Evaluation of the Use of Rectal Swabs for Laboratory Diagnosis of *Clostridium difficile* Infection

N. Jazmati,^{a,b} E. Kirpal,^a E. Piepenbrock,^a Y. Stelzer,^a M. J. G. T. Vehreschild,^{b,c} H. Seifert^{a,b}

^aInstitute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany

^bGerman Centre for Infection Research (DZIF), partner site Bonn-Cologne, Germany

^cDepartment I of Internal Medicine, University Hospital of Cologne, Cologne, Germany

ABSTRACT For the diagnosis of *Clostridium difficile* infection (CDI), microbiological testing is almost always accomplished through the analysis of stool specimens. We evaluated the performances of rectal swabs with liquid transport medium (FS) and nylon flocked dry swabs for the detection of *C. difficile*. Additionally, the impact on the diagnostic yield of storing swabs at -80°C for up to 3 months was evaluated. Sixty clinical stool samples positive for *C. difficile* by PCR were used for simulating rectal swabbing. FS and dry swabs were dipped into the stool and tested by PCR directly after swabbing at 1 and 3 months after storage at -80°C . Stool and the liquid medium of FS were additionally tested by a combination of glutamate dehydrogenase antigen (GDH) testing and toxin A/B enzyme immunoassay (EIA), as well as by toxigenic culture (TC). Using dry swabs, the PCR-based detection rate of *C. difficile* was equal to the rate using stool samples (30/30 [100%]), whereas the detection rate in FS was significantly lower (25/30 [83.2%]; $P = 0.019$). The sensitivities of FS for detecting *C. difficile* by PCR, TC, GDH testing, and toxin A/B EIA were 83.3%, 85.7%, 88%, and 68.9%, respectively. Storage of swabs at -80°C had no impact on the detection rate. FS cannot replace stool samples in the two-step laboratory diagnosis of CDI, as the sensitivities were too low, probably due to diluting effects of the fecal sample in the liquid medium. For simple PCR-based detection of *C. difficile*, dry swabs proved to be a suitable alternative to the use of stool samples.

KEYWORDS *Clostridium difficile*

Fast and accurate diagnosis of *Clostridium difficile* infection (CDI) is crucial for the management, infection control, and therapy of the disease. For the diagnosis of CDI, microbiological testing in Europe is almost always accomplished through the analysis of stool specimens, as recommended by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guideline (1).

Nevertheless, obtaining adequate stool specimens can be complicated, as patients may not deliver the sample on demand or, in case of ileus, patients may not produce stool at all. Additionally, a certain inconvenience for patients and nurses is associated with stool sample collection. Therefore, delays from the onset of diarrhea to the collection of stool specimens for testing are common (2, 3).

For surveillance and infection control studies (i.e., detection of asymptomatic *C. difficile* carriers), the use of rectal swabs for collection, transport, and storage of the fecal samples is common (4, 5).

The main limiting factor using rectal swabs for the laboratory diagnosis of CDI is the very small amount of stool obtained by rectal swabbing. Today in Europe, laboratory detection of *C. difficile* from clinical samples of symptomatic patients is based on a two-step combination of a highly sensitive with a highly specific testing procedure, such as PCR or a glutamate dehydrogenase antigen (GDH) test and toxin A/B enzyme

Received 14 March 2018 **Returned for modification** 2 April 2018 **Accepted** 30 April 2018

Accepted manuscript posted online 2 May 2018

Citation Jazmati N, Kirpal E, Piepenbrock E, Stelzer Y, Vehreschild MJGT, Seifert H. 2018. Evaluation of the use of rectal swabs for laboratory diagnosis of *Clostridium difficile* infection. J Clin Microbiol 56:e00426-18. <https://doi.org/10.1128/JCM.00426-18>.

Editor Andrew B. Onderdonk, Brigham and Women's Hospital

Copyright © 2018 American Society for Microbiology. All Rights Reserved.

