Part 2: Environmental Panel Organisms

2.1 Soil Testing

Airborne soil particles may constitute a significant challenge to aerosol collection p**olymerase chain reaction** (**PCR**) assays. Soils contain genomic materials or nucleic acid fragments of countless archaebacterial, bacterial, and eukaryotic organisms. Some of the more common soil organisms can be anticipated. Soils may also contain unanticipated inhibitors that interfere with extraction, denaturation, polymerization, or annealing reactions. Therefore, an investigative challenge of a PCR assay to variety of representative soils is an important first step to establish the specificity of the primers/probes, and the robustness of PCR assay against potential interfering compounds.

Using the primers/probe, and amplicon sequences specific for any given assay evaluate each regional soil type*† for any signs of positive response.

Samples of each regional soil type* should be spiked at **2x, 5x and** 10x AMDL with the archetype organism (usually specified in the SMPR for AMDL testing, such as strain CO92 for *Yersinia pestis*) and then the samples evaluated for inhibition. Inhibition testing should be done with intact target organisms so that potential interference of the DNA extraction can be determined.

* Arizona Test Dust is available as a baseline starting point.

† See section 2.2 “Bioinformatics Analysis of Signature Sequences” on probing all available data bases including that contains soil metagenome sequences generated from specific regions of operations (if available) for In Silico Analysis and further validation of the signature sequences.

2.2 Bioinformatics Analyses of Signature Sequences

In silico screening will be performed on signature sequences (eg: oligo primers/probes and amplicon) to demonstrate specificity to the target biological threat agent.

In silico results are suggestive of potential performance issues, so will guide necessary additions to the wet screening panels. *In silico* identification of potential cross-reactions (false positives) or non-verifications (false negatives) would require the affected strains be included in the exclusivity or inclusivity panels, respectively, if available.

A method developer-selected tool to carry out the bioinformatics evaluation should be able to predict hybridization events between signature components and a sequence in a database including available genomic sequence data, databases and/or published documents describing the genetic sequences found in soils that are representative of the regions of operation. The selected tool should be able to identify predicted hybridization events based on platform annealing temperatures, thus ensuring an accurate degree of allowed mismatch is incorporated in predictions. The program should detect possible amplicons from any selected database of sequence.

Potential tools for *in silico* screening of real-time PCR signatures include:

- http://sourceforge.net/projects/simulatepcr/files/?source=navbar
    - This program will find all possible amplicons and real time fluorescing events from any selected database of sequence.

- NCBI tools

The method developer submission should include:

- Description of sequence databases used in the *in silico* analysis
- Description of conditions used for *in silico* analysis
    - Stringency of *in silico* analysis must match bench hybridization conditions
- Description of tool used for bioinformatics evaluation
    - Data demonstrating the selected tool successfully predicts specificity that has been confirmed by wet-lab testing on designated isolates
        - These data can be generated retrospectively using published assays
- List of additional strains to be added to the inclusivity (Annex II) or exclusivity (Annex III) panels based on the bioinformatics evaluation

2.3 Environmental Organisms

Inclusion of all environmental panel organisms is not a requirement if a method developer provides appropriate justification that the intended use of the assay permits the exclusion of specific panel organisms. Justification for exclusion of any environmental panel organism(s) must be documented and submitted.

If bioinformatic analysis is completed then DNA from Group 1 organisms should be tested. If bioinformatic analysis is not completed then DNA from Group 1 and 2 organisms should be tested

Organisms and cell lines may be tested as isolated DNA, or as pools of isolated DNA. Isolated DNA may be combined into pools of up to 10 panel organisms, with each panel organism represented at 10 times the AMDL, where possible. The combined DNA pools are tested in the presence (at 2 times the AMDL) and absence of the target gene or gene fragment. If an unexpected result occurs, each of the individual environmental organisms from a failed pool must be individually re-tested at 10 times the AMDL with and without the target gene or gene fragment at 2x the AMDL in the candidate method DNA elution buffer.

DNA in this list that already appear in the inclusivity or exclusivity panel do not need to be tested again as part of the environmental factors panel.

Group 1

Aedes aegypti (ATCC /CCL-125(tm) mosquito cell line)
Aedes albopictus (Mosquito C6/36 cell line)
Dermatophagoides pteronyssinus (Dust mite -commercial source)
Xenopsylla cheopis Flea (Rocky Mountain labs)
Drosophilia cell line
Musca domestica (housefly) ARS, USDA, Fargo, ND
Gypsy moth cell lines LED652Y cell line (baculovirus)– Invitrogen
Cockroach (commercial source)
Tick (Amblyomma and *Dermacentor* tick species for *F. tularensis* detection assays)[1]
Mus musculus (ATCC/HB-123) mouse
Rattus norvegicus (ATCC/CRL-1896) rat
Homo sapiens (HeLa cell line ATCC/CCL-2) human

Group 2

Cultivatable bacteria identified as being present in air soil or water

Acinetobacter lwoffii
Agrobacterium tumefaciens
Bacillus amyloliquefaciens
Bacillus cohnii
Bacillus psychrosaccharolyticus
Bacillus benzoevorans
Bacillus megaterium
Bacillus horikoshii
Bacillus macroides
Bacteroides fragilis
Burkholderia cepacia
Burkholderia gladoli
Burkholderia stabilis
Burkholderia plantarii
Chryseobacterium indologenes
Clostridium sardiniense
Clostridium perfringens
Deinococcus radiodurans
Delftia acidovorans
Escherichia coli K12
Fusobacterium nucleatum
Lactobacillus plantarum
Legionella pneumophilas
Listeria monocytogenes
Moraxella nonliquefaciens
Mycobacterium smegmatis
Neisseria lactamica
Pseudomonas aeruginosa
Rhodobacter sphaeroides
Riemerella anatipestifer
Shewanella oneidensis
Staphylococcus aureus
Stenotophomonas maltophilia
Streptococcus pneumoniae
Streptomyces coelicolor
Synechocystis
Vibrio cholerae