PCR optimization protocol

Assumptions: the probability p that a swab is infected must be low (less than 30%) to make this method worthwhile compared with the current method, and the smaller p is the more convenient the method.

Brief summary: Assume that the probability p among our swabs of having an infected is 1/8 then we organize the genetic material from the swabs into groups of 4, that is, we take part of the liquids in which the swabs were soaked and mix them 4 by 4, and then to this mixture we do the genomic extraction in case your procedure for analysis requires it. It is FUNDAMENTAL that the genomic is not extracted patient by patient, but in aggregate form as described, you will see why later.

Now to this we take some of the mixture of genetic materials and proceed to do real time RT-PCR to identify the presence of viral RNA. If the result is negative (and it will happen about 60 percent of the time) we have established the negativity of four patients by occupying only one slot in the PCR machine, otherwise we go back to the liquids in which the swabs we had mixed in tubes that tested positive for the virus (an indication that at least one of the four patients is infected) , mix them two by two, extract the genomic*(see the note) and proceed to do PCR to the two groups from two from each group from four positive in the previous test. For those who still test positive you go back, and this time you extract the genomics of the individual patients, do the pcr, and determine which of them is infected.

note*: it actually pays off for time reasons to extract the genomic of the patients grouped two by two while you are doing the pcr of the groups of 4, so the two processes go in parallel and you save time.

Bonus of this method: if the probability of being sick p is 1/32 our method can analyze 4 times more patients in the unit of time, in the case where p=1/128 even 10 times more patients.

Malus: the method requires multiple PCR runs, with a delay in the time at which each person knows whether they are positive or negative (Although in reality more than half of those tested know after the usual 5 hours that they are negative, a quarter have to wait for another pcr run, an eighth another...

Criticality and mission of your laboratory: you need to find out whether the concentration of the viral titer in the tubes in the early stages of the procedure is high enough for the PCR to start amplifying the genome.

Proposed protocol for finding out: the proposal varies depending on whether it is convenient for you to occupy a large part of the real time RT-PCR machine with our experiment and put in a little time or occupy a very small part but devote more than a day of attempts.
What we need to find out is at what stage of the initial dilution (mix the prevenient liquid from 2,4,8...swabs) the false negatives start to occur. So concretely we propose a restrospective analysis (so where we know the positivity or negativity of all the swabs that we are going to use), we take a positive swab and mix it with 1,3,7,15 and possibly 31 and 63 negative swabs, then we do the genomic extraction and PCR, and we look at which samples come out positive that way. If all samples come out positive we have won because concentration is not an issue and the whole world will adopt this method. If, on the other hand, for example, only the samples up to 8 swabs together are positive and 16, 32 and 64 swabs together are negative, then it is proposed to try again to do the 16, 32 and 64 groups but this time concentrating the genetic material coming out of the extractor by centrifugation. To all those who might object that this lengthens the time we agree, but our method would still converge on the standard method even with centrifugations at each step.

At the point when we have determined the sensitivity threshold of our method both with and without the concentration by centrifugation, which we assume to be, for example, up to 16 swabs together with centrifugation and 4 without, we would propose to perform a prototype of the clinical analysis in the two cases, i.e., taking 3 negatives and one positive in one case and maybe 2 positives and 14 negatives in the other, and perform every step that would allow the diagnosis of the patients, so collective extraction, pcr, then again extraction of the genomic from the swabs into positive tubes, etcetra, until you get to determine who has the disease in each of the two cases.

If any steps in the process are unclear or you see critical issues you are strongly encouraged to call and we will discuss them, Marco Eterno, marco.eterno@sns.it , 340 98 05 787.