Short Communication

Impact of detector degradation on bacterial identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Abstract Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI−TOF MS) is an innovative tool that is easy to use, rapid, accurate, and cost-effective. It has revolutionized bacterial identification in clinical microbiology laboratories. Mass spectrometry involves three steps: ionization, mass separation, and detection. Few reports have focused on the function of the mass spectrometry itself, and hardware like the detector (discussed here), in improving the accuracy of bacterial identification. The detector plays an important role in determining the mass of biological macromolecules such as proteins and peptides. We herein describe an incident in which deterioration of the detector disturbed the bacterial identification.

The scores for identifying *Escherichia coli* and *Staphylococcus epidermidis* isolates were compared between immediately before (old detector) and immediately after detector replacement (new detector). Using the MALDI BioTyper software, we found that the identification score for *E. coli* was 1.755 (on a scale of 0−3) at the genus level before detector replacement. Contrarily, immediately after replacement using the new detector, the score was 2.410 at the species level. For *S. epidermidis*, the score was 1.931 at the genus level immediately before detector replacement and 2.042 at the species level immediately after replacement with the new detector; both scores were obtained using the MALDI BioTyper software. Moreover, intensity of MS peaks were obviously high after detector replacement. Thus, if bacterial identification using MALDI-TOF MS is not successful, the detector may need to be replaced.

Key words: bacterial identification, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, detector

Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption ionization−time-of-flight mass spectrometry; MS, mass spectrometry

Introduction

Matrix-assisted laser desorption/ionization−time-of-flight mass spectrometry (MALDI−TOF MS) is an innovative tool that is easy to use, rapid, accurate, and cost-effective. It has revolutionized bacterial identification in clinical microbiology laboratories[1−5]. Mass spectrometry separates ions by their mass-to-charge ratio (*m/z*). The ions reach a detector with different velocities according to their *m/z*. The sensitivity of conventional detectors such as microchannel plate detectors is highly dependent on the impact velocity of the ion. Detectors are incorporated with mass analyzers

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to detections[6,7].

A sample for analysis is mixed with a matrix, an organic compound that absorbs laser light and enhances the ionization of the sample[8–10]. The sample is ionized by irradiation with laser light which adds protons (H+). The ionized sample accelerates between the electrodes when a voltage is applied. It flies toward the detector through a vacuum tube called a flight tube. Since the voltage applied to the ionized sample is constant for all its ions, they all have the same kinetic energy ($E=1/2\,mv^2$). The ions with a lower *m/z* reach the detector faster, while ions with a higher *m/z* are slower. The MALDI-TOF MS performs mass analysis based upon the varying flight time of the ions, which depends on their *m/z*[11,12]. The mass spectra are plotted as detection intensity (peak) on the vertical axis and *m/z* on the horizontal axis. The distribution of the peaks in the mass spectrum indicates the composition and characteristics of the analyzed sample and is used to identify microorganisms[13,14].

The laser device in the MALDI-TOF MS apparatus is consumable and has a limit to the number of times it can be shot on a sample. When bacterial identification scores using MALDI-TOF MS deteriorate, it is common practice to replace the laser device. However, we have experienced an incident in which poor accuracy for bacterial identification was due to deterioration of the detector rather than laser device. In the present article, we describe about the incident.

Methods

The Department of Clinical Laboratory in the Chiba University Hospital initially purchased the analysis software from Bruker Daltonics GmbH (Bremen, Germany) and performed clinical applications using an Autoflex II TOF/TOF MS instrument (Bruker Daltonics), which had been used for research. Since April 2013, a MALDI Biotyper system (Bruker Daltonics), which combines a Microflex LT instrument and analysis software, has been used. The detector of the Microflex LT instrument was replaced for the first time on October 2017. Therefore, the detector would have been in use for about 4.5 years before replacement. The company performed maintenance at least once a year until the first detector replacement.

We examined strains from bacterial isolates stocked in the study institution. The strains included the gram-negative bacteria *Escherichia. coli* and the gram-positive bacteria *Staphylococcus epidermidis.* They were simultaneously assayed in the day before and immediately after the replacement of the detector.

The bacterial specimens were plated on an appropriate agar medium, chocolate agar, or trypticase soy agar II with 5% sheep blood (Nippon Becton Dickinson, Tokyo, Japan). We performed a formic acid extraction (on-plate) method generally used for preparing *E. coli* and *S. epidermidis*. If a specimen contains more than 10^5 CFU/mL of microorganisms, the specimen can be directly applied to the target plate for MS[15]. Therefore, in this study, we examined bacterial specimens containing more than 10^5 CFU/mL. A single colony adhering to a toothpick was transferred directly onto the spotting surface of a MALDI target plate (MSP 96 target-polished steel BC target plate, Bruker Daltonics) using an up-and-down rather than a sliding motion. Immediately after applying the sample, 1 μL of 70% formic acid was spotted onto the sample and overlaid with 1 μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution (Bruker Daltonics). The matrices we used were pre-dissolved Bruker Matrix HCCA, portioned (Bruker Daltonics). According to the manufacturer's instructions, the HCCA matrix was dissolved in acetonitrile 50%, water 47.5%, and trifluoroacetic acid 2.5% solvent to a final concentration of 5 mg/mL. This series of operations was performed immediately before and after detector replacement.

