Alexandria Journal of Veterinary Sciences
www.alexjvs.com

AJVS. Vol. 53: 11-20. April 2017
DOI: 10.5455/ajvs.256953

Development and Validation of Protein G Based Indirect ELISA Versus Lateral Flow Assay as Screening Immunoassays for Brucellosis in Camels *(Camelus Dromedarius)*

Nour H. Abdel-Hamid *[1], Rania I. Ismail[1], Eman I. M. Beleta [1], Hazem M. Ghobashy[1]

[1]Department of Brucellosis Research, Animal Health Research Institute, Nadi El-Seid Street, Dokki, Giza 12618, Egypt

Key words:
Brucellosis; LFA; iELISA; camel; ROCs; protein G.

Correspondence to:
Nour H. Abdel-Hamid: nour78_78@yahoo.com

Abstract

Two hundred selected positive and negative serum samples were collected from dromedary she-camels aged from 1.5-3 years during the period of January 2015 to February 2016 from the market and abattoirs in Halayeb-Shalateen and Abu-Simbel districts and with a history of *Brucella melitensis* biovar 3 isolation. This was aimed for validation of lateral flow assay (LFA) and in-house indirect enzyme-linked immunosorbent assay (iELISA) to be used in the diagnosis of camel brucellosis. The highest relative sensitivity (98%) was achieved by in-house iELISA coated with lipopolysaccharide antigen and with protein G conjugate -for the first time- in the diagnosis of camel brucellosis. LFA offered better performance in terms of relative sensitivity (92%), relative specificity (92.5%) and performance index (184.5). The overall performance of the LFA and in-house iELISA in camels based on both receiver operating characteristic curves (ROCs) and area under ROCs (AUCs) was very good being equal or closer to 0.9. LFA revealed better accuracy than other screening immunoassays tried. The highest positive predictive value was achieved by LFA (0.95), while the corresponding highest negative predictive value (NPV) was attained by in-house iELISA (0.97). All the screening serological tests agreed significantly with LFA and in-house iELISA (LPS) at p value < 0.05. Based on the large association between both LFA, in-house iELISA and the other immunoassays, along with their better diagnostic performance characteristics, authors concluded that both tests were fit for their purpose and valid to be used as rapid screening tests for camel brucellosis as well as useful additions tools to the control and eradication programs in such animal species.

1. INTRODUCTION

Camel population is rising as Egypt imports large numbers of camels from east African countries to compensate the gap in meat production. The final destination of imported camels from Somaliland, Ethiopia and Sudan is either markets like Birqash camel market in Giza, or slaughterhouses (Sayour et al., 2015a).

The symptoms of brucellosis in camels are not clearly defined. Consuming *Brucella* infected milk and meat from camels has led to a high number of human infected cases and serious public health problems (Gwida et al., 2011). In long lasting infected (serologically positive) dromedaries which give birth to healthy offspring, *Brucella* organisms are unlikely to be isolated from expelled placentas usually without *Brucella* shedding in milk and the blood of camel calves is often negative to both culture and polymerase chain reaction (Von-Hieber, 2010). Remarkably, all camel calves of serologically positive dams are almost serologically negative to RBT and cELISA techniques at the age of six months.

Rose-Bengal test (RBT), complement fixation Test (CFT), slow agglutination test (SAT), and ELISAs are commonly used for the detection of

antibodies to Brucella spp. The sensitivity of RBT achieves the requirements for surveillance of free areas at the flock level but it is believed that only the combination of RBT and CFT in infected herds/flocks can obtain accurate individual sensitivity in combined eradication (test-and-slaughter) programs (European Commission Regulation, 2002). It is presumed that serological tests used for the diagnosis of Brucella infection in cattle may also be adequate for brucellosis in camels (OIE, 2016). However, no validation for camel sera was done yet (Gwida et al., 2011).

The LFA is a simplified form of ELISA for the qualitative detection of specific antibodies in serum, whole blood and milk samples. The assay is based on the binding of bio-recognition molecules (specific antibodies) to an antigen immobilized on a test strip and bound antibodies are visualized using a secondary antibody conjugated to colloidal gold particles (Shome et al., 2015).

The aim of the current research was to evaluate the diagnostic performance of lateral flow assay (LFA) and home-made indirect enzyme-linked immunosorbent assay based on protein G for the diagnosis of camel brucellosis and to validate their performance statistically with their peers.

2. MATERIALS AND METHODS

2.1. Samples

A total of 2530 blood samples were collected from Sudanese she-camels at market and abattoirs located in Halayeb- Shalateen and Abu-Simbel districts, with age range from 1.5-3 years old, during the period of January 2015 to February 2016. Sera were separated and divided into aliquots and stored at -20°C until examined. Serum samples were serologically tested with BAPA and RBPT (3%). Two hundred selected positive and negative serum samples (95% confidence interval; 2% error) were chosen from the previously examined samples for the validation of different serological tests used in this current research according to (OIE, 2013) regulations. She-camels from which blood samples were collected, had no history of vaccination against brucellosis and a history of *Brucella melitensis* biovar 3 isolation (Abou-Eisha, 2000; Ibrahim et al., 2016).

