

Rapid, Accurate, and On-Site Detection of *C. difficile* in Stool Samples

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- OBJECTIVES:** A rapid test to diagnose *Clostridium difficile* infection (CDI) on hospital wards could minimize common but critical diagnostic delay. Field asymmetric ion mobility spectrometry (FAIMS) is a portable mass spectrometry instrument that quickly analyses the chemical composition of gaseous mixtures (e.g., above a stool sample). Can FAIMS accurately distinguish *C. difficile*-positive from -negative stool samples?
- METHODS:** We analyzed 213 stool samples with FAIMS, of which 71 were *C. difficile* positive by microbiological analysis. The samples were divided into training, test, and validation samples. We used the training and test samples ($n=135$) to identify which sample characteristics discriminate between positive and negative samples, and to build machine learning algorithms interpreting these characteristics. The best performing algorithm was then prospectively validated on new, blinded validation samples ($n=78$). The predicted probability of CDI (as calculated by the algorithm) was compared with the microbiological test results (direct toxin test and culture).
- RESULTS:** Using a Random Forest classification algorithm, FAIMS had a high discriminatory ability on the training and test samples (C-statistic 0.91 (95% confidence interval (CI): 0.86–0.97)). When applied to the blinded validation samples, the C-statistic was 0.86 (0.75–0.97). For samples analyzed ≤ 7 days of collection ($n=76$), diagnostic accuracy was even higher (C-statistic: 0.93 (0.85–1.00)). A cutoff value of 0.32 for predicted probability corresponded with a sensitivity of 92.3% (95% CI: 77.4–98.6%) and specificity of 86.0% (78.3–89.3%). For even fresher samples, discriminatory ability further increased.
- CONCLUSIONS:** FAIMS analysis of unprocessed stool samples can differentiate between *C. difficile*-positive and -negative samples with high diagnostic accuracy.

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INTRODUCTION

Clostridium difficile infection (CDI) is a common healthcare-associated infection, and is particularly prevalent in the elderly and in patients who have received antimicrobial agents. Symptoms range from mild diarrhea to severe pseudomembranous colitis and toxic megacolon. Since 2000, this infection has become increasingly prevalent, and more severe forms of the disease have emerged. Large hospital outbreaks have required ward closures and extensive infection control measures (1–3).

Early and rapid identification of CDI is important for outbreak prevention and containment (4). Many different tests are available,

used singularly or in combination for either the detection of the bacterium, its genes, its antigens, or its toxins. In general, these tests are performed in a laboratory environment. As a consequence, efficient diagnosis depends not only on timely clinical suspicion, but also on the logistics of getting stool samples to the laboratory and the time required to process samples. Inefficient logistics and laboratory procedures can contribute to diagnostic delay that leads to delay in treatment and infection control measures. Collectively, such factors contribute to a mean time from onset of symptoms to start of treatment that usually ranges from 2.8 to 7.7 days (5,6). During this time, contamination of other patients and hospital staff is a risk.

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Immediate detection of *C. difficile* in unprocessed stool samples on hospital wards would be ideal. Since *C. difficile* was identified as the major causative microorganism of antibiotic-associated diarrhea in the 1970s, its typical smell has been frequently described as resembling horse manure. Our sense of smell depends on the ability of specialized sensory cells of the nose to respond to gases and volatile organic compounds (VOCs). Diseases such as infections and malignancies can be associated with specific VOC profiles, for example, originating from microorganisms or from changes in host metabolism, and thus have a different odor. The ability of the human nose to identify CDI has been the subject of several studies. Although the smelling capacity of mankind is far from perfect, nurses' noses performed better than expected by chance alone (7,8). The smelling capacity of many animals, such as dogs, far exceeds that of humans. Recently, we published a study showing that a trained scent-detection dog is very capable of distinguishing *C. difficile*-positive from -negative stool samples (9). Furthermore, the dog could also be used in the clinic: he was capable of identifying hospital patients with CDI by simply walking past their beds (9). The use of dogs or other animals in hospitals, however, has obvious drawbacks.

