Corona Detective Application 2

JOGL project 181 - 18/10/2020

1.0 Introduction

1.1 Problem and Background (200 words max)

Neither diagnosis of cases of Covid-19, caused by SARS-CoV-2, nor detection of environmental contamination by the virus are yet simple. Complicated techniques of molecular amplification, requiring serious infrastructure and biosafety procedures, limit people everywhere from knowing whether the virus is really in their environment or not.

This open science molecular method of Corona Detective, inspired by GMO Detective, was awarded support in the first JOGL funding round, and much progress was made. Now we await the XPRIZE semi-finalist 'blinded proficiency kit' arrival, and hope to get just a bit more backing from the JOGL community for our production partner in Paris and struggling open public lab in Lausanne this round. This will help enable more shipment of tubes to our distant collaborators and testing of a triplex model (to include a flu viral target in a third color) at Hackuarium.

1.2 Solution summary in simple terms (150 words max)

The 'Do-It-Together SARS CoV-2 Detective' project has already developed a molecular amplification strategy detect both the virus causing Covid-19 and a cellular RNA as an internal extraction control. Done not only without complicated equipment but with a simple +/- readout, the #CoronaDetective is very specific. Furthermore, controls to ensure sensitive detection, without false positives or negatives, are intrinsic to this solution. The final product, strips of tubes with dry reagents, can be readily supplied anywhere, without cold-chain dependence; and monitoring tests run by ordinary people, without any background in medicine or biology, just some ability to follow simple instructions and common sense.

The original application template for JOGL funding (7April 2020) (https://docs.google.com/document/d/1Pou6TPO5heDZcvwUWGV_kbHdXnovnG7ruDu0kKJtiKI/edit?usp=sharing) and a more recent slide deck presentation (https://drive.google.com/file/d/1HJEcHi5_bl_AH3mbKFkoknujBguJFc8L/view?usp=sharing) are available for further information.

1.3 Solution summary in technical terms (200 words max)

Synthetic RNA detection is already very sensitive for the multiplex Corona Detective, containing the viral and cell mRNA control primer sets, down to 20 copies in a reaction, or one copy per microliter. Methods for not only the user protocol but also low cost local production of reactions tubes are already shared openly.

User Protocol (https://dx.doi.org/10.17504/protocols.io.bk43kyyn)

Production protocol (https://dx.doi.org/10.17504/protocols.io.bk44kyyw)

Now with flu season coming, we request more support for production and shipping of the current multiplex reaction tubes. Furthermore we will test whether triplex detection, including a flu specific set of primers, (https://docs.google.com/spreadsheets/d/13HHwiX_OqrXwudE1uWIDfi3iHoqWDrlcUmTzVfF6fEs/edit?usp=sharing) will work for a 'new and improved' Corona Detective v2.

Initial testing of another couple of sets of control RNA targests (18S and 16S RNAs) was promising, and they can be tried in case the current combination does not work well. Another viral RNA set (Lamb) also has been tested, and is another alternative to try in the triplex context, if there are problems.

Furthermore, we have developed an easy app for collaborators to send us their results (see news item from Tatiana, 30sept, in the front end JOGL page).

1.4 State of advancement of the project (100 words max)

Multiplex Corona Detective reactions were developed, which work very well to detect viral (NM) and internal control (RP) targets. This means that very sensitive and specific detection is possible. Furthermore, in the 8-tube format for the multiplex, 5 unknown samples can be tested, much better than just 2 unknowns per 8 tube set, as in the GMO Detective. (In the 96 well format, which can also be produced on the system in Paris, this means 93 unknowns could be tested - a boon for scaling up the procedure, once validation is complete.)

1.5 Project Timeline

- producing and shipping many Corona Detective multiplex tubes to international colleagues.
- ordering the flu primer set and optimising reaction conditions (relative primer concentrations and magnesium levels).
- testing these primers in Corona Detective reactions, in combination with the usual multiplex components, and alternatively with the other control RNA primer sets.
- analysing results of these validation attemps, gathered with the simple app from our international colleagues.
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These timeline items can all be fulfilled in weeks (to maximum two to three months for analysis of the validation results, perhaps), after orders are in hand for production (FR) and tri-plex (CH) tests.

