

identifies p53 binders including antibody classes other than IgG, and it requires about 10-fold less p53 antigen. We propose this method for screening patients with cancer and as a confirmatory test in combination with Method A.

17 **New methodology for measuring anti-p53 antibodies in human serum**

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Current methodology for measuring human antibodies against the p53 tumour suppressor gene product are based on the use of radioactive p53 antigen, immunoprecipitation and Western blot analysis. These methods are time-consuming, cumbersome and unsuitable for screening large numbers of samples. A recently developed assay is based on p53 antigen immobilization through a solid-phase monoclonal anti-p53 antibody and detection of immunoabsorbed anti-p53 human IgG with alkaline phosphatase (ALP)-labeled goat anti-human antibody. The ALP activity is then measured with time-resolved fluorometry (Method A). We here describe a new method (Method B) as follows: Human serum is incubated with a carefully selected amount of p53 antigen (derived from the tumour cell line COLO 320HSR which overproduces mutant p53 protein). During this step any antibodies present will bind the added p53 antigen. This mixture is then assayed for p53 by using a method based on a monoclonal capture anti-p53 antibody (PAb 240) and a polyclonal rabbit anti-p53 antibody. The signal is generated with a goat anti-rabbit antibody labeled with ALP. If the test serum does not contain any p53 antigen binders (e.g. antibodies) there will be generation of signal in the p53 assay due to the added p53 antigen. In the presence of human anti-p53 antibodies, the signal in the p53 assay will be very low because of p53 antigen binding prior to the assay for p53. This method was tested with p53-antibody-negative and positive human sera identified by Method A with excellent agreement between the two methods. Method B has some advantages over Method A: it