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Main topic : Surveillance and control of emerging diseases

Dried tube specimen preparation and stability validation for brucellosis serological external quality assessment and quality control materials in resource-limited settings

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Introduction: Brucellosis remains one of the major zoonotic diseases worldwide and requires a One Health approach for early detection and control. One of the crucial components for brucellosis control and spread is timely and reliable laboratory diagnosis. External quality assessment is a key component of laboratory quality assurance to evaluate performance and identify possible insufficiencies in laboratory practices. Implementation of brucellosis external quality assessment in resource-limited countries are rare and challenging due to logistical and financial constraints. The aim of this study was to evaluate the preparation and stability of dried tube specimens for external quality assessment of brucellosis serological testing that could be used in resource-limited countries to avoid logistical and financial limitations associated with use of sera.

Methods: Bovine serum (50 µL) was used to prepare dried tube specimen (DTS). The tubes were left open and allowed to dry at room temperature for 24 hours, then they were kept for 15 weeks at room temperature. The panel for external quality control consisted of 5 samples: one negative and 4 positive samples ranging from weak positive to strong positive. The Rose Bengal Test (RBT), complement fixation test (CFT), enzyme-linked immunosorbent assays (ELISA) and fluorescence polarization assay (FPA) methods were used for stability test and panel validation. The RBT (Biomérieux, France) was performed using a validated kit according to the manufacturer's instruction. The cutoff value for the CFT was >50% of hemolysis inhibition (++) at the 1/4 dilution, corresponding to 20 International Units (IU/mL). The indirect ELISA test for the detection of antibodies against *Brucella abortus*, *melitensis* or *suis* was performed according to the manufacturer (IDVet, France) instruction and test was calibrated according to OIE specifications. The FPA was performed using Brucella FPA test kit (Ellie LLC, USA) according to the manufacturer's instruction.

Results: It was shown that brucellosis DTS were stable at room temperature for 105 days (15 weeks) at minimum. Consistent results were observed for all levels of DTS compared with the liquid samples used to prepare the batch on the day of preparation by RBT during weekly stability testing and at the end of the stability period by CFT, ELISA, and FPA. These data indicate that anti-brucella agglutinating antibodies were not affected by air drying of sera for 24 hours and during indicated stability period of DTS at room temperature.

Conclusion: In conclusion, DTS maintains integrity of serum samples for serological testing of brucella infection and can be a powerful tool for external quality assessment providers, as it decreases huge shipping costs and avoids challenges in maintaining cold chain shipments between the provider and the recipient laboratories. Moreover, it has great prospects for enabling expansion of external quality assessment programs within one health to involve human health laboratories, as well as to include lower tier labs in resource-limited countries to monitor and improve the quality and accuracy of brucellosis testing. Additionally, DTS method could be used for preparation and transportation of clinical samples from remote areas without cold chain logistics.