2.2. Serological tests

2.2.1. Serum samples were serologically examined against brucellosis using screening tests, *viz*. buffered acidified plate antigen (BAPA), Rose-Bengal (RBT 8%), Rose-Bengal (RBT 3%), lateral flow assay and home-made indirect enzyme linked immunosorbent assay (iELISA), in addition to confirmatory tests; complement fixation (CFT) and competitive enzyme-linked immunosorbent assay (cELISA).

2.2.2. RB 8%, RB 3% and BAPA antigens were purchased from NVSL/DBL, USDA, USA. RB (8%), RB (3%) and BAPA tests were performed according to (Alton et al., 1988). Although qualitative, the BAPA and RBT (8% and 3%) results were recorded as scores from 0 to 4+ according to the degree of agglutination within 4 min. for RBPT and 8 min. for BAPA.

2.2.3. Antigen for the American CFT was imported from NVSL/DBL, USDA, USA. Complement and hemolysin were prepared and preserved according to (Alton et al., 1988) and were titrated according to (Hennager, 2004). Warm fixation of complement was performed according to American technique cited in (Hennager, 2004**;** Sayour et al., 2015b). Results of CFT were converted to ICFTU/ml and interpreted as positive at a cutoff point of $\geq$ 20 ICFTU/ml (OIE, 2016).

2.2.4. Multispecies competitive ELISA kit (SVANOVIR® Brucella-Ab C-ELISA), was produced by Svanova Biotech AB, Uppsala, Sweden. This kit uses *Brucella abortus* smooth lipopolysaccharide antigen, horseradish peroxidase conjugated goat anti-mouse IgG monoclonal antibodies and tetramethylbenzidine in substrate buffer containing H_2O_2. Validation of the kit was done according to the kit instructions, the validation guidelines of the (ISO/IEC 17025, 2005). The test was performed according to the kit instructions and the percent inhibition (PI) was estimated for competitive ELISA kit from the formula: PI = 100 – [(Mean OD samples×100)/ (Mean OD Conjugate control)]. The status of a test results was determined as follows: PI Status $\geq$ 30% was considered positive. If < 30%, the test was negative.

2.2.5. Anigen Rapid Camel Brucella Ab Test Kit was manufactured by BIONOTE, Inc. 22 samsung1ro 4-gil, Hwaseong-si, Gyeonggi-do 445170, Republic of Korea: It is a chromatographic immunoassay for the qualitative detection of *Brucella melitensis*, *abortus* or *suis* antibodies in camel serum, plasma, whole blood and milk. The test was performed and validated according the kit instructions and results interpreted as positive if test (T) line and

control (C) line appear within the result window to indicate the presence of the antibodies and interpreted as negative if only control (C) line appears in the result window.

2.2.6. In-house iELISA (LPS):

Brucella lipopolysaccharide (LPS) from *Brucella abortus* reference strain 544 was extracted by hot phenol method (Biancifiori et al., 1996; OIE, 2004). The procedure of in-house iELISA using LPS was performed and cutoff point was estimated according to (Crowther, 2009). Protein G, horseradish peroxidase conjugate 1/5000 dilution was obtained from Invitrogen, Ltd. Paisley, UK. OPD tablets, product number P9187 and phosphate-citrate buffer tablet, product number P4809 and 30% hydrogen peroxide (product No. H1009) were obtained from Sigma-Aldrich, Inc., Saint Louis, Missouri 63103, USA. For standardization, three OIE ELISA Standard Sera were used. These contained of a strong positive (OIEELISASPSS), a weak positive (OIEELISAWPSS) and a negative (OIEELISANSS) standard. The test was done and the cutoff point was estimated according to (Crowther, 2009).

2.3. Statistical analyses

All the following analyses were performed using IBM® SPSS® Statistics, Version 21, IBM Corporation, 2012.

2.3.1. Kappa (κ) agreement and relative sensitivity and specificity: The kappa (κ) agreement of LFA, in-house iELISA (LPS) and cELISA with screening serological tests used in diagnosis of camel brucellosis as well as relative sensitivity/ specificity pairs were also estimated using CELISA in place of the gold standard (OIE, 2013) and (Sayour et al., 2015b).

2.3.2. Diagnostic performance characteristics parameters of serological tests: This involved the calculation of PPV (positive predictive value), NPV (negative predictive value), LR+ (likelihood ratio of a positive result), LR- (likelihood ratio of a negative result), and PI (performance indices). These were calculated according to (Loong, 2003; McGee 2003; Gall and Nielsen, 2004; Macaskill et al., 2010).

