Automation of a throughput system for the diagnosis and the genotyping of Hepatitis C Virus

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Background

Hepatitis C Virus (HCV) is a single stranded RNA virus belonging to Flaviviridae Family and it is the etiologic agent of Hepatitis C disease in humans. The World Health Organization (WHO) evaluates 130-150 million cases of HCV infection worldwide, with 350-500 thousand deaths each year. The virus causes an acute infection which can spontaneously clear or in the 55-85% of the cases it can exacerbate in a chronic infection. The main symptoms of this chronic infection derive from a severe inflammation of the liver, which is the principal host of the virus. This hepatitis disease, called Hepatitis C disease, entails the reduction in the quality of the life for the patients (1) and it implies cirrhosis of the liver in the 50% of the cases and hepatocellular carcinoma in the 7% (2).
Early diagnosis of HCV infection is rare and frequently it remains undiagnosed until serious liver damages have developed. The detection of HCV infection is carried out in two different steps: firstly it is verified the presence of anti-HCV antibodies and secondly it is confirmed the presence of the viral RNA in the serum of the patient in order to discern the patients whose infection spontaneously clears.
Considering the high frequency of HCV infection, the difficulty in an early diagnosis and especially the absence of a vaccine, it is strongly recommended to subject population with an high risk of infection to a careful screening for the presence of HCV. Furthermore it is necessary to determine the genotype of HCV infecting patient, because it is demonstrated that different strains of the virus respond to the treatment in a heterogeneous way (3).

Aim

This work aims at the development of an easy-to-use system for the diagnosis and the genotyping of HCV. The project comprises the employment of simple and high-throughput technologies, in order to satisfy the requirements of both sanity and market. The system involves a first step consisting in the extraction of viral nucleic acids from the serum of the patient and a second step designed to detect and genotype the RNA of HCV in the extract.

Results

We have developed a process of automatical extraction of viral nucleic acids working with the CE-IVD instrument GeneQuality-X120 (AB ANALITICA srl, Italy). This is a workstation provided with thermo-shakers and four channels plus a 96-probe head for the multichannel liquid handling. The process of automation involves the use of a kit composed of cartridges and tubes containing reagents for the viral nucleic acids extraction based on their purification with magnetic beads. This kit used with this instrument allows extracting up to 96 samples simultaneously with high sensitivity, efficiency and reproducibility avoiding cross-contaminations. We have tested and validated this process for the extraction of both viral DNA and RNA starting from different materials, like blood, swab, urine, plasma and serum. Moreover, GeneQuality-X120 offers the possibility of preparing Real Time PCR plates for the analysis of the extracts avoiding manual passages.
Secondly, we are developing a protocol for the detection and the genotyping of the RNA of HCV in the extract. This system is based on the retro-transcription of viral RNA and Reverse Line Dot Blot.

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