第390回ガスクロマトグラフィー研究懇談会講演会・見学会

質量分析計を用いた細菌同定・疾患マーカー探索

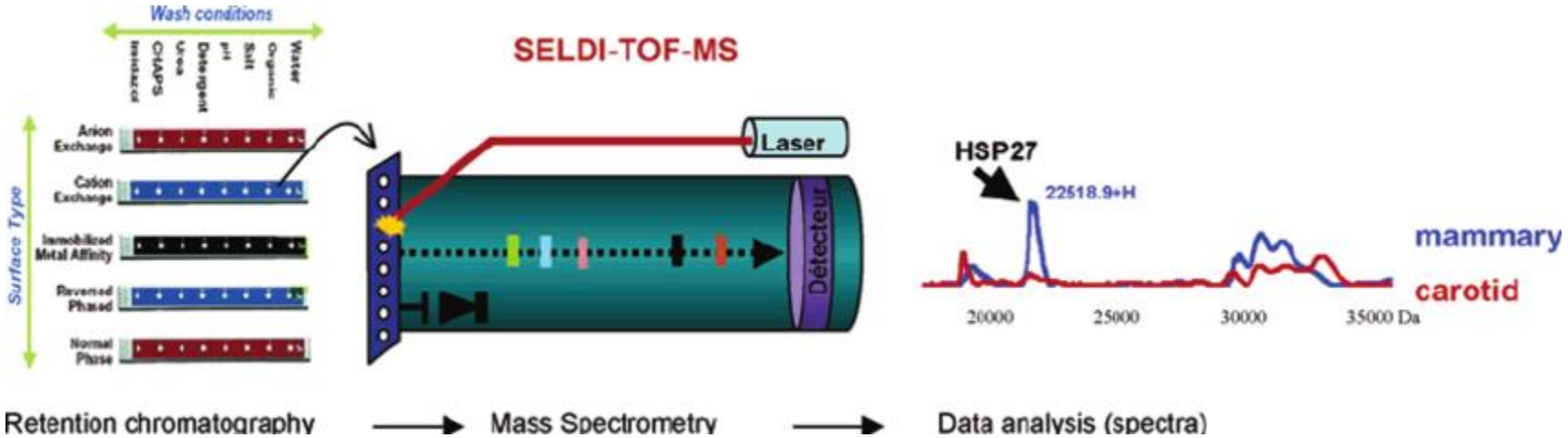
麻布大学生命環境科学部生化学研究室
麻布大学大学院環境保健学分子病態解析学

曽川一幸

質量分析計との出会い

サイファージェン・バイオシステムズ（株）

2001年3月に使用方法を練習



Identification of novel and downregulated biomarkersfor alcoholism by surface enhanced laser desorption/ionization-mass spectrometry

Proteomics. 2004, 4, 1187–1194.

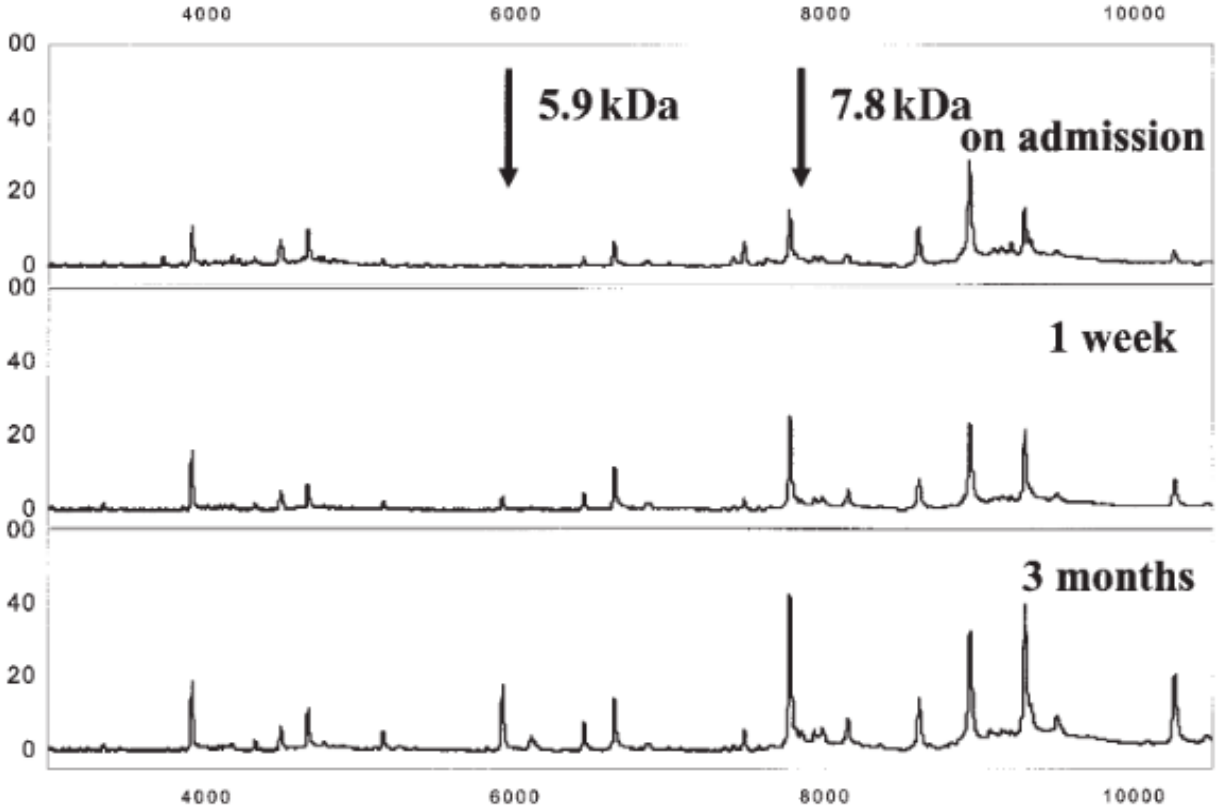


Figure 3. Representative view of the spectra of proteins retained on the WCX2 protein chips by SELDI-TOF-MS analysis. Note that the 5.9 kDa and 7.8 kDa peaks, downregulated on admission, remarkably increased during abstinence.

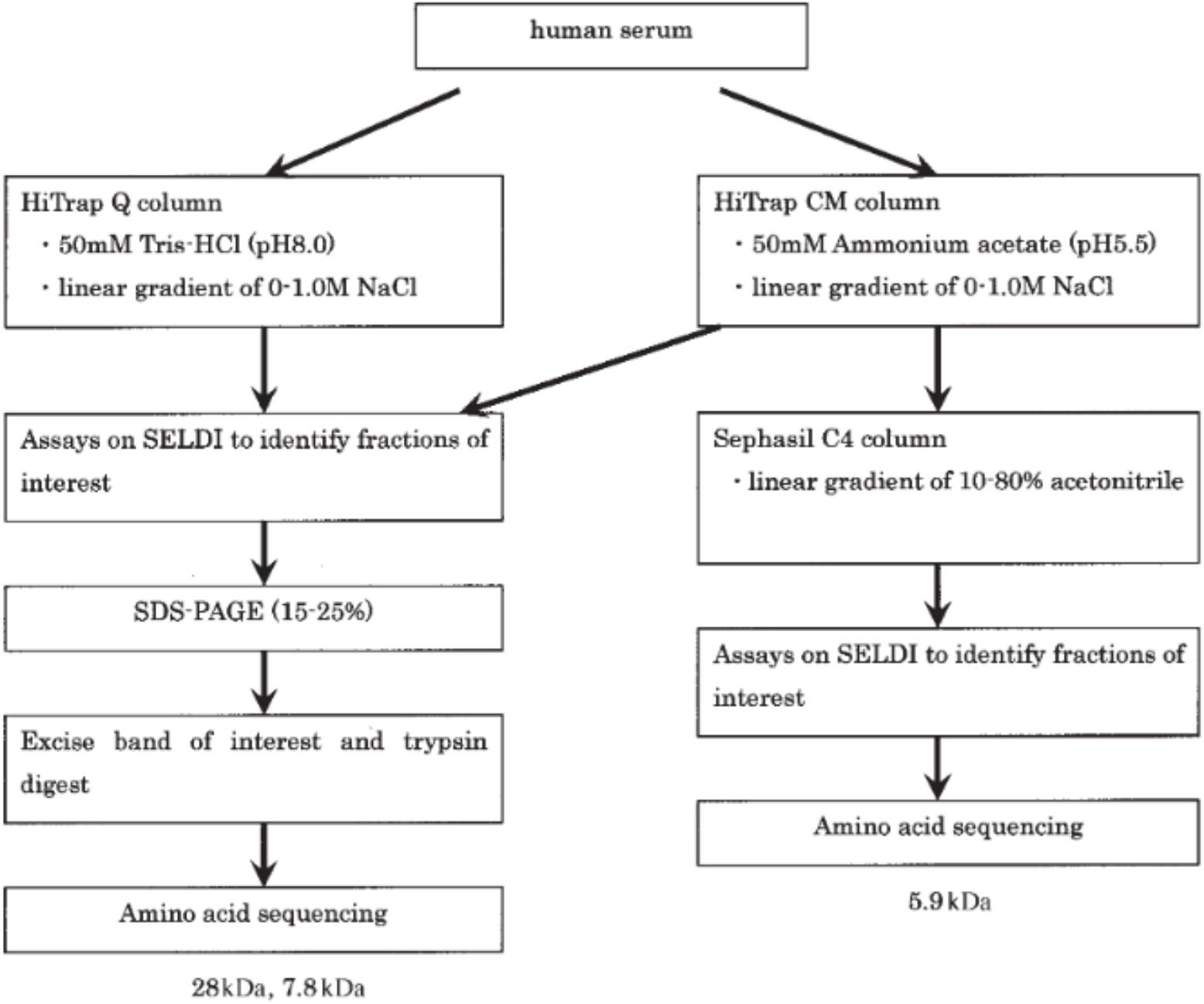


Figure 1. Schematic diagram of the protein purification protocol.

(5A) Estimated human Fibrinogen A α -chain fragment

1	MFSMRIVCLV	LSVVGTAWTA	DSGEGDFLAE	GGGVRGPRVV	ERHQSACKDS
51	DWPFCSDEDW	NYKCPSGCRM	KGLIDEVNQD	FTNRINKLKN	SLFEYQKNNK
101	DSHSLTTNIM	EILRGDFSSA	NNRDNTYNRV	SEDLRSRIEV	LKRKVIEKVQ
151	HIQLLQKNVR	AQLVDMKRLE	VDIDIKIRSC	RGSCSRALAR	EVDLKDYEDQ
201	QKQLEQVIAK	DLLPSRDRQH	LPLIKMKPVP	DLVPGNFKSQ	LQKVPPEWKA
251	LTDMPQMRME	LERPGGNEIT	RGGSTSYGTG	SETESPRNPS	SAGSWNSGSS
301	GPGSTGNRNP	GSSGTGGTAT	WKPGSSGPGS	TGSWNSGSSG	TGSTGNQNPG
351	SPRPGSTGTW	NPGSSERGSA	GHWTSESSVS	GSTGQWHSES	GSFRPDSPGS
401	GNARPNNPDW	GTFEEVSGNV	SPGTRREYHT	EKLVTSKGDK	ELRTGKEKVT
451	SGSTTTTRRS	CSKTVTKTVI	GPDGHKEVTK	EVVTSEDGSD	CPEAMDLGTL
501	SGIGTLDGFR	HRHPDEAAFF	DTASTGKTFP	GFFSPMLGEF	VSETESRGSE
551	SGIFTNTKES	SSHHPGIAEF	PSRGK**SSSYS**	**KQFTS**STSYN	**RGDSTFESKS**
601	**YKMADEAGSE**	**ADHEGTHSTK**	**RGHAKSRPV**R	GIHTSPLGKP	SLSP

(5B) Complete human Apo A1

1	**DEPPQSPWDR**	**VKDLATVYVD**	**VLKDSGRDYV**	**SQFEGSALGK**	**QLNLKLLDNW**
51	**DSVTSTFSKL**	**REQLGPVTQE**	**FWDNLEKETE**	**GLRQEMSKDL**	**EEVKAKVQPY**
101	**LDDFQKKWQI**	**EMELYRQKVE**	**PLRAELQEGA**	**RQKLHELQEK**	**LSPLGEEMRD**
151	**RARAHVDALR**	**THLAPYSDEL**	**RQRLAARLEA**	**LKENGGARLA**	**EYHAKATEHL**
201	**STLSEKAKPA**	**LEDLRQGLLP**	**VLESFKVSFL**	**SALEEYTKKL**	**NTQ**

(5C) Estimated human Apo A2 fragment

1	MKLLAATVLL	LTICSLEGAL	VRRQAK**EPCV**	**ESLVSQYFQT**	**VTDYGKDLME**
51	**KVKSPELQAE**	**AKSYFEKSKE**	**QLTPLIKKAG**	**TELVNFLSYF**	**VELG**THPATQ

Figure 5. Amino acid sequencing of the trypsin digests. Amino acid sequencing of the trypsin digests of the 28 kDa and the 7.8 kDa bands, and the final preparation of the 5.9 kDa protein revealed that the 5.9 kDa protein was a fragment of fibrinogen αE chain (A), and the 28 kDa was apoprotein A-I (B). The 7.8 kDa protein was identified as a fragment of the apoprotein A-II (C). The matched sequences are printed in bold and amino acids determined by the Edman method are underlined.

Diagnostic Values of Surface-Enhanced Laser Desorption/Ionization Technology for Screening of Habitual Drinkers

Alcohol Clin Exp Res. 2007;31:S22-S26.

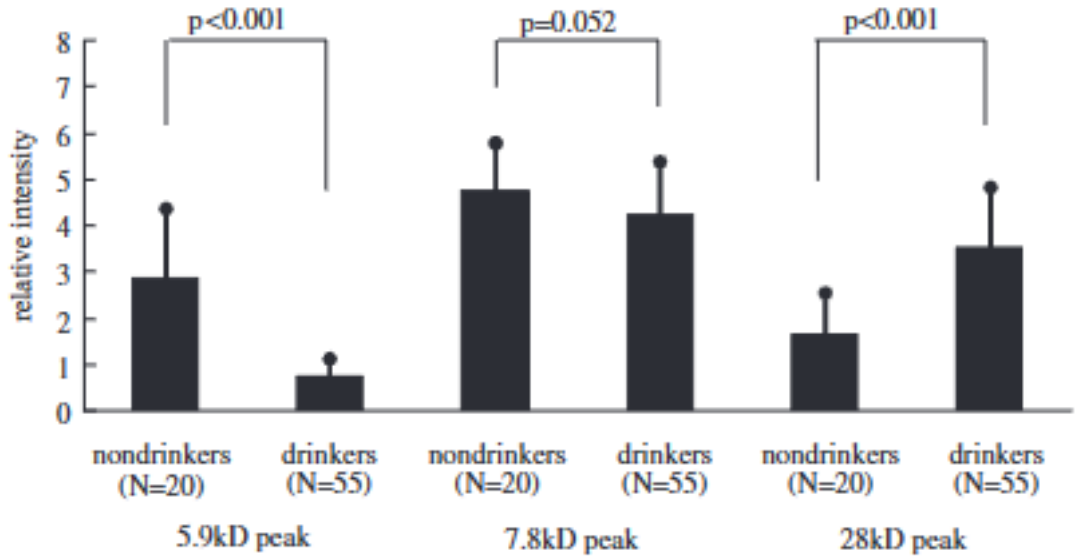


Fig. 1. Relative intensities of the 5.9, 7.8, and 28 kDa peaks obtained by the surface-enhanced laser desorption/ionization analysis in nondrinkers and habitual drinkers (values are means ± SD).