Address correspondence to N. Jazmati, nathalie.jazmati@uk-koeln.de.

immunoassay (EIA), as recommended by the ESCMID guidelines (1). Therefore, a certain amount of stool is needed, as the fecal sample has to be divided into aliquots for use in different testing procedures. Using conventional swabs, only one testing procedure can be performed from one swab (6). Recently, improvements in the swab tip material and the transport medium have greatly enhanced the recovery and viability of various microorganisms (7–10). This new generation of swabs elutes the entire fecal sample from the swab into the liquid medium. The medium enriched with the fecal sample can be split up into several aliquots. Thus, the requirements for a two-step laboratory diagnosis of CDI from rectal swabs are met. To our knowledge, the detection of *C. difficile* by all recommended testing procedures (PCR, combined GDH test toxin A/B EIA, and toxigenic culture) from only one swab with liquid transport medium has not been systematically investigated. Thus, in our study, we aimed to assess rectal swabs as an alternative to stool samples for the laboratory diagnosis of CDI. To this aim, we compared the detection rates of *C. difficile* for four different laboratory assays (PCR, GDH and toxin A/B detection, and toxigenic culture) in stool samples with the detection rates in swabs with liquid transport medium (FS). We additionally tested common nylon flocked dry swabs in comparison to stool samples using real-time PCR for the detection of *C. difficile*. Furthermore, the influence of storage of FS and dry swabs at -80°C for up to 3 months on the detection rate of *C. difficile* was investigated.

MATERIALS AND METHODS

Study design. The study was performed at the University Hospital Cologne in Germany. In July and August 2016, clinical stool samples sent to the laboratory of medical microbiology from patients suspected of having CDI were screened for *C. difficile* within 24 h of receipt by *C. difficile* real-time PCR. A total of 60 consecutive stool samples (one sample per patient) that tested positive for the toxin B gene (*tcdB*) by real-time PCR were used in this study. All PCR-positive clinical stool samples were additionally characterized by a combination of GDH testing and toxin A/B EIA, and by toxigenic culture (TC). For further characterization, capillary gel-based PCR ribotyping was performed on all recovered *C. difficile* isolates. The study was performed in two consecutive steps. The first 30 clinical stool samples that were positive for *C. difficile* in the study period were used for comparing stool samples to rectal swabs with liquid medium. The second set of 30 swabs were used for comparing stool samples and dry swabs. Even if testing “simulated” dry swabs by PCR is quite similar to testing stool samples, we added this part to our study design, as we aimed to analyze the durability and influence of storage conditions also for this type of swab.

Simulation of rectal swabbing. For a simulation of rectal swabbing, in a first round of tests, the tips of three (for testing at three different time points; see below) Copan FecalSwabs (Copan Italia, Brescia, Italy) with liquid Cary-Blair medium (FS) ($n = 30$) were gently dipped into one *C. difficile*-positive stool sample and wiped off carefully on the edge of the stool vial. In a second round of tests, 30 nylon flocked dry swabs (article number 552; Copan Italia) were used for simulating rectal swabbing in the same manner.

After “swabbing,” each swab was placed into the corresponding transport tube. For processing FS, the tube was vortexed for 10 s to allow the fecal sample to elute in the liquid medium. All swabs were processed by the same investigator, and care was taken to handle all swabs in the same manner to ensure that the amount of stool for each swab was similar. FS and dry swabs were then tested for *C. difficile* by real-time PCR immediately after simulating rectal swabbing and additionally after 1 and 3 months of storage at -80°C . FS were additionally tested by a combination of GDH testing and a toxin A/B EIA, and by TC at all three time points, and the results were compared to the results of the initial clinical stool sample used for simulating rectal swabbing (Fig. 1). Before testing frozen swabs, swabs were thawed at room temperature for 15 min, and the FS were vortexed for 30 s.

