Veterinary Medical Journal –Giza (VMJG), Vol. 60(2)B - , December 2014
http://www.vetmedjcu.com/
ISSN 1110-1423

Evaluation of Lateral Flow Immunoassay kits for diagnosis of bovine tuberculosis

Nasr, E.A*; Makharita, M.A.; Ereny S.L. **; Abdelrahman, M.* and Shereen A. M. ***

*Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. ** Central Laboratory for Evaluation of Veterinary Biologic Abbasia, Cairo, Egypt.
Email:essamnasr@yahoo.com

Abstract

Bovine tuberculosis is an enduring contagious disease of cattle that has caused substantial losses to the global livestock industry. In this study, two types of rapid tests (**IQRT** and **Vet-TB-STAT-PAK™**) have been evaluated using serum samples collected from positive reactor animals to tuberculin test.

M. bovis was isolated from those animals. Four thousands two hundred and fifty **(4250)** dairy cattle were tested by single intradermal comparative tuberculin skin test, **74 (1.7%)** animals reacted positively, **51 (68.9%)** of the slaughtered animals showed visible lesions on post mortem (PM) examination while the other **23 (31.1%)** showed non visible lesions. The bacteriological examination of the **74** samples revealed ***M. bovis*** from 44 (59.5%) of processed samples. The use of the **IQRT** that employed recombinant ***M. bovis*** MPB70 antigen as capture and detector, revealed that this anti *M. bovis* antibody test kit has detected 24.3% of tuberculin positive cattle against those confirmed by the bacteriological examination (59.9%) as *M. bovis* positive. On the other hand, using the other lateral flow assay **Vet-TB-STAT-PAK™** has detected 36.5 % of tuberculin positive cattle against 59.5% of them confirmed by bacterial isolation as ***M. bovis*** positive. Applying the **IQRT** on milk samples of tuberculin reactor cattle has detected 35% of tuberculin positive cattle against 7.5% of them confirmed by bacterial isolation from milk samples as *M. bovis* positive while, the other lateral flow assay **Vet-TB-STAT-PAK™** has detected 47.5 % of tuberculin positive cattle against 7.5% of them confirmed by bacterial isolation from milk as *M. bovis* positive. It is recommended that bovine tuberculosis Antibody Rapid Test Kit alone may not be reliable for screening cattle infected with tuberculosis especially in the developing countries as additional test is required to validate its results.

keywords: Diagnosis - Evaluation - Lateral flow - *Mycobacterium bovis.*

INTRODUCTION

Bovine tuberculosis (bTB) is not only an economic disease representing a barrier for free trade of livestock between countries but also a zoonotic disease with high prevalence in developing countries (Amanfu, 2006 and Feliciano et al., 2008). Tuberculin skin test though cumbersome, can effectively detect early stages of M. bovis

infection in cattle thereby allowing immediate removal of infected animals and limiting transmission of the disease and eventual eradication of bTB (Buddle et al., 2009). Serological assays are generally simple, rapid and inexpensive, but the development of improved serodiagnostic assays also require understanding of the bTB humoral immune mechanism as it is characterized by highly heterogeneous antigen recognition (Lyashchenko et al., 1998). Advances in humoral based responses tests have led to the development of lateral flow test kit among others, to capture and detect M. bovis antibodies (Garnier et al., 2003). The bound antibodies are visualized with the naked eye as colour band at the test device within some minutes of application (Lyashchenko et al., 2004, Wernery et al., 2007). In developing countries bTB is widely distributed and control measures are applied sporadically and pasteurization of milk is rarely practiced, this is because financial constraints and limitation of diagnostic test in detecting early exposures before the tubercle bacilli begin to shed in milk (Cosivi et al., 1998). In this study two rapid lateral-flow test assays were used the first **IQRT** Test kit that employed recombinant *M. bovis* MPB70 antigen (specific for *M. bovis*) as capture and detector is one of such newer serological test that is specific and sensitive to *M. bovis* antibodies, rapid and portable. The second rapid antibody detection assay, **Vet-TB-STAT-PAK™**, which uses the lateral-flow technology and a unique cocktail of recombinant antigens of *M. bovis* to detect specific antibodies of three immunoglobulin classes, IgG, IgM, and IgA, in dairy cattle.

