Chapter 6
Blood-Borne Disease

Dominic Dwyer and William Rawlinson

Abstract The use of nucleic acid tests (NAT) has dramatically enhanced the detection and management of blood-borne viruses. Some of the very first applications of NAT using polymerase chain reaction (PCR) were in the identification of HIV in blood. The recognition of other blood-borne viruses, in particular hepatitis B and hepatitis C, further prompted the application of molecular tests to clinical medicine. It was the ease of transmission of viruses through blood and blood products that was one of the main stimuli for the development of nucleic acid testing (NAT) in virology. The improvements in technology leading to automation, reduction in contamination, quantitation and increased sensitivity have enhanced this development and are likely to continue and expand, and so further improve patient management – hopefully with reductions in cost. The introduction of these more sensitive NAT assays has further reduced the likelihood of the acquisition of blood-borne viruses during transfusion or transplantation. The development of commercial viral load assays, or quantitative NAT, has revolutionised for example the clinical management of HIV infection and of other systemic diseases (e.g. cytomegalovirus, Epstein-Barr virus, HHV-6, BK polyomavirus) that are a particular clinical issue in immunocompromised individuals (Table 6.1). Also, resistance genotyping tests can now be performed by sequencing the relevant drug targets e.g. HIV – most commonly the reverse transcriptase and protease regions, but now including integrase and envelope gp 41. Infections in immunocompromised patients may occur in the absence of classical symptoms and the use of PCR to diagnose, in a targeted fashion, the most common infections has revolutionised treatment of these infections in the immunocompromised patient. Knowledge of the most likely agents – for example, CMV in the first 3 months following bone marrow transplant, is often used because such reactivation infection is extremely common (and often fatal) in this population. The availability of polyoma virus PCR means a clinical transplant service is able to monitor their patients for polyoma virus and adjust the level of immunosuppression

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appropriately. The availability of rapid diagnosis for fungi or Pneumocystis means transplant clinical services can often reduce the use of antimicrobial prophylaxis for these agents. Therefore, as a result of these rapid diagnostic nucleic acid testing services, clinical services can directly improve patient therapy, thereby reducing side effects and improving outcomes as well as saving money.

Keywords Virus · Immunocompromised · Transplant · Diagnosis · Quantitative PCR

6.1 PCR Use in the Diagnosis of Blood-Borne Viral Diseases

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6.1.1 Introduction

The ease of transmission of viruses through blood and blood products was one of the main stimuli to the development of nucleic acid testing (NAT) in virology. The improvements in technology leading to automation, reduction in contamination, quantitation and increased sensitivity have enhanced this development.

Following the recognition in 1984 of human immunodeficiency virus (HIV) as the cause of AIDS, serological assays to detect HIV-specific antibodies were developed as the main screening and diagnostic tests. However, their inability to detect either early HIV seroconversion or to measure the severity of infection drove the demand for NAT. In fact, some of the very first applications (published in 1987) of NAT using polymerase chain reaction (PCR) were in the identification of HIV in blood. The recognition of other new blood-borne viruses, in particular hepatitis C, as well as the knowledge of the already identified hepatitis B, further prompted the application of molecular tests to clinical medicine.

6.1.2 Qualitative NAT

NAT for blood-borne viruses has gone through various applications. Initially it was characterised by qualitative PCR directly on blood or other tissues. In HIV, this was done on whole blood or different blood components, e.g. plasma (reflecting 'free' RNA virions) and peripheral blood mononuclear cells (reflecting mainly integrated provirus DNA). Importantly, qualitative NAT has reduced the 'window period', the time between infection and HIV seroconversion, to less than 2 weeks. It has

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also allowed the resolution of incomplete serological results, as exemplified by the evolving Western blot in acute HIV infection. PCR for integrated HIV DNA is invariably positive in people who are HIV seropositive; it remains useful in confirming indeterminate serological results and determining the presence of infection in babies born to HIV seropositive mothers (where maternal HIV antibodies may circulate for up to 18 months).

Qualitative PCR can also be used to detect HIV in different body tissues, an important tool in the early days of understanding HIV pathogenesis.

