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Molecular and diagnostic clinical virology in real time

Bert Niesters' enthusiastic review of molecular methods in diagnostic virology [1] makes many valid and useful points, but omits some important viruses and is over-dismissive of the value of cell culture. His Table 2 lists viruses for which in-house molecular tests should be developed, but omits rotaviruses, parainfluenza viruses and flaviviruses. Vaccines against rotaviruses are under trial and tests for them will be essential in any evaluation of their efficacy. Parainfluenza viruses are important respiratory viruses, types 1 and 2 frequently active in the winter, and type 3 active in the spring and summer. If global warming progresses, flaviviruses, such as West Nile virus, which are prevalent in the tropics and have already caused epidemics in the USA, will become commonplace in temperate climes. Since the review was written, the SARS coronavirus has also emerged and its epidemic potential would also now rate a special mention.

Moreover, the unqualified entry of 'adenoviruses' gave me concern, and highlights the point Dr Niesters himself makes about the extra sensitivity of molecular methods and the need to evaluate the significance of finding amplified DNA or RNA. Adenoviruses were discovered originally in explant organ cultures of apparently normal tonsils in which they had persisted [2], especially in children, and may not therefore be causing disease. Both typing and quantification is necessary for such positive results [3]. In addition, adenoviruses of types 40 and 41 have been implicated in diarrhoeal disease; their presence in the nasopharynx might be revealed by amplification, but the significance of such a finding is unknown. Hence, some sero/genotyping of adenoviruses is essential.

The availability of high-quality cell culture as part of diagnostic virology will remain essential as long as new viruses or variants of old viruses (such as novel influenza A viruses) continue to appear. We must have a catch-all system which does not depend on primers prepared previously, or other reagents. Although random primers may pick up new viruses, this is not guaranteed, and a negative result does not mean that no virus is present. Personally, I would wish to add electron microscopy (EM) as another catch-all technique—morphology tells us what sort of virus is present, and other properties can be predicted with considerable confidence from knowing what it looks like. Nonetheless, both cell culture and EM depend on practised personal skills, and both techniques must be used regularly and consistently if they are to work reliably. It seemed to me that Dr Niesters felt that cell culture could be set up 'as-and-when' it was needed, and might, or even should, be phased out in the near future. This view is mistaken, and it should also be emphasised that cell culture must be in continuous and routine use to demonstrate that it can, and is, isolating viruses. Running cell culture is a skill analogous to top-class sport or music—practice makes (more) perfect, while absence of practice leads to disaster.

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RESPONSE FROM DR NIESTERS

Professor Madeley's comments address some points raised during recent discussions on how molecular testing and virus culture should be dealt with in the routine clinical virological laboratory. In principle, we do not disagree about this. The discussion should actually be directed towards the following points:

- Should we use molecular diagnostics in clinical virology by selecting primers and probes to detect all possible viruses? Definitely not, and Table 2 in my review was intended more to indicate the fact that, unfortunately, only a few commercially validated tests are available, and that this number has not increased significantly in the last few years. However, in the laboratory, tests are needed for a growing number of viral targets (whether such tests are based on molecular testing, serology or virus culture is not relevant). The implementation of tests for Epstein–Barr virus has been successful in our hospital for allogeneic bone marrow transplant patients, and the value of adenovirus detection in the same group of young patients has also been demonstrated [4].
- It is important that molecular testing should be quantitative, although one can argue that this

is not always of clinical value. However, the example of detecting adenovirus in the nasopharynx, as mentioned by Professor Madeley, demonstrates the importance of quantitative results. The technology is available, and the information is needed to understand the importance of viral load in relation to clinical outcome. The recent detection of almost 9% of double infections in respiratory samples, as reported by several groups at the recent meeting of the European Society for Clinical Virology in Copenhagen, strengthens the need for and value of this quantitative information.

• The list in Table 2 is far from complete, but it is not realistic to extend it to include all known viruses. I agree that parainfluenza viruses should be on the shortlist, but other important viruses are also missing. Technology is developing fast, and it is becoming possible to combine a positive and quantitative signal with typing for a growing number of viruses, including enteroviruses (and parechovirus) and adenoviruses.

• The discussion on the position of virus culture is still ongoing. We both agree that high-quality cell culture-based facilities are needed, not only to obtain epidemiologically relevant strains, but also to detect 'new' and emerging viruses. However, this is a problem for those laboratories that do not have any culture facilities, or for which transportation of clinical specimens to the laboratory takes a relatively long period. Is the molecular diagnostics approach the answer to better clinical virology? The answer 'yes' is too easy, simply because the technology itself can be rather difficult to implement, and information on the primers and probes needed is often limited with respect to their clinical performance. Unfortunately, studies that analyse only a limited number of clinical reference strains and samples are still in the majority. So, although it is necessary to remain critical, it cannot be denied that molecular testing is a significant aid to diagnosis.

• The question of whether virus culture is obsolete or should be phased out in the future is also influenced by the fact that there are now limited numbers of laboratories capable of performing virus culture. The number of laboratories with EM facilities is even smaller, with a consequent loss of knowledge. I agree with Professor Madeley in this respect, but while practice makes (more) perfect, it is important to realise that in a large number of laboratories, there is already no practice at all.

In my view, molecular diagnostics has had, and will continue to have, a great impact in clinical virology. However, there is still a lot to learn and, in a routine clinical virology laboratory, molecular testing, serology and virus culture are the major cornerstones. The continuation of EM is most in doubt, and in my own country is limited to those laboratories that are enthusiastic enough to send people for training. But without support from the government, it will be difficult to maintain.

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