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Cost-Effective Pooling of DNA from Nasopharyngeal Swab Samples for Large-Scale Detection of Bacteria by Real-Time PCR

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We investigated the potential of pooling DNA from nasopharyngeal specimens to reduce the cost of real-time PCR (RT-PCR) for bacterial detection. Lyophilization is required to reconcentrate DNA. This strategy yields a high specificity (86%) and a high sensitivity (96%). We estimate that compared to individual testing, 37% fewer RT-PCR tests are needed.

Real-time PCR (RT-PCR) is an essential tool for routine diagnostics and large epidemiological studies of infectious diseases (1, 2). However, its cost remains significant and limits its use. To reduce it, samples can be pooled (3–5). This idea was suggested by Dorfman in 1943 (6) and has been used for the serological diagnosis of infectious diseases. Pooling samples is also an efficient way to screen for the nucleic acids of viruses, bacteria, or parasites (4, 5, 7–15). However, pooling can decrease the sensitivity of assays due to the dilution of the samples, which is problematic in clinical diagnostics (16). Here, we investigated the potential of pooling DNA from clinical specimens using lyophilization to concentrate the pooled DNA and maintain the sensitivity of RT-PCR.

We screened 2,380 nasopharyngeal swabs (Remel, USA) by RT-PCR to detect their rates of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Klebsiella pneumoniae* carriage. DNA was extracted using a Macherey-Nagel NucleoSpin-96 kit. A total of 119 pools containing 80 μl of DNA from 20 patients was frozen for 4 h at −20°C, lyophilized using a Lyovac GTZ instrument (Leybold Heraeus, France), and redissolved in 80 μl of sterile water. The effectiveness of lyophilization was verified with pool controls, including one positive sample with a known threshold cycle (C_T) value for each bacterium. The specificities of the primers and probes (17, 18) were verified *in silico* by conducting a BLAST search in GenBank and performing RT-PCR on 10 to 15 closely related bacterial strains present in the respiratory tract (see Tables S1 and S2 in the supplemental material). RT-PCR was performed using 5 μl of DNA per reaction and a 7900HT thermocycler (Applied Biosystems). Nuclease-free water was used as a negative control, and DNA from a clinical strain was used as a positive control. The cutoff C_T value for positive results was ≤38. Each sample was also tested individually. The DNA extraction quality of each pool was verified by RT-PCR targeting the human beta-actin gene (19).

The prevalences for the individual samples were 9% (215/2,380) for *S. pneumoniae*, 7% (169/2,380) for *H. influenzae*, and 4% (95/2,380) for *K. pneumoniae*. Among the pools tested, 69% (82/119) were positive for *S. pneumoniae*, 53% (63/119) were positive for *H. influenzae*, and 53% (63/119) were positive for *K. pneumoniae* (Table 1). Among them, 184/357 (52%) were true-positive results and 142/357 (40%) were true-negative results. However, 24/357 (7%) were false positives (negative individual samples), and 7/357 (2%) negative pools contained positive individual specimens, yielding false negatives (see Table S3 in the supplemental material). All false-positive and false-negative results occurred at a C_T value of ≥35.

There was a mean of 3 (range, 1 to 8) positive samples per positive pool, and the mean C_T value of the pools was 32 (range, 22 to 38). This assay had a detection threshold equivalent to that of individual testing. The C_T value of the pool was similar to that of the individual sample with the lowest C_T value in the pool (Spearman coefficient, $r = 0.93$). We detected up to 8 positive samples per pool, and compared to the individual results, the presence of several bacteria in the same pool did not inhibit detection.

In this study, a total of 2,623 (37%) tests could have been saved by pooling groups of 20 samples rather than conducting 7,140 individual tests (Table 2). Time and cost were measured for these 2 strategies by calculating the costs of consumables, reagents, and personnel. We excluded DNA extraction because this step is common to the 2 strategies. We estimated that the time needed from plate preparation to the interpretation of results for one 384-well plate was 4.5 h; thus, 85 h is needed to test 7,140 individual samples versus 55 h with pooling, including repeat analysis of individual samples from positive pools. The cost was evaluated to 1.34 euros/reaction for consumables and reagents and 33 euros/h for a technician. The cost of performing RT-PCR on a pool of 20 samples was decreased by 37% compared to that of individual testing (Table 2).

