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# Evaluation of Using VITEK2 System against BRUCE-LADDER Assay for Rapid Identification of Brucella Field Isolates in Egypt

**Heba M. Soliman[1], Marwa S. Diab[1], Marwa Fathy Elsayed[1], Maha A. Elshazly[1]*, Manar F. Seioudy[1], Shaimaa Abdelall Elsaady[1], Lubna F Farahat[2] and Waleed Saad Eldin Shell[1]**

[1] *Central Laboratory for Evaluation of Veterinary Biologics (CLEVB)-Agriculture Research Centre (ARC), Cairo, Egypt.*
[2] *Veterinary Vaccine and Serum Research Institute (VSVRI)-Agriculture Research Centre (ARC), Cairo, Egypt.*

**Abstract**

THE current investigation was conducted to evaluate how well the VITEK 2 compact system identified Brucella strains utilizing Gram-Negative (GN) identification cards while utilizing the VITEK 2 automated microbial identification system. The 47 substrates or biochemical tests that measure inhibition and resistance, carbon source utilization, and related enzymatic activities form the basis of the VITEK 2 GN card. All Brucella isolates and strains, 3 Brucella vaccine strains, six reference strains, and 30 well-characterized field isolates were identified using traditional methods and the BRUCE-LADDER assay. Nine of the field isolates were identified as *Brucella abortus*, and the other 21 isolates were identified as *Brucella melitensis*. All Brucella isolates and strains were identified correctly using the VITEK 2 system as *Brucella melitensis*, with a different probability range from 97-99 %, with 100% sensitivity. The probability of identification of both *Brucella abortus* and *Brucella ovis* strains was 97%, where the probability of identification of *Brucella melitensis* strains and *Brucella suis* strains was 99% and 98%, respectively. According to these findings, the VITEK 2 GN system is a suitable automated technique that can identify Brucella isolates at the species level with accuracy, speed, and dependability.

**Keywords:** Brucella, BRUCE-LADDER, VITEK 2 system, Brucella vaccination.

## Introduction

Worldwide, brucellosis is a contagious bacterial infection and a significant bacterial zoonosis of livestock that has a significant economic impact. Both humans and animals, including pigs, cattle, sheep, goats, rodents, and marine mammals, can have Brucellae, which are Gram-negative intracellular bacteria. The disease mostly affects the reproductive system of food animals, resulting in a decrease in the animals' productivity and fertility. Infection in humans is characterized by frequent episodes of fever, which is why it is called "undulant fever". In humans, Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia, and prostration, and which, in the absence of specific treatment, may persist for weeks or months (economic, public, and one health importance). Effective and secure immunization programs and precise diagnostic instruments are essential to both animal and human brucellosis control and preventative efforts [1-2].

In addition to its significant zoonotic component with a bioweapon potential and posing a significant public health concern, *Brucella melitensis* is the most significant causative agent of ovine and caprine brucellosis, a disease that results in abortion in sheep and goat ewes and causes financial losses, especially in Mediterranean nations like Egypt [3-4]. Although an infection with *B. ovis* is not regarded as a zoonotic disease, it causes epididymitis, miscarriage, and infertility in sheep, resulting in significant financial losses Worldwide [5]. Controlling the disease in animals and, by extension, humans, depending on an accurate identification of Brucella spp. infection. Although clinical diagnosis is a tentative diagnosis that requires confirmation using clinical laboratory tests, it is typically based on clinical indicators and the history of reproductive failures in animals. There are 2 methods of diagnosis: either detection of antibodies against Brucella (serological tests) or

*Corresponding authors: Maha A. Elshazly; dr.mahaahmed2006@gmail.com Tel.: 01101009967
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detection of Brucella antigens (isolation and identification, and molecular identification). Still widely used are serological assays like the complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), and rose Bengal plate test (RBPT) [6].

The "gold standard" for diagnosing brucellosis involves isolating and identifying bacteria from clinical specimens, which necessitates extended cultivation times. All of these tests, however, take a lot of time, call for experts, and require some necessary reagents that are typically not sold commercially [7]. The purpose of this work was to identify Brucella strains and isolates at the species level utilizing a VITEK assay to get around the majority of these challenges [8-9]. Accurate diagnosis and timely, efficient treatment of these illnesses depend on VITEK's fast and accurate bacterial species identification. Numerous commercial and manual identification techniques have been created and are currently in widespread usage. The completely automated VITEK 2 system (bioMérieux, Inc., Hazelwood, Mo.) can identify gram-positive cocci in a few hours, concerning the fluorescence-based technology's better sensitivity. This feature is a significant advancement over previous iterations of the same system. The goal of the current investigation was to assess the VITEK compact 2 system's sensitivity and dependability in identifying Brucella isolates and strains at the species level as part of standard laboratory procedures in comparison to other traditional identification methods as biochemical and molecular identification which are very sensitive and accurate but are known to be very time-consuming and labor-intensive as well as material consuming and need well trained persons.