For bacterial identification, MALDI-TOF MS was performed on a Microflex LT mass spectrometry (Bruker Daltonics), with the measurement range set at 2–20 kDa. FlexControl ver. 3.3, Flex Analysis, ver. 3.3; and BioTyper® ver. 3.1 analytical software (Bruker Daltonics) were used. Version 4.0.0.1 of the database (Bruker Daltonics) was used for identification following the manufacturer's instructions, and as previously described[16].

The mass spectra from each isolate were imported into the Biotyper software (version 3.1, Bruker Daltonics) and analyzed by standard pattern matching. The results of the pattern-matching process were expressed (according to the manufacturer's instructions) with scores ranging from 0 to 3. Scores below 1.7 were regarded as unreliable identification; scores between 1.7 and 2.0 were regarded as genus-level identification, and scores $>$2.0 were regarded as species-level identification.

Results

Before detector replacement, the MALDI BioTyper software scored 1.755 for *E. coli*, identified at the genus level;

after replacement with the new detector, the score was 2.410, successfully identified at the species level (Fig. 1). The same software recorded a score of 1.931 for *S. epidermidis* at the genus level immediately before detector replacement and 2.041 at the species level immediately after replacement with the new detector (Fig. 2). The intensity of MS peaks were obviously high after detector replacement (Figs. 1 and 2). Additionally, we compared 7 representative *m/z* peaks of *E. coli* and 9 representative peaks of *S. epidermidis* before and after detector replacement. The signal intensities and the signal/noise (S/N) ratios of these 16 peaks exhibited generally increasing trend and improved after the detector replacement (Table 1).

Thus, the detector replacement appears to have affected MS spectrum acquisition and subsequently to the accuracy of the bacterial identification.

Discussion

A mass spectrometry is composed of three parts for: ionization, mass separation, and detection. In the MALDI-TOF MS, laser irradiation is used for ionization, time-of-flight (TOF) is used for mass separation, and a micro channel plate (MCP) is used as the detector. When a mass spectrometry is used for a long time, each device in the instrument deteriorates and should be replaced. In the MALDI-TOF MS, deterioration of the laser equipment often interferes with the acquisition of MS spectra, and the computer screen that operates the MALDI-TOF MS displays the cumulative number of laser irradiations, enabling researchers to be constantly aware of the degree of deterioration of the laser equipment. In the present study, however, we have shown that deterioration of the detector also has potential to disturb the acquisition of MS spectrum. Unfor-

