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Research Article

Recent Applications of Ion Mobility Spectrometry in Diagnosis of Vaginal Infections

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Vaginal infections (vaginosis) globally affect more than 15% of the female population of reproductive age. However, diagnosis of vaginosis and differentiating between the three common types: bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis are challenging. Elevated levels of the biogenic amines, trimethylamine (TMA), putrescine, and cadaverine have been found in vaginal discharge fluid of women with vaginosis. Ion mobility spectrometry (IMS) is particularly suitable for measurement of amines even in complex biological matrices due to their high proton affinity and has been shown to be suitable for the diagnosis of vaginal infections. Recent developments that have increased the accuracy of the technique for diagnosis of BV and simplified sample introduction are described here.

1. Introduction

There are three common types of vaginal infections (vaginosis): bacterial vaginosis (BV), vulvovaginal candidiasis (VVC or yeast infection), and trichomoniasis that affect women worldwide [1]. According to many studies the prevalence of these infections varies considerably from country to country, and between different populations within each country but according to a conservative estimate more than 15% of women of reproductive age are affected by one or more of these infections at any given time [2]. Symptoms may include copious discharge of vaginal fluid, fishy odor, pain, itching, or burning. However, in many cases the symptoms are not noticed, that is, an asymptomatic infection [1]. The cause of BV is generally attributed to an overgrowth of anaerobic microorganisms and it has been associated with several types like Gardnerella, Mobiluncus, Bacteroides, Mycoplasma hominis, Ureaplasma urealyticum, and so forth. These microorganisms may normally be present in vaginal discharge fluid, but their growth is inhibited by Lactobacilli that maintain a low pH and produce hydrogen peroxide. Vulvovaginal candidiasis is caused by some types of candida (fungi) while trichomoniasis is caused by trichomonads that are parasites. When the balance between the pathological microorganisms and the Lactobacilli is disturbed a vaginal infection may erupt. This could be due to the use of antibiotic medication that deleteriously affects the Lactobacilli and thus allows the fungi to thrive leading to a yeast infection. An infection can also result from practices like frequent douching or unhygienic habits, allergy, or even sensitivity to semen.

Correctly diagnosing the vaginal infection could be complicated due to the similarity of symptoms of the three common infections or when women are asymptomatic. Several laboratory methods have been proposed to overcome this problem. The Amsel test includes a visual inspection of the discharge, measuring its pH, a "whiff test" performed by adding a drop of alkaline solution, and a "wet mount" microscopic examination [3]. The Gram stain method involves staining the vaginal discharge sample with a dye and using a microscope to quantify three morphotypes (Lactobacilli, Gram variable rods and curved rods) and analyzing the results according to the Nugent score [4–6]. Cultures may be used to diagnose candidiasis and trichomoniasis but they are not effective for determination of bacterial vaginosis as some anaerobic microorganisms are normally present in the discharge fluid. Other methods involve Antigen-detecting immunoassays, the OSOM Trichomonas Rapid Test, DNA

probes, and polymerase chain reaction (PCR). These are useful for detecting trichomonads as concisely summarized recently [1, 7]. However, each of these methods may either have a low accuracy (low specificity or sensitivity), high cost, long response time or place demands of time, and skill on the physician.

The need for a rapid, reliable, inexpensive diagnostic test that can differentiate between BV and VVC has been delineated [8]. The fact that biogenic amines are produced by the action of the microorganisms has been well known and has served as the basis for several commercial kits that are used to diagnose these vaginal infections [9–11]. However, a simple analytical procedure that can differentiate between the biogenic amines and thus serve to accurately diagnose the vaginal infections and determine BV was only reported less than a decade ago [12, 13].

