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Evaluation of six culture media for supporting the isolation of *Brucella abortus* and *Brucella melitensis*

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Abstract:
Brucellosis is endemic in many developing countries and is caused by many species of the genus *Brucella* that affect man, domestic and some wild animals, and marine mammals, It is estimated at 500, 000 new human cases of brucellosis annually worldwide *Brucellosis* is the most important zoonosis and has gained prominence over the years since its discovery on the island of Malta.To facilitate monitoring of culture media, a simple quantitative streaking techniques in spiral plating was used. The procedure evaluates, in quantitative terms, the ability of media to support the formation of colonies by organisms under test. The procedure was named ecometric evaluation.. This study aimed to compare between six media for their degree in supporting the growth of *Brucella*. These media included Potato infusion agar, Serum Dextrose agar, Tryptose agar, Tryptose Soy agar, Thayer-Martin agar and Blood agar. All media in the study passed the ecometric assessment with different degrees, indicating that most of these formulations would provide an acceptable medium for isolation of *Brucella* even if the sample contains small numbers of viable organisms. Two isolates were used for conducting the tests, *Brucella abortus* (animal isolate) and *Brucella melitensis* (human isolate). , a duplicate of each media was used. A suspension of about 10^{10} organism's /ml of suspension for each organism under test was inoculated onto each of the media using the ecometric technique The plates were divided to four quarter(A,B,C and D) any quarter represented 25% of cultured 5 lines by full loop of the suspension was streaked 5 times on to medium represent 5% of the culture then incubated at anaeropic incubation by adding 10% CO_2 at 37°C for three to five days and at 37°C at aerobic condition. The growth of two isolate on tryptose agar serum dextrose agar thyr.martin agar potatoes agar and blood agar was 100% at anaerobic incubator by adding 10% CO_2 at 37^0C. In aerobic incubation at 37^0C the growth rates were lower and to varying degrees of 25-100 at *B.miletensis* and 55-100 at *B.abortus*. .
Ecometric evaluation is recommended for routine monitoring of the quality of growth culture media for brucella
Key words: culture media, *Brucella* isolate. Ecometric test

Introduction:

Brucellosis is endemic in many developing countries and is caused by many species of the genus Brucella that affect man, domestic and some wild animals, and marine mammals (Geresu et al., 2016). It is estimated at 500 000 new human cases of brucellosis annually worldwide (Pappas et al., 2006). Brucellosis is the most important zoonosis and has gained prominence over the years since its discovery on the island of Malta (Abubakar *et al.,* 2012). Brucella species are gram negative cocci bacilli, which are classified into species by various techniques such as growth patterns on media and phage susceptibility. There are six "classical" recognized species; *B.abortus, B. melitensis, B. suis, B. ovis, B. canis,* and *B.neotomae* (Hadush and Pal, 2013). lately, four new Brucella species have been recognized and classified, namely*, B. pinnipedialis, B. ceti, B. microti , B. inopinataand B.papionis* (Foster *et al*., 2007; Scholz *et al*., 2009 and Whatmore*et al*., 2014).The disease in humans is also known as "undulant fever" or "Malta fever," since the first isolation of the causative bacterium was made in the Mediterranean island of Malta from spleens of British soldiers who became infected as a consequence of drinking contaminated caprine milk. The most common Brucella species infecting humans are *B. melitensis, B. abortus and B. suis*. Among these, *B. melitensis* and *B. suis* are the most aggressive species for humans. These bacteria cause a severe syndrome, which if not treated may lead to death. *B. canis* and the marine Brucella strains have been reported sporadically to infect humans. *B. ovis* and *B. neotomae* have not been detected in humans. The marine strains may represent, however, a real hazard for human communities that hunt and gain continuation from whales and seals and for workers that are in close contact with marine mammals (Higgins, 2000).*Brucella* species are responsible for brucellosis, one of the world's most widespread zoonosis causing abortion and subsequent economic losses in meat and dairy farms of domestic animals, debilitating infection of humans and serious impediment to international trade (Whatmore, 2009

Materials and Methods:

Bacterial cultures:

Two *Brucella* isolates were used for conducting the tests, *Brucella abortus* isolated from bovine hygroma and *Brucella melitensis* isolated from human blood. The *Brucellae* were grown from a single isolated colony in Tryptose agar slants (Difco, Becton Dickinson and Co.) for 48 hours, at 37^{0}C prior to testing and were used as inocula on the differentmedia during the evaluation trials**.** Six culture media were used to test their support to the growth of the *Brucella* isolation. (Table 1)

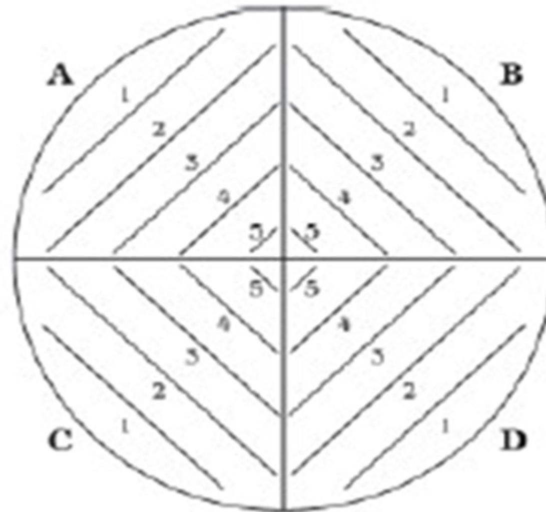
Table (1). Culture Media used for growth of *Brucella* isolates:

Culture Media	Preparation Formula/ manufacturer
Potatoes Infusion Agar	Alton *et al*., 1988
Tryptose Agar	Difco tm REF263400
Tryptone Soya Agar	Oxoid code CM131,
Serum Dextrose Agar	OxoidCM3 Basic Nutrient agar formula, serum (5%) and Dextrose 5%
Thayer-Martin Agar	OxoidGCCmo 367
Blood Agar base	Oxoid no .2 code M271

All media were prepared according to the manufacturers, a model was developed to standardize inoculation and the depth of the agar was controlled to within ±10%, the attributes of the inocula used were accurately defined.

Preparation of liquid suspensions and inoculation with loops:

Heavy well mixed cell suspensions were obtained by harvesting the cultures with sterile phosphate buffered saline (PBS). The plates were streaked with wire loops according to the design by Mossel *et al*., 1983, with care being taken to collapse the liquid bubble on each loop in the first steak on the first quadrant of each plate (a quarter of a circle; an arc of 90°). Each quarter was streaked by the same loop-full inoculums as by Jeffrey *et al*.,(2003) figure 1). Duplicates of plates were used for each sample for each media, one was incubated aerobically at 37°C, and the other was incubated anaerobically in 10% carbon dioxide. Four quadrant streaking of each plate (each line was calculated as 5% and each quadrant as 25%, the whole plate was 100%(Fig 1).



Figure(1) Schematic diagram of the ecometric test A, B, C and D= 25% of the culture plate.1, 2, 3,4 and 5 = 5% of the culture plate.

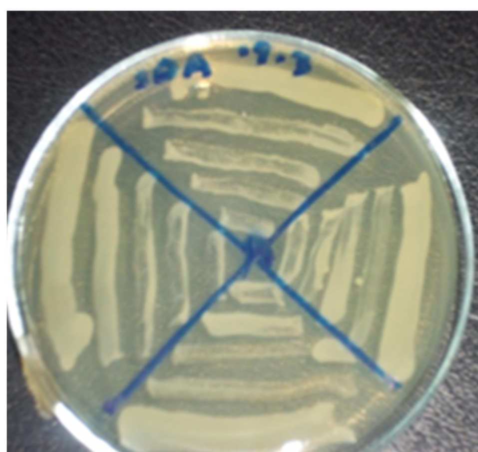
Results:

The growth of *B. abortusB. melitensis* was observed in the plates. (Table 2) shows the result of the ecometric test for two *Brucella*isolates in serum dexterose agar ,potatoes agar,tryptose agar, thyr. Martin agar, tryptonse soya agar and blood agar. At aerobic incubation at 37°C, the growth was 25-100% and at anaerobic incubation of 10% CO_2 at 37°C about 55-100% (Table 2 Fig 2,3,4,5.6.7).