Material and Methods

I) ***Animals*:** A total of 4250 cross-breed dairy cattle were tested by single intradermal comparative tuberculin skin test. (OIE, 2009).

II) Samples:

a) Serum Samples: The sera samples were collected from animal blood, before single comparative tuberculin test (Kennedy et al., 2003), for lateral flow assays.

b) Tissue samples: The tuberculin positive cattle were slaughtered and PM examination was conducted on them (Corner, 1994) and tissue samples showing gross lesions were taken for bacteriological examination according to Corner et al, 2012.

c) Fresh raw milk samples: milk samples were collected from lactating cows that tested positive for tuberculin test. Decontamination and examination of milk samples was done according to the method described by Petroff, 1915.

III) Rapid Bovine Tuberculosis detection Kits:

a) IQRT test kits

Specific for *M. bovis* antibodies containing the test devices and specimen droppers procured from Anigen Animal Genetics Inc. in South Korea were used in detecting *M. bovis* antibodies in the sera collected.

b) TB- STAT-PAK ™ test kits:

The test uses colored latex-based lateral flow technology and a cocktail of selected *M. bovis* antigens, including ESAT-6, CFP10, and MPB83. The test required one drop of

serum sample (30 µ) and 3 drops of sample buffer, which were added sequentially to the sample pad, results were read after 20 min.

Results and Discussion

BTB is a zoonotic disease with severe public health significance. Maximum detection of bTB in cattle populations in Egypt is vital to understand its epidemiology and zoonotic potentials and also achieve significant reduction and control of the disease in livestock. The tuberculin skin tests (TST) are currently the best available techniques for international field diagnosis of bTB in live animals (de la Rua-Domenech et al., 2006) and it is based on delayed hypersensitivity reactions (OIE,2009).

Table (1) showed the results of tuberculin skin test and PM findings of slaughtered tuberculin reactor cattle, out of **4250** tuberculin tested cross-bred dairy cattle, **74** were found to be reactors with a prevalence rate of 1.7 %. This is comparatively lower than that given by other investigators in Egypt [(Lotfy et al., 1960, 6.9%), (El Battawy, 2008, 4.6%) and Nasr et al., 2008, 2.2%)] and other countries of Africa (Ameni and Erkihun, 2007 in Ethiopia 11.6%; Borna et al., 2009, 8% in Chad) and this may be due to that these farms perform the tuberculin test regularly and applied test and slaughter strategy (Gonzalez et al., 1999). On the other hand, the prevalence rate recorded in the present study is comparatively higher than that given by other investigators (Shirma et al., 2003) and (Cleaveland et al., 2007), it was 1.3% and 0.9%, respectively in Tanzania.

Table (2) showed that the higher severity of lesion was observed in the pulmonary lymph nodes (24.3%), this is may be due to the intensive husbandry systems which make the respiratory excretion the main route by which animal-to-animal transmission occurs (Smyth et al., 2001). Also the same table showed the relation between PM findings in different ages of tuberculin reactor cattle, the percentage of reaction-positive animals increased with age, reaching a maximum in animals over 60 months of age. Similarly studies in Great Britain, an increase of incidence of bTB with increased age were indicated (Pollock et al., 2013).It was also suggested earlier by (Mackay and Hein, 1989) that the possible influence of γδ T cells which are predominantly found in the circulation of young calves and previous studies have shown the role of γδ T cells in anti mycobacterial immunity (Stamp, 1948). On the other side, it has been suggested that increased incidence of TB in older animals can be due to a waning of protective capability in aging animals (O'Reilly and Daborn, 1995) or, it may be due to the increase in the likelihood of encountering M. bovis over a longer period (Barwinek and Taylor, 1996). Rapid and simple immune-chromatographic assays for the sero diagnosis of bTB have been developed (Lyashchenko et al., 2004) and proposed as additional tests to the TST for ante mortem diagnosis (Pollock et al., 2005, Ameni et al., 2010). These chromatographic immunoassays employ unique cocktails of selected M. bovis antigens as both qualitative captures and detectors of specific antibodies against M. bovis in plasma, serum, and whole blood (Lyashchenko et al., 2004, Wernery et al., 2007). MPB83, ESAT-6, 14-kDa protein, CFP-10, MPB70, MPT63, MPT51, MPT32, MPB59, MPB64,