6.1.3 NAT in the Blood Supply

In addition to confirming infection in an individual, testing is needed to protect the blood supply and identify asymptomatic infections in blood donors. This was initially done by rejecting individuals with a clinical or social history that put them at risk, people with surrogate markers of blood-borne virus infections e.g. abnormal liver function tests, or people with serological evidence of infection. Despite these assessments, transmission of blood-borne viruses through the blood supply still occurred. The introduction of more sensitive NAT has further reduced the likelihood of the acquisition of blood-borne viruses during transfusion or transplantation. Of course, the availability of testing methodologies varies around the world, and there certainly is inequality in the access to NAT.

Other viruses may also be transmitted by blood or organ transplantation, although these may not reflect the most common modes of transmission. Examples include arboviruses (West Nile virus infection has been associated with transfusion in the USA), influenza A H5N1 infection, SARS, and other novel viruses such as hepatitis G, TTV, EBV, CMV, BK/JC, Dengue etc. NAT for these pathogens are available for both individual patient diagnosis and protection of the blood supply, but overall they are perhaps less clinically relevant than HIV, hepatitis B and hepatitis C.

6.1.4 Quantitative NAT

The development of commercial viral load assays, or quantitative NAT, has revolutionised the clinical management of HIV infection (Table 6.1). The plasma HIV load, expressed in HIV RNA copies/mL plasma or log copy number, allows the clinician to do a number of things. A high viral load, when combined with the CD4+ T cell count, allows the prognosis to be discussed with the patient by determining the likelihood of progressing to either AIDS or death within a defined time period. It facilitates discussion of transmission, as it is known that the higher the HIV load, the more transmissible the virus is to others. The HIV plasma load also guides when antiretroviral therapy should be started and perhaps most importantly, assesses the response to antiretroviral therapy. Reducing the HIV load with antiretroviral therapy not only provides clinical benefit, but also provides a public health benefit by reducing the likelihood of ongoing HIV transmission. The HIV plasma load is used

Table 6.1 Commercial kits, methods and technologies for HBV, HCV and HIV detection

Target	Company	Assay name and version	Dynamic range	Instrument	Technology
HCV viral load	Abbott Molecular	RealTi*me* HCV	12–100,000,000 IU(copies)/mL	*m*2000 system	Real-time PCR
	Bayer	Siemens Versant HCV RNA 3.0 Assay	615–7,690,000 IU/mL	Versant 440 Molecular System	Branched DNA
	Bayer	Siemens Versant HCV RNA 1.0 kPCR Assay	Unavailable	Versant kPCR Molecular System	Real-time PCR
	Roche	COBAS Ampliprep/COBAS Taqman HCV Test	43–69,000,000 IU/mL	COBAS Ampliprep/COBAS Taqman	Real-time PCR
	Roche	COBAS Taqman 2.0 (with HighPure)	25–391,000,000 IU/mL	COBAS Taqman	Real-time PCR
	Abbott Molecular	HCV Genotype II	Viral load and genotypes 1–6 and 1a or 1b	*m*2000 system	Real-time PCR
HBV viral load	Abbott Molecular	RealTi*me* HBV	10–1,000,000,000 IU(copies)/mL	*m*2000 system – *m*24sp and *m*2000rt	Real-time PCR
	Abbott Molecular	RealTi*me* HBV	10–1,000,000,000 IU(copies)/mL	*m*2000 system – *m*2000sp and *m*2000rt	Real-time PCR
	Bayer	Siemens Versant HBV DNA 3.0 Assay	2,000–100,000,000 copies/mL	Versant 440 Molecular System	Branched DNA
	Bayer	Siemens Versant HBV DNA 1.0 kPCR Assay	Unavailable	Versant kPCR Molecular System	Real-time PCR
	Qiagen	Artus HBV Assay	0.02–100,000,000 IU/mL	Multiple platforms and kits	Real-time PCR
	Roche	COBAS Ampliprep/COBAS Taqman HBV Test V2.0	54–110,000,000 IU/mL	COBAS Ampliprep/COBAS Taqman	Real-time PCR
	Roche	COBAS Amplicor HBV Monitor	60–38,000 IU/mL	Cobas Amplicor	PCR