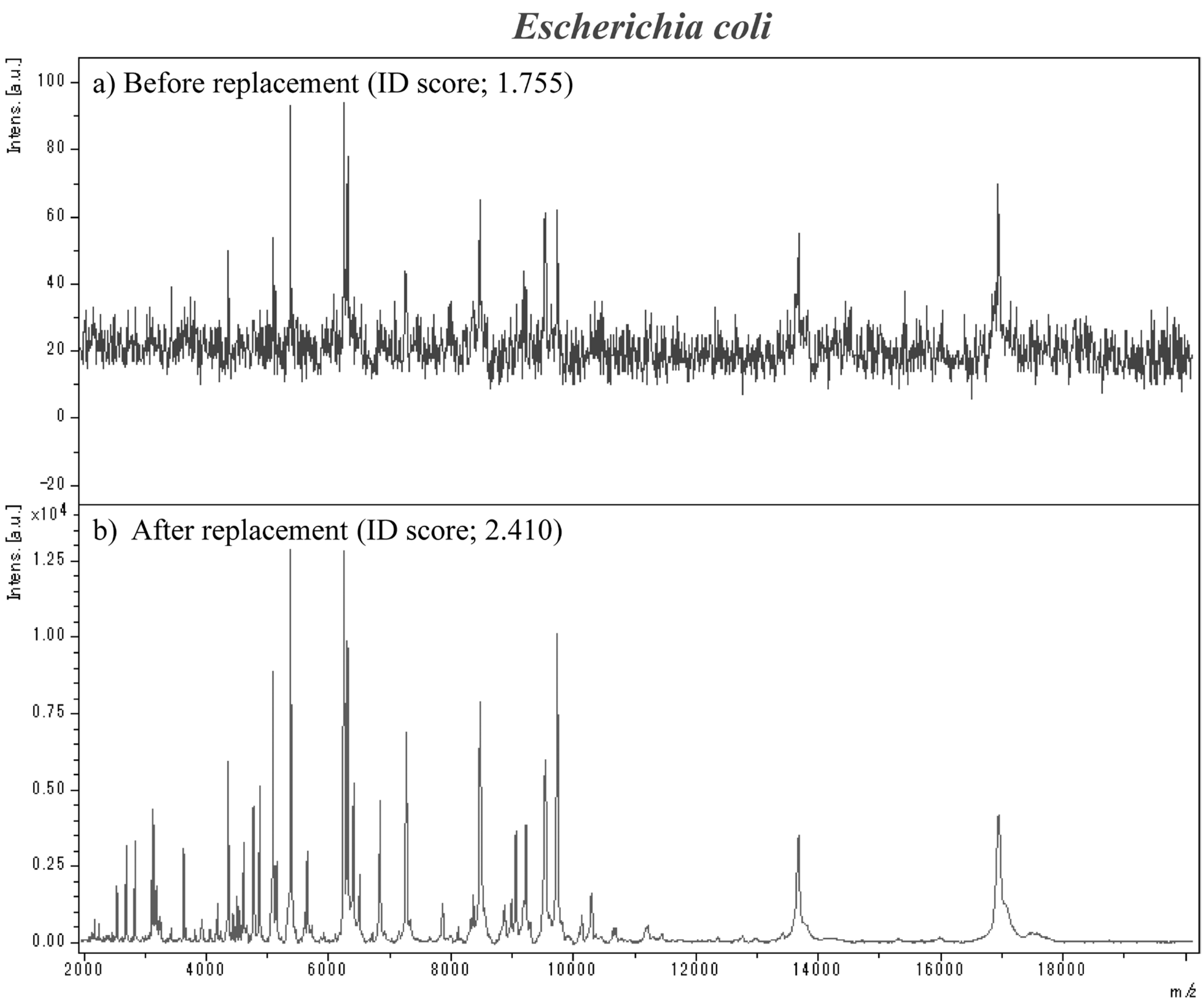

Fig. 1. Mass spectral pattern of *Escherichia coli*.

a) Mass spectral pattern of *E. coli* immediately before detector replacement; the identification score was 1.755. b) Mass spectral pattern of *E. coli* immediately after detector replacement; the identification score was 2.410.