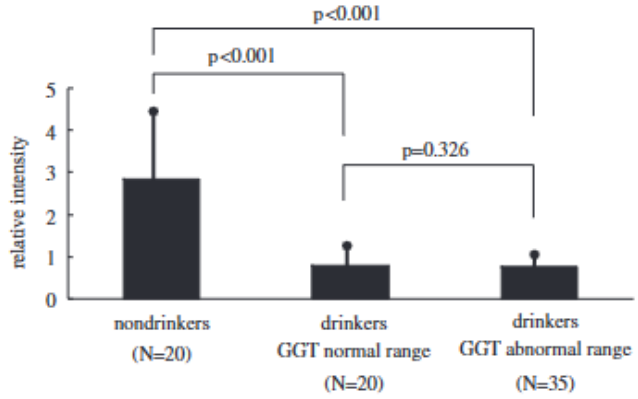


Fig. 2. Relative intensities of the 5.9 kDa peak obtained by the surface-enhanced laser desorption/ionization analysis in nondrinkers, habitual drinkers with or within elevated γ-glutamyltransferase (GGT) (values are means ± SD).

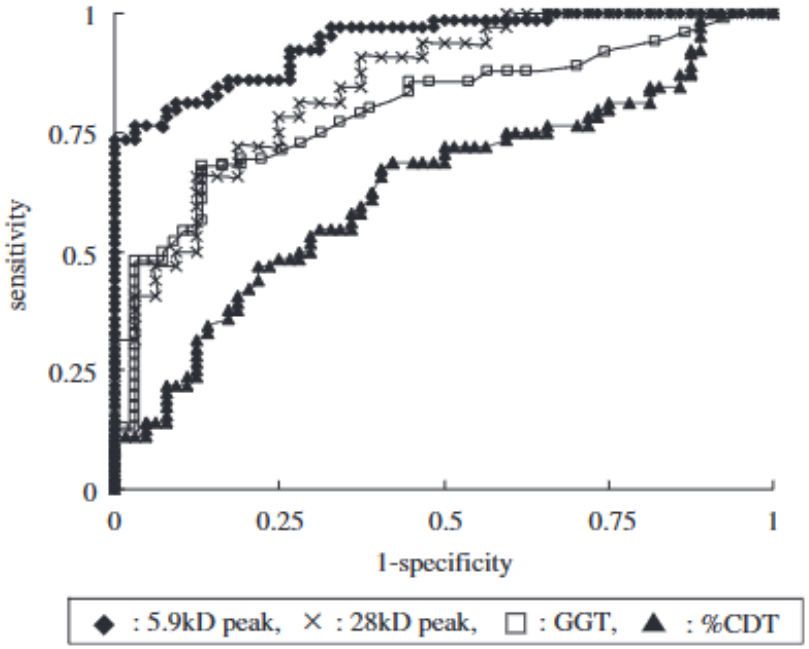
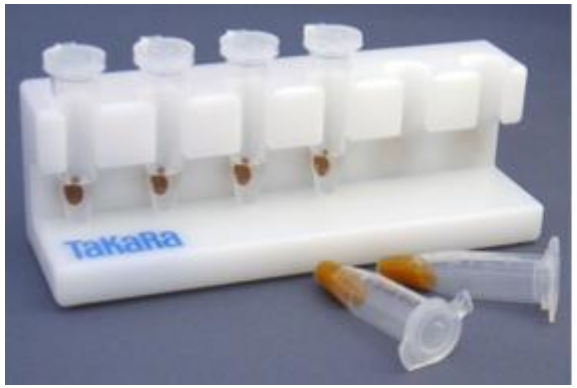


Fig. 4. Receiver-operating characteristic curve analysis obtained for the 5.9 and 28 kDa peaks and also for serum γ-glutamyltransferase (GGT) and carbohydrate-deficient transferrin (CDT) activities.

質量分析計との出会い

ブルカージャパン（株）

2007年9月に使用方法を練習



A search for novel markers of alcohol abuse usingmagnetic beads and MALDI-TOF/TOF massspectrometry

Proteomics Clin. Appl. 2009, 3, 821–828.

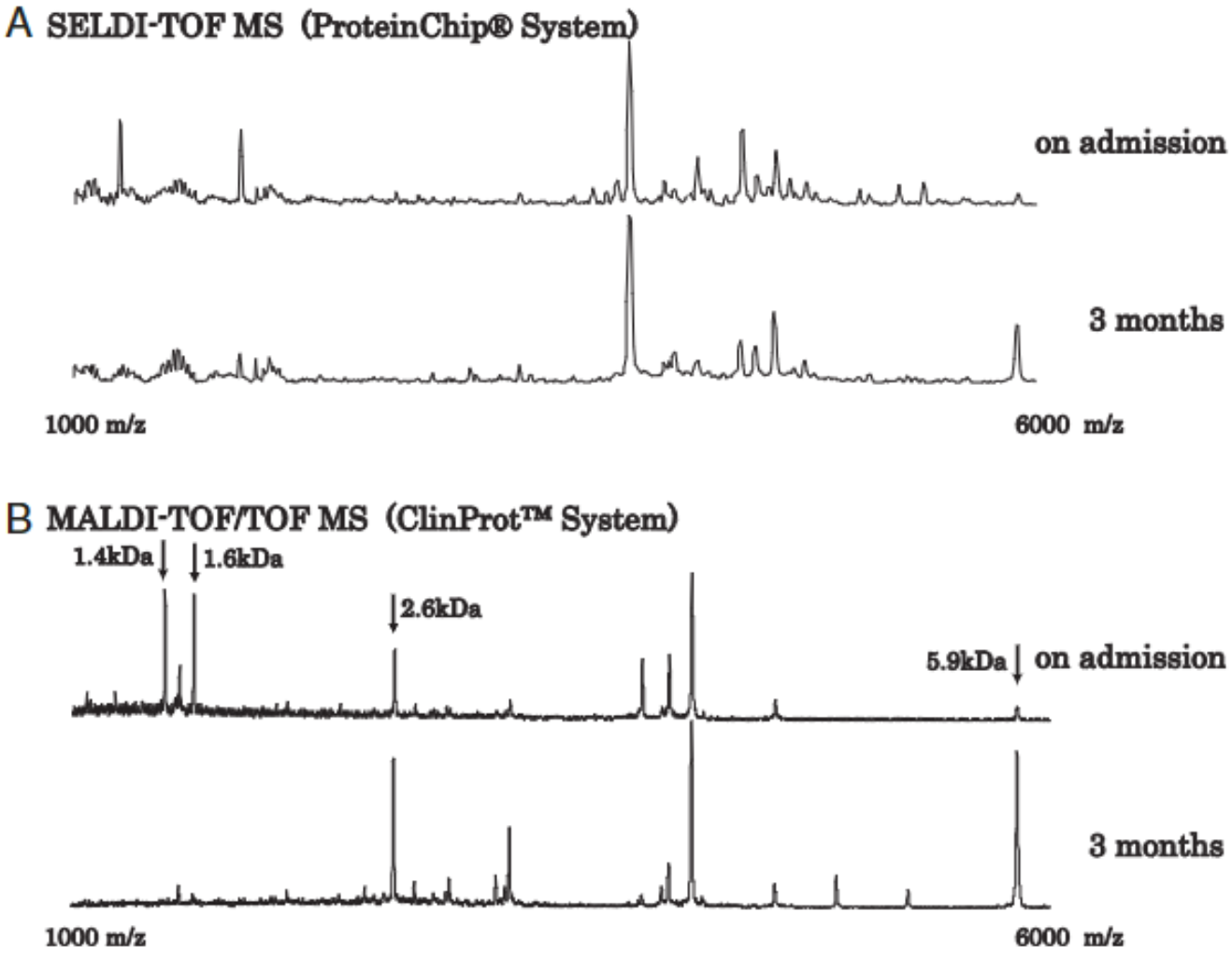


Figure 1. A representative view of the spectra of serum proteins produced by the ProteinChip® SELDI-TOF MS (A) and ClinProt™ MALDI-TOF MS (B) systems on admission and after 3 months abstinence in an alcoholic subject. Note that the 5.9 and 2.6 kDa peaks, which were downregulated on admission, increased markedly after abstinence. By contrast, the 1.4 and 1.6 kDa peaks, which were upregulated on admission, decreased after 3 months of abstinence.

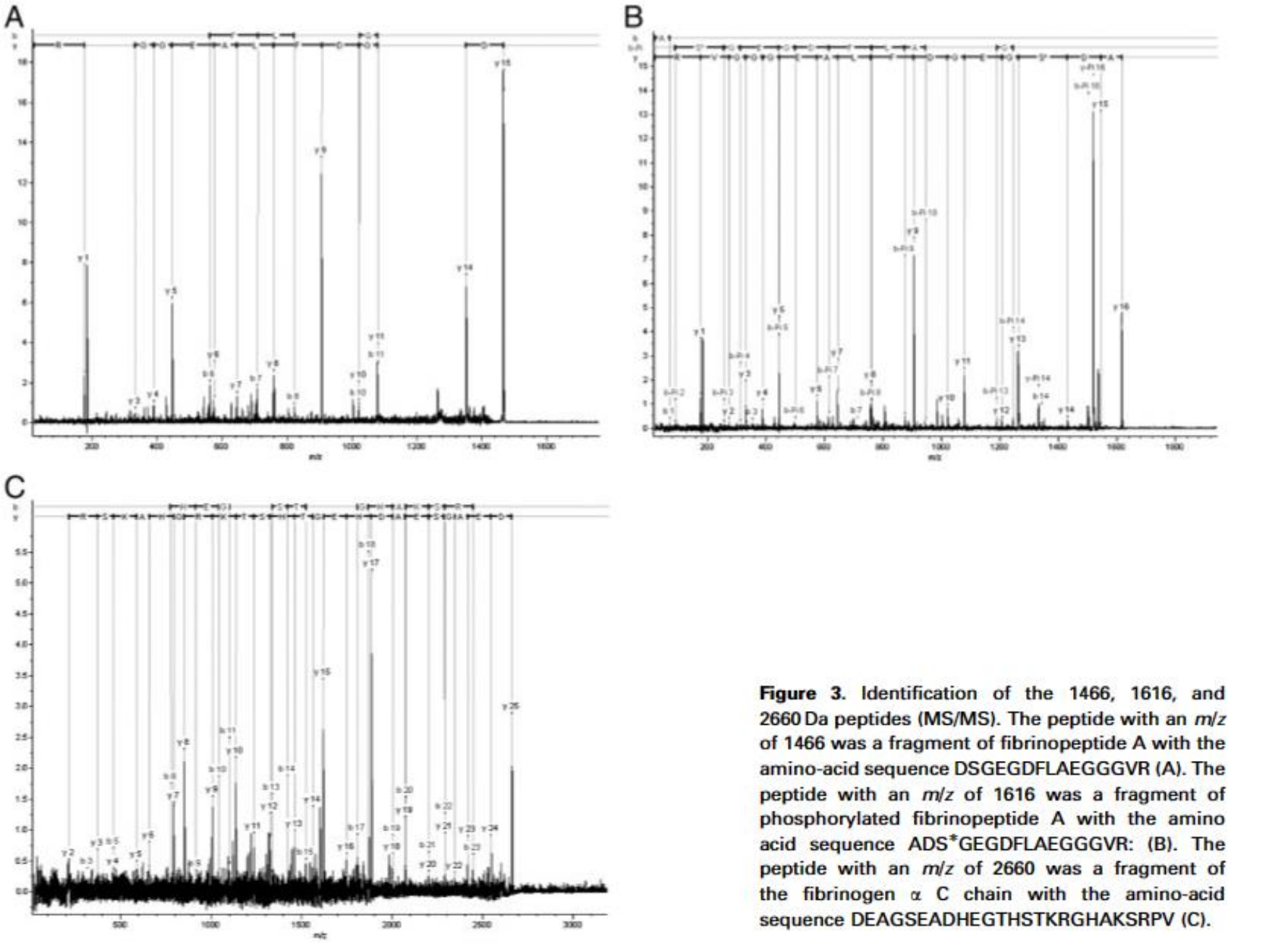


Figure 3. Identification of the 1466, 1616, and 2660 Da peptides (MS/MS). The peptide with an *m/z* of 1466 was a fragment of fibrinopeptide A with the amino-acid sequence DSGEGDFLAEGGGVR (A). The peptide with an *m/z* of 1616 was a fragment of phosphorylated fibrinopeptide A with the amino acid sequence ADS*GEGDFLAEGGGVR: (B). The peptide with an *m/z* of 2660 was a fragment of the fibrinogen α C chain with the amino-acid sequence DEAGSEADHEGTHSTKRGHAKSRPV (C).