Acr1, PstS-1,M.bovis purified protein derivatives, ESAT-6/CFP10 fusion protein, 16-kDa alpha-crystallin/MPB83 fusion protein, and M. bovis culture filtrate have been identified as the common sero reactive antigens in bTB (Lyashchenko et al., 2004, Waters et al., 2006).

In this study, the antibovine TB antibody detection **IQRT** kit that employed recombinant *M. bovis* MPB70 antigen (specific for *M. bovis*) as capture and detector was conducted prior to TST because TST can boost antibody responses in infected cattle and emphasizes the importance of timing of collection of blood samples on the interpreting the test (Palmer et al., 2006). This anti bTB antibody test kit has detected 24.3% of tuberculin positive cattle against 59.5% of them confirmed by bacterial isolation as *M. bovis* as shown in **Table (3).** While the other lateral flow assay **Vet-TB-STAT-PAK™** has detected 36.5 % of tuberculin positive cattle against 59.9% of them confirmed by bacterial isolation as *M. bovis* positive. The TB STAT-PAK ™ test is a single-directional lateral-flow serological test which can provide a quick determination of the presence of M. bovis antibody (Harrington et al., 2008 and Lyashchenko et al., 2008)

As shown in **Table (4),** the **IQRT** Test has detected 35% of tuberculin positive cattle against 7.5% of them were confirmed by bacterial isolation from milk samples as *M. bovis* positive while the **Vet-TB-STAT-PAK™** has detected 47.5 % of tuberculin positive cattle against 7.5% of them confirmed by bacterial isolation from milk as *M. bovis* positive.

It was clear from these results that apparently healthy lactating cows may shed viable *M. bovis* in milk thereby posing a serious public health problem where unpasteurized milk is consumed. This calls for the need to ensure that only non-positive milking cows are milked for human consumption (Danbrini et al., 2010)

It was concluded that comparative cervical tuberculin test, culture and isolation of the bacilli are recommended for the true prevalence of bTB in the herd to be established.

The recent kits (**IQRT** and **Vet-TB-STAT-PAK™**) could be used for initial tuberculosis screening in combination with TST for improving sensitivity of bovine tuberculosis screening, thereby leading to more successful control programs in developing countries; **Vet-TB-STAT-PAK™** assay can detect antibody responses after *M. bovis* infection in cattle. The rapid test is proposed as a potentially useful ancillary assay for bTB. In addition, **Vet-TB-STAT-PAK™** kit may be most suitable for surveillance, especially if an immediate result is needed.

It is recommended that the milk from reactor animals must not be used for human consumption and must be withheld from the bulk tank while the milk from inconclusive reactor may go for human consumption after having undergone heat treatment.

Veterinary Medical Journal –Giza (VMJG), Vol. 60(2)B - , December 2014
http://www.vetmedjcu.com/
ISSN 1110-1423

Table (1) Results of tuberculin skin test and PM finding of slaughtered tuberculin reactor cattle.

No Of Tested Animals	Positive Tuberculin skin test		PM Finding			
			VL		NVL	
	No	%	No	%	No	%
4250	74	1.7	51	68.9	23	31.1

VL → visible lesion NVL → Non visible lesion

Table (2) Results of correlation between age of animal and PM finding of slaughtered tuberculin reactor cattle