## Materials and Methods

*Strains of Brucella*

CZ Veterinaria S.A., Pontevedra, Spain, provided three lyophilized live attenuated vaccine strains: *B. abortus* biovar 1 (S19 and RB51) and *B. melitensis biovar* 1 (Rev-1). Prof. Dr. JM Blasco of the CITA Institute in Zaragoza, Spain, generously provided six reference strains: *B. melitensis* biovar 1 (16M), *B. melitensis* biovar 3 (ETHER), *B. abortus* biovar 1 (S544), *B. suis* biovar 1 (1330), *B. suis* biovar 2 (S2), and B. *ovis* (REO198). Together with 30 Brucella field isolates that have been recovered from different animal species (cattle, sheep, buffalo, and goats) by Prof. Dr. Ashraf E. Sayour (Animal Health Research Institute, Egypt) and Prof. Dr. Waleed S. Shell (CLEVB). Additionally, all of the strains included in this study were identified using standard techniques, and they were all cultivated on media that contained various antibiotics and stains. [10-11] to distinguish between Brucella species, particularly vaccine strains, and the outcomes were contrasted with those recorded using the multiplex PCR (BRUCE-LADDER ASSAY) technique.

*Genomic DNA extraction from strains of Brucella*

A kit (EasyPure®Bacteria Genomic DNA kit, TRANSgenbiotech, China) was used to extract genomic DNA. DNA extracts were kept at -20°C until they were needed.

*Bruce-Ladder Multiplex PCR*

This study utilized a previously standardized multiplex PCR assay known as Bruce-ladder, conducted following the methodologies outlined by [10-12-13], incorporating certain modifications. Five primer pairs (Metabion International AG, Germany) (Table 1) were utilized in multiplex for molecular typing of various Brucella species in Bruce-ladder PCR. These primer pairs were created based on strain-specific genetic variations. 39 multiplex PCR reaction mixes, each with a volume of 50 µl, were used in this investigation. These included 20 pmol of each primer, PCR master mix (2x EasyTaq® PCR SuperMix, TRANSgenbiotech, China), and template DNA. Thermo Cycler TC-TE, BOECO, Germany, was used to do the PCR amplification.

For the first denaturation, the cycling conditions were 7 minutes at 95 °C, 25 cycles of 30 seconds each at 94 °C, 30 seconds at 64 °C for primer annealing, 1 minute and 40 seconds at 72 °C for amplicon extension, and one cycle at 72 °C for 7 minutes for final extension. Ten microliters of the PCR results were run in a 1.5% agarose gel stained with ethidium bromide (0.5 µg/ml) to examine the PCR amplicons. The amplification pattern of each Brucella species was then identified based on the molecular size of the amplified products after gels were photographed under UV light.

*Compact Automated Identification System, VITEK 2:*

The standard Gram-negative bacteria identification card on the automated VITEK 2 system was applied to analyze 47 biochemical reactions of the Brucella strains in compliance with the specifications provided by the manufacturer.

Identification of strains and isolates was carried out utilizing the VITEK 2 Compact (bioMérieux). Using sterile cotton swabs, using a calibrated VITEK 2 DensiCheck instrument (bioMérieux), a bacterial suspension comprising several pure isolated colonies was aseptically prepared in 0.45% aqueous NaCl and adjusted to a McFarland standard of 0.5 needed for the GN reagent. The 47 biochemical tests or substrates on the card for Gram-negative (GN) bacterial biochemical tests (Tables 2 and 3) were utilized.

A variety of identification levels based on numerical probability calculations were taken into account when interpreting the results. These levels were as follows: acceptable (likelihood 85 to 88%), very good

(probability 93-95%), excellent (probability 96-99%), and good (probability 89-92%).

## Results

Results of traditional methods for identification of Brucella isolates (10, 11) and strains revealed that 9 of the field isolates were *Brucella abortus*, while 21 of the field isolates (70% of the field isolates) were identified as *Brucella melitensis.* All Brucella reference strains gave the expected outcomes.

All 30 field isolates that had previously been identified at the species level using traditional methods were verified by Bruce-Ladder PCR with 100% sensitivity, while all reference strains and negative controls of Brucella produced the anticipated results, which were identical to the results of conventional methods (Photos 1 and 2).

Bruce-ladder products were of 1682, 1071, 587 bp with 21 field isolates, ETHER, 16M and Rev.1 and with additional band at 218 bp with rev.1 strain, 9 field isolates and S544 react with BRUCE-LADDER and gave bands of 1682 and 587 bp where RB51 and S19 gave 2524 and 587 bp and 1682 bp respectively. Two *Brucella suis* (S1330 and S2) strains were confirmed by PCR as they generated 272, 587, 1071, and 1682 bp fragment sizes, while *Brucella ovis* REO198 gave fragments of 587 and 1071 bp on amplification (Photos 3 and 4).

