BG Pulse Journal USA
ISSN:1947-2536 http://www.bgpulseusa.com/index.php/bg
BG PULSE JOURNAL

LABORATORY DIAGNOSIS OF BRUCELLOSIS

Shukurova Shoxina Tuyg'unovna
Teacher of the "General Sciences" department of the Asia International University Bukhara Uzbekistan
e-mail:shukurovashoxinatuygunovna@oxu.uz
https://doi.org/10.5281/zenodo.20510203

Abstract. This article recommends the following culture media for isolating Brucella cultures: glucose-glycerol broth, blood agar, meat-peptone agar, erythrite agar, and Brucella agar base. Currently, erythrite agar, a commercial nutrient medium, is widely used in practice for the isolation of Brucella. It should be noted that this environment is highly effective in primary isolation of the pathogen. However, in subsequent re-cultures, the Brucella being studied on this agar may dissociate. Sowing should be done in pre-tested nutrient media. To test the agar, a suspension of 200 microbial cells (c.p.) per ml is initially prepared from a 2-day culture of vaccine strains of B. melitensis or B. abortus.

Keywords: Suspension, gension violet, polymyxin, Kozlovsky method, polyvalent (monoreceptor), glucose-glycerol broth, stereoscopic.

Introduction. Two different groups of methods are used for laboratory reporting of brucellosis in humans: the first - tests that allow the detection of the causative agent of the disease and its soluble antigens; The second is a method that detects specific antibodies in blood serum. 0.1 ml - 20 m.h. in 4 Petri dishes. is sown from suspension. The culture media are considered suitable for use if at least 18 colonies grow on the medium (average) after 5 days of incubation at 37ºC. It is recommended that blood cultures be performed when the patient has a fever, as the percentage of Brucella cultures isolated during this period is higher. However, the need for blood culture at normal body temperature is not denied. Blood for culture should be collected before antibiotic treatment. It is recommended to inoculate blood in 100–200 ml vials (rectangular), each of which is filled with 30–50 ml of erythrite agar. After sterilization, the vial is placed so that the agar solidifies on one side of the vial. Then, 25–30 ml of sterilized broth (glucose-glycerol broth) is poured into each vial and placed in a thermostat at 37ºC for 2–3 days (agar broth should be used within 5–7 days after preparation). At least 10 ml of blood is taken from the elbow vein with a sterile syringe and inoculated into two 5 ml vials. One of the vials is incubated with a high concentration of SO2 (5–10%), while the other is incubated under normal conditions. Starting 4 days after inoculation, the flasks are inspected and if the cultures have not grown, the surface is washed with broth. Positive results will show the appearance of Brucella colonies on the agar surface. If no Brucella colonies are detected within a month, the broth is inoculated onto solid nutrient media as a control. Brucella can also be isolated from bone marrow, urine, cerebrospinal fluid, bursal exudate, breast milk, bile, sputum, feces, cadaveric material, etc.

Main part. The seeds are sown in dense and liquid nutrient media. To reduce the growth of foreign microflora in the material being tested, gentian violet is added to the culture media at a ratio of 1:200,000. In addition, for this purpose, various antibiotics are added to the culture media, including polymyxin - 3 μg/ml and amphoglucamine - 3 μg/ml. Ready-made commercial "HI Media" culture media can also be used for this purpose. The following methods are used to determine whether isolated cultures belong to the Brucella genus: study of colony morphology,

Gram or Kozlovsky staining of preparations, luminescent microscopy, and agglutination reaction on a slide with a special serum. The colony of Brucella on agar is colorless, convex, with a smooth surface, homogeneous, and in some cases, fine grains are visible in the center of the colony. Delicate and clear colonies become dull over time. Colonies can vary in size. Along with colonies 3–4 mm in diameter and larger, very small colonies of 0.1–0.05 mm are also found. Various factors affecting the biology of cultures (pH of nutrient media, humidity, presence of bacteriophage in cultures) can change the appearance of colonies (granular, glassy, eroded, dry, slimy, radially striped, etc.). To study the structure of colonies, it is advisable to use a stereoscopic magnifier (MBS-1) or a simple microscope with the smallest objective lens.