***C. difficile* detection laboratory workup.** (i) ***C. difficile* real-time PCR.** For the detection of *C. difficile* in clinical stool samples by PCR, the GeneXpert *C. difficile*/Epi test (GX test) (Cepheid, CA, USA), a multiplex real-time PCR that detects the toxin B gene (*tcdB*), the binary toxin gene (*cdt*), and a deletion at nucleotide (nt) 117 in the *tcdC* gene indicating the hypervirulent *C. difficile* ribotype 027, was used according to the manufacturer’s instructions. Cycle threshold (C_t) values for the detection of *tcdB* as a marker for *C. difficile* bacterial density in the sample were documented. Low C_t values indicate a greater amount of target genes. For testing dry swabs, these were placed into the sample reagent of the GX test, and the swabs were cut right above the vial. Vials were then vortexed for 10 s. The swabs placed in the reagent vial were discarded after testing. For testing FS, 400 μl of the FS medium was added to the GX sample reagent. The sample reagent vial was then vortexed for 10 s before testing according to the manufacturer’s instructions. The remaining liquid medium of FS was used for further testing (see below).

(ii) **Combined GDH and toxin A/B enzyme immunoassay.** The C. Diff Quik Chek Complete (Alere, Cologne, Germany) (CDQCC), a combined GDH and toxin A/B enzyme immunoassay, was used for the detection of GDH and toxin A/B, according to the manufacturer’s instructions. Stool samples were processed using 25 μl of liquid stool or a portion of formed stool with 2 mm diameter and 750 μl of

A) RECTAL SWABS WITH LIQUID TRANSPORT MEDIUM (FS)

B) NYLON FLOCKED DRY SWABS (DS)

FIG 1 Study design. *C. difficile*-positive stool samples were used for simulating rectal swabbing. Either three swabs with fluid Cary-Blair medium (FS) (A) or three nylon flocked dry swabs (DS) (B) were gently dipped into the stool sample. FS and DS were then tested for *C. difficile* by PCR immediately after dipping the swab into the stool sample (T1), and 1 month (T2) and 3 months (T3) after storage at -80°C . FS were additionally tested by *C. Diff* Quik Chek Complete (CDQCC) for GDH and toxin A/B detection and by toxigenic culture (TC).

diluent. For testing FS, 100 μl of the liquid Cary-Blair transport medium with 650 μl of diluent were used, as recommended by the manufacturer.

(iii) Toxigenic culture and PCR ribotyping. For anaerobic culture of stool samples, a pea-sized portion of the stool sample was inoculated onto cycloserine cefoxitin fructose agar (CCFA; Oxoid Ltd., Thermo Fisher Scientific, Wesel, Germany), additionally subjected to alcohol shock, and inoculated onto nonselective Schaefer agar (Becton Dickinson, Heidelberg, Germany). Agar plates were incubated anaerobically at 37°C and examined after 48 h. Initial identification was based on the characteristic colony morphology, followed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MALDI Biotyper; Bruker Daltonics, Bremen, Germany). For the detection of toxin A/B production in the isolate, the *C. DIFFICILE* Tox A/B II test (Alere, Cologne, Germany) was performed according to the manufacturer's instructions. For testing FS, 50 μl of the liquid medium was used for inoculation of each agar plate. Plates were processed as mentioned above. Dry swabs were not tested by TC or the GDH and toxin A/B test. This was mainly because data for TC already exist (6), and for reliable detection of GDH and toxin A/B by lateral flow assays, dry swabs will not provide enough stool material (according to the manufacturer's instructions, a minimum of 2 mm diameter of stool material is required).

All recovered *C. difficile* isolates were further characterized using capillary gel-based PCR-ribotyping, according to published protocols (11). Analysis of the ribotyping results was done using WebRibo (<https://webribo.ages.at/>), an open-source software/database directed by the Austrian Agency for Health and Food Safety Ltd., as described before (12).

Statistical analysis. Continuous variables were summarized by mean and standard deviation values. Qualitative variables were described by count and percentage. Samples with missing values were excluded in the analysis of the corresponding variable. Comparison of means (C_T value) was done by Student's *t* tests (if two groups were compared, e.g., stool samples and swabs) and by one-way analysis of variance (ANOVA), if more than two groups were compared (data from three different time points). A comparison of proportions was performed using the Chi-square test. Sensitivity, specificity, negative predictive value, and positive predictive value for the detection of *C. difficile* in rectal swabs were assessed in comparison to the detection of *C. difficile* in stool. All statistical tests were two-tailed, and a *P* value of <0.05 was considered statistically significant.