Classical, or linear, ion mobility spectrometry (IMS) is an analytical technique used for separation of ions on the basis of their mobility, or drift velocity, under the influence of a weak electric field (E/N $< 2Td$). The main factors that affect the drift velocity, in addition to the mass of the ion, are the composition, pressure and temperature of the gas through which the ion drifts, and the magnitude of the electric field, as explained in detail previously [14]. These parameters are accounted for in calculation of the reduced mobility of the ion. The ion shape, size, and internal charge distribution also affect the drift velocity and are important mainly for differentiating between isomers and isobaric ions [14]. In most practical applications of IMS, as in the present study, the drift gas is air at ambient pressure. The fact that amines in general, and biogenic amines in particular, have high proton affinities [15] and are thus readily ionized through atmospheric pressure chemical ionization (APCI) conditions enables their sensitive and specific detection even in a complex biological sample like vaginal discharge fluid or food products [16–19]. The vapors of volatile biogenic amines like trimethylamine (TMA) or semi-volatile diamines like putrescine (PUT) and cadaverine (CAD) can be measured directly as they emanate from the sample. The emission of these vapors from biological samples can be enhanced chemically by application of an alkaline solution and physically by heating the sample, as shown previously [12, 13]. Two schematic representations of the structure of the three biogenic amines and triethylphosphate (TEP) are shown in Figure 1. On the left, the space-filling model is shown (blue-N atoms, black-C atoms, gray-H atoms, red-O atoms, and orange-P atoms). The schematics on the right show important features of the amines regarding the protonation site of the ions formed: the nitrogen lone-pair of electrons shown for TMA and a proton-bridged cyclic structure for the diamines where the proton is between the two amine groups [20].

Since the first publication of the method for diagnosing vaginal infections by measurement of biogenic amines with IMS [12, 13] there have been several developments that have helped increase the reliability and accuracy of the method, as well as making it simpler to perform. These will be described and discussed in the following article.

2. Experimental

Commercially available chemicals were used including Putrescine (1,4 Diamino-butane, Aldrich 99%), Cadaverine (1,5 Diaminopentane, Sigma > 97%), and trimethylamine (50% aqueous solution, Acros, New Jersey). Triethylphosphate (TEP) (Aldrich 99.8%) was used as the dopant. and 2, 4-Lutidine (Acros 99%) was used to calibrate the reduced mobility scale. Reduced mobility values were calculated relative to 2,4-lutidine (1.90 $cm^2\ V^{-1}\ sec^{-1}$ at 90°C) [21].

A linear ion mobility spectrometer with a corona discharge ionization source (VG-Test, 3QBD, Arad, Israel) was operated at 90°C as described in detail previously [22].

Ambient air, purified by passing over an activated charcoal filter, was used as the drift gas and carrier gas. The drift cell consisted of a stack of metal rings separated from one another by Teflon rings in order to form a linear electric field gradient of 270 $V\ cm^{-1}$. The dopant (TEP) was introduced continuously into the ionization region from a homemade permeation tube emanating vapors at a rate of about 1 $\mu g\ min^{-1}$. The samples were dripped onto a cotton swab (50 μL by calibrated pipette), and one drop of a 15% KOH alkaline solution was added. The swab was then placed in a sample holder that was inserted into the sample block of the IMS where the analysis was triggered by a microswitch. For determination of volatile TMA a halogen lamp was turned on for the duration of the measurement (25 secs). For determination of the semivolatile biogenic amines, putrescine, and cadaverine, a longer measurement time was used in order to enhance their emanation. The mobility spectrum was measured at a rate of 80 kHz, and every second the averaged spectrum is recorded and stored. The nominal resolution was 25, as calculated by dividing the drift time of the ion by the full width at half maximum (FWHM).

In some cases, particularly when samples are sent to a central laboratory for diagnosis, the swab with the sample of vaginal discharge fluid was stored in a gel test tube (Amies gel, Copan, Italy). Extended storage may lead to migration of biogenic amines from the swab to the gel, and this has been tested with samples of TMA that were deposited on a swab that was placed in the gel and stored for different periods at room temperature, refrigerator, and deep freeze (25°C, 4°C and −18°C, resp.).

3. Results and Discussion

3.1. Calibration. Needless to say that for ethical and practical reasons calibration could not be done with real vaginal discharge fluid so alternative approaches were deployed. First a dilute aqueous solution of TMA was used, and calibration of the system was carried out by adding a fixed volume (50 μL) of an aqueous solution containing between 125 and 2000 ng of TMA to a cotton swab and measuring the mobility spectrum as described above. The intrarun reproducibility was 15% which is quite typical for measurement of volatile samples by IMS. The interrun reproducibility was 30% so that by performing a daily calibration procedure a clear

