Validation and Cost-effectiveness of an in-house Complement Fixation Test Using locally prepared biological components Compared with a Commercial CFT for the Diagnosis of Bovine Brucellosis

Dalia Agamy[1*], Mahmoud Hashad[1], Mahmoud E. R. Hamdy[2], and Nour H. Abdel-Hamid[2]

1. Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt.
2. Department of Brucellosis Research, Animal Health Research Institute, Agricultural Research Center, Dokki, Giza 12618, Egypt.

* Corresponding author: Dalia Agamy, e-mail address: daliaagamy1998@gmail.com

1. Abstract

This study aimed to develop and validate an in-house Complement Fixation Test (CFT) using locally prepared, titrated, and standardized biological reagents (complement and hemolysin). Diagnostic performance was assessed in comparison with the competitive ELISA (cELISA) and Rose Bengal Plate Test (RBPT), while cost per reaction was compared against a commercial CFT. Between February 2022 and March 2025, blood samples (n = 1265) were collected from private cattle farms in Nile Delta governorates, following reports of late-stage abortions, confirmed human brucellosis cases, and brucellosis seropositive cattle. From these, serum samples (n =119) from bacteriologically confirmed positive and negative cows were used to validate the in-house against the commercial CFT. Diagnostic evaluation revealed that both in-house and commercial CFTs demonstrated superior diagnostic performance, with sensitivity of 91.5%, specificity of 96%, accuracy of 92%, performance index of 187.5, almost perfect kappa agreement (0.810 ± 0.065), positive predictive value (0.99), diagnostic odds ratio (DOR) (258), area under the ROC curve (0.937) at $p<0.05$, 95% CI, compared with the other immunoassays. The in-house CFT exhibited equivalent diagnostic performance to the commercial CFT, although it displayed 25–50% differences in complement fixation titers (1/4 to 1/128). These variations, however, did not affect the interpretation of positive or negative results. The estimated cost per one in-house CFT reaction was significantly lower than that of the commercial CFT. In conclusion, the in-house CFT matched the diagnostic performance of the commercial assay while substantially reducing cost and dependence on imported reagents. Local production of CFT components ensures sustainable availability, expanding the diagnostic capacity for brucellosis and other infectious diseases in resource-limited settings.

Key words: Brucellosis; In-house CFT; Validation; Diagnostic performance; Cost-effectiveness.

2. Introduction

Brucellosis, a critical zoonotic disease caused by *Brucella* species, poses significant animal welfare and economic burdens globally. Primarily affecting livestock and wildlife, it also endangers public health, particularly in regions with suboptimal sanitary conditions, food safety practices, and veterinary services [1]. Human infections typically arise

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from consuming contaminated animal products or direct contact with infected animals [2, 3]. To effectively combat brucellosis, it is imperative to refine diagnostic techniques and implement rigorous screening protocols for newly introduced animals. The isolation and identification of *Brucella* remain the gold standard [4]. Instead of a positive culture, diagnosis is primarily based on serological testing [5]. Advancements in diagnostic strategies are essential to streamline the process and enhance accuracy [2]. The serological diagnosis of brucellosis began over a century ago with a simple agglutination test [6, 4]. The underlying principle of these agglutination tests is the interaction between host antibodies and the smooth lipopolysaccharide (LPS) of the bacterial cell wall. Serological tests that primarily detect IgM antibodies are generally not preferred due to the elevated risk of false-positive results, compromising the test's specificity. As IgG2 and IgA antibodies appear later and in smaller, less consistent amounts, IgG1 is the primary isotype targeted for serological testing. Consequently, assays that measure IgG1 antibodies are considered the most valuable and confirmatory [7, 8].The CFT primarily detects the presence of IgG1, an immunoglobulin indicative of long-term *Brucella* infection. This serological test identifies anti-*Brucella* antibodies capable of activating complement. In cattle, IgG and IgM immunoglobulins can trigger complement activation [9, 10]. The RBPT, CFT, and ELISAs are routinely employed to detect antibodies against *Brucella* species. Nevertheless, CFT continues to be extensively employed as a quantitative confirmatory diagnostic method for brucellosis in reference laboratories globally. Its distinctive balance of sensitivity and specificity renders it the serological test of choice for brucellosis diagnosis [11, 12].Serological testing for brucellosis has long faced challenges related to limitations in sensitivity and specificity. Reduced sensitivity may fail to detect infected animals, which poses significant risks to the effectiveness of disease control programs. Conversely, decreased specificity can lead to the unnecessary culling of healthy animals.