FIG 2 Detection of *C. difficile* by PCR in nylon flocked dry swabs (DS) before and after storage at -80°C . (A) Detection of the *C. difficile* toxin B gene, binary toxin gene, and ribotype 027 by PCR (GeneXpert) in DS ($n = 30$) immediately after swabbing (T1), after 1 month (T2) of storage, and after 3 months of storage (T3). No significant difference (n.s.) was found. (B) Comparison of the *Clostridium difficile* toxin B PCR cycle threshold (C_T) values of DS at different time points ($n = 26$ for T1 to T3). The horizontal line in each box indicates the median, whereas the top and bottom lines represent the 75th and 25th percentiles, respectively. Patients with missing values were excluded ($n = 4$). No significant difference was found between the C_T values at different points of time.

RESULTS

Detection of *C. difficile* by PCR in dry swabs. The results of 30 stool samples that tested positive for the toxin B gene by the GX test were compared to the results of 30 dry swabs tested directly after simulation of swabbing. No difference in the recovery rates of *C. difficile* by PCR between stool samples (30/30) and dry swabs (30/30) was found when testing dry swabs directly after the swabbing simulation. Thus, the sensitivity for the detection of *C. difficile* in dry swabs was 100%. Using the C_T values of the toxin B gene (*tcdB*) as a quantification marker of *C. difficile* in the sample, there was no difference between the C_T values measured in dry swabs and in stool samples (mean $C_{T\text{swabs}}$ 27.4 ± 4.8 , versus mean $C_{T\text{stool}}$ 27.2 ± 4.6 ; $P = 0.83$).

Also, no difference was found for the detection of *C. difficile* binary toxin gene (*cdt*) (8/8; mean $C_{T\text{swabs}}$ 25.4 ± 5.2) versus mean $C_{T\text{stool}}$ 24.9 ± 5.3 ; ($P = 0.87$) or for the detection of presumptive ribotype 027 (RT027) (7/7; mean $C_{T\text{swabs}}$ 27.2 ± 5.4), versus mean $C_{T\text{stool}}$ 26.9 ± 5.2 ; ($P = 0.93$) between stool samples and dry swabs.

Influence of storage of dry swabs on the detection of *C. difficile* by PCR. We compared the detection rates of *C. difficile* by PCR in dry swabs before and after storage of the swabs at -80°C for 1 month and for 3 months. No significant differences were found in the detection of toxin B, binary toxin, or deletion at nt 117 (RT027) (Fig. 2A). C_T values (*tcdB*) were stable over the time of storage (Fig. 2B).

Detection of *C. difficile* by GX, CDQCC, and TC in FS. The results of 30 stool samples tested by GX, CDQCC, and TC were compared to the results of the 30 consecutive FS tested directly after the swabbing simulation. In FS, the detection rate of *C. difficile* by PCR was significantly lower than the detection rate in stool samples (25/30 [83.3%] versus 30/30 [100%]; $P = 0.02$) (Fig. 3A). Also, the C_T values of the toxin B gene (*tcdB*) in FS as a quantification marker of *C. difficile* in the sample were significantly higher than those in stool samples (mean $C_{T\text{swabs}}$ 28.4 ± 3.6 , versus mean $C_{T\text{stool}}$ 25.2 ± 2.8 ; $P = 0.004$) (Fig. 3B). Conversely, the comparison of proportions did not reveal any significant differences between *C. difficile* detection in FS and stool samples tested by CDQCC and by toxigenic culture (see Fig. 3A).

