

Evaluation of an overnight non-culture test for detection of viable Gram-negative bacteria in endoscope channels



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ABSTRACT

Background and study aims Prevention of infection transmission from contaminated endoscopes would benefit from a rapid test that could detect low levels of viable bac-

teria after high level disinfection. The aim of this study was to evaluate the rapid NOW! (RN) test's ability to detect endoscope contamination.

Materials and methods The RN test kit and the accompanying fluorometer were evaluated. The manufacturer states that a fluorometer signal >300 units is indicative of viable Gram-negative bacteria. Suspension testing of varying concentrations of *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* were used to determine the RN test limit of detection. Simulated-use testing was done using a duodenoscope inoculated with 10% blood containing approximately 35 CFU *E. coli* per channel. Samples were extracted from the duodenoscope instrument channel and tested using the manufacturer's instructions.

Results The RN test could consistently detect 10 CFU of *E. coli* and *P. aeruginosa* (fluorescent signal of 9,000 to 11,000 units) but not *E. faecalis*. Sensitivity and specificity for Gram-negative bacteria were 93% and 90%, respectively, using all of the suspensions in the study. Extraction of *E. coli* from an inoculated duodenoscope instrument channel repeatedly provided a positive signal (i. e. >2,000 units).

Conclusions The RN test can reliably detect low levels of Gram-negative bacteria in suspension as well as from samples extracted from endoscope channels. These preliminary findings are encouraging but further assessment of extraction efficacy, impact of organic residuals and clinical workflow are still needed.

Introduction

Transmission of multi-antibiotic-resistant bacteria from contaminated endoscopes has prompted healthcare facilities to culture their endoscopes in an attempt to detect contamination of organisms of high concern so that these endoscopes can be removed from use and reprocessed to eliminate infection transmission [1–7]. The newly released US Food and Drug

Administration/Centers for Disease Control and Prevention/American Society for Microbiology (FDA/CDC/ASM) duodenoscope sample collection and culture protocol has been validated by the endoscope manufacturers and provides a standardized approach to culture duodenoscopes [8].

Unlike endoscope culture protocols from other countries, the FDA/CDC/ASM culture protocol is similar to that of Ross et al. (2015) [3] and indicates that ideally once a culture sample has been taken, the endoscope should be quarantined until

the results of the culture are known, so that if the endoscope was contaminated, patient exposure during subsequent procedures could be avoided [8]. However, this culture and quarantine approach severely limits the number of clinical procedures that can be performed. Instead of being able to use an endoscope multiple times each day, with the quarantine process, it can only be used for one procedure every 2 to 3 days (depending on how long the culture results take). Culture of endoscopes is also fraught with additional standardization issues such as the sample collection method used (i. e. type of friction, type of extraction fluid, use of neutralizer) [9–13] and the culture protocol used (i. e. concentration of sample, culture media and duration of incubation) affect the sensitivity of cultures [9–17]. Although culture is recommended in many countries as a monitoring tool for endoscope reprocessing, [11, 18–19] it is not currently recommended in United States guidelines [20–22] and there is evidence that there can be false-negative culture results [3, 23]. Many of these issues might be avoided if rapid test methods could be used to reliably detect endoscope channel contamination post-high-level disinfection (HLD) but prior to patient use.

A recent review [24] listed a range of commercially available rapid tests that detect organic residuals (e. g. protein, hemoglobin) and those that detect adenosine triphosphate (ATP) for assessing adequacy of endoscope cleaning. The rapid ATP test kits have been most widely studied for monitoring cleaning adequacy of flexible endoscopes [2, 25–27]. Data suggesting cut-offs for adequate cleaning of flexible endoscopes using rapid ATP or organic residuals have been published [25–28]. These cleaning monitoring approaches allow the opportunity to re-clean endoscopes that have not been properly cleaned before they are disinfected. Some authors have questioned whether some of these rapid cleaning monitoring test methods can reliably detect contamination in endoscope channels after HLD [14–15]. There have been very limited assessments of organic residuals post-HLD and clinical studies have reported variable results using the currently available rapid ATP kits to detect potentially contaminated patient-used endoscopes post-disinfection [10, 27, 29–31]. The majority of published studies indicate that the ATP test is too insensitive to detect low levels of viable bacteria in samples extracted from endoscope channels after HLD [2, 10, 25–27, 29].

