Data/Metadata

**ShahSanjivkumar\_HS7-53-05-146\_Data-Metadata\_Ftularensis RVPCR Method\_ Manuscript \_20190830**

**Manuscript: “Rapid Viability Polymerase Chain Reaction Method for Detection of *Francisella tularensis*”**

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**Table 1. Nucleotide Sequences of the primer/probe sets used for *Francisella tularensis* RV-PCR analysis**

| **Assay** | **Forward Primera** | **Reverse Primera** | **Probea,b** | **Amplicon Length (bp)** | **Reference** |
| --- | --- | --- | --- | --- | --- |
| **F4** | TTGCTCCAGTAGCTGCAAGATT | CCAAGTGCTTGGTGGTGGTA | FAM-TGCTGCCGAGATGTTTTCATTATTAACTGATGC-BHQ | 125 | Kugeler et al. (2006) |
| **F5** | GAGACATCAATTAAAAGAAGCAATACCTT | CCAAGAGTACTATTTCCGGTTGGT | FAM-AAAATTCTGCTCAGCAGGATTTTGATTTGGTT-BHQ | 105 | This study |

**a** Sequences are listed in 5’ to 3’ orientation.

**b** Probes were labeled with labeled at the 5’ end with FAM (6-carboxyfluorescein) and at the 3’ end with Black Hole Quencher® (BHQ-1).

Bp, base pair; FAM, fluorescein; BHQ, Black Hole Quencher

Kugeler, K.J., Pappert, R., Zhou, Y., Petersen, J.M., 2006. Real-time PCR assays for Francisella tularensis Types A and B. Emerg. Infect. Dis. 12, 1799-1801.

**Nucleotide:** Nucleotides are the basic structure units of nucleic acids (DNA-Deoxyribonucleic acid; and RNA-Ribonucleic acid found in all living systems). In other words, DNA/RNA is a long polymer made from repeating units called nucleotides. There are generally four basic nucleotides that make the DNA. They are Adenine (A), Guanine (G), Cytosine (C), and Thymine (T).

**Nucleotide Sequence:** A specific manner of presence/repetition of the nucleotides in a DNA/RNA.

**Primer:** A specific manner of presence/repetition of the nucleotides in a short stretch/piece of DNA which initiates synthesis of a large stretch/piece. Two primers, a forward and a reverse primer, with the help of nucleotides and enzyme, polymerase, synthesize long stretches/pieces of DNA in a biochemical reaction, called Polymerase Chain Reaction (PCR). One end of each primer is 5’ and the other is 3’ for DNA binding and synthesis.

**Probe:** A specific manner of presence/repetition of the nucleotides in a short stretch of DNA/RNA which is labeled with fluorescent dye molecules on one end and fluorescence quenching molecules (quencher) on the other end. The fluorescence-labeled probe by emitting fluorescence, helps detection of long stretches of DNA/RNA (amplified nucleic acids) generated in a PCR. One end of each probe is 5’ and the other is 3’ for DNA binding and detection.

**PCR:** Polymerase Chain Reaction. This is an assay used to detect the presence of any living system including disease causing microorganisms/germs including bioterrorism agents. A PCR assay is a biochemical reaction in which the nucleic acid of a target organism is amplified millions of time by a catalyst called polymerase enzyme using the forward and reverse primers, and nucleotides. The amplified nucleic acid can be detected via increase in fluorescence measured by a specially designed instrument.

**Amplicon:** A long stretch of nucleic acid (DNA) generated in a PCR. It is of a specific length (bp-base pairs) depending upon the primers used.

**bp:** base pairs. In the DNA the nucleotides pair in a specific manner. A pairs with T and G pairs with C to form a double-stranded (two parallel threads) of DNA.

***Francisella tularensis*:** *Francisella tularensis,* name ofthe bacteria that cause tularemia.

**RV:** Rapid Viability

**RV-PCR:** Rapid Viability (RV) Polymerase Chain Reaction (PCR). This method includes first growing in a growth medium the pathogenic bacteria such as *Yersinia pestis* present in a water sample and then detecting the increase in the amount of the nucleic acid (DNA-deoxyribonucleic acid) of these bacteria using the PCR assay.

