Optimal Matrix Preparation Methods for Matrix-assisted Laser Desorption/ionisation Time-of-flight Mass Spectrometry Profiling of Low Molecular Weight Peptides in Human Saliva and Serum Samples

Lu GAO[1], Yan GU[1], Feng CHEN[2], Qing Wei MA[3]

Objective: *To develop standard experimental methods to minimise technical variance in matrix preparation for MALDI-TOF MS (matrix-assisted laser desorption/ionisation time-of-flight) profiling.*

Methods: *Matrix factors in saliva and serum samples of 20 healthy volunteers were examined, assuring their peptide components using seven different matrix type/preparation methods, HCCA(a-cyano-4-hydroxycinnamic acid)/SM(sample/matrix), SA(sinapinic acid)/DD(dried droplet), SA/SM, DHB(2.5-dihydroxyhenz-zoic acid)/DD, DHB/SM, DHAP(2.5-dihydroxy-acetophenone)/DD, DHAP/SM. Number of peaks, S/N(signal to noise) ratio and approximate range of target peaks were set as main selection criteria to find if these spell out any common regularity in results.*

Results: *Different methods perform differently. DHB/DD performed worst in both samples, with no effective peak detected. For saliva sample, the S/N ratios of other six methods were lower. M/z range distributed differently. DHB/SM and DHAP/DD performed best in number of peaks, m/z distributing in 1000 to 2000 account for the vast majority. For serum sample, S/N ratios and m/z range distribution were different in different methods. S/N ratio of SA/DD and SA/SM were higher, number of peaks and m/z distribution were not irreplaceable. S/N ratios of the other four methods were lower.*

Conclusion: *DHAP/DD and HCCA/SM performed best in number of peaks, m/z in 5000 – 7000 account for the vast majority in HCCA/SM and 1000 – 2000 in DHAP/DD. Further studies should focus on other characteristics of peptide components detected in different matrix methods to increase evidence when selecting matrix type/preparation methods.*

Key words: *MALDI-TOF MS, matrix preparation, proteomics, peptides, saliva*

Chin J Dent Res 2018;21(1):51–61; doi: 10.3290/j.cjdr.a39918

1 Department of Orthodontic, Peking University School and Hospital of Stomatology, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Beijing, P.R. China.

2 Central Laboratory, Peking University School and Hospital of Stomatology, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Beijing, P.R. China.

3 BioyongTech Technology Corporation, Beijing, P.R. China.

Corresponding author: Dr Yan GU, Department of Orthodontic, Peking University School and Hospital of Stomatology, 22# Zhongguancun South Avenue, HaiDian District, Beijing 100081, P.R. China. Tel: 86 13651078552; Fax: 86 10 62173402. Email: guyan96@126.com;
Dr Feng CHEN, Central Laboratory, Peking University School and Hospital of Stomatology, 22# Zhongguancun South Avenue, HaiDian District, Beijing 100081, P.R. China. Tel: 86 10 82195773; Fax: 86 10 62173402. Email: chenfeng2011@hsc.pku.edu.cn.

Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF MS) profiling of proteome and peptidome in human body fluids for disease-associated patterns is a new concept in clinical diagnostics[1-9]. The technique of MALDI-TOF MS is highly sensitive to external sources of variation leading to potentially unacceptable numbers of false positive and false negative results[1]. Before MS profiling can be confidently implemented in a medical setting, standard experimental methods must be developed to minimise technical variance. There are many deviation factors that can

This research is supported by National Natural Science Foundation of China 81771027.

Table 1 Factors at various stages of MALDI-TOF MS profiling workflow according to past studies.

Stage	Factor
Pre-analytical	Demographic bias[17-19]
	Sample collection conditions[18,20-23]
	Clotting times[17-21]
	Storage[18,23,24]
	Freeze/thaw cycles[17-20,24]
	Handling temperature[25]
	Humidity[25]
	Pre-fractionation[18,26,27]
Analytical	Chromatographic separation[22,28,29]
	MALDI support targets[28,30-32]
	Matrix[33,34]
	Data acquisition[28,35,36]
Post-analytical	Bioinformatics[36-39]

influence the accuracy of MALDI-TOF MS profiling workflow, including pre-analytical, analytical and post-analytical stages[1].

Here we list the possible deviation factors at various stages of MALDI-TOF MS experiments according to past studies, as shown in Table 1. There, we examined the matrix factor influence MS profiling. The goal of our study is to be of some help to MALDI-TOF MS profiling of proteome and peptidome in human body fluids in the future, especially saliva and serum.

Materials and methods

This study was approved by the Peking University Biomedical Ethics Committee. All subjects provided written consent before participating in the study.

Experimental procedures

Samples collection

Saliva sample[1]

Twenty healthy volunteers without periodontitis were randomly chosen at the Peking University School and Hospital of Stomatology. All individuals were asked to rest for 15 min before saliva collection at 8:30 am, and not to eat or drink after dinner the previous evening or to brush their teeth on the collection day morning. The subjects sat in an upright position in a quiet room, and were required to put the tip of their tongue against the sublingual caruncle without straining. Thus, saliva ran from the mouth and was collected into a paper cup for the first 5 min using a 50 mL centrifuge tube until 6 ml was collected. During the collection procedure, the subjects were asked not to speak. Immediately after collection, the 6 ml unstimulated whole saliva samples were kept on ice and then centrifuged at 9000 g for 7 min at 4°C to remove insoluble materials, cells and debris. The supernatant of each sample was obtained; 1 mM pepstatin (Sigma Aldrich, St Louis, MO, USA) and 0.1 mM phenylmethyl sulfonylfluoride (Sigma) was added to inhibit protease activity[10]. Each 10 uL of sample was taken and mixed as one 200 uL mixed sample. The mixed sample was kept at -80°C for further analysis[10].

Serum sample

The same 20 healthy volunteers were asked to rest for 15 min before serum collection at 8:30 am, and not to eat or drink after dinner the previous evening. The blood samples were collected in EDTA tubes, kept at room temperature for 30 min and then centrifuged at 3000 rpm for 5 min at room temperature to separate serum from whole blood. The supernatant of each sample was obtained, 0.25 uL of each sample was taken and mixed as one 5 uL mixed sample[10-16]. The mixed sample was kept at -80°C for further analysis.

Matrix types and preparations

Matrix types[2]

Solvents and chemicals were sourced from the following manufacturers: a-cyano-4-hydroxycinn-amic acid (HCCA) (YuanYe Technology, Shanghai, China), sinapinic acid (SA) (YuanYe), 2.5-dihydroxybenzoic acid (DHB) (YuanYe), and 2.5-dihydroxyacetophenone (DHAP) (YuanYe).