There are few other tests available that reliably detect viable bacteria post-HLD or sterilization prior to patient use. The only other commercially available test for evaluating endoscope channels for viable bacteria is the rapid NOW! (RN) test. The manufacturer's instruction for use (MIFU) indicates that this test can detect as low as 10 CFU of Gram-negative bacteria within endoscope channels. The MIFU indicates the test is not applicable to Gram-positive bacteria as it is based on detection of a specific enzyme found only in Gram-negative bacteria. The sample collected from endoscopes needs to be incubated approximately 12 hours so the MIFU suggests that samples be collected from fully reprocessed endoscopes at the end of the day, incubated overnight and read the following morning before any patient procedures. If there is a test failure detected in the morning, the endoscope can be reprocessed prior to patient

use. However (to the best of the authors knowledge), there is only one peer-reviewed publication on this technology [32] and they did not provide specific data for this test in their publication.

Therefore, the objectives of this study were to determine if after overnight incubation the RN test satisfies the 10-CFU limit of detection claim in the MIFU and whether the test can reliably detect low levels of viable Gram-negative bacteria within duodenoscope instrument channels using simulated-use testing.

Materials and methods

Bacterial strains

The bacterial strains used included *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 15442). Each isolate was cultured on blood agar (BA) media and incubated aerobically at 35 °C for 24 hours prior to use in an experimental test.

Simulated-use study

Duodenoscopes and automated endoscope reprocessors (AERs)

A TJF-Q180V model (sealed elevator guidewire) Olympus duodenoscope (Olympus Corporation of the Americas, Center Valley, Pennsylvania, United States) was used for simulated-use testing. After each test, the duodenoscope was cleaned as per Olympus MIFU and then processed using the STERIS SYSTEM 1E (SS1E) (STERIS Inc, Mentor, Ohio, United States) and stored in a channel purge storage cabinet (Torvan Inc, Toronto, Ontario, Canada).

The duodenoscope instrument channel was inoculated by injecting 1 mL of 10% blood containing 10⁴ CFU/mL or 10² CFU/mL of *E. coli* into the distal opening of the suction channel and then raising the distal end allowing the inoculum to flow towards the biopsy port. The inoculated duodenoscope was allowed to dry for 2 hours prior to sample collection.

Rapid NOW! (RN) test

Duodenoscope testing

Samples from the inoculated duodenoscopes were collected by flushing 5 mL of extraction fluid (sterile water) from the RN test kit through the biopsy port of the instrument channel and the extracted sample was collected from the distal end into a sterile container. A small volume of extraction fluid was used to ensure that the most concentrated channel sample was available for testing. A 0.5-mL portion of the extracted channel sample was inoculated into the test cuvette and incubated overnight as per MIFU. The following day, test reagent was added to the cuvette, which was then placed into the fluorometer and the numeric fluorometer value (NFV) recorded. Baseline testing (i. e. negative endoscope testing) of the fully reprocessed, uninoculated duodenoscope was also done.

The MIFU indicates that an NFV < 200 indicates no viable Gram-negative bacteria, an NFV 200 to 300 indicates the likely presence of viable Gram-negative bacteria and an NFV > 300 in-

► **Table 1** Simulated-use evaluation of the RN test for detection of *E. coli* in duodenoscope instrument channel.