**F4 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**F5 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**Table 2. RV-PCR results for different starting cell levels per sample with and without chemical and biological backgrounds**

| **Treatment** | **Starting CFU / 3-mL Sample\*** | **ΔCT Range** | **Positive RV-PCR Samples\*\*\*** |
| --- | --- | --- | --- |
| **Control** | 2500 | 7.2–9.3 | 3 of 3 |
| 1200 | 7.4–9.3 | 3 of 3 |
| 250 | 6.8–8.1 | 3 of 3 |
| 120 | 8.9–9.3 | 3 of 3 |
| 25 | 10.6–12.3 | 3 of 3 |
| 12 | 10.3–12.7 | 3 of 3 |
| **Fe/Humic acid** | 2500 | 11.5–17.2 | 3 of 3 |
| 1200 | 14.5–22.7 | 3 of 3 |
| 250 | 12.5–13.9 | 3 of 3 |
| 120 | 15.4–18.3 | 3 of 3 |
| 25 | 16.4–16.8 | 3 of 3 |
| 12 | 15.9–17.8 | 3 of 3 |
| **ATD\*\*** | 2500 | 9.2–10.7 | 3 of 3 |
| 1200 | 5.5–8.3 | 2 of 3 |
| 250 | 6.4–7.5 | 3 of 3 |
| 120 | 7.2–8.2 | 3 of 3 |
| 25 | 0–4.9 | 0 of 3 |
| 12 | 0 - 0 | 0 of 3 |

\* CFU values are averages corrected for dilution, expressed with 2 significant figure accuracy.

\*\* The ΔCT range includes the highest ΔCT value per ATD sample replicate, either from undiluted or 10-fold diluted DNA extracts.

\*\*\* The number of positive samples per three replicate samples.

ATD, Arizona Test Dust; Fe = Ferric ions; CT, cycle threshold.

**PCR:** Polymerase Chain Reaction. This is an assay used to detect the presence of any living system including disease causing microorganisms/germs including bioterrorism agents. A PCR assay is a biochemical reaction in which the nucleic acid of a target organism is amplified millions of time by a catalyst called polymerase enzyme using the forward and reverse primers, and nucleotides. The amplified nucleic acid can be detected via increase in fluorescence measured by a specially designed instrument.

**RV-PCR:** Rapid Viability (RV) Polymerase Chain Reaction (PCR). This method includes first growing in a growth medium the pathogenic bacteria such as *Francisella tularensis* present in a sample and then detecting the increase in the amount of the nucleic acid (DNA-deoxyribonucleic acid) of these bacteria using the PCR assay.

***Francisella tularensis*:** Name ofthe bacteria that cause tularemia.

**Treatment:** Addition of inhibitors such as Ferrous Sulfate (Fe), Humic Acid (Humics), and Arizona Test Dust (ATD) in experimental conditions.

**Control:** No Addition of inhibitors such as Ferrous Sulfate (Fe), Humic Acid (Humics), and Arizona Test Dust (ATD) in experimental conditions.

**Fe/Humics:** Addition of inhibitors, as Ferrous Sulfate (Fe), Humic Acid (Humics) in experimental conditions.

**ATD:** Addition of inhibitor, Arizona Test Dust (ATD) in experimental conditions.

**F4 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**F5 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**CT:** Cycle Threshold. A cycle number in a PCR assay at which the amount of fluorescence is above the baseline value.

**T0:** Time Zero Hour. Zero hour incubation for growth of bacteria. A sample aliquot is taken out for analysis before incubation for growth and PCR CT is generated.

**T30:** Time 30 hours. Thirty hours incubation for growth of bacteria

**∆CT:** A change (∆) of cycle threshold (CT). In the RVPCR method, PCR assay is performed before (T0) and after (T30) the growth of bacteria and the CT values are measured. The CT value of the PCR assay performed after the growth (T30) is deducted from the CT value of the PCR assay performed before the growth (T0). This difference in the CT values is called ∆CT. Therefore, a ∆CT = CT (T0) - CT (T30).

**The summarized data in Table 2 were derived from the following detailed results tables 3-8.**

**Table 3. RV-PCR Results for *F. tularensis* Schu S4 Cells (~1,200 CFU/3-mL Sample) in the Presence of Fe/Humics or ATD**

