Serum paraoxonase (PON1) is a Ca\textsuperscript{2+}-dependent esterase which in serum is almost exclusively associated with high-density lipoprotein (HDL) [1]. Oxidative modification of low-density lipoprotein (LDL) plays a central role in atherogenesis [2] and PON1 directly protects LDL from modification [3]. PON1 rapidly removes fatty acyl groups from the Sn2 position in glycerophospholipids such as phosphatidylcholine. Typically these groups are polyunsaturates and thus susceptible to subsequent breakdown to shorter carbon chain aldehydes and ketones which on LDL could damage apolipoprotein B making it a ligand for macrophage scavenger receptors. Enhancement of lysophosphatidylcholine formation may also increase HDL binding and HDL-mediated cholesterol efflux from macrophages [4]. Measurement of serum PON1 activity may also be important in minimizing the exposure of susceptible individuals in, for example, the agricultural industry, to organophosphates [5]. Organophosphates are still the most widely used insecticides throughout the world even though their use is declining in most prosperous countries. Serum PON1 may also be of importance in assessing the severity of acute organophosphate poisoning [6,7]. Although it is possible to determine PON1 activity using relatively non-toxic substrates such as phenylacetate, the hydrolysis of paraoxon seems most closely related to the inverse relationship with coronary heart disease [8]. Also, different polymorphisms of the enzyme display different substrate specificity towards organophosphates which they do not towards phenylacetate. Thus any industrial application of the assay of PON1 activity will require the use of an organophosphate. The use of an organophosphate substrate such as paraoxon, presents particular problems for automation, for example in a conventional analyzer such as the Cobas Mira [9]. In order to remove traces of the toxic substrate from the equipment high concentrations of NaOH must be employed which would be damaging and also make it difficult to do assays for other analyses on the same machine. The most widely used method for determination of PON1 activity in serum is the continuous recording spectrophotometric method [10,11] but this is not suited to large numbers of samples. Although automated and semi-automated methods for serum PON1 have been occasionally described [9,12,13], no direct comparison of automated methods with the standard recording spectrophotometric method has been performed. A microtitre plate method is attractive because of the ease of disposal of a considerable portion of the equipment used. However, it is important to realize that there is, for example, an early burst of hydrolytic activity when an organophosphate substrate is added to serum which is not due to PON1, but to type B esterase activities present on albumin which are subsequently blocked by irreversible substrate binding, but which if included in the measurement of activity do not represent PON1 activity. With growing interest in PON1 in cardiovascular disease and in identifying risks of exposure to organophosphates, we validated a microtitre plate method with potential for use in larger studies in which the early phase of non-specific paraoxon analysis is discounted. This overcame some of the problems posed by the extreme toxicity of substrates such as paraoxon. The method was relatively safe and inexpensive and permitted the determination of PON1 activity at rate of 120
samples/hour [14]. We also confirmed that PON1 activity, certainly with respect to organophosphate hydrolysis, must be measured in serum and not EDTA plasma with the presently reported method or with earlier ones.

**References**