



The end of cell culture in diagnostics: Is molecular diagnosis the Harry Potter or the Lord Voldemort of clinical virology as a specialty?

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The culture of virology

Diagnostic virology is classically defined by the use of cell culture to grow viruses. This technique involves the use of cells, either primary (derived from fresh tissue and propagated only a few times) or continuous, which are generally cancer cells. An example of a cancerous cell line is HeLa, which has been in use for decades and propagated in laboratories around the world. The major advantage of primary cell culture is that it closely resembles the cells that viruses infect naturally; consequently, many viruses grow best in these cells. The foremost examples are respiratory viruses such as influenza and parainfluenza. Some continuous cell lines are suitable for other viruses, which is advantageous for the laboratory because of their ease of use, their uninterrupted availability and their reliability.

Cell culture has its problems (Table I). Primary cells have a major disadvantage in that they need to be sourced from tissue which may be available only unreliably, such as fetal lung or foreskin. One of the stalwarts of an old-fashioned routine diagnostic virology laboratory is primary rhesus monkey kidney, which is subject to strict animal laws (dialysis services for monkeys are in short supply), is less easy to source, and is often infected with monkey viruses, resulting in a loss of efficiency for growing other viruses due to competition.

Cell culture has other general problems. Firstly, many viruses do not grow *in vitro*, examples being all the hepatitis viruses, coronavirus (one of the commonest causes of colds) and parvovirus B19. Other viruses, such as cytomegalovirus (CMV) and rhinoviruses, can take many days to grow (if at all). This is a particular disadvantage when a rapid negative result is as important as a positive result. Secondly, cell culture can be contaminated by fungi and bacteria. The third and often underestimated problem is the cost in both staff and consumables to generate what can be a relatively small number of positive results (see below).

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Table I. Is cell culture 'catch-all'?

Only if ...

1. the cells are known to be sensitive
2. there is continuous QA
3. freezers do not break down
- 4a. monkeys still get killed

Or ...

- 4b. we use many different cell lines
5. they don't have mycoplasma
6. we can keep trained staff
7. cells do not get infected with fungus
8. as long as you do not want to 'catch' more than one virus
Even then, they are globally insensitive

Of course, there have been alternative, rapid methods for diagnosing viruses for many years. First is antigen detection, which can be applied to a number of specimens, such as blood (for example, to detect HBsAg), stool (for example, to detect rotaviruses or noroviruses) and respiratory specimens (for example, direct fluorescence to detect influenza, adenovirus, parainfluenza viruses, CMV and respiratory syncytial virus (RSV)). The antigen detection method has the advantage of speed but can be insensitive and, in the case of respiratory viruses, does not detect all potential pathogens and requires specimens with intact cells.

Another method is electron microscopy, which is quick if only a few specimens are to be investigated, and allows visualisation of all viruses, although large numbers are required and identification is at the family level only (e.g. herpes simplex virus (HSV) or varicella zoster virus (VZV) or Epstein-Barr virus (EBV) or CMV). Lastly, antibodies can be detected for many viral infections but these are subject to some fundamental drawbacks. Not everybody makes antibodies during virus infections and not all people make the same antibody to the same antigens. Antibodies are unreliable in immunocompromised patients. Antibodies often do not rise until the illness is well under way or improved, by which time the diagnosis may be of historical interest only. Furthermore, re-infection cannot be reliably diagnosed by antibodies (the negative predictive value is particularly poor), and validated serological tests are not available for every virus. For all these reasons, it has been accepted for many years that if virology is to be a clinically useful specialty, diagnosis needs to be quicker and all-encompassing, and the *virus itself* needs to be identified,



not some surrogate marker of infection. When hybridisation techniques became available to detect the nucleic acid and thus the virus itself, these were seen as a potential answer to this problem, but have never lived up to their initial promise for diagnostic purposes. Sensitivity is poor, cross reactions are many and, until recently, some form of radioactively labelled probe was required. By the time hybridisation had 'come of age', various nucleic acid target amplification techniques had become available and substantial experience gained.