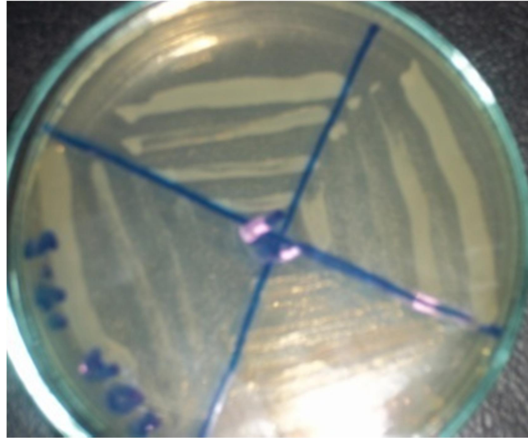
Table(2) The growth of Two isolates, *B.abortus* and *B. melitensis* in a different solid media

Media	Aerobic incubation at 37°C		Anaerobic incubation of 10% CO_2 at 37°C	
	B. melitensis	*B. abortus*	*B. melitensis*	*B. abortus*
1. Potato infusion agar	25%	60%	100%	100%
2. Serum dextrose agar	30%	65%	100%	100%
3. Tryptose agar	30%	75%	100%	100%
4. Thayer-Martin agar	No growth	55%	100%	100%
5. Tryptose soya agar	No growth	85%	100%	100%
6. Blood agar	100%	100%	100%	100%

Figures (2, 3) show the (100%) growth of both isolates in the presence of 10% CO_2 at 37^0C.



Figure(2). The growth 100% of *B. abortus* in serum dextrose agar at anaerobic atmosphere of 10% CO_2 at 37°C



Figure(3) The growth 100% of *B. melitensis* in serum dextrose agar at anaerobic atmosphere of 10% CO_2 at 37°C

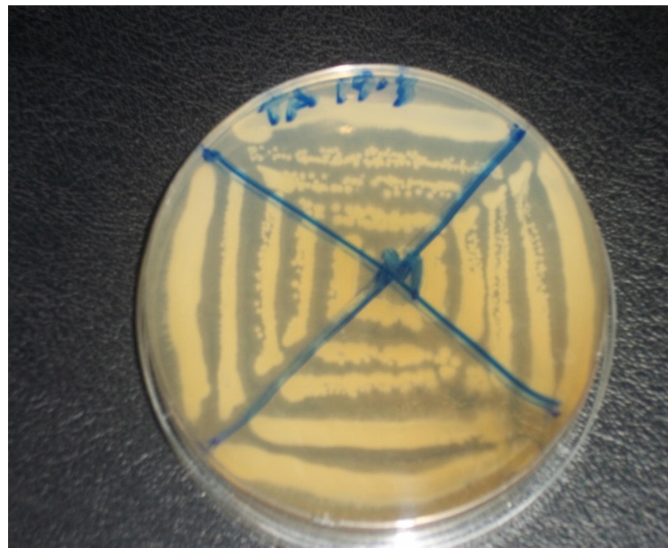


Fig (4) The100% growth of *B.miltensis* in tryptose agar at anaerobic atmosphere of 10% CO_2 at 37°c

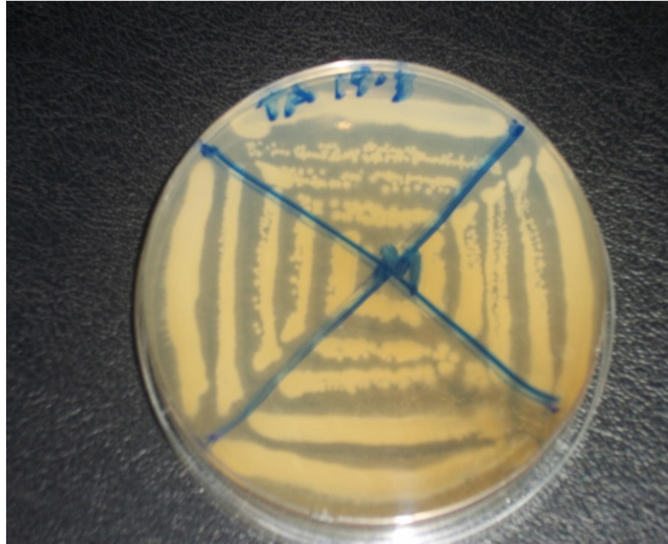


Fig (5) The 100% growth of *B.miltensis* in tryptose agar at anaerobic atmosphere of 10% CO_2 at 37°c

