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Detection of Meningococcal Carriage by Culture and PCR of Throat Swabs and Mouth Gargles

J. Zoe Jordens,[1,2]* Jeannette N. Williams,[2] Graeme R. Jones,[1,2] and John E. Heckels[2]

Public Health Laboratory[1] and University of Southampton Molecular Microbiology Group,[2] Southampton General Hospital, Southampton SO16 6YD, United Kingdom

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The standard method for detecting meningococcal carriage is culture of throat swabs on selective media, but the levels of carriage determined depend heavily on the skills of the individuals taking the swab and interpreting the cultures. This study aimed to determine the most sensitive detection method for meningococcal carriage. Throat swabs and saline mouth gargles, obtained from 89 university students, were processed in parallel by conventional culture and TaqMan *ctrA* PCR. Carriage of meningococci, as detected by the combined methods, was 20%. The sensitivities of throat swab culture, throat swab PCR, gargle culture, and gargle PCR were 72, 56, 56, and 50%, respectively, and the probabilities that these techniques would correctly identify the absence of carriage (negative predictive value [NPV]) were 93.4, 89.9, 89.9, and 88.8%. Culturing both throat swabs and gargles increased the NPV to 98.6%. The further addition of throat swab PCR increased this to 100%. Testing gargles by both culture and PCR was as sensitive as testing throat swabs by both methods, suggesting that gargles may be a suitable alternative for large-scale screening studies when throat swabs are difficult to obtain, although they required more lengthy laboratory processing. PCR was a useful adjunct to culture for detecting nasopharyngeal carriage, but it failed to detect some nongroupable strains. For maximum sensitivity, a combination of techniques was required. This study indicates the confidence with which health care professionals involved in meningococcal screening can regard laboratory results.

Infections with *Neisseria meningitidis* are a significant cause of mortality and morbidity in young children and adolescents. The majority of infections in the developed world are caused by serogroup B and C strains. The introduction of conjugate vaccines based on the group C capsule into the United Kingdom routine immunization schedule of infants in November 1999 with subsequent immunization of older children and adolescents has decreased the number of infections with group C meningococci in people under 20 years of age by 72% (4). However, the persistence of carriage in vaccinated individuals would provide a reservoir of infection for nonimmune individuals. Although the conjugate *Haemophilus influenzae* type b vaccine is known to reduce carriage of *H. influenzae* type b (14), leading to herd immunity, the effect of the meningococcal conjugate vaccine on nasopharyngeal carriage of meningococci is as yet unknown.

In contrast to the decline in the number of group C infections during 2000, the number of reported cases of confirmed group B infection in England and Wales increased by 12% (4). There is no current vaccine for group B meningococci, and the similarity between the capsular polysaccharide of group B organisms and polysialic acid-containing glycopeptides that are a component of human brain tissue precludes a capsule-based vaccine. Vaccine design is concentrated on subcapsular antigens of group B meningococci. Hence, it is essential to identify and characterize the contribution that subcapsular antigens of carried strains have on host immunity.

Sensitive meningococcal detection methods are therefore required to determine the contribution of carriage to host immunity, the effects of immunization on carriage, and the extent of carriage of highly virulent strains in putative outbreak situations. The standard method for detecting carriage is culture of throat swabs on selective media. This technique is subject to operator variation (11), and experienced operators are required to obtain good-quality throat swabs and to detect meningococci. Furthermore, culture of throat swabs is labor-intensive, cannot be delayed once the sample is taken, and often takes 4 days to obtain a result. For mass screening studies of older children and young adults, it would be more efficient for individuals to provide their own samples without the requirement for an experienced throat swab taker. The recent introduction of TaqMan PCR for the detection of meningococcal DNA from blood, plasma, serum, and cerebrospinal fluid from infected patients (8) has provided an alternative to culture for diagnosis and enhanced detection from antibiotic-containing samples. TaqMan PCR enables a high throughput of specimens in a microtiter plate format with rapid turnaround times. In this study, we have investigated the suitability of saline mouth gargles as an alternative to throat swabs and compared standard culture with TaqMan PCR for the detection of nasopharyngeal carriage of meningococci.

MATERIALS AND METHODS

Subjects. Volunteers were sought from first-year undergraduate medical students attending a practical class during week 6 of their first term. All had received the nonconjugate polysaccharide A/C vaccine prior to starting the course.