Attempts to replace the biological olfactory system by standardized hardware has resulted in several types of electronic noses and gas phase analyzers such as gas chromatography combined with mass spectrometry (GCMS). These analyze the volatile compounds in the air above a liquid or solid sample, also referred to as "headspace analysis." In the medical field, scent detection studies have, for example, been on pulmonary diseases (e.g., chronic obstructive pulmonary disease, asthma) and malignancies (e.g., lung and colorectal cancer) (10–13), and also on gastrointestinal diseases like *C. difficile* infection (14–18). VOCs emanating from feces samples of patients with CDI are found to differ from samples of healthy controls and patients with other gastrointestinal diseases. The diversity of VOCs of *C. difficile*-infected stool is considerably smaller, e.g., contains less (fatty) acids, esters, and organic sulfur compounds, but more butanol and furans (14,15). Both electronic noses and (solid-phase microextraction) GCMS have been used to study the production of VOCs in the headspace above stool samples in a specific culture broth or above pure cultures. Both could discriminate between different bacteria in pure culture (16,17) and even between *C. difficile*-positive and -negative samples (14,18). Automated headspace analysis of a stool sample may offer the ideal rapid, sensitive, and standardized method for detection of *C. difficile* in stool samples at the bedside. However, the techniques described have practical drawbacks, including the requirement of expensive and large equipment, drifting of sensors after calibration, and dependence on bacterial growth in a culture medium. These drawbacks compromise their suitability as a point-of-care diagnostic test.

Field asymmetric ion mobility spectrometry (FAIMS) is a mass spectrometry technique that can be used to separate complex mixtures of molecules based on their different mobilities (which relate to size and mass) in high and low electric fields. FAIMS has a number of advantages over more traditional analytical techniques (such as GCMS) as it operates at room temperature, does not

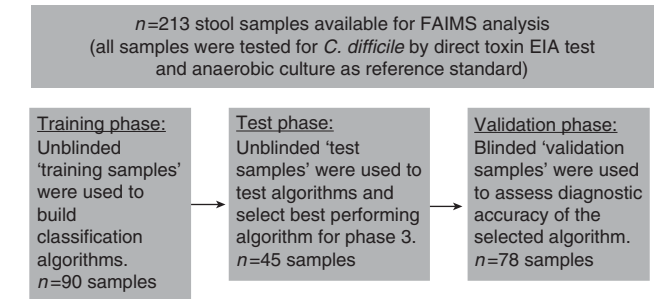


Figure 1. Flow diagram of the study design. EIA, enzyme immunoassay; FAIMS, field asymmetric ion mobility spectrometry.

require the use of specific gases, and does not suffer from sensor drift. Recently, a portable self-contained FAIMS instrument was developed, with its chemical detection system on a "dime-sized" chip. Whether unprocessed *C. difficile*-positive stool samples can be distinguished from negative stool samples by FAIMS through direct analysis of their headspace is not known. In this study, we aimed to determine the diagnostic accuracy of FAIMS for direct detection of *C. difficile* in stool samples.

METHODS

General study outline

In this study, we aimed to determine whether unprocessed *C. difficile*-positive stool samples can be distinguished from negative stool samples by FAIMS analysis of their headspace. All stool samples used were tested for *C. difficile* by direct toxin enzyme immunoassay (EIA) and anaerobic culture as reference standard. The study was done in three phases (see **Figure 1**). First, we analyzed a number of unblinded *C. difficile*-positive and -negative samples by FAIMS. The amount of datapoints acquired by FAIMS per analyzed sample is very large. For the interpretation of results we therefore tried several complex mathematical algorithms, to see whether samples can be categorized as either *C. difficile*-positive or -negative based on their FAIMS characteristics. The samples used to "build" the classification algorithms are referred to as the "training samples." Next, these algorithms were tried on a second, unblinded sample set to ascertain which algorithm performed best on new, different samples. The samples used here are referred to as the "test" samples. In the third phase, the best performing algorithm was validated on a new set of blinded stool samples (the validation samples) to formally assess its diagnostic accuracy on samples that were not used during algorithm development. The researcher training, testing and validating the algorithms was unaware of (blinded to) the *C. difficile* status of the validation samples.