| **Treatment** | **Sample Replicate** | **PCR Replicate** | **T0 CT** | **T30 CT** | **ΔCT (T0–T30)** |
| --- | --- | --- | --- | --- | --- |
| **Control** | **1** | 1 | 34.5 | 25.1 | **9.3** |
| 2 | 33.9 | 25.1 |
| 3 | 34.4 | 25.0 |
| **Avg. (SD)** | **34.3 (0.3)** | **25.0 (0.04)** |
| **2** | 1 | 33.7 | 26.6 | **7.4** |
| 2 | 33.8 | 26.6 |
| 3 | 34.5 | 26.6 |
| **Avg. (SD)** | **34.0 (0.5)** | **26.6 (0.03)** |
| **3** | 1 | 34.1 | 25.7 | **8.3** |
| 2 | 33.6 | 25.7 |
| 3 | 34.2 | 25.7 |
| **Avg. (SD)** | **34.0 (0.3)** | **25.7 (0.02)** |
| **Fe/Humics** | **1** | 1 | NDT | 22.3 | **22.7** |
| 2 | NDT | 22.3 |
| 3 | NDT | 22.3 |
| **Avg. (SD)** | **NDT** | **22.3 (0.04)** |
| **2** | 1 | 42.1 | 22.7 | **18.7** |
| 2 | 42.0 | 22.7 |
| 3 | 40.0 | 22.7 |
| **Avg. (SD)** | **41.4 (1.2)** | **22.7 (0.02)** |
| **3** | 1 | 37.3 | 21.6 | **14.5** |
| 2 | 35.7 | 21.6 |
| 3 | 35.4 | 21.6 |
| **Avg. (SD)** | **36.1 (1.0)** | **21.6 (0.01)** |
| **ATD** | **1** | 1 | NDT | 33.2 | **5.5** |
| 2 | 39.7 | 33.6 |
| 3 | 38.3 | 33.8 |
| **Avg. (SD)** | **39.0 (1.0)** | **33.5 (0.3)** |
| **2** | 1 | 38.2 | 33.1 | **5.3** |
| 2 | 38.8 | NDT |
| 3 | NDT | 33.4 |
| **Avg. (SD)** | **38.5 (0.4)** | **33.2 (0.2)** |
| **3** | 1 | 37.0 | 33.5 | **3.7** |
| 2 | 37.5 | 34.1 |
| 3 | NDT | 33.1 |
| **Avg. (SD)** | **37.2 (0.4)** | **33.5 (0.5)** |
| **ATD1:10\*** | **1** | 1 | 38.7 | 36.6 | **2.1** |
| 2 | NDT | 37.4 |
| 3 | 39.0 | 36.0 |
| **Avg. (SD)** | **38.8 (0.3)** | **36.7 (0.7)** |
| **2** | 1 | NDT | 37.3 | **8.1** |
| 2 | NDT | 36.6 |
| 3 | NDT | 36.6 |
| **Avg. (SD)** | **NDT** | **36.9 (0.4)** |
| **3** | 1 | NDT | 36.7 | **8.3** |
| 2 | NDT | 36.9 |
| 3 | NDT | 36.6 |
| **Avg. (SD)** | **NDT** | **36.7 (0.2)** |

\* The same ATD concentration was used with the DNA extract analyzed after 10-fold dilution with PCR H2O.

Acronyms: Avg., Average; SD, standard deviation; CFU, colony-forming units; CT, cycle threshold; NDT, Not Detected.

**PCR:** Polymerase Chain Reaction. This is an assay used to detect the presence of any living system including disease causing microorganisms/germs including bioterrorism agents. A PCR assay is a biochemical reaction in which the nucleic acid of a target organism is amplified millions of time by a catalyst called polymerase enzyme using the forward and reverse primers, and nucleotides. The amplified nucleic acid can be detected via increase in fluorescence measured by a specially designed instrument.

**RV-PCR:** Rapid Viability (RV) Polymerase Chain Reaction (PCR). This method includes first growing in a growth medium the pathogenic bacteria such as *Francisella tularensis* present in a sample and then detecting the increase in the amount of the nucleic acid (DNA-deoxyribonucleic acid) of these bacteria using the PCR assay.

***Francisella tularensis*:** Name ofthe bacteria that cause tularemia.

**Treatment:** Addition of inhibitors such as Ferrous Sulfate (Fe), Humic Acid (Humics), and Arizona Test Dust (ATD) in experimental conditions.

**Control:** No Addition of inhibitors such as Ferrous Sulfate (Fe), Humic Acid (Humics), and Arizona Test Dust (ATD) in experimental conditions.

**Fe/Humics:** Addition of inhibitors, as Ferrous Sulfate (Fe), Humic Acid (Humics) in experimental conditions.

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**F5 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**CT:** Cycle Threshold. A cycle number in a PCR assay at which the amount of fluorescence is above the baseline value.

**T0:** Time Zero Hour. Zero hour incubation for growth of bacteria. A sample aliquot is taken out for analysis before incubation for growth and PCR CT is generated.

