ARGENE

DEVELOPMENT OF A NEW DIAGNOSTIC TOOL FOR THE DETECTION OF *Bordetella pertussis* BY REAL TIME PCR.

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Introduction

Bordetella pertussis continues to circulate in populations where high vaccination coverage of infants and children is achieved, because the protection after natural infection wanes after 10 to 15 years and protection after vaccination lasts for 6 to 10 years.

Transmission of the disease in highly vaccinated populations occurs mainly from adolescents and adults to infants or among older vaccinated children, adolescents, and adults. Thus, most cases are now observed in unvaccinated infants, older schoolchildren, adolescents, and adults.

Bordetella pertussis is generally diagnosed by culture. Diagnosis by serology is also used but cannot differenciate immune response against wild or vaccinal strains. Real time PCR, sensitive specific and rapid technology, is an effective alternative for *Bordetella* diagnosis.

Materials and Methods

✗ Intra assay reproductibility: the intra assay reproductibility of BORDETELLA R-gene™ assay has been performed on differents samples of *Bordetella pertussis* positive cell cultures.
The experiment was repeated 8 times on the Rotor-Gene 6000™ (Corbett) after automatic extraction with MagNA Pure Compact (Roche) instrument.

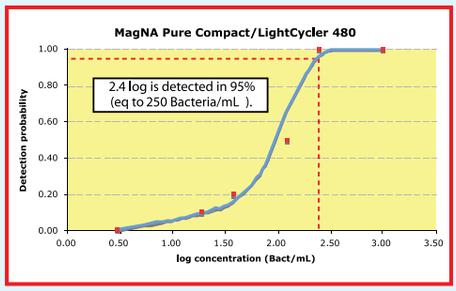
✗ Inter assay reproductibility: the inter assay reproductibility of BORDETELLA R-gene™ assay has been performed on differents samples of *Bordetella pertussis* positive cell cultures. The experiment was repeated 8 times on the SmartCycler (Cepheid) and Lightcycler 2.0 (Roche) after manual extraction with QIAamp DNA blood mini kit (Qiagen).

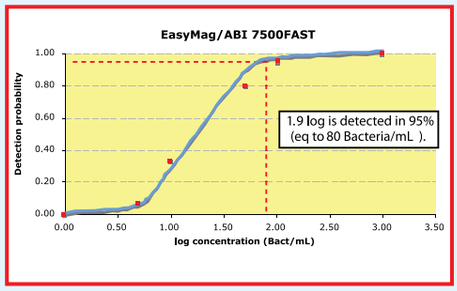
✗ Analytical sensitivity: analytical sensitivity of BORDETELLA R-gene™ assay has been evaluated with sample Quality Control (CNR Paris)(1). Sample contained 10^5 Bacteria/mL. After dilution, 3 extractions were performed, pooled, and amplified 20 times for each point. The experiment was performed on ABI 7500Fast (Applied Biosystem) after EasyMag (Biomerieux) extraction and on LightCyler 480 (Roche) after MagNA Pure Compact (Roche) extraction.

✗ Amplifications: For BORDETELLA R-gene™, 10µL of extracted sample were added to 15µL of ready-to-use amplification premix. An Internal Control, added before extraction step, allowed to check both extraction procedure and presence of inhibitors.

Results

Analytical sensitivity





The analytical sensitivity for *Bordetella pertussis* was **250 Bacteria/mL** with a 95% detection rate after extraction with MagNA Pure Compact instrument and amplification with LightCycler480. The analytical sensitivity for *Bordetella pertussis* was **80 Bacteria/mL** with a 95% detection rate after extraction with EasyMag instrument and amplification with ABI7500Fast.

Reproductibility

Reproductibility intra assay

	CT average	Standard deviation	Coefficient of variation
Dilution 1	25,31	0,09	0,37%
Dilution 2	30,27	0,17	0,56%
Dilution 3	34,02	0,15	0,44%

Reproductibility inter assay

	CT average	Standard deviation	Coefficient of variation
Dilution 1	23,02	0,343	1,49%
Dilution 2	26,45	0,326	1,23%
Dilution 3	29,87	0,642	2,15%

Depending on the quantity of Bacteria in the sample, the coefficient of variation varied from 0.37% to 0.56% for intra assay reproductibility and from 1.23% to 2.15% for inter assay reproductibility. These values demonstrate the good reproductibility of the kit.

Specificity

The specificity of BORDETELLA R-gene™ checked by real time PCR on cell culture and Bacteria strains(2) of the following pathogenics agents :

- *Legionella pneumophila*, *Mycoplasma pneumoniae* and *hominis*, *Chlamydia pneumoniae*, *Haemophilus influenzae*, *Ureaplasma urealyticum* and *Bordetella parapertussis.*
- Human Herpesvirus.
- Adenovirus type 5 and 8.
- JC/BK.
- Echovirus 9 and Poliovirus S1.

No cross reaction were observed with *Bordetella parapertussis*. None of the others virus or bacteria were amplified with BORDETELLA R-gene™ kit, wich clearly proved the specificity of the assay.

Conclusion

Analytical Sensitivity in titered bacteria culture was established below 250 and 80 Bacteria/mL (95%) and the limit of detection (5%) less than 10 Bacteria/mL. Specificity study showed no cross reaction with respiratory bacteria or viruses tested.
Reproductibility inter and intra-assay studies presented showed an excellent robustness and reliability of the kit.
This high quality associated with its compatibility with the major extraction and real time PCR platforms allows an immediate integration in most routine diagnostic laboratories.

(1)We acknowledge N. GUISO and M.L ROSSO (CNR Paris) for Quality control provided.

(2)We acknowledge F. GRATTARD (CHU St. Etienne) for specificity study.