Age of animal (month)	No	Positive animals		PM													
				VL												NVL	
				Local										Total			
		NO	%	General		Head		Pulmonary		Digestive		Mixed					
				No	%	No	%	No	%	No	%	No	%				
3-6M	450	5	1.1	0	0	2	40	2	40	0	0	0	0	4	80	1	20
6-16M	950	12	1.3	1	8.3	2	16.7	4	33.3	2	16.7	1	8.3	10	83.3	2	16.7
16-30M	1300	19	1.5	2	10.5	2	10.5	2	10.5	0	0	13	68.4	19	100	0	0
30-60M	850	20	2.4	1	5	2	10	4	20	1	5	0	0	8	40	12	60
Over 60 M	700	18	2.6	2	11.1	0	0	6	33.3	2	11.1	0	0	10	55.5	8	44.4
Total	4250	74	1.7	6	8.1	8	10.8	18	24.3	5	6.8	14	18.9	51	68.9	23	31.1

Table (3) Correlation between sites of infection, Mycobacterial isolation from tissue of tuberculin reactor cattle and lateral flow immunoassays.

PM findings	Site of lesion	No	Type of isolated mycobacteria				Type of Lateral Flow immunoassay			
			M. bovis		MOTT		IQRT		Vet-TB-STAT-PAK™	
			No	%	No	%	No	%	No	%
I- VL	1-General	6	6	100	0	0	5	83.3	6	100
	2-Local									
	a- Head	8	7	87.5	0	0	2	25	4	50
	b- Pulmonary	18	14	77.8	0	0	7	38.9	9	50
	c- Digestive	5	2	40	2	40	0	0	1	20
	d- Mixed	14	9	64.3	0	0	4	28.6	5	35.7
Sub total		51	38	74.5	2	3.9	18	35.3	25	49
II-NVL	Congestion in L.N.	23	6	26.1	2	8.7	0	0	2	8.7
Total		74	44	59.5	4	5.4	18	24.3	27	36.5

MOTT → Mycobacteria Other Than Tuberculosis.

Table (4) Correlation between site of lesion, Mycobacterial isolation from milk of tuberculin reactor cattle and lateral flow immunoassay.

PM findings	Site of lesion	No	Type of mycobacteria isolated from milk				Type of Lateral Flow			
			M. bovis		MOTT		IQRT		Vet-TB-STAT-PAK™	
			No	%	No	%	No	%	No	%
I- VL	1-General	4	2	50	0	0	4	100	4	100
	2-Local									
	a- Head	5	0	0	0	0	2	40	3	60
	b- Pulmonary	10	0	0	0	0	4	40	5	50
	c- Digestive	3	0	0	1	33.3	0	0	0	0
	d- Mixed	8	1	12.5	0	0	4	50	5	62.5
Sub total		30	3	10	1	3.3	14	46.6	17	56.6
II-NVL	Congestion in L.N.	10	0	0	2	20	0	0	2	20
Total		40	3	7.5	3	7.5	14	35	19	47.5

References

Amanfu W (2006): The situation of tuberculosis and tuberculosis controlling animals of economical interest: Tuberculosis, 86: 330-335.

Ameni, G. and Erkihun, A. (2007): Bovine tuberculosis on small-scale dairy farms in Adama town, central Ethiopia, and farmer awareness of the disease. Rev. Sci. Tech. Off. Int. Epiz.; 26(3):711–719.

G. Ameni, A. Aseffa, G. Hewinson, and M. Vordermeier (2010): "Comparison of different testing schemes to increase the detection *Mycobacterium bovis* infection in Ethiopian cattle," Tropical Animal Health and Production, vol. 42, no. 3, pp. 375– 383,.

Barwinek, F. and Taylor, N. M. (1996): Assessment of the socio-economic importance bovine tuberculosis in Turkey and possible strategies for control or eradication.

Turkish-German Animal Health Information Project, p. 3–45. General Directorate of Protection and Control, Ankara, Turkey.

Borna, M.; Penelope, V.; Bongo, N.; Colette, D.; Irene, S.; Beatrice, M; Bruno, O.; Esther, S. and Jakob, Z. (2009): Bayesian Receiver Operating Characteristic Estimation of Multiple Tests for Diagnosis of Bovine Tuberculosis in Chadian Cattle PLOS ONE 4 (12):1371-1381

Buddle BM, Livingstone PG, de-Lisle GW (2009): Advances in ante-mortem diagnosis of tuberculosis in cattle. New Zealand Vet Journal, 57 (4): 173-180.