**T30:** Time 30 hours. Thirty hours incubation for growth of bacteria

**∆CT:** A change (∆) of cycle threshold (CT). In the RVPCR method, PCR assay is performed before (T0) and after (T30) the growth of bacteria and the CT values are measured. The CT value of the PCR assay performed after the growth (T30) is deducted from the CT value of the PCR assay performed before the growth (T0). This difference in the CT values is called ∆CT. Therefore, a ∆CT = CT (T0) - CT (T30).

**Table 4. RV-PCR Results for *F. tularensis* Schu S4 Cells (~120 CFU/3-mL Sample) in the Presence of Fe/Humics or ATD**

| **Treatment** | **Sample Replicate** | **PCR Replicate** | **T0 CT** | **T30 CT** | **ΔCT (T0–T30)** |
| --- | --- | --- | --- | --- | --- |
| **Control** | **1** | 1 | 38.9 | 28.6 | **9.3** |
| 2 | 37.0 | 28.6 |
| 3 | 37.7 | 28.5 |
| **Avg. (SD)** | **37.9 (1.0)** | **28.6 (0.02)** |
| **2** | 1 | 37.0 | 28.2 | **8.9** |
| 2 | NDT | 28.3 |
| 3 | 37.1 | 28.2 |
| **Avg. (SD)** | **37.1 (0.07)** | **28.2 (0.04)** |
| **3** | 1 | 36.7 | 28.2 | **9.0** |
| 2 | 36.6 | 28.0 |
| 3 | 37.7 | 28.3 |
| **Avg. (SD)** | **37.0 (0.6)** | **28.2 (0.1)** |
| **Fe/Humics** | **1** | 1 | NDT | 26.8 | **16.2** |
| 2 | 42.9 | 26.4 |
| 3 | 42.5 | 26.4 |
| **Avg. (SD)** | **42.7 (0.3)** | **26.5 (0.2)** |
| **2** | 1 | NDT | 27.0 | **18.3** |
| 2 | NDT | 26.8 |
| 3 | NDT | 26.2 |
| **Avg. (SD)** | **NDT** | **26.7 (0.4)** |
| **3** | 1 | 38.3 | 23.6 | **15.4** |
| 2 | 40.3 | 23.7 |
| 3 | 38.4 | 23.7 |
| **Avg. (SD)** | **39.0 (1.1)** | **23.7 (0.06)** |
| **ATD** | **1** | 1 | NDT | 38.7 | **7.2** |
| 2 | NDT | 36.2 |
| 3 | NDT | 38.5 |
| **Avg. (SD)** | **NDT** | **37.8 (1.4)** |
| **2** | 1 | NDT | 37.8 | **8.2** |
| 2 | NDT | 35.7 |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **36.8 (1.5)** |
| **3** | 1 | NDT | 36.7 | **8.2** |
| 2 | NDT | 37.0 |
| 3 | NDT | 36.8 |
| **Avg. (SD)** | **NDT** | **36.8 (0.1)** |
| **ATD1:10\*** | **1** | 1 | NDT | 39.5 | **5.3** |
| 2 | NDT | 39.8 |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **39.7 (0.2)** |
| **2** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **3** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |

**\*** The same ATD concentration was used with the DNA extract analyzed after 10-fold dilution with PCR H2O.

Acronyms: Avg., Average; SD, standard deviation; CFU, colony-forming units; CT, cycle threshold; NDT, Not Detected.

**PCR:** Polymerase Chain Reaction. This is an assay used to detect the presence of any living system including disease causing microorganisms/germs including bioterrorism agents. A PCR assay is a biochemical reaction in which the nucleic acid of a target organism is amplified millions of time by a catalyst called polymerase enzyme using the forward and reverse primers, and nucleotides. The amplified nucleic acid can be detected via increase in fluorescence measured by a specially designed instrument.

**RV-PCR:** Rapid Viability (RV) Polymerase Chain Reaction (PCR). This method includes first growing in a growth medium the pathogenic bacteria such as *Francisella tularensis* present in a sample and then detecting the increase in the amount of the nucleic acid (DNA-deoxyribonucleic acid) of these bacteria using the PCR assay.

***Francisella tularensis*:** Name ofthe bacteria that cause tularemia.

**Treatment:** Addition of inhibitors such as Ferrous Sulfate (Fe), Humic Acid (Humics), and Arizona Test Dust (ATD) in experimental conditions.

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**F4 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**F5 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**CT:** Cycle Threshold. A cycle number in a PCR assay at which the amount of fluorescence is above the baseline value.