The CFT has been employed for the diagnosis of many bacterial and viral diseases in both human and veterinary medical fields [4].

Although the CFT is technically difficult and labor-intensive, it provides the best blend of sensitivity and specificity and is recommended by WOAH for brucellosis surveillance, control, eradication and is accredited as the test of choice for live animals' international trade. The CFT biological reagent is not always available throughout the year due to a shortage in hard currency, and test fees are often prohibitively expensive in developing countries. As a result, the availability of a nationally distributed CFT kit, as well as the manufacturing of CFT biological reagents, is mandatory for this test.

This study therefore aimed to (i) develop and standardize a in-house CFT using locally prepared biological components, (ii) validate its diagnostic performance in comparison with commercial CFT, RBPT, and cELISA using bacteriological culture as the reference standard, and (iii) assess its cost advantages relative to commercial kits.

It is expected that the sustainable local production of the CFT components will be valuable for performing the CFT as a routine test for brucellosis and facilitate performing the test in the diagnosis of many important animal diseases.

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3. Materials and Methods

3. 1. Study design and sampling

Between February 2022 and March 2025, blood samples (n = 1265) were collected from private bovine farms across the Nile Delta governorates (Egypt), following notification of late-stage abortions, confirmed human brucellosis cases, and seropositive cattle for brucellosis. To compare the in-house CFT based on locally prepared complement and hemolysin reagents with the commercial CFT, 119 serum samples out of 1265 blood samples were separated from animals that were bacteriologically positive and negative for brucellosis. The estimated number of samples from animals of known infection status required for establishing diagnostic sensitivity (DSe) and specificity (DSp) estimates and other diagnostic performance parameters based on 5% error margin and a confidence interval of 95% was 113 serum samples and adjusted to be 119 serum samples [13]. These blood samples were collected from cattle aged 1 to 5 years old. *B. melitensis* biovar 3 has been isolated from animals on these afflicted farms. These selected serum samples (n=119) from bacteriologically positive and negative animals were serologically screened for brucellosis using the RBPT. Four distinct categories were formed depending on the degree of RBPT positivity, which ranged from borderline (1+) to high positive results (4+), with groups 1 (1+) and 2 (2+) containing 25 serum samples and the other two groups (3, 3+, and 4, 4+) containing 24 serum samples. In addition to the four categories listed above, the final group was selected from farms free of brucellosis and with no previous history of *Brucella* infection. It comprises the negative RBPT samples (n= 21)..

3. 2. Serological tests and procedures

All the immune assays performed in this study, as well as the preparation and preservation of crucial components (the complement and hemolysin) used in the in-house CFT, were done in the Experimental Animal Center (BL3) and Brucellosis Research Department, WOAH Reference Lab, AHRI, Dokki, Egypt.

3.2.1. Complement fixation test

3.2.1.1. Locally prepared complement for in-house CFT:

Complement was prepared following the protocol outlined by Alton et al. [8]. Ten adult male guinea pigs (each weighing approximately 300 g) were maintained on a green vegetable diet supplemented with vitamin C for two weeks before blood collection. Blood samples were aseptically collected from each animal into sterile containers and promptly transported to the laboratory. Upon arrival, samples were incubated at 37 ± 2°C for approximately 10 minutes to facilitate partial serum separation. The serum was then clarified by centrifugation at 900 × g for 10 minutes. The clear serum samples were pooled and immediately preserved in Richardson's solution, then kept in a refrigerator at 4 °C for subsequent use.

3.2.1.2. Locally prepared hemolysin for in-house CFT:

Hemolysin was prepared according to the procedures described by Alton et al. [8]. A uniform suspension of washed sheep red blood cells (SRBCs) was prepared using established protocols. Five healthy adult male Balady (native) rabbits, each weighing approximately 3 kg, were selected and inoculated intravenously with the prepared SRBCs' suspension over two weeks to induce hemolysin production (*Rabbit anti-sheep red blood cells*). Rabbits demonstrating satisfactory Haemolysin titers were later exsanguinated under appropriate

conditions. The resulting haemolysin was preserved in glycerol (1:1) and stored at refrigeration temperature for future use.

3.2.1.3. Commercial CFT components:

Commercial Complement was purchased from IDvet, France, while the commercial hemolysin was obtained from Lillidale Diagnostics, UK.

Titration and standardization of in-house and commercial complement and hemolysin were carried out based on the SOP manual of the Brucellosis Reference Department, AHRI *(SOP 1.1.3.7 version 3, 2022)*.

