BRUCELLA CULTURE

Culture procedure for isolation and identification of *Brucella* spp. from diagnostic specimens in Veterinary diagnostic laboratories.

Brucella spp. are aerobic gram negative coccobacilli. Growth of *B . abortus and B. ovis* is enhanced in presence of CO_2. Each of the *Brucella spp*. has some established host preferences. Humans are not susceptible to *B. neotomae* and *B. ovis,* and are rarely susceptible to *B. canis*.

Species and their natural reservoirs are:
Brucella abortus: cattle
Brucella suis: swine
Brucella melitensis: goats and sheep
Brucella ovis: especially rams
Brucella canis: dogs
Brucella neotomae: wood rats

Acceptable Specimens:
Milk
Aborted fetal tissues
Blood
Female animals: Lymph nodes; supramammary, suprapharyngeal, lumbar, internal iliac
Other acceptable tissues: section of spleen, uterus or section of each quarter of the udder
Male animals: Lymph nodes; superficial inguinal, suprapharyngeal, lumbar, internal iliac
Other tissues, seminal vesicles. Testicles and sections of spleen

Unacceptable Specimens:
Heavily contaminated samples. Samples not transported appropriately.

Equipment and Materials Required:
Type II biohazard cabinet
Scissors
Forceps
Microscope
Microscope slides
Tissue grinder
37C incubator

Safety Precautions:
Personnel need to wear gloves and labcoats when handing potentially infectious specimens or culture material. All general laboratory safe practices should be followed when processing samples for *Brucella* culture. Infectious samples need to be plated in a bio-safety hood and BSL-2 practices are to be used. Work areas need to be disinfected after use.

Humans are highly susceptible to some *Brucella spp*, and laboratory infections are common. Due care must be given for safety procedures. Cultures that are suspected to be *Brucella* should only be manipulated under BSL-3 conditions. Center for Disease Control (CDC) recommends consultation with state public health laboratory if *Brucella* spp. is suspected in clinical specimens. If laboratory is not geared for handling suspect infectious material National Veterinary Services Laboratory at Ames, Iowa can be approached for help with culturing through USDA-Area Veterinary In-charge's office.

Methods:

Media.

The media described here are for convenience only and can be substituted by equivalent available media that are known to support growth of *Brucella* spp.

Trypticase soy agar with 5% Blood (BD 221239/221261)
Or Brucella Agar w/5% Blood (BD 221547)
Chocolate agar (221169/221267)
McConkey's agar (MAC)
Septi check blood culture system (BD 243178)

Processing/Plating, Incubation:

Sear tissue, macerate with a tissue grinder, plate the ground material onto agar media, and streak plate for isolation. Liquids may be concentrated for recovery of low number of organisms by centrifugation, or can be plated directly onto the agar. Milk samples most often submitted to veterinary diagnostic laboratories for *Brucella* detection should be centrifuged first at about 5000 rpm and 4^oC for 30 min. Milk cream from top and pellet from bottom of the tube should be plated and streaked to detect presence of the organism in the submitted specimen. Milk samples and tissues collected from field necropsies often carry a heavy microbial burden. For *Brucella* detection in such specimens, it is important that antibiotics in the form of selective supplement (Oxoid Code SR083) are included in the *Brucella* agar used for plating. Blood samples inoculated and incubated (aerobically at 37C) in a blood culture bottle can be subcultured to plates once positive growth is noticed for further characterization. Negative blood cultures should be discarded according to established laboratory procedures. CDC recommends minimum 21 day culturing for blood samples, with blind subculturing every 7^{th} day (a total ten day incubation is usually considered sufficient), and with terminal subculturing at 7 days. For direct plating, length of incubation for calling a sample negative is 7 days on primary plates at 37^oC in a 7.5% CO_2 incubator**.**

Results and Interpretation:

Suspect *Brucella* colonies are generally visible on the third or fourth day after inoculation, and are usually punctate, translucent, non-pigmented and non-hemolytic. The colonies are presumptively identified as *Brucella* spp. based on gram staining, colony morphology, positive oxidase test (*B canis* is oxidase variable) and urease production (*B ovis* is negative). *Brucella* colonies generally show poor or no growth on MAC. Other biochemical and identification tests that can be performed are H_2S production using a lead acetate strip (*B abortus, B. neotomae and B. suis* are positive), nitrate reduction (positive),

catalase test (positive), motility (negative), methyl red test (negative). Agglutination with specific antiserum also helps in presumptive identification. It should be noted that if *B. abortus* antiserum is not adsorbed would cross-react to other *Brucella spp* including *B. meletensis, B. suis and B. neotomae*. Presumptively identified *Brucella* spp. colonies should be sent for final bio-typing to the National Veterinary Services Laboratory (NVSL), Ames, Iowa. Cultures and contaminated materials must be disposed by autoclaving followed by incineration. Positive cultures recovered are maintained after recovery in the laboratory until the final results are available from NVSL and may need to be destroyed under current law based on registration status of the diagnostic laboratory.

B. abortus often encountered in veterinary laboratories appears as gram negative cocci, coccobacilli, or short rods. Most strains require supplementary CO_2 for growth, especially on primary isolation. *B. abortus* biotypes are catalase positive, oxidase positive, and non-motile. Methyl red test is negative, nitrate is reduced to nitrite, urease activity is usually observed within 1-2 hours. H_2S (small quantities) is produced by most biotypes in 2-5 days.

Quality Control/Quality Assurance:

Media purchased from commercial vendor need be tested according to NCCLS or laboratory recommendations. In house media for *Brucella* culture should be tested for growth of the *Brucella* type strain under appropriate biosecurity.

Reporting Results:

Results of presumptive identifications are reported as *Brucella spp* isolated or not isolated to appropriate authorities according to policy and recommendations of the State. Speciation if accomplished by performing necessary biochemicals and agglutination with monospecific serum can be reported. Final bio-typing results from NVSL are made available to interested parties subsequently upon test completion.

Other references (Web) for procedures on *Brucella* detection:

http://www.oie.int/eng/normes/mmanual/A_00048.htm

http://www.asmusa.org/pasrc/Brucella.pdf

http://www.bd.com/diagnostics/biodefense/brucella_cultureid.asp

Some differential characteristics of common Brucella spp.

	B abortus	**B. meletensis**	**B. Suis**	**B. canis**
Oxidase	+	+	+	--
H2S prod.	2-5d	--	1-6d	--
Urease	>90 min	>90 min	<90 min	<90 min
CO2 req.	+	--	--	--