2.3.3. Receiver operating characteristics (ROC) curves: Data obtained from ROC curves including the area under the curve (AUC) representing accuracy were done according to (Hanley and McNeil, 1982).

2.3.4. **Pearson's chi-square test** of independency (χ^2): it is used to test if there is a relationship between both LFA and in-house iELISA and the screening serological tests used in the diagnosis of camel brucellosis. The effect size of the significant associations were estimated by phi coefficient factor according to (Cohen, 1988).

3. RESULTS

3.1. Table 1 reveals the relative sensitivities of screening serological tests in camel which arranged in ascending order as follows: RBPT 8% (85%), LFA (92%), RBT 3% (93), BAPA (97%) and in-house iELISA LPS (98%). The corresponding diagnostic specificities among the same tests were recorded as follows in-house iELISA LPS (75%), RBPT (82.5%), BAPA (82.5%), RBPT 3% (85%) and LFA (92%). All PI values for each serological test was tabulated in Table 1 and charted in descending order as shown in Fig. (2) to be as follows LFA (PI=184.5), BAPA (PI=179), RBPT 3% (PI=178), in-house iELISA LPS (PI=173) and RBPT 8% (PI=167.5). While the accuracy % for all serological tests under the current study was calculated to be 92%, 91%, 90%, 89% and 84% for LFA, BAPA, RBPT 3%, in-house iELISA LPS and RBPT 8% respectively as shown by Table 1 and Fig. 2. Based on the ROCs and AUCS, the performance of the screening serological tests used in diagnosis of camel brucellosis can be arranged in descending order as follows, LFA, BAPA, RBPT 3%, iELISA, RBPT 8% of 0.921, 0.896, 0.892, 867 and 838. 3.2. Table 2 technically compares both LFA and in-house iELISA in dromedary camels with screening serological methods once again based on the determination of other performance characteristic parameters rather than relative Se and Sp, PI, accuracy%, ROCS and AUCS, *viz.* PPV and NPV, likelihood ratios. The PPVs as arranged in descending order of screening serological tests in camel were LFA (0.95), RBT 3% (0.9), BAPA (0.89%), RBPT 8% (0.88%) and in-house iELISA (0.86). The corresponding picture for the NPV as arranged in descending order of screening serological tests in camel were LFA in camel were in-house iELISA LPS (0.97), BAPA (0.94), RBPT 3% (0.89), LFA (0.88) and RBPT 8% (0.79). The highest LR+ in camel (Table 2) was achieved by LFA (12) followed by RBPT 3% (6.22). The smallest LR- in camel among screening tests (Table 2) achieved by in-house iELISA (0.02) and BAPA (0.04).

3.3. Table 3 assesses the matching of results through estimating the kappa (κ) agreement of LFA, in-house iELISA (LPS) and cELISA with the screening serological tests. The estimated κ agreement values with the LFA in camel indicated substantial agreement in case BAPA, RBPT (3% and 8%) and in-house iELISA (LPS), where the values ranged from 0.709 to 0.769 with slightly better agreement of LFA with BAPA (0.769). the corresponding κ agreement values in case of in-house iELISA (LPS) with the other screening tests indicated substantial agreement in case RBPT (3% and 8%) and LFA, where the values ranged from 0.681 to 0.758 with exception of almost perfect agreement with BAPA (0.819). almost perfect agreement of both LFA and in-house iELISA achieved with cELISA.

3.4. Table 4 includes Chi-square test of independency in camels revealed the following results. There is statistical significant association between LFA and BAPA, RBPT 8%, RBPT 3% and in-house iELISA (LPS) tests, χ^2 (1, N=200) 120.857, $p < 0.05$, χ^2 (1, N=200) 101.572, $p < 0.05$, χ^2 (1, N=202) 101.185, $p < 0.05$ and χ^2 (1, N=200) 110.667, $p < 0.05$ respectively. In the other side the estimated Pearson's chi-square test of independency (χ^2) between in-house iELISA and the other screening serological tests reveals statistical significant association between in-house iELISA (LPS) and BAPA, RBPT 8%, RBPT 3% and LFA, χ^2 (1, N=200) 135.392, $p < 0.05$, χ^2 (1, N=200) 98.016, $p < 0.05$, χ^2 (1, N=202) 117.682, $p < 0.05$ and χ^2 (1, N=200) 110.667, $p < 0.05$ respectively.