Replicate number	CFU Inoculum per channel	Maximum Total CFU Tested ¹	NOW! test Incubation time (Hrs)	Numeric fluorometer value (NFV)
Test 1: Low inoculum				
1	320.00	32.00	21.75	2376.00
2	360.00	36.00	21.67	2810.00
3	390.00	39.00	22.25	1832.00
Average:	356.70	35.67	21.89	2339.33
SD:	35.12	3.51	0.31	490.03
Test 2: Moderate inoculum				
1	15600.00	1560.00	20.00	2178.00
2	30000.00	3000.00	20.25	4534.00
3	22000.00	2200.00	21.92	4405.00
Average:	22500.00	2253.33	21.39	3705.67
SD:	7214.80	721.48	1.04	1324.57
<p>Bacteria were suspended in (10% sheep blood) and the instrument channel of a duodenoscope was inoculated and then allowed to dry for 2 hours before extraction as per MIFU (see Materials and Methods for details). The negative control from the test kit showed an NFV of 0 and 10% blood without any bacteria had an NFV of 3.</p> <p>¹ The inoculum per channel was extracted in 5 mL extraction fluid so the maximum CFU tested is calculated as (CFU inoculated/5 mL) × 0.5 mL (volume of extracted channel sample that is tested).</p>				

indicates presence of viable Gram-negative bacteria. The MIFU indicates that the RN test detects the activity of a specific enzyme found only in viable Gram-negative bacteria and therefore does not detect other types of microorganisms that are not Gram-negative bacteria. The MIFU indicates that the limit of detection for the RN test is 10 CFU in the sample tested.

Suspension testing

The RN test (Healthmark Industries, Fraser, MI) was evaluated following the MIFU. The limit of detection was assessed using varying separate concentrations of *E. faecalis*, *E. coli* and *P. aeruginosa* prepared in sterile reverse osmosis (sRO) water. A 0.5-mL portion of each concentration of bacteria was inoculated into the test cuvette and incubated overnight as per the MIFU. The following day test reagent was added to the cuvette, which was then placed into the fluorometer and the NFV was recorded.

Viable count for cultures

The inoculum for suspension testing and also for simulated-use testing were each serially diluted 1:10 using sterile phosphate buffered saline (PBS), and then 0.1 mL of the direct, as well as each dilution, was spread over the surface of BA plates. Plates were incubated at 35 °C and colony counts were performed at 24 hours. All tests were performed in triplicate.

Statistical analysis

The *t* test was used to assess the area under the Receiver Operator Characteristics (ROC) curve (AUROCC) to determine if AUROCC was significantly different from 0.5. The greater AUROCC, the more accurate the test. AUROCC of 0.5 suggests that cate-

gorization by an evaluated test is similar to random categorization and that the evaluated test is not a useful test. Detection cutoffs of 1 CFU as well as 10 CFU were evaluated. Sensitivity, specificity, positive and negative predictive values (NPV) were calculated.

Results

The data in ► **Table 1** are based on simulated-use testing to assess how well the RN test can detect bacteria inoculated into a duodenoscope instrument channel. The RN test MIFU endoscope sample collection consists of a squeezable plastic bottle containing 5 mL fluid that is flushed through the instrument channel (i.e. does not incorporate friction in the sample extraction protocol). The estimated maximum CFU recoverable in the 0.5-mL samples that were tested by the RN test ranged from 32 to 39 CFU and all three replicates gave a positive test result (► **Table 1**).

Detection results for the RN test in suspension testing are shown in ► **Table 2** for *E. coli*, *P. aeruginosa* and *E. faecalis*. As indicated in the MIFU, the Gram-positive bacteria *E. faecalis* did not generate consistent NFVs over 300 whereas the Gram-negative bacteria tested did have high NFVs even at very low CFU. All nine suspensions with *E. coli* and/or *P. aeruginosa* ≥ 10 CFU were correctly detected by the RN test. For the cut-off of 1 CFU there was only one of the 24 tests that was incorrect (i.e. *P. aeruginosa* at 2.35 CFU that was a false-negative). In contrast, for *E. faecalis*, only one of three suspensions with CFU ≥ 10 tested positive with the RN Test (NFV > 300) and none of the three suspensions with CFU between ≥ 1 and 10 tested positive.