We showed that this strategy is efficient and yields a high specificity (86%) and a high sensitivity (96%). Freeze-drying is required to concentrate pooled DNA because of the very small volumes used for PCRs. The volume of DNA constituting the pools and the final elution volume were calculated so that after lyophilization, the amount of DNA deposited into each well for RT-PCR was equivalent to that contained in 5 μl of DNA from individual samples that are typically used to avoid a loss of sensitivity. Lyophilization is an easy-to-perform one-step procedure. The main risk

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TABLE 1 Performance of pooled processed DNA compared to that of individually tested samples

Bacterium	No. of pools tested	Total no. (%) of presumptive positives in the pool	No. of positive pools with positive individual results (% true positives)	No. of positive pools with negative individual results (% false positives)	No. of negative pools with negative individual results (% true negatives)	No. of negative pools with positive individual results (% false negatives)	Specificity of pool (%)	Sensitivity of pool (%)	PPV[a] (%)	NPV[b] (%)
S. pneumoniae	119	82 (69)	78 (65)	4 (3)	33 (28)	4 (3)	89	95	95	89
H. influenzae	119	63 (53)	60 (50)	3 (2)	55 (46)	1 (1)	95	98	95	98
K. pneumoniae	119	63 (53)	46 (39)	17 (14)	54 (45)	2 (2)	76	96	73	96
Total	357	208 (58)	184 (52)	24 (7)	142 (40)	7 (2)	86	96	88	95

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

of this strategy is that PCR inhibitors may be concentrated; however, in our assay, the pooling of 20 samples did not affect the detection of bacterial DNA compared to that of the individual tests.

To prevent a loss of sensitivity, it may also be necessary to limit pool sizes to limit sample dilutions (7, 10, 14) or to use special kits for extracting DNA from larger sample volumes (4, 16). With our strategy, it is possible to pool a larger number of specimens (up to 20) and to lyophilize DNA specimens to a volume of 1.6 ml, while commercial DNA extraction kits are limited to 1 ml (4, 16). This strategy is particularly useful when searching for a large number of pathogens (>10) in the same clinical specimens compared to multiplex PCR, for which fewer pathogens can be detected.

The pooling of clinical samples is especially profitable if the bacterial prevalence is low because each sample in a positive pool is retested individually; thus, the optimum pool size should be determined for each study (4). We estimated that 50%, 54%, and 37% of tests could have been saved by pooling 10, 5, and 2 samples, respectively (Table 2). Pools of 5 samples would likely have been more profitable for this study.

Our RT-PCR targeting *S. pneumoniae* also amplified *Streptococcus pseudopneumoniae*; however, the prevalence of *S. pseudo-*

TABLE 2 Evaluation of the number of RT-PCR tests saved by pooling 20 samples and extrapolation of the number of RT-PCR tests saved by pooling 10, 5, and 2 samples

Characteristic of RT-PCR tests performed and saved by pooling samples	Value for RT-PCR				Delay in performing RT-PCR (h)	Cost[a] (cost of consumables/personnel costs) (euros)
	S. pneumoniae	*H. influenzae*	*K. pneumoniae*	Total organisms		
No. of individual specimens tested	2,380	2,380	2,380	7,140	85	12,372 (9,567/2,805)
No. (%) of positive individual specimens	215 (9)	169 (7)	95 (4)	479 (20)		
Total no. (%) of tests performed by pooling 20 samples	1,759 (74)	1,379 (58)	1,379 (58)	4,517 (63)	54	7,834 (6,052/1,782)
Total no. (%) of tests saved by pooling 20 samples	621 (26)	1,001 (42)	1,001 (42)	2,623 (37)		
Extrapolation of total no. (%) of tests performed by pooling 10 samples	1,470 (62)	1,170 (49)	920 (39)	3,560 (50)	42	6,123 (4,770/1,386)
Extrapolation of total no. of tests saved (%) by pooling 10 samples	910 (38)	1,210 (51)	1,460 (61)	3,580 (50)		
Extrapolation of total no. (%) of tests performed by pooling 5 samples	1,275 (54)	1,130 (47)	861 (36)	3,266 (46)	40	6,156 (4,376/1,320)
Extrapolation of total no. (%) of tests saved by pooling 5 samples	1,105 (46)	1,250 (53)	1,519 (64)	3,874 (54)		
Extrapolation of total no. (%) of tests performed by pooling 2 samples	1,575 (66)	1,520 (64)	1,374 (58)	4,469 (63)	54	7,77 (5,988/1,782)
Extrapolation of total no. (%) of tests saved by pooling 2 samples	805 (34)	860 (36)	1,006 (42)	2,671 (37)		