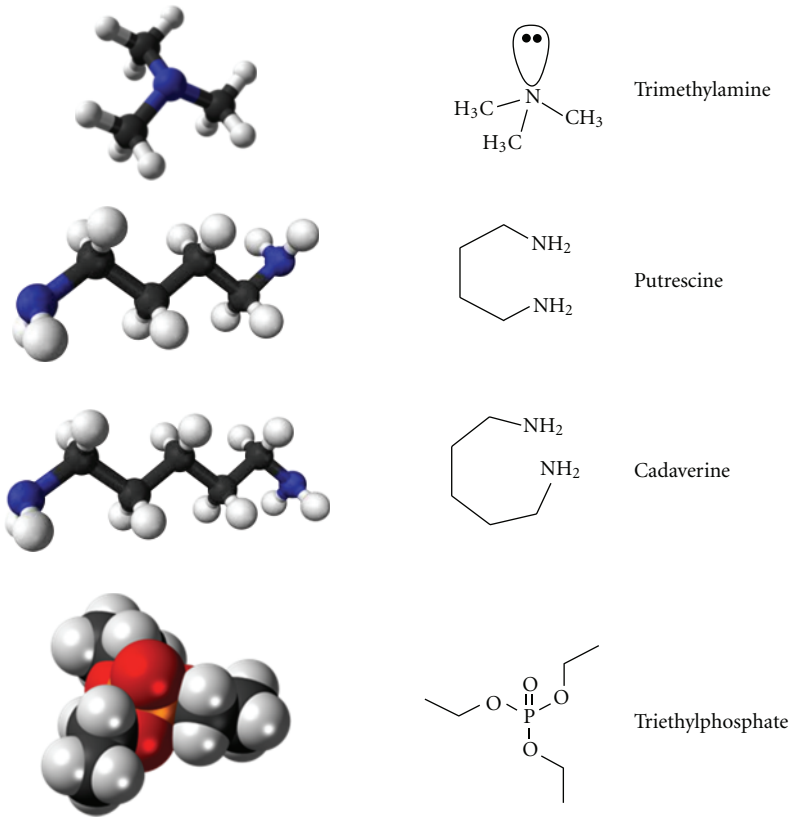


Figure 1: The structure of trimethylamine (TMA), putrescine, cadaverine and triethylphosphate (TEP). Note the lone pair of electrons on the nitrogen atom of TMA and the cyclic representation of the structure of putrescine and cadaverine.

distinction was found between samples collected from BV-negative and BV-positive patients.

One of the main features of IMS is that when product ions are formed the population of the reactant ions is depleted. The use of the ratio TMA/(TMA + TEP) somewhat provides a correction for this and also allows all the quantitative results to be displayed conveniently on an axis between zero and one. In the present study the level of the other biogenic amines (putrescine and cadaverine) was low, so they had little influence on the BV diagnosis. In cases where their level is significant the approach is to calculate and display the ratio between each amine and the total intensity of the ions, for example, TMA/(TMA + PUT + CAD + TEP).

Figure 2(a) shows the calibration curve obtained by measuring the ratio of the areas of the product ion peak (TMA) divided by the sum of the product ion and reactant ion (TEP) as a function of the amount of TMA placed on the swab. Each point represents the average of three measurements. The second approach was adopted in order to better simulate a biological sample by the use of the solution in canned tuna (in water not in oil) that contained about 0.4 mg mL^{-1} of TMA [23]. The tuna water was diluted so that the actual TMA concentrations were equivalent to the concentrations used in aqueous solution calibration curve. Figure 2(b) depicts the calibration curve thus obtained for TMA in water from canned tuna.

The calibration curves in Figures 2(a) and 2(b) are typical of the quantitative response of IMS instruments, that is, the response approaches saturation at high concentrations or large amounts of the analytes. In this case, after about 2500 ng of TMA the calibration curves start to level out. This happens when the intensity ratio of the analyte TMA/(TMA + TEP) reaches about 0.6 and is indicative of depletion of the TEP reactant ions.

3.2. New Instrumental and Sample Introduction Techniques. The VG-Test differs in several aspects from the PT-IMS (Rotem Industries, Israel) that was used in the early tests for diagnosing vaginal infections [12, 13]. The radioactive ^{63}Ni source that was used in the PT-IMS has been replaced by a corona discharge ionization source. The operating temperature of the drift tube was reduced from 130°C in the PT-IMS to 90°C in the VG-Test without deleterious consequences. The pump that was used to generate the flow of the carrier and drift gas streams in the PT-IMS was reinforced by a second pump that shortened clearance times of the system and reduced "memory effects". Nonylamine that was the dopant in the PT-IMS that formed the reactant ion was replaced by triethylphosphate (TEP) that is safer and easier to handle as it is not degraded by reaction with atmospheric moisture. However, the most radical change was in the sample introduction technique: in the PT-IMS the

