Editorial comments and responses:

1. The editor has formatted the manuscript to match the journal's style. Please retain the same.

Formation checked.

2. Please address specific comments in the manuscript.

The comments and responses are visible in the manuscript and under these comments.

3. Please reword the title to more clearly reflect the protocol.  
The title has been reword.

4. Please remember that the highlighted steps are the steps which are shown in the video. Please ensure these are the core steps of the protocol and reflective of the title and manuscript. The manuscript emphasizes the identification of microbial loads by qPCR while none of the RNA, DNA, qPCR steps are highlighted.  
The extraction of nucleic acids and qPCR was left out because these steps are very familiar to anyone doing molecular techniques and it is not interesting to follow someone pipeting from one tube to another. The idea was to explain these steps verbally. However, you have a good point and a step where the qPCR reactions are pipetted is now added. In this step, also the homogenization of the sample and nucleic acid extraction could be briefly mentioned.

5. Please remember that the highlight should not be more than 2.75 pages including heading and spacings.  
The highlighted text does not exceed 2.75 pages.

6. The protocol needs more clarity.  
The specific clarity comments has been addressed in the manuscript file.

7. Please proofread the manuscript for any spelling and grammar issues.

Checked

Comments within the manuscript:

Please change the title to reflect the protocol clearly. Please be more specific. Please do not use the term model of Human tuberculosis … please tone this down a bit.

Title modified

Citation?

Citations added

Do you work under BL-2 guideline? Please mention the same as well, if yes.

There is no ethical approval needed for handling BSL2 pathogen. In this case, the ethical permissions are needed only when animals are involved. This part refers to biosafety levels (not laboratory animals). The handling of pathogens is regulated locally. Please note, that the Finnish guidelines, that we are following, may not be the same in other laboratories.

Please mention if you needed any local ethics committee approval for your work. Please do not use personal pronouns in the protocol section.

The permits have been mentioned at the beginning of the protocol section. No other ethical permits were required. The text has been combined with the note below.

Converted to imperative tense, please check.

Checked, OK

We cannot have commercial language in the manuscript. Please move this part to the table of materials.

Commercial language removed and added to the table of materials

We cannot have paragraphs of text in the protocol section. Please consider moving some parts to the discussion.

Some text was moved to discussion

Till O.D what?

Added

We cannot have one note after another. So I have combined both. Please check.

Checked, OK

Since not in imperative tense, this is converted to a note.

OK

What is the day of infection- you grow bacteria for 3-4 days in liquid medium till O.D .., then dilute to 0.07 O.D, then grow again for two days to reach early log phase…. Then when is the day of infection?

Please write steps as if you the doing the protocol.

You followed the protocol correctly. The beginning of the sentence has now been removed

So, bacteria form clumps in the steps above? What is the O.D. at this time?

Many bacteria have a natural tendency to form aggregates and so does M. marinum. The bacteria form clumps even when tween is added to the culture medium (as it is in this protocol). The settling step does not change the OD-value much but gives a better correlation between OD600 and cfu/ul. So, the OD-value remains approximately at 0.5.

Please provide the desired concentration used by you in your experiment. This is important for the person who reads to have a sense of what concentrations and volumes are used throughout.

The infection dose depends on the experimental setup. In this article, we show results that have been produced by a low-dose infection (35 cfu) and high-dose infection (2000 cfu). The infection outcome is very different with these infection doses and has to be chosen by the reader. Also, adding a range from 35 to 2000 cfu would give a false impression that any infection dose can be used.

Notes cannot be filmed. Please convert this to steps in imperative tense if this needs to be filmed.

Not necessary to film

Needs citation. Or else how do you predetermine this?

A citation added

How do you do this.

The correlation between OD and cfu/ul has been determined by first measuring OD600-value of the bacterial suspension and then plating a sample of the same suspension on 7H10 plate and counting the bacterial colonies formed on the plate. The measurements and platings have been repeated in different OD-values with a minimum of three replicates.

What does AB wild-type represent and rag1-/- stands for. Either provide a reference or provide details in the materials table.

AB removed and rag explained in the note below

This detail should be included either in the introduction or in the protocol section (as a note) where the term is introduced.

Moved from the results

How do you maintain sterility in this case.

The injection of the bacterial suspension is not sterile. It would be technically hard to wipe a small zebrafish with ethanol. Also, it would do extra harm to the fish.

Please do not use personal pronouns in the protocol text.

Also, at which step did you add the red tracer to the bacterial solution?

Red tracer is added at step 2.4., as mentioned

The sentence has been modified

Take samples of bacterial suspension from where? Do you take samples from the fish?

The samples are taken from the bacterial suspension used for infecting the fish. We do not take samples from the fish at this point.

How do you verify the infection dose? What is the infection dose in this case.

We count the colonies that form on the plates. This has been now explained in the protocol. The infection dose has to be confirmed with this plating method, we cannot know the exact infection dose beforehand.

What are the symptoms of infection?

The symptoms are listed in the following sentence.

Is there a specific concentration used for euthanisa

Unfortunately, there is not. It is unethical to test, which concentration of the anesthetic is enough for euthanizing the fish. Therefore, we cannot say more specifically.

Please clarify? What symptoms what 30% ?

This has now been clarified.

Needs more clarity. Please reword better.

Sentence modified

Please do not use commercial language and use generic term instead. Also use imperative tense throughout.

Tri reagent changed to guanidine thiocyanate-phenol solution

Please make this a note or rewrite in imperative tense.

Added as a note

Please do not use commercial language in the manuscript. Please use generic terms instead. Please refer to the commercial language in the table of materials.

ROX is an abbreviation of carboxy-X-rhodamine which is now mentioned in the text

Wouldn’t this section 8 and 9 come before DNA isolation after step 5?

No, this is in the correct place. We want to remove any possible traces of contaminating genomic DNA in our RNA samples.

Please do not use commercial language in the manuscript. Please use generic term instead. Please refer to the commercial language in the Table of materials.

Modified

Same primers as before?

No, the primers are found from the table 1

This detail should be included either in the introduction or in the protocol section (as a note) where the term is introduced.

Added as a note in the protocol section

As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

Please do not make numbered steps in the discussion. Please use paragraph style instead.

Discussion modified

Please do not abbreviate journal titles.

The reference list was made with the jove’s own template found in refworks (proquest). Please consider editing the template according to your instructions.