Matrix preparations[2]

Seven different matrix solution groups, designated as matrix preparations, were prepared as described in Table 1. The solutions were freshly made each day. The dried-droplet (DD) preparation method was originally described by Karas and Hillenkamp[2]. The sample/wash (SM) method was based on the sample/matrix/wash (SMW) method described by Zhang et al[3], with the exclusion of the wash step. The names, compositions and spotting instructions for the seven matrix preparation groups are shown in Table 2.

Table 2 Preparation methods for the seven matrix preparation groups.

Name	Preparation	Spotting instructions
HCCA/SM	Stock solution: 10 mg/mL in 70% ACN, 0.1% TFA (prepared fresh) Working solution: 1:20 dilution of stock in 90% ACN, 0.1% TFA	Add 1 μL of sample to spot, allow to dry. Add 1 μL of matrix and allow to dry.
SA/DD	Working solution: 10 mg/mL in 90% ACN, 0.1% TFA	Mix sample and matrix 1:1, apply 1 μL to spot and allow to dry. Recrystallize with 0.5 μL matrix and allow to dry.
SA/SM	Working solution: 10 mg/mL in 90% ACN, 0.1% TFA	Add 1 μL of sample to spot, allow to dry. Add 0.5 μL of matrix and allow to dry
DHB/DD	Working solution: 10 mg/mL in 0.1% TFA	Mix sample and matrix 1:1, apply 1mL to spot and allow to dry.
DHB/SM	Working solution: 10 mg/mL in 0.1% TFA	Add 1 μL of sample to spot, allow to dry. Add 1 μL of matrix and allow to dry
DHAP/DD	Working solution: 15.2 mg/mL in 75% EtOH, 25% diammonium hydrogen citrate solution (27 mg/1.5mL water)	Mix sample 1:1:1 with 2% TFA, then matrix. Triturate thoroughly. Apply 1 μL to spot and allow to dry.
DHAP/SM	Stock solution: 15.2 mg/mL in 75% EtOH, 25% diammonium hydrogen citrate solution (27 mg/1.5mL water, 80mM) Working solution: 1:10 dilution of stock in 90% ACN, 2% TFA	Add 1 μL of sample to spot, allow to dry. Add 1 μL of matrix and allow to dry

Samples processing

For the 200 uL saliva mixed sample and the 5 uL serum mixed sample, 900 uL of ethanol was added to each sample, vibrated, and centrifuged at 12,000 rpm for 5 min at RM (room temperature), The supernatant was then discarded, then centrifuged at 12,000 rpm for 2 min at RM. After drying at RM 50 uL 70% methanoic acid was added to each sample and vibrated fully. Next, 50 uL acetonitrile was added to each sample, and again vibrated fully. Each sample was centrifuged at 12,000rpm for 5 min at RM. The supernatant of each sample was saved for spotting.

MS data acquisition

MALDI-TOF mass spectra were acquired using Clin-TOF-II (Bioyong Technology, Beijing, China) operating in positive linear ion mode between m/z 1000 and10,000 under the control of the MALDI Control software. MS data were collected manually by Bioexplorer software. The parameters were set as follows: pulsed ion extraction, 2.5 ns; frequency of laser, 60 Hz; laser power, 50% (fuzzy control on, weight ¼ 2.00, maximal resolution at six times above threshold); laser shots, 1000 in 40 different positions; movement, random walk with 15 shots per raster spot; peak selection, centroid (80% height); smoothing, on; block width, 2; alignment, on; normalisation, on; baseline subtraction, on; minimal resolution, 300.

Results

Comparative assessment of number of peaks in seven groups

Four matrix types were compared in our study using two different preparation methods. For ease of understanding, we made the necessary abbreviation as follows: dried droplet (DD); sample/matrix (SM); a-cyano-4-hydroxycinnamic acid (HCCA); sinapinic acid (SA); 2,5-dihydroxyhenz- zoic acid (DHB); 2,5-dihydroxy-acetophenone (DHAP).

According to past studies, HCCA/DD method (working solution: 3 mg/mL in 2:1 EtOH/acetone)[1] could not produce enough surface tension on the target plate because the solvent is an organic solution. Thus, we spotted and analysed the results of the other seven groups. Representative images showing the typical crystallisation patterns of the seven groups are shown in Figure 1. The DD preparations produced more homogenous crystallisation patterns than SM preparations for