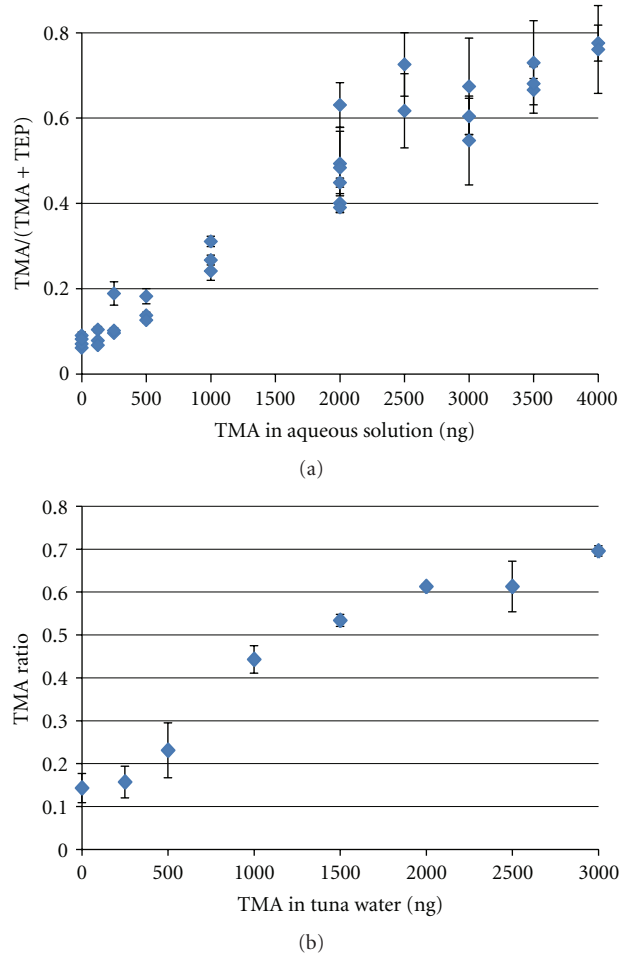


Figure 2: (a) The ratio TMA/(TMA + TEP) as a function of the amount of TMA in aqueous solution that was deposited on a cotton swab. (b) The ratio TMA/(TMA + TEP) as a function of the amount of TMA in water from canned tuna that was deposited on a cotton swab.

swab with the sample of vaginal discharge was stirred in a vial with distilled water, followed by addition of alkaline solution, and then the vial was connected to the carrier flow stream. For enhanced emanation of the semivolatile amines the vial had to be placed in a beaker with hot water. The inlet system of the VG-Test simplified the sample introduction considerably: the swab was placed in a module (housing), and a drop of alkaline solution was added directly to the swab followed by insertion of the housing into the inlet where heating by a halogen lamp started automatically according to the analytical procedure that was selected. This also diminished the "memory effects". Typical mobility spectra obtained for a blank swab and a swab upon which 1 μg of TMA for calibration was deposited are shown in Figure 3. Note the TEP peak at a drift time of 7.2 ms and the TMA peak at 5.9 ms.

3.3. Sensitivity and Limit of Detection. The limit of detection (LOD) and limit of quantification (LOQ) of the method were calculated from the uncertainty in the spectrum of

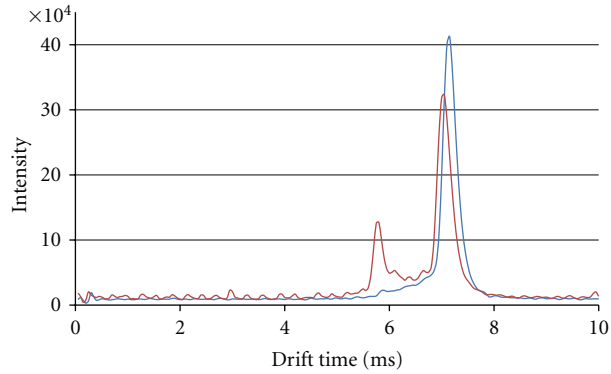


Figure 3: Typical mobility spectra of a blank swab (blue trace) and a swab upon which 1 μg of TMA was deposited for calibration (red trace). Note the TEP peak at a drift time of 7.2 ms and the TMA peak at 5.9 ms.

