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THE USEFULNESS OF GLUTARALDEHYDE COAGULATION TEST AS A CONJUNCTURE TEST IN THE DIAGNOSIS OF TUBERCULOSIS IN HUMANS AND ANIMALS

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ABSTRACT

A comprehensive study was conducted to investigate the effect of temperature variations of water bath along with the sample's storage duration on the results of the glutaraldehyde coagulation test (GCT), being developed for the diagnosis of tuberculosis. Furthermore, association of GCT results and duration of tuberculosis, tuberculosis + Hepatitis C virus positive, hepatitis C virus infected and control negative humans was also assessed. The blood samples from tuberculosis positive as well as negative humans and animals were collected and carried GCT with the variation of storage duration and temperature at which test performed (V1-V6). The demographic data of all individuals along with blood samples was also collected. Hematological and serum biochemical analysis was also performed. The results showed the significantly ($P<0.05$) lower time for coagulation of blood samples by the glutaraldehyde in TB positive than negative cases in all variations of temperature and samples storage time, with the remarkable difference in humans than animals. Time taken for coagulation was less than 10min when tuberculosis disease was of less than six months' duration and was around 10min when the disease was of more than six months. The coagulation time for tuberculosis + HCV and HCV alone were non-significantly ($P>0.05$) different from the control group. Increased fibrinogen level in tuberculosis positive humans and animals and decrease in hepatitis patients confirmed the relationship of fibrinogen level with coagulation time of the glutaraldehyde test. The diagnostic value of the glutaraldehyde test for human and animal tuberculosis has been highly speculated.

***Keywords*:** Tuberculosis, Glutaraldehyde test, Condition variation, Disease association

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1. INTRODUCTION

Tuberculosis (TB) is an infectious disease of humans and animals caused by bacteria of the genus Mycobacterium. Tuberculosis is a disease of the respiratory system, but it can be extra-pulmonary as well (Krishnamoorthy and Singh 2009). It is caused by Mycobacterium tuberculosis in humans and by Mycobacterium bovis in bovines (Agnihotri et al. 2013). The main route of disease transmission is inhalation of infected droplets, but feed, water and other inanimate particles are also responsible for pathogen transfer. This public health disorder has always been remained one of the main causes of mortality in both humans and animals and has caused 1.3 million human deaths in the year 2017, among which 5% of the deaths were in Pakistan. The 9-11 million people are annually infected with TB, which is 10% of people exposed to the pathogen. The highest incidence rate is seen in economically poor countries (WHO 2018). The TB in bovines reduces the productive capabilities by up to 25% (Rodostits et al., 2000). Bovine tuberculosis is known to be one of the important bovine diseases that caused significant economic loss across 176 countries (Javed et al. 2011; Ejeh et al. 2014; Bennett 2017).

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There are many conventional methods available for the diagnosis of disease such as microscopic examination of stained blood smears, culture test, tuberculin skin test (TST), nucleic acid amplification test through PCR or LAMP and Gene-Expert (Laraque et al. 2009). For the diagnosis of TB, the glutaraldehyde coagulation test (GCT) was first time reported by Larsson et al. (1990). The sensitivity and specificity of GCT for the diagnosis of tuberculosis were reported to be 89 and 95%, respectively (Larsson et al. 1990). It has been reported that GCT is an easy and fast technique to determine the semi-quantitative level of fibrinogen and immunoglobulin in the blood (Clabough et al. 1989). The test has been conducted to indicate the inflammatory disease condition in cattle and thus is considered important in the herd health surveillance program (Tøllbøll and Jørgensen 2003). Keeping in view of these facts, a comprehensive study was carried out to investigate the effect of temperature variations of water bath along with duration of storage on the outcome of GCT results. Furthermore, the duration of TB and its effect on the outcome of GCT results in tuberculosis positive, tuberculosis + Hepatitis C virus positive, hepatitis C virus infected and control negative humans were also carried out.