► **Table 2** Performance of the RN test for various bacterial concentrations suspended in sRO water.

Numeric fluorometer value (NFV) for various CFU in suspension					
Test 1 ¹		Test 2 ¹		Test 3 ¹	
Total CFU tested	NFV	Total CFU tested	NFV	Total CFU tested	NFV
<i>Enterococcus faecalis</i>					
13.50	178.00	20.00	331.00	15.50	43.00
1.35	84.00	2.00	145.00	1.55	255.00
0.14	240.00	0.20	320.00	0.16	270.00
0.01	193.00	0.02	121.00	0.02	246.00
<i>Escherichia coli</i>					
150.00	14373.00	225.00	15966.00	105.00	15348.00
15.00	10825.00	22.50	10052.00	10.50	11891.00
1.50	8389.00	2.25	9753.00	1.05	2109.00
0.15	117.00	0.23	1.00	0.11	124.00
<i>Pseudomonas aeruginosa</i>					
16.00	9551.00	23.50	24196.00	22.00	10896.00
1.60	13333.00	2.35	51.00	2.20	12036.00
0.16	13.00	0.24	38.00	0.22	27.00
0.02	70.00	0.02	0.00	0.02	5.00

¹ Tests 1, 2 and 3 were independent bacterial suspensions prepared on three different days and each of the three organisms was evaluated separately (i. e. each microbe tested separately). Any Total CFU tested for a Gram-negative bacterium that is <1 CFU would be considered to be a negative sample. Negative control test using sRO water showed an NFV of 92, 40 and 0 on three separate test days.

Therefore, sensitivity for detection of CFU ≥ 10 was 100% for both *E. coli* and *P. aeruginosa* and for CFU ≥ 1 was 100% for *E. coli*, 83% for *P. aeruginosa* and 93% for either. The AUROCC for *E. coli* was 1 for both CFU ≥ 1 as well as CFU ≥ 10 and for *P. aeruginosa* was 0.85 for CFU ≥ 10 and 0.97 for CFU ≥ 1 ($P < 0.0001$). Importantly, when the *E. faecalis* suspensions were included as negative controls in the analysis, the sensitivity of NFV > 300 for detection of CFU ≥ 1 Gram-negative bacteria was 93% and specificity was 90%, NPV was 95.0% and positive predictive value was 88%; the AUROCC was 0.95 ($P < 0.0001$).

Discussion

Because it was not feasible to form biofilm within a flexible endoscope, we chose to perform simulated-use testing whereby an organic matrix containing 10% blood containing low to moderate levels of *E. coli* was used to inoculate a duodenoscope instrument channel that was then allowed to dry for 2 hours. Collecting and testing samples extracted from the inoculated instrument channel confirmed that RN test provided a very strong NFV of over 2300. It should be noted that this signal was generated by a maximum of 32 to 39 CFU in the 0.5 mL of extracted sample that was used for the RN test. Furthermore, we were able to demonstrate that up to 10% blood did not interfere with the ability of the test to generate a high NFV.

Our study is the first to demonstrate that in suspension testing, the RN Test does detect very low levels of Gram-negative bacteria but (as stated in the MIFU) cannot reliably detect Gram-positive bacteria. Our data support the manufacturer's claim that the RN test can detect as low as 10 CFU of Gram-negative bacteria in the sample tested. In addition, most of the samples with Gram-negative bacteria CFU between 1 and 10 were also detected as positive by the RN test.