blank samples. The uncertainty was 10.3 mV, and based on the sensitivity calibration (0.86 mV ng^{-1}) the LOD and LOQ were calculated as 39 and 120 ng, respectively. Thus, the linear range for quantification of TMA in aqueous solution or in tuna water (that represents a biological matrix) is between 120 to 2500 ng of TMA deposited on cotton swab. This was certainly sufficiently sensitive to readily distinguish between samples collected from a patient with a BV infection where the TMA level in a sample of vaginal discharge fluid exceeded 2000 ng and a sample collected from a healthy woman where the TMA level was typically well below 1000 ng. The mobility spectra shown in Figure 4 were obtained from actual swabs with vaginal discharge fluid collected from a patient without a vaginal infection (blue trace) and a patient with bacterial vaginosis (red trace).

3.4. Effect of Storage in Gel. As expected, the storage temperature strongly affects the rate of TMA migration from the swab into the gel. After three days at room temperature (25°C) about 90% of the TMA was lost from the swab but could be detected in the gel. A similar period in a refrigerator (4°C) led to loss of about 84% of the TMA while storage in a deep freeze (−18°C) resulted in loss of about 50% of the TMA originally deposited on the swab. Figure 5 shows the calculated peak area of the TMA retained on the swab after deposition of 1 μg of TMA as a function of the storage time in a refrigerator. Initially, after one day about 75% of the deposited TMA migrated from the swab to the gel, but after one week the amount of TMA retained on the swab remained almost constant.

Therefore, a calibration curve for swabs stored in gel would differ significantly from that obtained from a fresh swab, and corrections must be made in consideration of the storage time and temperature. In case the swab contains elevated levels of TMA then a BV-positive diagnosis is unmistakable. However, in order to confirm a BV-negative diagnosis, particularly for swabs that have been stored for several days in gel, the TMA level in the gel should also be tested.

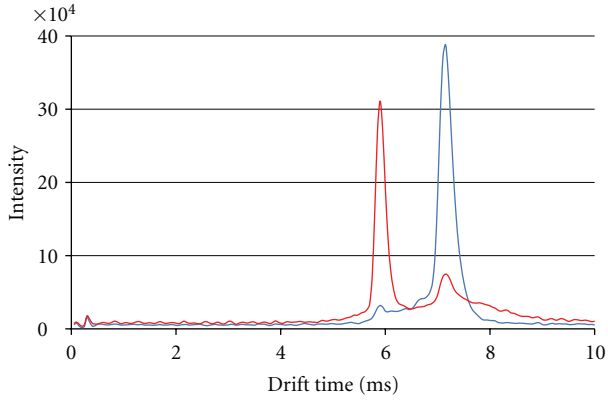


Figure 4: Typical mobility spectra of a swab collected from a patient without a vaginal infection (blue trace) and a swab from a patient with BV (red trace).

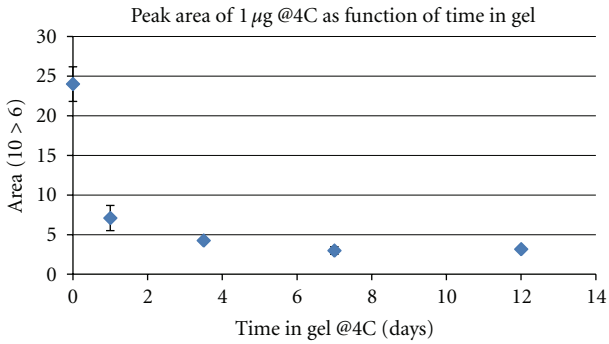
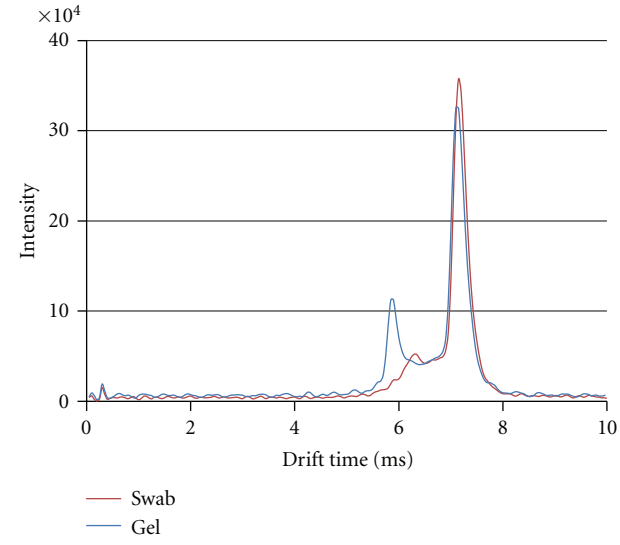