Specimens. Plain throat swabs (medical wire) were taken from 89 students by a single experienced medical practitioner. The volunteers then gargled with 10 ml of sterile phosphate-buffered saline. Specimens were transported to the laboratory and processed within 3 h of collection. Ethical permission for the study was

* Corresponding author. Mailing address: Public Health Laboratory, Level B South Block, Southampton SO16 6YD, United Kingdom. Phone: 44 2380 794810. Fax: 44 2380 702530. E-mail: jzj@soton.ac.uk.

TABLE 1. Primer and probe sequences used in the present study

Name	5′-3′ sequence
ctrA forward 1	TTGTGTGGAAGTTTAATTGTAGGATGC
ctrA reverse 1	TCAGATTGTTGCCCTAAAGAGACA
ctrA TaqMan probe 1	TCCTTCATCAGGCCCCAGCG[a]
ctrA forward 2	GCTGCGGTAGGTGGTTCAA
ctrA reverse 2	TTGTCGCGGATTTGCAACTA
ctrA TaqMan probe 2	CATTGCCACGTGTCAGCTGCACAT[a]
Group B forward	TGCATGTCCCCTTTCCTGA
Group B reverse	AATGGGGTAGCGTTGACTAACAA
Group B TaqMan probe	TGCTTATTCCTCCAGCATGCGCAAA[a]
Group C forward	GATAAATTTGATATTTTGCATGTAGCTTTC
Group C reverse	TGAGATATGCGGTATTTGTCTTGAAT
Group C TaqMan probe	TTGGCTTGTGCTAATCCCGCCTGA[a]

[a] Labeled with carboxyfluorescein (FAM) at 5′ position and carboxytetramethyl rhodamine (TAMRA) at 3′ position.

obtained from the Southampton and South West Hampshire local research ethics committee and the Public Health Laboratory Service.

Bacterial culture. Throat swabs were used to inoculate modified New York City (MNYC) selective media (Wessex Media Services, Dorchester, United Kingdom) for the growth of *Neisseria* species. For culture, 1.5 ml of gargle fluid was centrifuged at 10,000 × *g* for 7.5 min, and the supernatant fluid was discarded, leaving approximately 100 μl of residue. This was mixed and used to inoculate MNYC media. The media were incubated for 48 h at 37°C in 5% CO_2 and then examined. Potential meningococcal colonies were Gram stained and tested with oxidase reagent (BDH Laboratory Supplies, Poole, England). Gram-negative cocci which were oxidase positive were subcultured for biochemical identification with apiNH (BioMerieux s.a., Lyon, France). All confirmed *N. meningitidis* isolates were stored in 10% glycerol in proteose peptone broth at −80°C for subsequent typing.

Sample preparation for PCR. Immediately after culture, the throat swabs were agitated in 200 μl of sterile water (Sigma tissue culture grade). The swabs were then broken off, inverted, returned to the tube, and then centrifuged for 15 min at 15,000 × *g* so that fluid trapped in the swab would be drawn out (12). The swab was then discarded, and the washings were stored at −80°C.

Swab washings (50 μl) were thawed, boiled for 10 min, cooled on ice, and then centrifuged for 10 min at 12,500 × *g* (12). The supernatant solution (45 μl) was transferred to a fresh tube and used immediately for PCR or stored at −80°C until it was required. Gargles (1.5 ml) were thawed and then treated with dithiothreitol (33 μM) for 15 min at room temperature to liquefy the sample. After centrifugation for 10 min at 12,500 × *g*, the supernatant fluid was discarded and the pellet was resuspended in 200 μl of sterile water. This was boiled for 10 min and centrifuged as described above. About 180 μl of supernatant fluid was transferred to a fresh tube and used immediately for PCR or stored at −80°C until it was required.