The CFT *B. abortus* antigen S99 antigen was obtained from the Veterinary Laboratories Agency in New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom. Sheep RBCs were obtained from an adult healthy ram using Alsever's solution and standardized to 3% suspensions in complement fixation diluent (CFD). The British version of CFT was carried out utilizing both in-house and commercial components (Complement; IDVet, France, hemolysin; Lillidale Diagnostics, UK). The British CFT version was performed according toWOAH [4].

3.2.2. Rose Bengal Plate Test (RBPT):

Rose Bengal antigen was bought obtained from Lillidale Diagnostics, UK. The RBPT was performed according to the guidelines stated in Alton et al. and WOAH [8, 4]. Any noticeable agglutination within 4 minutes was declared positive for RBPT. The RBPT is a qualitative test, with results recorded as scores ranging from 1+ to 4+ based on the degree of agglutination for comparison with other quantitative tests.

3.2.3. Competitive Enzyme-Linked Immunosorbent Assay (cELISA):

A multi-species (cELISA) was conducted using the AsurDx^{TM} *Brucella* Multispecies Antibodies cELISA Test Kit for the detection of IgGs antibodies against *Brucella abortus*, *Brucella Ovis*, *Brucella melitensis*, and *Brucella suis*. This kit uses purified *Brucella abortus* smooth lipopolysaccharide antigen-coated plates, anti-*Brucella* antibody-HRP horseradish conjugate, and tetramethylbenzidine substrate. The assay was performed and validated according to the kit instructions. The inhibition percentage (IP) was calculated using the formula: 1 - OD (test sample) / mean OD negative control * 100. The interpretation of test results was determined as follows: the sample was considered positive if the IP ≥ 50%. If IP < 50%, the sample was considered negative.

3.3. Statistical Analysis

The diagnostic performance of commercial and in-house CFT, as well as RBPT (screening test) and cELISA (confirmatory test), was estimated using bacteriological examination as the gold standard. The parameters used included diagnostic sensitivity (DSe), diagnostic specificity (DSp), Kappa agreement, receiver operating characteristics (ROC), area under the ROC (AUC) curve, positive predictive value (PPV), and negative predictive value (NPV) at $p \leq 0.05$ with a 95% confidence interval. The parameter values were estimated with SPSS Statistics for Windows version 21.0 (IBM Corp., Armonk, N.Y., USA). The remaining diagnostic performance measures (performance indices, DOR, and accuracy %) were determined using a method previously reported [14,15].

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3.4. Ethical approval

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Cairo University (process number Vet CU11/05/2025/1197), in compliance with national and international ethical standards for animal experimentation

4. Results

4.1. Validation pathway: diagnostic performance of the in-house CFT biologicals versus those of a commercial kit via CFT proper alongside with RBPT and cELISA.

Considering bacteriological examination as the gold standard, diagnostic performance characteristics were estimated in terms of performance indices, diagnostic sensitivity and specificity, receiver operating characteristic (ROC) curves, area under the curve (AUC), positive and negative predictive values (PPV and NPV), diagnostic odd ration (DOR), Pearson correlation coefficients, and Kappa agreement as illustrated by Table (1) and Figures (1) and (2). The diagnostic performance parameters, beginning with the diagnostic sensitivity of the serological tests, were listed in descending order as follows: In-house CFT (91.5%), commercial CFT (91.5%), RBPT (90.3%), and CELISA (89.3%). The matching diagnostic specificities among the same tests were likewise organized in descending order, and they are provided below: in-house CFT (96%), commercial CFT (96%), CELISA (92%), and RBPT (73%). All the performance indices (PI) values for each serological test were recorded (Table 1 and Figure 2) as follows: in-house CFT (187.5), commercial CFT (187.5), CELISA (181.3), and RBPT (163.3). Then again, the accuracy percentage for all serological tests in the current study was calculated to be 92% for in-house and commercial CFT, 90% for RBPT, and 87% for CELISA. Predictive values of the test results are another key tool assessed and documented in Table 1 and Figure 2, such as the positive predictive value (PPV) and negative predictive value (NPV). The PPVs of the serological tests used under the umbrella of this study, arranged in ascending order, were: RBPT (0.92), CELISA (0.98), in-house CFT (0.99), and commercial CFT (0.99). In descending order, the NPVs were in-house CFT and commercial CFT (0.75), CELISA (0.70), and RBPT (0.68).

The ROC curves, which depict graphs of sensitivity on the Y-axis against the false positive rate on the X-axis, were produced and displayed in Figure 1 to compare the findings of the handmade CFT with commercial ones, as well as other serological tests. The AUC, a single alternative accuracy metric for various approaches, was calculated and arranged numerically (Table 1 and Figure 2) as follows: RBPT (0.869), CELISA (0.912), and (0.937) for both handmade and commercial CFT.