Table 6.1 (continued)

Target	Company	Assay name and version	Dynamic range	Instrument	Technology
	Roche	Amplicor HBV Monitor Test, v2.0	190–7,604,563 IU/mL	Cobas Amplicor	Microwell plate format PCR
HIV viral load	Abbott Molecular	RealTi*me* HIV-1	40–10,000,000 IU(copies)/mL	*m*2000 system	
	Bayer	Siemens Versant HIV-1 RNA 3.0 Assay	50–500,000 copies/mL	Versant 440 Molecular System	Branched DNA
	Bayer	Siemens Versant HIV-1 RNA 1.0 kPCR Assay	35–11,000,000 copies/mL	Versant kPCR Molecular System	Branched DNA
	Biomerieux	Nuclisens EASYQ HIV-1 V2.0	10–10,000,000 copies/mL	Nuclisens EasyQ	NASBA Real-time PCR
	Roche	Ampliprep/Cobas Taqman HIV-1	40–10,000,000 copies/mL	COBAS Ampliprep/COBAS Taqman	Real-time PCR
		TAQman HIV-1 (with HighPure)	47–10,000,000 copies/mL	COBAS Taqman	Real-time PCR
HIV Genotyping	Abbott	ViroSeq HIV-1 Integrase Genotyping Kit	An 864 base pair region of the entire IN gene is amplified and sequenced	ABI PRISM 3100 OR 3130xl	Sequencing based-mutations in the integrase region of the pol gene in RNA
	Bayer	Siemens TruGene HIV-1 Genotyping Assay	A 1038 base sequence of HIV-1 DNA is determined	PCR sequencing based run on the OpenGene DNA System	Sequencing based-mutations in the protease and part of the RT region of HIV

to monitor the response to new antiviral agents, in both treatment experienced and treatment of naive individuals. A rise in HIV plasma load is a surrogate for HIV drug resistance, as well as other therapy-related problems such as compliance and drug absorption, and is an indication for changing therapy.

Following evidence of the clinical value of HIV plasma load assays, quantitative NAT has also proved clinically useful in addressing treatment and antiviral drug resistance issues in hepatitis C and B infection. The management of other systemic diseases (e.g. cytomegalovirus, Epstein-Barr virus, HHV-6, BK polyomavirus) that are a particular clinical issue in immunocompromised individuals is also guided by quantitative NAT on blood, and will be enhanced as multiplex quantitative assays become available.

6.1.5 Genotyping

Genotyping for the detection of mutations associated with HIV drug resistance and clinical failure of therapy is now 'standard of care' in HIV management. A resistance genotyping test is most commonly performed by sequencing the relevant drug targets – most commonly the reverse transcriptase and protease regions, but now including integrase and envelope gp 41. Genotyping is recommended prior to the commencement of the first antiviral regimen to ensure that the individual is not already infected with drug resistant virus. Resistance testing is also indicated when people are failing antiretroviral therapy. HIV phenotype assays are also available, but their cost precluded their routine use in clinical practice and methods have been excluded from this section. The same principles apply to other blood-borne viruses, and assays are available for the detection of hepatitis B and CMV drug resistance mutations. As hepatitis C-specific therapies evolve, it is likely that genotyping will assist clinicians in the best use of antiviral drugs in both acute and chronic hepatitis C.

6.1.6 Molecular Epidemiology

Molecular epidemiology studies are undertaken with HIV, hepatitis B and hepatitis C, and to a lesser extent with the other blood-borne viruses. Here, sequencing of the viral genome allows comparison of a patient's viral sequences with those in large public sequence databases, allowing one to determine in which viral subgroup the patient's virus falls. Molecular subtyping of viral strains assists with understanding how viruses move in a community, and indicate potential subtype-specific responses to antiviral therapy and clinical outcomes. NAT has also been used in medico-legal cases, where HIV or other blood-borne viruses have been transmitted to other individuals

Another molecular advance is the use of NAT to determine the presence of human gene alleles associated with adverse drug reactions. This has been most clearly

demonstrated in HIV-infected individuals with abacavir hypersensitivity in the presence of HLA-B5701. This test is now performed as part of 'standard of care' in HIV-infected individuals where abacavir therapy is being considered.