Results of VITEK 2 revealed that out of 47 substrates, only seven biochemical indicators, APPA, ELLM, SUCT, ProA, TyrA, URE, and GlyA, could be utilized to identify and differentiate between species of Brucella. All Brucella strains, either field isolates or nine reference and vaccine strains of Brucella, were tested positive for ProA, GlyA, and TyrA, where all *Brucella melitensis* references and vaccine strains and traditionally identified *Brucella melitensis* field isolates reacted positively also with URE substrate, and where all *Brucella abortus*-characterized strains and isolates reacted negatively with URE as with rough vaccine strain RB51 or positively as with smooth reference strain S544 with SUCT. *Brucella ovis* strain REO198 gives an additional positive biochemical reaction, which was ELLM. *Brucella suis* 1330 and S2 were also positive in URE, ELLM (Table 4).

Every strain used in this investigation was accurately identified as *Brucella melitensis* at high probability levels (excellent), ranging from 97%-99% and with 100% sensitivity. The probability of identification of both *Brucella abortus* and *Brucella ovis* strains was 97%, whereas the probability of identification of strains of *Brucella melitensis* was 99% and 98%. The probability of identification of strains of *Brucella suis* ranges from 98% to 99% according to the reaction with APPA in case of positive reaction, as with *Brucella suis* reference strain 1330 the Probability was 98% where in case of negative reaction the probability was 99% as with *Brucella* suis reference strain S2 (Tables 5, 6 and 7).

## Discussion

Traditional techniques like culture, biochemical characterization, and serological identification have been used to identify the many Brucella species. Additionally, new techniques are presented in the area of Brucella laboratory identification. Using both conventional and modern techniques, 39 strains of Brucella were found in this investigation (30 local isolates, 3 vaccine strains, and 6 reference virulent strains).

The local field isolates were obtained from several animal species and were serologically identified as having brucellosis positive by the Rose Bengal screening test. S19 and RB51 were the vaccine strains, which are live attenuated Brucella vaccines used for control of bovine brucellosis, while vaccine strain Rev.1 is a live attenuated vaccine used for eradication and control of ovine brucellosis. The six reference virulent strains were, namely, 16M, ETHER, S544, S1330, S2 (*B. suis* biovar 2), and REO198. Neither REO198 nor S2 is a zoonotic Brucella strain.

While the culture and cultural characteristics of the thirty-three strains were in line with those of the normal Brucella species, the strains were conventionally recognized using the most widely used techniques. Within 72 hours, all strains developed, and they appeared pink when stained with modified Ziehl-Neelsen and Gram-negative when stained with Gram stain.

RB51 may develop in a culture with 250 µg/ml of rifampicin and resist it [10]. Conversely, vaccination strain S19 is sensitive to media containing thionin blue (2 µg/ml), i-erythritol (1 mg/ml), and penicillin (5 IU/ml), whereas vaccine strain Rev-1 is the only strain that can withstand and thrive on culture containing streptomycin (2.5 µg/ml).

Both REO198 and RB51 showed rough colonies (uptake crystal violet) in contrast to all other Brucella species in this study, which are smooth strains, while all other Brucella strains, except RB51 and REO198, revealed smooth colonies and crystal violet had not been taken up by these smooth colonies.

The biochemical methods confirmed that vaccine strains were *Brucella abortus* S19 and RB51, while the other vaccine strain was *Brucella melitensis* Rev 1. Two of the reference strains behaved as *Brucella melitensis* biovar 3, which were 16M and ETHER 16M, while one of the reference strains reacted as *Brucella abortus* biovar 1 (S544). Strains 1330 and S2 behaved as *Brucella suis* biovar 1 and 2, respectively. On the other hand, one reference strain was identified as *Brucella ovis* (REO198), and ETHER strains exhibited biochemical characteristics similar to those of *B. melitensis.*

Based on their biochemical identification, all Egyptian field isolates were determined to be *B. melitensis* biovar 3. Serological identification using 3 monospecific antisera (A, M, and R) had confirmed the results of the biochemical identification. The identification process employed three monospecific antisera (A, M, and R). Where strain S19 and S544 reacted with the monospecific antiserum A, confirming their *B. abortus* behavior, the vaccine strain RB51 and reference strain REO198 reacted with the monospecific antiserum R, as they are rough strains and devoid of O-chain lipopolysaccharide. The monospecific antiserum M was used to agglutinate the Rev-1, ETHER, and 16M strains as well as all other Brucella isolates, which are *B. melitensis*. Strains S2 and S1330 were agglutinated with both monospecific antiserum M and A, as they are *Brucella suis* reference strains.

As a DNA-dependent technique, molecular characterization trials have been used to identify and distinguish between the many species and biovars of Brucella that depend mainly on the detection of genetic materials of Brucella in specimens or culture of isolated organisms on enriched media containing selective supplements, which consist of cocktail of antibiotics to which Brucella is resist. Recently, DNA-dependent or molecular diagnosis techniques have been proposed as global laboratory tools for Brucella isolate identification and differentiation.