In our opinion, nucleic acid testing (NAT) (examples being the polymerase chain reaction (PCR), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA) and strand displacement amplification (SDA)) is the current saviour – the Harry Potter – of the speciality of clinical virology; paradoxically, it may also be the harbinger of its doom – its Lord Voldemort. Below, we focus on PCR as this is the technique which is most in use and in which we have the most experience. We focus particularly on in-house methodologies.

Precepts of PCR

Essentially, PCR is the process by which a known sequence of nucleic acid is enzymatically amplified to generate millions of copies of DNA of the same length and sequence. Details of the process can be found in many standard textbooks. The process can be applied to DNA viruses (such as HBV, adenoviruses, HSV) and RNA viruses (such as hepatitis C virus (HCV), HIV, influenza and rotaviruses) as long as the RNA is first converted into DNA using reverse transcriptase. Any sort of clinical sample can be used as source material for the nucleic acid, including blood, tissue, respiratory secretions, stool and CSF. The major advantage of the technique is that it is generic; fundamentally the same technology is applied to any sort of clinical sample to detect any sort of virus. However, there are a number of potential disadvantages, which are discussed below. Cost is often also cited as a major impediment to its widescale introduction; we also briefly discuss this.

In the last few years, major progress has been made in three areas. Automation is the first. There are now machines for nucleic acid extraction and others which can load up and amplify the nucleic acid and analyse the result. Nucleic acid extraction used to be a cumbersome process involving multiple operator processes to digest and remove all proteinaceous material, leaving only the nucleic acid in a reasonably pure state. Not only are the results more reliable, but in laboratories where large numbers of samples are tested manual extraction becomes impossible. The time has almost come when the entire process, from sample to result, will be automated. The second area is real-time PCR. By using fluorescence, either incorporated in a primer, into the growing DNA chain, or in the form of an oligonucleotide probe, the accumulation of amplified product can be monitored on a computer screen.

This means that products do not have to be detected in an additional step called gel electrophoresis, and no manual manipulations are necessary, such as opening of reagent tubes that may be a potential source of contamination. In the recent past, a technique called nested PCR was commonly performed, whereby two rounds of PCR were done sequentially on each sample – this had the potential for contamination of the second round with products from the first round from a different patient, giving false-positive results. It was also more difficult to optimise and to run reliably. Real-time PCR also means that, by including viruses of known amount as controls, one can now quantitate the viral load of the input sample. With certain forms of real-time PCR, the process can take as little as 20 minutes instead of 2 hours once the nucleic acid has been isolated. In our laboratories, the process takes just over an hour. The third is multiplexing. Multiple sets of primers and probes can be mixed together in one tube, allowing detection of one pathogen without the need for selecting which pathogens might be of interest. Technology now exists to allow detection of up to 20 or more potential pathogens¹ without the need for one tube per target. This brings the cost per sample down considerably and, we would argue, actually makes NAT an economic prospect for any clinical sample. This approach also allows detection of more than one pathogen per sample, which occurs in up to 20% of respiratory samples.²

Real-time PCR has made the wholesale introduction of NAT an inevitability – and even obligatory – routine in laboratories. In the next few paragraphs, we present some experiences from a comprehensive molecularisation programme at our virus laboratories. Virus culture has consequently been relegated to specialist activities. Although the process began haltingly, the majority of this change was completed 2 - 3 years ago.

Cerebrospinal fluid

It has been established for many years that HSV encephalitis is essentially a clinical diagnosis, because the virus (HSV1 in most cases) will rarely be found by culture in CSF, and antibody titres take a week at least to appear, which is not very useful, and require a second CSF sample to be taken. Detection of HSV in CSF was one of the first clinical applications of PCR.³ Protocols for enteroviruses rapidly followed.⁴ In our laboratories an overall doubling at least in overall detection rates followed. With the decline in mumps incidence, this was not perceived to be an issue in Western Europe and the USA, but a surprising number of HSV2 and VZV infections were detected in patients with meningitis. Now that mumps is enjoying a resurgence, mumps PCR of CSF has become useful. Overall, it is rare for a culture-positive sample to be missed by PCR, but some two to three times more diagnoses can be made using PCR. Although the expected pick-up rate will be lower, some laboratories have broadened the range of viruses targeted, such as CMV, EBV, JCV and human herpesvirus



(HHV) 6/7. HHV6 is particularly important in young children with febrile convulsions, and CMV and EBV can occasionally be found in the immunosuppressed patient. They either grow slowly or not at all in tissue culture, so CSF is a good example of PCR widening the range of detectable pathogens.