The use of NAT has dramatically enhanced the detection and management of blood-borne viruses. This is likely to continue and expand, and so further improve patient management – hopefully with reductions in cost.

In Australia, assays for the detection of HIV and HCV require approval by the Therapeutic Goods Administration [see Therapeutic Goods Regulations 1990. Schedule 3. Therapeutic goods required to be included in the part of the Register for registered goods (regulation 10)]. The legislative basis for the regulation of in-vitro diagnostic devices (IVDs) (see In vitro diagnostic devices – definitions and links at http://www.tga.gov.au/devices/definitions.htm) involves inclusion on the Australian Register of Therapeutic Goods (ARTG). The ARTG is a database of therapeutic goods approved for supply in Australia. IVDs for the diagnosis of patients infected with the HIV or with the HCV are required to be registered in the ARTG. If the application is acceptable, the Conformity Assessment Branch (CAB) will advise the sponsor of the evaluation fee, any additional data required and the number of test kits required for evaluation. All data for evaluation must be sent to the CAB who will coordinate the evaluation. The TGA undertakes the evaluation of kit integrity while the quality and efficacy aspects are evaluated by the National Serological Reference Laboratory (NRL) located in Melbourne.

Every IVD approved for listing or registration in the ARTG is subject to standard and specific conditions under Section 28 of the Act. http://www.tga.gov.au/docs/pdf/dr4/dr4app04.pdf

IVDs approved for use as screening or as supplemental tests for the diagnosis of infection with Human Immunodeficiency Virus [HIV] (viral load assays exepted) and also for supplemental tests for the diagnosis of infection with Hepatitis C Virus [HCV] may be supplied to authorised laboratories only.

Sponsors and manufacturers also have ongoing responsibilities once a product has been approved for supply in Australia. All IVDs supplied in Australia (including exempt IVDs) are subject to post market requirements and IVD users can report any problems relating to the safety, quality or performance of an IVD supplied for commercial use.

Extract from *http://www.tga.gov.au/docs/pdf/dr4/dr4v1s2.pdf*:

> Conditions of Registration HIV Test Kits
> Test kits will be entered in the ARTG and specified as being suitable for routine screening or supplemental purposes. The conditions relating to the registration will specify the appropriate category.
> Sponsors will be advised by the TGA of the marketing approval, which will include details of the ARTG registration number, conditions of approval and the certificate of registration.
>
> HCV Test Kits
> Test kits are categorised as being suitable either for routine screening or for supplemental purposes only, and the condition of entry in the ARTG will specify the category of supply.

There is no restriction to the supply of HCV test kits approved for screening once entered in the ARTG. However, it is Commonwealth policy that all HCV test kits approved for use as supplemental assays and those using newer technology (such as polymerase chain reaction, branched DNA amplification or procedures currently in developmental stages) be supplied only to laboratories approved by State/Territory health authorities.

Use of Unapproved HIV/HCV Test Kits
Provisions exist for the supply of unapproved HIV/HCV test kits either for research use or under the Clinical Trials / Special Access Schemes in the Therapeutic Goods Act 1989. Refer to Chapter 1.24 Access to Unapproved Therapeutic Devices. . ..
Sponsors are permitted to supply their kits for research purposes prior to the evaluation procedures having been completed. The kits may only be supplied to bona fide research institutes as well as to the designated testing laboratories. Such products cannot be used for diagnostic purposes, i.e. specimens must not be identifiable. . ..

6.2 PCR Use in the Diagnosis of Infection in the Immunocompromised Host

William Rawlinson

6.2.1 Background

The prevention of infection is dependent upon existing (innate) and responsive (humoral and cell mediated) immune functions. All these host functions are critical to preventing infection in the host. However, in most clinical settings we see immune compromise as immunodeficiencies resulting from abnormalities of humoral and cell-mediated components. Ranulocytopaenia results in predominantly bacterial infections and neutropaenia is associated with infection by bacterial species predominantly, although the types of bacterial species differ slightly. Changes in humoral immunity resulting from absence of antibody predominantly cause bacterial infection, whilst impaired cellular immunity results predominantly in infection with intracellular organisms – bacteria such as *Nocardia*, mycobacteria including, atypical mycobacteria, *Pneumocystis jirovecii* and fungal infections. All viral infections have an intracellular component, as viral reproduction is critically dependent upon intracellular infection, and therefore impaired cellular immunity is particularly associated with infection with viruses – either due to reactivation of latent herpesviruses or the more severe infections from exogenous viruses such as respiratory or gastrointestinal agents.