The two most used techniques for molecularly diagnosing Brucella isolates are AMOS PCR which used for identification of *B. abortus*, *B. melitensis*, *B. suis* and *B. ovis*, Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA) which can identify Brucella on biovar level and also vaccine strains, BRUCELADDER ASSAY which can identify Brucella on species level and also vaccine strains REAL TIME PCR which can identify Brucella generally and *Brucella abortus*, *Brucella melitensis* and *Brucella sui*s and Whole Genome Sequencing (WGS) can identify any genetic mutations or newly isolated species.

In the current investigation, field isolates and Brucella vaccine strains were identified and separated from other Brucella species and biovars using a multiplex PCR assay and genomic DNA extraction. This work used five multiplex primer sets that were previously created to identify the distinct genomic variations of Brucella species based on the entire genome sequences of *B. melitensis*, *B. suis*, *B. ovis*, and B. *abortus* that have been published (Table 1). Based on the size of the generated amplicons, the Brucella species were distinguished.

A total of 27 *B. melitensis* strains consisting of the reference strains 16M, along with ETHER, the Rev1 vaccine strain, and 24 field strains, where the infecting strains were biochemically identified as *B. melitensis.* Three bands with molecular weights of 1682 bp, 1071 bp, and 587 bp were amplified by the BRUCELADDER test for both *Brucella melitensis* reference strains 16M and ETHER reference, and also for field-recovered isolates of DNA. Ovine Brucella vaccine strain (Rev-1) had differentiated from other Brucella spp. especially *Brucella melitensis* isolates and stains with an additional specific fragment of molecular weight 218 bp generated by the BMEI0752 primer pair which represent a S12, gene rpsLwhich is a distinct point mutation in the vaccine strain *B. melitensis* Rev-1's rpsL gene, which codes for the ribosomal protein S12 and causes Streptomycin resistance in Rev-1 [14]. The reference strain *B. ovis* REO198 produced two bands measuring 1071 and 587 bp and was identified by the lack of the 1682 bp fragment. This was caused by the BMEI0998 and BMEI0997 primers' inability to anneal to the target sequence due to a 15-kb deletion that included both the wboA-wboB omp25b genes in the *B. ovis* species, which makes the strains of *Brucella ovis* rough [15]. *B. abortus* isolates and strains were differentiated from other Brucella species by the absence of the gene coding for omp31. On the other hand, these strains identified by 2 characteristic bands represent the gene coding for Glycosyltransferase (gene wboA), which is responsible for the production of O-chain of lipopolysaccharide (1682 bp) and D-erythrulose-1-phosphate dehydrogenase (eryC) gene and erythritol catabolism (587 bp) [16].

All Brucella species possess eryC gene except vaccine strain *B. abortus* S19 so it can be identified from other Brucella species by the absence of 587 bp band that represent for Ery C. the absence of eryC gene may be the reason that S19 do not cause complete catabolism for Erythritol and so it is less abortifacient than other Brucella species including other vaccine strain as RB51 and Rev.1 [17].

However, the *B. abortus* RB51 vaccine strain experienced interlude of the wboA gene due to an addition sequence 711 (IS711) element led to that RB51 DNA react with the primers set giving higher band than other smooth Brucella species (2524 bp) and this in contrast with other rough strains which miss this gene in its genome. Disruption by an IS711 led to that although presence of this gene, but it is not functioning, so there is no production of the O chain of lipopolysaccharide [18].

*Brucella suis* strains (S2 and 1330) have the same profile as that of *Brucella melitensis*, with an additional fragment at 272 bp representing a gene ABC-type transporter, which is deleted in both *Brucella melitensis* and *Brucella abortus* [19]. Pervious mentioned results concluded that the Bruce-ladder PCR assay is recommended for laboratory routine work for detection of brucellosis, and additionally, testing and assessing the seed cultures that are frequently employed in quality control labs to produce live Brucella vaccines (Rev-1, S19, and RB51 vaccines)

According to the previously reported findings, all of the investigated Brucella species and the vaccine strains could be successfully identified and distinguished in a single test using Bruce-ladder multiplex PCR.

In addition, with conferring many benefits over the current methods of Brucella identification, the major advantage is that it is a rapid method in which it just requires the direct addition of 104 bacteria to the reaction mixture, allowing the experiment to be carried out with little sample preparation.

Using growth-based technologies, the VITEK 2 is an automated microbiological system. Brucella isolates were identified and confirmed using the GN colorimetric reagent card, which is available for the identification of fermenting and non-fermenting bacilli that are Gram-negative bacilli.

Each of the 64 wells on the reagent cards can hold a different test substrate. Acidification, alkalinisation, enzyme hydrolysis, and growth in the presence of inhibitory chemicals are only a few of the metabolic processes that substrates assess.

Every strain used in this investigation was accurately identified as *Brucella melitensis* at high probability levels (excellent), ranging from 97%-99% and with 100% sensitivity. The probability of identification of both *Brucella abortus* and *Brucella ovis* strains was 97%, where the probability of identification of *Brucella melitensis strains* and *Brucella suis* strains was 99% and 98%, respectively.