Genital samples

Chlamydia trachomatis (CT), human papillomavirus (HPV) and HSV are common and perhaps increasing in incidence. Culture for CT is slow and insensitive; enzyme-linked immunosorbent assay (ELISA) and immunofluorescence on swab material have low sensitivity, though acceptable specificity. Introduction of amplification procedures has dramatically increased the detection rate of these pathogens: by 30 - 40% for CT and 20% for HSV.^{5,6} HPV is a pathogen whose time is coming because of the ability to detect the virus using NAT. There are even debates as to whether cervical cytology should be abandoned in favour of NAT for HPV because of the sensitivity of NAT, its high negative predictive value and the finding that essentially all cervical cancers and the pre-cancerous lesions contain the virus.⁷ HPV cannot be cultured without heroic efforts. However, the ability to multiplex has allowed expansion of the targets that one can detect in a genital specimen. Why stop at CT when one can detect *Neisseria gonorrhoeae* and *Mycoplasma genitalium* in the same tube? Why bother with wet mounts for *Trichomonas vaginalis* when PCR is as sensitive⁸ and can be done in the same tube as other STDs on a vaginal swab for marginal additional cost? Syphilis, as with mumps, is making a comeback, and detection in vaginal swab increases the diagnostic rate significantly when added to antibody testing in blood.⁹

Respiratory secretions

Cell culture established the culturable causes of upper and lower respiratory tract infection. These include influenza, para-influenza, RSV and adenoviruses. However, not only has PCR increased the sensitivity of detection of all these pathogens, but it has also enabled us to detect viruses which we knew about but which were very seldom detected. With the advent of this technology, it has become apparent that these are significant causes of morbidity and, in some age groups,

mortality. For example, rhinoviruses and coronaviruses 229E and OC27 were known to be the cause of the common cold in immunocompetent adults (even if they were only detected in less than 20% of cases) but they are now also known to be the major precipitating factor of asthma in children (found in 70% of samples) and, to a lesser degree, adults (perhaps 50%).¹⁰ They are responsible for apnoeic episodes in infants, pneumonia in the immunocompromised and in the elderly, and for exacerbations of COPD. They also cause severe (sometimes fatal) disease in the immunocompromised. But PCR has brought an additional dimension to the diagnosis of these common conditions: it has enabled us to widen the range of viruses detected to include human metapneumovirus,¹¹ influenza C,¹² a novel parvovirus (Bocavirus),¹³ the relatively new coronaviruses (SARS, NL63 and HKU)¹⁴⁻¹⁶ and two new polyomaviruses – WU and KI.^{17,18} Whether they are worth detecting is discussed below, but why culture and immunofluorescence are inadequate for this sample type in modern practice is absolutely clear.

Stool/faeces

Gastroenteritis, whether in outbreaks or sporadic, is most commonly caused by viruses. Epidemiological surveillance of these illnesses is highly insensitive, not only because a small proportion of cases are reported, but also because laboratory diagnosis is usually inadequate. The major cause is noroviruses (previously termed small, round structured viruses and then Norwalk-like viruses), but other significant causes are rotavirus, astroviruses, adenoviruses and sapoviruses (part of the Calicivirus family). None of these grow well in cell culture, but all can be detected by electron microscopy. Electron microscopy has the advantage of being quick on individual samples, but has the dual disadvantages of lack of sensitivity and the inordinate amount of technical time required to look at a large series of samples. Our, and others', experience shows clearly that all of these viruses can be detected with greater sensitivity using PCR (Table II).^{19,20} However, it cannot be denied that electron microscopy is a very useful asset for the detection of new viruses or the exploration of unknown changes in cell cultures.

