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**Analytical calibration of batches of ELISA kits used for the indirect diagnosis of Q fever in ruminants: work in progress**

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Introduction
Q fever is a zoonosis whose main reservoir is ruminants. In the latter, Q fever is responsible for abortions. Serological analyzes are carried out for screening, and provide information that is useful for the diagnosis of abortions (in addition to qPCR results) and for epidemiological investigations as well as surveillance. To date, three commercially available ELISA kits are widely used. However, a significant discrepancy rate was observed among them, and no gold standard has yet been established worldwide (1, 2). A global project was therefore undertaken to assess their diagnostic and analytical performances. The primary results revealed that diagnostic performances are variable: some tests are more sensitive but less specific and vice versa (3). Here, the objective is to define additional decision rules in the validation of kit batches to better calibrate the analytical performances around the interpretation cut-off (ICO), which is the critical zone where positive and negative results are segregated.
Methods
The steps consisted in: 1/ providing an experimental scheme and a reference material (RM) supplied by a Reference National Laboratory (RNL), common to the three suppliers, in order to assess the analytical performances (trueness, repeatability, reproducibility) around the ICO; 2/ carrying a preliminary study out, in order to acquire data from theses quality control assays on several batches; 3/ defining statistical criteria to control measurement uncertainty in the ICO area for each of the three kits.
Results
A common procedure was applied by kit producers (30 replicates analyzed on three independent tests for a given batch) to monitor two tracers around the ICO, prepared from the RM. Variability data obtained from at least 10 batches per kit were analyzed. They allowed to specify a single tracer used as a control process for each kit (RM 1:2 for kits A and B, RM at 1:4 for kit C). The means of %S/P obtained for each selected antibody titer were fixed as reference values (26, 58 and 51 % S/P, respectively). Acceptance criteria, such as control limits and a maximum coefficient of variation, were set in order to maintain both trueness and repeatability or reproducibility of the ICO zone for each of the 3 kits.
Conclusions
Methods were defined to standardize the analytical performances of the three ELISA tests, a first step in the harmonization of their results. The process, currently being adopted by the three suppliers of the kits, needs to be monitored and adjusted if necessary. Such knowledge of the ICO critical zone will also allow user laboratories to set modalities for acceptance of a new batch and to develop a control chart based on the assigned value of one RM per kit. This control chart, taking into account all the sequential batches, will constitute a major advance: it guarantees that the kit in use is a standardized operating procedure.