All immunoassays in this investigation showed significant agreement with bacteriological examination ($p<0.05$). The calculated k agreement value for CFT (commercial and in-house) was 0.810 ± 0.065, while cELISA and RBPT had equivalent agreement values of 0.747 ± 0.071 and 0.785 ± 0.073, respectively (Table 1 and Figure 2).

Another performance characteristic metric is the diagnostic odds ratio (DOR), which is the ratio of the odds of positivity in diseased animals to the odds of positivity in healthy animals. Commercial and handmade CFT (258) had the highest total DOR values, followed by cELISA (97) and RBPT (25), as shown by Table 1 and Figure 2.

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4.2. Conformity of cELISA, RBPT, and commercial and in-house CFT with the gold standard, bacteriological examination

The proportion of true positives of the evaluated serological tests versus bacteriology, considered as the gold standard in the group, revealed positive results for both bacteriology and serology; the values were estimated as follows: 72.2% for both the in-house CFT and commercial CFT, followed by RBPT and cELISA of 71% each.

The proportion of true negatives of the investigated serological tests versus bacteriology in the group of negative bacteriology and serology was estimated as follows: 20.2% for both the in-house CFT and the commercial CFT, followed by CELISA (19.3%), and (16%) for RBPT.

The proportion of false negatives of the evaluated serological tests versus bacteriology (positive bacteriology + negative serology) was estimated to be: 6.7% for both in-house CFT and the commercial CFT, 7.6% for RBPT, and 8.4% for cELISA.

The proportion of false positives of the evaluated serological tests versus bacteriology, in the group of negative bacteriology and positive serology, was recorded as follows: 0.8% for the in-house CFT and the commercial CFT, 1.7% for CELISA, and 6% for RBPT.

4.3. The comparison of in-house and commercial CFT in terms of costs and benefits

The commercial complement costs in Egyptian pounds for a single reaction are fourteen times as much as the locally made complement (Table 3). Compared to the commercial complement (385 reactions), the locally made complement in a 5 ml volume can accomplish 480 reactions. Additionally, commercial hemolysin was projected to be six times more expensive than locally prepared hemolysin. Locally made hemolysin can execute 9600 CFT reactions in a 2 ml capacity, which is more than commercial hemolysin can. The ultimate cost projected per one CFT reaction based on the locally manufactured complement and hemolysin was less than half the price compared with the commercial CFT (Table 3) after additional charges (60 EGP) were added to both CFTs (commercial and hemolysin).

5. Discussion

Diagnosis of brucellosis is based on clinical history, symptomatic presentation, bacteriological isolation and identification, and serological assays. Nonetheless, each diagnostic serological method presents distinct advantages and limitations [2, 4].

CFT is complex and labour-intensive; it necessitates daily component titration, anti-complementary results, prozoning, false positive reactions in samples collected from vaccinated animals with smooth vaccines, and detection of cross-reacting antibodies resulting from exposure to other microorganisms such as *Salmonella urbana O:30, Escherichia coli O:157, and Yersinia enterocolitica O:9* [15].

However, CFT is widely recognized as a confirmatory test with minimal occurrence of nonspecific reactions and thus prescribed by WOAH for international trade and recommended by WOAH for the contribution of disease eradication and herd/flock disease surveillance [4].

A consistent and sustainable supply of diagnostic reagents is essential for reliable detection and surveillance. However, in developing countries like Egypt, nearly all diagnostic materials are imported. This dependency imposes a significant burden on national reserves of

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foreign currency, which must be maintained throughout the year. Consequently, due to foreign currency shortages, we prepared and preserved two crucial components essential for performing the CFT, complement and hemolysin, according to the procedure adopted by Alton et al. [8], with some modifications as an initial step towards the future manufacture of all the CFT reagents. This allows decreasing the financial burden of performing the sophisticated confirmatory test, CFT, on a wide scale via central labs for brucellosis diagnosis, as well as the WOAH, Egypt's brucellosis reference lab. Furthermore, assuring the availability and sustainability of CFT reagents throughout the year is essential. Thus, facilitates performing the confirmatory test on a routine daily basis and not being restricted to research purposes only.

The first step after preparing the complement and hemolysin locally is to validate the in-house CFT to confirm that its performance does not differ significantly from that of the commercial reagent-based CFT used in our labs. The second step is to ensure that the test's cost is no longer a burden on national foreign currency reserves and that test components are available throughout the year.