When stained by the Gram stain, Brucella are Gram-negative polymorphic cocci (stain red). Staining of preparations according to Kozlovsky: preparations fixed in alcohol are held in a 0.5% aqueous solution of safranin until bubbles appear, then washed with distilled water, and re-stained for 40–50 seconds in a 0.5% aqueous solution of malachite or brilliant blue. Brucella retains the red color of safranin, while other bacteria remain green. Test with acriflavine solution (in the product window). Gram-negative bacteria contain lipopolysaccharides (LPS). Smooth-shaped microorganisms (S-shaped) have LPS consisting of lipid A, a core, and an O-polysaccharide. These microorganisms (R-form) agglutinate with neutral acriflavine due to the charge on their outer membrane. The neutral acriflavine solution must be freshly prepared daily (10 mg of acriflavine dissolved in 10 ml of distilled water - 0.1%). A small amount of the solution is taken and dripped onto the glass of the object. A sterile swab or loop is used to remove the colony and prepare a suspension with a drop of acriflavine. Agglutination in suspension is observed. Coarse colonies are clustered together or form clusters in acriflavine.

Research observations: In the thermoagglutination reaction, a two-day, 2 billion culture of Brucella in 2–3 ml of saline is heated in a water bath at 90º C for 30 minutes in a test tube. Results are recorded at room temperature after 30 minutes, 1 hour, and finally after 24 hours. If dissociation occurs during these periods, clear agglutination of Brucella is evident, while the suspension of non-dissociated (S) strains remains homogeneous. Only S-shaped Brucella cultures are differentiated. Growth in the presence of SO2, production of hydrogen sulfide, resistance to fuchsin and thionine, urease, catalase, and oxidase activity, and agglutination with polyvalent (monoreceptor) sera are tests for the differentiation of Brucella.

In a test method with a special serum. For initial rapid identification, an agglutination reaction is performed on the specimen window. Specific serum diluted 1:25 in 0.5% carbolic saline is dripped onto the glass of the device, a loop is taken from the culture being tested and mixed with it. In positive results, agglutination occurs with the appearance of well-defined streaks within 1 minute, while in negative cases, the suspension remains homogeneous. To control the culture being tested, the culture is mixed with a drop of normal serum or saline. The Brucella culture is tested for dissociation in parallel with agglutination. For this purpose, the following tests are used: acriflavine test or thermoagglutination reaction (heat test). Differentiation by hydrogen sulfide formation - An aqueous solution of lead in acetic acid is used as a reagent, which is soaked in 1x8 cm filter paper. These filter papers are then dried. Dried filter paper pieces can be stored in dark jars with lids closed for 1–2 months. Commercial reagents are used when available. A mixture of 2 billion of the culture under test in physiological saline is inoculated onto an agar slant using a standard loop (2mm) (Ph – 6.8–7.2). Then, pieces of filter paper are taken and squeezed between the test tube and a cotton pad so that its lower end hangs above the agar medium. The cotton plug should be porous enough to not prevent the carbonic anhydrite from escaping from the test tube. The cultured tubes are placed in a 37ºC thermostat. An indicator of the intensity of hydrogen sulfide formation is the

darkening of the lower part of the paper, above the grain. The darkening of the paper is measured in mm. The results are recorded for 6 days with an interval of 2 days. With each recording, the darkened papers are replaced with new ones. All three outcome measures are summed to provide a final assessment of the culture's ability to produce hydrogen sulfide. The total hydrogen sulfide production rate for B. suis (biovar 1) is approximately 12–20 mm, for B. abortus (biovar 1) it is about 5–7 mm, and B. melitensis (biovar 1) usually does not produce hydrogen sulfide at all or turns lead paper slightly brown. The average hydrogen sulfide production rate for B. neotomae strains is 5–8mm. B. ovis and B. canis cultures do not produce hydrogen sulfide. The recommended solid medium for this differentiation method is meat-peptone agar. Basic fuchsin and thionine are used in the following concentrations: thionine 1:25000, 1:50000, 1:100000, fuchsin 1:50000 and 1:100000 (preparation of basic solutions of the dyes is given in the appendix). Stained vials are stored in a dark place for 6–10 months. Stained culture media should be stored in a refrigerator (+ 4º C) and are considered suitable for use for 10–15 days. Discolored nutrient media cannot be used. Brucella suspensions are prepared from two-day agar cultures. This suspension is streaked onto the agar surface using a loop. A Petri dish can be divided into 4–6 sectors and 4–6 cultures can be grown at the same time. The cultures are placed in a thermostat at 370 C. After incubation, the results are recorded after three days. Recording scheme: intensive growth across the entire bar 4+;

intensive at the beginning and much slower growth along the bar at the end 3+;

weakly intensive at the beginning and slow growth throughout the entire streak 2+;

Very weak growth or isolated colonies in the direction of the bar +.

