most laboratories and thus others should evaluate their technical service.

Systems such as middleware and LIS are perfect for overcoming such technical problems as they allow laboratories to approach a given problem in a timely, consistent and standardised fashion. Thus, once the appropriate programming is in place, analytical problems that occur several times a day are dealt with in the same appropriate way as are those that occur only monthly or even less frequently.

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Detection of HCV antibody-negative donations: Saudi experience with nucleic acid testing

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Currently, the risk of contracting hepatitis C virus (HCV) or human immunodeficiency virus (HIV) in the blood transfusion setting is very low. The sensitivity of donor screening using serological tests and donor selection practices ensures that transfused blood is an extremely safe product. However, there is a small residual risk of infection from individuals who test serologically negative during the so-called window period or from immunosilent infectious donors.

In 1999, the European authorities required all plasma for fractionation to be tested for HCV RNA, which led many European blood services to implement HCV nucleic acid amplification testing (NAT) in small pools. Subsequently, the UK and other European countries implemented HCV NAT as a release criterion for all blood components (including platelets). In the USA, the American Association of Blood Banks (AABB) recommended mandatory screening for blood donors from March 1999.[1]

Jarvis *et al*.[2] reviewed the yield of serologically negative, nucleic acid-positive window-period donations and described lower than expected detection rates following NAT for antibody-negative HCV and HIV-1 donations. They suggest that the donor health assessment questionnaire has improved the safety of the blood supply greatly, hence the low rates of detectable infection.[2] Similar results have been obtained in Canada.[3]

The prevalence of HCV antibodies in Saudi blood donors is estimated at 0.6–1%.[4] However, results suggest that the majority of blood donors who are anti-HCV-positive by the screening method are negative by recombinant immunoblot assay (RIBA).[5] Blood donors who attend the authors' hospital mainly comprise male National Guard soldiers. Prior to donation, donors undergo strict selection criteria as laid down by the AABB.

Currently, all blood donations in the authors' hospital are tested for HCV and HIV-1 using the Abbott Architect serology screening platform and also by NAT, with HCV and HIV NAT implemented in April 2006.

The present short study describes the NAT methodology used in the Saudi National Guard Health Affairs programme and describes the first NAT-positive, seronegative HCV case.

All blood units were tested routinely for HBV, hepatitis C virus (HCV), HIV, syphilis and human T-lymphotropic virus (HTLV) by serology. Sera with a signal-to-cut-off ratio (S/CO) ≥1.0 were considered reactive, whereas sera with a S/CO <1 were considered non-reactive. All initial reactive specimens were retested in duplicate.

A plasma preparation tube (K3 EDTA, Greiner Bio-One, Germany) was collected from each donor who demonstrated HCV and HIV seronegative results and submitted for NAT testing. A minipool of 24 donors was prepared by mixing equal quantities of plasma from seronegative donors. Positive primary pool results were resolved in four secondary pools of six donors. If a secondary pool proved to be positive then all six pool samples were tested individually.

Nucleic acid was extracted from a 1 mL plasma pool using the AmpliPrep total nucleic acid isolation kit and the Cobas AmpliPrep instrument (Roche Molecular System, Branchburg, NJ, USA). An internal control was added to each pool specimen in order to monitor the extraction and amplification efficiency. Following the manufacturer's recommendation, a 50 µL sample of the extracted nucleic acid was mixed with an equal volume of the Cobas AmpliScreen HCV (version 2.0)/HIV-1 (version 1.5) reagents to amplify and detect HCV/HIV RNA. The final amplified products for both targets and the internal controls were determined by measuring colorimetric absorbance at a wavelength of 660 nm using the Cobas Amplicor analyser (Roche).

Total HCV nucleic acid (RNA) was extracted from 1050 µL patient plasma using the Cobas AmpliPrep instrument and the Cobas TaqMan HCV test kit (Roche), following the

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manufacturer's recommendations. Amplification and detection of HCV RNA were performed using the Cobas TaqMan 48 analyser (Roche). The final viral load was reported in international units (iu)/mL. Genotyping was performed using the HCV Genotyping ASR kit (Abbott Molecular Diagnostics, Abbott Park, IL, USA).

A 5 µL sample of the extracted RNA was added to 11 µL of each master mix. Amplification and detection were performed using an ABI 7900 real-time polymerase chain reaction (PCR) system (Applied Biosystems, Foster City, CA, USA). Data analysis was performed using the Celera sequence genotyping software (SGS v2.0).

The methodology described above identified the first Saudi blood donor who was seronegative but positive for HCV RNA. This is the first case out of 21,306 donors tested. The HCV genotype found in this patient was genotype 4, and the initial HCV viral load result obtained was 51,303 iu/mL. When repeated six weeks after the initial test, screening serology for HCV was positive and the viral load was 2,052,120 iu/mL. Clearly, more blood donors need to be tested in order to have a better idea about the prevalence of NAT-positive, seronegative donors. The HCV genotype 4 is consistent with the known HCV genotype in Saudis and is the predominant genotype in the Saudi population.[6]

Anti-HCV antibodies are usually detected three to 20 weeks after initial exposure to the virus, but this can be delayed from six to nine months in rare cases.[7,8] There are examples in the literature of blood donors who have remained viraemic for up to five years without developing anti-HCV antibodies.[9,10] This was attributed in part to the presence of subgenomic HCV in the plasma of infected patients as a result of the absence of immunological pressure and the presence of a high viral load.[11]

Immunosilent donors can be responsible for transfusion-related infections, although the introduction of NAT methodology in blood banks has achieved a reduction in the level of risk. However, even when NAT methods are negative, there remains a small risk of contracting HCV from blood donations.[12]

The introduction of NAT in the authors' hospital has reduced the incidence of transfusion-related HCV and HIV infections. Clearly, the introduction of this methodology in all blood banks in the region is recommended.

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Comparison of clustered, regularly interspaced short palindrome repeats (CRISPRs) in viridans streptococci (*Streptococcus gordonii*, *S. mutans*, *S. sanguinis*, *S. thermophilus*) and in *S. pneumoniae*

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Clustered, regularly interspaced short palindrome repeats (CRISPRs) have been described in several archaea and bacteria. An *in silico* analysis of pneumococcal and viridans group streptococci (VGS) demonstrated the presence of

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