Cleaveland S. Shaw D.J. Mfinanga S.G. Shirima G. Kazwala R.R. Eblate E. Sharp M. (2007): *Mycobacterium bovis* in rural Tanzania: risk factors for infection in human and cattle populations. Tuberculosis; 87(1):30–43.

Cosivi, O., J. M. Grange, C.J. Daborn, M. C. Raviglione and T. Fujikura *et al.*, 1998. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. Emerg. Infect. Dis., 4:59-70.

Corner, L. A. (1994): Post mortem diagnosis of *Mycobacterium bovis* infection in cattle. Vet. Microbiol. 40:53-63.

Corner, L.A.L., Gormley , E., Pfeiffer , D.U. (2012): Primary isolation of *Mycobacterium bovis* from bovine tissues: Conditions for maximizing the number of positive cultures, Veterinary Microbiology, Volume 156, Issues 1–2, 23 April 2012, Pages 162-171, ISSN 0378-1135

S. Danbrini ; A. K. B. Sackey ; J. O. Ayo ; E. K. Bawa ; A. C. Kudi ; S. O. Okaiyeto and S. B. Pewan (2010): Expouser and sheding in milk of *Mycobacterium bovis* in dairy herds using one- step Anigen rapid bovine tuberculosis antibodies test and Ziehl- Neelsen stain. Medwell Journals Veterinary Research 3(3): 38-42.

de la Rua-Domenech, R., A. T. Goodchild, H. M. Vordermeier, R. G. Hewinson, K. H. Christiansen, and R. S. Clifton-Hadley(2006): Ante mortem diagnosis of tuberculosis in cattle: A review of the tuberculin tests, -interferon assay and other ancillary diagnostic techniques. Res. Vet. Sci. 81:190– 210.

El battawy, E. H. (2008): Bacteriological Characterization, Molecular, and Immunological Diagnostic Assays for Mycobacterium Infection in Some AnimalsThesis Ph.D. Minufiya University, Sadat city branch Faculty of Veterinary Medicine Department of Bacteriology, Immunology and Mycology

Feliciano MS, Beth H, Camila AD, Cecilia RT, Tod S, Genoveva AO, Alberto ML, Martina PS, Janet BP, (2008): Molecular epidemiology of *Mycobacterium bovis*: usefulness in international trade. Preventive veterinary medicine, 87: 261-271.

Garnier T, Eiglmeier K, Cadmus JC, Medina M, Mansoor H (2003): The complete genome sequence of *Mycobacterium bovis*. Proc. Nat. Acad. Sci. USA., 100: 7877-7882.

Gonzalez-Llamazares, O.R.; Gutierrez-Martin, C.B.; Alvarez-Nistal, D.; Puente-Redondo, V.; Dominguez-Rodriguez, L.; Rodriguez-Ferri, E.F.; De-La-Puente, R.V.A.(1999): Field evaluation of the single intradermal cervical tuberculin test and the interferon-gamma assay for detection and eradication of bovine tuberculosis in Spain Vet. Microbiol. 70 (1):55-66.

Harrington, N. P., O. P. Surujballi, J. F. Prescott, J. R. Duncan, W. R.

Waters, K. Lyashchenko, and R. Greenwald (2008): Antibody responses of cervids (*Cervus elaphus*) following experimental *Mycobacterium bovis* infection and the implications for immunodiagnosis. Clin. Vaccine Immunol. 15:1650–1658.

Kennedy, H.E.; Welsh, M.D.; Cassidy, J.P.; Bryson, D.G.; Forster, F.; McNair, J.; Gangadharan, B.; Howard, C.J. and Pollock, J.M. (2003):The role of WC1+δ T- cells in the delayed type hypersensitivity (DTH) skin- test reaction of *Mycobacterium bovis* infected cattle. J. Vet. Immunol. Immunopathol.;93:169-176.