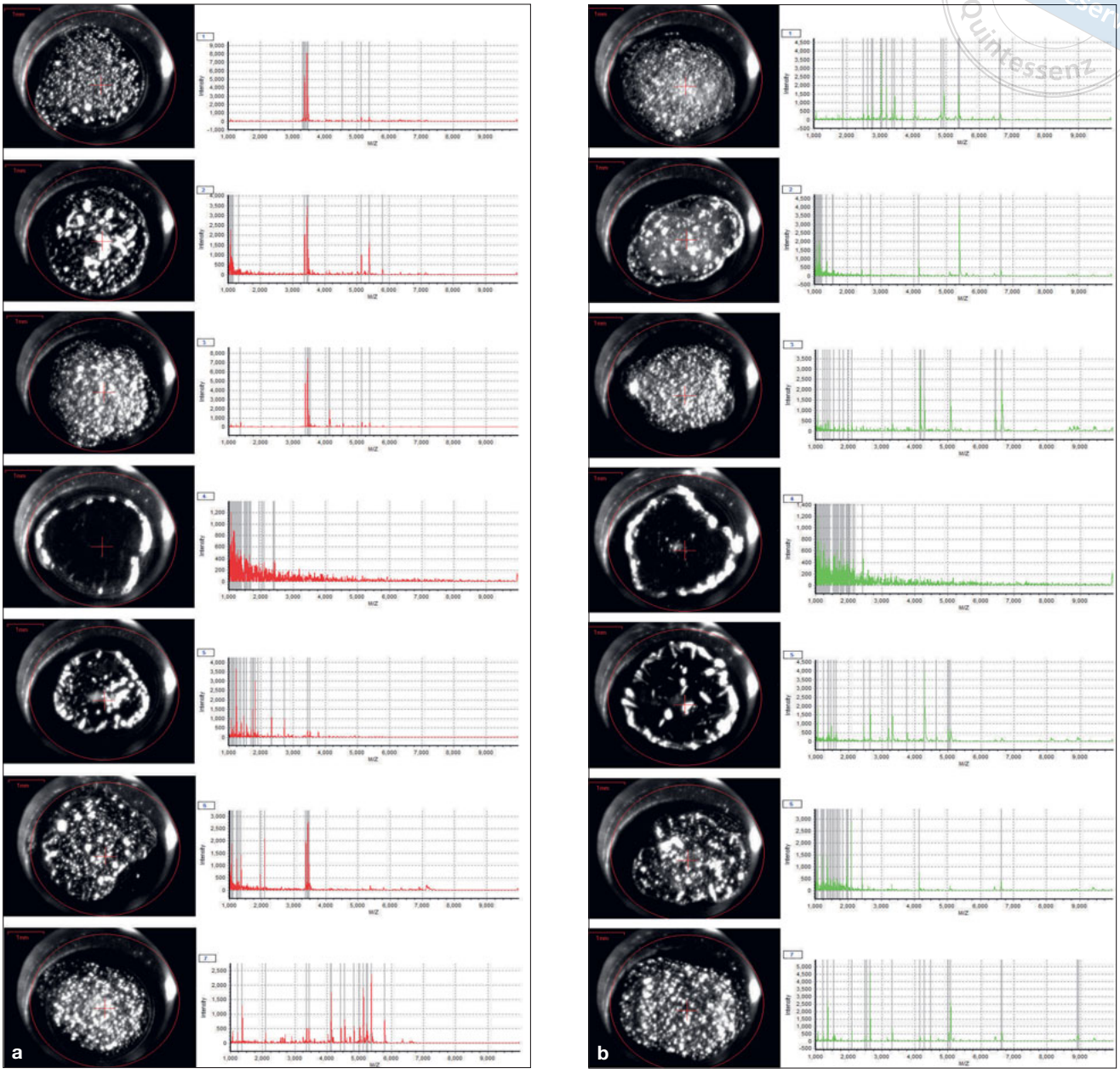

Fig 1 **(a)** Saliva; **(b)** Serum. Appearance of crystallised spots and average spectra in the seven matrix preparation groups.

SA and DHAP matrix. There was no significant difference in DHB matrix, which are both rare in the DD and SM methods. DHB/DD performed worst of all the seven groups, and there was almost no effective peak that could be detected in DHB/DD spotting sites, with high noise and an unsteady baseline. The average MS spectrums for each matrix type/preparation group are also shown in Figure 1.

DHAP/DD performed best if we set the number of peaks detected as the selection criterion in the serum sample, and HCCA/SM was second best. In the saliva sample, DHAP/DD was the best and DHB/SM came second. By incorporating with the S/N ratio (signal to noise ratio) factor, which turned out to be obviously lower in the saliva sample than the serum sample, DHAP/DD and HCCA/SM performed best – significantly better than the other five groups in the serum sample, and DHB/SM and DHAP/DD in the saliva sample. The numbers of peaks detected in the seven matrix type/preparation groups are shown in Table 3.

Comparative assessment of peptide components in seven groups

We analysed the m/z range distribution of peptide components in the seven matrix preparation groups; the m/z range distributed differently in different groups and there were some clues in them. We divided m/z of peptide components in 1000 – 10000 into 10 zones: 1000 – 2000, 2000 – 3000, etc, to 9000 – 10000.

In the saliva mixed sample, m/z of peptides components' distributing in 3000 – 4000 accounted for the vast majority (70%) in HCCA/SM group, and all of the peptide components' m/z distributed in 3000 – 6000. In the SA/DD group, m/z distributing in 1000 – 2000 accounted for the vast majority (65.52%), and all of the peptide components' m/z distributed in 1000 – 6000. In SA/SM group, m/z distributed more uniformly than other groups, mostly in 3000 – 4000 (33.33%), 4000 – 5000 (27.78%) and 1000 – 2000 (27.78%), and all of the peptide components' m/z distributed in 1000 – 2000 and 3000 – 6000. The DHB/DD group performed worst and no effective peak could be detected, so no analysis was done for this group. In the DHB/SM group, m/z distributing in 1000 – 2000 accounted for the vast majority (72.73%), and all of the peptide components' m/z distributed in 1000 – 6000. In the DHAP/DD group, m/z distributing in 1000 – 2000 accounted for the vast majority (79.31%), and m/z of all of the peptide components distributed in 1000 – 4000, 7000 – 8000 and 9000 – 10,000. In the DHAP/SM group, m/z distributed mostly in 5000 – 6000 (30.43%), 4000 – 5000 (26.09%) and 2000 – 3000 (21.74%), and the m/z of all of the peptide components distributed in 1000 – 6000. The range distribution is also shown as a Venn diagram in Figure 2 and as pie charts in Figure 3.

Table 3 Numbers of peaks in the seven matrix type/preparation groups.

Method	Serum	Saliva
HCCA/SM	64	10
SA/DD	39	29
SA/SM	37	18
DHB/DD	0	0
DHB/SM	37	44
DHAP/DD	73	58
DHAP/SM	24	23

In the serum mixed sample, m/z of peptides components distributing in 6000 – 7000 accounted for the vast majority (40.63%) in the HCCA/SM group, and m/z of all of the peptide components distributed in 1000 – 7000. In the SA/DD group, m/z distributing in 1000 – 2000 accounted for the vast majority (76.92%), and m/z of all of the peptide components distributed in 1000 – 3000 and 4000 – 7000. In the SA/SM group, m/z distributing in 1000 – 2000 accounted for the vast majority (40.54%), and m/z of all of the peptide components distributed in 1000 – 7000 and 8000 – 10000. The DHB/DD group also performed worst and no effective peak could be detected, so there is no analysis for this group. In the DHB/SM group, m/z distributing in 1000 – 2000 accounted for the vast majority (45.95%), and m/z of all of the peptide components distribute in 1000 – 7000. In the DHAP/DD

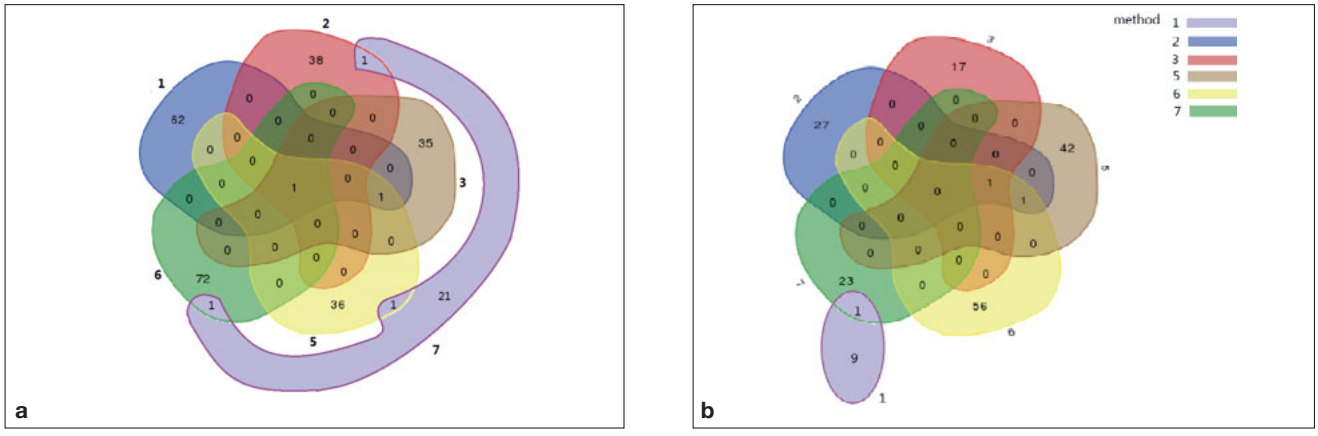

Fig 2 **(a)** Serum; **(b)** saliva. Venn diagram comparison of peptide components detected in the seven matrix preparation groups.