質量分析計との出会い

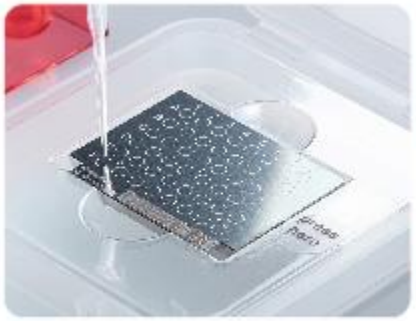
ブルカージャパン（株）

2011年3月に使用方法を練習

コロニーを釣菌

プレートへ塗布

パネルを選ばずに同定へ

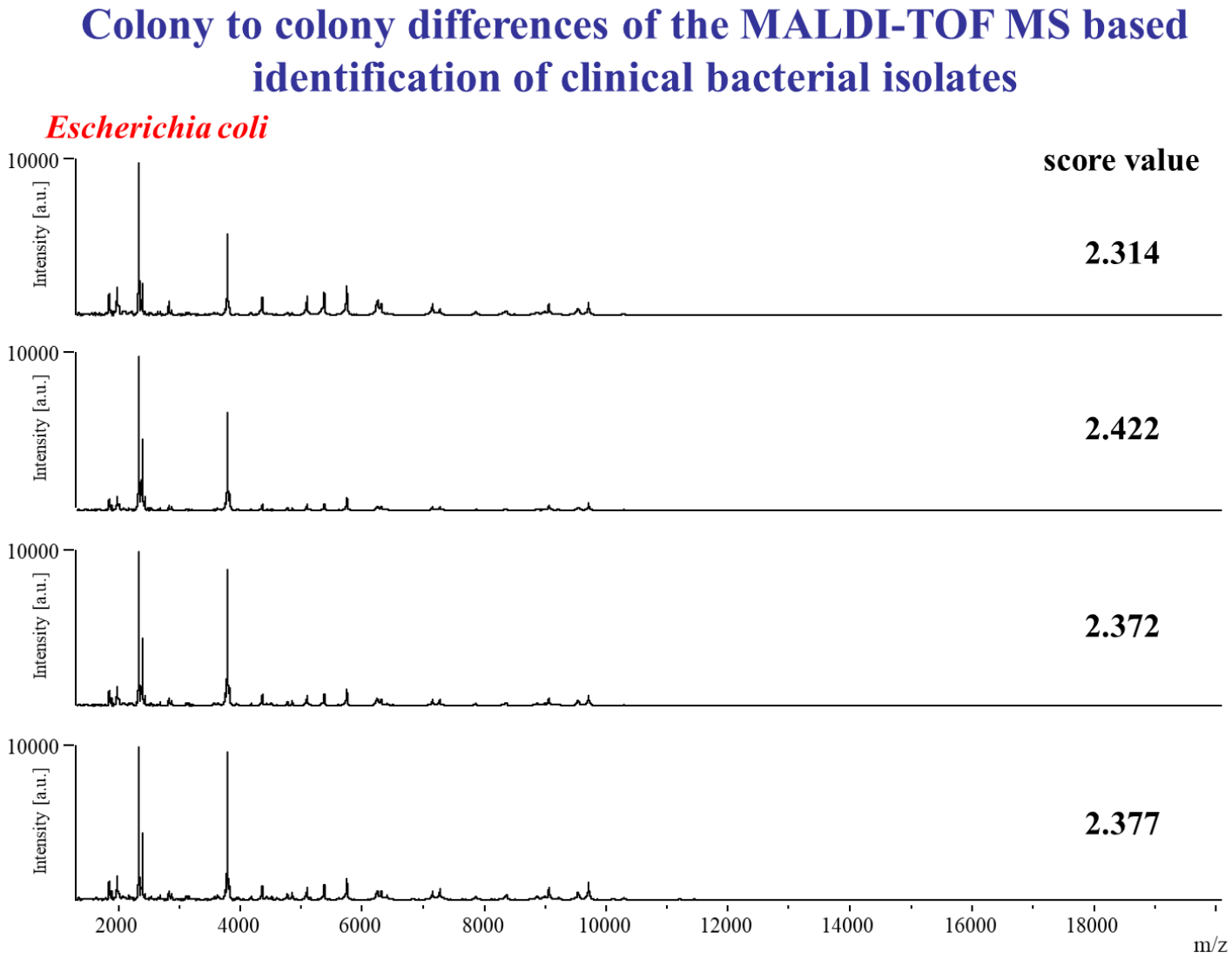


マトリックスを滴下

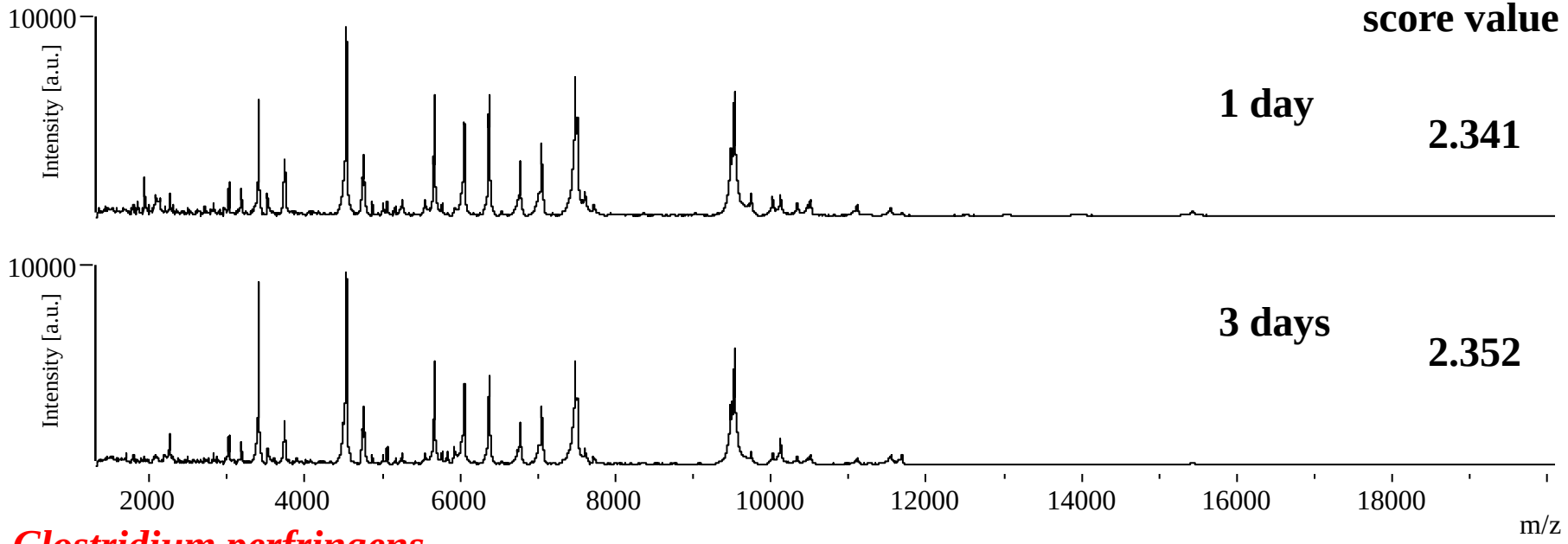
乾燥後、装置に装填

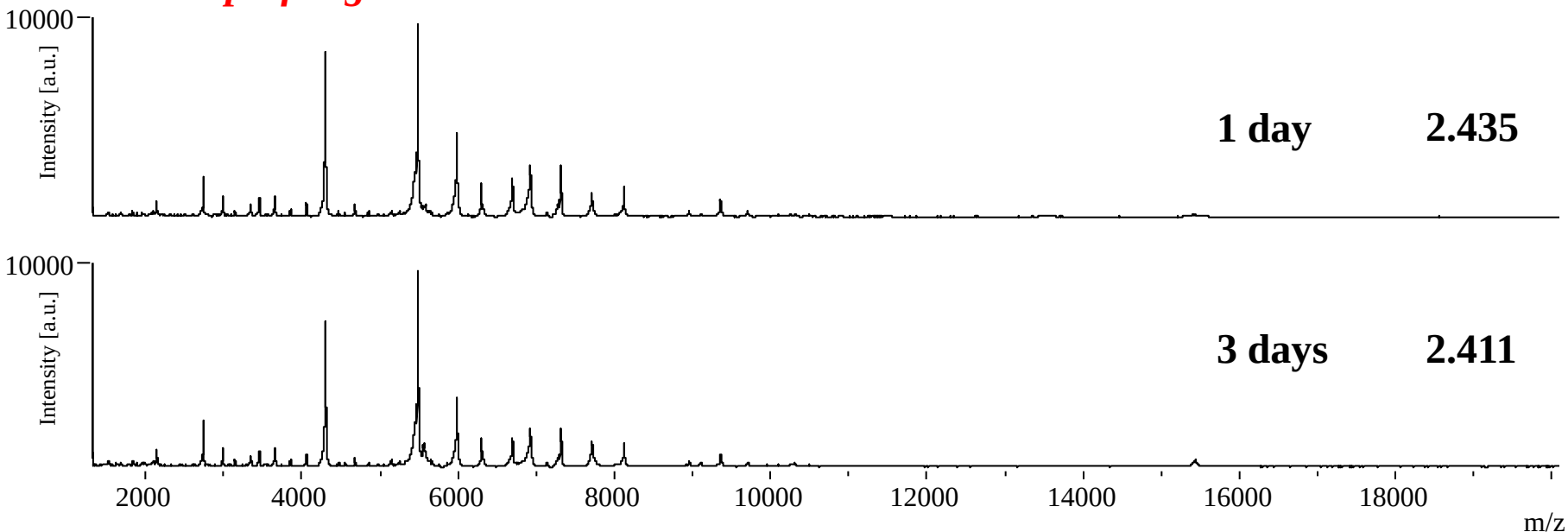
Use of the MALDI BioTyper system with MALDI-TOF mass spectrometry for rapid identification of microorganisms

Anal Bioanal Chem. 2011, 400, 1905-1911.



Influence of culture time on MALDI-TOF MS identification





Comparison of the results obtained by the conventional and the MALDI-TOF MS-based method with the 16S rRNA method

Conventional method	MALDI Biotyper method	16S rRNA method
Aeromonas hydrophila group	*Aeromonas caviae*	*Aeromonas caviae*
	Aeromonas hydrophila	*Aeromonas hydrophila*
Bordetella parapertussis	*Bordetella bronchiseptica*	*Bordetella bronchiseptica* or *Bordetella parapertussis*
Enterobacter cloacae	*Enterobacter asburiae*	*Enterobacter cloacae*
	Enterobacter ludwigii	*Enterobacter cloacae*
Enterococcus casseliflavus	*Enterococcus phoeniculicola*	*Enterococcus casseliflavus*
Haemophilus parahaemolyticus	*Haemophilus parainfluenzae*	*Haemophilus parainfluenzae*
Haemophilus parainfluenzae	*Haemophilus parahaemolyticus*	*Haemophilus parahaemolyticus*
Lactobacillus acidophilus	*Lactobacillus gasseri*	*Lactobacillus gasseri*
Stenotrophomonas maltophilia	*Pseudomonas geniculata*	*Stenotrophomonas maltophilia*
	Pseudomonas hibiscicola	*Stenotrophomonas maltophilia*
Streptococcus bovis	*Streptococcus pasteurianus*	*Streptococcus pasteurianus*
Vibrio fluvialis	*Vibrio furnissii*	*Vibrio furnissii*

質量分析計との出会い

APPLIED BIOSYSTEMS

QSTAR

サーモフィッシャーサイエンティフィック株式会社

リニアイオントラップ質量分析計『LTQ XL』

Increased serum levels of pigment epithelium-derived factor by excessive alcohol consumption －Detection and identification by a three-step serum proteome analysis－

Alcoholism: Clinical and Experimental Research, 2011.

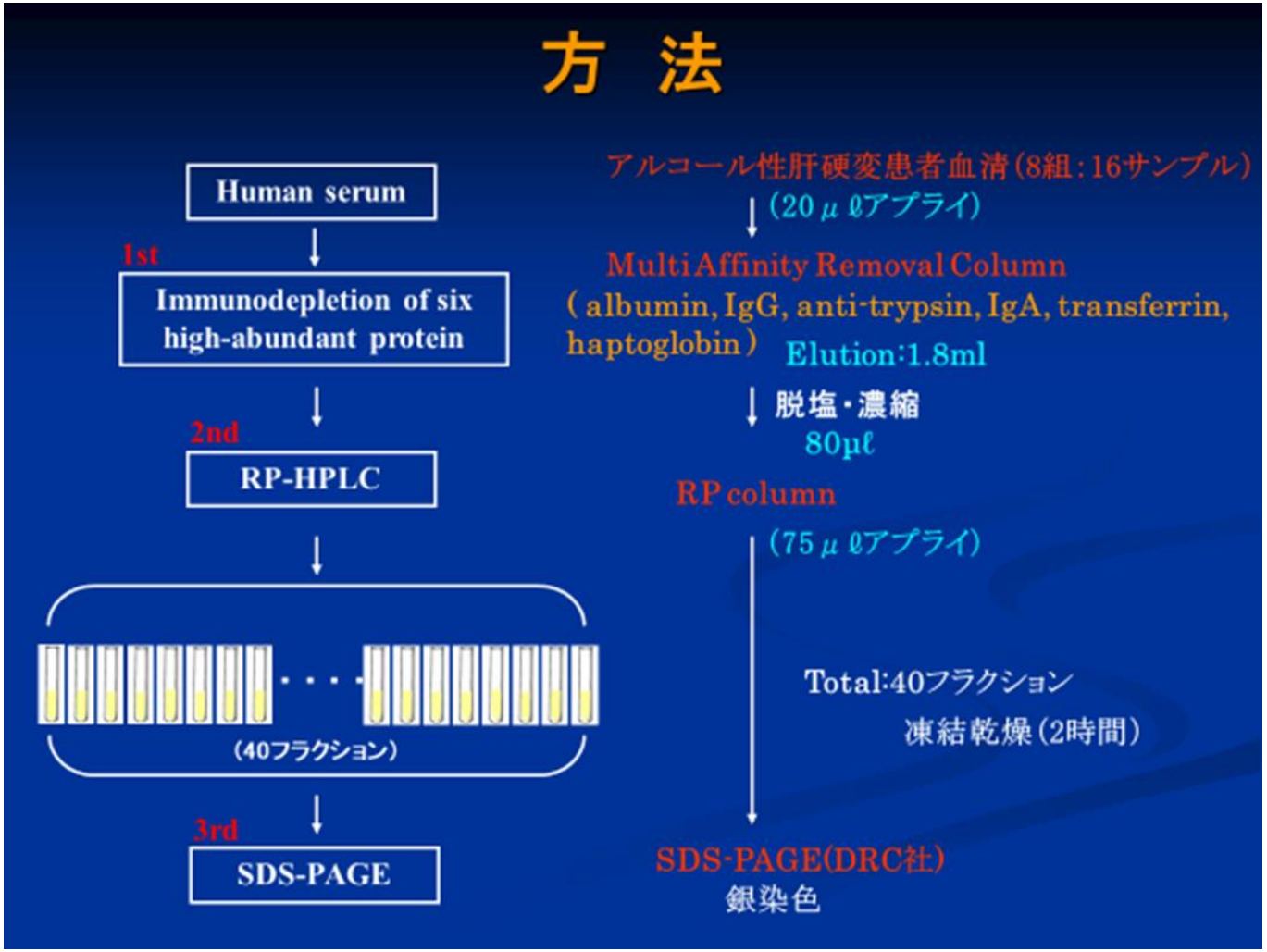


Table 2. Serum Proteins Upregulated (A) and Downregulated (B) in Alcoholic Patients on Admission, as Detected by Three-Step Proteome Analysis

No.	Database accession no.	ID	MW	Score	Number of matching peptides	Sequence coverage (%)
A. Upregulated serum proteins						
1	gi2521981	Alpha2-HS glycoprotein	35,641	112	5	8
2	gi90108664	Apolipoprotein A-I	28,061	1,409	43	70
3	gi121672	Glutathione peroxidase 3	25,489	82	2	6
4	gi23200172	Heparin cofactor II	57,034	161	3	5
5	gi189778	PEDF	46,300	491	9	22
B. Downregulated serum proteins						
1	gi224917	Apolipoprotein C-III	8,759	118	3	24

MW, molecular weight; PEDF, pigment epithelial-derived factor.

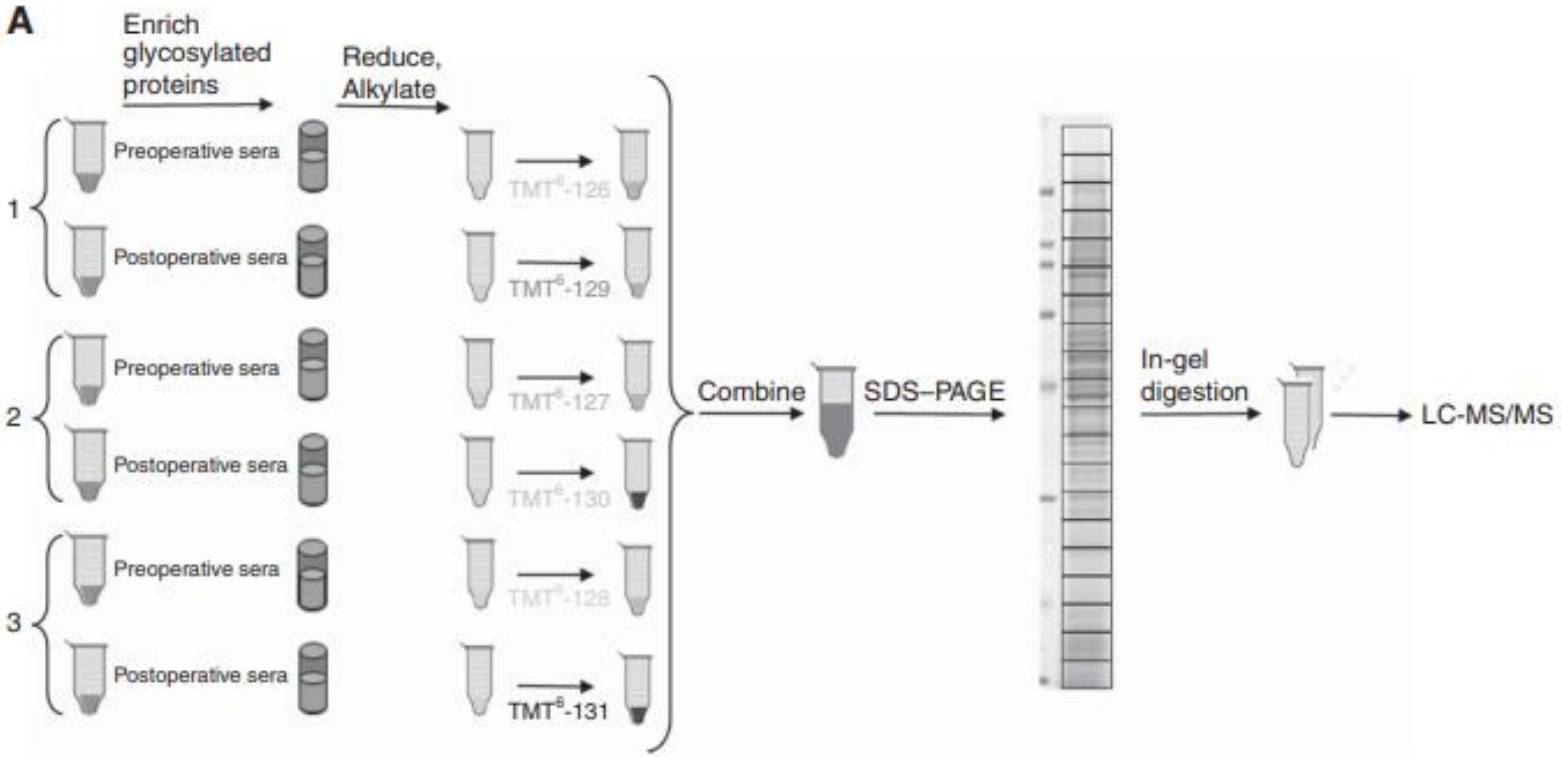
質量分析計との出会い

サーモフィッシャーサイエンティフィック株式会社

リニアイオントラップ質量分析計『Q Exactive』

Identification of a novel serum biomarker for pancreatic cancer, C4b-binding protein α-chain (C4BPA) by quantitative proteomic analysis using tandem mass tags

Br J Cancer. 2016;115:949-956.

