



**Project SOP D-JRP17-
WP5.SOP3 – Brucella PCR -
Bcsp31, IS711, Per genes
Workpackage 5**

Responsible Partner: WBVR

Contributing partners: ANSES, APHA, BfR,
FLI, IZSAM



GENERAL INFORMATION

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DOCUMENT MANAGEMENT

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Dissemination <i>Author's suggestion to inform the following possible interested parties.</i>	OHEJP WP 1 <input type="checkbox"/> OHEJP WP 2 <input type="checkbox"/> OHEJP WP 3 <input type="checkbox"/> OHEJP WP 4 <input type="checkbox"/> OHEJP WP 5 <input type="checkbox"/> OHEJP WP 6 <input type="checkbox"/> OHEJP WP 7 <input type="checkbox"/> Project Management Team <input checked="" type="checkbox"/> Communication Team <input checked="" type="checkbox"/> Scientific Steering Board <input checked="" type="checkbox"/> National Stakeholders/Program Owners Committee <input checked="" type="checkbox"/> EFSA <input checked="" type="checkbox"/> ECDC <input checked="" type="checkbox"/> EEA <input checked="" type="checkbox"/> EMA <input checked="" type="checkbox"/> FAO <input checked="" type="checkbox"/> WHO <input checked="" type="checkbox"/> OIE <input checked="" type="checkbox"/> Other international stakeholder(s): Social Media: Other recipient(s):



BRUCELLA PCR - BCSP31, IS711, PER GENES

According to:

Bounaadja, L., Albert, D., Chénais, B., Hénault, S., Zygmunt, M. S., Poliak, S., & Garin-Bastuji, B. (2009). Real-time PCR for identification of *Brucella* spp.: A comparative study of IS711, bcsp31 and per target genes. *Veterinary Microbiology*, 137(1–2), 156–164. <https://doi.org/10.1016/J.VETMIC.2008.12.023>

Materials

- Taqman Fast Universal PCR master mix (2x)
- Primer Forward 10 µM
- Primer Reverse 10 µM
- Probe 10 µM
- UDG 5U/ul
- PCR-grade water

Stocks: Dissolve primers and probes in H₂O to 100 uM

Work batch: Dissolve stocks 1/10 in H₂O to 10 uM

Primers (for sequences of primers and probes see Reference above)

	Gene		
	IS711	bcsp31	per
Primer FW	IS421	BCSP1163	Per525
Primer RV	IS511	BCSP1199	Per575
Probe	ISTq	BCSPTq	PerTq

PCR pipetting scheme

	Final concentration	volume (ul) per sample
Taqman Fast Universal PCR master mix (2x)	1x	10
Primer Forward 10 µM	0.3 µM	0.6
Primer Reverse 10 µM	0.3 µM	0.6
Probe 10 µM	0,2 µM	0.4
UDG 5U/ul	1U	0.2
PCR-water		3.2
Total:		15

➔ Add 5 ul DNA to 15 ul PCR mix



Tagman RT-PCR Protocol

10' 95C	
15" 95C] 45x
1' 60C	
data collection	