Table II. Increased sensitivity of PCR for viral gastroenteritis

Virus	EM	%	PCR/RT-PCR	% change (PCR-EM/EM)x100
Rotavirus	70	25.8	86	+22.9
Norovirus	6	2.2	46	+666.7
Adenovirus	12	4.4	40	+233.4
Sapovirus	1	0.4	8	+700
Astrovirus	3	1.1	7	+133.3
Virus detected	92	33.9	187	+103.3
No virus detected	179	66.1	111	-38.0
Total	271	100.0	298	

EM = electron microscopy; PCR = polymerase chain reaction; RT = reverse transcriptase.



Blood

This has not been considered a useful sample for detecting viruses, except hepatitis B (an antigen test), HIV (an antigen test and growth in lymphocyte culture) and CMV in transplant patients (detection of antigen in lymphocytes and the occasional culture). The hepatitis viruses have never been detected in cell culture. PCR has changed all this. Now, we know that: enteroviruses can be detected in blood as a surrogate for CSF; HSV, EBV and adenovirus can be detected in plasma in immunosuppressed persons with serious disease; the amount of CMV, EBV, HIV, HCV and HIV in blood correlates with treatment outcome; and the best way of diagnosing HHV6 is by PCR of a plasma sample.

Pros and cons

So: PCR is comprehensive. However, many reasons are cited for not introducing PCR (Table III). We discuss these issues in turn.

It is too complicated

This used to be true. Tests used to involve multiple steps with transfer of small volumes of reagents between vials, gel running and (often poor) interpretation of multiple bands. However, reagents are now available which allow a test to be set up – even for detection of RNA – by adding aliquots from only two pre-supplied vials. Also, with the advent of real-time technology, a generic method has become increasingly suitable for a wide range of pathogens. Automated extraction has made it even easier. Results are now read by examining a curve on a screen. However, although it sounds ridiculously easy, there is a relatively slow learning curve that is not apparent for the introduction of other assays such as direct immunofluorescence (DIF) and ELISA tests. This is partly due to the need for small-volume pipetting, the need to interpret results at the boundary of sensitivity, and the manipulation of electronic datasets on screen. On the other hand, culture was never easy!

PCR just stops working for no good reason

This, unfortunately, remains true though is less common as reagents improve. There are a number of reagents which, if

missing from the 'molecular soup', or if inactive, will lead to failure of that assay. In our experience, primers, nucleotides and Taq polymerase can all 'go off' or, surprisingly commonly, manufacturers send a batch of reagents which do not work as well as the previous batch. The only way to circumvent this problem is, firstly, to have skilled staff with substantial experience in PCR who can sort out the problem and, secondly, be well prepared for such eventualities by having stocks of quality-controlled reagents in small volumes for at least 2 months' worth of assays in the freezers. In this way, the service is not interrupted while the problem is sorted out.

Contamination is a real problem

This is the single most important problem with large-scale introduction of PCR. Because of the sensitivity of the assay, either very small amounts of virus that may not have been in a particular patient's specimen, plasmids containing the amplified sequence or the PCR product itself, can, if they find their way into the wrong tube, lead to a positive signal in a patient who does not have that virus. All laboratories and all personnel (as a generalisation) will have contamination problems during the learning phase. It can be very frustrating and, often, the offending contaminant is never identified. However, if a strict clean-up procedure and adherence to rules regarding gloves, gowns and separate pre-PCR and post-PCR working areas are adhered to, contamination is rarely an issue in an experienced laboratory. It has also become less of a problem with real-time PCR as tests are performed in vessels that are sealed and do not need to be opened after amplification.

It takes too long compared with antigen detection

Table IV and Figs 1 and 2 compare the time taken to achieve a positive result by PCR compared with culture and antigen detection and how a real-time assay can take a similar amount of time to an antigen test, but can detect many more pathogens in that interval. On the other hand, a common time to achieve a true negative result by culture is 15 days. A typical run of 50 CMV real-time PCR tests would now take 5 hours from the beginning of nucleic acid extraction to result generation. This is irrespective of whether the result is negative or positive.