2. MATERIALS AND METHODS

2.1. Ethical Approval

The study plan was approved by the Institutional Biosafety/Bioethics Committee (IBC), University of Agriculture, Faisalabad, Pakistan (No. Path/699; Dated: 26-12-2018).

2.2. Experiment 1

2.2.1. Sampling in Humans and Animals: The experiment was conducted on 100 human subjects with confirmed TB status by use of ZN-staining and GeneXpert MTB/RIF techniques. The blood samples from randomly selected 100 TB patients were collected with their verbal consent. The blood samples were also collected from 50 apparently healthy TB negative people. The patients were informed about the study objectives and usefulness. Three to five ml of the blood sample from each patient was collected in EDTA coated vacutainers and was transported by placing in special double wall leakproof box. In an analogous manner, the blood samples from all the TB positive animals (n=16) and the same number of negative animals (n=16) were collected in EDTA coated vacutainers. The GCT was performed on all the samples collected from humans and animals with test variations V1, V2, V3, V4, V5 and V6.

The tuberculin testing was done on a total 84 number of cattle and buffalo of more than two years of age at the local farm. The test was carried out by injecting purified protein derivatives (PPD) containing antigens of M. bovis on the cervical region of the animal. The thickness of the skin fold was measured after 48-72 hours and results were interpreted as previously described by Aagaard et al. (2003).

2.3. Glutaraldehyde Coagulation Test

The glutaraldehyde test was performed in two experiments by using 2.5% the glutaraldehyde solution as performed by Larsson et al. (1990) with two replicates. A few variations regarding environmental temperature and sample storage were also made. The temperature of the water bath, the storage temperature of the samples before the performance of the test and the duration of the sample storage were changed to check the effect of these variations on the results of the glutaraldehyde test. The variations were categorized as V1-V6.

Variation 1 (V1): the samples were used fresh and were not stored before the performance of the test and water bath temperature was maintained at 22°C.

Variation 2 (V2): the storage duration of the sample before test performance was 2 hours at 4°C and the water bath temperature was maintained at 22°C.

Variation 3 (V3): the storage duration of the sample before test performance was 168 hours or 1 week at 4°C and the water bath temperature was maintained at 22°C.

Variation 4 (V4): the storage duration of the sample before test performance was 336 hours or 2 weeks at 4°C and the water bath temperature was maintained at 22°C.

Variation 5 (V5): the storage duration of the sample before test performance was 2 hours at 4°C and the water bath temperature was maintained at 25°C.

Variation 6 (V6): the storage duration of the sample before test performance was 2 hours at 4°C and the water bath temperature was maintained at 30°C.

2.4. Experiment 2

2.4.1. Sampling of Humans and Animals: Blood samples from 99 randomly selected TB patients from District TB Hospital Faisalabad, were collected by venipuncture with an anticoagulant and without an anticoagulant to perform the glutaraldehyde coagulation test as described in Experiment 1 and for plasma and serum separation, respectively. Patients were confirmed for TB in the TB hospital diagnostic laboratory by carrying out a chest X-ray examination,

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Ziehl-Nelson (ZN) staining and GeneXpert MTB/RIF. The history of hepatitis C virus infection was also collected along with other demographic information about the patients. The time period of patients undergoing DOT (directly observed therapy) treatment was also noted.

Fresh sampling from TB positive animals (n=12) as described above was also done. Animals showing a negative tuberculin test were considered as controls (n=12). It may be mentioned here that the second experiment was carried out after one year of the first experiment.

Samples were placed in leak proof double walled containers having ice for transportation. Serum samples were stored at -40°C till further used.

2.5. Hematological Parameters

The following hematological parameters were also studied (Benjamin 1978) including Total Erythrocytic Count (TEC), Total Leukocytic Count (TLC), Differential Leukocytic Count (DLC) and Erythrocyte Sedimentation Rate (ESR).