FAIMS technology

For this study a commercially available FAIMS instrument was used (Lonestar, Owlstone, Cambridge, UK) (**Figure 2a**). FAIMS uses an oscillating electric field to separate different gaseous ions based on how they move through this electrical field (their

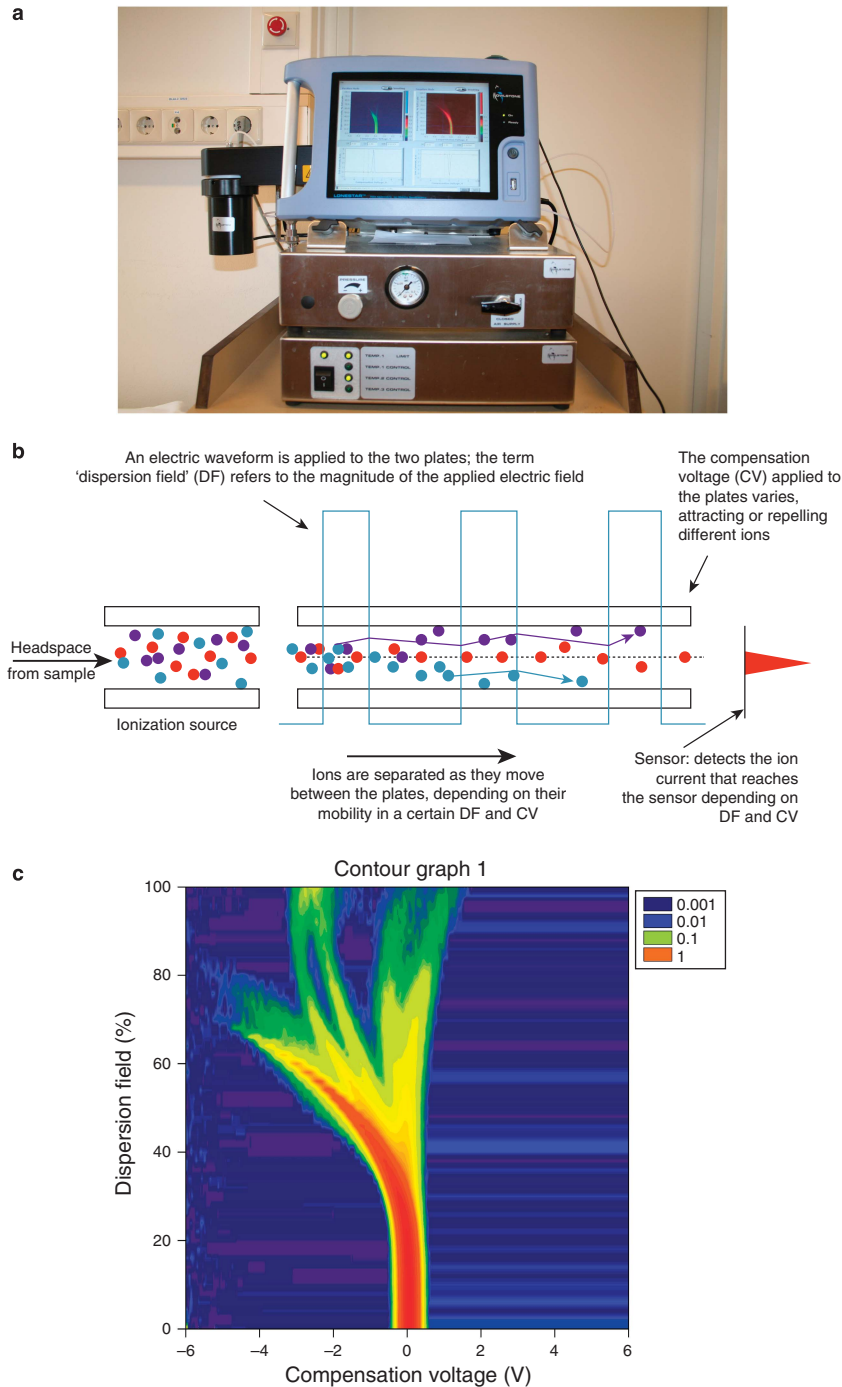


Figure 2. Field Asymmetric Ion Mobility Spectrometer (FAIMS): what is it and how does it work? **(a)** Lonestar FAIMS. The (stool) sample is placed in a glass jar in the black bottle holder on the left; the headspace gas is guided to the ionization source, separation plates, and sensor that are located on a “dime-sized” chip inside the computer. **(b)** FAIMS separates complex mixtures of different gaseous ions based on how they move through an oscillating electric field. Volatile compounds and gases released from a sample (in this case the headspace of a stool sample) are transported by a carrier gas (dry, clean air) into the FAIMS unit. Here, the sample is first ionized by a nickel-63 beta radiation source. Ionized molecules then pass between two plates to which an electric field waveform is applied. The applied peak-to-peak voltage of the electric field (or dispersion field (DF)) varies with a proportionate effect on an ion’s mobility. The electric field waveform is asymmetric, and thus a high positive voltage is applied for a short period of time and a low negative voltage is applied for a longer period. However, the net force over a time period is zero. Ions that have the same mobility in high and low electric fields create a “saw tooth” path on their way through the two plates and drift toward the sensor at the end (red molecules in figure). If an ion is pulled to one side in the electric field, it contacts the plate on which its charge is lost and the molecule does not reach the sensor (blue and purple molecules). To counter this effect a voltage is applied to the plate, known as the compensation voltage (CV). Depending on their charge, ions are again repelled or attracted to the plate; therefore, the CV is another determinant of which molecules reach the sensor. Both the DF and CV are stepped through a range of values that results in different ions reaching the sensor as a function of applied DF and CV potential. This creates a three-dimensional (3D) map of chemical components (or the “chemical fingerprint”) of a sample, as shown in **(c)**. The whole procedure of sample analysis takes <10min.