Lotfy, O; Khater, A.R and Guindis, S.M. (1960) : an investigation on tuberculosis in Umkenan village. Egypt. U.A.R.J. Egypt .Vet. Med. Ass., 20:105-112

Lyashchenko, K. P., J. M. Pollock, R. Colangeli, and M. L. Gennaro (1998): Diversity of antigen recognition by serum antibodies in experimental bovine tuberculosis. Infect. Immun. 66:5344–5349.

Lyashchenko, K.P., A. O. Whelan, R. Greenwald *et al.*,(2004): "Association of Tuberculin-Boosted Antibody Responses with Pathology and Cell-Mediated Immunity in Cattle Vaccinated with *Mycobacterium bovis* BCG and Infected with *M. bovis*," Infection and Immunity, vol. 72, no. 5, pp. 2462–2467.

Lyashchenko, K. P., R. Greenwald, J. Esfandiari, M. A. Chambers, J. Vicente, C. Gortazar, N. Santos, M. Correia-Neves, B. M. Buddle, R. Jackson, D. J. O'Brien, S. Schmitt, M. V. Palmer, R. J. Delahay, and W. R. Waters (2008): Animal-side serologic assay for rapid detection of *Mycobacterium bovis* infection in multiple species of free-ranging wildlife. Vet. Microbiol. 132: 283–292.

Mackay, C. R., and Hein, W. R. (1989): A large proportion of bovine T cells express the γδ T cell receptor and show a distinct tissue distribution and surface phenotype. Int. Immunol. 1:540–545.

Nasr, E.A.; Abdel Twab, A.A.; Abdel Rahman, M.; Abdel Aziz, A. M. (2008): A comparative study between the performance of a noval rapid lateral flow test and the conventional tuberculin skin test in recognizing cattle with tuberculous lesions in Egyptian herds. 13th. Sci. Cong. Fac. Vet. Med. Assiut Univ. Egypt: 637 - 652

O'Reilly, L. M. and Daborn, C. J. (1995): The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. Tuberc. Lung Dis. 76:1–146.

Palmer, M. V., Waters, W. R., Thacker, T. C., Greenwald, R., Esfandiari, J., and Lyashchenko, K. P., (2006): "Effects of different tuberculin skin-testing regimens on gamma interferon and antibody responses in cattle experimentally infected with *Mycobacterium bovis*," Clinical and Vaccine Immunology, vol. 13, no. 3, pp. 387–394,.

Petroff,S.A., (1915): A new and rapid methodfor the isolation and cultivation of the tubercle bacillus directly from sputum and faeces, J. Exp. Med., xxi, 38

Pollock, M. D. Welsh, and J. McNair (2005): "Immune responses in bovine tuberculosis: towards new strategies for the diagnosis and control of disease," Veterinary Immunology and Immunopathology, vol. 108, no. 1-2, pp. 37–43,

Pollock Ellen Brooks-, Andrew JK Conlan , Andy P Mitchell, Ruth Blackwell, Trevelyan J McKinley and James LN Wood (2013): Age-dependent patterns of bovine tuberculosis in cattle http://www.veterinaryresearch.org/content/44/1/97

Shirma G.M. Kazwala R.R. Kambarage D.M. (2003): Prevalence of bovine tuberculosis in cattle in different farming systems in the Eastern zone of Tanzania. Prev. Vet. Med.; 57(3):167–172.

Smyth, A. J.; Welsh, Girvin, R. M.; and Pollock, J. M. (2001): In vitro pensiveness of γδ T cells from *Mycobacterium bovis* infected cattle to mycobacterial antigens: predominant involvement of WC1 cells. Infect. Immun. 69:89–96.

Stamp, J. T. (1948): Bovine pulmonary tuberculosis. J. Comp. Pathol. 58:9–23.

Vordermeier M., S. V. Gordon, and R. G. Hewinson (2011): "*Mycobacterium bovis* antigens for the differential diagnosis of vaccinated and infected cattle," VeterinaryMicrobiology, vol. 151, No. 1-2, pp. 8–13,.