**T0:** Time Zero Hour. Zero hour incubation for growth of bacteria. A sample aliquot is taken out for analysis before incubation for growth and PCR CT is generated.

**T30:** Time 30 hours. Thirty hours incubation for growth of bacteria

**∆CT**: A change (∆) of cycle threshold (CT). In the RVPCR method, PCR assay is performed before (T0) and after (T30) the growth of bacteria and the CT values are measured. The CT value of the PCR assay performed after the growth (T30) is deducted from the CT value of the PCR assay performed before the growth (T0). This difference in the CT values is called ∆CT. Therefore, a ∆CT = CT (T0) - CT (T30).

**Table 5. RV-PCR Results for *F. tularensis* Schu S4 Cells (~12 CFU/3-mL Sample) in the Presence of Fe/Humics or ATD**

| **Treatment** | **Sample Replicate** | **PCR Replicate** | **T0 CT** | **T30 CT** | **ΔCT (T0–T30)** |
| --- | --- | --- | --- | --- | --- |
| **Control** | **1** | 1 | NDT | 35.3 | **10.3** |
| 2 | NDT | 33.9 |
| 3 | NDT | 34.8 |
| **Avg. (SD)** | **NDT** | **34.7 (0.7)** |
| **2** | 1 | NDT | 34.5 | **10.9** |
| 2 | NDT | 34.3 |
| 3 | NDT | 33.5 |
| **Avg. (SD)** | **NDT** | **34.1 (0.6)** |
| **3** | 1 | NDT | 32.4 | **12.7** |
| 2 | NDT | 32.3 |
| 3 | NDT | 32.4 |
| **Avg. (SD)** | **NDT** | **32.3 (0.07)** |
| **Fe/Humics** | **1** | 1 | NDT | 27.3 | **17.8** |
| 2 | NDT | 27.2 |
| 3 | NDT | 27.1 |
| **Avg. (SD)** | **NDT** | **27.2 (0.07)** |
| **2** | 1 | NDT | 29.1 | **15.9** |
| 2 | NDT | 29.2 |
| 3 | NDT | 29.0 |
| **Avg. (SD)** | **NDT** | **29.1 (0.08)** |
| **3** | 1 | NDT | 28.5 | **16.5** |
| 2 | NDT | 28.5 |
| 3 | NDT | 28.6 |
| **Avg. (SD)** | **NDT** | **28.5 (0.07)** |
| **ATD** | **1** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **2** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **3** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **ATD1:10\*** | **1** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **2** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **3** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |

**\*** The same ATD concentration was used with the DNA extract analyzed after 10-fold dilution with PCR H2O.

Acronyms: Avg., Average; SD, standard deviation; CFU, colony-forming units; CT, cycle threshold; NDT, Not Detected.

**PCR:** Polymerase Chain Reaction. This is an assay used to detect the presence of any living system including disease causing microorganisms/germs including bioterrorism agents. A PCR assay is a biochemical reaction in which the nucleic acid of a target organism is amplified millions of time by a catalyst called polymerase enzyme using the forward and reverse primers, and nucleotides. The amplified nucleic acid can be detected via increase in fluorescence measured by a specially designed instrument.

**RV-PCR:** Rapid Viability (RV) Polymerase Chain Reaction (PCR). This method includes first growing in a growth medium the pathogenic bacteria such as *Francisella tularensis* present in a sample and then detecting the increase in the amount of the nucleic acid (DNA-deoxyribonucleic acid) of these bacteria using the PCR assay.

***Francisella tularensis*:** Name ofthe bacteria that cause tularemia.

**Treatment:** Addition of inhibitors such as Ferrous Sulfate (Fe), Humic Acid (Humics), and Arizona Test Dust (ATD) in experimental conditions.

**Control:** No Addition of inhibitors such as Ferrous Sulfate (Fe), Humic Acid (Humics), and Arizona Test Dust (ATD) in experimental conditions.

**Fe/Humics:** Addition of inhibitors, as Ferrous Sulfate (Fe), Humic Acid (Humics) in experimental conditions.

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**F4 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**F5 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**CT:** Cycle Threshold. A cycle number in a PCR assay at which the amount of fluorescence is above the baseline value.

**T0:** Time Zero Hour. Zero hour incubation for growth of bacteria. A sample aliquot is taken out for analysis before incubation for growth and PCR CT is generated.