The patient groups demonstrating immunocompromise are many. However, we predominantly focus on immunocompromised patients who present often to hospitals, with severe immune compromise such as results from organ transplantation,

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haematological transplantation (bone marrow transplants are a highly immunocompromised population), compromised immune systems as a result of therapy – such as cancer chemotherapy, and immune compromise resulting from infection with human immunodeficiency virus (HIV) with the development of acquired immune deficiency syndrome (AIDS). As increased numbers of transplants are done each year, and additional transplants (such as bone marrow matched unrelated donor transplantation) are performed, then the number of immune compromised patients has increased. Furthermore, more aggressive immune suppression associated with some of these transplants and longer survival of transplant patients with better medical and surgical therapy, has meant that the number of severely immune compromised patients has increased significantly. The annual cost to the health care system of transplantation transplant complications are also significant – transplants cost $65,000 to $75,000 per transplant with an annual average cost of care of $11,000 (www.transplant.org.au). This cost is significantly increased by post-transplant viral infection – for example, liver transplant recipients who develop cytomegalovirus (CMV) disease have significantly (24–49%) higher costs than patients with no, or asymptomatic CMV infection.

Diagnosis of these infections in immunocompromised patients is critical to proper therapy. Empirical therapy is often begun urgently, due to the tendency of such infections in the immune compromised host to progress, but such empiric therapy often has side effects, requires the patient to remain in hospital, and is costly. Therefore more targeted therapy is needed. Diagnosis, particularly using nucleic acid tests, is critical to allow narrowing of therapy to the minimum required to give the best clinical outcomes. Importantly, much infection may go undiagnosed due to the need for urgent empirical therapy. The febrile neutropenic patient with an undiagnosed infection and treatment with multiple antibiotics, often including antifungal and antiviral agents, is a recurring clinical scenario. Up to one in five infections will remain undiagnosed, and it is apparent that treatment for these different infections, bacterial, fungal or viral is completely different. Therefore diagnosis of infection in the immune compromised host, utilising nucleic acid tests such as PCR is critical.

6.2.2 Issues to Consider in the Diagnosis of Infection in the Immunocompromised Host

The source of the organism in transplant infection may be exogenous or endogenous. The exogenous organisms are those commonly seen in other hosts – for example influenza during winter season, respiratory syncytial virus (RSV) in lung transplant recipients, bacterial agents in neutropenic patients that are normal colonisers of the skin – such as *Staphylococcus aureus* and nosocomial agents introduced via central venous catheter related infections (such as coagulase negative staphylococci, *Staphylococcus aureus*, *Candida albicans*, *Candida parapsilosis*). These agents are often antibiotic resistant as they may be acquired in hospital, and the immunocompromised patient often is treated with multiple antibiotics.

Significant infection exposures of transplant recipients may be derived from the donor organ, preexisting latent infections (herpesviruses, HIV, HCV) in the recipient or donor, the hospital environment (e.g. nosocomial MRSA), and community exposures (e.g. influenza). These have contributed to reducing long term patient survival through death from infection and cancer (ANZDATA and ANZOD transplant data in www.transplant.org.au) and improved diagnosis is vital in reducing these infections.