3.5. Fig. 3 reveals negative (right strip) and positive reactions (left strip) of the lateral flow assay

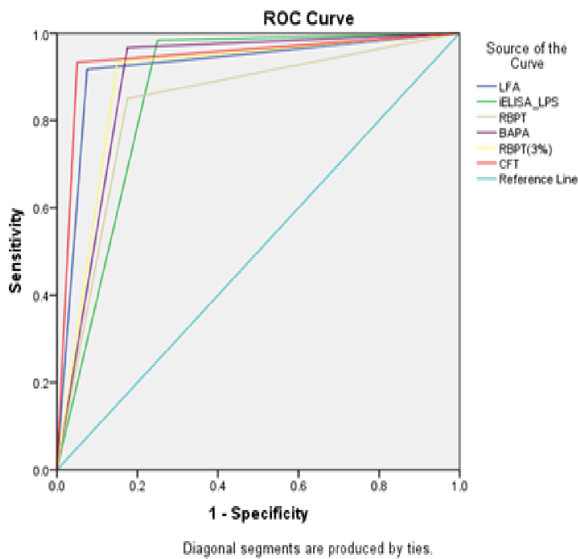

Fig. (1) ROC curves viewing diagnostic performance of serological test categories in camel Immunoassay accuracy = area under the ROC curve

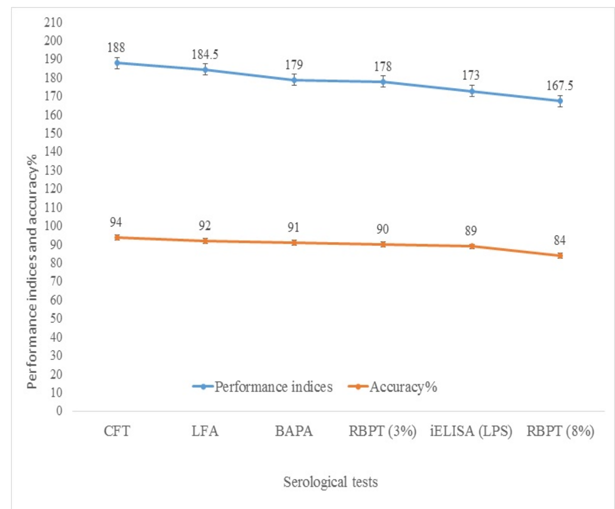

Fig. (2) Performance indices and accuracy % of different serological tests used in the diagnosis of camel brucellosis arranged in descending order

Table (1). Relative sensitivity/ specificity, performance indices, accuracy% and AUCs of different serological tests used in the diagnosis of camel brucellosis

Diagnostic performances	BAPA				RBPT (8%)				RBPT (3%)				LFA				iELISA (LPS)				CFT			
	TP	TN	FP	FN	TP	TN	FP	FN	TP	TN	FP	FN	TP	TN	FP	FN	TP	TN	FP	FN	TP	TN	FP	FN
	116	66	14	4	102	66	14	18	112	68	12	8	110	74	6	10	118	60	20	2	112	76	4	8
Relative sensitivity %	97%				85%				93%				92%				98%				93%			
Relative specificity %	82.5%				82.5%				85%				92.5%				75%				95%			
Performance index (PI) (sensitivity + specificity)	179				167.5				178				184.5				173				188			
Accuracy % (TP+TN)/ (TP+TN+FP+FN)	91%				84%				90%				92%				89%				94%			
AUC	0.896				0.838				0.892				0.921				0.867				0.942			

The abbreviations TP, FP, FN, and TN denote the number of respectively, true positives, false positives, false negatives, and true negatives considering cELISA as gold standard. Accuracy = Proportion correctly identified subjects. PI summarizes test accuracy in a single numeric value

Table (2). Performance parameters of screening serological tests used in the diagnosis of camel brucellosis considering cELISA as gold standard

Serological tests	PPV	NPV	LR+	LR-
	TP/(TP+FP)	TN/(TN+FN)	Se / (1-Sp)	(1-Se)/ Sp.
BAPA	0.89	0.94	5.53	0.04
RBPT (8%)	0.88	0.79	4.9	0.18
RBPT (3%)	0.9	0.89	6.22	0.078
LFA	0.95	0.88	12	0.09
iELISA (LPS)	0.86	0.97	4	0.02

PPV=positive predictive value (proportion diseased among subjects with a positive test result). **NPV**= negative predictive value (proportion of non-diseased among subjects with a negative test result). **LR+** = likelihood ratio of a positive test (the probability of an animal who has the disease testing positive divided by the probability of an animal who does not have the disease testing positive). **LR-** = likelihood ratio of a negative result (the probability of an animal who has the disease testing negative divided by the probability of an animal who does not have the disease testing negative). The abbreviations TP, FP, FN, and TN denote the number of respectively, true positives, false positives, false negatives, and true negative

Table (3): Agreement of screening serological tests with cELISA as well as LFA and in-house iELISA (LPS)

Serological tests	Kappa agreement with LFA (* κ value)	Kappa agreement with iELISA LPS (* κ value)	Kappa agreement with cELISA (* κ value)
BAPA	**0.769 ± 0.046	**0.819 ± 0.043	**0.809 ± 0.043
RBPT (8%)	**0.713 ± 0.050	**0.681 ± 0.052	**0.669 ± 0.053
RBPT (3%)	**0.709 ± 0.051	**0.758 ± 0.048	**0.790 ± 0.044
LFA		**0.723 ± 0.049	**0.835 ± 0.040
iELISA (LPS)	**0.723 ± 0.049		**0.802 ± 0.047

*: agreement with LFA, iELISA (LPS) and cELISA at $p < 0.05$ with confidence interval of 95%, **: κ value ± standard error

Table (4). Pearson's chi-square test of independency (χ^2) and the effect size (phi coefficient) between LFA, in-house iELISA (LPS) and screening test categories in camel.

