



## Development and analytical validation of a genus-specific *Brucella* real-time PCR assay targeting the 16S-23S rDNA internal transcribed spacer

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### Abstract:

Brucellosis is an economically important bacterial disease of both animals and humans. Annually, about 500,000 cases are reported for human brucellosis. However, true incidence is estimated to be 5,000,000 to 12,500,000 cases per year. The diagnosis of the disease remains a challenge and is underreported, due to inconsistency in reports of bacteriological and serological tests, which lack adequate sensitivity and specificity in the diagnosis of the disease. They also are ineffective in confirming brucellosis during early stages of the disease.

The aim of this study was to develop a 16S-23S ribosomal deoxyribonucleic acid (rDNA) internal transcribed spacer (ITS) quantitative polymerase chain reaction (qPCR) assay for early diagnosis of brucellosis and as a rapid screening tool. To achieve this, blood, milk and tissue samples were spiked with *B. abortus* biovar (bv.) 1 (B01988-18 strain) to determine the analytical sensitivity and specificity of the assay. The efficiency was 105% in tissue, 99% in blood, and 93% in milk. The 95% limit of detection (LOD) of the ITS qPCR assay was highest in tissue, followed by blood, then milk; thus (1.45, 13.30 and 45.54 bacterial genome copies/PCR reaction).

The assay was efficient, sensitive and specific. It detected as little as 1.45 bacterial genome copies/PCR reaction in tissue, making this assay a valuable tool in early detection of the presence of the *Brucella* pathogen. It is sensitive and specific in the diagnosis of brucellosis.

### Biography:

Rejoice Esenam Nyarku graduated with Doctor of Veterinary Medicine (DVM) degree from the School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana in 2015. She completed her Masters degree at the University of Pretoria, South Africa. Rejoice Nyarku has worked with the Veterinary Services Department of Ministry of Food and Agricul-



ture, Ghana as a Project Assistant on a PREDICT-USAID project in collaboration with the wildlife department, VSD. She is currently a PhD student at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria.

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