2.6. Serum Biochemistry Analysis

The serum albumin concentration and total serum proteins were determined in the samples by methods as described earlier (McEwan et al. 1970; Varley et al. 1980). The serum globulins and fibrinogen concentrations were determined by subtraction methods (Benjamin 1978).

3. RESULTS

3.1. Experiment 1

3.1.1. GCT Variations and Coagulation Time in Humans and Animals: The results in Table 1 represent the GCT values at variations 1-6 (V1-V6) in humans and animals. The results showed significantly ($P<0.05$) lower time for coagulation (jellification) of blood samples by the glutaraldehyde in TB positive than negative cases in all variations. It was less than 5min for positive animals compared with more than seven minutes for positive cases. However, in humans the difference was very high as it was less than 10 minutes in positive cases, while being greater than 21min in negative cases.

Table 1: The value of Glutaraldehyde coagulation test time in variation 1-6 (V1-V6)

Specie	V1 (mi./sec)	V2 (min/sec)	V3 (min/sec)	V4 (min/sec)	V5 (min/sec)	V6 (min/sec)
Humans						
TB Negative (n=50)	40.71±3.30	39.10±11.78	42.03±4.18	46.85±3.53	21.00±3.60	19.00±9.64
TB Positive (n=100)	7.37±2.42*	5.31±2.81*	11.55±8.20*	7.34±6.19*	4.46±2.06*	4.84±2.29*
Animals						
TB Negative (n=16)	7.429±1.897	8.892±2.298	8.907±4.538	7.806±1.405	9.023±2.941	8.990±1.752
TB Positive (n=16)	3.620±0.494*	3.989±1.285*	3.646±1.062*	3.833±0.925*	4.336±0.106*	5.007±1.251*

Values (mean±SD) bearing asterisks differ significantly ($P<0.05$) from TB Negative as compared to TB positive in respective species.

3.2. Experiment 2

Results of jellification (coagulation) time for the glutaraldehyde test conducted at 3 different conditions (performed on fresh samples at room temperature, on fresh samples at 22°C, on overnight stored samples in a refrigerator at 22ºC) are shown in Table 2. Results of tests conducted at three variations showed almost comparable results in cases of blood collected from TB patients (with disease duration of 0-1, 1-6 and 6+ months), tuberculosis + HCV, HCV patients and control groups. However, the time taken for jellification was less than 10min when TB disease was of less than six months and was around 10min when the disease was of more than six months. All of these results were significant as compared to those of the control group. The jellification time for tuberculosis + HCV and HCV alone were non-significantly different from the control group. Similarly, the jellification time for tuberculin positive animals was significantly ($P<0.05$) less than the control negative animals.

3.3. Biochemical Analysis

Results of serum biochemical parameters including total proteins, albumin, globulins, and fibrinogen in human and animals' samples are shown in Table 3. Serum total proteins, globulins and fibrinogen were significantly higher in human patients having less than 6 months TB than the values in the control group. Similarly, the values of serum total proteins and globulins were significantly higher, while the fibrinogen was significantly lower in humans having Tuberculosis+ HCV or HCV only than the control group. In humans having TB disease of more than six months' duration had a significantly higher globulin level than the control group. The results for all the serum biochemical parameters in animals were significantly higher in Tuberculin positive animals than tuberculin negative animals.

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Table 2: Jellification time (seconds) for human fresh samples at various conditions

Disease Group	Tested at Room temperature	Tested (water bath: 22°C)	Tested (water bath: 22°C; overnight storage)
Humans			
Tuberculosis 0-1 Month (n=17)	7.24 ±2.23*	7.57±2.28*	7.99±2.27*
Tuberculosis 1-6 Month (n=16)	7.64±1.59*	8.09±1.48*	8.47±1.45*
Tuberculosis 6+ Month (n=15)	10.97±1.04*	11.25±1.09*	11.81±1.05*
Tuberculosis+ HCV (n=26)	18.75±3.87	19.79±2.14	21.01±2.22
HCV (n=25)	21.56±6.89	22.38±2.76	22.97±2.76
Control (n=25)	17.74±4.96	19.05±2.08	19.04±2.09
Blood coagulation time of animals at room temperature			
Negative control (n=12)	7.426±1.145	8.021±1.168	8.586±1.263
Tuberculosis positive (n=12)	2.950±0.257*	3.241±0.269*	3.782±0.354*

Values (mean±SD) bearing asterisks differ significantly (P<0.05) from TB Negative as compared to TB positive in respective species.