[a] The costs of RT-PCR were measured by adding the costs of specific consumables and reagents (including plates, plastic film, primers and probes, tips, and PCR mix) based on prices set by providers in France in January 2014 and the costs of paying personnel.

pneumoniae in the respiratory tract is low (1% to 12%) (20–22), and it is more frequently present in sputum than in the nasopharynx (23). Moreover, our purpose here was not to evaluate this RT-PCR assay but to assess the lyophilization of DNA.

Our assay, which combines pooling and lyophilization of DNA, is simple, efficient, and cost-effective and increases the feasibility of large epidemiological studies of infectious diseases by PCR without a loss of sensitivity.

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REFERENCES

1. **Maurin M.** 2012. Real-time PCR as a diagnostic tool for bacterial diseases. Expert Rev Mol Diagn **12:**731–754. http://dx.doi.org/10.1586/erm.12.53.
2. **Fournier PE, Drancourt M, Colson P, Rolain JM, La Scola B, Raoult D.** 2013. Modern clinical microbiology: new challenges and solutions. Nat Rev Microbiol **11:**574–585. http://dx.doi.org/10.1038/nrmicro3068.
3. **Emmanuel JC, Bassett MT, Smith HJ, Jacobs JA.** 1988. Pooling of sera for human immunodeficiency virus (HIV) testing: an economical method for use in developing countries. J Clin Pathol **41:**582–585. http://dx.doi.org/10.1136/jcp.41.5.582.
4. **Van TT, Miller J, Warshauer DM, Reisdorf E, Jernigan D, Humes R, Shult PA.** 2012. Pooling nasopharyngeal/throat swab specimens to increase testing capacity for influenza viruses by PCR. J Clin Microbiol **50:**891–896. http://dx.doi.org/10.1128/JCM.05631-11.
5. **Currie MJ, McNiven M, Yee T, Schiemer U, Bowden FJ.** 2004. Pooling of clinical specimens prior to testing for *Chlamydia trachomatis* by PCR is accurate and cost saving. J Clin Microbiol **42:**4866–4867. http://dx.doi.org/10.1128/JCM.42.10.4866-4867.2004.
6. **Dorfman R.** 1943. The detection of defective numbers of large populations. Ann Math Statist **14:**436–440. http://dx.doi.org/10.1214/aoms/1177731363.
7. **Singer RS, Cooke CL, Maddox CW, Isaacson RE, Wallace RL.** 2006. Use of pooled samples for the detection of *Salmonella* in feces by polymerase chain reaction. J Vet Diagn Invest **18:**319–325. http://dx.doi.org/10.1177/104063870601800401.
8. **Paixao P, Almeida S, Videira PA, Ligeiro D, Marques T.** 2012. Screening of congenital cytomegalovirus infection by real-time PCR in urine pools. Eur J Pediatr **171:**125–129. http://dx.doi.org/10.1007/s00431-011-1496-4.
9. **Hourfar MK, Jork C, Schottstedt V, Weber-Schehl M, Brixner V, Busch MP, Geusendam G, Gubbe K, Mahnhardt C, Mayr-Wohlfart U, Pichl L, Roth WK, Schmidt M, Seifried E, Wright DJ, German Red Cross NAT Study Group.** 2008. Experience of German Red Cross blood donor services with nucleic acid testing: results of screening more than 30 million blood donations for human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus. Transfusion **48:**1558–1566. http://dx.doi.org/10.1111/j.1537-2995.2008.01718.x.
10. **Westreich DJ, Hudgens MG, Fiscus SA, Pilcher CD.** 2008. Optimizing screening for acute human immunodeficiency virus infection with pooled nucleic acid amplification tests. J Clin Microbiol **46:**1785–1792. http://dx.doi.org/10.1128/JCM.00787-07.