Table III. Potential problems with PCR

It is too complicated
It just stops working for no good reason
Contamination is a real problem
It takes too long compared with antigen detection
How to process many samples in a reasonable time – multitip 1
Do all positives mean something? – transient infections
Cost
Quality assurance is insufficient
What's the point if you can't do anything about it?
Whole viruses are needed for epidemiology and new virus discovery
Coping with genetic variability



Table IV. Time comparison between a positive culture and NAT

Culture		NAT
Hours - 1 - 2 days	Influenza A, B	1 - 2 days
Hours - 3 days	RSV	1 - 2 days
Hours - 3 days	Paraflu 1, 2, 3	1 - 2 days
?	Paraflu 4	1 - 2 days
?	Influenza C	1 - 2 days
?	Metapneumovirus	1 - 2 days
2 - 3 days	SARS CoV	1 - 2 days
5 days	Rhinovirus	1 - 2 days
1 - 2 days	CMV	1 - 2 days
1 - 2 days	HSV	1 - 2 days
Days	Measles	1 - 2 days
Days	Adenoviruses	1 - 2 days
?	Coronaviruses	1 - 2 days

NAT = nucleic acid testing.

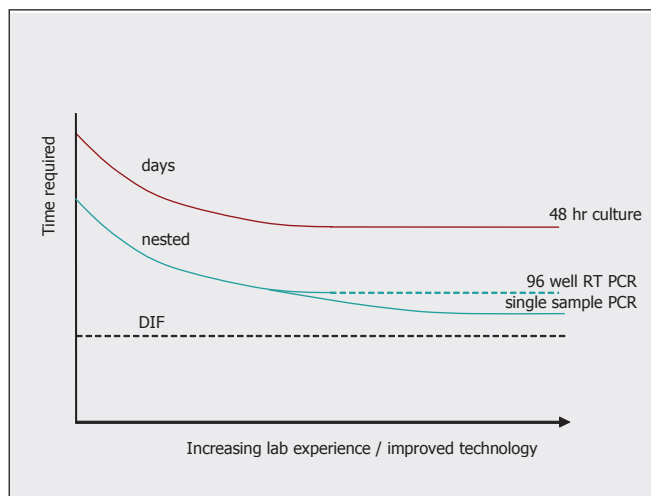


Fig. 1. Improved timings with technological development.

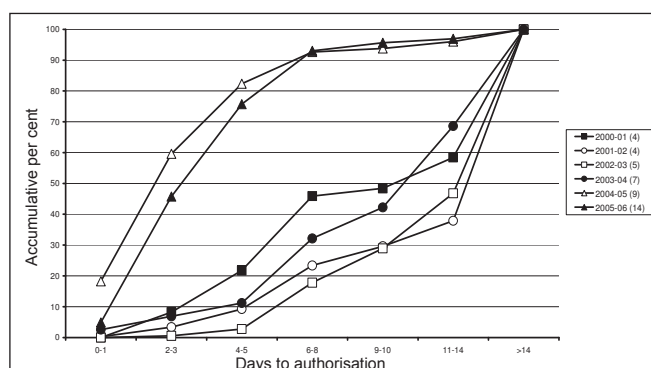


Fig. 2. Further improvement with real-time PCR. This details the full turnaround time from receipt until final printing of the report. All the results were available at least 24 hours before this; intervening weekends, when no work was done, are included. Only respiratory PCR results are detailed. The introduction of real-time PCR (on left of figure, from 2004) led to a significant reduction of processing time.

How to process many samples in a reasonable time

There are a number of approaches to this potential quandary. Firstly, one can multiplex, which means that reagents for multiple targets are placed within a single tube. Up to 4 targets can be simultaneously reliably assayed, limited by the number of dyes attached to probes that can be clearly distinguished (without cross-talk) by the electronics of the real-time instrument. It is unusual for more than two pathogens to be present at any one time, even in a stool or respiratory sample. Fig. 3 shows how two respiratory viruses can be tested for in one tube: a different fluorescent dye is used for each, allowing separate interpretation of the curves. An alternative is to test for the most likely pathogen first, followed by less likely causes if negative; this works particularly well during the influenza season, when 50% or so of all samples can be positive. Or one only tests for the 3 or 4 most common pathogens linked to a clinical scenario in a multiplex format. The disadvantage of the latter is that many viruses cause similar disease, so one will miss diagnoses. A newer approach is Luminex technology that can detect 20 or more targets using specific probes in a single tube.^{1,21} The approach that one of us has taken is to have a fixed menu of targets in a number of multiplexes for each specimen type, almost without taking the clinical picture into account. This minimises mistakes and improves workflow as the staff have a relatively fixed work pattern every day.