2.0 Project Implementation

2.1

- **Solution**: Method of viral RNA amplification

This solution, the #CoronaDetective, allowing SARS CoV-2 detection, would benefit anyone that wants to know whether a given sample contains viral RNA. Environmental monitoring would be facilitated for anyone, although any patient sample tests should be run in an appropriate laboratory setting (BSL3). The tubes, containing dried reagents, can be shipped anywhere for

analyses, along with the stable reaction buffer solution. Furthermore, the fluorescence detector for the 8-strip tubes can be simply built at a very low cost. Project partners as far away as Africa and Latin America are ready to help validate the system after shipment, following simple instructions analogous to those from the GMO Detective protocol. Thus, implementation of this project could have a very broad impact.

2.2 Methodology (500 words max)

Description of tools and methods used.
The methodology must allow the full reproduction of the the results

Complete details for following a basic isothermal amplification protocol can be found here (https://www.pnas.org/content/117/39/24450 -Rabe and Cepko method)

The Corona Detective methods for not only the user protocol but also low cost local production of reactions tubes are already shared openly.

User Protocol (https://dx.doi.org/10.17504/protocols.io.bk43kyyn)

Production protocol (https://dx.doi.org/10.17504/protocols.io.bk44kyyw)

Synthetic control viral RNA detection, as standard in the Hackuarium lab, is not sufficient for complete validation, and the use of inactivated viral preps is our great current focus (particularly in Paris where several methods are being evaluated. The eagerly anticipated 'blinded proficiency kit' set for the semi-final round of XPRIZE is anticipated to allow great validation already in the next two weeks. Sample preparation and reliable prevention of cross contamination are key, which the teams in France and Sri Lanka will ensure in that effort.

Whatever the result of these tests, however, the plan remains to send hundreds of reaction tubes to our various international collaborators, including medical practitioners in Germany and France, and we have had further confirmations from teams in Chile and Camaroon in the last week.

Production and shipping costs have so far been taken care of primarily by funding to the Paris team, it should be noted. Token support by funding this grant would be appreciated nonetheless.

2.3 Results/Expected results (500 words max)

Being able to produce and ship off 1000s of the current multiplex Corona Detective tubes and collect data from colleagues using the new app will provide more validation to supplement that obtained from the XPRIZE semi-final tests. More R&D for the triplex detection version of Corona Detective (as flu season is coming) and optimisation of a few other possibilities for sample preparations will be very beneficial.

The results of the Corona Detective test are readily scored by eye on the fluorescence detector, but we have also made a table for all possible result interpretations (i.e. if the negative control comes up positive, the reactions are null and must be repeated). This will be further updated, if the triplex works well, of course!

3.0 Safety, quality assurance and regulation

3.1 What steps have you taken to ensure your solution's safety? How advanced are you in this process **(if applicable)**? Please check the Biosafety and Biosecurity guideline of OpenCovid19

Human or clinical samples should only be run in settings with access to appropriate biosafety facilities, of course. This project is in full compliance with the OpenCovid-19 Initiative's Biosafety and Biosecurity Guidelines, and is fortunate to include appropriate labs for all levels of validation.
Risk assessment has been reviewed. Use of not only synthetic RNAs, but inactivated virus, have been successfully validated in partner labs. For most proposed tests no live virus would be utilised, with only parts of genes either as DNA or RNA used as the positive controls, except when a partner has access to a BSL3 lab

3.2 Have you planned the conduct of your manufacturing process that ensures quality, what are the steps you have taken? How advanced are you in this **(if applicable)**?

Dependence on molecular biology companies for primers and reagents means there is some guarantee applicable.
Furthermore, the robotic/manufacturing pipeline of one partner (FR) has already been validated for the GMO Detective kit, and other projects.

