia*	R	S	R	
150	Trimethoprim Sulfa	*S. maltophilia*	R	R	R	S
			Major errors	4	3	1
			Very Major errors	2	0	3

* MicroScan actual Mic value same as reference value; interpretation different

^ Reference broth microdilutions performed at Clinical Microbiology Institute, Wilsonville, OR 97070

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Alfred 60/AST

- Alfred 60AST : kultur bakteri, *residual antimicrobial activity test* (RAA test), AST secara automatis
- *Light Scattering Technology*
- Hasil Kuantitatif dalam CFU/ml
- AST automatis dengan panel antibiotik dapat diatur sesuai keinginan
- Deteksi pertumbuhan bakteri secara *real time*
- Turbidimeter terintegrasi dengan monitor Mc Farland

Alfred 60/AST

Organisms Detected by Alfred

Organism Type	Average Time to Detection
Enteric Gram negative bacilli (*E. coli*; *Klebsiella* / *Enterobacter* / *Serratia* / *Citrobacter*; *Proteus* / *Providencia* / *Morganella*)	1 hr. 59 mins. 23 secs. (< **2 hrs**)
Gram positive (Enterococci; Streptococci; Staphylococci; *Aerococcus*)	2 hrs. 30 mins. 46 secs. (~ **2.5 hrs**)
Yeast (*Candida*)	2 hrs. 20 mins. (**2.25-2.5 hrs**)
Afermenting Gram negative bacilli (*Pseudomonas*; *Acinetobacter*)	2 hrs. 23 mins. 20 secs. (**2.25-2.5 hrs**)
Mixed growth of doubtful significance; Non-urinary tract pathogens [coagulase negative Staphylococci (other than *S. lugdunensis* & *S. saprophyticus*), corynebacteria (other than *C. urealyticum*), Viridans group streptococci, lactobacilli]	2 hrs. 58 mins. 08 secs. (~ **3 hrs**)
No growth (< 1,000 CFU/mL)	**4 hrs. 25 mins.**
No significant growth (< 10,000 CFU/mL)	**4 hrs. 25 mins.**

Lab. Connections, April 2013, Issue#124

Keuntungan penggunaan Alfred 60/AST

- Klinisi dapat memulai terapi antibiotik yang benar sesegera mungkin
- Evaluasi harian pasien
- Menurunkan *hospital length of stay (LOS)*
- Mengurangi beban biaya perawatan
- Menurunkan penggunaan antibiotik spektrum luas yang dapat berakibat peningkatan resistensi dan infeksi nosokomial.

MALDI-TOF MS

- *Matrix Assisted Laser Desorption/Ionization (MALDI)- Time of Flight Analysis (TOF)*
- *Mass Spectrometry*
- *Contoh :*
  - *Vitek2 MS*
  - *Bruker MALDI® Biotyper System*

MALDI-TOF MS

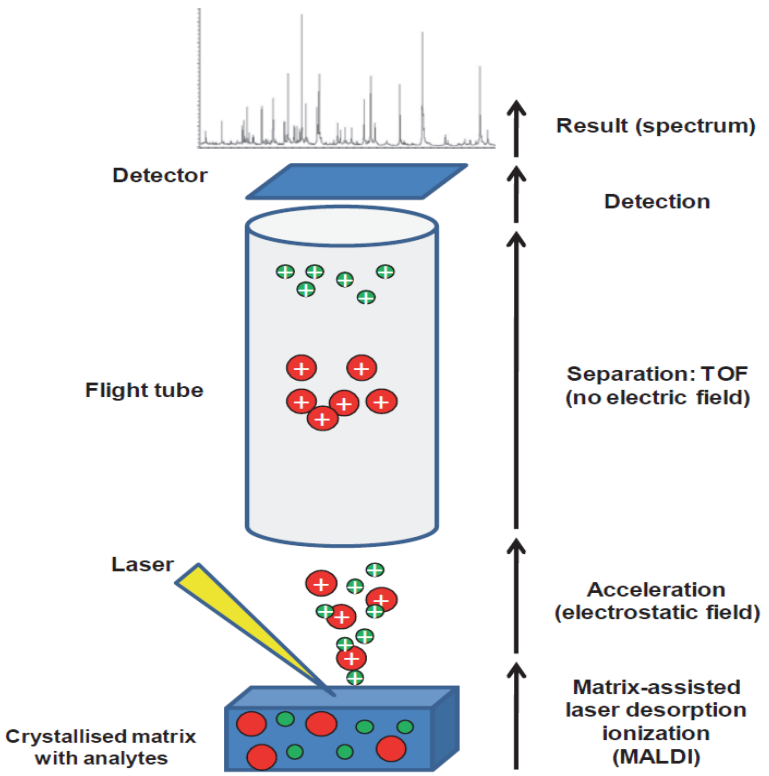


Fig. 1. Technical description of MALDI-TOF MS. The sample is mixed with a matrix on a conductive metal plate. After crystallization of the matrix and microbial material, the metal plate is introduced in the mass spectrometer and is bombarded with brief laser pulses. The desorbed and ionized molecules are accelerated through an electrostatic field and ejected through a metal flight tube subjected to vacuum until they reach a detector, with smaller ions traveling faster than larger ions. Thus, bioanalytes separated according to their TOF create a mass spectrum that is composed by mass to charge ratio (*m/z*) peaks with varying intensities. A spectrum is thus a microbial signature that is compared with a database for the identification at the species or genus level.