**T30:** Time 30 hours. Thirty hours incubation for growth of bacteria

**∆CT:** A change (∆) of cycle threshold (CT). In the RVPCR method, PCR assay is performed before (T0) and after (T30) the growth of bacteria and the CT values are measured. The CT value of the PCR assay performed after the growth (T30) is deducted from the CT value of the PCR assay performed before the growth (T0). This difference in the CT values is called ∆CT. Therefore, a ∆CT = CT (T0) - CT (T30).

**Table 6. RV-PCR Results for *Francisella tularensis* Schu S4 Cells (~2,500 CFU/3-mL Sample) in the Presence of Fe/Humics or ATD**

| **Treatment** | **Sample Replicate** | **PCR Replicate** | **T0 CT** | **T30 CT** | **ΔCT (T0–T30)** |
| --- | --- | --- | --- | --- | --- |
| **Control** | **1** | 1 | 34.7 | 25.3 | **9.3** |
| 2 | 34.0 | 25.1 |
| 3 | 34.7 | 25.2 |
| **Avg. (SD)** | **34.4 (0.4)** | **25.2 (0.07)** |
| **2** | 1 | 34.2 | 26.2 | **7.9** |
| 2 | 33.8 | 26.3 |
| 3 | 34.3 | 26.2 |
| **Avg. (SD)** | **34.1 (0.3)** | **26.2 (0.04)** |
| **3** | 1 | 33.6 | 26.2 | **7.2** |
| 2 | 33.4 | 26.1 |
| 3 | 33.0 | 26.1 |
| **Avg. (SD)** | **33.4 (0.3)** | **26.1 (0.04)** |
| **Fe/Humics** | **1** | 1 | 33.7 | 22.0 | **11.5** |
| 2 | 33.4 | 21.9 |
| 3 | 33.1 | 21.9 |
| **Avg. (SD)** | **33.4 (0.3)** | **21.9 (0.03)** |
| **2** | 1 | 33.8 | 20.5 | **13.3** |
| 2 | 34.0 | 20.6 |
| 3 | 33.7 | 20.6 |
| **Avg. (SD)** | **33.8 (0.1)** | **20.5 (0.02)** |
| **3** | 1 | 37.5 | 19.9 | **17.2** |
| 2 | 37.8 | 19.9 |
| 3 | 36.3 | 20.0 |
| **Avg. (SD)** | **37.2 (0.8)** | **20.0 (0.03)** |
| **ATD** | **1** | 1 | 37.6 | 38.3 | **-0.1** |
| 2 | 39.6 | 39.3 |
| 3 | NDT | 38.4 |
| **Avg. (SD)** | **38.6 (1.4)** | **38.7 (0.5)** |
| **2** | 1 | 38.0 | 37.5 | **2.4** |
| 2 | 38.8 | 35.1 |
| 3 | 37.8 | 34.8 |
| **Avg. (SD)** | **38.2 (0.6)** | **35.8 (1.5)** |
| **3** | 1 | NDT | 34.6 | **10.7** |
| 2 | NDT | 33.5 |
| 3 | NDT | 34.7 |
| **Avg. (SD)** | **NDT** | **34.3 (0.7)** |
| **ATD1:10\*** | **1** | 1 | NDT | 35.8 | **9.2** |
| 2 | NDT | 36.3 |
| 3 | NDT | 35.1 |
| **Avg. (SD)** | **NDT** | **35.8 (0.6)** |
| **2** | 1 | NDT | 35.9 | **9.2** |
| 2 | NDT | 35.5 |
| 3 | NDT | 36.1 |
| **Avg. (SD)** | **NDT** | **35.8 (0.3)** |
| **3** | 1 | NDT | 37.8 | **8.3** |
| 2 | NDT | 36.1 |
| 3 | NDT | 36.1 |
| **Avg. (SD)** | **NDT** | **36.7 (1.0)** |

**\*** The same ATD concentration was used with the DNA extract analyzed after 10-fold dilution with PCR H2O.

Acronyms: Avg., Average; SD, standard deviation; CFU, colony-forming units; CT, cycle threshold; NDT, Not Detected.

**PCR:** Polymerase Chain Reaction. This is an assay used to detect the presence of any living system including disease causing microorganisms/germs including bioterrorism agents. A PCR assay is a biochemical reaction in which the nucleic acid of a target organism is amplified millions of time by a catalyst called polymerase enzyme using the forward and reverse primers, and nucleotides. The amplified nucleic acid can be detected via increase in fluorescence measured by a specially designed instrument.

**RV-PCR:** Rapid Viability (RV) Polymerase Chain Reaction (PCR). This method includes first growing in a growth medium the pathogenic bacteria such as *Francisella tularensis* present in a sample and then detecting the increase in the amount of the nucleic acid (DNA-deoxyribonucleic acid) of these bacteria using the PCR assay.