Do all positives mean something?

Table V gives an indication of the relative value of PCR compared with culture. However, as PCR is so sensitive, a positive result could theoretically mean that the virus is just 'passing through', or that non-viable nucleic acid is lingering on long after the active infection. As with any relatively new assay, the clinical utility has to be established by prospective testing linked to patient details. This is more difficult than it sounds because it is often not easy to get all relevant clinical information. Further, some virus infections are obviously

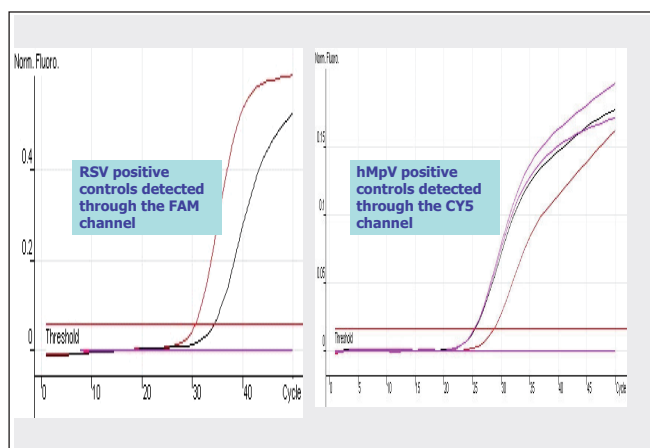


Fig. 3. Real-time multiplex PCR. Two dyes with different wavelengths are added to the probes. The reaction takes place in one tube. By analysing results using one wavelength only, each result can be noted individually.

Table V. How useful are culture and real-time PCR for disease diagnosis?

Positive predictive value?	
Culture	100%
NAT	We do not know – ?90%
Negative predictive value?	
Culture	30%
NAT	80%

asymptomatic and so it is only from experience that we will fully understand whether the finding of a viral sequence is always, or usually, clinically relevant. Rhinoviruses are a good example of this. Since introducing this assay, the number of rhinovirus detections has increased 20-fold, and clinicians often ask what it means when they receive a positive result. Clearly, the samples were sent to us in most cases because the patient was ill and we have to assume that it is causing the illness unless an alternative pathogen is identified (which is unusual).

What is required is exhaustive testing of asymptomatic persons to get some idea of the relative specificity of virus detection for clinical illness (the positive predictive value). The increased sensitivity of PCR means that viral nucleic acid will be present for a number of days longer than the viable virus (as detected in cell culture). In our experience, influenza RNA can be detected up to 14 days after the disease began, but after 7 or 8 days, the detection rate drops. Clinicians are keen to send follow-up samples to monitor disappearance of virus in the mistaken belief that this correlates well with clinical recovery. One approach to this is to perform NAT as the initial diagnostic assay but then do an alternative assay such as DIF to monitor response to therapy (as one would expect this less sensitive test to become negative quickly with recovery). Parainfluenza virus can be positive intermittently for years without illness. However, when influenza is not around, we do not pick up any influenza virus positives in throat swabs, indicating that,

at least for influenza, this is not an issue. For CMV in blood of bone marrow transplant recipients, the PCR usually becomes negative a few weeks after the institution of ganciclovir. A further, minor problem is that the primers used for a pathogen may detect related viruses which are not pathogenic. Some methods used to detect rhinoviruses also amplify vaccine strains of poliovirus. It is however clear that not only the qualitative result of the technique is important, but also that the quantification of viral samples will contribute significantly in solving these key questions regarding the presence of the virus and risk for disease. It is shown that with increasing viral levels, or changes of viral levels in time, a substantial increased risk for developing disease has been shown for viruses like CMV and EBV.

Cost

As with most things in the laboratory, the more you do, the cheaper it becomes. This is particularly applicable to equipment. Table VI provides a rough estimate of the relative cost per result in Europe of PCR compared with culture and DIF. The cost per positive result is particularly illuminating: this effect is because the number of positives is greatly enhanced by PCR. One also needs to include in any cost-effectiveness analysis an estimate of the clinical and laboratory value of a negative result that is reliable and quick (and so does not require further costly investigations). An issue that currently has a negative effect on overall costs is the licensing fees that hospitals and companies have to pay to patent holders of new techniques such as real-time technology.