Performance characteristics for *C. difficile* detection from FS compared to stool were calculated for the different testing procedures (PCR, GDH, toxin A/B, and TC) and are described in Table 1. In total, 24 *C. difficile* isolates recovered by TC (18 isolates matching in rectal swabs and in stool samples, 3 isolates recovered only from stool samples, and a further 3 isolates recovered only from rectal swabs) were successfully characterized by PCR-ribotyping. RT027 (5/24 [21%]) was the most prevalent ribotype,

FIG 3 Detection of *C. difficile* by PCR, CDQCC, and TC in rectal swabs with liquid medium (FS) compared to stool samples. (A) Detection of *C. difficile* by PCR, C. Diff Quik Chek Complete (GDH and toxin A/B) (CDQCC), and by toxigenic culture (TC) in clinical stool samples and in FS. *, the detection rate of *C. difficile* by PCR was significantly lower when using FS ($P < 0.05$). (B) Comparison of the *Clostridium difficile* toxin B PCR cycle threshold (C_T) values of stool samples ($n = 25$) and FS ($n = 25$). The horizontal line in each box indicates the median, whereas the top and bottom lines represent the 75th and 25th percentiles, respectively. Patients with missing values were excluded ($n = 5$). **, a significant difference was found between the C_T values of stool samples and FS ($P = 0.004$).

followed by RT014/6 (3/24 [13%]). No correlation of ribotypes and diagnostic yield was found for any of the tests used.

Influence of storage of FS for up to 3 months on the detection rate of *C. difficile* by PCR, CDQCC, and TC. Storage of the FS at -80°C for up to 3 months had no significant impact on the detection rate of *C. difficile* in any of the tests used (Fig. 4).

DISCUSSION

This study was conducted to evaluate the use of Copan FecalSwabs, a rectal swab system with liquid Cary-Blair medium, for the laboratory diagnosis of CDI. Furthermore, the performance of nylon flocked dry swabs (no medium) for the detection of *C. difficile* by PCR and the impact of storage of rectal swabs at -80°C on the detection rate were evaluated.

We have shown that the use of FS is not a good alternative to the use of stool samples for the laboratory diagnosis of CDI, as the calculated sensitivities for the

TABLE 1 Performance of 30 rectal swabs with liquid medium in the detection of *Clostridium difficile* compared to performance of stool samples

Result of <i>C. difficile</i> detection in FS by assay ^a	Result of <i>C. difficile</i> detection in stool sample (no.)		Detection of <i>C. difficile</i> using FS compared with stool samples (% [95% CI]) ^b			
	Positive	Negative	Sensitivity	Specificity	PPV	NPV
GX			83.3 (64.5–93.7)		100 (83.4–100)	
Positive	25	0				
Negative	5	0				
Toxigenic culture			85.7 (62.6–96.2)		66.7 (30.9–91)	
Positive	18	3				
Negative	3	6				
GDH (by CDQCC)			88 (67.7–96.8)		100 (46.3–100)	
Positive	22	0				
Negative	3	5				
Toxin A/B (by CDQCC)			68.8 (41.5–87.9)		100 (67.9–100)	
Positive	11	0				
Negative	5	14				

^aFS, rectal swab with liquid medium; GX, GeneXpert *C. difficile*/Epi test; CDQCC, C. Diff Quik Chek Complete.

^bCI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

FIG 4 Detection of *C. difficile* by PCR, CDQCC, and TC in rectal swabs with liquid medium (FS) before and after storage at -80°C . (A) Detection of *C. difficile* by PCR, *C. Diff* Quik Chek Complete (GDH and toxin A/B), and by toxigenic culture (TC) in rectal swabs with FS ($n = 30$) immediately after swabbing (T1), after 1 month of storage (T2), and after 3 months of storage (T3). No significant difference was found before and after storage. (B) Comparison of the *Clostridium difficile* toxin B PCR cycle threshold (C_T) values of FS ($n = 25$) at T1, T2, and T3. The horizontal line in each box indicates the median, whereas the top and bottom lines represent the 75th and 25th percentiles, respectively. Patients with missing values were excluded ($n = 5$). No significant difference was found between the C_T values of FS before and after storage.

detection of *C. difficile* were unacceptably low for the detection of *C. difficile* and its toxins in rectal swabs compared to those with the use of stool samples.

For the detection of *C. difficile* by PCR (for example, in surveillance studies), the use of dry swabs is possible without any loss of sensitivity. In that respect, our results are in good accordance with those of other studies showing that PCR from rectal swabs is suitable for the detection of *C. difficile* (10, 13, 14). Storage of swabs (FS and dry swabs) at -80°C for up to 3 months did not have any impact on the detection rate of *C. difficile* by any testing procedure used in this study.