***Francisella tularensis*:** Name ofthe bacteria that cause tularemia.

**Treatment:** Addition of inhibitors such as Ferrous Sulfate (Fe), Humic Acid (Humics), and Arizona Test Dust (ATD) in experimental conditions.

**Control:** No Addition of inhibitors such as Ferrous Sulfate (Fe), Humic Acid (Humics), and Arizona Test Dust (ATD) in experimental conditions.

**Fe/Humics:** Addition of inhibitors, as Ferrous Sulfate (Fe), Humic Acid (Humics) in experimental conditions.

**ATD:** Addition of inhibitor, Arizona Test Dust (ATD) in experimental conditions.

**F4 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**F5 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**CT:** Cycle Threshold. A cycle number in a PCR assay at which the amount of fluorescence is above the baseline value.

**T0:** Time Zero Hour. Zero hour incubation for growth of bacteria. A sample aliquot is taken out for analysis before incubation for growth and PCR CT is generated.

**T30:** Time 30 hours. Thirty hours incubation for growth of bacteria

**∆CT:** A change (∆) of cycle threshold (CT). In the RVPCR method, PCR assay is performed before (T0) and after (T30) the growth of bacteria and the CT values are measured. The CT value of the PCR assay performed after the growth (T30) is deducted from the CT value of the PCR assay performed before the growth (T0). This difference in the CT values is called ∆CT. Therefore, a ∆CT = CT (T0) - CT (T30).

**Table 7. RV-PCR Results for *F. tularensis* Schu S4 Cells (~250 CFU/3-mL Sample) in the Presence of Fe/Humics or ATD**

| **Treatment** | **Sample Replicate** | **PCR Replicate** | **T0 CT** | **T30 CT** | **ΔCT (T0–T30)** |
| --- | --- | --- | --- | --- | --- |
| **Control** | **1** | 1 | NDT | 29.9 | **7.8** |
| 2 | 38.3 | 29.7 |
| 3 | 36.8 | 29.8 |
| **Avg. (SD)** | **37.6 (1.1)** | **29.8 (0.1)** |
| **2** | 1 | 38.8 | 28.9 | **8.1** |
| 2 | 36.3 | 29.2 |
| 3 | 36.3 | 28.9 |
| **Avg. (SD)** | **37.1 (1.4)** | **29.0 (0.1)** |
| **3** | 1 | 36.1 | 29.6 | **6.8** |
| 2 | 37.0 | 29.5 |
| 3 | 35.7 | 29.2 |
| **Avg. (SD)** | **36.3 (0.7)** | **29.4 (0.2)** |
| **Fe/Humics** | **1** | 1 | 37.2 | 24.9 | **12.5** |
| 2 | 37.8 | 24.9 |
| 3 | 37.2 | 24.8 |
| **Avg. (SD)** | **37.4 (0.3)** | **24.9 (0.05)** |
| **2** | 1 | 38.7 | 25.5 | **12.6** |
| 2 | 38.4 | 25.5 |
| 3 | 37.1 | 25.5 |
| **Avg. (SD)** | **38.1 (0.9)** | **25.5 (0.01)** |
| **3** | 1 | 38.0 | 24.9 | **13.9** |
| 2 | NDT | 24.8 |
| 3 | 39.4 | 24.8 |
| **Avg. (SD)** | **38.7 (1.0)** | **24.8 (0.08)** |
| **ATD** | **1** | 1 | NDT | 38.4 | **7.5** |
| 2 | NDT | 37.3 |
| 3 | NDT | 36.7 |
| **Avg. (SD)** | **NDT** | **37.5 (0.9)** |
| **2** | 1 | NDT | 40.6 | **5.2** |
| 2 | NDT | 40.3 |
| 3 | NDT | 38.5 |
| **Avg. (SD)** | **NDT** | **39.8 (1.1)** |
| **3** | 1 | NDT | 37.6 | **7.0** |
| 2 | NDT | 39.2 |
| 3 | NDT | 37.2 |
| **Avg. (SD)** | **NDT** | **38.0 (1.1)** |
| **ATD1:10\*** | **1** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **2** | 1 | NDT | NDT | **6.4** |
| 2 | NDT | 38.0 |
| 3 | NDT | 39.2 |
| **Avg. (SD)** | **NDT** | **38.6 (0.8)** |
| **3** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |

**\*** The same ATD concentration was used with the DNA extract analyzed after 10-fold dilution with PCR H2O.

Acronyms: Avg., Average; SD, standard deviation; CFU, colony-forming units; CT, cycle threshold; NDT, Not Detected.