Table 3: Total serum proteins, Albumin, Globulin and Fibrinogen concentration in various human disease groups

Disease group	Total serum proteins (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Fibrinogen (mg/dL)
Humans				
Tuberculosis 0-1 Month	8.22±0.45*	4.0±0.3	4.20±0.32*	319.1±82.05*
Tuberculosis 1-6 Month	7.9±0.24*	4.07±0.19	3.75±0.34*	308.5±15.5*
Tuberculosis 6+ Month	7.05±0.24	3.94±0.25	3.94±0.25*	262.5±25.45
Tuberculosis+ HCV	7.41±0.46*	4.19±0.33	3.21±0.25*	212.34±21.5*
HCV	7.35±0.34*	3.28±0.27*	3.93±0.48*	156.32±15.13*
Control	6.82±0.63	3.99±0.52	2.83±0.24	250.52±38.2
In animals				
Control negative	6.929±0.315	3.479±0.210	3.450±0.237	582.41±51.557
Tuberculosis positive	8.303±0.504*	3.900±0.255*	4.152±0.876*	1149.42±71.329*

Values (mean±SD) bearing asterisks differ significantly (P<0.05) from TB Negative as compared to TB positive in respective species.

3.4. Hematology

The results of hematological study carried out in humans and animals are presented in Table 4. The results indicated a significant difference in hematocrit and hemoglobin concentration in humans but none in animals. These were significantly (P<0.05) lower in TB positive than TB negative patients.

Table 4: The hematological variations recorded in TB positive, and TB negative humans and animals are presented in a tabulated form.

Specie	RBC (10^{12}/L)	MCV (fL)	HCT (%)	PLT (10^9/L)	WBC (10^9/L)	HGB (g/dL)	MCH (Pg)	MCHC (g/100mL)	LYM (%)	GRA (%)
Humans										
TB -ve	5.36±0.10	91.36±1.55	49.50±0.50	49.50±0.50	9.53±0.85	14.06±0.86	28.20±1.21	28.40±1.40	24.60±10.2	71.43±8.82
TB +ve	4.78±0.55	86.34±9.66	41.12±5.47*	41.12±5.47	11.21±3.68	10.75±1.65*	26.13±1.22	26.14±1.41	18.98±7.59	74.12±8.71
Buffalo										
TB -ve	7.26±0.03	53.4±0.05	38.8±0.11	8.0±0.02	13.70±0.05	10.70±0.11	14.70±0.12	27.50±0.10	37.80±0.11	38.80±0.13
TB +ve	6.76±0.48	56.22±1.78	38.07±3.35	25.25±24.83	14.12±6.84	10.10±0.79	14.95±0.67	26.67±0.82	33.60±7.32	42.37±5.14
Cattle										
TB -ve	6.21±0.96	47.62±2.82	29.65±5.16	160.0±71.72	11.30±1.68	8.05±1.48	12.90±0.63	27.15±0.47	48.75±6.59	43.42±6.66
TB +ve	6.22±0.46	48.40±7.49	29.94±3.06	108.0±33.66	12.20±1.99	8.08±0.69	13.02±1.51	27.10±1.48	35.34±12.0	48.30±11.06

Values (mean±SD) bearing asterisks differ significantly (P<0.05) from TB Negative as compared to TB positive in respective parameter and species.

4. DISCUSSION

The present study was conducted to check the usefulness of a cheap and easy-to-use conjuncture test to diagnose tuberculosis (TB) in both humans and animals.