Amplification by TaqMan PCR. The sequences of all primers and probes used are shown in Table 1. The primer-probe combination for the capsule transfer *ctrA* gene detects serogroups B, C, Y, and W135 (8) (Table 1, *ctrA* forward 1, *ctrA* reverse 1, and *ctrA* probe 1). Amplification reaction mixtures contained 1 μM primers (Cruachem); 0.5 μM probe (Scandinavian Gene Synthesis); and 1× buffer A; 3 mM $MgCl_2$; 200 μM (each) dATP, dCTP, dGTP, and dUTP; 0.025 U of AmpliTaq Gold/μl; 0.01 U of AmpErase UNG/μl (all provided in the TaqMan Core reagent kit [Applied Biosystems]), as well as 5 μl of target DNA in a total volume of 25 μl. Dilutions of plasmid containing the cloned *ctrA* gene were included in every experiment and were used to construct standard curves from which the number of copies of target DNA could be calculated.

A modified *ctrA* primer-probe combination (Table 1, *ctrA* forward 2, *ctrA* reverse 2 and *ctrA* probe 2) which detects serogroups A, B, C, X, Y, Z, W135, and 29E and some nongroupable strains was also used (5). Reaction mixtures with this primer-probe combination contained 1× TaqMan Universal Mix, 0.3 μM each primer, 0.2 μM probe (all supplied by Applied Biosystems), and 5 μl of target DNA in a total volume of 25 μl. Dilutions (10-fold) of bacterial DNA covering the range 10^3 to 10^7 copies/ml were included with these *ctrA* assays. The specificities of both assays were confirmed with extracts from *Neisseria gonorrhoeae* as well as *Neisseria lactamica* and *Neisseria sicca* (the last two were obtained from the National Collection of Type Cultures, Central Public Health Laboratory, London, England).

Amplification parameters for both primer-probe combinations consisted of 2 min at 50°C and 10 min at 95°C followed by 50 cycles of 15 s at 95°C and 1 min at 60°C on a sequence detector system 7700 (Applied Biosystems). Positive controls (containing cloned *ctrA* or DNA extracted from group B *N. meningitidis* and group C *N. meningitidis*, as described above) and negative controls (water only) were included in every experiment. The threshold cycle (the cycle at which sample fluorescence exceeds a threshold value, indicating a positive result, and which is proportional to the number of genome copies present) was reported for each sample.

Typing. Bacterial cultures were characterized by group, type, and subtype antigens by standard methods at the Meningococcal Reference Unit (Manchester Public Health Laboratory, Manchester, United Kingdom). Samples which were positive with either TaqMan *ctrA* primer-probe combination were characterized by TaqMan *siaD* PCR (8) with primer-probe combinations which detect group B- and group C-associated DNA (Table 1, group B forward, reverse, and probe; group C forward, reverse, and probe).

Analysis. All positive tests (culture and PCR) were deemed to have a specificity of 100% and a positive predictive value of 1.0. Sensitivity and negative predictive values (NPVs) were calculated for each sample type (throat swab and gargle) and test (culture and PCR) combination as follows: sensitivity = $a/(a + c)$ and NPV = $d/(c + d)$, where a, b, c, and d represent the number of true positives, false positives, false negatives, and true negatives, respectively. Sensitivity and NPV results were expressed as percent values.

RESULTS

Sensitivity. The results of culture and TaqMan PCR amplification of throat swabs and mouth gargles for the detection of nasopharyngeal carriage of *N. meningitidis* from 89 students are shown in Table 2. There was not a 1:1 correlation between the results from throat swabs and gargles for either detection method. Standard culture detected meningococci in both throat swabs and gargles from six students, but meningococci were also detected in seven throat swabs from students with culture-negative gargles and in four gargles from students in whom throat swabs were culture negative. The original *ctrA* TaqMan PCR procedure detected meningococcal DNA in nine throat swabs and six gargles, with both samples being positive in five students; four positives were detected only in the throat swab and one only in the gargle.

Similarly, there was not a 1:1 correlation between the culture and the original *ctrA* PCR results within each of the two specimen types. For throat swabs, culture and PCR results were both positive for seven students, but culture detected six positives which were not detected by PCR while PCR detected two positives from throat swabs that were culture negative. Similarly for gargles, the culture and PCR results were both positive for four students, but culture detected meningococci from six specimens which were negative by PCR while PCR detected meningococcal DNA in two gargles which were culture negative.