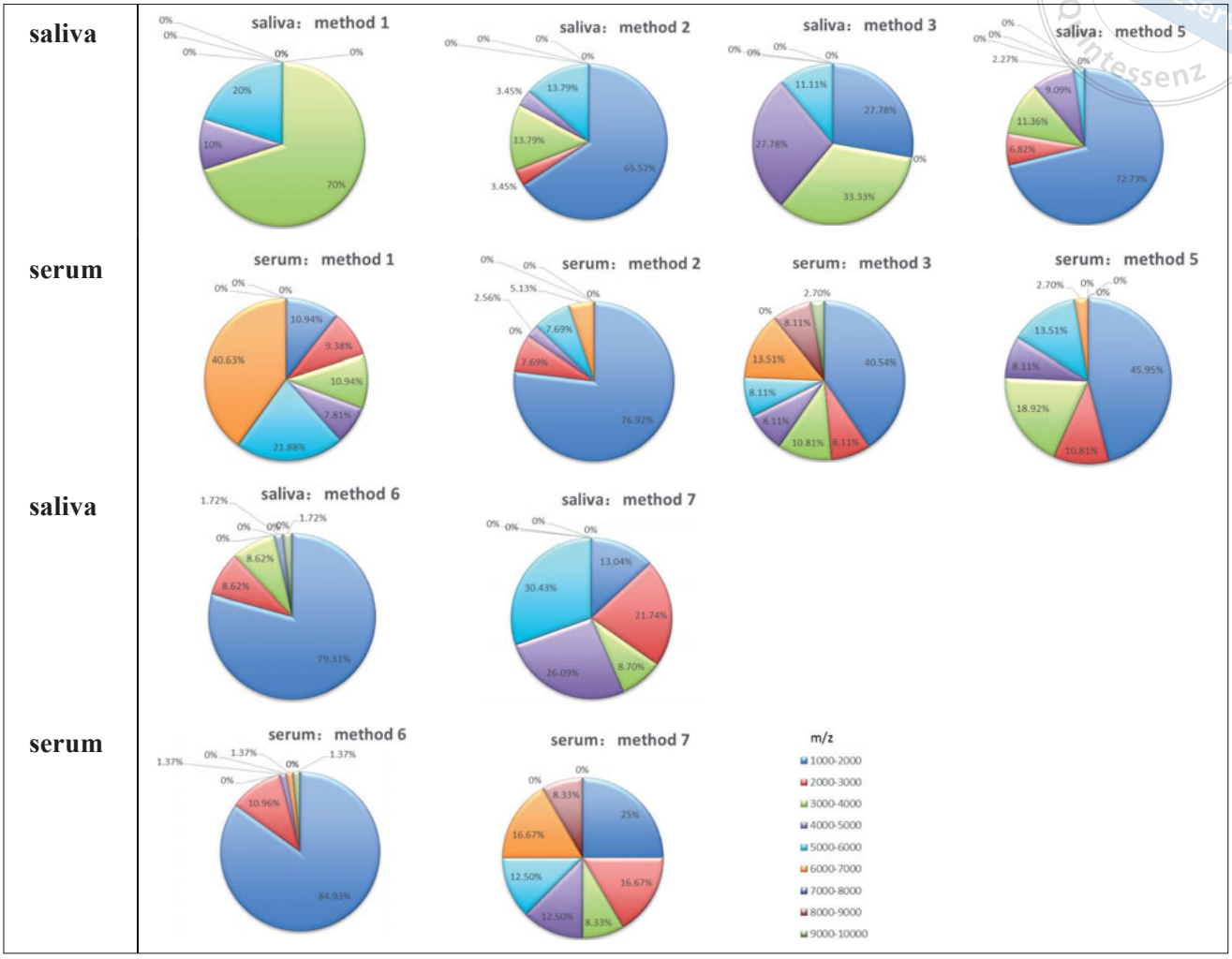

Fig 3 Pie charts comparison of m/z distribution of peptide components in seven matrix preparation groups.

group, m/z distributing in 1000 – 2000 accounted for the vast majority (84.93%), and m/z of all of the peptide components distributed in 1000 – 2000, 4000 – 5000, 6000 – 7000 and 9000 – 10,000. In the DHAP/SM group, m/z distributed more uniformly than other groups, mostly in 1000 – 2000 (25.00%), 2000 – 3000 (16.67%) and 6000 – 7000 (16.67%), and m/z of all of the peptide components distributed in 1000 – 7000 and 8000 – 9000. The range distribution is also shown as a Venn diagram in Figure 2 and as pie charts in Figure 3.

As our data shown, m/z of the peptide components detected in saliva and serum sample in our study mostly distributed in 1000 – 7000 in all of the seven groups. 1000 – 2000 was the most intensively distributed zone. In the SA/DD group, m/z distributing in 1000 – 2000 accounted for the vast majority (saliva: 65.52%, serum: 76.92%), and it was similar in the DHB/SM group (saliva: 72.73%, serum: 45.95%) and DHAP/DD (saliva: 79.31%, serum: 84.93%). In the DHAP/SM group, m/z of peptide components distributed more uniformly than other groups, mostly in 1000 – 3000 and 4000 – 6000 (saliva total: 91.30%, serum total: 83.34%). In the HCCA/SM and SA/SM groups, m/z distributions of peptide components were different in the saliva and serum samples.

Comparative assessment of peptide components in different samples

We detected peptide components of saliva and serum samples in seven different matrix type/preparation method groups, and they were not all the same in each group. We compared the total peptide components of each sample. Here we list the total statistics of peptide components of saliva and serum samples in Table 4 and show the overlap as a Venn diagram in Figure 4A.

Table 4 Comparison of peptide components in saliva and serum samples.