Waters W. R., M. V. Palmer, T. C. Thacker et al., (2006): "Early antibody responses to experimental *Mycobacterium bovis* infection of cattle," Clinical and Vaccine Immunology, vol. 13, no. 6, pp. 648–654.

Wernery U., J. Kinne, K. L. Jahans et al., (2007): "Tuberculosis outbreak in a dromedary racing herd and rapid serological detection of infected camels," Veterinary Microbiology, vol. 122, no. 1-2, pp. 108–115.

World Organisation for Animal Health (OIE), (2009): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2009). OIE Terrestrial Manual (2008), World Organization for Animal Health, Paris, France, (2009).

تقييم استخدام الاختبارات السريعه لتشخيص السل البقرى فى مزارع الابقار

عصام امين نصر، محمدعلى مخاريطه، ايرينى صادق لبيب، محمدعبدالرحمن و شيرين عزيز محمد
معهدبحوث الامصال واللقاحات البيطريه بالتعاون مع المعمل المركزى للرقابه

ان السل البقرى مرض معدى دائم يصيب الماشيه متسببا فى خسائر جسيمه لصناعة الثروه الحيوانيه فى العالم. على الرغم من الجهود المبذوله للقضاء عليه فانه مازال قائما والاختبارات الحاليه تعتمد على قياس الاستجابه المناعيه لمرض السل داخل جسم الحيوان باستخدام اختبارات الجلد. فى هذه الدراسه تم تقييم نوعين من الاختبارات السريعه على عينات سيرم من حيوانات ايجابيه لاختبار التيوبركلين وتم عزل الميكوبكتريا بوفس منها وكانت النتائج كالاتى: ٧٤بقره ايجابيه من بين ٤٢٥٠ بنسبة١.٧ % وقد تم ذبحهم وعند اجراء الصفه التشريحيه لهم كانت الحيوانات التى بها اصابه سليه ظاهريه ٥١ بنسبة ٦٨.٩% بينما كانت ال٢٣ الباقيه بنسبة ٣١.١% لم يظهر بها اصابه سليه ظاهريه. وبالفحص البكتريولوجى لهم تم عزل وتصنيف عدد ٤٤ عينه بنسبة ٥٩.٩% عترة الميكوبكتريا بوفس. وكانت نتائج استخدام اطقم ال **IQRT** احادى الانتجين التى تحتوى على انتيجينات ذات خصوصية لميكروب السل البقرى (MPB70) وبمقارنة استخدامها على عينات سيرم مأخوذة من حيوانات ايجابيه لاختبار التيوبركلين البقرى فى الجلد مع العزل البكتريولوجى للميكوبكتريا" بوفس" من انسجة مصابة اظهرت النتائج الاتي ان نتيجة الاختبار بال kit كانت ٢٤.٣% مقارنة بالعزل البكتريولوجى للميكوبكتريا بوفس كانت ٥٩.٩% بينما باستخدام الاطقم الثانيه **Vet-TB- STAT-PAK™** متعدد الانتجين اظهرت النتائج الاتي 36.5% مقارنة بالعزل البكتريولوجى للميكوبكتريا" بوفس كانت ٥٩.٩%. كما تم ايضا فى هذه الدراسة مقارنة العزل البكتريولوجى للميكوبكتريا" بوفس من عينات الالبان الماخوذة من الحيوانات الايجابيه لاختبار التيوبركلين مع ال kit اظهرت النتائج ان الاختبار بال (IQRT) احادى الانتجين كانت ٣٥% مقارنة بالعزل البكتريولوجى للميكوبكتريا" بوفس كانت ٧.٥% بينما باستخدام **Vet-TB-STAT-PAK™**متعدد الانتجين اظهرت النتائج الاتي ان نتيجة الاختبار بال kit كانت 47.5% مقارنة بالعزل البكتريولوجى للميكوبكتريا" بوفس كانت ٧.٥ %.

يوصى البحث أن طقم اختبار الأجسام المضادة السريعة للسل البقري وحدها قد لا تكون كافيه لفحص الماشية المصابة بالسل وخاصة في البلدان النامية لذا كان المطلوب اختبار إضافي للتحقق من صحة نتائجه.