Quality assurance is insufficient

The recent European directive makes it clear that any laboratory which uses an in-house assay for *in vitro* diagnostics has to set up an internal quality assurance system, to be certain that the technique is performing at an acceptable level. This is one of the main reasons why large-volume in-house PCR assays need to be performed at large centres to begin with before they become routine and properly controlled and can then be done at smaller laboratories. There are good quality assessment systems, such as Quality Control for Molecular Diagnostics (qcmd.org), which enable individual laboratories to compare their performance in an anonymous manner against others. Unfortunately, the QA systems do not cover all the

Table VI. Back-of-an-envelope costs of diagnostic virology techniques for a respiratory sample

Consumables	DIF	54 euros
	Culture	61 euros
	PCR	16 euros
+ staff costs		
Costs per positive result	DIF	54 euros
	Culture	388 euros
	PCR	50 euros
What is the clinical and monetary value of a 'true negative'??		



viruses tested for in many laboratories. However, one should also realise that quality control issues are relevant for all kinds of viral diagnostics, including serology and virus culture.

What's the point if you can't do anything about it?

Information in itself is valuable. Knowing that a sample is positive for a virus should change the management of that patient. An implication of the increased sensitivity is that a negative result becomes more believable to the clinician, i.e. it has a high negative predictive value. For example, a negative HSV result in a genital swab indicates to the clinician that further investigation is required and that the illness may be due to some previously unrecognised illness. In addition, although HSV grows well in cell culture (it is the virological weed), it requires some 7 - 10 days of continuous culture before an experienced operator would be willing to declare the sample negative. With blind immunofluorescence techniques, this can be reduced to 2 - 3 days. PCR brings this date forward by 5 - 7 days in large laboratories, giving definite results in 1 - 2 days. We live in the information age, and both doctors and patients should know what the diagnosis is if the illness is severe enough to warrant the time and cost involved in an office visit. In these days of requiring a financial justification for every technological advance, we propose that the cost of the test will be offset by savings on X-rays, return visits and, most importantly, misguided use of antibiotics. The introduction of an increasing number of antivirals has meant that one can now actively intervene if one has a positive result within a short time after the illness begins. Finally, the Public Health depends critically upon reliable information on infections provided in a useful timeframe. PCR delivers this.

Whole viruses are needed for epidemiology and new virus discovery

This is a more difficult criticism to rebuff, but it is only true for a few viruses. PCR protocols can be chosen so that the type of virus can be analysed for genotype or serotype by restriction enzyme digest of the PCR product. Similarly, tracing an outbreak is easily achievable by sequencing of PCR products. Characterisation of antigenicity cannot be done easily; however, for influenza, the most cited example of the value of cell culture, reverse genetic approaches are now available to clone the PCR product into a vector and subsequently produce RNA, and then viruses, in tissue culture. New viruses have been discovered by both culture and molecular approaches.

We suggest that such activities are highly specialist and should only be offered by large central reference facilities where suitably trained staff, the requisite skills and significant numbers of samples are available.

Coping with genetic variability

Virus culture should not be abolished completely – only for routine clinical diagnosis. It will be particularly important

to monitor the sequence of circulating viruses, after culture, to ensure that point mutations do not arise that decrease the sensitivity of NAT assays (Fig. 4). An alternative approach is to use PCR protocols that amplify a long stretch of nucleic acid to include the primer and probe binding regions and then sequence those critical sites. Any variability that has arisen can be detected and the reagents suitably altered.