mobility) that relates to size and mass (Figure 2b) (19). It can be used for the real-time analysis of complex gases, looking at the pattern of total chemical composition (the “chemical fingerprint”) of a sample rather than the individual components. Separate spectra are obtained for positive and negative ions. For this study, we used the following settings: the dispersion field (which relates to the magnitude of the applied electric field) was varied between 0 and 100% in 51 steps and the compensation voltage (to regulate which ions (i.e., with what mobility) reach the sensor) from -6 V to +6 V in 512 steps. This generates two matrixes (one for positively and one for negatively charged ions) with a total of 52,224 data points per sample run. Each sample was run three times. The first run can be affected by the environment that the sample was collected in (i.e., the hospital ward) and this matrix is therefore discarded; thus, for the analysis we used only the second and third matrix. An example of FAIMS output of a stool sample analysis is shown in Figure 2c.

Samples

In this study we used stool samples of patients with a clinical suspicion of CDI that were sent to the microbiology laboratory to test for *C. difficile*. Between March 2013 and July 2013, we prospectively collected all consecutive *C. difficile*-positive samples, plus one to three negative samples (per positive sample). Samples were taken from patients in two Amsterdam hospitals, the VU University medical center (a tertiary clinical care center) and the Onze Lieve Vrouwe Gasthuis (a large teaching hospital). The vast majority of samples came from patients admitted to the hospital on either a medical or surgical ward. Patients were 54% male and 46% female with a median age of 64 years (interquartile range (IQR) 50–75 years).

The negative samples were randomly chosen from all samples that were available for *C. difficile* testing in the microbiology laboratory on that day. We aimed to collect two negative samples per positive sample from the same day. On the rare occasion that no two negative samples were available, we compensated this with an extra negative sample on the next occasion. Negative samples were chosen at random but, if available, we preferred the fuller vials over those with only little stool. The selection of negative samples was done after and based on the toxin EIA test result. As the culture results were often not available yet at this moment, it did occur on some occasions that a sample with a negative toxin EIA result was later classified as a positive sample based on a positive culture result (7 samples in the training and test phase and 8 in the validation phase; see Table 1).