TB is one of the most important diseases of animals and humans, in animals it impacts on the economy of the country, where livestock are an important contributor in the economic status of that country and that the public health is also connected with animal tuberculosis (Torgerson and Torgerson, 2010). Among the most conventional diagnostic tools, Zeihl-Neelsen (ZN) staining was most widely used and is still in practice in many developing countries to diagnose human TB on sputum samples. According to Gounder et al. (2002) and Ben-Selma et al. (2009), the responsiveness of ZN staining is not more than sixty to seventy percent. We evaluated the glutaraldehyde coagulation test (GCT) to be used for the diagnosis of TB in both humans and animals. As reported by Larsson et al. (1990), the specificity and sensitivity of GCT were 95 and 89%, respectively. According to Alavi-Naini et al. (2009), the particularity and responsiveness of GCT were 89 and 85%, respectively. The sensitivity and specificity of the GCT increased in case of discriminating pulmonary TB cases from the control group (Alavi-Naini et al. 2009).

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We tried different variations to see if those have an impact on the outcome of the results of GCT, both in humans and animals, and we found that the test can be performed on fresh or stored samples and can be carried out at room temperature or if a water bath is available then in a water bath at 20-25°C without impacting on the outcome of the test result. Furthermore, the test is suitable for both humans and animals. Though, in these studied the animal number was small, but the test showed similar results as in humans, however, it is suggested that a larger sample size may be selected to have a better understanding about the test results. Mathur and Sachdev (2005) conducted an experimental study to observe the effect of temperature change on the result of the GCT and they concluded that the temperature changes have a notable impact on the GCT. Mahin et al. (1985) checked the effect of changed the glutaraldehyde concentration on the results of GCT and found that the impact of these changes was significant and concluded that the GCT is an unreliable test due to variation in the results, at different concentrations of the solution. However, the results of the present study showed the valid outcome of GCT to diagnose TB, at different variations pertaining to storage of samples and temperature variation to carry out the test. However, it may be true that the variation in concentration of the glutaraldehyde solution has an impact on the outcome of test results.

This study results showed that the coagulation time for the glutaraldehyde test was shorter in TB patients than the healthy control, which may be attributed to increased fibrinogen level in TB positive individuals. Increased fibrinogen level has also been seen in this study in TB positive humans and animals. There was no significant difference in coagulation time between negative controls and hepatitis patients. A decrease in fibrinogen level in hepatitis patients has already been reported by Gallus et al. (1972). This also confirms the relationship of fibrinogen level with coagulation time of the glutaraldehyde test. Apart from fibrinogen, other serum proteins were also studied which showed an inverse relationship with coagulation time in animals but not in humans. An increase in polyclonal gamma globulins and fibrinogen in the blood of TB patients was also suggested by Ahmed et al. (2017). Their results are in line with the findings of the present study. The coagulation time gradually increased as the treatment proceeded and after six months of treatment, the coagulation time started returning to normal. The results showed the success of the currently used DOTS method of anti-tuberculosis treatment. The coagulation time in the patients having both TB and hepatitis C patients was considerably higher and was comparable to the control group. This was attributed to the opposing effects of TB and hepatitis C on blood protein concentrations. The coagulation time in patients positive only for hepatitis C was considerably higher as compared to all other groups. This was linked to a decreased plasma fibrinogen associated with chronic infections. The results in animals were also similar and were in confirmation with those given by De-Kantor et al. (1993). The temperature was found to have an insignificant effect on the results of the test. However, the coagulation time was lowest for the blood samples that were processed immediately after collection without any storage. When the test was conducted in the water bath having a temperature of 22°C, the time was found to be slightly elevated which can be attributed to slower reaction at this temperature. The decrease in coagulation time with increasing temperature was also observed by Mathur and Sachdev (2005). The coagulation time was found to be highest in the blood samples that were given overnight storage. The increase in the coagulation time can be attributed to decomposition and denaturation of various serum proteins by overnight storage. The reaction of these proteins with glutaraldehyde is responsible for the coagulation of blood. Cooling and warming during the storage and subsequent thawing tends to have a negative impact on the concentration and activity of blood proteins. The coagulation time was found to be an effective semi-quantitative measure of hepatitis linked to decrease in fibrinogen concentration. Hence, the fibrinogen concentration can be guessed by the coagulation time with the glutaraldehyde test. A similar pattern was also observed in animals. The increase in serum proteins was also reported by Jemikalajah et al. (2014). Fibrinogen is the main plasma protein responsible for clotting of blood outside the body. It is produced by liver hepatocytes. Its elevation in TB is thought to be responsible for the shortening of jellification time on reaction with the glutaraldehyde. Its level is markedly decreased in hepatitis. This decrease is due to hepatic impairment associated with hepatitis.