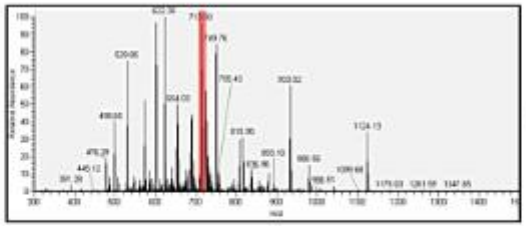


【解析フロー：TMT sixplex Reagents による6検体の網羅的タンパク質発現量比較】

＊試料中の全ペプチドに対して、TMT標識を行います。

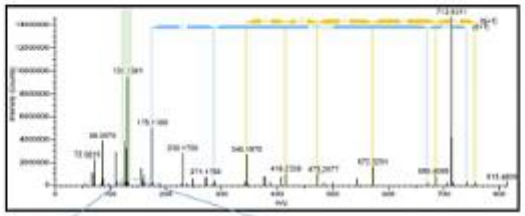
各試料由来のペプチド
溶液を 等量混合

質量分析

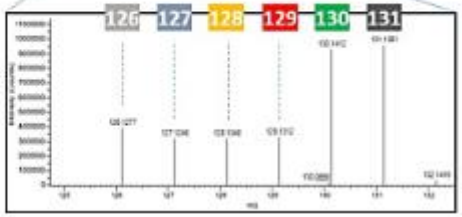


TMT 1分子あたりの分子量は同じのため、各試料由来の同一ペプチドは、
TMT標識されても同一の質量スペクトルを示します。

MS/MS 解析



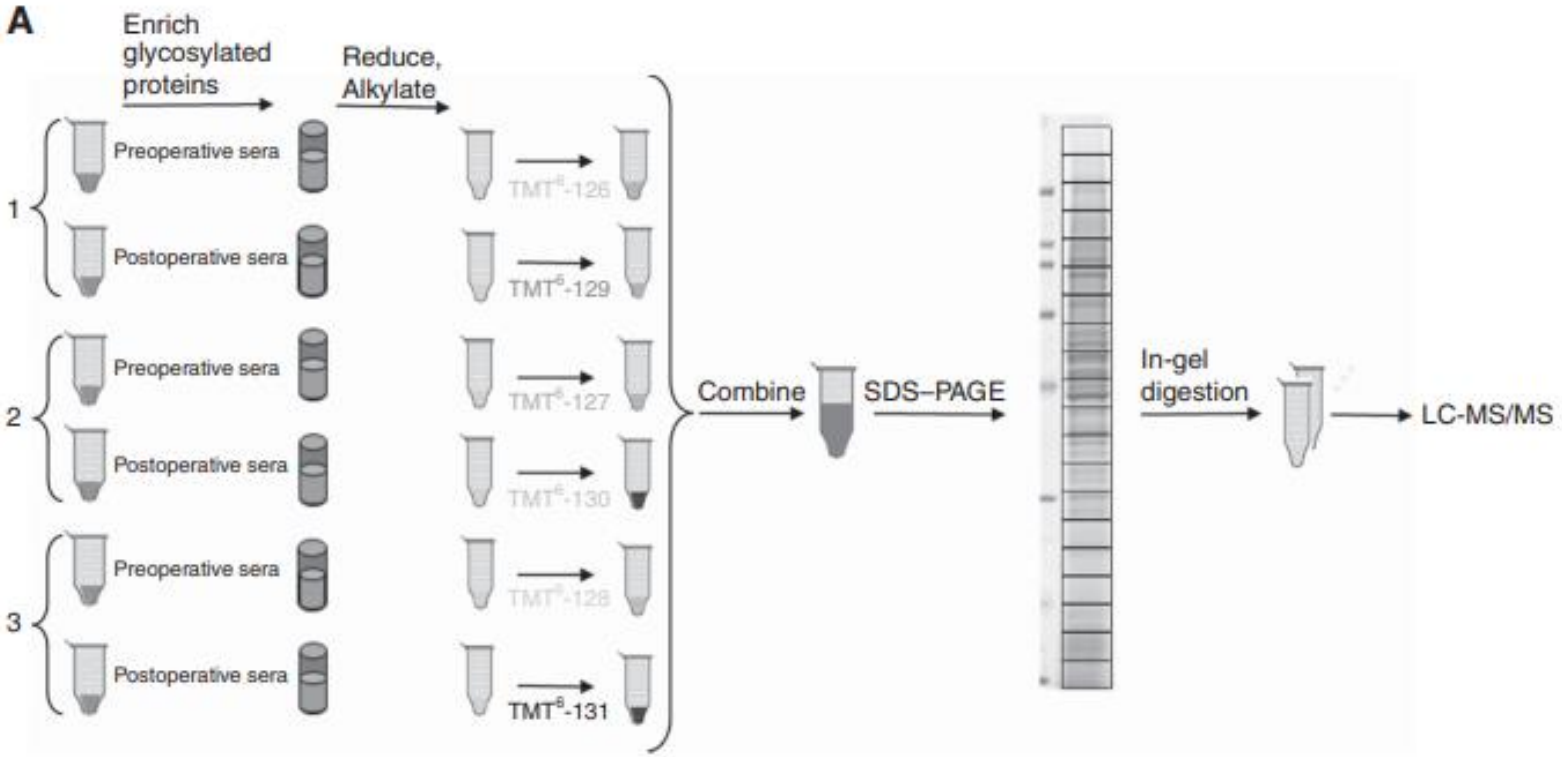
MS/MS により得られたフラグメントイオンの
スペクトルから、各ペプチド配列を同定します。



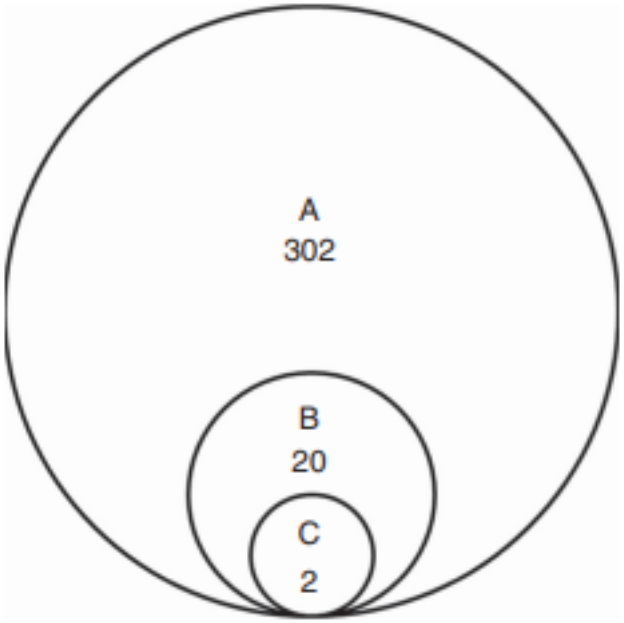
さらに、レポーター部のMSスペクトル強度を比較する
ことで、各ペプチドの相対発現量の比較を行います。

Identification of a novel serum biomarker for pancreatic cancer, C4b-binding protein α-chain (C4BPA) by quantitative proteomic analysis using tandem mass tags

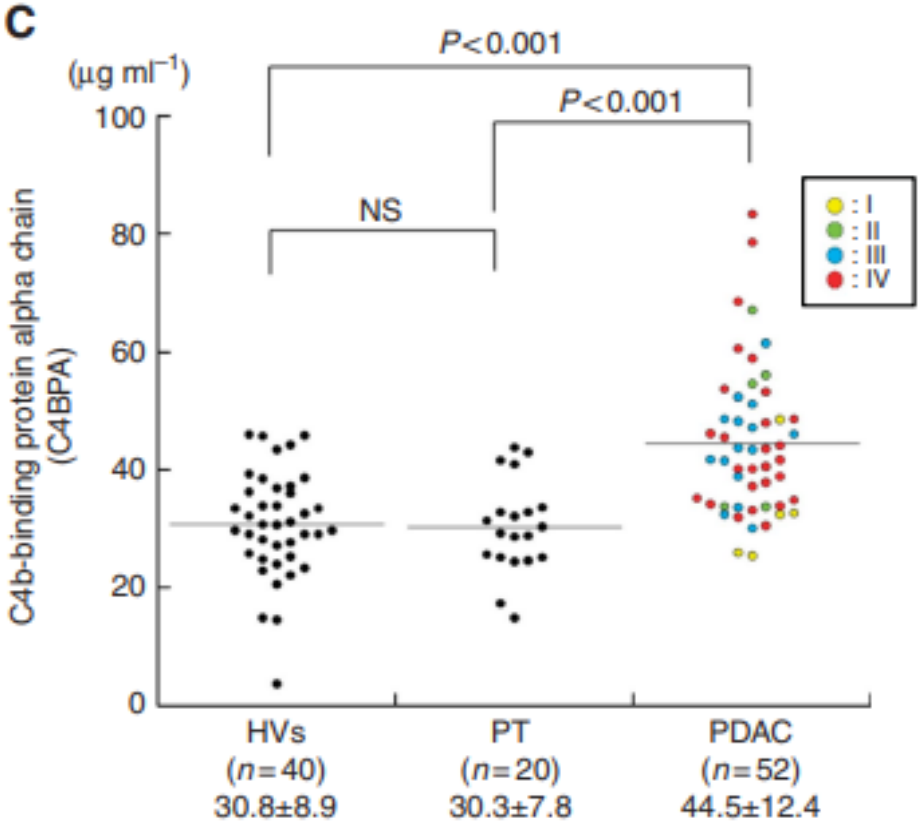
Br J Cancer. 2016;115:949-956.



B

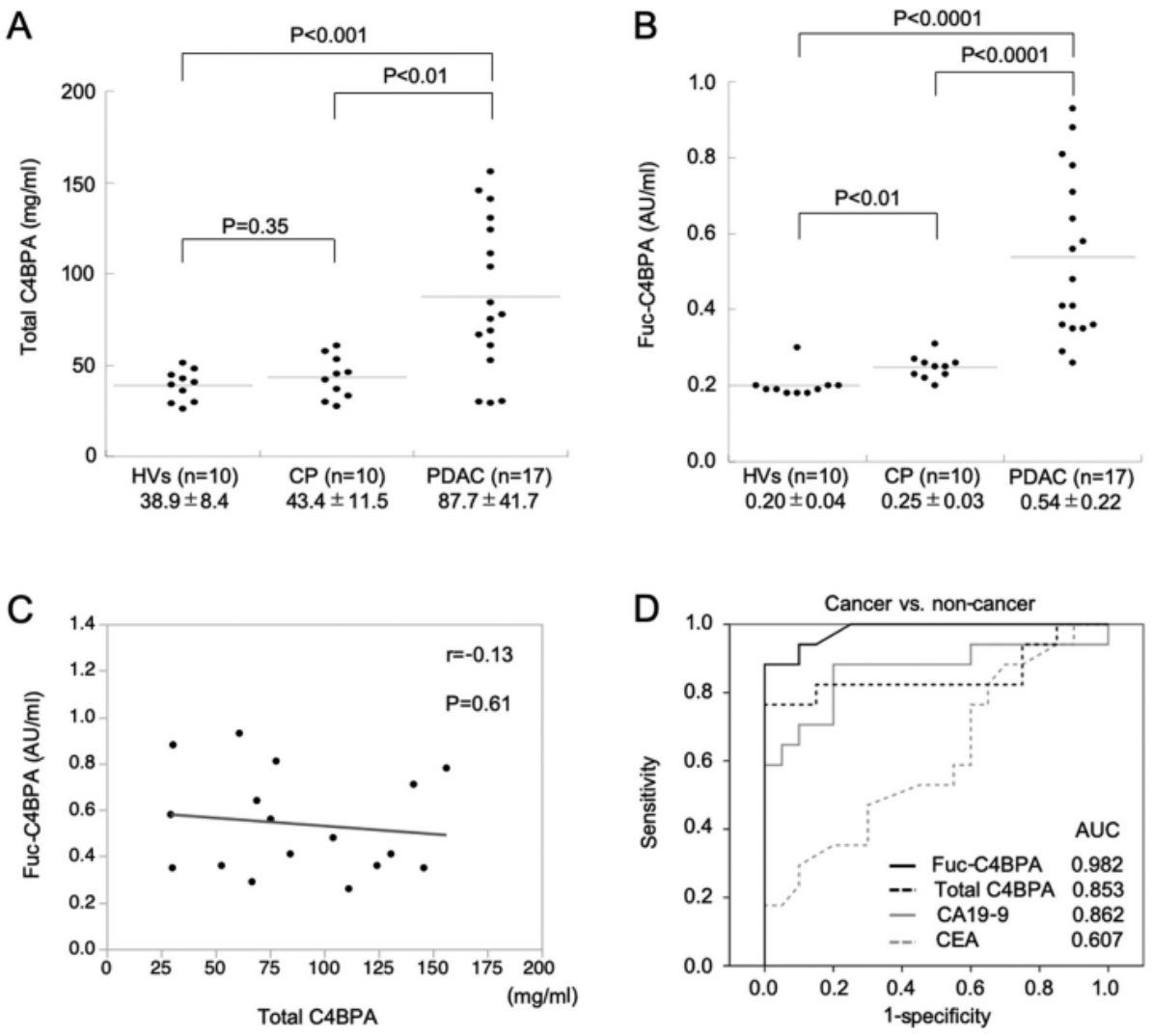

A
302
B
20
C
2

C


P<0.001
P<0.001
NS
(μg ml−1)
C4b-binding protein alpha chain (C4BPA)
100
80
60
40
20
0
: I
: II
: III
: IV
HVs
(n=40)
30.8±8.9
PT
(n=20)
30.3±7.8
PDAC
(n=52)
44.5±12.4

Fucosylated C4b-binding protein α-chain, a novel serum biomarker that predicts lymph node metastasis in pancreatic ductal adenocarcinoma

Oncol Lett. 2021;21:127. doi:10.3892/ol.2020.12388.