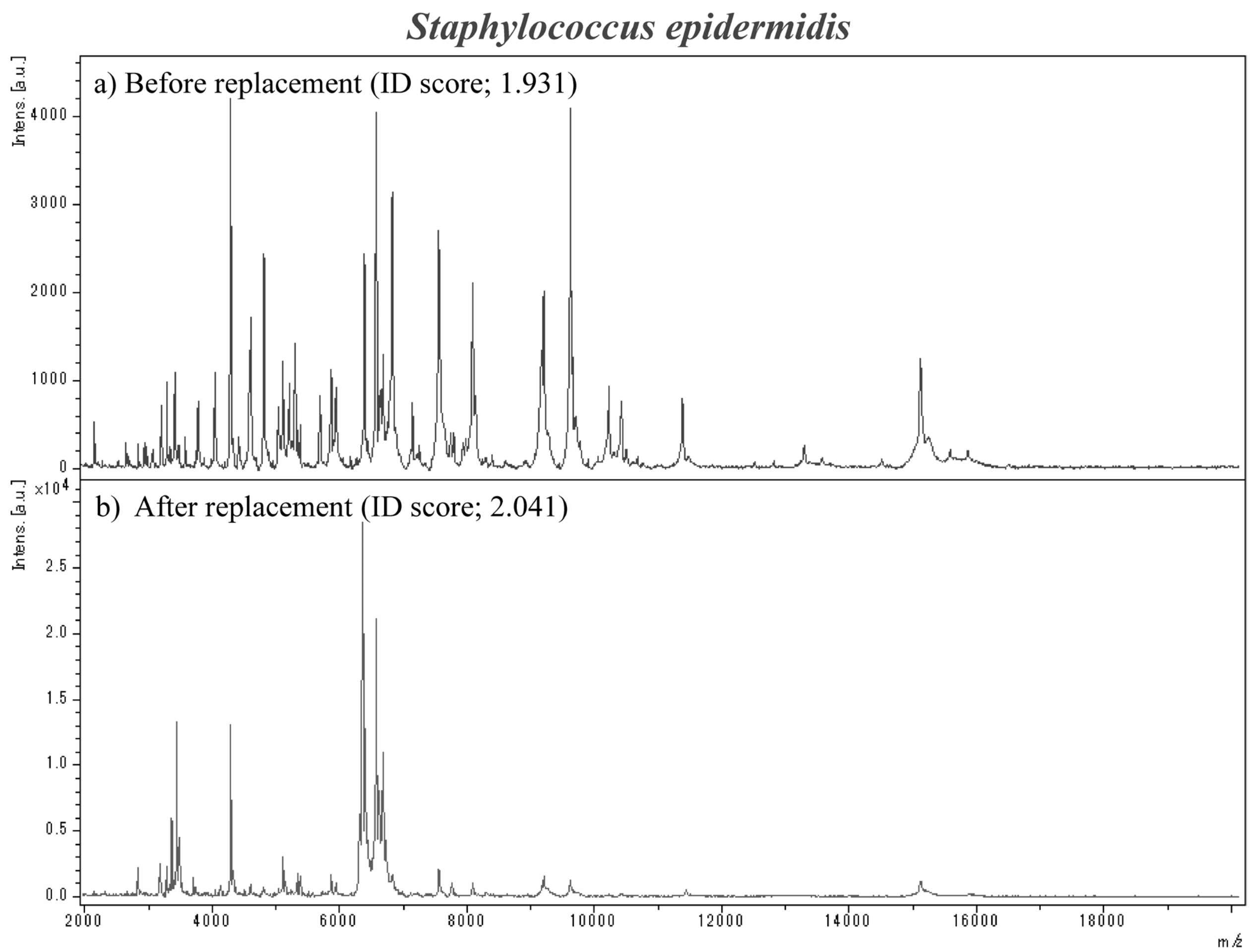

Fig. 2. Mass spectral pattern of *Staphylococcus epidermidis*.

a) Mass spectral pattern of *S. epidermidis* immediately before detector replacement; the identification score was 1.931. b) Mass spectral pattern of *S. epidermidis* immediately after detector replacement; the identification score was 2.041.

tunately, unlike lasers, there is no way for researchers to routinely check for detector deterioration. Therefore, users need to pay attention to the detector as well as the laser when the accuracy of bacterial identification by MALDI-TOF MS is compromised.

It is difficult for medical personnel who handle microorganisms to examine the deterioration of the detector in the mass spectrometry and determine when to replace it. However, it is necessary to control the accuracy of the detector and other components of the mass spectrometry to improve the accuracy of bacterial identification. In the present study, we demonstrated that the identification score is clearly higher both in *E. coli* and *S. epidermidis*, and signal intensities and S/N ratios generally increased for the representative *m/z* peaks after detector replacement. The quality control for MALDI-TOF MS bacterial identification using identification scores and representative *m/z* peaks will be a relatively easy method for researchers to implement in daily clinical practice.

Generally, the S/N ratios of the spectrum should be higher for *E. coli*, a gram-negative bacterium, than for *S. epidermidis*, a gram-positive bacterium, and the identification score should also be higher. However, *S. epidermidis* showed a higher identification score than *E. coli* before the detector replacement (Figs. 1 and 2), and 4 (4293, 4606, 5871, and 9630 *m/z*) out of 9 peaks in *S. epidermidis* showed higher S/N ratios before the replacement. The reason for this is unknown, but deterioration of the detector may reduce reproducibility as well as absolute sensitivity.

A wide range of indicators, such as accumulated shots of the laser, acceleration voltage of ionization, detector threshold, and vacuum level of the mass separation section, can be employed to more precisely determine the performance of mass spectrometry. Additionally, general quality control methods, such as the Xbar-R score value control, the twin-plot, and the delta-check methods, may be utilized for

Table 1. Signal intensities and signal/noise ratios of representative *m/z* peaks before and after detector replacement

Escherichia coli (*m/z*)	Before replacement		After replacement	
	Intensity	Signal/ noise ratio	Intensity	Signal/ noise ratio
4363	50	1.89	5919	24.18
5097	54	2.02	8862	33.76
7273	44	1.67	6871	23.95
8477	65	2.49	7871	31.62
9740	62	2.37	10095	45.28
13680	55	2.07	3503	24.86
16947	70	2.50	4185	45.44
Staphylococcus epidermidis (*m/z*)	Before replacement		After replacement	
	Intensity	Signal/ noise ratio	Intensity	Signal/ noise ratio
2843	281	2.74	3076	5.96
3415	1099	9.98	39342	74.22
4293	4203	33.61	16249	27.76
4606	1716	13.03	6802	10.91
5871	1133	8.31	4075	7.61
6582	4049	29.99	36975	72.18
7566	2708	21.09	14041	31.31
9630	4095	39.57	8091	27.54
15131	1247	25.20	5112	54.90

inspection. Future studies should aim for manualization using similar conventionally used quality control methods. Observation of analytical results using quantified variables is essential to confirm the high quality of analytical results. Development of such processes will allow the wider implementation of MS analysis as a clinical laboratory method.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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