Kang *et al.*, 2015 agreed with our results as they used VITEK 2 for identification of Brucella isolates, and the results showed that four biochemical indicators, ProA, TyrA, URE, and GlyA, could be utilized to distinguish between several species of Brucella, but according to the results of the current study, all eight reference strains of *B. abortus* and twenty-one field strains tested positive for ILATk (L-lactate alkalisation), while three reference strains of *B. melitensis*, ninety-two field strains, and strain 8416 tested negative.

Melzani *et al.*, 2023 noted that laboratory misidentification is problems as VITEK® 2 regularly misidentify Brucella as the closely related Ochrobactrum species, which may be due to related to the recent reclassification of Ochrobactrum, Falsochrobactrum, and Pseudochrobactrum as Brucella. Therefore, identification of Ochrobactrum in a clinical laboratory should be followed by serological tests for brucellosis and molecular analysis for identification confirmation, wherein the VITEK 2 system was utilized to recognize every strain of Brucella and isolates in the current investigation as *Brucella melitensis.*

Most of the research, including our results, used the VITEK 2 system to identify Brucella on the genus level, and all Brucella strains were identified as *Brucella melitensis*, but the ratio of probability and some differences in biochemical patterns may help in identifying Brucella on the species level. [22] According to the NRL's differential testing, strain 08RB3647 is classified as a non-motile, Gram-negative coccoid rod.

The strain was positive for the synthesis of catalase, oxidase, and urease but negative for haemolysis and H2S. It also did not require CO2. VITEK GN ID card biochemical identification indicated *Brucella melitensis.* Also, [23-24] highlighted that MALDI-TOF MS was shown to accurately determine Brucella spp. to the genus level with minimal correction for species-level identification, but the VITEK® 2 automated system Gram-negative card can only identify culture as *B. melitensis* or Brucella species, respectively.

## Conclusion

In conclusion and according to above mentioned results, the VITEK 2 system is recommended assay for rapid, sensitive, and reliable identification of Brucella at the species level and can be implemented as a routine laboratory work in association with serological tests and epidemiological situation of the area under test.

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*Declaration of Conflict of Interest*

The authors declare that there is no conflict of interest.

*Ethical of approval*

There is no need for Ethical approval in our research, as we did not use any laboratory animals in our experiment.

*Authors' Contributions*

The contribution of the authors was as follows: H.M.S. was the owner of the main idea and design of the work, M.A.E., M.S.D., and L.F.F. performed the experiments and laboratory work, M.F.E., M.F.S., and S.A.E. collected reviews, followed up on the work and interpretations, W.S.S. was the supervisor and wrote the original manuscript, M.A.E. and H.M.S. edited and revised the manuscript. All authors have read and approved the final manuscript.

**TABLE 1. Primer sets for Bruce-ladder multiplex PCR**

| Primer | Sequence (5'–3') | Amplicon size (bp) | DNA targets | Source of genetic difference |
|---|---|---|---|---|
| **BMEI0998F** | ATC-CTA-TTG-CCC-CGA-TAA-GG | 1682 | Glycosyltransferase, gene wboA | S711 insertion in BMEI0998 in *B. abortus* RB51, and deletion of 15,079 bp in BMEI0993-BMEI1012 in *B. ovis* |
| **BMEI0997R** | GCT-TCG-CAT-TTT-CAC-TGT-AGC | | | |
| **BMEII0843F** | TTT-ACA-CAG-GCA-ATC-CAG-CA | 1071 | Outer membrane protein, gene omp31 | deletion of 25,061 bp in BMEII826- BMEII0850 in *B. abortus* |
| **BMEII0844R** | GCG-TCC-AGT-TGT-TGT-TGA-TG | | | |
| **BMEII0428F** | GGA-ACA-CTA-CGC-CAC-CTT-GT | 587 | Erythritol catabolism, gene eryC (D-erythrulose-1 phosphate dehydrogenase) | deletion of 702 bp in BMEII0427- BMEII0428 in *B. abortus* S19 |
| **BMEII0428R** | AAT-GAC-TTC-ACG-GTC-GTT-CG | | | |
| **BR0953F** | GGA-ACA-CTA-CGC-CAC-CTT-GT | 272 | ABC transporter binding protein | deletion of 2653 bp in BR0951 BR0955 in *B. melitensis and B. abortus* |
| **BR0953R** | GAT-GGA-GCA-AAC-GCT-GAA-G | | | |
| **BMEI0752F** | CAG-GCA-AAC-CCT-CAG-AAG-C | 218 | Ribosomal protein S12, gene rpsL | point mutation in BMEI0752 in *B. melitensis* Rev.1 |
| **BMEI0752R** | GAT-GTG-GTA-ACG-CAC-ACC-AA | | | |