Samples were analyzed in the microbiology laboratory with a direct toxin enzyme immunoassay (VIDAS EIA *Clostridium difficile* A and B; Marcy l’Etoile, France), and by anaerobic culture followed by toxin EIA on the cultured strain, if applicable. Negative stool samples had both a negative result in the direct toxin test on the stool sample and a negative culture (or culture of a non toxin-producing *C. difficile* strain). In our clinical practice, samples are considered positive if a toxin-producing *C. difficile* strain is cultured from a stool sample, even if a direct toxin test on the stool sample is negative, although this could indicate colonization rather than infection. In the building of our algorithm (using our train-

Table 1. Laboratory test results for the different sample sets

Stool sample characteristics	Training and test set	Validation set	Total
Toxin negative, culture negative	81	43	124
Toxin positive, culture positive	45	26	71
Toxin negative, culture positive	7	8	15
Toxin intermediate, culture positive	2	1	3
Total	135	78	213

“Toxin” refers to the direct toxin enzyme immunoassay (EIA) test on the stool sample; “culture” refers to whether or not a toxin-producing *Clostridium difficile* strain was cultured.

ing and test samples), we made the same assumption, i.e., positive cultures with negative direct toxin tests were classed as positive. The research protocol was approved by the institutional review boards in both hospitals.

The samples were stored at 4 °C until analysis. For FAIMS analysis, we mixed ~0.5 ml of stool sample with 10 ml tap water in a clean glass jar. Clean compressed room air (0.1 MPa) was used as the carrier gas to move the sample headspace into the FAIMS instrument. The flow rate over the sample was 2l/min, with the temperatures set at 35 °C for the sample/ bottle holder, 70 °C for the lid, and 100 °C for the filter region. Each sample was analyzed three times sequentially, producing three matrixes; one matrix typically takes 180 s. Between samples, we refreshed the air in the FAIMS by analyzing the headspace of a clean jar with only water.

Analyzing the training set: training, testing, and validating a classification algorithm

Because of the large amount of data points, the data were pre-processed to aid in extracting signals from the data. The data were first concatenated into a one-dimensional array formed of 52,224 data points to which a wavelet transform was applied (Daubechies D4). Wavelet transforms are a common method of data reduction used for audio and image compression (e.g., in JPEG 2000, <http://www.jpeg.org/jpeg2000/>). It is particularly suited to separate small/hidden signals of one frequency swamped by a much larger signal of a different frequency. Once the wavelet transform was applied, coefficients below a given threshold were removed on the basis that these are dominated by noise. Next, a Wilcoxon rank-sum test was used on the training set to identify which features were most informative in discriminating between positive and negative samples. The variance threshold and the number of features kept from the Wilcoxon analysis were optimized using cross-validation on the training set, in order to optimize discrimination. Three machine learning classification algorithms, which are computational algorithms capable of identifying important patterns in large data sets, were then applied to the training and test set data: Sparse Logistic Regression (using R package “glmnet”; open source software by the R Foundation for Statistical Computing, Vienna, Austria), Random Forests (using the R package “randomForest”), and a Support Vector

Machine (using the R package “kernlab”) (20). The best performing algorithm was subsequently validated on a new, blinded set of stool samples (the validation samples) to assess its diagnostic accuracy on samples that were not used during algorithm development. The algorithm’s diagnostic accuracy was then determined by comparing the predicted probability (as calculated by the algorithm) with the microbiological test results with IBM SPSS Statistics version 20 (IBM, New York, NY). Medians of probability were compared between groups (e.g., between the known positive and known negative group) by means of a Wilcoxon rank-sum test.

RESULTS

Sample characteristics

In total, 213 stool samples were collected. The training and test set included 135 samples (of which 45 were *C. difficile* positive); 78 samples (of which 26 were *C. difficile* positive) were used for blinded validation (see **Table 1**). The majority of the samples (65%) were analyzed within 2 weeks after collection.