Validation is the process used to assess whether an assay, having been properly developed, improved, and standardized, is suitable for its intended application [13]. Validation includes estimations of a test's analytical and diagnostic performance [13]. The gold standard for diagnosing brucellosis is bacterial typing of *Brucella* [4,13]. To validate the in-house CFT, 119 serum samples (2% error, 95% confidence interval) from culture-positive and -negative animals were selected as negative and positive reference samples. These samples have been used to compare the diagnostic performance parameters of in-house CFT with commercial CFT, as well as other immunoassays used in this study (RBPT and cELISA) for the diagnosis of brucellosis in cattle. Validation was performed according to the principles and methods of validation of Diagnostic Assays for infectious diseases [13].

The evaluation of diagnostic performance parameters revealed distinct differences among the assays tested in this study; results were tabulated in Table 1 and Figure 2. Starting with the most critical parameters, the diagnostic sensitivity and specificity, which are particularly reveal for identifying infected herds or confirming the absence of infection in herds declared free from brucellosis [4]. Where the in-house and commercial CFT both gave the highest diagnostic sensitivity and specificity values (DSe = 91.5%, and DSp = 96%), followed by CELISA with (DSe = 89.3% and DSp = 92%), and (RBPT) with (DSe = 90.3% and DSp = 73%). The RBPT demonstrated higher diagnostic sensitivity at the expense of its lower specificity compared with the CELISA. Hence, it matches its use as a screening test, making it a frontlineinitial screening tool for brucellosis diagnosis under field conditions [4, 8].

The European RBT antigen, employed in this study, was standardized to be positive with WOAH international standard serum (WOAHISS) at 1/45 dilution, and to give a negative reaction at 1/55 dilution. However, the above standardization against the WOAHISS is probably a cause of the reduced sensitivity of some RBPT antigen batches for diagnosing brucellosis [4], and this explains the slight decrease in the sensitivity of the RBPT (90.3%) compared with the commercial or in-house CFT (91.5%) used in this study.

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CFT, whether commercial or in-house, provides a superior balance of DSe and DSp compared to the immunoassays used in this investigation [16, 17, 18]. This could be attributable to the CFT's preference for detecting IgG1, which is indicative of long-term (chronic) *Brucella* infection [8]. Since only the IgG1 isotype of antibody fixes complement efficiently, the CFT specificity is high [19]. On the contrary, these findings contradict that published by Pfukenyi et al. and Nazir et al. [20, 21], because some commercially available cELISA kits' methods are less sensitive or specific than others. Thus, the data from different assays are not always comparable [4].

The Diagnostic Odds Ratio (DOR) is a single statistic that summarizes the performance of a diagnostic test, especially in the setting of binary categorization (positive or negative). DOR values range from 0 to infinity, with higher values reflecting discriminatory test performance [14]. A value of 1 indicates that a test cannot distinguish between diseased and non-diseased animals. Values less than 1 show improper test interpretation (a more negative test among the ill) [14]. When DSe or DSp approaches perfection, the DOR increases dramatically. The DOR results for both commercial and in-house CFT, as reported in Table 1, demonstrated the highest DOR (258) among the immunoassays utilized in this investigation. This indicates CFT's capacity to distinguish between diseased and healthy animals. The reason for improved DOR results is that CFT (commercial and in-house) had better DSe and DSp in the diagnosis of brucellosis in cattle than the immunoassays used in this study.

While in terms of the performance indices and accuracy%, which represent the average of the test DSe and DSp indicating the overall accuracy of the test, the higher the test value, the more balance the test gives between sensitivity and specificity, and CFT (in-house and commercial) gave the highest PI and accuracy% value (PI =187.5 and accuracy = 92%), followed by cELISA (PI =181.3 and accuracy = 90%), and the lowest values were for RBPT 8% (PI =163.3 and accuracy = 87%

The PPV is the possibility that an animal that has tested positive is positive concerning the true diagnostic status [13]. Predictive values are not inherent characteristics of a specific diagnostic test but are a function of DSe and DSp in a defined population at a given point in time. Predictive values are of foremost importance to field veterinarians for the interpretation of results. For example, a PPV of CFT (commercial and in-house) in this study, as shown in Table 1, is 0.99, meaning that an animal with a positive test result to CFT (commercial and in-house) has a 99% chance of indeed being infected and a 1% probability of testing falsely positive. The PPV of RBPT is 0.92 (Table 1), meaning that an animal reacting positively to RBPT has a 92% chance of indeed being infected and an 8% probability of testing falsely positive. The PPV of cELISA is 0.98 (Table 1), meaning that an animal reacting positively to RBPT has a 98% chance of indeed being infected and a 2% probability of testing falsely positive.