The idea of streamlining *C. difficile* diagnostics with user-friendly rectal swabs (instead of stool samples) is not new. McFarland et al. stated in 1987 for the first time that the use of rectal swabs with semisolid Amies transport medium for culturing *C. difficile* was comparable to the use of stool samples (15). Since then, the use of rectal (or perirectal) swabs for the detection of *C. difficile* in asymptomatic carriers by PCR and/or toxigenic culture has been shown to be helpful (6, 13). Thus, rectal swabs are commonly used for the detection of asymptomatic carriers in surveillance studies (4, 5). One small study with 22 symptomatic patients suspected of having CDI indicated that the use of rectal swabs might be applicable for the diagnosis of CDI (16). In that study, the detection of PCR was only done by real-time PCR. No systematic data exist on the utility of rectal swabs for the laboratory diagnosis of CDI in symptomatic patients using a two-step algorithm, as recommended by European ESCMID guidelines, including a sensitive screening test (PCR or GDH) and a more specific confirmation assay (*C. difficile* toxin A/B EIA) (1). Recently, FS were shown to enhance the recovery and viability of diarrheagenic bacteria (10). This swab system provides a flocced tip that, according to the manufacturer, dispenses the entire fecal sample into the liquid Cary-Blair medium. Thus, all tests required for the diagnosis of CDI can potentially be performed from the liquid medium of one swab.

In our study, however, the detection rate of *C. difficile* by PCR from the liquid Cary-Blair medium of FS was significantly lower than that from stool samples. Analysis of the toxin B gene C_T values confirmed this to be a result of diluting effects (small amount of fecal sample diluted in 2 ml of liquid medium). Although in our small study, the detection rates of *C. difficile* by CDQCC (GDH and toxin A/B detection) and by toxigenic culture were not significantly different between FS and stool, we would not recommend using FS for the diagnosis of CDI, as the diagnostic accuracy was not high

enough compared to that obtained by the use of stool samples (the sensitivity in our study being as low as 63.3% for toxin A/B testing from FS). Furthermore, it is rather likely that diluting effects also compromise the performance of GDH and toxin A/B EIA as well as of TC. Probably, we were just not able to reach statistical significance due to small sample size and the generally lower sensitivity of the mentioned testing procedures. On the other hand, FS is FDA cleared for transport and culture of gastrointestinal (GI) pathogens, but it is not FDA cleared for use with any molecular GI assays. Newer studies conclude that FS with Cary-Blair medium can be used for molecular detection of pathogens without cross-reaction or inhibitory effects (10, 17). Nevertheless, a cross-reaction of the Cary-Blair medium and the GeneXpert *C. difficile* test as an explanation for the lower detection rate seen in our study cannot completely be ruled out.

In contrast to the ESCMID guidelines, the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) guidelines recommend a single-step algorithm for the laboratory diagnosis of *C. difficile* based on PCR (18). Therefore, the use of dry swabs would be appropriate and can probably speed up and facilitate the diagnosis of CDI. Nevertheless, using single-step PCR-based detection of *C. difficile* may lead to overdiagnosis of CDI due to the high sensitivity but lower specificity of PCR (19). Thus, careful clinical evaluation of the patient to distinguish between an asymptomatic carrier, an excretor (i.e., a patient with other reasons for diarrhea), and a patient with true CDI is even more important when using only PCR for *C. difficile* detection.

Our data clearly show that the storage of FS and dry swabs at -80°C for up to 3 months had no impact on the detection rate of *C. difficile* by any of the methods used. This is in accordance with data from a recent publication investigating the cultural recovery of *C. difficile* from FS. Hirvonen and Kaukoranta showed that storage of FS at -70°C did not affect the cultural recovery rate of *C. difficile*, whereas storage of FS at 4°C or storage at room temperature was found to result in a significantly lower recovery rate of *C. difficile* (10).