**PCR:** Polymerase Chain Reaction. This is an assay used to detect the presence of any living system including disease causing microorganisms/germs including bioterrorism agents. A PCR assay is a biochemical reaction in which the nucleic acid of a target organism is amplified millions of time by a catalyst called polymerase enzyme using the forward and reverse primers, and nucleotides. The amplified nucleic acid can be detected via increase in fluorescence measured by a specially designed instrument.

**RV-PCR:** Rapid Viability (RV) Polymerase Chain Reaction (PCR). This method includes first growing in a growth medium the pathogenic bacteria such as *Francisella tularensis* present in a sample and then detecting the increase in the amount of the nucleic acid (DNA-deoxyribonucleic acid) of these bacteria using the PCR assay.

***Francisella tularensis*:** Name ofthe bacteria that cause tularemia.

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**F4 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**F5 Assay:** A PCR assay in which a specific primer-probe set is used to specifically detect the amplification/synthesis of a short stretch/piece of DNA in the pathogenicity determinant protein D gene on the *pdp*D plasmid (a small circular DNA structure separate from the genome/chromosome DNA) of *Francisella tularensis* bacteria.

**CT:** Cycle Threshold. A cycle number in a PCR assay at which the amount of fluorescence is above the baseline value.

**T0:** Time Zero Hour. Zero hour incubation for growth of bacteria. A sample aliquot is taken out for analysis before incubation for growth and PCR CT is generated.

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**∆CT:** A change (∆) of cycle threshold (CT). In the RVPCR method, PCR assay is performed before (T0) and after (T30) the growth of bacteria and the CT values are measured. The CT value of the PCR assay performed after the growth (T30) is deducted from the CT value of the PCR assay performed before the growth (T0). This difference in the CT values is called ∆CT. Therefore, a ∆CT = CT (T0) - CT (T30).

**Table 8. RV-PCR Results for *F. tularensis* Schu S4 Cells (~25 CFU/3-mL Sample) in the Presence of Fe/Humics or ATD**

| **Treatment** | **Sample Replicate** | **PCR Replicate** | **T0 CT** | **T30 CT** | **ΔCT (T0–T30)** |
| --- | --- | --- | --- | --- | --- |
| **Control** | **1** | 1 | NDT | 34.3 | **10.6** |
| 2 | NDT | 34.7 |
| 3 | NDT | 34.0 |
| **Avg. (SD)** | NDT | **34.4 (0.4)** |
| **2** | 1 | NDT | 33.6 | **11.4** |
| 2 | NDT | 33.6 |
| 3 | NDT | 33.6 |
| **Avg. (SD)** | **NDT** | **33.6 (0.02)** |
| **3** | 1 | NDT | 32.6 | **12.3** |
| 2 | NDT | 32.9 |
| 3 | NDT | 32.6 |
| **Avg. (SD)** | **NDT** | **32.7 (0.2)** |
| **Fe/Humics** | **1** | 1 | NDT | 28.5 | **16.4** |
| 2 | NDT | 28.6 |
| 3 | NDT | 28.6 |
| **Avg. (SD)** | **NDT** | **28.6 (0.08)** |
| **2** | 1 | NDT | 28.3 | **16.8** |
| 2 | NDT | 28.2 |
| 3 | NDT | 28.1 |
| **Avg. (SD)** | **NDT** | **28.2 (0.07)** |
| **3** | 1 | NDT | 28.6 | **16.5** |
| 2 | NDT | 28.4 |
| 3 | NDT | 28.4 |
| **Avg. (SD)** | **NDT** | **28.5 (0.08)** |
| **ATD** | **1** | 1 | NDT | 44.4 | **1.2** |
| 2 | NDT | 43.3 |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **43.8 (0.8)** |
| **2** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **3** | 1 | NDT | 41.4 | **4.9** |
| 2 | NDT | NDT |
| 3 | NDT | 38.8 |
| **Avg. (SD)** | **NDT** | **40.1 (1.8)** |
| **ATD1:10\*** | **1** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **2** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |
| **3** | 1 | NDT | NDT | **0.0** |
| 2 | NDT | NDT |
| 3 | NDT | NDT |
| **Avg. (SD)** | **NDT** | **NDT** |

**\*** The same ATD concentration was used with the DNA extract analyzed after 10-fold dilution with PCR H2O.

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