The NPV is the possibility that an animal that has tested negative has a true negative diagnostic status [13]. If the aim is to establish evidence for freedom from disease, the NPV is the most important measure. The highest NPV revealed by PCR techniques (Table 1) targeting *Brucella* DNA in cattle sera was achieved by CFT (commercial and in-house) (0.75), followed by cELISA (0.70) and RBPT (0.68). The better NPV of CFT (commercial and in-house) and cELISA

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indicates that, among those who had negative test results, the probability of being disease-free was 75% and 70% for CFT and cELISA, respectively. The reason for this finding may be attributed to the better DSe offered by the CFT (commercial and in-house), as the NPV critically depends on DSe.

The ROC curves, which exhibit plots of sensitivity on the Y-axis against the false positive rate on the X-axis, were constructed (Figure 1) to evaluate the results of CFT (commercial and in-house), RBPT, and cELISA. The closer the curve is to the y-axis and top boundary, the better the test results. Both commercial and in-house CFT demonstrate superior performance based on the ROC curve, followed by CELISA and RBPT. The AUC can therefore be estimated as a single alternative accuracy metric for the employed immunoassays [22]. The AUC values range from 0.5 (no apparent accuracy) to 1 (perfect accuracy), with higher values suggesting better test performance [22]. In the context of this study, CFT (commercial and in-house) has a high accuracy (Table 1 and Figures 1 and 2) based on ROC and AUC values (0.937) when compared to cELISA (AUC = 0.912) and RBPT (0.869) as evidenced by the lower FP (1) and FN (8) results shown by the CFT (commercial and in-house) if compared with the other serological test, as shown in Table 1.

According to the classification proposed by Landis and Kock [23], kappa (κ) values are interpreted as follows: values less than 0 indicate no agreement; 0.00–0.20 indicate slight agreement; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, substantial; and 0.81–1.00, almost perfect agreement.

Based on this classification, commercial and in-house CFT displayed a better agreement with bacteriological examination (almost perfect) over RBPT (0.785 ± 0.073), and CELISA (0.747 ± 0.071) (substantial agreement).

No single serological test is adequate for all epidemiological conditions or animal species; all tests have limitations, such as false positives and negative results, particularly when testing individual animals [4]. The CFT, either using commercial reagents or the CFT using locally prepared biological components, displayed the lowest false-positive result (n = 1) (FPR) and false-negative result (n = 8) (FNR) compared with the other immunoassays employed in this study (Table 2). FPR and FNR may be attributed to recent immunization with smooth *Brucella* vaccines (e.g., S19 & Rev1) that can yield false-positive results.

Comparing the cost-effectiveness of locally prepared CFT components to the commercial CFT, the locally prepared complements in a 5ml volume can accomplish 95 CFT reactions more than the commercial ones (Table 3). The locally prepared hemolysin in a 2 ml capacity could do 5760 CFT reactions more than the commercial ones. The predicted cost per one CFT reaction using locally made complement and hemolysin was less than half that of the commercial CFT (Table 3).

6. Conclusions

The in-house CFT, using locally prepared reagents (complement and hemolysin), demonstrated the same performance compared with the commercial counterpart using the same version of the CFT (British version). Both offer superior diagnostic performance and the highest balance between sensitivity and specificity among all the demonstrated tests. However, there are 25% to 50% complement fixation differences in the titres ranging from 1/4 to 1/128 between the commercial and in-house, without affecting the interpretation of results, positive or

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negative, with 20 ICFTU/ml as a cut-off point. An international standard positive serum sample expressed as international unit/ml anti-brucella antibodies was dealt with as a sample. Therefore, in spite of differences in the test titres, the IU would be the same as related to the standard.

The in-house CFT offers a cost-effective solution by decreasing the ultimate cost projected per one CFT reaction, less than half the price compared with commercial ones, and no longer a burden on national reserves of foreign currency, especially if all CFT biological reagents are locally manufactured. Thus, ensure the availability and sustainability of CFT reagents all year round and widen the scope of the laboratories that confirm brucellosis results by CFT. The production of standardized in-house complement and hemolysin could be applied in CFT for the diagnosis of other highly infectious diseases, namely, Dourine, Glanders, and EIA. Overall, the local production and validation of diagnostic reagents represent a sustainable approach to enhancing disease surveillance and control, particularly in resource-limited settings.