3.3 Will you need assistance with the regulation system? If not, which regulatory system do you plan on using to distribute the product? Please elaborate (please see: Regulatory-Strategies) **(if applicable)**

At least at first, this project will be either WeProvideNonMedicalDevices-Public or WeProvideInstructions: and it will be for research use only, for viral detection, not a medical diagnostic. (at least initially - if get through to finals of the XPRIZE, it may well be that their validation will allow more use-cases.)
To get Corona Detective reaction tubes used is already half way there, in terms of the regulatory landscape, as the viral primer set we use, NM is already FDA Approved.
(Link to EUA announcement: https://www.fda.gov/media/139937/download)

3.4 Have you talked to medical staff about the feasibility of your project? What did they say?
Enthusiasm was obvious when we talked to others, including medical professionals, about this project. One German doctor from the Open Covid-19 Initiative already asked about possibilities for clinical tests in Dortmund with this proposed solution to the testing problem.

3.5 Have you planned the testing, verification and validation of your solution? How advanced are you?

We have done many tests and some verification, but the further validation and the possibility for detecting a third important target is still in question. Funding this request will definitely help.

4.0 Impact, issues and risks

4.1 What impact do you feel your project could have? (100 words max)
The impact of this project will potentially be very high, as molecular detection tools for the virus causing Covid-19 are only available in specialised laboratory settings now. The 'DIT SARS CoV-2 Detective' solution could allow average people to test for the presence of virus, wherever they might be.

4.2 What do you think would make your project a success?(100 words max)
Getting the #CoronaDetective kit out to the world after collaborative experiments for parallel tests and validation by multiple partners in different countries involved in this open participatory research and development work would really make this project a success.

4.3 Please list the known issues, potential risks, grey-areas, etc in your project
***Making sure people know to **never open kit tubes after the reaction has run**, particularly ones that gave a positive result, is the biggest issue around using this method. The worst would be if samples got contaminated by end-product. If such contamination occurs, people might panic, believing there is much more virus around than there really may be. This risk is mitigated, however, by the requirement to always have a negative control for each reaction set. If the negative control shows up positive, cleaning well with diluted bleach and alcohol before running any new set of tests is essential.*

**Another issue is the fact that all the molecular reagents, in particular the enzymes needed, must be purchased from biotech companies like New England Biolabs. In the Open Covid-19 Initiative, however, a group (FreeGenes) is working towards open alternatives. However, even in the short term, we hope that the companies might be convinced to help make even the initial #CoronaDetective kit a feasible option.*

5.0 Originality

5.1 What other projects on JOGL are like yours?
The other three collaborative projects in JOGL that are similar to Corona Detective, also semi-finalists for XPRIZE, are:
163: One Hour Covid Test using LAMP (#1HourCovidTest)

187: COVID-ALERT: Accessible LAMP-Enabled Rapid Test (#COVIDALERT)
392: Corona Hunter (#CoronaHunter)

Other JOGL OpenCovid-19 Initiative participants in the Diagnostics and Detection Challenge, who could well affect further even more open source iterations of the detection system, include members of the projects 174 Open Enzyme Production for Covid-19 Diagnostics, 188 Cell-Free systems for seq-specific sensing of SARS-CoV-2, and 241 Reclone.org - establishing a reagent collaboration network, among others.

5.2 Is this an innovative project? What makes this project different if it's unique on JOGL?

Yes, this is innovative primarily because of the lack of cold chain dependence for shipping of the freeze-dried reaction tubes. Local production of kits with non-commercial enzymes are also a long term goal of the project.

5.3 Is there already an open source version of this project?

In a way, yes.
As our methods for use and production of Corona Detective multiplex tubes are already published and it is analogous to GMO detective, it is indeed already basically open source.

6.0 Team experience

6.1 Please cite your team members and their roles in the project.

Guy Aidelberg developed the GMO Detective assay and has worked on similar systems for ZIKAV/DENGV and Rachel Aronoff worked with this method for various projects.