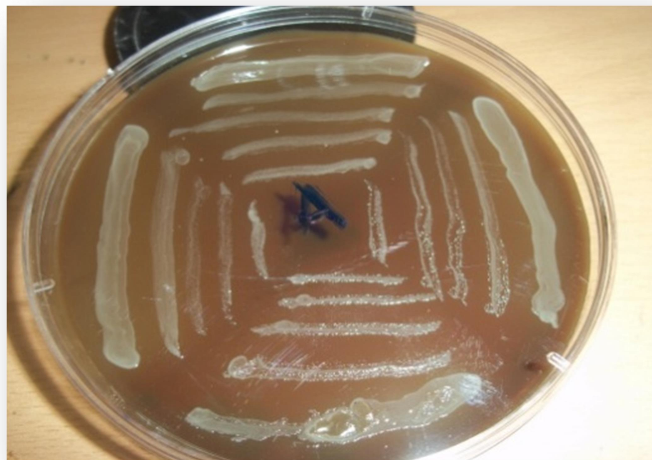


Fig (6) The 100% growth of *B.abortus* at anaerobic incubation of 10% CO_2 at 37°c in thyer –martin medium

Fig (7) The 100% growth of *B.militensis* in tryptose soya agar at anaerobic atmosphere of 10% CO_2 at 37°c

Discussion:

Brucellae are fastidious microorganisms and relatively slow growing cultured from clinical material that is often grossly contaminated. It is necessary to use culture media that support the growth for attempting isolation. There are many selective media available for the isolation of *Brucella* from contaminated material. Farrell's medium is currently widely recognized and used (MacMillan and Stack, 2000).

Solid media such as dextrose agar, tryptose agar, and trypticase soy agar, are recommended for primary isolation of *Brucella*, but some species, i.e. *B. ovis* and *B. canis* require addition of 5-10% of sterile bovine or equine serum to the culture media. In the case of blood or milk, biphasic media such as Castañeda's medium is recommended for improving sensitivity (Poester *et al*., 2010).

All media in this study passed the ecometric assessment, indicating that these formulations will provide an acceptable medium for isolation of *Brucella* even although the two samples contain small numbers of viable organisms.

The effect of growth in CO_2 atmospheric showed great enhancement of the ecometric test. This is attributed to The CO_2-dependence of *Brucella abortus biovars* and*Brucella ovis* caused by defective carbonic an hydrases, the enzymes catalyzing the hydration of CO_2 to the bicarbonate used by an aplerotic and biosynthetic carboxylases (Pérez Etayo*et al*., 2018).

Optimal temperature for culture and growth is 37^{0}C, but the organism can grow under temperatures ranging from 20^{0}C to 40^{0}C, whereas optimal pH ranges from 6.6 to 7.4. Some *Brucella* species require CO_2 for growth. Typical colonies appears after 2 to 30 days of incubation, but a culture can only be considered negative when there are no colonies after 2 to 3 weeks of incubation. False negative results should be considered in the absence of bacterial growth since the sensitivity of culture is low (Juliana *et al*., 2012).

Other parameters for isolation and subsequent characterization to identify species and for those species that have biotypes, as *B. abortus*, *B. melitensis* and *B. suis*, to the biotype level should studied. Bacteriological parameters such as, H_2S production, growth in the presence of Thionin and basic fuchsin and serum agglutination against anti-A, anti-M and anti-R. Such characterization is based on approximately 25 phenotypic features. this study contrast with Huda at growth of *B.abortus* in a different solid media at anaerobic incubation of 10% CO_2 at 37°C were 60% in potatoes agar, 65% in serum dextrose agar, 75% at Tryptose agar, 85% at Tryptose soya agar and 100% at Blood agar. Huda find the growth of Brucella abortus(S.19) at aerobic incubation, potatoes agar was 100%, serum dextrose agar was 85% ,Tryptose soya agar70%, Blood agar 50% and Brain heart infusion 30%

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