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HCV Donation Tests Confirmation

Dear Professor Cash

Re: RECOMBINANT IMMUNOBLOT ASSAY (RIBA) FOR HCV

Ortho Diagnostic Systems is pleased to announce the introduction of the Chiron RIBA-HCV test system, the first Recombinant Immunoblot Assay (RIBA) for the detection of antibodies to hepatitis C virus (HCV).

This exciting new assay is designed to detect the presence of antibodies to hepatitis C virus in samples that have given a positive result with the ORTHO* HCV antibody ELISA test.

The antigens used in the ORTHO* HCV antibody ELISA test and the Chiron RIBA-HCV assay are cloned from segments of the HCV viral polyprotein (see figure 1).

FIGURE 1:

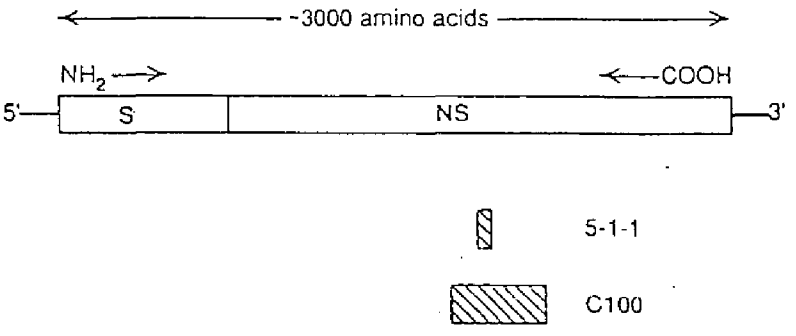


Figure 1. Basic organization of the HCV polyprotein. Virion structural proteins (S) are situated at the N-terminus while the majority of the polyprotein is responsible for the synthesis of a large variety of non-structural (NS) proteins.[1] The approximate positions of the polypeptides encoded by clones 5-1-1 and C100 are indicated.

In order to manufacture an assay for HCV antibody, viral antigen is synthesized in recombinant yeast. The orginal 5-1-1 clone and two adjacent clones are used for expression in yeast. The continuous running through these three clones is reconstructed and fused to the gene encoding superoxide dismutase (SOD). This fusion facilitates the efficient expression of foreign proteins in recombinant yeast and bacteria. The components of this fusion protein (C100-3) are illustrated in figure 2

(2)

FIGURE 2:

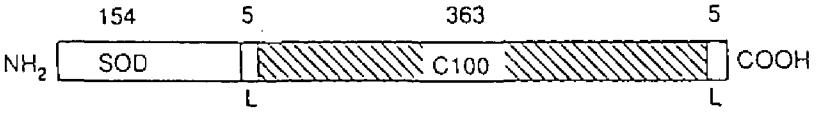


Figure 2. Composition of the yeast recombinant HCV antigen (C100-3) used to capture circulating antibodies. The antigen comprises 154 amino acids of human superoxide dismutase (SOD) and 363 amino acids from the non-structural part of the HCV polyprotein (C100). Due to the cloning procedures, there are an extra 10 amino acids derived from adjoining linker (L) DNA.

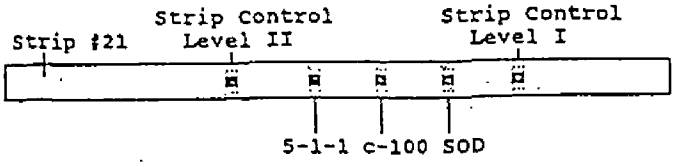
Following fermentation of the recombinant yeast cultures C100-3 is purified and used to coat the microwells in the ORTHO* HCV antibody ELISA test.

The Chiron RIBA-HCV assay employs three recombinant antigens (produced in yeast or E.coli): c100-3 and 5-1-1 which are SOD-fusion polypeptide related to HCV, and human superoxide dismutase (SOD), which is included as a control to detect the presence of antibodies against SOD.

Reactivity of samples are determined by visually comparing the colour of a recombinant antigen band with positive controls included on each strip (see figure 3).

The solid phase and conjugate used in the RIBA-HCV assay are different from those used in the ELISA assay. Furthermore, the addition of a second antigen expressed in a different organism offers the potential of verifying that the reactivity seen in the first antigen is not due to a non specifric reaction.

FIGURE 3:



The RIBA-HCV assay can be performed in 5 hours and does not require specialised instrumentation.

RIBA-HCV is now available as a 30 test kit, product code number 933430, price £625.00; discounts are available for bulk purchase or standing orders.

Should you wish to obtain this product could you please mark your order for my attention.

If you require further information, please contact me.

Yours faithfully
for **Ortho Diagnostic Systems Limited**

PETER SAVAGE
Marketing Manager -
Infectious Diseases/Immunology

Reference: [1]Choo Q-L et al. Manuscript in preparation.