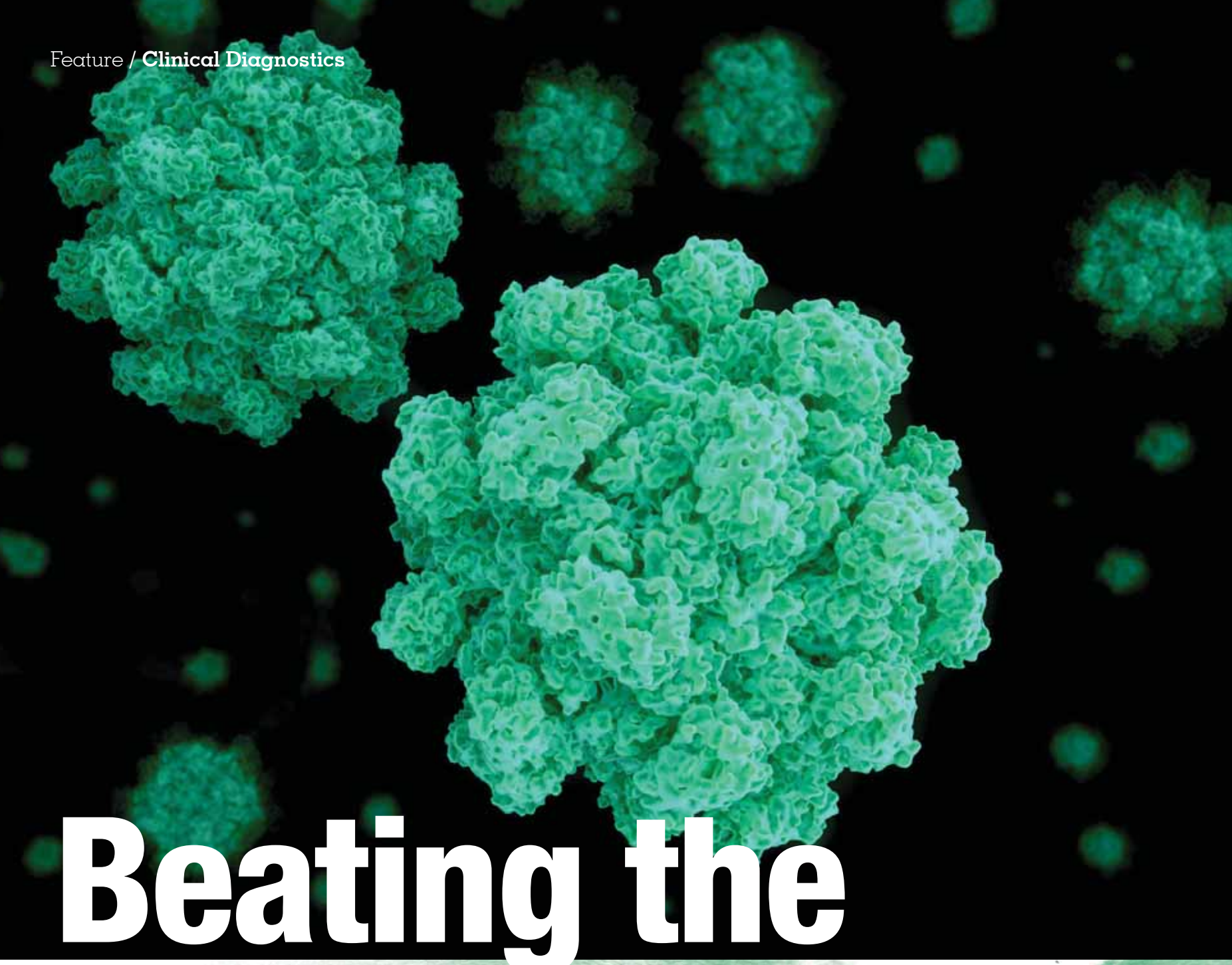

Beating the swarm

Tony Sackville discusses how innovative diagnostic approaches to HIV and Norovirus characterisation can defeat the drug resistance which occurs as a result of "quasispecies swarm"

The RNA viruses such as HIV-1 lack a "proof-reader" in their replication systems, leading to the accumulation of mutations and the persistence of a "quasispecies swarm", that is the virus population exists as a mixture of genetically distinct variants within the host. This enables the virus to constantly probe its environment for a replicative advantage, although most mutations lead simply to the production of non-functioning viral proteins that have little clinical significance. However, some mutations do result in changes to the proteins that are targeted by antiviral drugs. The result is drug resistance, a major global health issue with important clinical consequences.