11. **Lindan C, Mathur M, Kumta S, Jerajani H, Gogate A, Schachter J, Moncada J.** 2005. Utility of pooled urine specimens for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in men attending public sexually transmitted infection clinics in Mumbai, India, by PCR. J Clin Microbiol **43:**1674–1677. http://dx.doi.org/10.1128/JCM.43.4.1674-1677.2005.
12. **Bharti AR, Letendre SL, Patra KP, Vinetz JM, Smith DM.** 2009. Malaria diagnosis by a polymerase chain reaction-based assay using a pooling strategy. Am J Trop Med Hyg **81:**754–757. http://dx.doi.org/10.4269/ajtmh.2009.09-0274.
13. **Tan HH, Chan R.** 2005. Use of polymerase chain reaction on pooled cervical swabs to detect *Chlamydia trachomatis* infections in female sex workers in Singapore. Singapore Med J **46:**215–218.
14. **He YL, Gao HH, Ye LY, Guo ZH, Wang P, Zhu ZY.** 2013. Multiplex polymerase chain reaction with DNA pooling: a cost-effective strategy of genotyping rare blood types. Transfus Med **23:**42–47. http://dx.doi.org/10.1111/j.1365-3148.2012.01198.x.
15. **Mota M, Dezan M, Valgueiro MC, Sakashita AM, Kutner JM, Castilho L.** 2012. RHD allelic identification among D-Brazilian blood donors as a routine test using pools of DNA. J Clin Lab Anal **26:**104–108. http://dx.doi.org/10.1002/jcla.21489.
16. **Rours GI, Verkooyen RP, Willemse HF, van der Zwaan EA, van Belkum A, de Groot R, Verbrugh HA, Ossewaarde JM.** 2005. Use of pooled urine samples and automated DNA isolation to achieve improved sensitivity and cost-effectiveness of large-scale testing for *Chlamydia trachomatis* in pregnant women. J Clin Microbiol **43:**4684–4690. http://dx.doi.org/10.1128/JCM.43.9.4684-4690.2005.
17. **Shannon KE, Lee DY, Trevors JT, Beaudette LA.** 2007. Application of real-time quantitative PCR for the detection of selected bacterial pathogens during municipal wastewater treatment. Sci Total Environ **382:**121–129. http://dx.doi.org/10.1016/j.scitotenv.2007.02.039.
18. **Cohen-Bacrie S, Ninove L, Nougairede A, Charrel R, Richet H, Minodier P, Badiaga S, Noël G, La Scola B, de Lamballerie X, Drancourt M, Raoult D.** 2011. Revolutionizing clinical microbiology laboratory organization in hospitals with *in situ* point-of-care. PLoS One **6:**e22403. http://dx.doi.org/10.1371/journal.pone.0022403.
19. **Morel AS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta JP, Fenollar F, Fournier PE, Drancourt M, Raoult D.** 28 October 2014. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis http://dx.doi.org/10.1007/s10096-014-2263-z.
20. **Harf-Monteil C, Granello C, Le Brun C, Monteil H, Riegel P.** 2006. Incidence and pathogenic effect of *Streptococcus pseudopneumoniae*. J Clin Microbiol **44:**2240–2241. http://dx.doi.org/10.1128/JCM.02643-05.
21. **Laurens C, Michon AL, Marchandin H, Bayette J, Didelot MN, Jean-Pierre H.** 2012. Clinical and antimicrobial susceptibility data of 140 *Streptococcus pseudopneumoniae* isolates in France. Antimicrob Agents Chemother **56:**4504–4507. http://dx.doi.org/10.1128/AAC.06374-11.
22. **Mohammadi JS, Dhanashree B.** 2012. *Streptococcus pseudopneumoniae*: an emerging respiratory tract pathogen. Indian J Med Res **136:**877–880.
23. **Rolo D, Simoes S, Domenech A, Fenoll A, Linares J, de Lencastre H, Ardanuy C, Sá-Leão R.** 2013. Disease isolates of *Streptococcus pseudopneumoniae* and non-typeable *S. pneumoniae* presumptively identified as atypical *S. pneumoniae* in Spain. PLoS One **8:**e57047. http://dx.doi.org/10.1371/journal.pone.0057047.