During the course of the study, a modified primer-probe combination for *ctrA* TaqMan PCR which detected a wider range of meningococcal groups and some nongroupable strains became available. As this modified *ctrA* PCR had not been widely used, the present study compared it with the original *ctrA* PCR for detecting meningococcal carriage. The modified *ctrA* TaqMan PCR detected meningococcal DNA from two throat swabs and four gargles which were negative with the original TaqMan PCR but failed to detect two samples; these two samples contained low numbers of target DNA ($<10^4$ copies/ml, determined by comparison with known standards). The modified TaqMan *ctrA* PCR detected meningococcal DNA in more students than the original TaqMan PCR, although the threshold cycle values were slightly higher (about 2 cycles), indicating that the modified PCR was about fourfold

TABLE 2. *N. meningitidis* culture and PCR results for throat swabs and mouth gargles from 89 students[a]

Volunteer no.	Throat swab					Gargle				
	Culture	Type	PCR			Culture	Type	PCR		
			Original *ctrA*	New *ctrA*	*siaD*[b]			Original *ctrA*	New *ctrA*	*siaD*[b]
1	+	NG; NT; P1.16	−	+	−	−		−	+	−
3	+	NG; NT:P1.3,P1.6	−	−	ND	−		+	+	−
6	+	NG; NT:P1.16	+	+	B	−		−	−	ND
9	+	NG; NT:P1.15	−	+	−	−		−	+	−
13	+	W135; NT:P1.3,P1.6	+	+	−	+	NG; NT:P1.3,P1.6	−	+	−
15	+	NG; 1:P1.6	+	+	−	+	NG; 1:P1.6	+	+	−
16	−		−	−	ND	+	NG; NT:P1.15	−	−	ND
33	+	NG; NT:P1.7	−	−	ND	+	NG; NT:P1.7	−	−	ND
34	+	W135; NT:P1.3,P1.6	+	+	B	+	NG; 1:P1.13	±	−	−
36	+	NG; NT:P1.16	+	+	B	+	NG; NT:P1.16	+	+	B
49	−		+	+	B	−		−	+	−
59	−		+	+	B	+	NG; NT:P1.14	+	+	−
63	+	W135; NT:P1.3,P1.6	±	−	−	−		−	−	ND
64	+	NG; 15:P1.6	−	−	ND	+	NG; 15:P1.6	−	−	ND
69	+	NG; NT:P1.15	−	−	ND	−		−	−	ND
72	−		−	−	ND	+	NG; 15:P1.6	−	−	ND
75	−		−	−	ND	+	NG; NT:P1.14	−	−	ND
82	+	NG; NT:P1.3,P1.6	+	+	−	−		+	+	−
Others			−	−	ND	−		−	−	ND
Total no. of positives	13		9	10		10		6	9	

[a] −, negative test result; ±, weak positive result; +, positive result; ND, test not done; NG, not groupable; NT, not typeable.
[b] Negative *siaD* PCR result does not exclude the possibility of group B or C.

less sensitive for detecting meningococcal DNA. This difference in sensitivity was confirmed by comparing serial dilutions of meningococcal DNA. On the basis of the increased ability to detect carriers and the wider range of groups detected with the new primer-probe combination, only the PCR results from this assay were included in the subsequent analysis, despite the fourfold-lower sensitivity.

Comparison of culture and modified *ctrA* PCR results for each of the two specimen types also lacked a 1:1 correlation. Throat swab culture and PCR results were both positive for eight students, but culture detected five positives which were not detected by PCR while PCR detected two positives from throat swabs that were culture negative. For gargles, the culture and modified *ctrA* PCR results were both positive for four students, but culture detected meningococci from six specimens which were negative by PCR while PCR detected meningococcal DNA in five gargles which were culture negative.

NPVs. The number of true positives (i.e., carriers), defined as the number of students detected as positive by any test (culture or PCR) in any sample (throat swab or gargle), was 18. Thus, the carriage rate was 20%. Similarly, the number of true negatives, defined as the number of students with no detectable meningococci by culture or PCR in throat swab or gargle specimens, was 71. The sensitivity was calculated for every detection method (specimen type plus test method), alone and in combination. The most sensitive single detection method was standard throat swab culture, with a sensitivity of 72% (Table 3). Culturing both throat swab and gargle increased the sensitivity to 94%, and the addition of throat swab PCR increased this further to 100% (Table 3).