Globulins were the other protein, which was significantly different in all the groups as compared to the control group, as these were significantly different even in tuberculosis + HCV and HCV groups. Thus, levels of globulins and the outcome of GCT were unrelated.

The complete blood count (CBC) was also performed on the positive and negative samples of both humans and animals. As reported by Karademir (2006), the values of total leukocyte count (TLC) and differential leukocyte count (DLC) changed significantly ($P<0.05$) in accordance with the glutaraldehyde coagulation test result and concluded that TLC and DLC could be helpful for the diagnostic point of view as well. The CBC values of the present study did not support the statement of Karademir (2006). The blood parameters did not show significant difference in positive than negative animals, while in the case of humans, the hematocrit (HCT) and hemoglobin

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(HGB) showed significantly ($P<0.05$) different values than those in the negative patients, but based on this, the relationship cannot be considered as meaningful.

Keeping in view the results of GCT in the present study, we can state that the time of less than 10 min in humans may be considered as a positive test, between 10 and 20min as doubtful and more that 20min as a negative test. These suggestions are strengthened by the findings of Brink et al. (2005) who states that a positive test within 3 minutes indicates a high level of the fibrinogen and immunoglobulins, between 3 and 6 min indicate a moderate increase in the fibrinogen and immunoglobulins and between 6 and 15min indicate a low level of these components. However, further studies are required before finalizing these timings. However, we also noticed that the TB patients who were observing the DOT treatment for at least nine months, their test appeared negative (data not shown, only a few cases were having treatment of nine months). This finding needs further investigation and can be very helpful in monitoring the TB patients who are under DOT treatment. It is our observation that the TB patients remain under treatment sometime for more than 1 year without clinical indication of the disease, just as a prophylactic measure. Thus, the GCT can be used as a test to decide when to stop the DOT treatment and patient should not undergo unnecessary therapy with hepatotoxic drugs for a long time, when actually it is not required.

Further, the result of the study also indicated that GCT can be used as a screening test on animals and can replace the most cumbersome tuberculin skin test which requires highly trained technicians to perform the test and the wait for the result of the test is also long (72 hours). Furthermore, tuberculin testing in animals leads to drop in milk production and animal remain under stress, therefore, GCT can replace the tuberculin testing in animals.

5. CONCLUSION

The diagnostic value of the glutaraldehyde test for human and animal tuberculosis has been highly speculated. The GCT test results showed statistically significant association in tuberculosis patients.

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Conflict of Interest: There was no conflict of interest.

Authors' Contribution

MTJ ZA, AZ and QHF collected the samples and were involved in sample testing and/or lab activity. MTJ conceived the idea of research, drafted the skeleton, preparation of manuscript; NT, SZ, SUKB and MHA drafted the details of the manuscript helped in data editing, data analysis and construction of tables; IJ and RH did proof reading, added references and checked reference styling etc.; all authors read and approved the final manuscript.