Conflict of interest: Nothing to declare

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Table (1): Performance parameters' estimates for the commercial and in-house CFT versus screening and confirmatory immunoassays used for the diagnosis of bovine brucellosis

Serological tests					Diagnostic sensitivity (%) (SPSS)	Diagnostic specificity (%) (SPSS)	Area under the ROC curve (AUC) (SPSS)	Diagnostic odds ratio (DOR) (TP/FN)/(FP/TN)	Kappa agreement (* κ value) (SPSS)	Positive predictive values (PPV) TP/(TP + FP)	Negative predictive values (NPV) TN/(TN + FN)	Accuracy % (TP+TN)/ (TP+TN+FP+FN)	performance indices (PI)
RBPT	**TP**	**TN**	**FP**	**FN**	90.3%	73%	0.869	25	0.785 ± 0.073**	0.92	0.68	87%	163.3
	84	19	7	9									
CELISA	**TP**	**TN**	**FP**	**FN**	89.3%	92%	0.912	97	0.747 ± 0.071**	0.98	0.70	90%	181.3
	84	23	2	10									
CFT (Commercial)	**TP**	**TN**	**FP**	**FN**	91.5%	96%	0.937	258	0.810 ± 0.065**	0.99	0.75	92%	187.5
	86	24	1	8									
CFT (in-house)	**TP**	**TN**	**FP**	**FN**	91.5%	96%	0.937	258	0.810 ± 0.065**	0.99	0.75	92%	187.5
	86	24	1	8									

*: agreement with CFT at $p < 0.05$ with confidence interval of 95%, **: κ value ± standard error. The abbreviations TP, FP, FN, and TN symbolize the number of respectively, true positives, false positives, false negatives, and true negatives in view of bacteriology as a gold standard. DOR = diagnostic odds ratio, AUC = area under the ROC curve estimated at a confidence interval of 95%.

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Table (2): Agreement of commercial and in-house CFT, CELISA, and RBPT used for the diagnosis of bovine brucellosis versus bacteriological examination.

Groups	CFT commercial		CFT in-house		CELISA		RBPT	
	No.	%	No.	%	No.	%	No.	%
Positive bacteriology + positive serology	86	72.2	86	72.2	84	71	84	71
Positive bacteriology + negative serology	8	6.7	8	6.7	10	8.4	9	7.6
Negative bacteriology + positive serology	1	0.8	1	0.8	2	1.7	7	6
Negative bacteriology + negative serology	24	20.2	24	20.2	23	19.3	19	16

Table (3): Cost benefits profits offered by in-house CFT versus the commercial CFT used for the diagnosis of bovine brucellosis

Cost benefits' parameters	CFT (Commercial)	CFT (In-house)
Cost of complement/Rx (EGP)	70 EGP	5 EGP
Number of reactions/5ml complement	385 reactions	480 reactions
Cost of hemolysin/Rx (EGP)	20 EGP	3.5 EGP
Number of reactions/ 2ml hemolysin	3840 reactions	9600 reactions
Other costs (EGP)	60 EGP	60 EGP
Cost of CFT/ reaction	150 EGP	68.5 EGP