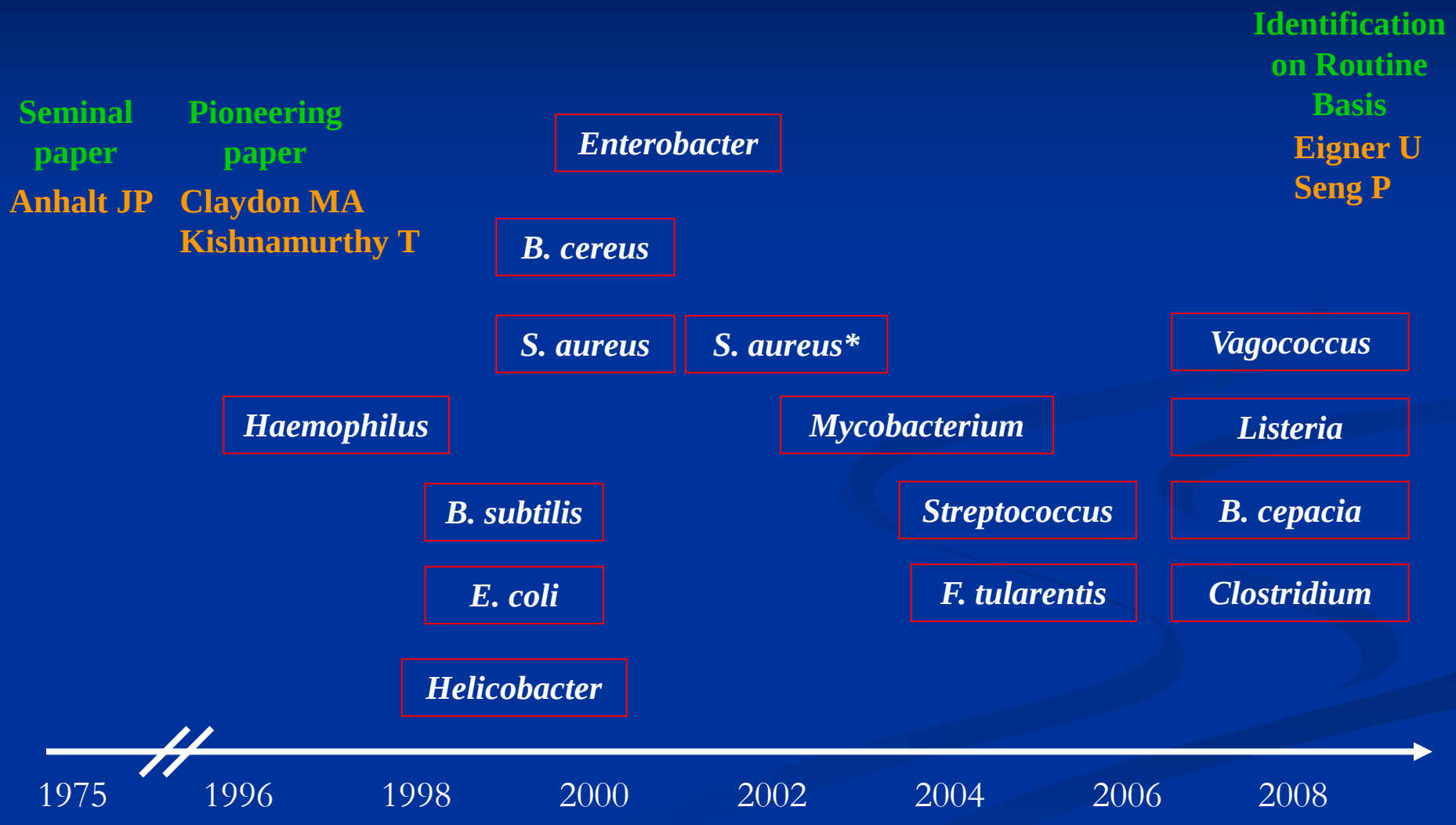


質量分析計との出会い

サーモフィッシャーサイエンティフィック株式会社

Orbitrap Fusion Lumos Tribrid

質量分析計を用いた細菌同定



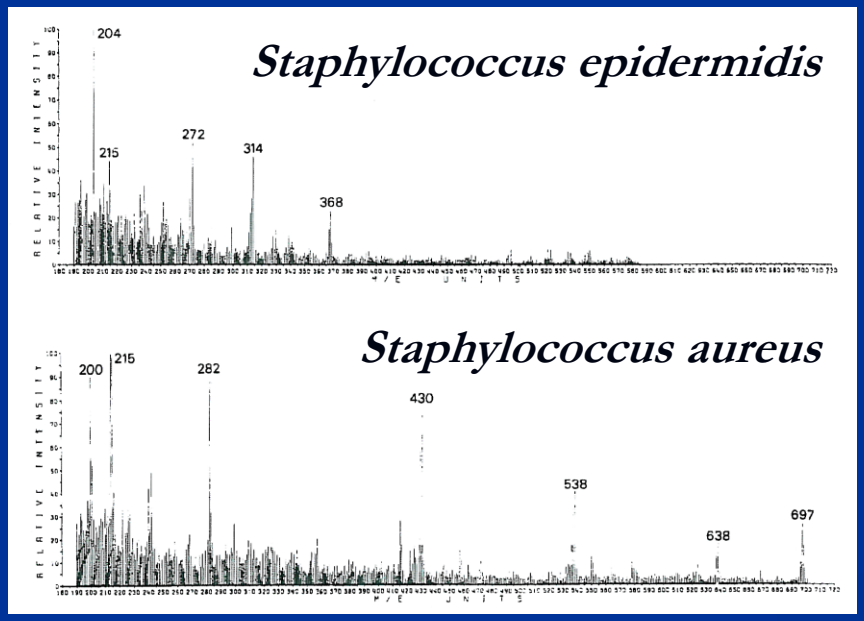
（Seng P, et al. *Clinical Infectious Diseases*. 2009　一部改訂）

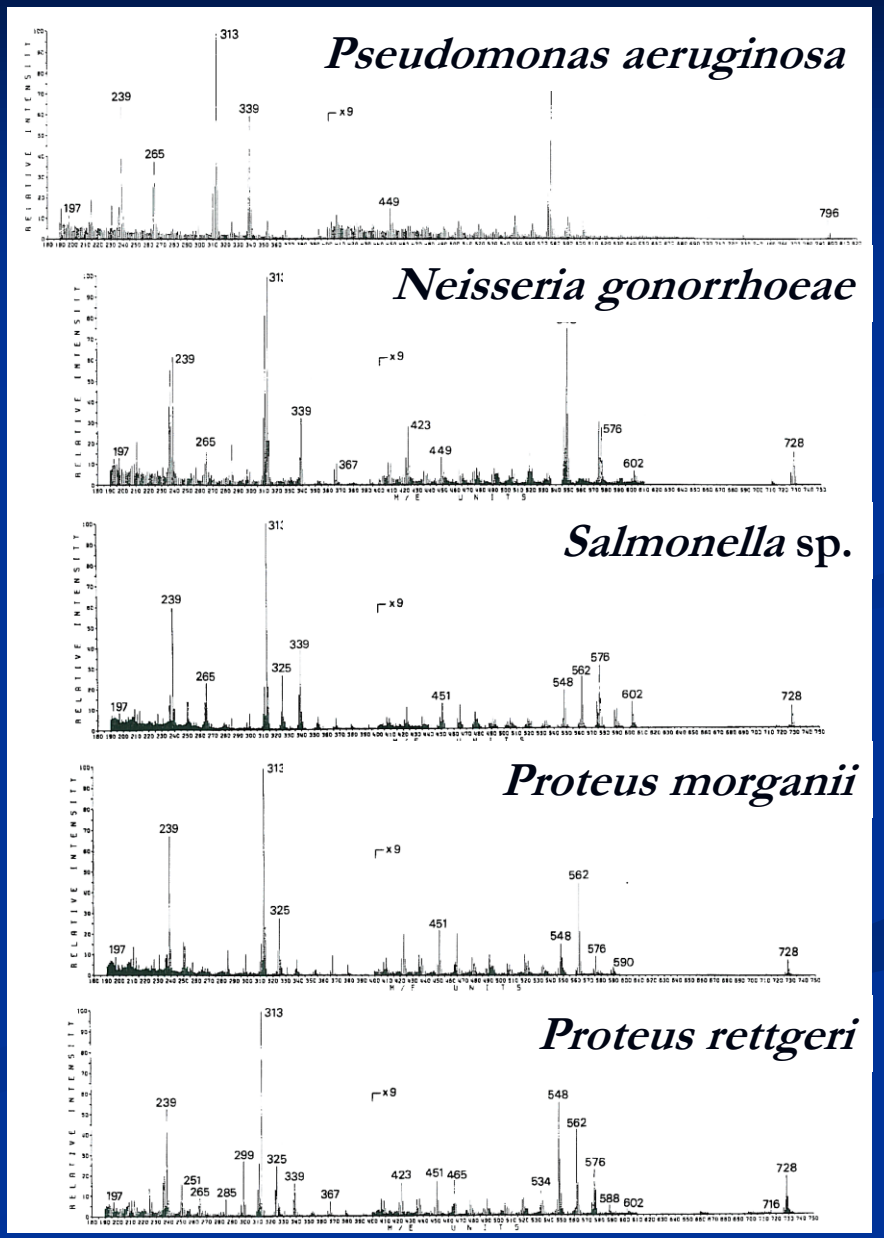
Seminal paper

Anhalt JP and Fenselau C

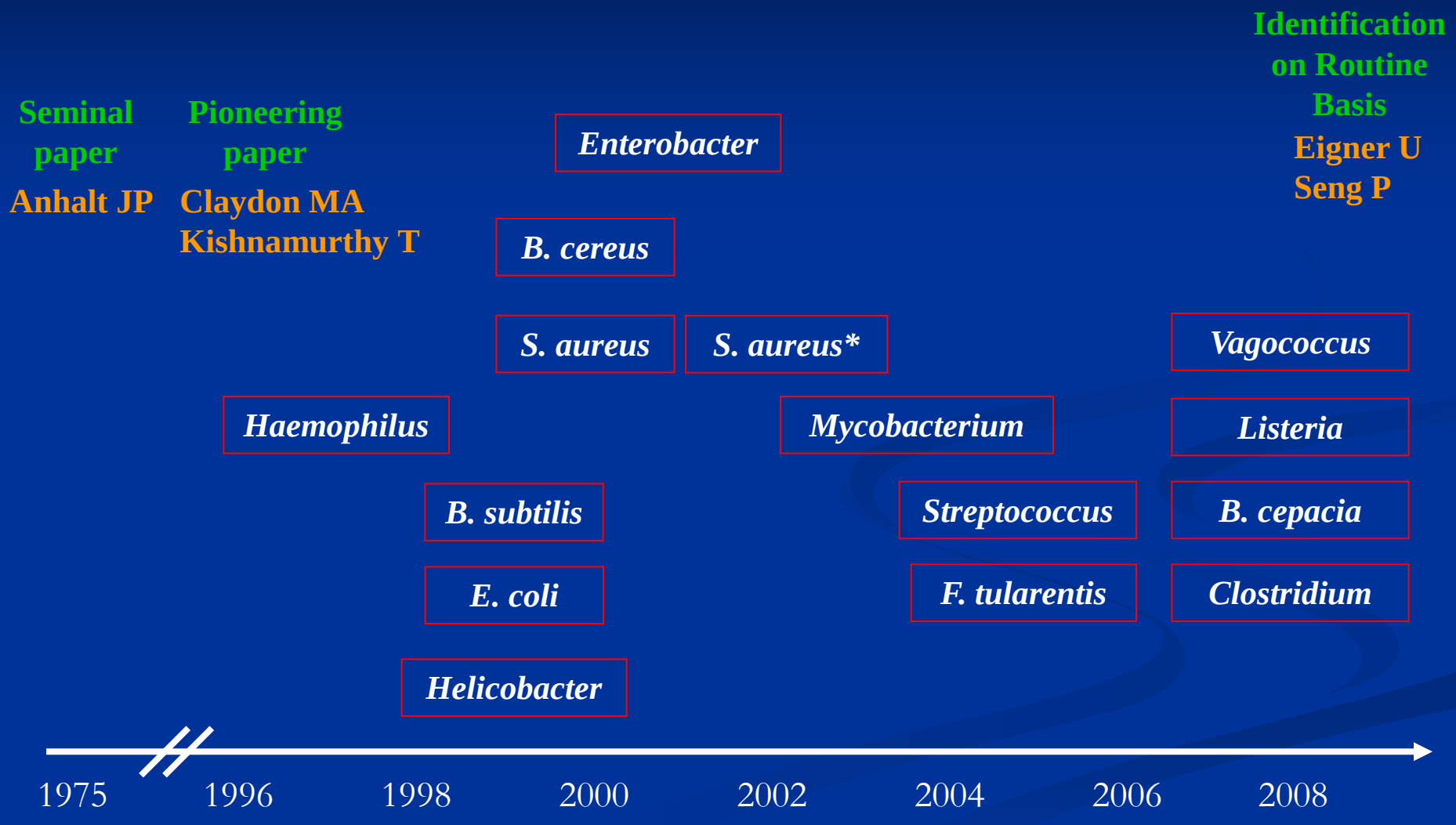
***Analytical Chemistry*. 1975**

Identification of bacteria using mass spectrometry





質量分析計を用いた細菌同定



（Seng P, et al. *Clinical Infectious Diseases*. 2009　一部改訂）

Identification on Routine Basis

Eigner U, et al. *Clinical laboratory*. 2009

Performance of a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system for the identification of bacterial isolates in the clinical routine laboratory.

臨床分離株：1116株　同定率　種レベル：95.2%, 属レベル：100%

MALDI-TOF MS system:Bruker Daltonics GmbH

Seng P, et al. *Clinical Infectious Diseases*. 2009

Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

臨床分離株：1660株　同定率　種レベル：84.1%, 属レベル：94.5%

MALDI-TOF MS system:Bruker Daltonics GmbH

MSACL 2010 EU

MALDI-TOF MS system
Bruker Daltonics GmbH

BRUKER

お問合せ

製品とソリューション　アプリケーション　サービス　ニュースとイベント　キャリア　企業情報

MALDI-TOF/TOF

microflex® LRF

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質量分析計 -- 簡便で多くの分析に理想的です

ベンチトップクラスのパフォーマンス

幅広いアプリケーション

Anal Bioanal Chem (2011) 400:1905–1911
DOI 10.1007/s00216-011-4877-7

ORIGINAL PAPER

Use of the MALDI BioTyper system with MALDI–TOF mass spectrometry for rapid identification of microorganisms

Kazuyuki Sogawa · Masaharu Watanabe · Kenichi Sato · Syunsuke Segawa · Chisato Ishii · Akiko Miyabe · Syota Murata · Tomoko Saito · Fumio Nomura

Received: 29 November 2010 / Revised: 3 March 2011 / Accepted: 6 March 2011 / Published online: 26 March 2011

Abstract In a clinical diagnosis microbiology laboratory, the current method of identifying bacterial isolates is based mainly on phenotypic characteristics, for example growth pattern on different media, colony morphology, Gram stain, and various biochemical reactions. These techniques collectively enable great accuracy in identifying most bacterial isolates, but are costly and time-consuming. In our clinical microbiology laboratory, we prospectively assessed the ability of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS) to identify bacterial strains that were routinely isolated from clinical samples. Bacterial colonies obtained from a total of 468 strains of 92 bacterial species isolated at the Department of Clinical Laboratory at Chiba University were directly placed on target MALDI plates followed by addition of CHCA matrix solution. The plates were then subjected to MALDI–TOF MS measurement and the microorganisms were identified by pattern matching with the libraries in the BioTyper 2.0 software. Identification success at the species and genus levels was 91.7% (429/468) and 97.0% (454/468), respectively. MALDI–TOF MS is a rapid, simple, and high-throughput proteomic technique for identification of a variety of bacterial species. Because colony-to-colony differences and effects of culture duration on the results are minimal, it can be implemented in a conventional laboratory setting. Although for some pathogens, preanalytical processes should be refined, and the current database should be improved to obtain more accurate results, the MALDI–TOF MS based method performs, in general, as well as conventional methods and is a promising technology in clinical laboratories.

Keywords Rapid microorganism identification · Clinical sample · MALDI–TOF MS · MALDI BioTyper

Published in the special issue *Biomedical Mass Spectrometry* with Guest Editors Hisao Oka and Mitsutoshi Setou.

Electronic supplementary material The online version of this article (doi:10.1007/s00216-011-4877-7) contains supplementary material, which is available to authorized users.

K. Sogawa · F. Nomura
Clinical Proteomics Research Center, Chiba University Hospital,
1-8-1 Inohana, Chuo-ku,
Chiba City, Chiba 260–8670, Japan

細菌同定結果

Number of isolate tested	Manufacturer of the MALDI-TOF MS system	Overall correct identification at the species level (%)	Overall correct identification at the genus level (%)
468	**Bruker Daltonics GmbH**	**91.7**	**5.3**

Conventional method	MALDI BioTyper method	16S rRNA method
Aeromonas hydrophila group	*Aeromonas caviae*	*Aeromonas caviae*
	Aeromonas hydrophila	*Aeromonas hydrophila*
Bordetella parapertussis	*Bordetella bronchiseptica*	*Bordetella bronchiseptica* or *Bordetella parapertussis*
Enterobacter cloacae	*Enterobacter asburiae*	*Enterobacter cloacae*
	Enterobacter ludwigii	*Enterobacter cloacae*
Enterococcus casseliflavus	*Enterococcus phoeniculicola*	*Enterococcus casseliflavus*
Haemophilus parahaemolyticus	*Haemophilus parainfluenzae*	*Haemophilus parainfluenzae*
Haemophilus parainfluenzae	*Haemophilus parahaemolyticus*	*Haemophilus parahaemolyticus*
Lactobacillus acidophilus	*Lactobacillus gasseri*	*Lactobacillus gasseri*
Stenotrophomonas maltophilia	*Pseudomonas geniculata*	*Stenotrophomonas maltophilia*
	Pseudomonas hibiscicola	*Stenotrophomonas maltophilia*
Streptococcus bovis	*Streptococcus pasteurianus*	*Streptococcus pasteurianus*
Vibrio fluvialis	*Vibrio furnissii*	*Vibrio furnissii*

Anal Bioanal Chem 2011; 400: 1905-1911.