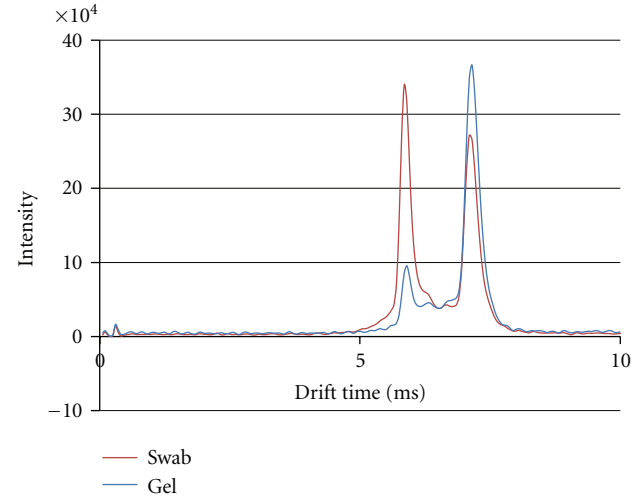
Figure 5: The calculated peak area of the TMA retained on the swab after deposition of 1 μg of TMA as a function of the storage time in a refrigerator.

3.5. Preliminary Tests of Vaginal Samples Stored in Gel. Samples that were stored in gel were diagnosed according to the Nugent score either as positive for BV or as negative for BV and were subsequently analyzed by the VG-Test. Of 18 samples that had Nugent scores of 7–10 (i.e., BV-positive samples) 15 were observed to have elevated TMA levels, two had slightly elevated levels of cadaverine, and one was stored for over a week which probably led to migration of almost all the TMA from the swab to the gel. Of 21 samples that were with Nugent scores of 1–3 (i.e., BV-negative samples) according to the VG-Test analysis 19 had little or no TMA and the remaining two had elevated cadaverine levels indicating a vaginal infection but not BV. As mentioned above the calibration for a swab stored in gel differs from a fresh swab, but nevertheless the accuracy of the BV diagnosis was impressive, especially as other biogenic amines could be seen in the few samples where there was a disagreement between the laboratory diagnosis and IMS analysis.

Figure 6 shows the mobility spectra recorded from a swab with vaginal discharge fluid and from a fraction of the gel in which the swab was stored. Figure 6(a) depicts the mobility



(a)



(b)

Figure 6: The signal intensity as a function of drift time for the swab (red trace) and gel (blue trace) of a vaginal discharge fluid that was stored in gel. (a) After storage in gel for one week; (b) after storage in gel for less than two days.

spectra of the swab and a sample of the gel after storage in the gel of one week while Figure 6(b) shows the spectra after storage of less than two days. Evidently, the spectra show that the long storage time resulted in migration of the TMA from the swab to the gel, while after a short storage period only a small fraction of TMA was transferred to the gel.

These studies made it possible to extend the diagnosis of bacterial vaginosis that had previously been demonstrated for fresh swabs of vaginal discharge fluid [12, 13] to the diagnosis of vaginitis in swabs that have been stored in gel. Thus, the method can be of use to central laboratories that use this storage method to ship the samples from the clinic to the laboratory.

4. Summary

Recent developments in the application of ion mobility spectrometry (IMS) for diagnosing vaginal infections, or specifically bacterial vaginosis (BV), were described. The main features are improvements in the sample introduction methodology that make the system more user friendly, replacement of the radioactive ionization source by a corona discharge source, and changing the n-nonylamine dopant by triethylphosphate (TEP) that is more stable and easy to use. In addition, calibration techniques have been improved, and the use of water from canned tuna chunks as a biological matrix provides a better simulant for body fluids. The linear range is typical of the response of IMS instruments spanning a range of 120 ng to 2500 ng, but is suitable for diagnosis of BV on the basis of the TMA level in a sample vaginal discharge fluid. Finally, the study of the effects of storage in gel of vaginal discharge fluid samples makes it possible to extend the diagnostic method to samples that are tested in central laboratories.

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