Guy Aidelberg is in Paris at the CRI (FR), Ali Bektas is at UC Berkeley in California, Rachel Aronoff is in Lausanne working with the open public lab Hackuarium (CH). Thomas Mboa, Stephane Fadanka and Nadine Mowoh are in Cameroon with the open science MboaLabs (CM). Fernan Federici is in Chile, with his own academic lab (https://federicilab.org/ CL), and finally there are at least 3 partners in the United States (USA): Chris Monaco at the CDC in Atlanta, Ellen Jorgenson with the company Aanikabio in NY, Sarah Ware, founder of two independent labs in the Chicago area: BioBlaze Community Bio Lab and Lizzy Blossom Ag Services, and Isabella Zorra also working with the BioBlaze Community BioLab.

7.0 Funding and Costs

7.1 Please provide a costing of your project be as detailed as you can, all funding requests must be transparent and be for specific needs. The maximum grant is 1500 euros. Smaller grants are more likely to be funded. If no grant is required, request no funds in the form.

We are requesting 1400 from JOGL for the project, 700 for Guy in Paris and 700 for Hackuarium

7.2 How is your project being funded so far?

JOGL awarded initial funding of 1800 euros to Hackuarium (which also provided a microgrant of 500chf for the project) and to our Madrid partner, who ended up passing their funding on to Hackuarium, when the initial plan to use a real time machine in Madrid fell through due to Covid-19 restrictions. The work in Paris has been entirely funded by Ariel Lindner's lab in the CRI (where Guy is currently finishing his PhD), to date.

7.3 How much funding do you need and how do you plan to use that funding?

The production work in Paris essentially costs about 3000 USD just for ordering all the components for the Corona Detective tubes (3258 on table including some RNA extraction options too) - and ultimately costs about 2 USD per reaction - so for 1000 tubes, 2000USD is really needed on that end.

(See table X
https://drive.google.com/file/d/1EV4E07NU0961eNNIi2VXJiywz8KzFsrz/view?usp=sharing)

For the further R&D in Switzerland, the cost of a primer set, with one primer tagged fluorescently (Texas Red is a likely choice, to go with the Fam and Hex targets) and a complementary quencher for the QUASR detection costs at least 250 euros for the tag and quencher and about 70 euros for the rest of the primers (i.e. for the Flu target), while Hackuarium has already spent almost 4000 chf for all the various enzymes, nucleotides, etc. (and should include costs for sample extraction tests.)

(see table Y - go to bottom of 'partnerRA_CH' sheet.
https://docs.google.com/spreadsheets/d/1ZMayB2jx3VDAT2iO9VGjujWdCtD_ATq-V_IBWPE3s90/edit?usp=sharing)

3981chf total for reagents and 1038chf total for primers, already spent for project by Swiss team.

To help us out, for production and the further R&D costs, 700 euros each (1400 total) would thus be really great to obtain.

for Paris team at CRI for some contribution to tube production
for Swiss team at Hackuarium for primers, extraction reagents (glass milk and NaI)

8.0 Achievement and Benefits of funding [only for projects already funded by JOGL]

• **Add Your latest results, development and methods in the About section of your page**. In the case that you previously already used this section for hosting your proposal for a micro grant, please copy the proposal

section into a pdf and attach it as a doc instead for archiving purposes. It's Open Science!
Done.

• **Include a special paragraph where you indicate what part of your project JOGL and its micro-grant has enabled you to accelerate your research or project**
In particular the JOGL contributions kick-started the R&D on this project at Hackuarium, and we are extremely grateful!

• **A post on the wall of your project page indicating that you've updated your page with the latest results and development**! Post some cool results if you have any, links to papers and news articles to check out are also great to see!
Done

--- In the "Needs" section ---

Needs
Please use the "Needs" section of your JOGL project page to indicate your needs in people / skills / equipment / data / ... [do not use this section to ask for funding]
Done

--- In the "Documents" section ---

[Optional] Additional documents

Esp this Document!
Done

thank you very much for your kind consideration!