世界的な同定の比較

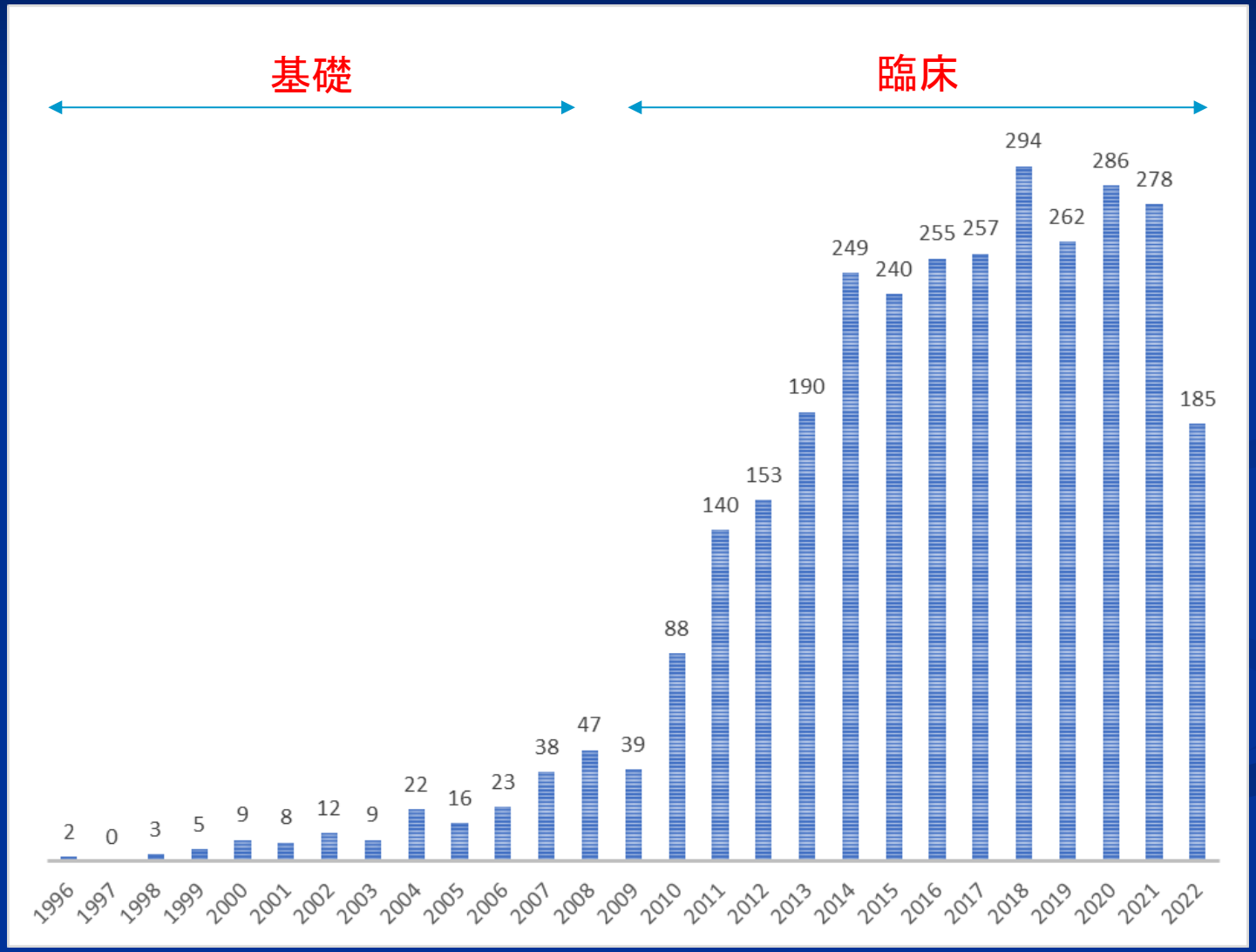
Table 1. International experience with performance of the Bruker Biotyper MALDI-TOF mass spectrometry system for routine bacterial (and yeast) identification in the clinical laboratory.

Isolates			% Identification				
No.	Type	Period of isolation	Genus level	Species level	Country	Comparator	Reference
1371	Bacteria, yeast	1 month	98%	93%	Switzerland	VITEK2, API, Biochemical	Bizzini A et al. *(6)*
980	Bacteria, yeast	5 weeks	99%	92%	The Netherlands	VITEK2, API, Biochemical	Veen SQ et al. *(7)*
1013	Bacteria	2 months	99%	97%	France	Phoenix, API, Biochemical	Bessède E et al. *(8)*
468	Bacteria	3 months	97%	92%	Japan	MicroScan, API, Phoenix	Sogawa K et al. *(9)*
2781	Bacteria	1 month	96%	85%	Australia	VITEK2, API, Biochemical	Neville SA et al. *(10)*

（Patel R. *Clinical Chemistry*. 2012）

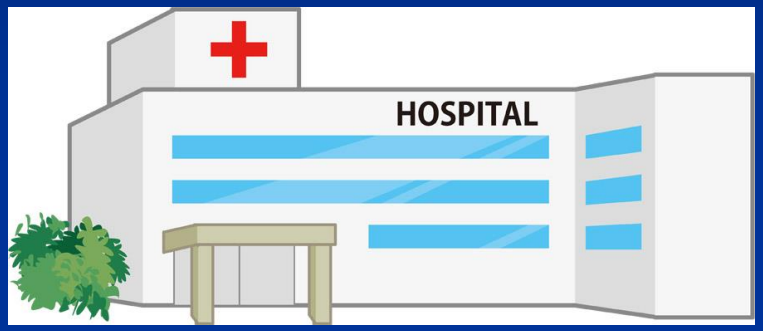
Pubmed 論文数

（MALDI-TOF MS, bacteria, identification）



日本の現状

病院



検査センター

約450台が稼働

一般社団法人
日本医療検査科学会
The Japan Association for Clinical Laboratory Science
（旧：日本臨床検査自動化学会）

検索する

新規入会申込み　会員マイページ

ホーム　本学会について　会員の皆様へ　委員会　書籍販売　COI　受賞関連

日本医療検査科学会
第53回大会情報はこちら
会期：2021年10月8日（金）～10日（日）　会場：パシフィコ横浜

精度管理の構築

- 2017年6月に検体検査の品質・精度確保に関する医療法等改正法案が可決・公布され、それに伴い改正された厚生労働省令（医療法施行規則、臨検法施行規則）が2018年7に公布、同年12月1日に施行された。この改正により、臨床検査を実施している施設には精度管理の法的基準が導入されることとなった。

- 近年、臨床微生物検査室を含む臨床検査室認定の国際規格「ISO 15189」（ISO；International Organization for Standardization, 国際標準化機構）の認定を取得する施設が増加している。臨床微生物検査は、測定対象が微生物であるため、菌株、市販生培地、測定方法で変動が生じやすいという特徴がある。内部精度管理として、菌株は*Escherichia coli*を使用している施設が多いが、MALDI-TOF MSの質量の校正として使用するため不適当である。

- 質量分析装置による細菌同定の内部精度管理について、菌株、市販生培地、測定方法に注目し、同定率（Score Value）で評価する内部精度管理実施要領の作成。

日本臨床検査自動化学会
遺伝子・プロテオミクス技術委員会
MALDI-TOF MSによる細菌同定精度管理の標準化WG

- 九州大学病院検査部（清祐麻紀子）
- 千葉大学医学部附属病院検査部（村田正太）
- 旭川医科大学医学部微生物学講座（渡　智久）
- 日本大学医学部病態病理学系臨床検査医学分野（中山智祥）
- ビオメリュー・ジャパン株式会社（奥村 元、関口幸恵）
- ブルカージャパン株式会社（藤永あずみ）
- 麻布大学（曽川一幸）

医療検査と自動化 Vol.45 No.3 2020

質量分析装置Matrix-assisted laser desorption/ionization time-of-flight mass spectrometryの細菌同定における内部精度管理実施要領の検討

Examination of Conditions for Internal Quality Control in Identification of Microorganism from Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry

曽川一幸[1)]　清祐麻紀子[2)]　服部佳奈子[2)]　村田正太[3)]　渡　智久[4)]
奥村　元[5)]　関口幸恵[5)]　藤永あずみ[6)]　中山智祥[7)]

要旨　高い迅速性と正確性を有し，しかも低コストの細菌同定手法として，Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry（MALDI-TOF MS）質量分析装置が，2011年に医療機器として認定され，細菌同定のツールとして運用されている。国内における微生物検査ならびにその内部精度管理は主に米国の臨床検査標準協会の基準の基で実施されている。しかしながら，MALDI-TOF MSによる細菌同定の内部精度管理を実施している施設はわずかである。日本臨床検査自動化学会遺伝子・プロテオミクス技術委員会MALDI-TOF MSワーキンググループにおいて，日常業務に適したMALDI-TOF MSによる細菌同定における内部精度管理の実施要領の作成に資する検討を行った。

Key words　identification of microorganism, internal quality control, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

MALDI-TOF MSによる細菌同定の内部精度管理実施要領

① 使用菌株

Enterobacter aerogenes（ATCC13048）

Enterococcus faecalis（ATCC19433）

② 培養方法

マイクロバンクから常に同じ血液寒天培地に接種し、18～24時間培養する

③ マトリックス試薬

MALDI Biotyper：HCCA porsion試薬

VITEK MS：MS-CHCAマトリックス試薬

④ ファインチューニングの期間

MALDI Biotyper：6か月～1年

VITEK MS：約2500検体に1回

Biocontrol Science, 2021, Vol. 26, No. 2, 99–104

Note

Examination of conditions for regular internal quality control in identification of microorganisms using MALDI-TOF MS

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was approved for medical use in 2011, and is currently used as a rapid, accurate and low-cost technique for bacterial identification. Microbiological testing and internal accuracy control in Japan are mainly implemented in accordance with the standards of the Clinical and Laboratory Standards Institute (CLSI). However, few facilities perform internal accuracy control of bacterial identification by MALDI-TOF MS. Therefore, we examined the procedures for internal accuracy control of bacterial identification using MALDI-TOF MS in daily work at clinical laboratories in the seven hospitals.

Key words :Identification of microorganisms / Internal quality control / Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

MALDI-TOF MSによる細菌同定の外部精度管理実施要領

① 使用菌株

Enterobacter aerogenes（ATCC13048）

Enterococcus faecalis（ATCC19433）

② 培養方法

マイクロバンクから常に同じ血液寒天培地に接種し、18～24時間培養する

③ マトリックス試薬

MALDI Biotyper：HCCA porsion試薬

VITEK MS：MS-CHCAマトリックス試薬

④ 施設数

40施設

TABLE 2. MALDI Biotyper score values for *E. aerogenes* and *E. faecalis*

	MALDI Biotyper: score values (Mean ± SD)							
	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
Chiba University Hospital								
E. aerogenes	2.520±0.062	2.533±0.022	2.539±0.019	no data	2.532±0.037	2.561±0.049	2.540±0.047	2.480±0.018
E. faecalis	2.437±0.019	2.408±0.027	2.411±0.015	no data	2.441±0.025	2.433±0.036	2.403±0.038	2.442±0.034
Saga University Hospital								
E. aerogenes	2.542±0.026	2.528±0.014	2.519±0.026	2.505±0.038	2.519±0.023	2.539±0.014	2.505±0.042	2.517±0.038
E. faecalis	2.399±0.056	2.345±0.035	2.368±0.021	2.392±0.037	2.413±0.044	2.394±0.024	2.367±0.040	2.398±0.021
Shinshu University Hospital								
E. aerogenes	2.594±0.045	2.522±0.043	2.512±0.063	2.524±0.015	2.613±0.052	2.554±0.046	2.589±0.029	2.538±0.048
E. faecalis	2.337±0.166	2.366±0.101	2.394±0.059	2.421±0.060	2.349±0.067	2.428±0.014	2.279±0.102	2.439±0.038
Miyazaki University Hospital								
E. aerogenes	2.542±0.032	2.504±0.034	2.530±0.024	2.526±0.020	2.528±0.025	2.540±0.044	2.518±0.061	2.524±0.010
E. faecalis	2.364±0.038	2.438±0.043	2.378±0.037	2.386±0.031	2.355±0.027	2.378±0.030	2.348±0.092	2.228±0.073

As reliability indexes that indicates accuracy at the bacterial species level, a score of 2.000-3.000 was used for the MALDI Biotyper.

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Note

External quality control survey on identification of microorganisms using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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[5] *Department of Clinical Laboratory, Chiba University Hospital, 1-8-1 Inohana, Chuo-ku, Chiba, Japan*
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[7] *Department of Clinical Laboratory and Blood Transfusion, University Hospital of the Ryukyus, 207 Uehara, Nishihara-cho, Okinawa, Japan*
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Received 13 September 2023 / Accepted 6 December 2023

Abstract: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a bacterial typing tool that was approved as a medical device in 2011. However, external accuracy control examination of bacterial typing using mass spectrometry is still only performed on a small scale. In this study, *E. faecium* and *S. maltophilia* were selected and tested according to established procedures using Score Values at 228 institutions. The Score Values for MALDI Biotyper were 2.43±0.08 for *E. faecium* and 2.38±0.08 for *S. maltophilia*; and those for VITEK MS/PRIME were 99.9±0.0 for *E. faecium* and *S. maltophilia*. These results suggest that it is useful to evaluate external accuracy control with Score Values using the procedures we have developed.

Keywords : external quality control / identification of microorganisms / matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Bacterial typing is an easy-to-use method with regard to sample preparation and measurement that permits identification after about 5 minutes per strain (Freiwald and Sauer, 2009; Seng et al., 2009). This technique has attracted attention as a procedure that makes it easy to identify species and strains without cumbersome pretreatment of samples (van Veen et al., 2010; Bizzini et al., 2010; De Bel et al., 2010; Sauer and Kliem, 2010; Sogawa et al., 2011; Sogawa et al., 2012). In Japan, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was approved in 2011 as a medical device for use as a tool for bacterial typing.