Keunggulan :

- TAT cepat (< 20menit)
- Jumlah m.o yang dibutuhkan sedikit (< 10^4 organisme/ satu koloni tunggal)
- Tidak membutuhkan probe/target spesifik dari m.o untuk identifikasi

REVIEW ARTICLE

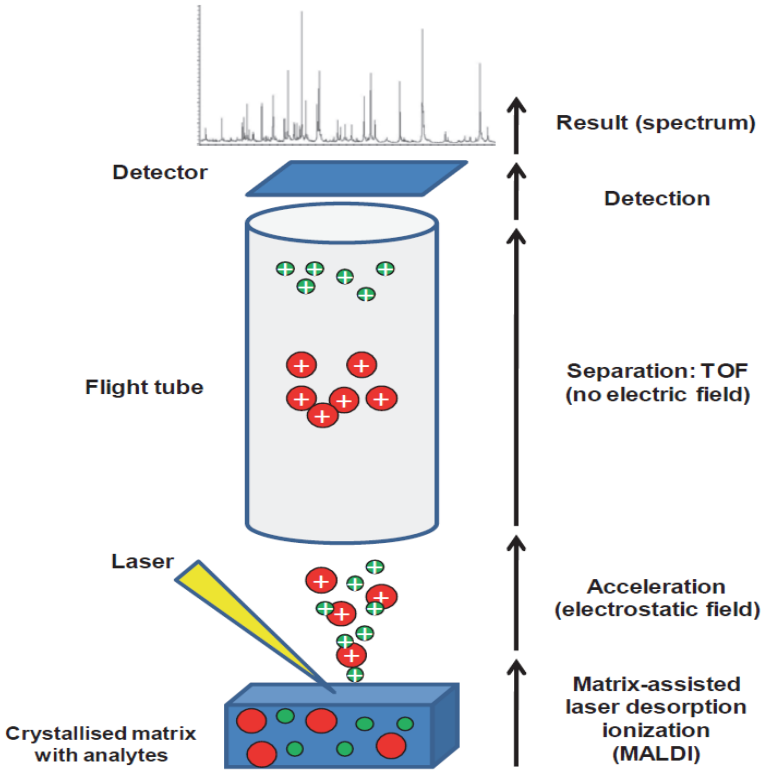
Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology

Antony Croxatto, Guy Prod'hom & Gilbert Greub

Institute of Microbiology, University Hospital Center and University of Lausanne, Lausanne, Switzerland

FEMS Microbiol Rev 36 (2012) 380–407

MALDI-TOF MS



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Keterbatasan :

- Harga instrumen mahal
- Masih dibutuhkan proses kultur untuk mendapatkan isolat murni
- Kesulitan membedakan m.o yang dekat secara genetik
- Jumlah m.o yang dapat diidentifikasi masih dalam tahap pengembangan.

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Total Laboratory Automation

Kelengkapan Instrumen Meliputi

- *conveyor/track systems* untuk memindahkan lempeng agar keluar masuk inkubator
- Kamera digital yang menangkap gambar lempeng agar pada waktu tertentu secara periodik
- *automated smart incubators*
- software yang menunjang seluruh proses
- Lengan robotik yang diatur oleh komputer untuk menjalankan semua proses.
- Instrumen pelengkap (preparasi inokulum)

Total Laboratory Automation

- *Kiestra TLA (BD Kiestra B.V., Drachten, Netherlands)*
- *full microbiology laboratory automation (FMLA; bioMérieux, Inc., La Balme, France)*
- *the WASPLab (Copan Diagnostics, Murrieta, CA).*

Total Laboratory Automation

FIG 5 Kiestra TLA system.

PP Beurbou, JCM.ASM.ORG, 2016

Total Laboratory Automation

FIG 6 FMLA system.

PP Beurbou, JCM.ASM.ORG, 2016

Total Laboratory Automation

FIG 7 WASPLab.

PP Beurbou, JCM.ASM.ORG, 2016

Pencitraan Digital

- Mengamati semua lempeng agar dari satu pasien pada layar yang sama
- Gambar diambil secara berkala selama inkubasi
- Software menganalisis hasil pertumbuhan m.o
- Gambar dapat disimpan untuk kepentingan konsultasi, pendidikan, uji kompetensi
- Dapat diaplikasikan untuk identifikasi dengan Chrom Agar.
- Koloni murni dipilih untuk proses selanjutnya (ID/AST, MALDI-TOF MS)

Keuntungan TLA

- Efisiensi >>
- Penghematan biaya untuk SDM
- Menurunnya cedera krn pekerjaan berulang
- Keamanan dan Keselamatan kerja >>

Kekurangan :

- Evaluasi "Mean time to failure" terbatas karena populasi mesin masih sedikit dan penyebarannya terlokalisasi.

Faktor yang perlu diperhatikan dalam memilih automatisasi mikrobiologi

- Reliabilitas
- Akurasi
- Kapasitas
- *Technical support*
- Fleksibilitas :

  Jenis spesimen; loops; protokol; media ; LIS
- *Modularity*
- Biaya awal, bahan habis pakai, tenaga operasional

Greub and Prod'hom.Clinical Microbiology and Infection ª2011 European Society of Clinical Microbiology and Infectious Diseases, CMI, 17, 655–660

Keuntungan Automatisasi Mikrobiologi

- Produktivitas meningkat
- Beban kerja menurun → menurunkan tingkat cedera ergonomi
- TAT lebih cepat
- Menurunkan angka positif palsu atau negatif palsu ok kesalahan manusia
- Peningkatan sensitivitas dalam mendeteksi pertumbuhan koloni
- Lebih mudah dilacak dan dilakukan pengulangan
- Kualitas pelayanan pasien lebih baik

Terima Kasih

Terima Kasih