Sample	Total	Elements
Common	6	1000.5 1060 1071.4 1126.8 1328.2 1368.3
Serum	256	1012.4 1014.2 1020.2 1023.4 1026.7 1030 1039 1042.1 1043.9 1052.4 1058.5 1062.6 1071.2 1087.2 1087.8 1095.9 1100.3 1100.6 1107.6 1119.9 1128.6 1141.6 1143.8 1145.2 1154.8 1165.3 1169.3 1177.3 1182.9 1191 1204.1 1204.3 1209.5 1220.3 1220.9 1223.9 1227.6 1230.9 1235.6 1236.1 1241.6 1250.6 1256.7 1263.5 1264.2 1266.6 1280.1 1286.6 1295.9 1297.6 1300.1 1308.3 1309.5 1318.1 1327.6 1339.3 1351.5 1352.2 1353.6 1366.1 1366.7 1367.1 1368.6 1381.8 1396.2 1400 1412.3 1418 1428.5 1436.2 1439.9 1456.2 1457.2 1468.1 1470.4 1484.2 1485 1492.5 1502.8 1513 1519.2 1520.4 1528.2 1529 1544.1 1544.5 1545.3 1550.5 1563.1 1571.7 1572.1 1573.3 1574.8 1585.6 1597.7 1615.9 1622.1 1630.7 1640.9 1660.2 1695.4 1704 1714.1 1721.1 1723.3 1747.7 1769.8 1778.5 1787.2 1806.5 1835.7 1843.1 1876.3 1881.9 1894.9 1930.1 1954.8 1957 1968.5 1972.8 1986.7 1996.4 2008.2 2043 2086.4 2093.4 2094.1 2095 2096.2 2096.8 2209 2287.4 2302.4 2427.2 2429.5 2433.4 2457.4 2462.3 2492.4 2542.5 2648.4 2651.7 2667.8 2692.6 2744 2782.1 2988.3 3036.1 3050.9 3162.5 3192.5 3202.4 3219.3 3317.1 3317.8 3321.4 3322 3337.8 3353.7 3369.8 3376.9 3442 3448.8 3473.6 3667.1 3766.5 3767.1 4062.7 4151.5 4152.9 4154.9 4157 4159.3 4190.8 4287.7 4288.5 4291.3 4293 4314.1 4471.5 4650.3 4849.9 4930.5 5008.2 5017.6 5020.9 5036 5080.8 5084.1 5084.3 5085.6 5086 5112.3 5173.5 5283.5 5378.3 5381.8 5386.4 5403.4 5438.1 5438.4 5587.9 5631.8 5676.2 5720.1 5764.4 5807.6 5851.9 5896.6 5940 5984.6 6027.6 6071.8 6081.2 6115.9 6160.4 6204.3 6248.4 6257.9 6292.3 6335.8 6380.7 6425.8 6427.8 6436.4 6439.2 6441.3 6451.2 6468.6 6478.1 6513.1 6523.2 6557.1 6601.3 6626.7 6627 6630.4 6633 6635 6635.6 6638.5 6639 6644.7 6648.7 6651.3 6698.5 6699.1 6734.5 6743.6 8808.7 8829.2 8913.1 8923.3 8946.5 9421 9968.4
Saliva	170	1011.7 1013.9 1021.4 1022.3 1026.2 1031.3 1044.6 1045.2 1049.1 1074.1 1074.2 1088.5 1089.3 1101.4 1105.7 1110.3 1118.7 1126.2 1128.9 1139.7 1140.2 1144.2 1144.7 1145.1 1158.9 1160.4 1161 1161.5 1180.9 1183.9 1184.1 1204.9 1213.9 1225.8 1227.4 1227.8 1228.3 1242.7 1251.3 1261.4 1266.7 1274.5 1281.3 1284.5 1291.5 1301.6 1314.6 1323.3 1331.8 1339.1 1344.4 1352.1 1358.3 1366.8 1367 1367.7 1390.1 1391.3 1400.5 1443.4 1464.7 1465 1466.1 1475 1479.1 1507.8 1554.7 1559.6 1572.6 1591.7 1593 1609.5 1611.8 1642.3 1667.5 1684.6 1687.1 1702.9 1722.4 1735.6 1770.3 1783.6 1788.3 1822.6 1860.8 1879.5 1898.7 1902.9 1932.3 1949.1 1956.1 1966.5 1993.9 2094.7 2094.8 2096.1 2215.5 2317.5 2327.8 2554.7 2568.4 2588.1 2648.8 2692.8 2727.3 2882.4 2900.3 3314.9 3327.9 3370.8 3372 3373.1 3373.9 3374.9 3376.4 3400.5 3411.2 3440.6 3443.2 3444.8 3445.1 3445.9 3446.1 3473.2 3485 3487 3490.3 3491.2 3496.7 3502.6 3523.7 3525.3 3526.1 3527.2 3544.3 3781.6 4034.8 4048.3 4122.9 4125 4138.3 4138.4 4140.4 4360.5 4392.5 4435.8 4550.2 4552.4 4552.9 4789.5 4841.3 4920.8 4928.9 5017.4 5134.1 5136.3 5136.8 5139.6 5143.6 5238.2 5271.4 5383.4 5384.2 5385.7 5387.6 5793.1 5795.2 5801.7 7127.1 9969

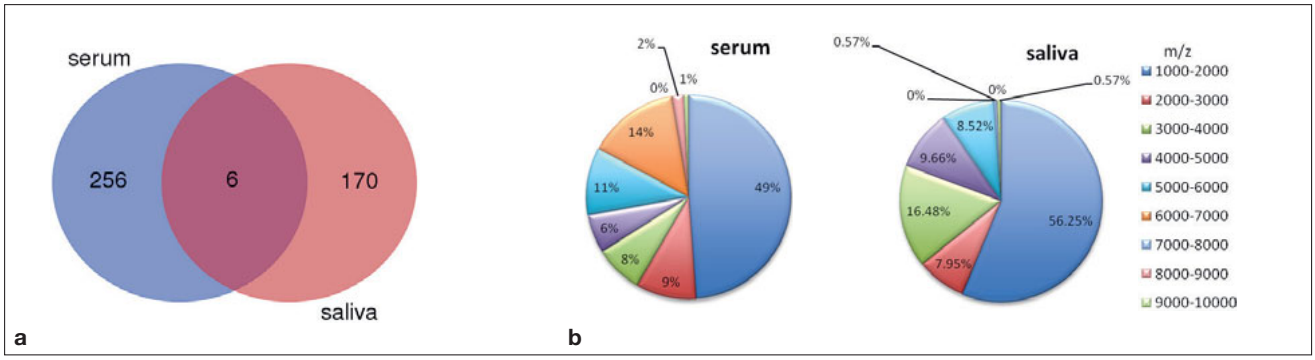

Fig 4 **(a)** Venn diagram comparison of peptide components in saliva and serum sample; **(b)** Pie charts comparison of m/z distribution of peptide components in saliva and serum sample.