Monitoring urease activity. Some bacteria produce urease, an enzyme that converts urea into ammonia, water, and carbon dioxide. Urease breaks down urea into ammonia, water, and carbon dioxide, ammonia being alkaline in nature. This alkaline compound is detected in the pH indicator, turning the basic nutrient medium pink. To determine urease activity, Christensen medium (commercial Hi Media or according to the recipe in the appendix) is used. A culture of Brucella grown on agar is inoculated onto a nutrient medium using a single-loop streak method. The seeds are stored at room temperature. They are monitored for 5 hours and stopped after 24 hours. All types of Brucella have urease activity and hydrolyze urea in nutrient media, but at different times. Br. suis changes the color of the culture medium in 15 minutes, Br. melitensis in 2 hours, and Br. abortus after a day. The differential characteristics of species and biovars of the Brucella genus are presented in Tables 1 and 2.

Conclusion. Depending on the purpose of the test, it is recommended to use several methods for brucellosis. In epidemiological examinations of the population in outbreaks, the following tests are recommended: agglutination reaction on a glass slide (Heddelson), Wright, and IFT. Serological and bacteriological tests are performed for acute and subacute brucellosis. IFT is recommended for the diagnosis of chronic brucellosis and during dispensary observation of the population with brucellosis. From the initial period of the disease (in the first 6 months), the diagnostic value of serological reactions is higher than that of allergic methods; Serological reactions during this period give a positive result in 98 percent of cases. As the disease progresses, the percentage of positive serological results decreases. In the late stages of the disease, IFT is of important diagnostic value. It is worth noting that the agglutination reaction with Brucella antigens also gives a positive result with antibodies against other microorganisms (E. coli, V. cholerae, Fr. tularensis, Y. enterocolitica 0–9) that have common antigenic determinants with Brucella antigens.

References

1. Shukurova, s. (2024). Optimizing synergies: Effective strategies for integrating economic and environmental interests in sustainable development. In e3s web of conferences (vol. 587, p. 04007). Edp sciences.
2. Tuyg‘unovn, s. S. (2025). Trigonella foenum-graecum vegetatsiya davri va undagi foydali moddalarining vegetatsiya davriga bog’liq ko’payish dinamikasi. *Central asian journal of academic research*, *3*(3), 174-178.
3. Qizi amonova, g. R., tuygunovna, s. S., & rashidovna, o. G. (2025, november). Growing medicinal plants (example of silybum marianum) in bukhara region. In *proceeding of international conference on biology education, natural science, and technology* (pp. 340-348).
4. Tuyg’unovna, s. S. (2024). Medicinal plants that are widely used in nature, rich in vitamins. Образование наука и инновационные идеи в мире, 39(3), 242-247.
5. Tuyg’unovna, s. S. (2024). The process of packaging medicinal plants. Образование наука и инновационные идеи в мире, 39(3), 248-256.
6. Tuyg’unovna, s. S. (2024). About useful medicinal plants rich in lipids used in medicine. *Образование наука и инновационные идеи в мире*, *39*(3), 235-241.
7. Tuyg’unovna, s. S. (2024). Bakteriyalar genetikasi. Bakteriyalarda genetik almashinuv mikroorganizmlarning o ‘zgaruvchanligi. *Masters*, *2*(5), 183-192.
8. Tuyg’unovna, s. S. (2024). Tarkibida efir moylar bo’lgan dorivor o’simliklar. Ta'lim va rivojlanish tahlili onlayn ilmiy jurnali, 4(3), 164-167.
9. Tuyg’unovna, s. S. (2024). Medicinal plants containing essential oils. Образование наука и инновационные идеи в мире, 41(4), 62-69.
10. Shukurova, s. T. U. The structure of bacteriological, virological and immunology laboratories. Groups of micro-organisms. Simple painting techniques. *American journal of botany and bioengineering*.
11. Tuyg’unovna, s. S. (2024). Cultivation of medicinal plants and forms of preparation. European journal of modern medicine and practice, 4(5), 71-75.
12. Tuyg’unovna, s. S. (2024). Systematic analysis of medicinal plants. *Лучшие интеллектуальные исследования*, *19*(5), 159-164.
13. Tuyg’unovna, s. S. (2024). Dorivor o’simliklarning sistematik tahlili. Ta'lim va rivojlanish tahlili onlayn ilmiy jurnali, 4(4), 180-184.
14. Tuyg’unovna, s. S. (2023). Useful properties of the medicinal product and use in medicine. *Gospodarka i innowacje.*, *40*, 179-181.
15. Tuyg’unovna, s. S. (2023). Chemical composition of medicinal plants and classification. *European journal of modern medicine and practice*, *3*(11), 33-35.
16. Tuyg’unovna, s. S. (2023). Dorivor na’matakning foydali xususiyatlari va tibbiyotda qo’llanilishi. *Ta'lim va rivojlanish tahlili onlayn ilmiy jurnali*, *3*(9), 11-13.
17. Tuygunovna, s. S. (2023). Ways to use mint and peppermint. *European journal of business startups and open society*, *3*(12), 20-23.