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Overview of *Brucella* spp. Isolation and Identification

- Acceptable Samples types updated, Section 2.a.1 (pg 1)

Acceptable samples
(1) Appropriate clinical specimens (e.g. Blood, serum, bone marrow, joint fluid, cerebrospinal fluid, abscess fluid, tissue masses, breast milk)
(2) Environmental/nonclinical samples
(3) Culture isolates

- Table 1 updated to include RB51 (pg 2)

Table 1. Confirmation of *Brucella* spp. by biochemical and molecular tests

Strain	H_2S production	Urease		Gel formation	Lysis by Tbilisi		Real-time PCR (2 of 3 targets)
		<5 min	>5 min		RTD	RTD x10^4	
B. melitensis	No	Variable	Yes	No	No	No	Positive
B. abortus	Variable	No	Yes	No	Yes	Yes	Positive
B abortus RB51	Yes (may be weak)	No (may be weak)	Yes	No	No[1]	No[1]	Positive
B. suis[2]	Variable	Yes	Yes	No	No	Yes	Positive
B. canis	No	Yes	Yes	Yes	No	No	Positive

[1]Different from *B. melitensis*, there is no "ghost lysis"
[2]Some *B. suis* demonstrate no lysis (i.e. resistance) at the RTD x 10^4 concentration of Tbilisi phage

Isolation of *Brucella* spp. from Human, Animal and Environmental Samples

- Breast Milk added to sample type, Section 2.a.1.b (pg 1)
- Updated tissue processing- now use tissue grinder, Section 5.b. (pg 3)

Gel Formation Test (*Brucella canis*)

- Older culture works better should use a 48-72 hr old culture for test, Section 2.a. (pg 1)
- Phenolized Buffered Saline now stored at room temperature and will be good for 5 years, Section 3.a.
- Changed control to B. canis from NVSL order number BRU-NS, Section 4.a. (pg 2)

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Hydrogen Sulfide Production

- **Updated QC**
  Quality control Section 4.a.1,2 (pg 2)
  a. Controls
    (1) Positive control: B. abortus, strain 19 or Escherichia coli (ATCC 25922) or equivalent
    (2) Negative control: Pseudomonas aeruginosa (ATCC 27853) or equivalent
  b. Quality control testing
    (1) Test each new lot of lead acetate paper with positive and negative control strains.
    (2) Use both a negative and a positive control organism with each run.

Susceptibility to *Brucella* phage Tbilisi

- Changed controls used, Section 3.b.(2).a,b,c (pg1)
- Changed protocol – Now use 0.5McFarlandas inoculum, Section 5.a.(2) & 5.b.(1)
- Changed observation period/intervals, Section 5.b.(7) (pg 3)
  - Examine at 48 hrs and daily thereafter for up to 4 days.

Urease Test (Christensen's Method)

- Added a note to positive control reaction, Section 6.a.
  - NOTE: All *Brucella* spp. except *Brucella ovis* should produce a positive reaction after overnight incubation.

Slide Agglutination for Detection of F. tularensis Cellular Antigens

- Updated control that is used Section 3.2.a.
  - Francisella tularensis LVS cells

Antimicrobial Susceptibility Testing of Yersinia pestis by Etest

- Changed Etest order numbers Section 3.b.a-d
  - Reagents

    (1) Etest strips (bioMerieux) for the following antimicrobial agents
      (a) Ciprofloxacin (catalog #412310)
      (b) Gentamicin (catalog # 412367)
      (c) Levofloxacin (catalog #412392)
      (d) Tetracycline (catalog #412470)

    Store Etests according to manufacturer's recommendations. Do not use after the expiration date.

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The Non-variola *Orthopoxvirus* Real-time PCR Assay

- Has 510(k) clearance by the FDA as a Class II medical device
- Added RP positive control to table 1, Section 4. Table 1

Table 1. Use of primer and probe sets with samples and controls

		VAC1	RP	16S
1	**Clinical Specimen DNA**	Yes	Yes	Optional (See section 6.a.(4))
2	**Viral culture lysate DNA**	Yes	No	Yes
3	**No Template Control (NTC)**	Yes	Yes *If DNA from clinical specimens is included in the run*	Yes *If DNA from viral cell culture lysates is included in the run*
4	**Agent Positive Control**	Yes	No	No
5	**RNase P Positive Control**	No	Yes *If DNA from clinical specimens is included in the run*	No

- RP positive control should be <30 CT, Section 4(5) (pg 7)
- Quanta mastermix is now approved details on pages 9-13
- Flow chart added for interpretation of results page 19
- Added RP Positive requirements samples must be <40 CT page 22

Note: This assay can't be run until a validation is complete