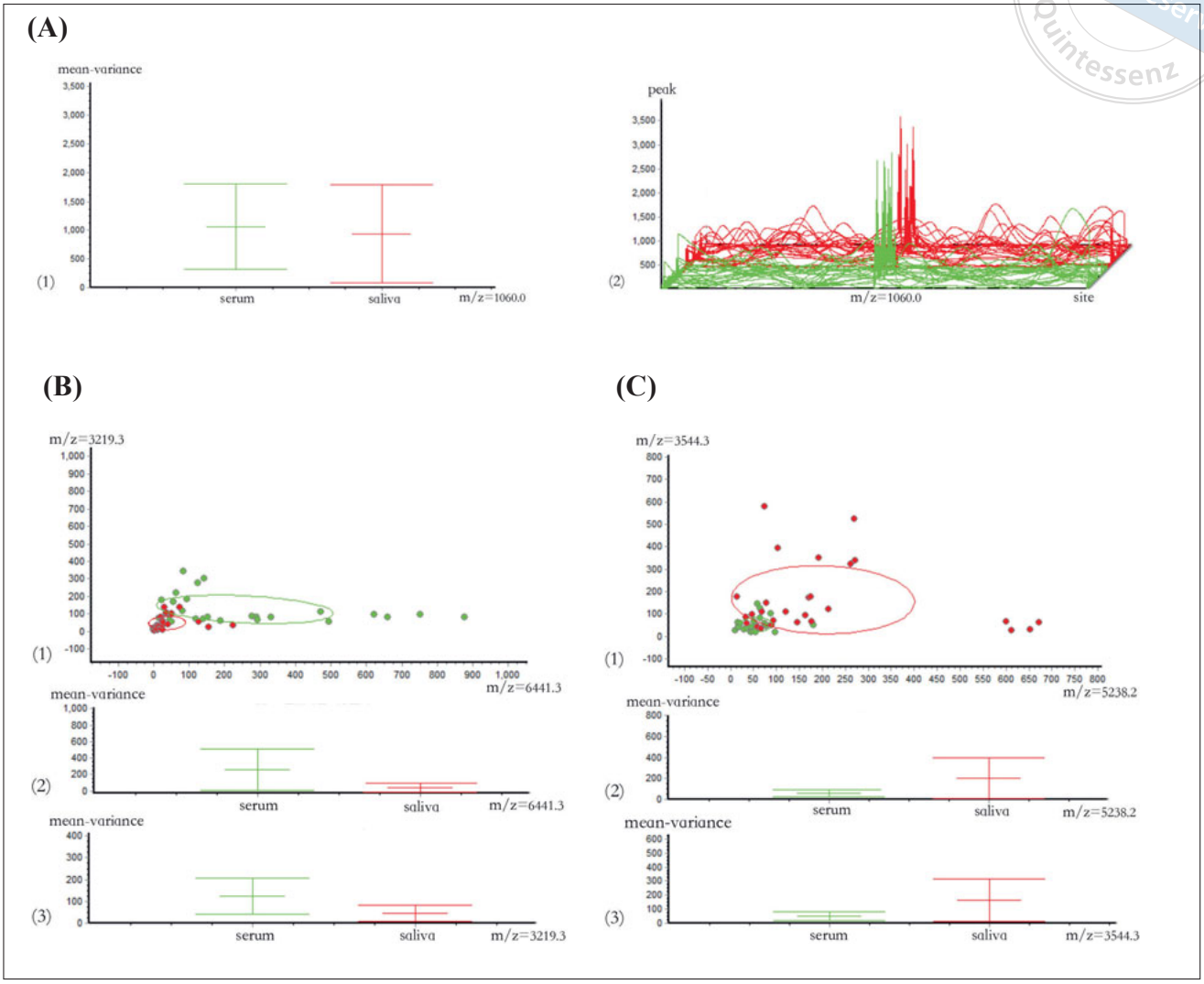

Fig 5 Red: saliva; green: serum.

(A) 1) M/z=1060.0, mean-variance figure. **2)** M/z=1060.0, site-peak figure.
(B) 1) Sample distribution figure; **2)** M/z1=6441.3 mean-variance figure; **3)** M/z2=3219.3 mean-variance figure.
(C) 1) Sample distribution figure; **2)** M/z1=5238.2 mean-variance figure; **3)** M/z2=3544.3 mean-variance figure.

As our data show, it was also verified that m/z of the peptide components detected in saliva and serum sample mostly distributed in 1000 – 7000 (saliva: 98.86%, serum: 97.33%). 1000 – 2000 was the most intensively distributed zone (saliva: 56.25%, serum: 48.85%), and 3000 – 4000 in saliva sample (16.48%), 6000 – 7000 in serum sample (14.50%) was the second distributed zone. M/z of peptide components distributed more uniform in other zones, e.g. 2000 – 3000, 4000 – 5000 and 5000 – 6000.The statistics comparisons are shown as list in Table 5 and as pie charts in Figure 4B.

As shown in Table 4 and Figure 4, six overlapped peptide components were detected in the saliva and serum sample. The contents of each overlapped peptide component in different sample were different. We randomly chose m/z = 1060.0 as an example and the results are shown as site-peak figure and as mean-variance figure in Figure 5 ($P < 0.01$). The DHB/DD method performed the worst, and was not a suitable method for MALDI-TOF MS experiment for saliva and serum.

The remaining peptide components were all non-overlapped. We randomly chose: a) $m/z_1 = 6441.3$, $m/z_2 = 3219.3$; b) $m/z_1 = 5238.2$, $m/z_2 = 3544.3$ as two comparison example groups and the results are shown as sample distribution figures and as mean-variance figures in Figure 5 ($P < 0.01$).

Discussion

The goal of our study is to find if there is any common regularity in MS results of different types of matrix and preparation methods, and further analysed raw data with two main evaluation indexes: number of peaks and m/z distribution of peptide components detected. MS results of different matrix type/preparation methods are indeed different.

The nature of the matrix was one of the most important parameters affecting the quality of the spectrum[11]. The matrix was believed to serve two major functions: absorption of energy from the laser and isolation of the biopolymer molecules from each other[11]. They all require physical and chemical properties: 1) an efficient absorbance at the laser wavelength; 2) an efficient ionization; 3) an important stability not to interfere with the mass spectrum of the sample. The choice of the matrices depended on the nature of the sample studied. DHB allowed the study of oligosaccharides, glycopeptides, and glycoproteins. Generally, DHB was more efficient for peptide/protein components of smaller m/z, SA and HCCA especially allow the study of proteins. For the same sample, mass spectral fingerprints are different depending on the matrices used. These observations emphasised the need for careful attention in the preparation of the sample to obtain optimum reproducibility[11].