In the thirty years since HIV was identified, a significant amount of knowledge has been acquired about the virology and pathogenesis of infection. The development of highly active antiretroviral therapy (HAART) in the mid-1990s transformed HIV from a fatal infection to what is now a chronic manageable condition. Despite this success, however, drug resistance remains a major cause of treatment failure. The high mutation rate of HIV-1 can lead to continued evolution of drug resistant variants. With one infected individual potentially playing host to a range of quasispecies, strains exhibiting drug resistance invariably flourish if virus replication is not fully suppressed by anti-retroviral treatment. HIV genotyping is now standard of care to identify drug resistant variants but current assays do have limitations as they cannot detect low levels of drug resistant variants, which may contribute to treatment failure. A key area of HIV research is therefore focusing on development of highly sensitive diagnostic techniques capable of detecting mutations in even the minor sequence variants that exist within the quasispecies population

and determining their clinical significance. Such innovative technologies could potentially provide additional molecular assays to support and optimise patient management.

Development of HIV diagnostics has established HIV genotyping as part of the molecular repertoire supporting both optimum patient management and cost effective use of antiretroviral drugs. HIV genotyping is recommended for all new diagnoses; in cases of treatment failure as well as being standard of care for HIV infected pregnant women. GSTS currently carries out approximately 1,000 genotyping assays annually in order to provide clinicians with crucial information on the presence of drug resistant strains. To do this, traditional genotyping techniques focus on sequencing the HIV-1 reverse transcriptase (RT) and protease (PR) genes comparing them to an HIV-1 reference sample. This method, widely established throughout healthcare services, combines two techniques, reverse transcriptase polymerase chain reaction (RT-PCR) and Sanger dideoxy sequencing of the HIV template produced by RT-PCR.

Current assays will not detect variants if they represent less than 20% of the viral population. This lack of sensitivity also raises more general issues in attempts to answer current questions about HIV. For example, does HIV evolve differently depending on where it is located within the body e.g. central nervous system, genital tract, blood; do drug resistant variants differ in the blood and other body compartments and, if they do, what is the clinical significance, both in terms of individual patient treatment and to a wider understanding of HIV virology? In both cases strains may differ only subtly, so subtly that traditional Sanger sequencing may not detect any differences.

To answer some of these questions GSTS scientists, together with clinical colleagues, have investigated two innovative, highly sensitive genotyping techniques – Allele Specific Polymerase Chain Reaction (ASPCR) and Next Generation Sequencing (NGS). Both techniques have potential in improving our understanding of HIV, and allowing clinicians greater insight into patient management.

The first mutation to be targeted was M184V, which can confer resistance to some antiretroviral drugs, intended to reduce the risk of mother to child transmission (MTCT) of HIV. When routine genotyping was conducted on samples from 23 HIV infected women following birth, none of the women were positive for this mutation. However, when the same samples were analysed using the more sensitive ASPCR, 30% of women were positive for low levels of M184V (0.5-14%). Fortunately, in these cases, none of the women transmitted virus to their infants and future maternal treatment options were unaffected by the presence of this minority variant. However, a further study on non-pregnant individuals focussing on detection of the K103N mutation, which confers resistance to Nevirapine (NVP) and Efavirenz (EVP), demonstrated how valuable this sensitive assay can be. Again, ASPCR detected low levels (1-14.5%) of minority variants in approximately 30% of individuals with no evidence of K103N by conventional genotyping. Importantly, for some individuals, the presence of low levels of K103N was associated with subsequent treatment failure.