**TABLE 2. Biochemical tests included in the VITEK 2 GN card**

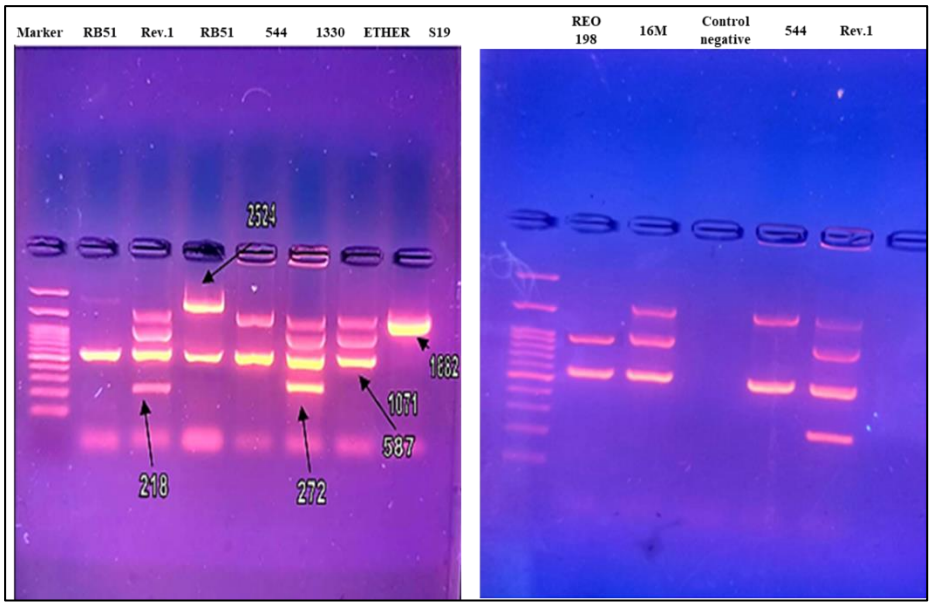
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | 3 | ADO | 4 | PyrA | 5 | IARL | 7 | dCEL | 9 | BGAL |
| 10 | H2S | 11 | BNAG | 12 | AGLTp | 13 | dGLU | 14 | GGT | 15 | OFF |
| 17 | BGLU | 18 | dMA | 19 | dMAN | 20 | dMNE | 21 | BXYL | 22 | BAlap |
| 23 | ProA | 26 | LIP | 27 | PLE | 29 | TyrA | 31 | URE | 32 | dSOR |
| 33 | SAC | 34 | dTAG | 35 | dTRE | 36 | CIT | 37 | MNT | 39 | 5KG |
| 40 | ILATk | 41 | AGLU | 42 | SUCT | 43 | NAGA | 44 | AGAL | 45 | PHOS |
| 46 | GlyA | 47 | ODC | 48 | LDC | 53 | IHISa | 56 | CMT | 57 | BGUR |
| 58 | O129R | 59 | GGAA | 61 | IMLTa | 62 | ELLM | 64 | ILATa | | |

**TABLE 3. Abbreviation of biochemical tests included in the VITEK 2 GN card**

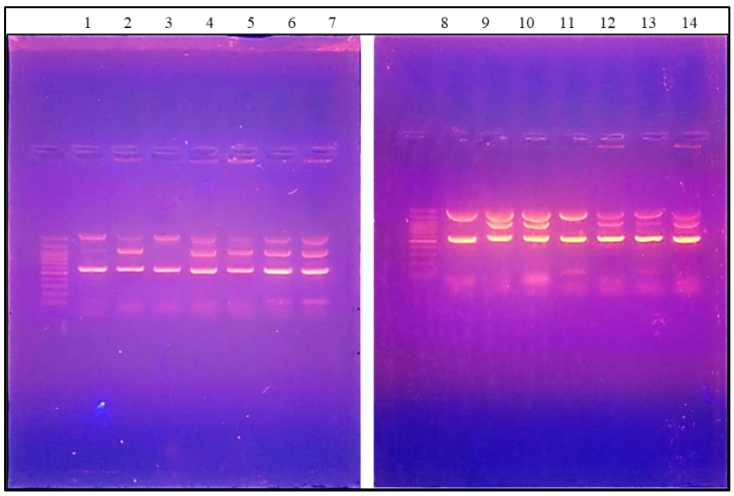
| No | Symbol | Chemistry name | No | Symbol | Chemistry name |
|---|---|---|---|---|---|
| 2 | APPA | *Ala-Phe-Pro Arylamidase* | 5 | IARL | *L-Arabitol* |
| 10 | H2S | Produksi H2S | 13 | dGLU | *d-Glucose* |
| 17 | BGLU | *Beta-Glucose* | 20 | dMNE | *d-Mannose* |
| 23 | ProA | *L-Prolin Arylamidase* | 29 | TyrA | Tyrosine Arylamidase |
| 33 | SAC | *Saccharose/Sucrose* | 36 | CIT | *Citrate/Sodium* |
| 40 | ILATk | *L-Lactate alkalinization* | 43 | NAGA | *Beta-N-Acetyl* |
| 46 | GlyA | *Glycine Arylamidase* | 53 | IHISa | *Histidine assimilation* |
| 58 | O129r | O/129 *Resistance* | 62 | ELLM | *Ellman* |
| 3 | ADO | *Adonitol* | 7 | dCEL | *D-Cellobiose* |
| 11 | BNAG | *Beta-N-Acetyl-Glucosaminidase* | 14 | GGT | *Gamma-Glutamyl-Transferase* |
| 18 | dMAL | *D-Maltose* | 21 | BXYL | *B-Xylose* |
| 26 | LIP | *Lipase* | 31 | URE | *Urease* |
| 34 | dTAG | *D- Tagatosa* | 37 | MNT | *Malonate* |
| 41 | AGLU | *Alpha-Glucosidase* | 44 | AGAL | *Alpha-Galactosidase* |
| 47 | ODC | *Ornithine Decarboxylase* | 56 | CMT | *Coumarate* |
| 59 | GGAA | *Glu-Gly-Arg-Arylamidase* | 64 | ILATa | *L-Lactate assimilation* |
| 4 | PyrA | *L-Pyrrolydonyl-Arylamidase* | 9 | BGAL | *Beta-Galactosidae* |
| 12 | AGLTp | *Glutamyl Arylamidase Pna* | 15 | OFF | *Fermentation Glucose* |
| 19 | dMAN | *D-Mannitol* | 22 | BAlap | *Beta-Alanine Arylamidase* |
| 27 | TyrA | *Tyrosine Arylamidase* | 32 | dSOR | *D-Sorbitol* |
| 35 | CIT | *Citrate/Sodium* | 39 | 5KG | *5-Keto-D-Gloconate* |
| 42 | NAGA | *Beta-N-Acetyl* | 45 | PHOS | *Phospatase* |
| 48 | IHISa | *Histidine assimilation* | 57 | BGURr | *Beta-Glucuronidase* |
| 61 | ELLM | *Ellman* | | | |

