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The impact of laboratory characteristics on molecular detection of respiratory syncytial virus in a European multicentre quality control study.

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ABSTRACT

The performance of nucleic acid amplification techniques for detecting respiratory syncytial virus (RSV) was evaluated in 25 laboratories across Europe by an external quality assessment study. In addition, factors related to the diagnostic performance of laboratories were explored. The results of this quality control study show that the performance of laboratories for RSV diagnosis in Europe is good, with an overall correct score of 88%. The type of assay (nested or real-time PCR vs. commercial tests) was identified as a significant factor (OR 8.39; 95% CI 1.91–36.78) in predicting a correct result.

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infections, particularly in infants and the elderly [1–3], and leads to hospitalization and excess mortality [4]. Nucleic acid amplification techniques used for the detection of RSV can be validated by external quality assessment studies [5,6]. The aim of this study was to assess the laboratory performance of RSV nucleic acid amplification techniques and to identify laboratory-related factors that may be used to improve the quality of molecular diagnostic methods for RSV.

Panels with samples were distributed on dry ice by courier service to 33 laboratories (in 23 European countries) in June 2006 by the QCMDNeutral Office in Glasgow, UK. The panel consisted of nine coded samples containing RSV-A and ⁄ or RSV-B (ATCC strain RSV-A-2; ATCC strain RSV-B ⁄WV⁄ 14617 ⁄ 1985 wild type) and one sample negative for RSV. Semiquantitative information was available in the form of sample dilutions. The sample preparation procedure guaranteed uniformity and reproducibility of the panel members. To ensure confidentiality, all participating laboratories received a code number.

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Information on the type of assay (commercial test, single PCR, nested PCR, real-time PCR), number of RSV tests performed per year (<500 and ≥500) and the accreditation of the laboratory was collected in a QCMD questionnaire. An additional questionnaire included information on the date of receipt of the panel (no delay, ≥1 week delay), the target gene, whether the sample was tested for inhibition of the amplification reaction, whether the participating laboratory was a national reference laboratory for RSV, the European region (east, west), and the training level of the laboratory staff (no training, personnel without training or doctoral students; training, personnel with general training and experienced personnel; PCR training, personnel with specific training for performing PCR). It was hypothesized that these factors could be determinants of the performance score, and they were therefore included in an exploratory analysis to determine whether they were indeed related to the performance of the laboratories.

A sum score was defined (i.e. the number of correct results; range 0–10) and used to analyse the performance of the laboratories. Multilevel logistic regression was used [7] (MLwiN) to determine which laboratory characteristics were the best predictors of a correct result. For the dependent variable (sample score: incorrect = 0; correct = 1), a logistic regression model was calculated with the following laboratory characteristics as independent variables: type of assay (two dummy variables (real-time PCR or nested PCR and single PCR); reference category 'commercial assay'), level of training (two dummy variables; reference category 'no training') and number of RSV tests (<500 or ‡500). The real-time and nested PCR were combined in the multilevel analysis, as they were similarly efficient. The data were analysed at the sample level, with ten samples for each laboratory for which all data were available in the questionnaires (n = 18).

The number of participating laboratories was 25 (response rate 76%) from 18 countries, with 20 laboratories from western Europe and five from eastern Europe. The overall mean percentage of correct results was 88%, and ranged from 50% to 100%. The percentage of correct results decreased in correspondence with the decreasing sample concentration (Table 1), and RSV-B was less often correctly detected than RSV-A by a number of laboratories. One false-positive test result (4%) was reported for the negative panel sample. The rate of false-negative results was 14%. The lowest correct performance scores (60% and 72%) at the sample level were observed for samples containing RSV-B only.

The laboratory performance score and its relation to laboratory characteristics is presented in Table 2. Statistically significant differences in means were observed for the type of assay (commercial, single PCR, nested PCR, and realtime PCR: p 0.026). These results suggest that there is a difference in performance scores across the different types of assay, with the highest performance scores coming from laboratories using real-time PCR and nested PCR. Most laboratories used the nucleoprotein gene as target (n = 12) for the PCR, or this gene in combination with the phosphoprotein gene (n = 3) or the surface glycoprotein gene (n = 1). Other PCR targets were the fusion protein (n = 5), surface glycoprotein (n = 1) and polymerase (n = 1) genes. One laboratory used the nonstructural protein-1 gene for RSV-B detection. The performances of laboratories that used two target genes (n = 4) did not differ significantly (p 0.076) from those that used one target gene (n = 19).

[TABLE 1 AND 2]

To study the effect of each of the relevant variables corrected by other variables on the performance score, multilevel logistic regression analysis was performed. The type of assay, level of training and the number of swabs tested were included in the model. The results showed an OR of 8.39 (95% CI 1.91–36.78) for the in-house PCR (nested or real-time PCR) vs. commercial PCR, indicating that the in-house PCRs perform better than the commercial PCRs.