Diagnosis may be straightforward, as evidence of infection (for example an infected centrovenous catheter) is clinically apparent, a swab can be taken, and the organism will often grow, albeit that antibiotics may inhibit culture in some cases. Endogenous infections, arising from reactivation of latent viruses such as the herpesviruses (HSV1, HSV2, EBV, CMV, HHV6, HHV7, HHV8), the polyoma viruses (BK/JC virus) may be more difficult to detect. This is because serological assays are often of little use in this setting, as IgG and IgM assays, or even avidity assays, are not accurate because immune suppression reduces production of IgM. Therapy for these infections is often toxic (for example ganciclovir for CMV) and decisions regarding prophylaxis versus pre-emptive therapy often need to be made. It is here that nucleic acid tests, particularly quantitative PCR is extremely useful in deciding whether a (viral) pathogen is reactivating, whether the level is increasing (suggesting active infection), and whether the level continues to increase on therapy, suggesting antiviral resistance has developed.

The clinical presentation of the infected immunocompromised patient is often atypical. Infections may occur in the absence of classical syndromes – fever is often used to indicate active bacterial infection, although this may be absent in the immunocompromised patient. Different pathologic agents (bacterial, viral and fungal infecting agents), may present in a very similar fashion. For example, pneumonia with diffuse, bilateral lung infiltrates in the immunocompromised bone marrow transplant recipient may be due to a virus (RSV, CMV, influenza, adenovirus, parainfluenza virus, VZV, HSV), *Mycoplasma pneumoniae*, *Pneumocystis jirovecii*, bacterial and fungal agents although the latter would normally result in localised rather than diffuse infiltrates. Sampling of the respiratory tract in this setting, by bronchoalveolar lavage with or without bronchial biopsy is often useful, when accompanied by specific PCRs for these agents.

Neurological infections, with encephalitis and/or meningitis can also result in non-specific symptoms (confusion, headache, seizures) and symptoms may initially be non-specific. Infections of the CNS with reactivated HSV (HSV encephalitis), *Toxoplasma gondii* (cerebral toxoplasmosis) or intracellular organisms such as *Listeria monocytogenes* or *Cryptococcus neoformans* may all result in this syndrome. Fungal infection may also cause non-specific symptoms, although again focal infection with cryptococcus, aspergillus or *Nocardia* or mycobacteria species may occur. The obvious difficulty with neurological infection in the immunocompromised patient is that access to clinical samples (cerebrospinal fluid or very rarely brain biopsy) is limited when compared with other organ system infections.

Gastrointestinal infections are occasionally troublesome in immunocompromised patients, although long-standing, ongoing infections are usually due to a small number of agents – *Strongyloides stercoralis*, *Clostridium difficile*, and cytomegalovirus (CMV) can infect the lower gut resulting in ongoing diarrhoea. Oesophagitis due to CMV, HSV, or candida may occur. A more serious problem can be for a hospital unit treating immunocompromised patients, where infection with one of the epidemic viruses such as norovirus or astrovirus results in an acute, severe gastroenteritis which may be associated with widespread infection (including of the staff) that can cause severe fluid and electrolyte depletion. Infections of other parts of the gut such as oropharyngeal gingivostomatitis, severe mucositis and oesophagitis may occur although often specific agents (such as HSV in gingivostomatitis, candida in oesophagitis) are limited to a small number of agents. Again, in all these cases, directed PCR to identify the agent will result in successful treatment strategies. Treatment of infections in immunocompromised patients is critically dependent upon accurate diagnosis. The use of PCR to diagnose, in a targeted fashion, the most common infections has revolutionised treatment of these infections in the immunocompromised patient. Knowledge of the most likely agents – for example, CMV in the first 3 months following bone marrow transplant, is often used because such reactivation infection is extremely common (and often fatal) in this population. This means that other agents then become more likely in the individual patient on prophylaxis, and nucleic acid testing can be more directed towards these other agents. The laboratory services are of enormous benefit in directing the approach to treatment taken by a clinical service. The availability of routine CMV viral loads for example, done two to three times weekly means a clinical service is able to undertake pre-emptive treatment with ganciclovir, rather than relying on prophylaxis. The availability of polyoma virus PCR means a clinical transplant service is able to monitor their patients for polyoma virus and adjust the level of immunosuppression appropriately. The availability of rapid diagnosis for fungi or Pneumocystis means transplant clinical services can often reduce the use of antimicrobial prophylaxis for these agents. As a result of these rapid diagnostic nucleic acid testing services, clinical services can directly improve patient therapy, thereby reducing side effects and improving outcomes.