Our study has several limitations. First, we simulated rectal swabbing using *C. difficile*-positive stool samples and did not collect rectal swabs from patients. Thus, our data should be confirmed in a prospective clinical study using both fecal swabs and stool samples in parallel. It was our aim to evaluate the practical potential of rectal swabs for the diagnosis of CDI in this study. Furthermore, we only included *C. difficile*-positive stool samples in our study. This might have influenced the sensitivity analysis in our study.

In conclusion, the use of FS is no alternative to the use of stool samples for the two-step laboratory diagnosis of CDI, as the sensitivities were lower for rectal swabs than for stool samples for all the assays used. For PCR-based single-step laboratory diagnosis of CDI, as well as for the simple detection of asymptomatic carriers in surveillance studies or for targeted surveillance in infection control interventions, the use of dry swabs is a suitable alternative to stool samples without any loss of sensitivity. Even if small studies on patients have already shown that PCR for the detection of *C. difficile* from rectal swabs is reliable (16), our data should be confirmed in a prospective clinical study on a larger scale using both fecal swabs and stool samples from patients in parallel. Storage of dry swabs and FS at -80°C for up to 3 months is possible without compromising the detection rate of *C. difficile*.

ACKNOWLEDGMENTS

This study was partially financed by Da Volterra, Paris, and by the Innovative Medicines Initiative Joint undertaking under grant agreement 115523, with financial contribution from the EU Seventh Framework Programme (FP7/2007-2013) and EFPIA companies in kind contribution.

The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication.

N.J. has received payment for lectures from MSD Sharp & Dohme and travel support from IMDx (Qiagen). H.S. reports research grants from Accelerate and personal fees

from 3M, Becton Dickinson, bioMérieux, and Thermo Fisher. M.J.G.T.V. has served at the speakers' bureaus of Pfizer, Merck/MSD, Organobalance, Gilead Sciences, and Astellas Pharma, received research funding from 3M, Astellas Pharma, Seres Therapeutics, Organobalance, and Gilead Sciences, and is a consultant to Berlin Chemie, Merck/MSD, and Maat Pharma. All other authors declare no conflicts of interest.