Our previous data showed that for 6639 CFU/mL of *P. aeruginosa* extracted from biofilm, there was only 1 RLU by the ATP test [9]. Furthermore, it took 7,424,795 CFU/mL extracted from biofilm to generate 1 $\mu\text{g/mL}$ protein [9]. These data demonstrated that although rapid ATP and rapid protein testing are very useful for monitoring manual cleaning compliance, they are very insensitive and cannot act as a substitute for culture post-HLD [2, 10, 27, 29]. Unlike rapid ATP and protein testing that do not detect low levels of viable bacteria [9, 23, 25–27, 29] the RN test was sensitive enough to reliably detect nine of nine suspension tests when there were ≥ 10 CFU of viable *E. coli* and *P. aeruginosa* in the 0.5-mL sample tested. This is an excellent limit of detection but it must be borne in mind that all the current guidelines for interpretation of endoscope cultures [8, 18–19] indicate that even 1 CFU per channel of a Gram-negative bacteria is considered unacceptable. Of interest, the RN test correctly detected 14 of 15 of the tests for both of these

Gram-negative bacteria when there was ≥ 1 CFU in the 0.5-mL sample tested. All nine suspensions with Gram-negative bacteria < 1 CFU (i.e. considered as a negative sample) had NFV readings < 300 . Although there are a number of Gram-positive bacteria that are organisms of concern [8, 18–19], there have been no infectious outbreaks attributed to such bacteria arising from contaminated endoscopes, so utilizing a test that only detects Gram-negative organisms of concern maybe a reasonable alternative option to routine universal culturing. Potential advantages of RN test compared to cultures are a more rapid test (overnight compared with 48- to 72-hour hold for cultures), lack of need for addition of a neutralizer and concentration of the samples (neutralizer and sample concentration increase sensitivity of cultures but have no role with RN test) and ability to process in the endoscopy unit itself (compared with cultures which require transportation to a Microbiology laboratory).

Although 10% blood did not generate a NFV (▶ **Table 1**), it may affect the optical NFV reading as the signal generated from samples with 10% blood (▶ **Table 1**; 2,253.33 CFU of *E. coli* had NFV of 3,705) were not as high as those generated when the same organism was in sRO water (▶ **Table 2**; 105 CFU *E. coli* had NFV of 15,348). This aspect did not affect the ability of the RN test to detect low levels of viable Gram-negative bacteria, however, further assessment of the effect of organic residuals on the RN test is needed.

A limitation of this study is that we only evaluated two Gram-negative bacteria so further studies to show the applicability of the RN test for a wider range of Gram-negative bacteria would be valuable. The MIFU does not indicate the extraction efficacy of the 5-mL flush with extraction fluid. In addition, we did not evaluate the extraction efficacy of flushing 5 mL through an instrument channel containing dried organic debris, biofilm or build-up biofilm. Future clinical studies are needed to evaluate the feasibility of implementation of the RN test for patient-used endoscopes in a busy endoscopy clinic. The RN test cannot be used for testing endoscopes used more than once during the day as this test requires overnight incubation. In addition, it is unclear if testing endoscopes used on a Friday would require reading of RN test results on Saturday or whether the test could still provide reliable results if the completion of testing was done Monday morning. Despite these limitations, the RN test is the only currently available test that can provide reliable detection of 1 to 10 CFU with an overnight turnaround time. Our data support the recent study by Washburn et al. who reported that the RN test had similar results compared to culture [32].

Conclusion

In conclusion we have demonstrated that the RN test can reliably detect ≥ 10 CFU (and most of ≥ 1 CFU) of Gram-negative bacteria which is superior to what current rapid ATP and protein test kits can detect. Our encouraging preliminary data suggest that the RN test may be a useful alternative to culture for assessing contamination with Gram-negative bacteria in patient-ready endoscopes but further evaluation of extraction efficiency, effect of organic residuals and clinical testing is needed.

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Competing interests

Harminder Singh: Advisory Board; Ferring Canada & Takida Canada, Research funds; Merck Canada
Michelle Alfa: Advisory board; 3M, J&J, Consultant for Olympus, Novaflux, Ofstead Associates, Royalty through University of Manitoba for license to HealthMark Industry for ATS test soil patent, Invited speaker for Olympus, 3M, Ambu & Sealed Air
Donald Duerksen: Advisory Board for Ferring Canada
All other authors have no conflicts of interest

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