Rx; reaction, EGP; Egyptian pound

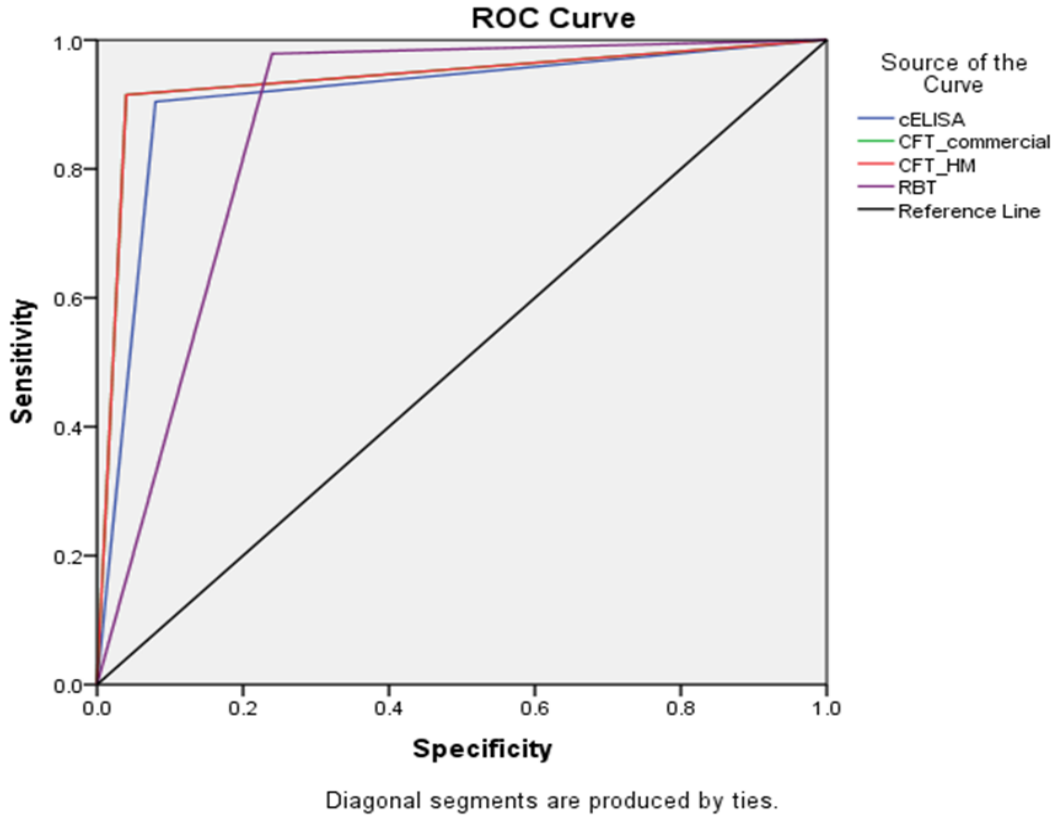

Fig. (1): ROC curves reflecting the diagnostic performance of CFT (commercial and in-house) in comparison with RBPT and cELISA

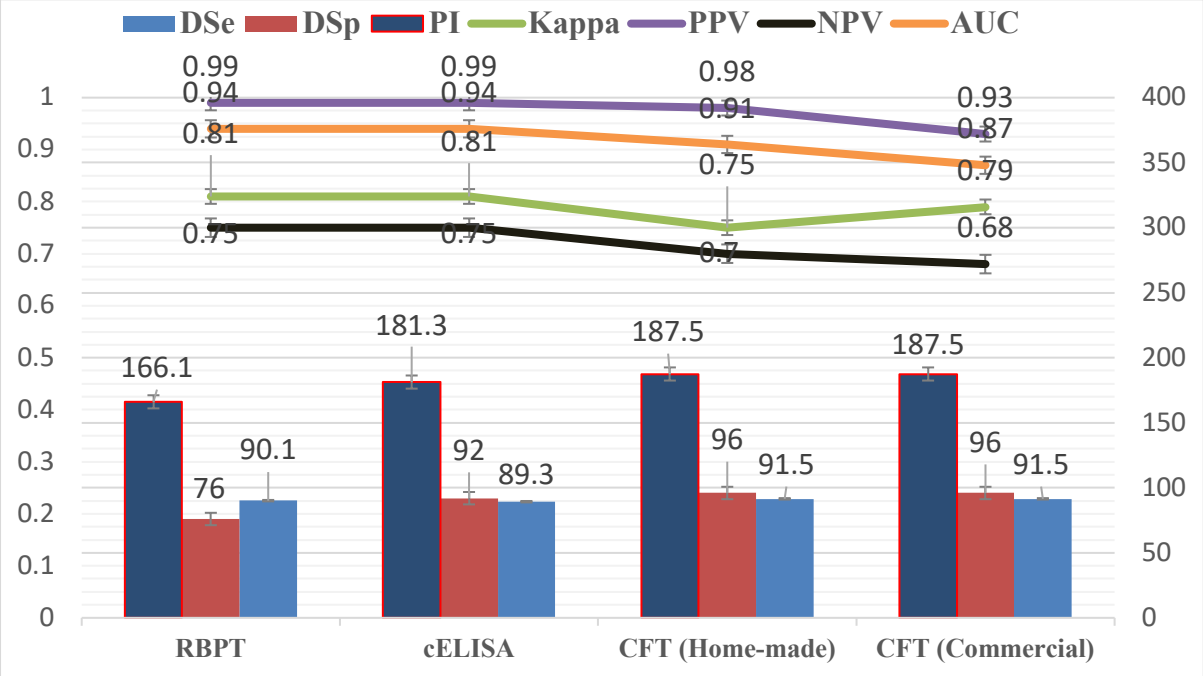

Fig (2): Diagnostic performance figures of the CFT (commercial and in-house) versus RBPT and cELISA in terms of Sensitivity, Diagnostic Specificity, Performance index, Kappa agreement, Positive predictive value, Negative predictive value, and area under the ROC curve.