Serological tests	ICA Vs screening test categories	Chi-square values	Degree of freedom (df)	* Sig. (p value)	Phi coefficient values	Effect size
LFA	LFA Vs BAPA	120.857	1	0.000	0.777	Large
	LFA Vs RBPT (8%)	101.572	1	0.000	0.713	Large
	LFA Vs RBPT (3%)	101.185	1	0.000	0.711	Large
	ICA Vs iELISA (LPS)	110.667	1	0.000	0.744	Large
In-house iELISA (LPS)	iELISA (LPS) Vs BAPA	135.391	1	0.000	0.823	Large
	iELISA (LPS) Vs RBT (8%)	98.016	1	0.000	0.7	Large
	iELISA (LPS)Vs RBT (3%)	117.682	1	0.000	0.767	Large
	iELISA (LPS) Vs LFA	110.677	1	0.000	0.744	Large

*Chi-square is significant at $p < 0.05$ with confidence interval of 95%, Vs = versus

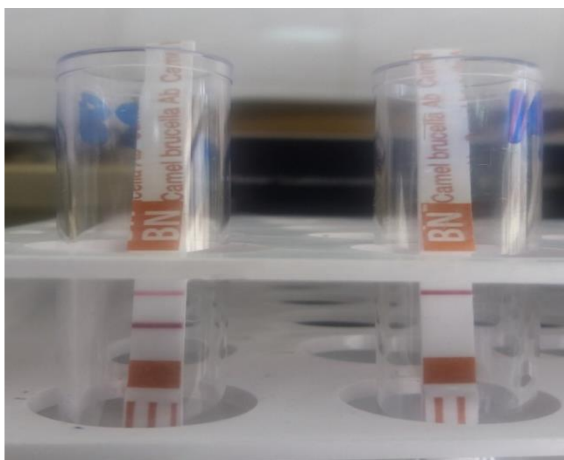

Fig. 3. Negative (right strip) and positive reactions (left strip) lateral flow assay.

4. DISCUSSION

In other species rather than cattle such as camels (*Camelus bactrianus* and *C. dromedarius*), the same serological assays used in the diagnosis of brucellosis may be used but every test shall be validated for its fitness (OIE, 2016).

Validation is a method that detect the fitness of an assay, which has been appropriately developed, optimized and standardized, for an intended purpose (OIE, 2013). Therefore all diagnostic assays performed under the field of the current investigation undergo a validation in term of estimation of their diagnostic performance characteristic parameters.

As it is generally problematic to find sufficient numbers of true positive reference camels, as determined by isolation of the pathogen, it was necessary to resort to samples from animals that have been identified by another test of adequately high accuracy as a gold standard model (OIE, 2013**).** The cELISA was preferred by the authors for the following reasons; 1- The two tests under validation (LFA and in-house iELISA) in the current study belong to the same category of primary binding assays as the cELISA. Primary binding assays are superior to secondary binding ones as the formers detect all antibodies regardless of their biological activity (Crowther, 2001). 2- Since there is no single serological test specified by the OIE (2016) as gold standard for the diagnosis of camel brucellosis, where all of them need to be validated and have their cutoff point estimated. 3- The frequent occurrence of CFT prozones in camel sera (MacMillan, 1990). 4- Very high CFT titers of 35840 ICFTU/ml have been recorded by Sayour et al. (2015a), a matter which may indicate a need for special cutoff determination in camels that sometimes produce tremendous amount of antibodies revealed in the form of shooting CFT titers. On the other hand, the cELISA was less vulnerable to such titers (Sayour et al., 2015a).

The screening serological tests as shown by Table (1) were highly sensitive identical with their use as screening tests especially when used for detecting infected herds or for ensuring the absence of infection in brucellosis-free herds (OIE, 2009). Better sensitivity of RBPT 3% version over RBPT 8% may be attributed in part to low packed cell volume which improves the test sensitivity (Alton et al., 1988).