The Partial Amendment of the Medical Practitioners' Act on quality and accuracy assurance of specimen examination was passed and promulgated in June 2017. The relevant amended ordinances of the Ministry of Health, Labour and Welfare (Ordinance for Enforcement of the Medical Care Act and Regulation for Enforcement of the Act on Clinical Laboratory Technicians) were promulgated in July 2018 and put into effect on December 1, 2018 (Nishiyama, 2018). Legal criteria for accuracy control were introduced into clinical laboratory sites by this amendment. In April 2017, the Clinical and Laboratory Standards Institute (CLSI) devel-

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第2回MALDI-TOF MSによる細菌同定の外部精度管理調査実施要領

① 使用菌株

Enterococcus faecium

Stenotrophomonas maltophilia

② 培養方法

マイクロバンクから常に同じ血液寒天培地に接種し、18～24時間培養する

③ マトリックス試薬

MALDI Biotyper：HCCA porsion試薬

VITEK MS：MS-CHCAマトリックス試薬

④ 施設数

MALDI Biotyper（ブルカージャパン）使用施設：184施設

バイテックMS（ビオメリュー・ジャパン）使用施設：41施設

調査報告

MALDI Biotyper（ブルカージャパン）

菌名	評価		
	A （SDI ±5）	B （SDI<-5）	D （スコア＜2.000）
Enterococcus faecium			
1回目	179施設	4施設	1施設
2回目	175施設	7施設	2施設
Stenotrophomonas maltophilia			
1回目	181施設	3施設	0施設
2回目	174施設	0施設	10施設

バイテックMS（ビオメリュー・ジャパン）

菌名	評価		
	A （SD ±3）	B （SD<-3）	D （同定出来ず）
Enterococcus faecium			
1回目	40施設	0施設	1施設
2回目	40施設	0施設	1施設
Stenotrophomonas maltophilia			
1回目	41施設	0施設	0施設
2回目	41施設	0施設	0施設

第3回MALDI-TOF MSによる細菌同定の外部精度管理調査実施要領

① 使用菌株

Enterococcus faecium（ATCC 8459）
Moraxella catarrhalis（ATCC8176）
Candida albicans（ATCC2091）

② 培養方法

マイクロバンクから常に同じ血液寒天培地に接種し、18～24時間培養する

③ マトリックス試薬

MALDI Biotyper：HCCA porsion試薬
VITEK MS：MS-CHCAマトリックス試薬

④ 施設数

MALDI Biotyper（ブルカージャパン）使用施設：141施設
バイテックMS（ビオメリュー・ジャパン）使用施設：22施設

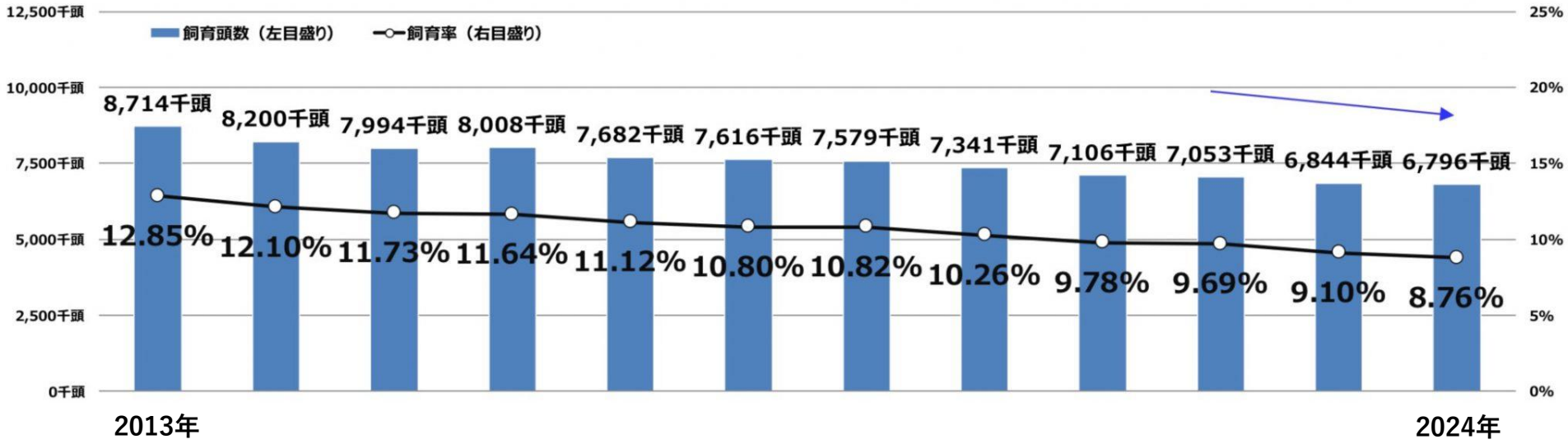
⑤ 参加費：5500円

調査報告

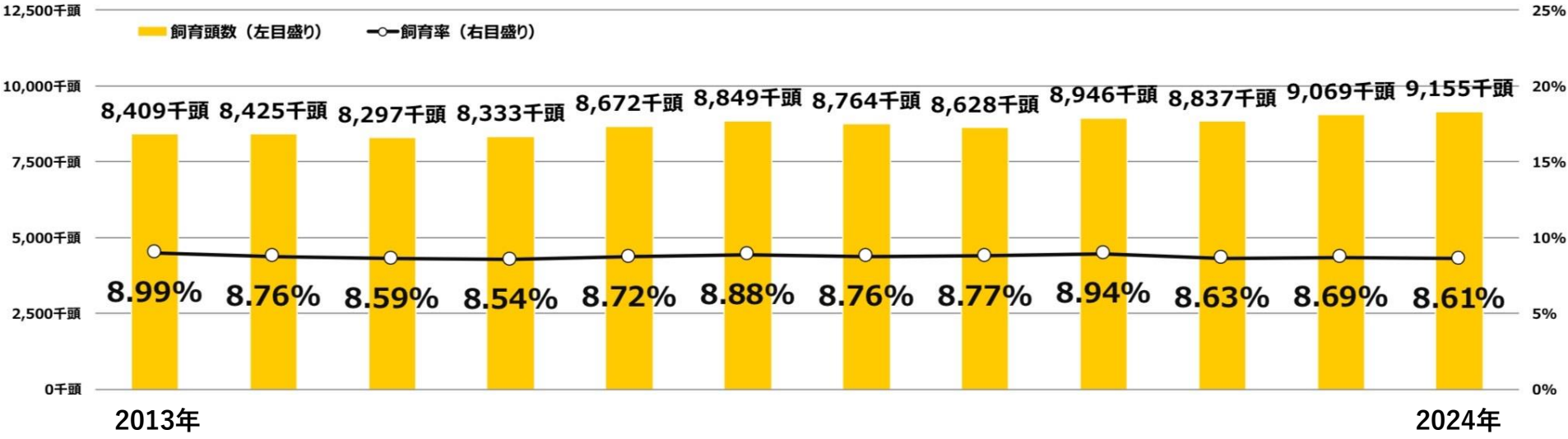
Table 1. Evaluation by MALDI Biotyper and VITEK MS

Bacterial strain	Score values		
	Average ± 2SD	Below average - 2SD	Not identification
Enterococcus faecium (ATCC8459)			
MALDI Biotyper	127	14	0
VITEK MS	22	0	0
Moraxella catarrhalis (ATCC8176)			
MALDI Biotyper	139	1	1
VITEK MS	22	0	0
Candida albicans (ATCC2091)			
MALDI Biotyper	110	0	31
VITEK MS	22	0	0

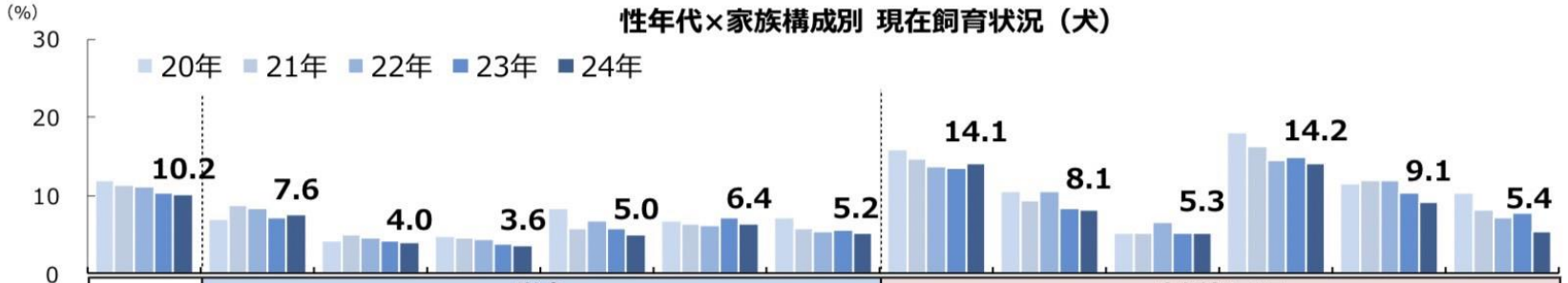
犬の飼育頭数推移



猫の飼育頭数推移



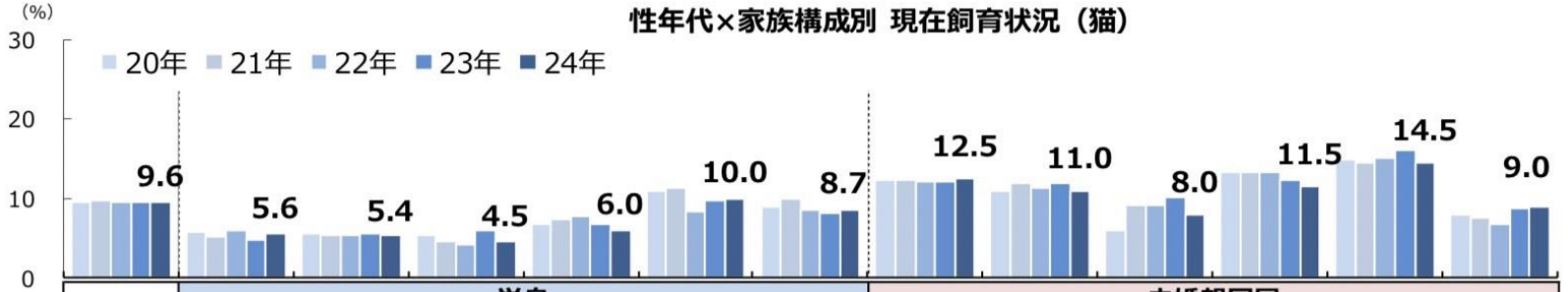
性年代×家族構成別 現在飼育状況（犬）



	TOTAL	単身						未婚親同居					
		男性			女性			男性			女性		
		20-30代	40-50代	60-70代	20-30代	40-50代	60-70代	20-30代	40-50代	60-70代	20-30代	40-50代	60-70代
n=	(50,000)	(2,434)	(2,160)	(1,378)	(1,668)	(1,430)	(1,779)	(3,026)	(1,916)	(246)	(2,397)	(1,244)	(226)
20年	11.9	7.0	4.2	4.8	8.3	6.7	7.3	15.9	10.6	5.3	18.0	11.5	10.4
21年	11.3	8.8	4.9	4.5	5.7	6.3	5.9	14.8	9.3	5.1	16.3	12.0	8.2
22年	11.1	8.4	4.7	4.4	6.7	6.2	5.3	13.7	10.6	6.6	14.5	11.9	7.1
23年	10.4	7.3	4.3	3.9	5.8	7.1	5.5	13.6	8.4	5.3	15.0	10.3	7.8
24年	10.2	7.6	4.0	3.6	5.0	6.4	5.2	14.1	8.1	5.3	14.2	9.1	5.4

※集計母数は24年のみ掲載

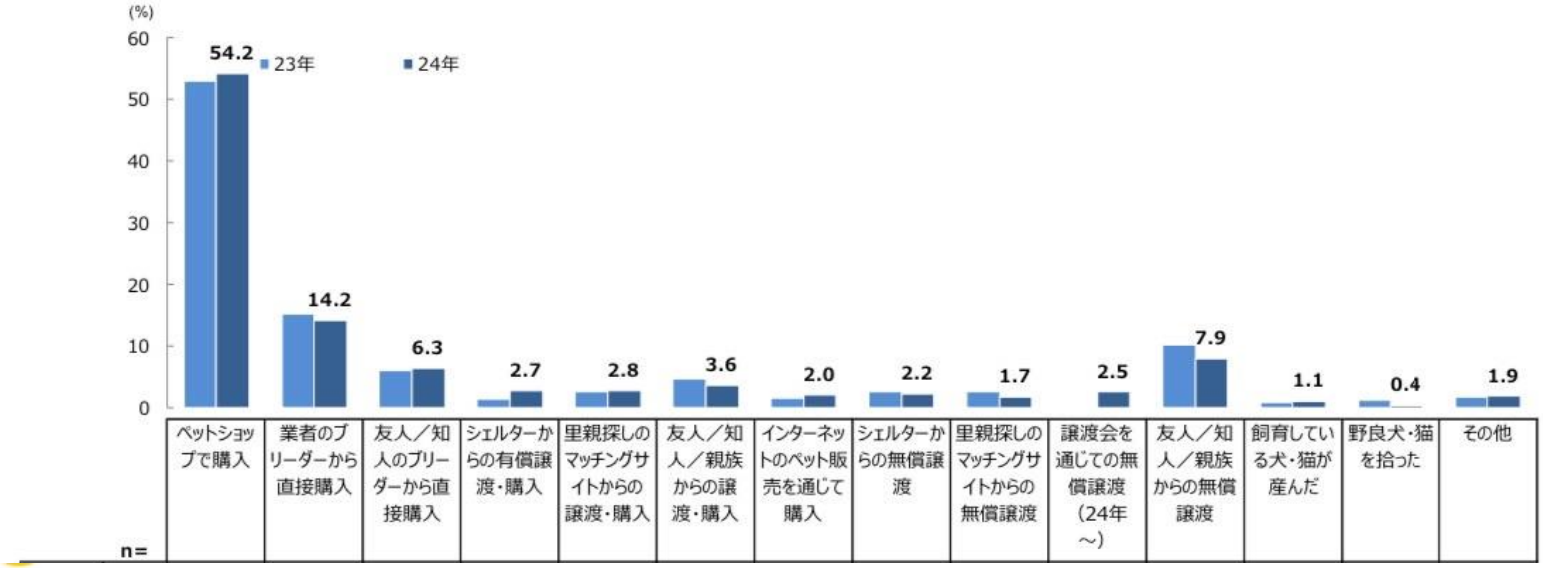
性年代×家族構成別 現在飼育状況（猫）



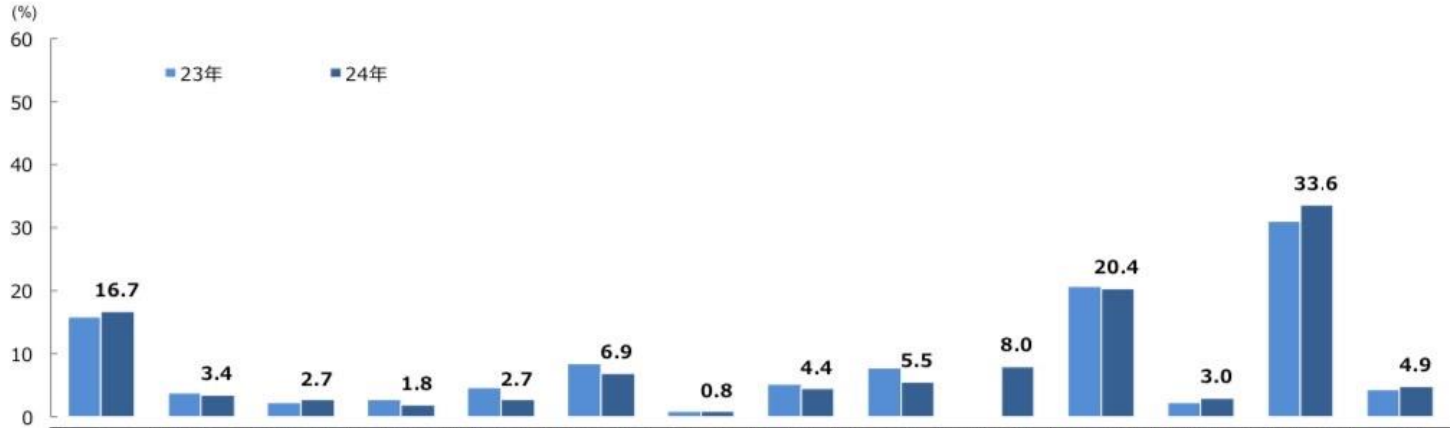
	TOTAL	単身						未婚親同居					
		男性			女性			男性			女性		
		20-30代	40-50代	60-70代	20-30代	40-50代	60-70代	20-30代	40-50代	60-70代	20-30代	40-50代	60-70代
n=	(50,000)	(2,434)	(2,160)	(1,378)	(1,668)	(1,430)	(1,779)	(3,026)	(1,916)	(246)	(2,397)	(1,244)	(226)
20年	9.6	5.8	5.6	5.4	6.8	11.0	9.1	12.3	11.0	5.9	13.3	14.8	8.0
21年	9.8	5.2	5.4	4.7	7.4	11.3	9.9	12.4	12.0	9.2	13.2	14.5	7.5
22年	9.6	6.0	5.3	4.3	7.8	8.5	8.5	12.1	11.4	9.1	13.3	15.1	6.8
23年	9.6	4.8	5.6	6.0	6.9	9.7	8.2	12.2	12.0	10.1	12.3	16.0	8.8
24年	9.6	5.6	5.4	4.5	6.0	10.0	8.7	12.5	11.0	8.0	11.5	14.5	9.0

※集計母数は24年のみ掲載

Q27 ペットの入手先(MA)【ベース：現在犬猫各飼育者】：犬 [Q27(1)]



Q27 ペットの入手先(MA)【ベース：現在犬猫各飼育者※猫は野良猫・地域猫給餌あり含む】：猫 [Q27(2)]



	n=	ペットショップで購入	業者のブリーダーから直接購入	友人／知人のブリーダーから直接購入	シェルターからの有償譲渡・購入	里親探しのマッチングサイトからの譲渡・購入	友人／知人／親族からの譲渡・購入	インターネットのペット販売を通じて購入	シェルターからの無償譲渡	里親探しのマッチングサイトからの無償譲渡	譲渡会を通じての無償譲渡（24年～）	友人／知人／親族からの無償譲渡	飼育している犬・猫が産んだ	野良犬・猫を拾った	その他
23年	782	15.9	3.8	2.3	2.7	4.6	8.4	0.9	5.1	7.7	-	20.6	2.3	31.1	4.3
24年	886	16.7	3.4	2.7	1.8	2.7	6.9	0.8	4.4	5.5	8.0	20.4	3.0	33.6	4.9