The different types of assay were also assessed separately and compared using a contrast test for fixed effects in MLwiN, resulting in a chi-square test statistic. Significant differences

in performance scores between commercial and nested PCR ($\chi2 = 12.92$; $p < 0.001$) and between commercial and real-time PCR ($\chi2 = 14.62$; $p < 0.001$) were observed. Additionally, significant differences were observed between single PCR and nested PCR ($\chi2 = 3.86$; $0.02 < p < 0.05$) and between single PCR and real-time PCR ($\chi2 = 4.82$; $0.02 < p < 0.05$). The type of method was a significant factor in predicting a correct result. Limitations of the study were the number of non-respondents (24%) and of incomplete questionnaires (28%).

Additionally, no clinical isolates were used and, therefore, only the technical performance could be determined. Although the absolute virus quantity in the samples was unknown, it was possible to use the dilution factors as a semiquantitative measure. Finally, it may be possible that the primers of the molecular assays matched well with the ATCC strains of the panel, but whether this is also true for the circulating viruses that are actually present in clinical samples could not be assessed in this study.

In conclusion:

(1) with an overall correct score of 88%, the laboratories involved in the study are considered to be performing well in the diagnosis of RSV infection; and

(2) the type of assay applied was the only significant factor in predicting a correct result, with real-time and nested PCR performing better than conventional single- step and commercial PCR.

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TRANSPARENCY DECLARATION

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TABLES

Table 1. Performance score and type of assay according to laboratory

	Score by sample type and target dilution						Score total	Type of assay	
Randomized laboratory code	RSV-A 1.0×10^{-5} (n = 2)	RSV-A 2.0×10^{-6} (n = 2)	RSV-A 1.0×10^{-6} (n = 1)	RSV-B 2.0×10^{-5} (n = 2)	RSV-A/B $2.0 \times 10^{-5}/2.0 \times 10^{-4}$ (n = 2)	RSV-negative (n = 1)	% correct score		
2	2	2	1	2	2	1	100	In-house	Real-time PCR
4	2	2	1	2	2	1	100	In-house	Real-time PCR
10	2	2	1	2	2	1	100	In-house	Real-time PCR
15	2	2	1	2	2	1	100	In-house	Real-time PCR
16	2	2	1	2	2	1	100	In-house	Real-time PCR
29	2	2	1	2	2	1	100	In-house	Real-time PCR
7	2	2	1	2	2	1	100	In-house	Nested PCR
12	2	2	1	2	2	1	100	In-house	Nested PCR
28	2	2	1	2	2	1	100	In-house	Nested PCR
32	2	2	1	2	2	1	100	In-house	Nested PCR
6	2	2	1	2	2	1	100	In-house	Multiplex nested PCR
5	2	2	0	2	2	1	90	In-house	Real-time PCR
27	2	2	0	2	2	1	90	In-house	Real-time PCR
3	2	2	1	1	2	1	90	In-house	Nested PCR
1	2	2	0	2	2	1	90	In-house	Semi-nested PCR
11	2	2	1	1	2	1	90	In-house	Two-step RT-PCR
20	2	2	1	0	2	1	80	In-house	Single PCR
31	2	1	1	1	2	1	80	In-house	Real-time PCR
13	2	2	1	0	2	1	80	In-house	Nested with RT second round
22	2	2	1	0	2	1	80	Commercial	Arrow diagnostic fast set RSV-A/B
26	2	0	0	2	2	1	70	In-house	Real-time PCR
9	2	1	1	0	2	1	70	In-house	Nested PCR
21	2	2	1	0	2	0	70	In-house	Single PCR
18	2	1	0	0	2	1	60	Commercial	Euroclone RSV-check kit
33	2	0	0	0	2	1	50	Commercial	Finnzymes RobusT II RT-PCR Kit

RSV, respiratory syncytial virus.

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Table 2. Percentage of correct results for the different laboratory characteristics

		Percentage of correct results (%)		
Laboratory characteristics	*n*	Median	Range	p-value
Type of assay				
Commercial	3	60	50–80	0.026
Single PCR	3	80	70–90	
Nested PCR	9	100	70–100	
Real-time PCR	10	100	70–100	
Number of swabs tested				
<500	9	90	50–100	0.086
>500	10	100	70–100	
Laboratory accreditation				
No	7	100	50–100	0.723
Yes	12	90	70–100	
Delay in receipt panel				
No delay	15	100	60–100	0.199
>1 week delay	10	85	50–100	
Training level				
No training	5	70	50–100	0.080
Training	13	90	70–100	
PCR training	5	100	90–100	
Target gene				
Single target gene	19	90	50–100	0.076
Two target genes	4	100	90–100	
Samples tested for inhibition				
No	18	90	50–100	0.944
Yes	6	95	70–100	
National reference laboratory				
No	17	90	50–100	0.539
Yes	5	90	80–100	
Region[a]				
East	5	100	50–100	0.567
West	20	90	60–100	

Significance of differences was evaluated using the Mann–Whitney *U*-test or Kruskall–Wallis test.

[a]On the basis of the United Nation Statistics Division, the following countries were defined as 'east': the Czech Republic, Lithuania, Latvia, Poland, Romania, Slovenia, Slovakia, Estonia. Western European countries were: Austria, Denmark, Finland, France, Germany, Great Britain, Greece, Ireland, Italy, Malta, The Netherlands, Norway, Portugal, Spain, Sweden and Switzerland.

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