**TABLE 4. Seven biochemical indicators for the identification of different Brucella strains and isolates**

| Substrate | *B. abortus* | *B. melitensis* | *B. suis* | *B. ovis* |
|---|---|---|---|---|
| APPA | - | - | +/- | - |
| ProA | + | + | + | + |
| GlyA | + | + | + | + |
| ELLM | - | - | + | + |
| TyrA | + | + | + | + |
| URE | - | + | + | - |
| SUCT | (+) / (-) | - | - | - |
| Probability (*B. melitensis*) | 97% | 99% | 98% | 97% |




**Photos 1 and 2. BRUCE-LADDER results of reference strains.**




**Photos 3 and 4. Examples of profiles of field *Brucella* isolates (from 1 to 14) obtained after Bruce-Ladder PCR and gel electrophoresis.**

**TABLE 5. VITEK 2 report of results of reference strain Brucella *abortus* S544 showing 97% probability as *Brucella melitensis***

***McFarland: (0.50- 0.63)***

| | | | |
|---|---|---|---|
| Identification Information | Card: GN | Lot Number: 2411784103 | |
| | Status: Final | Analysis Time: 9.78 hours | |
| Organism Origin | VITEK 2 | | |
| Selected Organism | 97 % Probability | **Brucella melitensis** | |
| | Bionumber: 0000001100401000 | Confidence: Low discrimination | |

Biochemical Details

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | - | 5 | IARL | - | 7 | dCEL | - | 9 | BGAL | - |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | - | 13 | dGLU | - | 14 | GGT | - | 15 | OFF | - |
| 17 | BGLU | - | 18 | dMAL | - | 19 | dMAN | - | 20 | dMNE | - | 21 | BXYL | - | 22 | BAlap | - |
| 23 | ProA | + | 26 | LIP | - | 27 | PLE | - | 29 | TyrA | + | 31 | URE | - | 32 | dSOR | - |
| 33 | SAC | - | 34 | dTAG | - | 35 | dTRE | - | 36 | CIT | - | 37 | MNT | - | 39 | 5KG | - |
| 40 | ILATk | - | 41 | AGLU | - | 42 | SUCT | (+) | 43 | NAGA | - | 44 | AGAL | - | 45 | PHOS | - |
| 46 | GlyA | + | 47 | ODC | - | 48 | LDC | - | 53 | IHISa | - | 56 | CMT | - | 57 | BGUR | - |
| 58 | O129R | - | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

**TABLE 6. VITEK 2 report of results of reference strain *Brucella suis* S2 showing 99% probability as *Brucella melitensis.***

***McFarland: (0.50- 0.63)***

| | | | |
|---|---|---|---|
| Identification Information | Card: GN | Lot Number: 2411926503 | |
| | Status: Final | Analysis Time: 7.78 hours | |
| Organism Origin | VITEK 2 | | |
| Selected Organism | 99 % Probability | **Brucella melitensis** | |
| | Bionumber: 0000001300001001 | Confidence: Excellent identification | |