The future

Viral diagnostics are now reliable in that they are sensitive and specific and more broad-ranging than before. Results should now be available while the patient is still in hospital or within 36 hours of a GP visit. Critically, a negative result is now useful because it is believable, and can radically change the clinical perception. The case is surely made for its wholesale introduction into laboratories dealing with tertiary care facilities. The article by Gunson *et al.*²² is recommended for further reading. However, we believe that our goal should be 'virology for the masses'. Currently, family practices do not submit many samples for virology; this is completely understandable, considering the long time to any result (positive or negative), the low detection rate and the

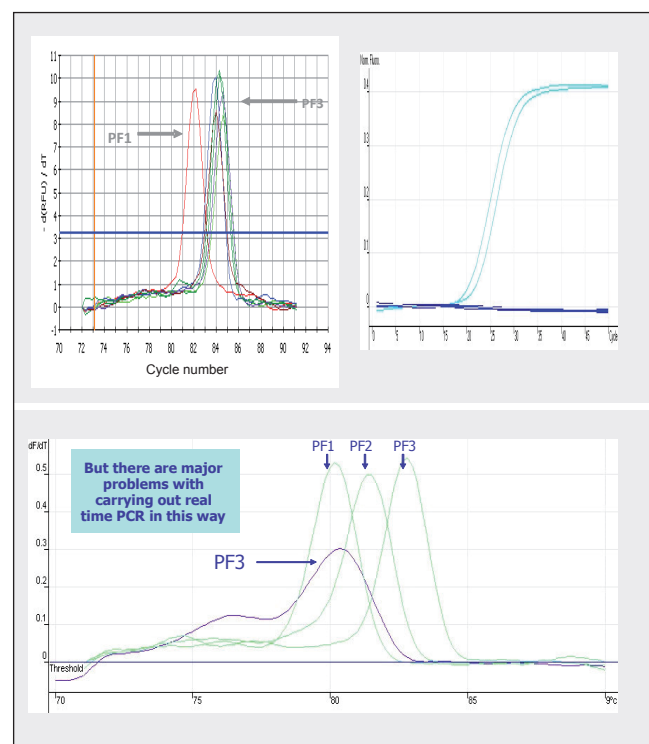


Fig. 4. The effect of genetic variability on real-time PCR. Top box: A probe based on a parainfluenza strain in The Netherlands was used on British strains. Although a peak was apparent (so the primers bound) (top left), the probe did not bind (top right). One base mismatch was found on sequencing. Bottom box: Sybr green was incorporated into the growing nucleic acid chain and the melt temperature was measured. Initially, laboratory examples of parainfluenza viruses (1, 2 and 3) were clearly distinguished. However, a clinical sample (arrowed) showed that genetic variability in the wild-type led to failure to correctly type this strain.

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regular irritating request to 'submit a second sample'. It is our contention that virological examination should become a regular part of patient diagnosis and management in the community. Samples can be submitted in viral lysis buffer (which stabilises nucleic acid); this can be stored for a few months and makes the sample non-infectious and thus suitable for posting.

There are other advantages to having large numbers of samples sourced from primary care. First, Public Health would be able to base policy on fact rather than presumption. Second, epidemiology of infectious diseases can be studied more reliably. Third, with increased understanding of how common specific viruses are and what diseases they cause, emphasis may shift in research into the most useful vaccines and antiviral therapies. Fourth, as antiviral therapies become broader in scope and more widely available, it is going to be very important to know which viruses are circulating in the community and whether or not your patient has that virus.

In conclusion: many disadvantages have been cited about this molecular revolution, but, in our opinion, it is almost unethical not to introduce the most sensitive and specific assays and to be able to make the most reliable diagnosis. Harry Potter came as a saviour of the magical world as PCR has waved its wand over clinical virology (although he can be as irritating as PCR!). However, the technique does require skill and experience, and it is not our contention that it should be introduced into every district general hospital, but there should be a nationally co-ordinated effort in virology to set up specialist centres linking closely with microbiologists in district general hospitals. Some molecular assays could be done in district general hospitals whereas others would be done at the regional centres.

This arrangement, however, leads to the paradox that this technology could also be virology's Lord Voldemort. Molecular diagnostics has been driven largely by the world of viruses, but it may become so streamlined, efficient and foolproof that pressure will mount on all laboratories, however small and inexperienced, to introduce such assays. There will be substantial commercial pressure to do so. We must be wary and ensure that training on how to deal with the

inevitable failed runs and difficulties in interpretation and quality assurance is in place. It will also be important to avoid reducing sample numbers submitted to virologists to such a degree that it becomes impossible to sustain the momentum in the PCR-driven blossoming of virology as a clinically relevant speciality.

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