The most remarkable parameter in Table (1) is the highest sensitivity of in-house iELISA using protein G conjugate with horseradish peroxidase as non-species specific and it is –up to our knowledge- the first time ever to use conjugated protein G based camel ELISAs in the diagnosis of camel brucellosis. Better sensitivity of iELISA may be attributed to the high affinity of protein G to camel immunoglobulin classes IgG_1, and IgG_3 (Hamers-Casrterman et al., 1993; Lange et al., 2001). In addition protein G reduces the background reactivity induced by anti-species conjugate when testing sera from *Brucella*-free animals, resulting in improving both sensitivity and specificity in LPS based iELISA versions (Ficapal et al., 1995). Higher relative sensitivity of iELISA (LPS) are assumed to be due to the detection of all immunoglobulins regardless to their isotype or biological activity (Crowther, 2001). Moreover, for indirect ELISA versions, the enzyme substrate reaction consequences in intensification of the signal indicating the presence of the analyte (Crowther, 2009), where a single molecule of enzyme can act on several molecules of the substrate.

The superior relative sensitivity of BAPA in the diagnosis of brucellosis in camel may be attributed to the low final packed cell volume (3%) and pH (4.02) after the addition of serum and also due to enhancement of the agglutinability of IgG_1 and reducing the agglutinability of IgM responsible for non-specific reaction by the acidic pH of lactate buffer where the stained cells of the antigen are preserved (Alton et al., 1988).

LFA as a simple form of ELISA offers a better performance in terms of relative Se (92%), Sp (92.5) and PI (184.5) as shown by Table 1 and Fig. (3) over the other screening tests specially iELISA including simplicity, possibility to performed in different samples, low cost if locally prepared, and rapid result, enabling easy portability, allowing testing at any time and at any place especially in remote and nomadic areas where the camel herds are located and laboratory facilities are inadequately equipped and the assay neither require professional personnel, nor expensive equipment in addition to the stability of the assay at different environmental conditions. (Abdel-Hamid et al., 2015; Sajid et al., 2015).

The performance index (PI) was used as a single tool for measuring the accuracy of screening

serological tests and was calculated by summing the relative sensitivity (Se) and specificity (Sp) values of each test under the validation. Better relative Se / or Sp stands behind the better PI and Accuracy % of LFA, BAPA and in-house iELISA (LPS) over the other screening tests

The receiver operating characteristic curves were produced by plotting the true positive rate (sensitivity) against the false positive rate (1-specificity) at different possible cutoff values of the tests under evaluation as shown in (Table 1) and (Fig. 1). The closer the ROC curve to the Y axis, the better the overall test performance (Fawcett, 2006). The area under the curve obtained (AUC) can be used as an alternative single pointer of test performance and a measure of how well a parameter can distinguish between infected and healthy group of animals (Hanley and McNeil, 1982). The AUC takes values between 0 and 1, with higher values indicating better test performance.

The overall performance of the LFA and in-house iELISA in camel based on both ROCs and AUCs (Table 1) is very good being equal to or closer to 0.9 and is a reflection of how good the both tests are in discriminating between *Brucella* infected and healthy animals. However LFA revealed better accuracy based on the superior AUC than the other screening immunoassays including in-house iELISA (LPS) as a result of both better relative Se and Sp offered by the test. Better performance of iELISA compared with other screening serological tests matches the results of (Azwai et al*.,* 2001).

Complement fixation test (confirmatory test) reveals a better performance over all the screening tests evaluated in this study in term of better Se (93%), Sp (95%), PI (188), accuracy% (94%) and AUC (0.942) as shown by (Table 1) and (Fig. 1 and 2.) No wonder why the test is recommended by the (OIE, 2016) to contribute in eradication policies and to estimate herd prevalence of infection as well as a suitable method to confirm suspected or clinical brucellosis cases.

Predictive values of test results: The positive predictive value (PPV) is the probability that an animal which has been tested positive by an assay is in fact positive with regard to the true diagnostic status. The negative predictive value (NPV) is the probability that an animal which has been tested negative by an assay is in fact negative with regard to the true diagnostic status (OIE, 2013). The PPVs as arranged in descending order of screening serological tests (Table 2) in camel indicating that among those who had a positive screening test, the probability of disease was 95%, 90, 89%, 88%, 86% for LFA, RBT 3%, BAPA, RBPT 8% and in-house iELISA respectively. The corresponding picture for the NPV as arranged in descending order of screening serological tests (Table 2) in camel indicating that among those who had a negative screening test, the probability of being disease-free was 97%, 94%, 89%, 88% and 79% for in-house iELISA LPS, BAPA, RBPT 3%, LFA and RBPT 8% respectively. Overall results reveal that both LFA and in-house iELISA behave well in term of both PPV and NPV especially when compared with other screening serological tests. The predictive value of a positive result also has great importance for the veterinary services in charge of the management of control or eradication programs, as the inverse of the PPV (i.e. 1/PPV) gives the information on how much money is spent in the culling of true and false positives for each true positive animal detected by the surveillance activity (Crowther et al., 2006).