Biochemical Details

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | - | 5 | IARL | - | 7 | dCEL | - | 9 | BGAL | - |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | - | 13 | dGLU | - | 14 | GGT | - | 15 | OFF | - |
| 17 | BGLU | - | 18 | dMAL | - | 19 | dMAN | - | 20 | dMNE | - | 21 | BXYL | - | 22 | BAlap | - |
| 23 | ProA | + | 26 | LIP | - | 27 | PLE | - | 29 | TyrA | + | 31 | URE | + | 32 | dSOR | - |
| 33 | SAC | - | 34 | dTAG | - | 35 | dTRE | - | 36 | CIT | - | 37 | MNT | - | 39 | 5KG | - |
| 40 | ILATk | - | 41 | AGLU | - | 42 | SUCT | - | 43 | NAGA | - | 44 | AGAL | - | 45 | PHOS | - |
| 46 | GlyA | + | 47 | ODC | - | 48 | LDC | - | 53 | IHISa | - | 56 | CMT | - | 57 | BGUR | - |
| 58 | O129R | - | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | + | 64 | ILATa | - | | | |

**TABLE 7. VITEK 2 report of results of reference strain *Brucella melitensis* ETHER showing 99% probability as *Brucella melitensis***

***McFarland: (0.50- 0.63)***

| | | | |
|---|---|---|---|
| Identification Information | Card: GN | Lot Number: 2411784103 | |
| | Status: Final | Analysis Time: 7.85 hours | |
| Organism Origin | VITEK 2 | | |
| Selected Organism | 99 % Probability | **Brucella melitensis** | |
| | Bionumber: 0000001300001000 | Confidence: Excellent identification | |

Biochemical Details

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | - | 5 | IARL | - | 7 | dCEL | - | 9 | BGAL | - |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | - | 13 | dGLU | - | 14 | GGT | - | 15 | OFF | - |
| 17 | BGLU | - | 18 | dMAL | - | 19 | dMAN | - | 20 | dMNE | - | 21 | BXYL | - | 22 | BAlap | - |
| 23 | ProA | + | 26 | LIP | - | 27 | PLE | - | 29 | TyrA | + | 31 | URE | + | 32 | dSOR | - |
| 33 | SAC | - | 34 | dTAG | - | 35 | dTRE | - | 36 | CIT | - | 37 | MNT | - | 39 | 5KG | - |
| 40 | ILATk | - | 41 | AGLU | - | 42 | SUCT | - | 43 | NAGA | - | 44 | AGAL | - | 45 | PHOS | - |
| 46 | GlyA | + | 47 | ODC | - | 48 | LDC | - | 53 | IHISa | - | 56 | CMT | - | 57 | BGUR | - |
| 58 | O129R | - | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

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## تقييم استخدام جهاز الفيتك 2 مقابل اختبار Bruce Ladder في التعرف السريع لعترات البروسيلا الحقلية بمصر.

**هبة الله محمد سليمان[1] ،مروة صلاح الدين دياب[1]، مروة فتحي السيد[1]، مها أحمد السيد الشاذلي[1]، منار فتحي حسن سعودي[1]، شيماء عبد العال[1]، لبني فادي فرحات أحمد[2] و وليد سعد الدين عبد اللطيف[1]**

[1] المعمل المركزي للرقابة علي المستحضرات البيطرية - مركز البحوث الزراعية ، القاهرة ، مصر.
[2] معهد بحوث الأمصال واللقاحات البيطرية ، مركز البحوث الزراعية ،القاهرة ، مصر.

**الملخص**

تم إجراء هذه الدراسة لتقييم كفاءة نظام VITEK Compact 2 في تحديد سلالات البروسيلا باستخدام بطاقة التعريف علي البكتريا سالبة الجرام (GN) ضمن نظام VITEK 2 الآلي لتحديد الميكروبات. تعتمد بطاقة VITEK 2 (GN) علي 47 اختبارا كيميائيا حيويا تقيس مقاومة وتثبيط البكتريا، واستخدام مصادر الكربون ، والأنشطة الإنزيمية المرتبطة بها.

تم تحديد جميع عزلات وسلالات البروسيلا بما في ذلك 3 سلالات لقاحية و6 سلالات مرجعية و30 عزلة ميدانية محددة باستخدام الطرق التقليدية واختبار Bruce Ladder. من بين العزلات تم تحديد 9 عزلات علاي انها *Brucella abortus* بينما تم تحديد 21 عزلة علي انها *Brucella melitensis*. تمكن نظام VITEK 2 من تحديد جميع عزلات وسلالات البروسيلا بدقة حيث تم التعرف عليها علي انها *Brucella melitensis* بنسبة 97%-99% مع حساسية 100%.كانت نسبة تحديد *Brucella abortus* و *Brucella ovis* 97%. بينما كانت نسبة تحديد *Brucella melitesis* كانت 99% و *Brucella suis* 98% . تشير هذة النتائج إلي أن نظام VITEK 2 (GN) هو طريقة آلية مناسبة يمكنها تحديد عزلات البروسيلا علي مستوي النوع بدقة وسرعة وموثوقية .

**الكلمات الدالة:** Bruce Ladder ، بروسيلا ، VITEK 2 system، تلقيح البروسيلا.