Question Concerning CRC Project No. AV-31-22, 'Microbial Test Kit Evaluation'

Q: The assay kits required for this study and/or associated reagents/consumables required; is any of that being provided by CRC for this evaluation/validation study or is the contractor expected to include those assay prices into our cost proposal?

A: Test kits will be supplied by the test kit manufacturers free of charge.

Exhibit G, Module I, Table1

Q: Are the test kits going to be provided by manufacturers, or they need to be purchased by contractor?

A: Test kits will be supplied by the test kit manufacturers free of charge.

2. qPCR/NGS : 1.2.2.8.5, 1.2.4.5.4, 1.2.4.5.4

Q: These 3 sections specify that the subsamples for qPCR/NGS need to be processed and preserved immediately. What does this mean? The winning contractor will run these tests later, or who else?

A: A number of protocols are available which enable DNA to be stabilized in samples so that testing can be conducted at a later time. For example; addition of sample to alcohol with suitable commercially available stabilizing agents. Once stabilized DNA should remain stable for at least several days, even weeks which enables flexibility in scheduling the various testing. The DNA should be stabilized as soon as each sub-sample is withdrawn from the microcosms, and no later than 1 hour.

The qPCR testing of stabilized samples can be sub-contracted to an appropriate laboratory, if necessary. Full details of the subcontract laboratory and information on protocols and timings of transit of samples should be provided in the submission.

3. Test Protocol, 1.2.1 Note

Q: Is CRC/IATA going to facilitate /provide the jet fuel for the study?

A: The bidder is invited to procure jet fuel for the study **– this is preferred.** A total of 250 litres would be needed for the study. The fuel should be a commercial grade jet fuel compliant with ASTM D1655 Jet A / A1 or DEFSTAN 91-91 and should be free of FSII and any biocidal additives. **If a bidder cannot do this, please state very clearly in the proposal that fuel procurement is not included.**

4. Test protocol, 2. Evaluating the kits against field samples

Q: Is CRC/IATA going to facilitate /provide the two field jet fuel samples for this section?

A: Yes, IATA can facilitate the two field jet fuel samples.

5. Test Protocol

Looks like section 3. of Test Protocol section of the RFP is missing. Is it just a miss numbering of subsections?

A. This is an error in the numbering in the protocol. There is no section missing.

6. Test Protocol, 5. Reference-Should be updated to 6th edition

A. The reference to 5th Edition is provided as this was the current edition at time of the protocol development and is the edition which provides details of previous recommended tests.

7. Table A3- Sample Volumes required for each test-

Q: For Easicult TTC, Easicult M and San- Air Biochecker FC there is no water sample volume specified. The methods can be performed by dipping the slide in the liquid or using a swab to wet the surface of the slide:

a) Does this mean the swab method is preferred?
b) If not, reconsider the total volume of water phase needed.

A: 200 mL of water phase will be available from each secondary microcosm for testing. After taking subsamples for all other tests, there should be 60 mL free water remaining to conduct the dip-slide type tests by dipping test paddles into the remaining fluid. However, water may also be "squirted onto each surface of the dipslides using a pipette.

8. Test Protocol 1.2.4.4.1: Reads: "Shake the separated water phase vigorously by hand for 10 seconds and immediately decant 20 mL into 2 x sterile 100 mL HDPE containers and 70 mL into a third sterile 100 mL HDPE container. Water phase in these containers will be used to complete triplicate testing using the LuminUltra QGO-M, qPCR and Hy-Lite tests, respectively (sample volumes required for each test are provided in Table A3 in the Appendix)."

Q: Clarify how each of the 2 x 20 mL and 70 mL samples are to be used. 20 mL is insufficient for QGO-M + HY-LiTE + qPCR testing.

a) The volumes listed in Table A3 do not correlate with the sample volumes listed in 1.2.4.4.1.
b) Please reconcile the differences between the subsection and the table.

Table A3 is correct but the associated text should read 30 mL (and not 70 mL) would be required to complete triplicate tests using the HY-LiTE test. It is recommended slightly more water phase is transferred than is actually needed for triplicate testing.
For clarity,

- 20 mL of water phase decanted into one container is to be used for LuminUltra QGO-M testing; 15 mL of this will be used to complete triplicate tests of 5 mL.
- 20 mL of water phase decanted into another container is to be used for qPCR testing; 15 mL of this will be used to complete triplicate tests of 5mL.
- 35 mL water phase decanted into another container for HY-LiTE testing; 30 mL of this will be used to complete triplicate tests of 10 mL.

A total of 200 mL of water phase will be available from each pooled microcosm. There will be ~60 mL free water remaining after all subsamples have been taken for the various tests; this should be enough to conduct the dip-slide type tests.

9. If a single analyst runs all of the tests, and that analyst is required to complete all heavy, moderate, and low bioburden tests for the fuel and water phases of **one microcosm** on a given day, that analyst will have to perform about 234 tests on one day(not including qPCR/NGS).