GSTS scientists and their clinical colleagues have has also addressed the issue of HIV-1 drug resistance using Next Generation Sequencing (NGS), a technique that is generating significant interest in its application to infectious diseases. NGS has an incredibly high sample throughput capable of generating several billion bases of sequence from millions of amplicon reads, depending on the platform used. The sheer number of copies of the viral sequences produced makes it both powerful and highly sensitive.

Samples of paired blood and cerebrospinal fluid from HIV positive patients were evaluated by NGS. Genetic differences were demonstrated in HIV from the different sites, which was not apparent with routine genotyping. NGS therefore indicated different evolution of HIV in the CSF compared to blood from the same individual, which may reflect differences in immune or drug pressure in these compartments.

Another application of NGS was in the investigation of HIV transmission, testing maternal and infant samples from a case of mother to child transmission, and samples from partners in a case of recent heterosexual transmission. In these cases not only did NGS sequencing detect a number of major and minor variants transmitted from mother to child, additional drug resistant variants were found to be transmitted between the heterosexual couple. None of these differences were apparent with routine genotyping which showed no differences in terms of virus populations detected between the transmitting pairs.

Another disease that annually reaches significant levels within the UK is gastroenteritis, which presents a very different set of challenges to health care services as they try to control the disease. Each year up to 20% of the population suffers with gastroenteritis, an unpleasant condition characterised by vomiting and diarrhoea which persists for around 1-3 days before resolving itself, usually without treatment. Many infectious pathogens can cause gastroenteritis, one of which is the highly contagious norovirus (NoV). However, not all infections will require the same level of response. For example, an outbreak of NoV in a hospital setting requires rapid infection control and management to avoid ward closures and reduce the risk to vulnerable patients. Rapid and accurate detection of the virus is therefore crucial, but with such a wide range of bacterial, viral and parasitic enteric agents potentially responsible, traditional diagnostic tests struggle to provide results with the speed and sensitivity required for effective care.

Multi-pathogen detection approaches are becoming an increasingly important element in pathology diagnostics, offering the ability to identify a broader range of human pathogens involved in highly infectious respiratory, enteric, sexually transmitted or central nervous system syndromes. In response to this GSTS has conducted a wide ranging pilot study on a novel commercial approach to NoV diagnosis. The technique, known as multiplex RT-PCR, can simultaneously target 15 of the pathogens known to cause gastroenteritis in hospitalised patients in a single test.

When compared to the routine rapid diagnostic procedure using enzyme immunoassay for detection, the commercial multiplex approach results in a significant increase in detection of NoV. This indicates that NoV is potentially the causative pathogen in a much higher percentage of gastroenteritis patients than originally thought.

In light of this, a further research project compared the two techniques on a larger scale, running the new multi-pathogen panel against the routine screening technique on a number of hospital in-patients at several locations. The study was carried out to coincide with the NoV season from November to June. After running over 3000 tests with both techniques in parallel, more than twice as many cases of NoV were detected using the multiplex procedure compared with the enzyme immunoassay.

This research not only has immediate implications for how we respond to NoV, it also presents huge potential for further epidemiological research. Following the in-patient study, 80 of the 219 clinical samples were sequenced to characterise the species responsible for causing several hospital ward outbreaks. This identified five distinct viral genotypes responsible for outbreaks of which the GLL.4 'New Orleans' strain was the most prevalent, responsible for 77% of cases. It also allowed researchers to trace contamination back to the most likely original source. For one ward closure this led to the identification of the individual patient from whom the virus most likely originated.

Developing new, more rapid and efficient sample preparation and characterisation techniques are at the forefront of the struggle for better understanding and control of infectious diseases. While these techniques are still in development, continued research that adapting and extending advanced technologies such as ASPCR, NGS together with greater levels of multiplexing PCR and RT-PCR methods will have a significant impact on the diagnosis and optimum treatment of infectious diseases. LN

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