LR+ is the probability of a positive test in animals with disease (Se)/probability of a positive test in animals without disease (1- Sp). LR+ is one of the best ways to measure and express diagnostic accuracy (McGee, 2003) as it is simply includes Se and Sp of a test into a single measure. The best test to be used for ruling in the disease is the one with the largest positive likelihood ratio (Altman et al., 2000). The overall LR+ results of LFA (12) seem to be better than in-house iELISA (4) and this may be attributed to the overall better Sp of LFA. LR- is the probability of the test to be negative in animals with disease (1-Se)/ probability of negative test in animals without disease (Sp). The best test to rule out disease is the one with the smallest negative likelihood ratio (Altman et al., 2000). The overall LR- results (Table 2) of in-house iELISA (0.02) seem to be better than LFA (0.09) and this may be attributed to superior Se of in-house iELISA over the other screening tests including LFA.

Table (3) Landis and Koch (1977) considered the kappa (κ) values < 0 as indicating no agreement and 0- 0.20 as slight, 0.21- 0.40 as fair, 0.41- 0.60 as moderate, 0.61- 0.80 as substantial, and 0.81- 1 as almost perfect agreement. All the screening

serological tests used to diagnose brucellosis in camel brucellosis agreed significantly with LFA, in-house iELISA (LPS) and cELISA at $p < 0.05$ (Table 3). The main reason behind the very good agreement recorded between in-house iELISA and LFA (Table 3) is the ability of this test to apparently detect IgG1 and IgG2 (Angus and Barton, 1984; Crowther, 2009; Nielsen, 2010). Despite the two immunoassays are technically the same (Shome et al., 2015), but use different labels (HRP and gold nanoparticles) and biorecognition molecules (secondary antibodies IgG and Protein G).

In order to estimate the association between serological tests (Table 4) and each of LFA and in-house iELISA (LPS). Phi coefficient values of 0.1, 0.3, and 0.5 correspond to effect sizes that could be described as small, medium and large respectively (Cohen, 1988). The effect of these significant associations (Phi coefficient values) were estimated to be large between LFA as well as in-house iELISA and the other screening serological tests as shown in Table 4.

Based on the large association between the adopted screening serological and LFA as well as in-house iELISA, along with their better diagnostic performance characteristics, authors concluded that both tests are fit for their purpose and valid to be used as rapid screening test in the diagnosis of camel brucellosis and to be incorporate in the control and eradication programs in this species.

Like the other adopted diagnostic screening tests, a definitive diagnosis should not be based on the result of a single test as every test has its own limitation, so authors suggest that the results of both tests shall be confirmed with a reliable confirmatory test.

5. REFERENCES

Abdel-Hamid, N.H., Beleta, E.I.M., Ismail R.I. 2015. Performance characteristics of a rapid qualitative immunochromatographic assay for antibody detection in small ruminant brucellosis. Animal Health Res. J. 3(3): 84-98.

Abou-Eisha, M.J. 2000. Brucellosis in camels and its relation to public health. Assiut Vet. Med. J. 44: 54-64.

Altman, D.G., Machin, D. Bryant, T.N., Gardners, M.J. 2000. Statistics with confidence: confidence intervals and statistical guidelines. London: BMJ Books. ISBN 07279-1375.

Alton, G.G., Jones, L.M., Angus, R.D., Verger, J.M. Techniques for the brucellosis laboratory. INRA Publications, Paris, France: 1988.

Angus, R.D., Barton, C.B. 1984.The production and evaluation of the buffered plate antigen for use in a presumptive test for brucellosis. Dev. Biol. Stand. 56: 349-356.

Azwai, S.M., Carter, S.D., Woldehiwet, Z., MacMillan, A. 2001. Camel brucellosis: evolution of field sera by conventional serological tests and ELISA. J. Camel Practice and Research. 8(2): 185-193.

Biancifiori, F., Giovannini, A., Di-Matteo, A., Urbani, G., Nannini, D.1996. Standardizazione di una tecnica ELISA per la ricerca degli anticorpi brucellari nel latte bovino. Vet. Ital. 32: 42-46.

Cohen, J. Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, NJ: Lawrence Earlbaum Associates: 1988.

Crowther J.R. The ELISA Guidebook, (2nd Edition). Humana Press. New York, USA: 2009.

Crowther J.R., Unger, H., Viljoen, G.J. 2006. Aspects of kit validation for tests used for the diagnosis and surveillance of livestock diseases: producer and end-user responsibilities. Rev. Sci. tech. off. Int. Epiz. 25 (3): 913–935.

Crowther, J.R. The ELISA Guidebook. Humana Press Inc., Totowa, New Jersey, USA: 2001.

European Commission Regulation, 2002: No 535/2002: Official Journal of the European Communities. 80: 22-28.

Fawcett, T. 2006. "An introduction to ROC analysis". Pattern Recognition Letters, 27 (8): 861–874.

Ficapal, A., Alonso-Urmeneta, B., Velasco, J., Moriyón, I., Blasco, J.M. 1995. Diagnosis of *Brucella* ovis infection of rams with an ELISA using protein G conjugate, Vet. Rec. 137: 145-147.