Test	Water Phase Testing	Fuel Phase Testing
Easicult TTC	1	0

Easicult M	1	0
FuelStat Resinae	1	1
IP 385	1	1
Hy-LiTE	1	1
MM2	1	1
San-Ai Biochecker FC	1	0
QGO-M	1	1
No of tests Sum/bioburden/phase	8	5
triplicate testing	24	15
@ 3 levels bioburden	72	45
2 secondary microcosms (1.2.4.1)	144	90
Water + fuel phases		**234**

A. Please see response to Question 10 below

10. Table A3 lists the time from the initiation of the test to data availability. However, if we are to add all together, not including IP385 (which depends on the analyst's experience with the method), not even considering the time for supporting tasks, or time needed for 1.2.4.4.4 , it will look more like:

TEST	**Water Phase**		**Fuel Phase**	
	Min	**Min x 3**	**Min**	**Min x 3**
Easicult TTC	2	6	0	0
Easicult M	2	6	0	0
FuelStat Resinae	10	30	0	0
IP 385	-	-	-	-
Hy-LiTE	10	30	10	30
MM2	5	15	5	15
San-Ai Biochecker FC	2	6	0	0
QGO-M	10	30	10	30
Sum/bioburden/phase	61	183	45	135
@ 3 levels bioburden	183	549	135	405
@ 2 secondary microcosms (1.2.4.1)	366	1,098	270	810
Water + fuel phases				1,908 minutes
Total time/day (h)-minimum				**32 h**

From 9 and 10 is clear that a single analyst cannot cover all testing in one given day. Even if fuel and water phases are tested on different days, the time spent running tests will be more 10 h. If each bioburden level is run on a different day then time spent testing more than 10 h as well.
Thus, the testing workload is rather unreasonable for 1 analyst. Fatigue is likely to affect analyst's performance and thereby become a significant source of variation.
A: Comment on points 9 and 10 together. It is envisaged that the successful tenderer would assign several analysts to enable timely analysis. Using timings suggested in the question, which are reasonable, it is believed 3 analysts could complete water phase analysis (one microcosm, one bioburden level) within two hours. More analysts could mean that water phase and fuel phase tests could be conducted concurrently. Consideration could be given to staggering testing to consecutive days (e.g. different bioburden level each day) depending on availability of analysts.

11. The project could be challenging even for big commercial labs or research institute labs. In my opinion, a successful outcome stands in need of the participation of one of the lead fuel microbiologists in the industry. However, it is clear that most of them, if not all, have an interest in the test kits intended to be tested in this project. Is it permissible to bring one of them into the team, as a subcontractor, with a clear statement that they will be involved only to the point where testing of the kits commences?

A. It is recognized that many laboratories and experts in this field have commercial interest in the various methods under evaluation. However, it is considered essential the project is conducted impartially and therefore involving one or more experts who has a vested interest in the technologies would not be permitted. However, the contractor will be able seek guidance from the IATA Microbial Panel, if needed.

Q: Is the CONTRACTOR responsible for sourcing the various test Method kits or will they be supplied by the vendors?

- And if so, is this free of charge?

A: Test kits will be supplied by the test kit manufacturers free of charge.
Q: Is the CONTRACTOR responsible for sourcing the two field samples mentioned under 2.1 ("one sourced from a heavily contaminated aircraft fuel tank and one sourced from a heavily contaminated aviation fuel storage tank.")?
A: Yes, IATA can facilitate the two field jet fuel samples
Q: Any sample freight will need to be included in the commercial proposal or will be covered direct by CRC?

- Given the high deviations seen in global freight will a cost-plus basis be accepted for this line item?

A: Any sample freight will need to be included in the commercial proposal. Provide an estimate of any variable cost elements that will ensure a cost modification will not be required, if pursuing a cost-plus approach.

Q: Should our proposal only cover Module I, or should we also elaborate on Module II also (taking into account "a technical protocol for field trial evaluation will be developed and presented separately.")?

- If only for Module I, does our work of this part of the scope influence our potential role in scope of Module II?

A: The purpose of this RFP is to cover Module I, however elaborating on Module II is welcome

Q: Regarding the 8 week incubation period: Is there a requirement to plan for contingency for failure to meet required growth density to be included in the project timeline?

A. We believe the 8 week period will be sufficient but the incubation could be extended to allow for sufficient growth to occur if necessary. The overall time allowed for the project already provides sufficient contingency for this.

Q: Please can CRC provide more insight on the cost plus fixed fee commercial basis outlined?

- Will this basis be applied to the baseline proposal forecasted cost basis OR actual project execution costs?

A. Whether a contract is a cost-plus or fixed price, CRC must set aside funding to support it. Increasing the cost of a contract at a later date is uncertain due to the need for funding availability and approvals. Bidders should provide the best price for their proposal to perform the research at the highest quality without anticipating the need for additional funding to complete the described scope of work.

Q: Regarding Exhibit D: It there potential to discuss the 'unlimited liabilities' as stipulated there? [Bidder} legal department have policies around such clauses.

A. The Request for Proposals provides CRC's preferred contracting language, but alternative approaches may be possible. Any concerns with CRC's contract terms should be described in the proposal so that can be known to the proposal reviewers. If a proposal is initially selected for award but an acceptable contracting solution can not be found, an alternate bidder may receive the award.