The training and test samples

Out of the three different classification algorithms, the Random Forest classification algorithm proved most accurate on the training and test set. The median predicted probability for negative samples was 0.17 (IQR: 0.20; $n=81$) and for the (direct toxin and culture) positive samples was 0.94 (IQR 0.44; $n=45$; $P<0.001$). The C-statistic (or area under the receiver operating characteristic curve) for these samples was 0.91 (95% confidence interval (CI): 0.86–0.97). For the samples with inconsistent direct toxin test and culture results, probabilities were as follows: “toxin negative/culture positive”: median 0.33 (IQR: 0.19; $n=7$) and “toxin intermediate/culture positive”: median 0.70 ($n=2$).

The validation samples

Next, we tested the Random Forest classification algorithm on the blinded validation samples. The predicted probabilities for the different samples in the validation set were as follows: median for the negative samples: 0.18 (IQR: 0.18; $n=43$) and median for the (direct toxin and culture positive samples) positive samples: 0.82 (IQR: 0.50; $n=26$), respectively ($P<0.001$); with a C-statistic of 0.86 (95% CI: 0.75–0.97). For the samples with inconsistent direct toxin test and culture results, probabilities were as follows: “toxin negative/culture positive”: median 0.31 (IQR: 0.56; $n=8$), “toxin intermediate/culture positive”: 0.46 ($n=1$).

Effect of sample age

When the analysis was restricted to stool specimens that were analyzed within 7 days of sampling (from both the training and test and validation set), the overall diagnostic accuracy further improved, as is demonstrated in **Figure 3a, b**. The median of the negative samples was 0.19 (IQR: 0.18; $n=50$) compared with a median of 0.94 (IQR: 0.38; $n=26$) in the (direct toxin and culture) positive samples ($P<0.001$), with a C-statistic of 0.93 (95% CI: 0.85–1.00). By definition, sensitivity and specificity vary with the

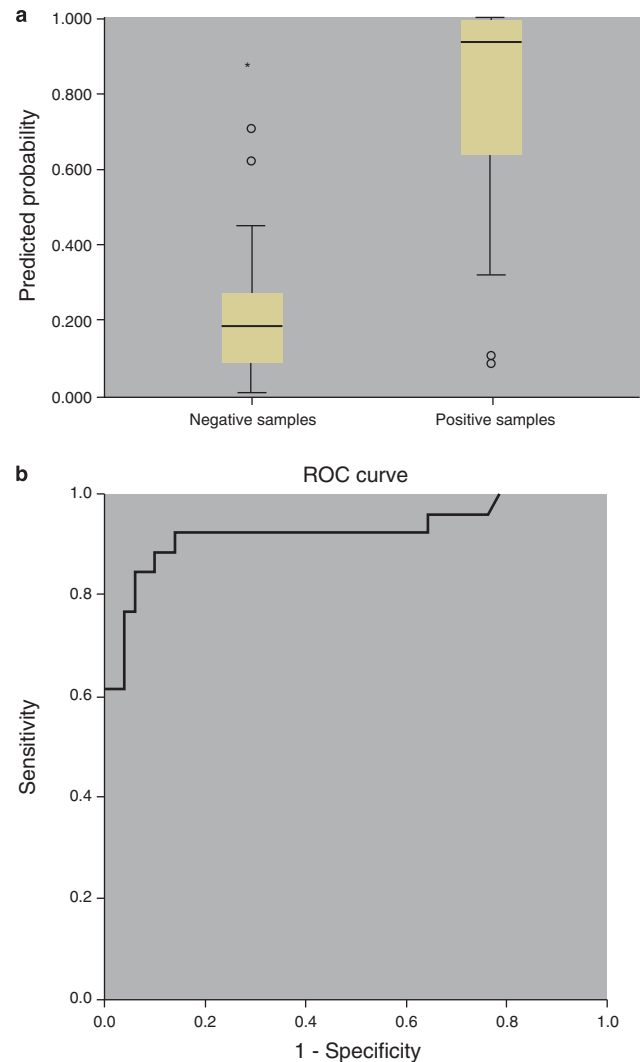


Figure 3. Results of the samples analyzed ≤ 7 days (from training, test, and validation sample sets). **(a)** Boxplot for the FAIMS results, presented on the y-axis as the ‘predicted probability’ of a sample being positive, on a scale of 0–1. The boxplot is presented for the predicted probability of a sample being positive in the (toxin and culture) negative samples and (toxin and culture) positive samples, with the median and its quartiles. Outliers (points) are values between 1.5 interquartile range (IQR) and 3 IQRs from the end of a box. Extreme outliers (asterisks) represent cases with values over three times IQR. **(b)** The receiver operating characteristic (ROC) curve for the (toxin and culture) negative and (toxin and culture) positive samples.