For single detection methods, the NPV (i.e., the probability of a true negative among apparent negatives) was highest for standard throat swab culture (93.4%) (Table 3). Culturing both throat swab and gargle increased this to 98.6%, and the addition of throat swab PCR increased this further to 100% (Table 3).

Characterization of *N. meningitidis*. The groups, types, and subtypes of the 23 isolates and the results of the TaqMan *siaD* typing PCR are given in Table 2. By traditional methods, the group could be determined for only three isolates; these were all group W135. The remaining isolates were nongroupable. However, six samples from five students gave a positive *siaD* PCR for group B, suggesting that these were genotypically

TABLE 3. Sensitivities and NPVs of culture and modified PCR of throat swabs and gargles for the detection of meningococcal carriage

Method(s)[a]	Sensitivity (95% CI[b])	NPV (%)
TS culture	72 (51–93)	93.4
TS PCR	56 (33–79)	89.9
Gargle culture	56 (33–79)	89.9
Gargle PCR	50 (27–73)	88.8
TS culture + TS PCR	83 (66–100)	95.9
TS culture + gargle culture	94 (83–100)	98.6
TS culture + gargle PCR	83 (66–100)	95.9
TS PCR + gargle culture	83 (66–100)	95.9
TS PCR + gargle PCR	61 (38–84)	91.0
Gargle culture + gargle PCR	83 (66–100)	95.9
TS culture + TS PCR + gargle culture	100 (82–100)	100
TS culture + TS PCR + gargle PCR	83 (66–100)	95.9
TS PCR + gargle culture + gargle PCR	89 (75–100)	97.3
TS culture + TS PCR + gargle culture + gargle PCR	100 (82–100)	100

[a] TS, throat swab.
[b] CI, confidence interval.

group B but were not expressing capsular material. The two isolates from volunteer no. 13 were both NT:P1.3,6, but the throat isolate expressed group W135 whereas the gargle isolate was not groupable, suggesting a difference in capsule expression. Where traditional immunological and TaqMan *siaD* PCR data were obtained for a sample, the results from the two methods were consistent, with the exception of volunteer no. 34. In this individual, a group B reaction was detected by PCR in the throat swab whereas culture detected a W135 strain. In addition, isolates with different subtypes were cultured from the throat swab and gargle of this individual, suggesting carriage of at least two strains simultaneously. The results indicated a group B carriage rate of 5.6%. No group C organisms were detected.

DISCUSSION

Meningococcal carriage is detected by selective culture from throat swabs, but the sensitivity of this technique is unknown. This study compared culture and PCR of throat swabs and gargles for detecting nasopharyngeal carriage of meningococci and determined the sensitivity of each method. A single swab was used for culture and PCR, as it would not have been acceptable to the volunteers to take two swabs which could then have been randomized. A single swab is not ideal, as it is cultured first and hence the sensitivity of the subsequent PCR may be reduced, as some organisms will be removed when the swab is plated out. For culture (the standard method) to be reliable, plating out needed to precede washing for PCR. Throat swabbing always preceded gargling, as it was the standard specimen and the sample size was not large enough to randomize into swab or gargle first.

Detected carriage was defined as a positive test result in any specimen; culture and PCR were assumed to be 100% specific. This is likely to be an underestimate of real carriage, as false negatives cannot be enumerated. Standard throat swab culture was the most sensitive single method tested (72% of total detected carriage). Gargle culture alone was less sensitive but increased the detection of carriage to 94% when combined with throat swab culture. Thus, despite the convenience of gargle specimens, their culture alone cannot replace throat swabs. The discrepancy between some of the culture results from the two specimens may reflect differences in meningococcal colonization of the sites sampled or the ease with which the bacteria can be dislodged from the epithelia.

Performing both culture and modified *ctrA* TaqMan PCR on a single specimen (throat swab or gargle) increased detection to 83% of carriers. However, the processing of gargles for PCR was relatively labor-intensive and time-consuming, so it is only warranted if throat swabs cannot easily be obtained. The threshold cycle was near the limit of detection of the assay and indicated very low numbers of meningococci ($<10^4$ per ml) in most gargles, while throat swab samples contained 10^5 to 10^7 meningocci per ml.