REFERENCES

Aagaard, C., Govaerts, M., Meng Okkels, L., Andersen, P., & Pollock, J. M. (2003). Genomic approach to identification of Mycobacterium bovis diagnostic antigens in cattle. Journal of clinical microbiology, 41(8), 3719–3728. https://doi.org/10.1128/JCM.41.8.3719-3728.200

Agnihotri I, Joshi PK and Tiwari N, 2013. Geographical distribution and surveillance of tuberculosis (TB) using spatial statistics. International Journal of Applied Geospatial Research 4, 39–53.

Ahmed, B. H. H., Manel, M., Mohamed, D., & Jalel, B. (2017). Glutaraldehyde test for the rapid diagnosis of pulmonary and extra-pulmonary tuberculosis in an area with high tuberculosis incidence. Memorias do Instituto Oswaldo Cruz, 112(11), 779–784. https://doi.org/10.1590/0074-02760160541

Alavi-Naini, R., Hashemi, M., Mohagegh-Montazeri, M., Sharifi-Mood, B., & Naderi, M. (2009). Glutaraldehyde test for rapid diagnosis of pulmonary tuberculosis. The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease, 13(5), 601–605.

Benjamin MM, 1978. Outline of Veterinary Clinical Pathology. 3rd Ed, The LoCrtate University Press, Ames, Iowa, USA.

Bennett R. M. (2017). The political economy of bovine tuberculosis in Great Britain. Revue scientifique et technique (International Office of Epizootics), 36(1), 105–114. https://doi.org/10.20506/rst.36.1.2614

Ben-Selma, W., Ben-Kahla, I., Marzouk, M., Ferjeni, A., Ghezal, S., Ben-Said, M., & Boukadida, J. (2009). Rapid detection of Mycobacterium tuberculosis in sputum by Patho-TB kit in comparison with direct microscopy and culture. Diagnostic microbiology and infectious disease, 65(3), 232–235. https://doi.org/10.1016/j.diagmicrobio.2009.07.021

Brink, P., Wright, J. C., & Schumacher, J. (2005). An investigation of the ability of the glutaraldehyde test to distinguish between acute and chronic inflammatory disease in horses. Acta veterinaria Scandinavica, 46(1-2), 69–78. https://doi.org/10.1186/1751-0147-46-69

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Open Access Journal

Clabough, D. L., Conboy, H. S., & Roberts, M. C. (1989). Comparison of four screening techniques for the diagnosis of equine neonatal hypogammaglobulinemia. Journal of the American Veterinary Medical Association, 194(12), 1717–1720.

de Kantor, I. N., Lopez, B., Torres, P., Nader, A., & Garcia, V. (1993). Preliminary evaluation of a simple method for detection of bovine tuberculosis: the glutaraldehyde test. Zentralblatt fur Veterinarmedizin. Reihe B. Journal of veterinary medicine. Series B, 40(1), 27–30. https://doi.org/10.1111/j.1439-0450.1993.tb00105.x

Ejeh, E. F., Raji, M. A., Bello, M., Lawan, F. A., Francis, M. I., Kudi, A. C., & Cadmus, S. I. (2014). Prevalence and direct economic losses from bovine tuberculosis in makurdi, Nigeria. Veterinary medicine international, 2014, 904861. https://doi.org/10.1155/2014/904861

Gallus, A. S., Lucas, C. R., & Hirsh, J. (1972). Coagulation studies in patients with acute infectious hepatitis. British journal of haematology, 22(6), 761–771. https://doi.org/10.1111/j.1365-2141.1972.tb05720.x

Gounder, C., De Queiroz Mello, F. C., Conde, M. B., Bishai, W. R., Kritski, A. L., Chaisson, R. E., & Dorman, S. E. (2002). Field evaluation of a rapid immunochromatographic test for tuberculosis. Journal of clinical microbiology, 40(6), 1989–1993. https://doi.org/10.1128/JCM.40.6.1989-1993.2002

Javed, M. T., Irfan, M., Ali, I., Farooqi, F. A., Wasiq, M., & Cagiola, M. (2011). Risk factors identified associated with tuberculosis in cattle at 11 livestock experiment stations of Punjab Pakistan. Acta tropica, 117(2), 109–113. https://doi.org/10.1016/j.actatropica.2010.10.009

Jemikalajah JD, Okogun GRA, Adu M and Okolie GC (2014). Evaluation of serum proteins in pulmonary tuberculosis. African Journal of Cell Pathology, 3, 20-24.