cutoff chosen for predicted probability. For the samples analyzed ≤ 7 days of collection, a cutoff value of 0.32 for predicted probability corresponded to a sensitivity of 92.3% (95% CI: 77.4–98.6%) and specificity of 86.0% (78.3–89.3%). At a cutoff value of 0.37, sensitivity was 88.5% (73.5–96.5%) and specificity was 90.0% (82.2–94.2%).

For samples analyzed within 5 days, the C-statistic is 0.94 (0.85–1.00; $n=45$, of which 16 positive); and for samples analyzed within 2 days the C-statistic is better still: 0.98 (0.92–1.00; $n=16$, of which 4 are positive). The discriminatory power for the different “sample age” groups is given in **Table 2**.

Table 2. Discriminatory power of FAIMS, depending on sample age

Sample age in days (median)	N (positive)	C-statistic (95% confidence interval)
All (10)	195 (71)	0.89 (0.84–0.95)
≤14 (7)	132 (48)	0.88 (0.81–0.95)
≤7 (5)	76 (26)	0.93 (0.85–1.00)
≤5 (3)	45 (16)	0.94 (0.85–1.00)
≤2 (2)	16 (4)	0.98 (0.92–1.00)

FAIMS, field asymmetric ion mobility spectrometry.

Sample age refers to the number of days between sample collection and sample analysis. “N” represents the total number of samples in the group; only samples with consistent toxin test and culture results (see **Table 1**) are presented (i.e., toxin-negative/culture-negative samples and toxin-positive/culture-positive samples).

Other infections found in the samples

As CDI is the major infectious cause of hospital-acquired diarrhea, in our hospital it is custom to test stool samples of hospitalized patients only for *C. difficile*, unless explicitly requested otherwise. Other infectious causes of diarrhea that were tested included: cultures for *Salmonella*, *Shigella*, *Yersinia* and/or *Campylobacter* species ($n=82$ samples), O157 *Escherichia coli* ($n=1$), an antigen test and/or PCR for rotavirus ($n=3$), norovirus ($n=4$), and adenovirus ($n=3$), and microscopic examination for presence of parasites ($n=11$). Only four samples had a positive result: 2× *Campylobacter jejuni*, 1× *Salmonella* group D in 3 *C. difficile*-negative samples with low predicted probabilities of 0.04, 0.14, and 0.05, respectively, and 1× *Aeromonas hydrophila/caviae* on the *Yersinia spp* culture plate in a *C. difficile*-positive sample with a high predicted probability of 0.68.

DISCUSSION

This article demonstrates the feasibility of rapid and accurate point-of-care *C. difficile* testing in unprocessed stool samples. The test is fast (<10 min) and can be performed on hospital wards.

What does FAIMS detect? There is probably not a single unique VOC that distinguishes *C. difficile* samples from stool of healthy donors or other gastrointestinal diseases. More likely, *C. difficile* samples have their own unique VOC fingerprint. This is not surprising, as in patients with infection, the bacterial composition of the gut flora alters. As different bacteria have different metabolic pathways, these alterations affect the proportions of metabolic compounds they consume and produce, and hence affect the mixture VOCs in the headspace. It is probably these differences that enable discrimination by FAIMS.

Study limitations leave room for improvement. The number of samples was sufficient to obtain impressive results, but still relatively limited in absolute terms.

