Comparison of the Mycoplasma Duo Test with PCR for Detection of *Ureaplasma* Species in Endotracheal Aspirates from Premature Infants

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We compared the Mycoplasma Duo kit (Sanofi Diagnostics Pasteur) with PCR for detection of *Ureaplasma* spp. in endotracheal aspirates from 60 premature neonates. The overall agreement between the two tests was 96%. The Mycoplasma Duo assay is a useful alternative to culture and PCR for detection of neonatal *Ureaplasma* infection.

Ureaplasma spp. are an important cause of chorioamnionitis and infections in premature infants (4, 5, 12). Detection of *Ureaplasma* spp. has traditionally relied on culture on special media. The development of a commercially available diagnostic kit (Mycoplasma Duo; Sanofi Diagnostics Pasteur, Marnes la Coquette, France) offers a simpler alternative for the detection of *Ureaplasma* spp. in urogenital and neonatal respiratory samples. With this kit, identification of *Ureaplasma* spp. is based on the hydrolysis of urea with the release of ammonia, signaled by a color change of a pH indicator (phenol red), and results are read within 24 to 48 h. Reports on similar commercial kits have shown high sensitivity compared with culture (1, 6). Recently, PCR has been shown to be a rapid and sensitive alternative test for the detection of *Ureaplasma* spp. in endotracheal aspirates from mechanically ventilated newborns (2, 3, 7, 8, 11). The aim of this study was to compare the performance of the Mycoplasma Duo kit with PCR for the detection of *Ureaplasma* spp. in neonatal endotracheal secretions.

Sixty-eight paired endotracheal aspirates were obtained from 60 premature infants (median gestational age, 26.5 weeks; median birth weight, 805 g) ventilated due to hyaline membrane disease. The paired aspirate samples were all collected within 24 h of each other and were tested for *Ureaplasma* spp., with one sample from each pair being tested by the Mycoplasma Duo assay and the other sample being tested by PCR. Parents gave informed written consent for participation in this study, which was approved by the Canterbury Ethics Committee.

For the Mycoplasma Duo assay, endotracheal secretions were removed under aseptic conditions by repetitive and gentle flushing of the suction catheters with 2 ml of the suspension medium provided in the kit. The specimens were then processed according to the manufacturer's recommendations, and

results were read after incubation for 24 h at 37°C. All results remained unchanged after a second reading performed after a 48-h incubation. For PCR analysis, endotracheal secretions in suction catheters were flushed with 1 ml of phosphate-buffered saline and then centrifuged at a relative centrifugal force of 9,200 for 2 min, and the supernatants were stored at –80°C. DNA was extracted from 200 µl of these aliquots by using a QIAamp DNA mini kit (QIAGEN, Hilden, Germany) and eluted from silica gel matrix by using 100 µl of AE buffer. Ten microliters of the DNA eluate was added to a PCR mixture to give a final reaction volume of 50 µl. The reaction mixture contained 1× PCR buffer without MgCl₂ (Roche Diagnostics), 1.5 mM MgCl₂, a 100 µM concentration of each deoxynucleoside triphosphate, a 0.3 µM concentration of each primer, and 1.25 U of *Taq* DNA polymerase (FastStart; Roche Diagnostics). The primer sequences (coding for a 429-bp urease gene product) used were 5'-CAA TCT GCT CGT GAA GTA TTA C-3' (sense) and 5'-ACG ACG TCC ATA AGC AAC T-3' (antisense), based on the method employed by Blanchard et al. (3). PCRs were visualized by 2% agarose gel electrophoresis and UV illumination after ethidium bromide staining.

All PCR-negative samples were tested for PCR inhibitors with a unique artificial oligonucleotide and primers derived from a novel synthetic construct sequence based on the human beta-actin gene (GenBank accession number U17140). The oligonucleotide was created as the PCR control template. The primer sequences were 5'-AGCGGTGACGCATGCCTTCC A-3' and 5'-CAAAGGAGACATTCTCACGCTACAGTT-3', and the presence of PCR inhibitors was demonstrated by the absence of the expected 92-bp product.

The results of Mycoplasma Duo and PCR testing are shown in Table 1. One tracheal aspirate sample contained PCR inhibitors and was excluded from the data analysis. The paired sample for this particular specimen was negative by the Mycoplasma Duo test. Of the infants who had two pairs of endotracheal aspirates each, five of the six who were initially positive with Mycoplasma Duo and PCR became negative with both tests after receiving erythromycin therapy for an average of 7 days.

We found that there was 96% agreement between the Mycoplasma Duo and PCR assays for detection of *Ureaplasma*

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TABLE 1. Comparison of the Mycoplasma Duo test with PCR for detection of *Ureaplasma* spp. in paired endotracheal aspirates from premature infants

PCR assay result	No. of specimens with the following Mycoplasma Duo result:		Total
	Positive	Negative	
Positive	11	1	12
Negative	2	53	55
Total	13	54	67

spp. in endotracheal samples. This level of agreement is similar to that recorded between culture and PCR (3, 7). For the two samples that were Mycoplasma Duo positive but PCR negative, we speculate that the nature of the processing of our endotracheal aspirate samples may contribute to the outcome. The aspirates allocated for PCR assay had previously been centrifuged at high speed to sediment all cellular material and debris so that clear supernatants could be removed for various other tests as part of a larger study. The availability of only supernatants in our study, rather than cell pellets, may have reduced the quantity of DNA available for PCR analysis and contributed to false negative results.

Culture on semisolid media is considered the "gold standard" for the detection of *Ureaplasma* spp. from clinical samples. We did not include culture in this study, as a previous evaluation in our laboratory showed that the Mycoplasma Duo assay compared well with culture (unpublished data). Briefly, of the 98 female genital swab samples that were tested by both culture on A7 media and the Mycoplasma Duo assay, 39 (40%) were positive by both assays and 52 (53%) were negative by both assays. The remaining seven samples were positive by Mycoplasma Duo but negative by culture. Of these, four were also positive by PCR. Clegg et al. have also reported high sensitivity by using a similar commercial detection kit (Mycoplasma IST; bioMérieux) when validated against culture with vaginal specimens (6).

The Mycoplasma Duo assay is a commercially available kit that is simple to use and has a sensitivity comparable to PCR for the detection of *Ureaplasma* spp. in neonatal endotracheal aspirates. These characteristics make this test suitable for use in diagnostic laboratories that do not currently test for *Ureaplasma* spp. Furthermore, many research proposals are also currently being designed to further clarify the positive or negative associations between *Ureaplasma* spp. and lung inflammation in the development of neonatal chronic lung disease (9, 10, 13, 14). Based on the findings of this study, many more centers may be able to embark on these studies by using the

Mycoplasma Duo kit to detect *Ureaplasma* spp. in neonatal endotracheal aspirates.

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