REFERENCES

- Debast SB, Bauer MP, Kuijper EJ, European Society of Clinical Microbiology and Infectious Diseases. 2014. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for *Clostridium difficile* infection. *Clin Microbiol Infect* 20(Suppl 2):S1–S26. <https://doi.org/10.1111/1469-0691.12418>.
- Scheurer D. 2008. Diagnostic and treatment delays in recurrent *Clostridium difficile*-associated disease. *J Hosp Med* 3:156–159. <https://doi.org/10.1002/jhm.277>.
- Frenz MB, McIntyre AS. 2003. Reducing delays in the diagnosis and treatment of *Clostridium difficile* diarrhoea. *QJM* 96:579–582. <https://doi.org/10.1093/qjmed/hcg098>.
- Longtin Y, Paquet-Bolduc B, Gilca R, Fortin E, Longtin J, Trottier S, Gervais P, Roussy JF, Levesque S, Ben-David D, Cloutier I, Loo VG. 2016. Effect of detecting and isolating *Clostridium difficile* carriers at hospital admission on the incidence of *C. difficile* infections: a quasi-experimental controlled study. *JAMA Intern Med* 176:796–804. <https://doi.org/10.1001/jamainternmed.2016.0177>.
- Johnson S, Clabots CR, Linn FV, Olson MM, Peterson LR, Gerding DN. 1990. Nosocomial *Clostridium difficile* colonisation and disease. *Lancet* 336:97–100. [https://doi.org/10.1016/0140-6736\(90\)91605-A](https://doi.org/10.1016/0140-6736(90)91605-A).
- Rogers DS, Kundrapu S, Sunkesula VC, Donskey CJ. 2013. Comparison of perirectal versus rectal swabs for detection of asymptomatic carriers of toxigenic *Clostridium difficile*. *J Clin Microbiol* 51:3421–3422. <https://doi.org/10.1128/JCM.01418-13>.
- Van Horn KG, Audette CD, Sebeck D, Tucker KA. 2008. Comparison of the Copan ESwab system with two Amies agar swab transport systems for maintenance of microorganism viability. *J Clin Microbiol* 46:1655–1658. <https://doi.org/10.1128/JCM.02047-07>.
- Van Horn KG, Audette CD, Tucker KA, Sebeck D. 2008. Comparison of 3 swab transport systems for direct release and recovery of aerobic and anaerobic bacteria. *Diagn Microbiol Infect Dis* 62:471–473. <https://doi.org/10.1016/j.diagmicrobio.2008.08.004>.
- Nys S, Vijgen S, Magerman K, Cartuyvels R. 2010. Comparison of Copan eSwab with the Copan Venturi Transystem for the quantitative survival of *Escherichia coli*, *Streptococcus agalactiae* and *Candida albicans*. *Eur J Clin Microbiol Infect Dis* 29:453–456. <https://doi.org/10.1007/s10096-010-0883-5>.
- Hirvonen JJ, Kaukoranta SS. 2014. Comparison of FecalSwab and ESwab devices for storage and transportation of diarrheagenic bacteria. *J Clin Microbiol* 52:2334–2339. <https://doi.org/10.1128/JCM.00539-14>.
- Fawley WN, Knetsch CW, MacCannell DR, Harmanus C, Du T, Mulvey MR, Paulick A, Anderson L, Kuijper EJ, Wilcox MH. 2015. Development and validation of an internationally-standardized, high-resolution capillary gel-based electrophoresis PCR-ribotyping protocol for *Clostridium difficile*. *PLoS One* 10:e0118150. <https://doi.org/10.1371/journal.pone.0118150>.
- Indra A, Huhulescu S, Schneeweis M, Hasenberger P, Kernbichler S, Fiedler A, Wewalka G, Allerberger F, Kuijper EJ. 2008. Characterization of *Clostridium difficile* isolates using capillary gel electrophoresis-based PCR ribotyping. *J Med Microbiol* 57:1377–1382. <https://doi.org/10.1099/jmm.0.47714-0>.
- Curry SR, Schlackman JL, Hamilton TM, Henderson TK, Brown NT, Marsh JW, Shutt KA, Brooks MM, Pasculle AW, Muto CA, Harrison LH. 2011. Perirectal swab surveillance for *Clostridium difficile* by use of selective broth preamplification and real-time PCR detection of *tcdB*. *J Clin Microbiol* 49:3788–3793. <https://doi.org/10.1128/JCM.00679-11>.
- Kundrapu S, Sunkesula VC, Jury LA, Sethi AK, Donskey CJ. 2012. Utility of perirectal swab specimens for diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 55:1527–1530. <https://doi.org/10.1093/cid/cis707>.
- McFarland LV, Coyle MB, Kremer WH, Stamm WE. 1987. Rectal swab cultures for *Clostridium difficile* surveillance studies. *J Clin Microbiol* 25:2241–2242.
- Shakir FA, Thompson D, Marlar R, Ali T. 2012. A novel use of rectal swab to test for *Clostridium difficile* infection by real-time PCR. *Am J Gastroenterol* 107:1444–1445. <https://doi.org/10.1038/ajg.2012.162>.
- Silbert S, Gostnell A, Kubasek C, Widen R. 2017. Evaluation of the new FecalSwab system for maintaining stability of stool samples submitted for molecular tests. *J Clin Microbiol* 55:1588–1590. <https://doi.org/10.1128/JCM.00273-17>.
- Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH, Society for Healthcare Epidemiology of America, Infectious Diseases Society of America. 2010. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). *Infect Control Hosp Epidemiol* 31:431–455. <https://doi.org/10.1086/651706>.
- Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, Nguyen HH, Huang B, Tang YW, Lee LW, Kim K, Taylor S, Romano PS, Panacek EA, Goodell PB, Solnick JV, Cohen SH. 2015. Overdiagnosis of *Clostridium difficile* infection in the molecular test era. *JAMA Intern Med* 175:1792–1801. <https://doi.org/10.1001/jamainternmed.2015.4114>.