As CDI is the major infectious cause of hospital-acquired diarrhea, in our hospital it is custom to test stool samples of hospitalized patients only for *C. difficile*, unless explicitly requested

otherwise. Therefore, the majority of samples were only tested for CDI. For the samples that tested positive for another infection (e.g., *Campylobacter jejuni*) the predicted probability for *C. difficile* was accurate, but the number of positive results ($n=4$) is too few to draw any definite conclusions with regard to FAIMS findings. Therefore, we cannot rule out any crossreactivity with other infectious causes of diarrhea. On the other hand, all our patients were tested on the basis of a clinical suspicion of CDI (e.g., because it involved a hospitalized patient with antibiotic-associated diarrhea) and therefore the results are very generalizable to daily practice. Second, previous research involving headspace analysis of various causes of diarrhea has shown that different infections and diseases have their own distinct VOC characteristics, as described before.

In the training and testing phase, we made the assumption that samples with a negative toxin EIA but a positive culture (with a *C. difficile* strain that itself has a positive toxin EIA and is thus considered “toxin-producing”) are positive for toxigenic *C. difficile*, and these patients are therefore considered to have CDI. However, it is likely that this group of patients are a mixture of those with actual CDI with a relatively low colonic toxin load, and those merely colonized with *C. difficile*, who have a different explanation for their diarrhea. With regard to FAIMS results, this heterogeneous group (direct toxin negative, culture positive) has a very intermediary predicted probability (median 0.31 with a wide IQR: 0.56) that is around the suggested cutoff value (0.32–0.37). Future research will show whether FAIMS can actually discriminate the colonized from the *C. difficile*-infected patients, as suggested by this finding.

In addition, although we aimed to analyze the stool samples as quickly as possible, most samples were kept at 4°C for up to 2 weeks before they were analyzed. We cannot exclude the possibility that the VOC composition changes during this time. However, despite the time delay, the overall results are promising. It is particularly relevant in this respect that the fresher the samples were (≤14 days vs. ≤7 days vs. ≤5 days vs. ≤2 days), the more accurate the algorithm. So with point-of-care testing on a daily basis on a hospital ward, the results may even more positive. Technically, the step from our findings to automated routine scent analysis on the many stool samples produced on wards is relatively small. The mathematical analysis of the FAIMS reading, which was now performed afterwards with extra software on a separate computer, can be integrated into the FAIMS computer to make the complete stool sample analysis and test result instantly available. Besides compressed air, clean glass jars, and water, the sample and device do not require specialized preparations, gases, or solutions; the device is compact, it does not suffer from significant sensor drift and it operates quickly and at room temperature. Theoretically, integrating such a VOC analyzer in a bedpan drain washer and thus equipping the machine with a “C-diff alarm” would be feasible.

In conclusion, FAIMS analysis of stool samples can accurately discriminate *C. difficile*-positive from -negative samples. Moreover, it is easy to use, quick, and accurate. It has the potential of a very useful tool in the diagnosis of *C. difficile* infections, not only in the laboratory, but even on hospital wards.

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CONFLICT OF INTEREST

Guarantor of the article: Marije K. Bomers, MD.

Specific author contributions: M.K.B., M.A.v.A., C.M.J.E.V.-G., and Y.M.S. together developed the concept and outlined the methods. F.P.M., J.A.C., R.S.S., and Y.M.S. provided conceptual and technical guidance. M.K.B. and F.P.M. operated FAIMS/ analyzed the samples; J.A.C., R.S.S., and M.K.B. performed the data analysis. M.K.B. drafted the paper. All authors critically appraised the paper, revised where appropriate, and approved the final version of the manuscript.

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Study Highlights**WHAT IS CURRENT KNOWLEDGE**

- ✓ Early and rapid identification of *Clostridium difficile* infection (CDI) is important for outbreak prevention and containment.
- ✓ The time between start of CDI symptoms and start of treatment ranges from 3 to 7 days.
- ✓ Field asymmetric ion mobility spectrometry (FAIMS) is a portable mass spectrometry instrument that analyses gaseous mixtures, e.g., the air emanating from stool samples.

WHAT IS NEW HERE

- ✓ FAIMS has high diagnostic accuracy for detecting *C. difficile* in stool samples of CDI patients.
- ✓ FAIMS analysis of stool samples is quick, easy to use, relatively cheap, and can be used on the ward.
- ✓ FAIMS may be the ideal point-of-care test for *C. difficile* in stool samples, reducing diagnostic and treatment delay.

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