Gall, D., Nielsen, K. 2004. Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. Rev. sci. tech. Off. int. Epiz. 23(3): 989-1002.

Gwida, M.M., El-Gohary, A. H., Melzer, F., Tomaso, H., Rösler, U., Wernery, U., Wernery, R., Elschner, M.C., Khan, L., Eickhoff, M., Schöner, D., Neubauer, H. 2011. Comparison of diagnostic tests for the detection of *Brucella* spp. in camel sera. BMC Res Notes. 6(4): 525.

Hamers-Casterman, C., Atarhouch, S., Muyldermans, S., Robinson, G., Hamers, C., Songa, B.E., Bendahman, N., Hamers, R. 1993. Naturally occurring antibodies devoid of light chain. Nature. 363: 446-448.

Hanley, J.A., McNeil, B.J. 1982. The meaning and use of the area under a receiver operating characteristic (ROC) curve". Radiology. 143: 29-36.

Hennager, S.G. 2004. Reagent Production Protocol - Guinea Pig Complement Preparation for the Complement Fixation Test. USDA, APHIS, National Veterinary Services Laboratories (NVSL), Ames, IA, USA.

Ibrahim, H.H., Rouby, S., Menshawy, A., Ghazy, N. 2016. Seroprevalence of camel brucellosis and molecular characterization of *Brucella melitensis* recovered from dromedary camels in Egypt. Research Journal for Vet. Practitioners 4(1): 17-24.

ISO/IEC 17025 2005. General Requirements for the Competence of Testing and Calibration Laboratories.

Landis, J.R., Koch, G.G. 1977.The measurement of observer agreement for categorical data. Biometrics 33(1): 159-174.

Lange, I.G., Daxenberger, A., Meyer, H.H.D. 2001. Studies on the antibody response of Lama glama-evaluation of binding capacity of different IgG subtypes in ELISAs for clenbuterol and BSA. Vet. Immunol. Immunopathol. 83: 1–9.

Loong, T., 2003. Understanding sensitivity and specificity with the right side of the brain. BMJ. 327: 716–719.

Macaskill, P., Gatsonis, C., Deeks, J.J., Harbord, R.M., Takwoingi, Y. 2010. Analysing and presenting results. In: Cochrane Handbook for systematic reviews of diagnostic test accuracy version 1.0. The Cochrane Collaboration, 2010, Eds., Deeks, J.J., P.M. Bossuyt, and C. Gatsonis. Available from: http://srdta.cochrane.org/.

MacMillan, A. 1990. Conventional serological tests. Research Journal, 2(4): 79-90. In: Animal Brucellosis (K. Nielsen & J.R. Duncan, 34. Wernery, U., 2014. Camelid brucellosis: a review. Rev. eds.), CRC Press, Boca Raton, Florida, USA, sci. tech. Off. int. Epiz., 33(3): 153-197.

McGee, S., 2003. Simplifying likelihood ratios. JGIM. 17: 647-650.

Nielsen, K., 2010. Serological diagnosis of brucellosis. Contributions, Sec. Biol. Med. Sci. 1: 65–89.

OIE, 2004. Manual of the Diagnostic Tests and Vaccines for Terrestrial Animals, Mammals, Birds and Bees. 5th ed. OIE, Paris, 409-436.

OIE, 2009. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 2.4.3. Bovine Brucellosis. OIE, Paris, France.

OIE, 2013. Principles & Methods of Validation of Diagnostic Assays for Infectious Diseases. Chapter 1.1.5. OIE, Paris, France.

OIE, 2016. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 2.1.4. Bovine Brucellosis. OIE, Paris, France.

Sajid, M., Kawde, A., Daud, M. 2015. Designs, formats and applications of lateral flow assay: a literature review. Journal of Saudi Chemical Society. 19: 689-705.

Sayour, A.E., Elbauomy, E.M., Abdel Hamid, N.H., Abdel-Haleem, M.H. 2015b. Selection of a unified standard complement fixation method for nationwide application to restore inter-laboratory harmony to the diagnosis of ruminant brucellosis. Global Veterinaria. 14(1): 83-96.

Sayour, A.E., Elbauoumy, E.M., El-Kholi, M.K., Shehata, A.A.E. 2015a. Brucellosis prevalence and serologic profile of male one-humped camels reared in Somaliland and eastern Ethiopia for meat production. Global Veterinaria. 14(1): 67-76.

Shome, F., Filia, G., Padmashree, B.S., Krithiga, N., Sahay, S., Triveni, K., Shome, B.R., Mahajan, V., Singh, A., Rahman, H. 2015. Evaluation of lateral flow assay as a field test for investigation of brucellosis outbreak in an organized buffalo farm: A pilot study. Veterinary World, EISSN: 2231-0916.

Von Hieber, D., 2010. Investigation of occurrence and persistence of brucellosis in female camel dams (*Camelus dromedarius*) and their calves. Thesis, Universität Ulm, Germany.