Diagnostic accuracy of digital RNA quantification versus real-time PCR for the detection of respiratory syncytial virus in nasopharyngeal aspirates from children with acute respiratory infection

Maiara L. Bouzas,[1] Juliana R. Oliveira,[1] Artur Queiroz[2], Kiyoshi F. Fukutani,[1,3] Aldina Barral,[1, 2, 4] Annabel Rector,[5] Elke Wollants,5 Els Keyaerts,[5,6] Winke Van der Gucht,[5] Marc Van Ranst,[5,6] Camila I. de Oliveira,[1,2] Johan Van Weyenbergh,[5*] Cristiana M. Nascimento-Carvalho,[1, 7*] and The Acute Respiratory Infection and Wheeze Study Group Phase I and II.

[1]Postgraduate Program in Health Sciences, Federal University of Bahia School of Medicine, Salvador, Brazil; [2]Centro de Pesquisas Gonçalo Moniz (CPqGM), Fundação Oswaldo Cruz (FIOCRUZ), Salvador, Brazil; [3]Department of Biochemistry, Immunology, and Cell Biology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto; [4]Department of Pathology, Federal University of Bahia School of Medicine, Salvador, Brazil; [5]Department of Microbiology and Immunology, Laboratory for Clinical and Epidemiological Virology, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium; [6]Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium; [7]Department of Pediatrics, Federal University of Bahia School of Medicine, Salvador, Brazil; *JVW and CMN-C are both senior authors.

Respiratory syncytial virus (RSV) is one of the most common etiological agents of acute respiratory infections (ARI) among children such as bronchiolitis and pneumonia.[1] Virus-specific molecular assays such as real-time polymerase chain reaction (RT-PCR) are now considered the gold standard in the diagnosis of viral respiratory tract infections, but simultaneous (multiplex) detection of different pathogens is limited which are considered majors limitations.[2] A multiplex digital method of RNA quantification, nCounter (NanoString Technologies), can overcome this disadvantage and identify, in a single reaction, the presence of different respiratory viruses.[3] We aimed to evaluate the accuracy of nCounter (Nanostring Technologies) to identify and quantify RSV-A and RSV-B in NPA of children with ARI using real-time PCR as the reference method.

1. Nascimento-Carvalho CM, Cardoso MRA, Barral A et al. Seasonal patterns of viral and bacterial infections among children hospitalized with community-acquired pneumonia in a tropical region. Scand. J. Infect. Dis. 42 (2010) 839-844.
2. Thorburn F, Bennett S, Modha S et al. The use of next generation sequencing in the diagnosis and typing of respiratory infections. J. Clin. Virol. 69 (2015) 96-100.
3. Geiss GK, Bumgarner RE, Birditt B et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol. 2008 Mar;26(3):317-25.

Funding
This work was supported by Fundação de Amparo à pesquisa do Estado da Bahia (FAPESB; grant PNX0019/2009). MLB was supported by fellowships from FAPESB. KFF was supported by fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), FAPESB and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP no. 2017/03491-6). JRO was supported by fellowships from FAPESB and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). AB, CIO, CMN-C are senior investigators from CNPQ. JVW was supported by FWO (grant G0D6817N).

NPA was collected at enrolment in a prospective cross-sectional study conducted in an Emergency Department from September 2009 to October 2013, in Salvador, Brazil. A quantitative RT-PCR with a subgroup-specific primer and probeset for RSVA and RSV-B was performed in parallel with a customized nCounter probeset containing viral targets in NPA.

Table 1A. Diagnostic accuracy of nCounter to detect RSV-A

		RT-PCR		*Total*
		Yes	No	
nCounter	Yes	55 (74.3%)	8 (1.6%)	63 (11.3%)
	No	19 (25.7%)	477 (98.4%)	496 (88.7%)
	Total	74 (13.2%)	485 (86.8%)	559 (100%)

Table 1B. Diagnostic accuracy of nCounter to detect RSV-B

		RT-PCR		*Total*
		Yes	No	
nCounter	Yes	52 (77.6%)	11 (2.2%)	63 (11.3%)
	No	15 (22.4%)	481 (97.8%)	496 (88.7%)
	Total	67 (12.0%)	492 (88.0%)	559 (100%)

This study group comprised 559 cases and RSV was detected by RT-PCR in 139 (24.9%) cases, RSV-A in 74 (13.2%) cases, and RSV-B in 67 (12.0%) cases. Two (1.4%) were co-infected.

Digital quantification by nCounter, the new diagnostic method, detected RSV in 122 (21.8%) samples, RSV-A in 63 (11.3%) and RSV-B in 63 (11.3%). Co-infections were detected in 4 (3.3%) cases. Interestingly, detection of RSV-A and RSV-B by any method occurred in 158 (28.3%) cases, indicating both methods might be complementary in detecting the complete RSV epidemic.
The validation of nCounter as a qualitative measure (presence vs. absence), considering RT-PCR as the reference standard is shown in Table 1A for RSV-A and in Table 1B for RSV-B.

Overall, accuracy was 95.2% (95%CI:93.1%-96.7%) for RSV-A and 95.3% (95%CI:93.3%-96.9%) for RSV-B. Moreover, quantification of both RSV-A and RSV-B viral RNA was significantly correlated between nCounter and RT-PCR, as shown in Fig. 1A-B.
Again, using RT-PCR as a reference, a significant correlation (Spearman r=0.44, $p=8x10^{-5}$) was found in RSV-A-positive samples (n=74), between quantitative detection by nCounter (measured as normalized counts) and RT-PCR (measured as copies/ml). Similarly, in RSV-B RT-PCR-positive samples (n=67), a significant correlation (Spearman r=0.73, $p=3x10^{-12}$) was found between RSV-B quantitative detection by nCounter and RT-PCR (Fig. 1B

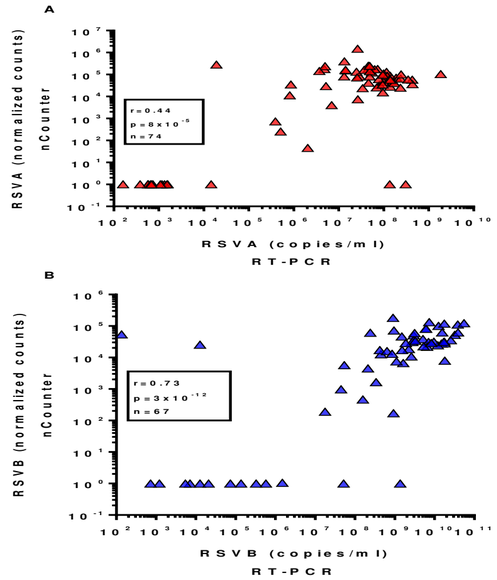

Figure 1: Correlation between RSV-A and RSV-B levels quantified by nCounter and Real-Time PCR. (A) In RT-PCR-positive samples (n=74), a significant correlation (Spearman r=0.44, p=8x10-5) was found between RSV-A quantitative detection by nCounter (measured as normalized counts) and RT-PCR (measured as copies/ml). (B) In RT-PCR-positive samples (n=67), a significant correlation (Spearman r=0.73, p=3x10-12) was found between RSV-B quantitative detection by nCounter (normalized counts) and RT-PCR (copies/ml).

In conclusion, digital RNA quantification of RSV-A and RSV-B by nCounter is highly accurate (>95%), using real-time PCR as a reference. Its robustness, high-throughput multiplex capacity and detection of cases undetected by real-time PCR indicate its suitability for large-scale epidemiological studies.