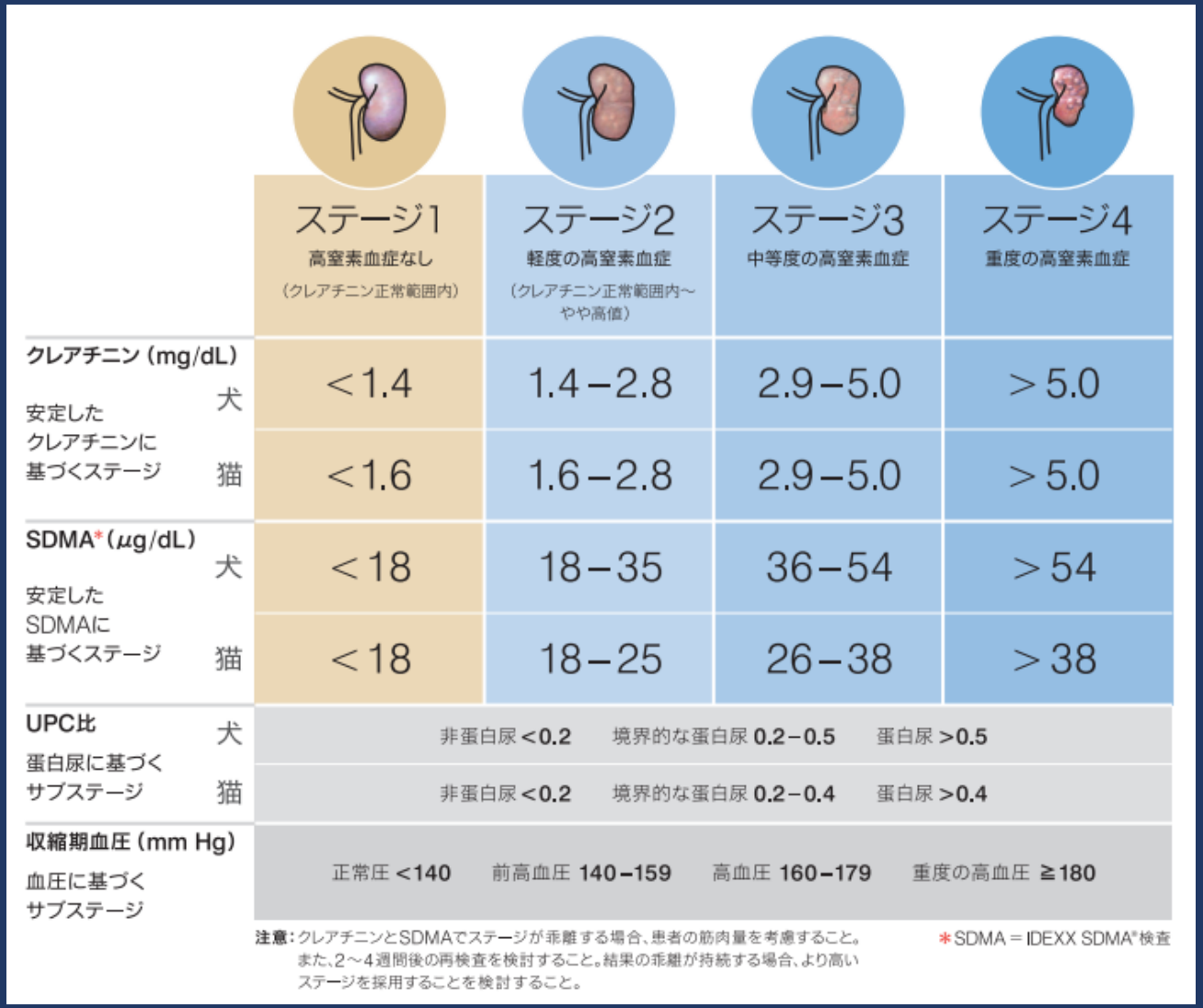
猫で多い病気TOP10と年間平均診療費

順位	病名	請求数（件）	金額（円）
1	慢性腎臓病（腎不全含む）	61,923	272,598
2	嘔吐/下痢/血便（原因未定）	20,523	37,601
3	膀胱炎	14,620	45,741
4	胃炎/胃腸炎/腸炎	11,934	36,334
5	心筋症	7,377	164,135
6	結膜炎（結膜浮腫含む）	7,354	18,647
7	原因未定の外耳炎	6,630	28,166
8	元気喪失（食欲不振含む、原因未定）	6,922	48,947
9	糖尿病	6,084	321,831
10	原因未定の皮膚炎	6,072	24,592

※猫の保険金請求289,196件のうち、請求件数が多かった病気TOP10を掲載

アニコム ホールディングス株式会社

国際腎臓病研究グループ（IRIS）病気分類



		ステージ1 高窒素血症なし （クレアチニン正常範囲内）	ステージ2 軽度の高窒素血症 （クレアチニン正常範囲内～やや高値）	ステージ3 中等度の高窒素血症	ステージ4 重度の高窒素血症
クレアチニン（mg/dL） 安定したクレアチニンに基づくステージ	犬	＜1.4	1.4－2.8	2.9－5.0	＞5.0
	猫	＜1.6	1.6－2.8	2.9－5.0	＞5.0
SDMA*（μg/dL） 安定したSDMAに基づくステージ	犬	＜18	18－35	36－54	＞54
	猫	＜18	18－25	26－38	＞38
UPC比 蛋白尿に基づくサブステージ	犬	非蛋白尿 ＜0.2	境界的な蛋白尿 0.2－0.5	蛋白尿 ＞0.5	
	猫	非蛋白尿 ＜0.2	境界的な蛋白尿 0.2－0.4	蛋白尿 ＞0.4	
収縮期血圧（mm Hg） 血圧に基づくサブステージ		正常圧 ＜140	前高血圧 140－159	高血圧 160－179	重度の高血圧 ≧180

注意：クレアチニンとSDMAでステージが乖離する場合、患者の筋肉量を考慮すること。また、2～4週間後の再検査を検討すること。結果の乖離が持続する場合、より高いステージを採用することを検討すること。

＊SDMA＝IDEXX SDMA®検査

日本獣医腎泌尿器学会HPより

対象・方法

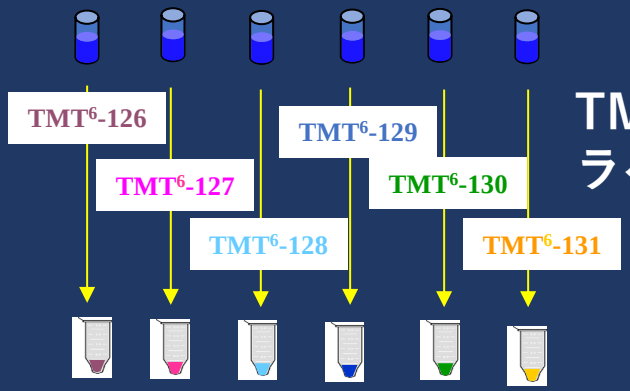
血漿(10μL)

健常ネコ3匹
(尿中Alb/Cr比 <10mg/g)

慢性腎障害ネコ3匹
(尿中Alb/Cr比 >30mg/g)

Con A (Canavalia ensiformis)
(N型糖蛋白質の抽出)

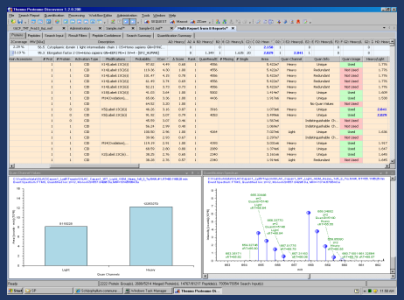
トリプシン消化



TMT試薬のラベル化

Combine

LC-MS/MSで測定



同定・定量比較

結果

No	Description	腎症群 / 正常群
1	Acetylcholinesterase	2.3
2	Aminopeptidase N	1.4
3	Angiotensin-converting enzyme 2	1.8
4	Apolipoprotein A-IV	1.2
5	Beta-2-microglobulin	1.3
6	Beta-galactosidase	1.4
7	Beta-glucuronidase	1.5
8	Beta-hexosaminidase subunit beta	1.2
9	Beta-lactoglobulin-1	1.1
10	Beta-lactoglobulin-2	1.6
11	Carboxylesterase 5A	2.5
12	CD9 antigen	1.2
13	CD63 antigen	1.3
14	Coagulation factor IX	1.2
15	Cystatin-A	1.3
16	Cystatin-C	1.5
17	Dipeptidyl peptidase 4	1.6
18	Ferritin light chain	1.2
19	Fibrinogen alpha chain (Fragment)	1.1
20	Haptoglobin	2.2
21	Hemoglobin subunit alpha	1.6
22	Junctional adhesion molecule A	1.2
23	Kidney injury molecule-1	3.4
24	Lysosomal alpha-mannosidase	1.3
25	Macrophage colony-stimulating factor 1 receptor	1.4
26	Malate dehydrogenase, cytoplasmic	1.1
27	Pro-epidermal growth factor	1.3
28	Prostaglandin-H2 D-isomerase	2.6
29	Retinol binding protein 4	1.2
30	Serum albumin	1.5
31	Stromal cell-derived factor 1	1.4
32	Toll-like receptor 4	1.2

ヒトにおける慢性腎障害診断マーカー

Biomarker	Sample	Origin	Biological function
NGAL	Urine	Distal tubule, collecting duct	Promotes tubule cell survival and proliferation, limits tubule cell apoptosis
KIM-1	Urine	Proximal tubule	Promotes epithelial regeneration, regulates tubule cell apoptosis
L-FABP	Urine	Proximal tubule	Endogenous antioxidant, suppresses tubulointerstitial damage
NAG	Urine	Proximal tubule	Glycosidase activity in lysosomes of proximal tubule cells
NGAL	Blood	Liver, lung, neutrophils	Acute phase reactant, marker of organ cross-talk following kidney injury
ADMA	Blood	Methylated proteins	Inhibitor of nitric oxide synthase, promotes endothelial damage and sclerosis
Adiponectin	Blood	Adipose tissue	Improves insulin sensitivity, anti-inflammatory, anti-atherogenic
ApoA-IV	Blood	Intestinal enterocytes	Reverse cholesterol transport pathway, anti-atherogenic
FGF23	Blood	Osteocytes, osteoblasts	Phosphaturic, reduction of circulating calcitriol, inhibition of PTH secretion
ANP, BNP	Blood	Stretched heart walls	Natriuresis, diuresis, vasodilation, regulation of cardio-renal axis, anti-fibrotic

Devarajan P. Adv Chronic Kidney Dis. 17: 469–479. 2010.

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ApoA-IV	Blood	Intestinal enterocytes	Reverse cholesterol transport pathway, anti-atherogenic
FGF23	Blood	Osteocytes, osteoblasts	Phosphaturic, reduction of circulating calcitriol, inhibition of PTH secretion
ANP, BNP	Blood	Stretched heart walls	Natriuresis, diuresis, vasodilation, regulation of cardio-renal axis, anti-fibrotic

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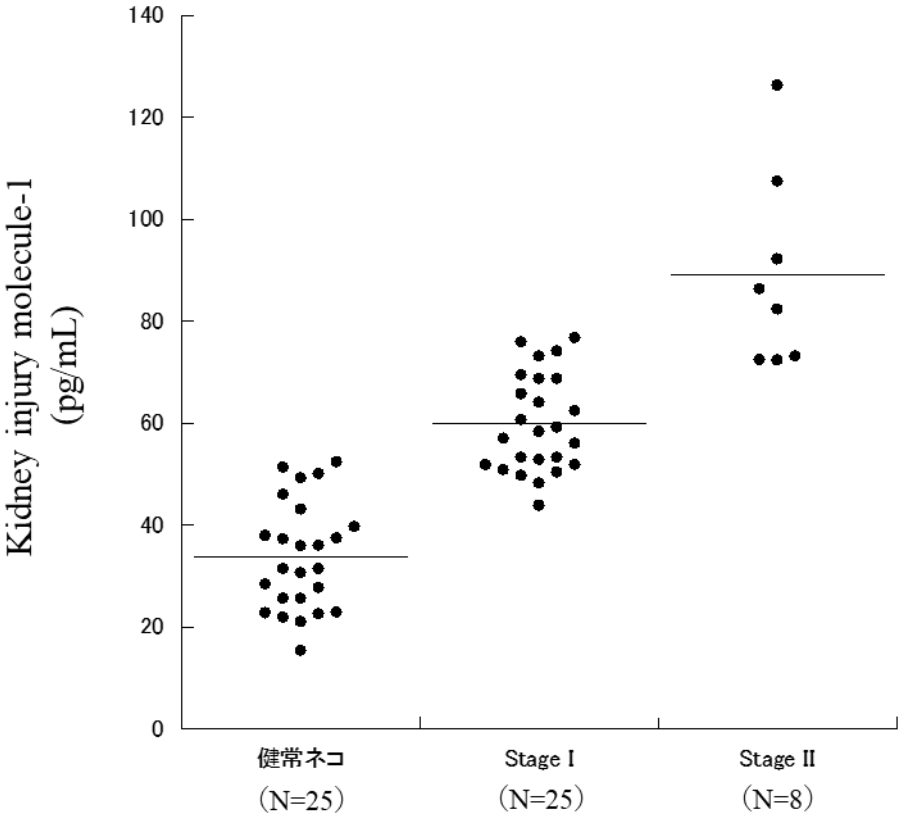
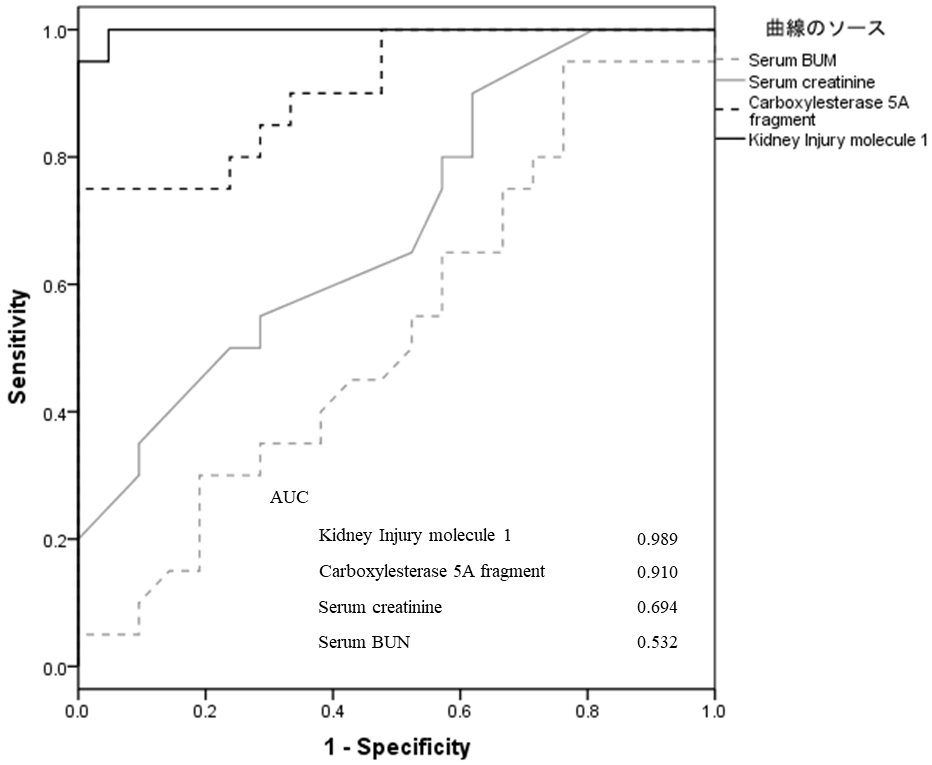
KIM-1測定



ROC曲線
（正常 vs IRIS分類stage I）



Medical Mass Spectrometry. 2022.