The modified *ctrA* TaqMan PCR was convenient for handling large numbers of samples, but it detected only approximately half of the carriers. Of the five culture-positive but PCR-negative throat swabs, two contained nongroupable strains not detected by this assay, one had low numbers of W135 organisms and was detected only weakly with the original assay, one had low numbers of a nongroupable strain as shown by a weakly positive result on subsequent testing, and the remaining discrepant result may have been due to lower sensitivity of the PCR. The results of the two culture-negative but PCR-positive throat swabs may reflect differences in sampling the swab. Similarly, for discrepant results from culture and PCR of gargles, one contained a nongroupable strain which could not be detected by this PCR, three had low copy numbers of target DNA as indicated by weakly positive results with the original PCR or weakly positive results on subsequent testing with the modified PCR, and the remaining two contained nongroupable strains which were poorly detected with this PCR. The five culture-negative and PCR-positive gargles may reflect the different portions tested, the use of dithiothreitol to emulsify specimens for PCR but not for culture (viscous samples may prevent successful culture), or the detection of nonviable organisms.

The modified PCR failed to detect 10 samples from eight volunteers from which nongroupable meningococci were cultured, illustrating that specimen culture is essential. PCR of boiled supernatants prepared from the strains confirmed that six nongroupable strains could not be detected or were poorly detected with this assay (data not shown). No inhibition was detected in the specimens (data not shown), suggesting that the lack of detection in the four remaining specimens was due to low copy numbers of target DNA. Ideally, PCR would provide a rapid result, but primers different from those used to confirm infection would be required to reflect the different capsular and nongroupable strains involved in carriage.

A recent study compared nasopharyngeal swabbing with PorA-based immunohistochemical staining of tonsillar tissue for the detection of meningococcal carriage in 32 patients undergoing tonsillectomy (13) and concluded that swabbing detects only one-quarter of carriers and that carriage is more frequent than previously thought. As the tissue was diseased (necessitating removal) and the function of lymphoid tissue is to capture microorganisms, the detection of meningococci within the tonsillar material may not necessarily reflect carriage or provide a reservoir of infection. However, *porA* may be a more appropriate target than *ctrA* for PCR-based detection of meningococcal carriage. In addition, *porA* PCR would enable the determination of subcapsular antigens, which have been associated with the development of specific immunity (9).

Characterization of strains by standard immunological methods showed the majority (20 of 23) to be nongroupable and the remaining 3 strains to be group W135. However, PCR for group B- and group C-specific DNA detected six genotype B strains, three of which were from samples from which nongroupable meningococci were isolated, suggesting that these organisms were potentially capsulate but were not expressing in the nasopharynx. The isolation of nongroupable and group W135 strains with the same subtype from the throat swab and gargle from an individual also suggests that capsule expression is variable, as reported elsewhere (1, 6). Although not specifically sought in this study (isolates were from standard single-colony subcultures), simultaneous carriage of multiple strains (differing in PorA subtype) was detected in one student. Such carriage could facilitate genetic transfer between strains and explain how organisms may change their capsules. This may have significant implications for immunization planning if an

outbreak-associated or hypervirulent group C strain switched its serogroup to one not included in a current vaccine. It is interesting that such a mechanism may account for the group W135 strain associated with the Hajj in 2000, which is closely related to the hypervirulent ET-37 complex (T. Popovic et al., *Neisseria meningitidis* Global Village 12th Int. Pathog. *Neisseria* Conf., abstr. 222, 2000) more commonly associated with group C strains (3). Similarly, a switch from group C to group B in a 2a:P1.2,5 strain has been reported from the Czech Republic (10).

The carriage rate in this study was 20%. This is similar to that reported during an outbreak at the same university 2 years previously (7). In outbreak situations, it is often the absence of carriage of specific strains which requires confirmation. The NPV of a single throat swab culture for excluding meningococcal carriage in this population was 93.4%. The NPV of throat swab culture to detect meningococci is dependent upon its sensitivity and the true prevalence of carriage in the population under test. The estimated prevalences of meningococcal carriage are 2 and 25% in age groups 0 to 4 years and 15 to 19 years, respectively (2). Assuming that the efficiency of throat swabbing is the same in each group, the calculated NPVs of a single throat swab culture for meningococcal carriage for age groups 0 to 4 and 15 to 19 years are 99 and 88%. Hence, this study indicates the confidence with which health care professionals involved in meningococcal screening can regard diagnostic laboratory culture results.

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