DHB/DD performed worst in our study, corresponding to past studies[11,12]. No effective peak was detected by DHB/DD. Big and non-homogeneous crystalline are shown in the crystallised spots, also verifies the result. The average spectra were not smooth, with loud noise and floating baseline, the typical characteristic of spotting directly on target plate areas where not enough enough matrixes covered the sample. Comparing with other methods, it was clear that shape and quality of crystalline significantly influenced quality of MS experiment results. Hence, we compared characteristics of matrixes in our study[1] and previous studies, and found the results were in accordance: smaller crystalline and larger covering surface are required for ideal matrix.

Crystalline of other methods in our studies meet the characteristic and their MS results are stable. Results of the study suggest that when researchers are doing pre-experiments, one useful and sample-saving method to check out is if the matrix is suitable to spot only matrix solutions on target plate spots and check crystalline by camera. Crystalline of good quality prompting basically stable spectrum and peak results, and crystalline of bad quality is a hint for changing matrix preparation methods, either the type or preparation of instructions.

Table 4 Comparison of m/z distribution of peptide components in saliva and serum samples.

m/z	Serum		Saliva	
Total	262	100%	176	100%
1000 – 2000	128	48.85%	99	56.25%
2000 – 3000	25	9.54%	14	7.95%
3000 – 4000	20	7.63%	29	16.48%
4000 – 5000	16	6.11%	17	9.66%
5000 – 6000	28	10.69%	15	8.52%
6000 – 7000	38	14.50%	0	0%
7000 – 8000	0	0%	1	0.57%
8000 – 9000	5	1.91%	0	0%
9000 – 10000	2	0.76%	1	0.57%

For saliva MALDI-TOF MS experiments, S/N ratio in all the other six matrix type/preparation methods are relatively low, therefore the number of peaks and approximate range of the target peaks are main selection criteria. M/z of peptide components detected range distribute differently according to different methods. So if the approximate range of the target peaks of study is not very clear, it is better to repeat experiment with different matrix type/preparation methods to assure larger comprehensive detecting range. Considering that it is complex and difficult to prepare various matrix and our results that DHB/SM and DHAP/DD perform best in number of peaks, m/z detected distributing in 1000 – 2000 account for the vast majority (> 70%), we recommend researchers use either DHB/SM or DHAP/DD in MS experiments. If the approximate range of the target peaks of study is basically clear, researchers can choose the relevant matrix type/preparation method according to our results – see details in Results.

For serum MALDI-TOF MS experiments, S/N ratios were different in different matrix type/preparation methods, thus they should also be considered as one deviation factor. Therefore, the number of peaks, S/N ratio and approximate range of the target peaks are the main selection criteria. M/z of peptide components detected range also distribute differently in different methods. Apart from DHB/DD that performed worst, the S/N ratio of SA/DD and SA/SM are higher than the other four methods. The number of peaks and m/z distribution of peptide components detected by these two methods

are not special or irreplaceable, so these two methods were unsuitable for in MS experiments. S/N ratios for the other four matrix type/preparation methods are relatively low. Hence, if the approximate range of the target peaks of study is not very clear, it is better to repeat experiment with these four different matrix type/preparation methods to assure larger comprehensive detecting range. Results of the study also suggest using both HCCA/SM and DHAP/DD in MS experiments. If the approximate range of the target peaks of study is basically clear, researchers can choose relevantly matrix type/preparation method in these four methods according to our results – more details in the Results section.

In our study, we analysed the MS results of different types of matrix and preparation methods, setting number of peaks, S/N ratio and approximate range of the target peaks as our main selection criteria.

Further studies should be warranted to analyse other characteristics of peptide components detected in different matrix methods, like hydrophila/hydrophobicity, electric charging, etc. to increase evidence when selecting matrix type/preparation methods in MALDI-TOF MS experiments on peptide components of human body fluids.

Conclusion

- Among the seven different matrix type/preparation methods, HCCA/SM, SA/DD, SA/SM, DHB/DD, DHB/SM, DHAP/DD, DHAP/SM, different methods performed differently. DHB/DD performed worst in both samples.
- M/z range was distributed differently in different methods. DHB/SM and DHAP/DD performed best in number of peaks, m/z distributing in 1000 – 2000 account for the vast majority.
- Further studies should focus on other characteristics of peptide components detected in different matrix methods to increase evidence when selecting matrix type/preparation methods.

Acknowledgements

The authors acknowledge the support of the clinicians from the Orthodontic Department at Peking University Hospital of Stomatology for their assistance in the sampling procedure.

Conflicts of interest

The authors reported no conflicts of interest related to this study.

Author contribution

Dr Lu GAO carried out sample collection, sample preparation, data analysis, and paper preparation; Drs Yan GU and Feng CHEN provided study design, technical assistance, preparation of the figures and polishing the manuscript; Dr Qing Wei MA provided instrument technology support and participated in data analysis; All authors reviewed the results and approved the final version of the manuscript.

(Received Sep 26; accepted Dec 05, 2017)

References

1. Penno MA, Ernst M, Hoffmann P. Optimal preparation methods for automated matrix-assisted laser desorption/ionization time-of-flight mass spectrometry profiling of low molecular weight proteins and peptides. Rapid Commun Mass Spectrom 2009;23:2656–2662.
2. Karas M, Hillenkamp F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem 1988;60: 2299–2301.
3. Zhang X, Shi L, Shu S, et al. An improved method of sample preparation on AnchorChip targets for MALDI-MS and MS/MS and its application in the liver proteome project. Proteomics 2007;7: 2340–2349.
4. Li YH, Wang J, Zheng XL, et al. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry combined with magnetic beads for detecting serum protein biomarkers in Parkinson's disease. Eur Neurol 2011;65:105–111.
5. Voortman J, Pham TV, Knol JC, Giaccone G, Jimenez CR. Prediction of outcome of non-small cell lung cancer patients treated with chemotherapy and bortezomib by time-course MALDI-TOF-MS serum peptide profiling. Proteome Sci 2009;7:34.
6. Ying X, Han SX, Wang JL, et al. Serum peptidome patterns of hepatocellular carcinoma based on magnetic bead separation and mass spectrometry analysis. Diagn Pathol 2013;8:130.
7. van der Werff MP, Mertens B, de Noo ME, et al. Case-control breast cancer study of MALDI-TOF proteomic mass spectrometry data on serum samples. Stat Appl Genet Mol Biol 2008;7:Article2.
8. Hsieh SY, Chen RK, Pan YH, Lee HL. Systematical evaluation of the effects of sample collection procedures on low-molecular-weight serum/plasma proteome profiling. Proteomics 2006;6:3189–3198.
9. Fiedler GM, Baumann S, Leichtle A, et al. Standardized peptidome profiling of human urine by magnetic bead separation and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Clin Chem 2007;53:421–428.
10. Zhang J, Zhou S, Zheng H, Zhou Y, Chen F, Lin J. Magnetic bead-based salivary peptidome profiling analysis during orthodontic treatment durations. Biochem Biophys Res Commun 2012;421:844–849.
11. Lu J, Huang Y, Wang Y, et al. Profiling plasma peptides for the identification of potential ageing biomarkers in Chinese Han adults. PLoS One 2012;7:e39726.
12. Wang Y, Adua E, Russell A, et al. Glycomics and its application potential in precision medicine, Precision medicine in China. American Association for the Advancement of Science(AAAS), 2013:36. Available at: https://www.researchgate.net/publication/318761536_Glycomics_and_its_application_potential_in_precision_medicine
13. Wang H, Luo C, Zhu S, et al. Serum peptidome profiling for the diagnosis of colorectal cancer: discovery and validation in two independent cohorts. Oncotarget 2017;8:59376–59386.