Karademir B, 2006. Evaluation of glutaraldehyde test in traumatic reticulo-peritonitis in cattle. *Indian Veterinary Journal, 83,* 996-998.

Singh, S. K., & Bhargava, R. (2010). Urine as an adjunct specimen for the diagnosis of active pulmonary tuberculosis. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases, 14 Suppl 3, e345–e346. https://doi.org/10.1016/j.ijid.2009.08.003

Laraque, F., Griggs, A., Slopen, M., & Munsiff, S. S. (2009). Performance of nucleic acid amplification tests for diagnosis of tuberculosis in a large urban setting. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 49(1), 46–54. https://doi.org/10.1086/599037

Larsson S, 1988. A preliminary study on an easy and inexpensive method for the detection of tuberculosis in cattle using the glutaraldehyde test. Journal of Nepal Medical Association, 26, 9-17.

Mahin, L., Chadli, M., Marzou, A., Maach, L., & Ychou, M. (1985). Differences in coagulability of three glutaraldehyde solutions in the glutaraldehyde test on bovine whole blood. Zentralblatt fur Veterinarmedizin. Reihe A, 32(2), 151–154. https://doi.org/10.1111/j.1439-0442.1985.tb01928.x

Mathur, M. L., & Sachdev, R. (2005). Temperature affects the results of the glutaraldehyde test in the diagnosis of pulmonary tuberculosis. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease, 9(2), 200–205.

McEwan, A. D., Fisher, E. W., Selman, I. E., & Penhale, W. J. (1970). A turbidity test for the estimation of immune globulin levels in neonatal calf serum. Clinica chimica acta; international journal of clinical chemistry, 27(1), 155–163. https://doi.org/10.1016/0009-8981(70)90390-6

Radostits OM, Gay CC, Blood DC, Hinchcliff KW, Gay C and Hinchcliff K (2000). Disease caused by bacteria -Mycobacterium. In: Veterinary Medicine: A Textbook of Diseases of Cattle, Sheep, Pig, Goat and Horses. (9th ed.), Harcourt Publisher Ltd, London. pp. 909-918.

Tøllbøll, T., Jørgensen, R.J. (2003). The Glutaraldehyde Coagulation Test (GCT) Used to Predict Health Status in Dairy Herds. Acta Veterinaria Scandinavica 44 (Suppl 1), P113. https://doi.org/10.1186/1751-0147-44-S1-P113

Torgerson, P. R., & Torgerson, D. J. (2010). Public health and bovine tuberculosis: what's all the fuss about?. Trends in microbiology, 18(2), 67–72. https://doi.org/10.1016/j.tim.2009.11.002

Varley H, Gowenlock AH and Bell AH, 1980. Practical clinical biochemistry. (5th Ed). Williams Heinemann Medical Books Ltd, London. pp. 395-535.

World Health Organization 2018. Global tuberculosis report. 2018. WHO/CDS/TB/-2018.20 Geneva, Switzerland.

Citation: Afzal Z, Javed MT, Mohsin M, Ahmad HMW, Saeed Z, Taimoor M, Aleem RA, Raza A, Ayub A, Israr F, Abdeen ZU, Sarfaraz T, Zarnab S, Bahadur SUK and Ullah A, 2024. THE usefulness of glutaraldehyde coagulation test as a conjuncture test in the diagnosis of tuberculosis in humans and animals. Agrobiological Records 15: 34-40. https://doi.org/10.47278/journal.abr/2023.046