14. Meng Q, Ge S, Yan W, et al. Screening for potential serum-based proteomic biomarkers for human type 2 diabetes mellitus using MALDI-TOF MS. Proteomics Clin Appl 2017;11.
15. Wenzel T, Sparbier K, Mieruch T, Kostrzewa M. 2,5-Dihydroxyacetophenone: a matrix for highly sensitive matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis of proteins using manual and automated preparation techniques. Rapid Commun Mass Spectrom 2006;20:785–789.
16. Callesen AK, Vach W, Jørgensen PE, et al. Combined experimental and statistical strategy for mass spectrometry based serum protein profiling for diagnosis of breast cancer: a case-control study. J Proteome Res 2008;7:1419–1426.
17. Villanueva J, Philip, J, Chaparro, CA, et al. Correcting Common Errors in Identifying Cancer-Specific Serum Peptide Signatures. J Proteome Res 2005;4:1060–1072.
18. West-Nørager M, Kelstrup CD, Schou C, Høgdall EV, Høgdall CK, Heegaard NH. Unravelling in vitro variables of major importance for the outcome of mass spectrometry-based serum proteomics. J Chromatogr B Analyt Technol Biomed Life Sci 2007;847:30–37.
19. Findeisen P, Sismanidis D, Riedl M, Costina V, Neumaier M. Preanalytical impact of sample handling on proteome profiling experiments with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Clin Chem 2005;51:2409–2411.
20. Hong H, Dragan Y, Epstein J, et al. Quality control and quality assessment of data from surface-enhanced laser desorption/ionization (SELDI) time-of flight (TOF) mass spectrometry (MS). BMC Bioinformatics 2005;6:S5.
21. Schuerenberg M, Luebbert C, Eickhoff H, Kalkum M, Lehrach H, Nordhoff E. Prestructured MALDI-MS sample supports. Anal Chem 2000;72:3436–3442.
22. Pelikan R, Bigbee WL, Malehorn D, Lyons-Weiler J, Hauskrecht M. Intersession reproducibility of mass spectrometry profiles and its effect on accuracy of multivariate classification models. Bioinformatics 2007 Nov;23:3065–3072.
23. Semmes OJ, Feng Z, Adam BL, et al. Evaluation of serum protein profiling by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry for the detection of prostate cancer: I. Assessment of platform reproducibility. Clin Chem 2005;51:102–112.
24. Cairns DA, Thompson D, Perkins DN, Stanley AJ, Selby PJ, Banks RE. Proteomic profiling using mass spectrometry--does normalising by total ion current potentially mask some biological differences? Proteomics. 2008;8:21–27.
25. Baumann S, Ceglarek U, Fiedler GM, Lembcke J, Leichtle A, Thiery J. Standardized approach to proteome profiling of human serum based on magnetic bead separation and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Clin Chem 2005;51:973–980.
26. Callesen AK, Mohammed S, Bunkenborg J, et al. Serum protein profiling by miniaturized solid-phase extraction and matrix-assisted laser desorption/ionization mass spectrometry. Rapid Commun Mass Spectrom 2005;19:1578–1586.
27. Xiao D, Meng FL, He LH, Gu YX, Zhang JZ. Analysis of the urinary peptidome associated with Helicobacter pylori infection. World J Gastroenterol 2011;17:618–624.
28. Fu G, Du Y, Chu L, Zhang M. Discovery and verification of urinary peptides in type 2 diabetes mellitus with kidney injury. Exp Biol Med (Maywood) 2016;241:1186–1194.
29. Yang J, Song YC, Dang CX, et al. Serum peptidome profiling in patients with gastric cancer. Clin Exp Med 2012;12:79–87.
30. West-Nørager M, Bro R, Marini F, et al. Feasibility of serodiagnosis of ovarian cancer by mass spectrometry. Anal Chem 2009;81:1907–1913.
31. Pawlik TM, Fritsche H, Coombes KR, et al. Significant differences in nipple aspirate fluid protein expression between healthy women and those with breast cancer demonstrated by time-of-flight mass spectrometry. Breast Cancer Res Treat 2005;89:149–157.
32. Coombes KR, Tsavachidis S, Morris JS, Baggerly KA, Hung MC, Kuerer HM. Improved peak detection and quantification of mass spectrometry data acquired from surface-enhanced laser desorption and ionization by denoising spectra with the undecimated discrete wavelet transform. Proteomics 2005;5:4107–4117.
33. Gatlin-Bunai, CL, Cazares, LH, Cooke, WE, Semmes, OJ, Malyarenko, DI. Optimization of MALDI-TOF MS Detection for Enhanced Sensitivity of Affinity-Captured Proteins Spanning a 100 kDa Mass Range. J Proteome Res 2007;6:4517–4524.
34. Callesen AK, Mohammed S, Bunkenborg J, et al. Serum protein profiling by miniaturized solid-phase extraction and matrix-assisted laser desorption/ionization mass spectrometry. Rapid Commun Mass Spectrom 2005;19:1578–1586.
35. Pietrowska M, Marczak L, Polanska J, et al. Mass spectrometry-based serum proteome pattern analysis in molecular diagnostics of early stage breast cancer. J Transl Med 2009;7:60.
36. Gatlin-Bunai CL, Cazares LH, Cooke WE, Semmes OJ, Malyarenko DI. Optimization of MALDI-TOF MS detection for enhanced sensitivity of affinity-captured proteins spanning a 100 kDa mass range. J Proteome Res 2007;6:4517–4524.
37. Villanueva J, Martorella AJ, Lawlor K, et al. Serum peptidome patterns that distinguish metastatic thyroid carcinoma from cancer-free controls are unbiased by gender and age. Mol Cell Proteomics 2006;5:1840–1852.
38. Cairns DA, Thompson D, Perkins DN, Stanley AJ, Selby PJ, Banks RE. Proteomic profiling using mass spectrometry – does normalising by total ion current potentially mask some biological differences? Proteomics. 2008;8:21–27.
39. Carbonnelle E, Mesquita C, Bille E, et al. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. Clin Biochem 2011;44:104–109.