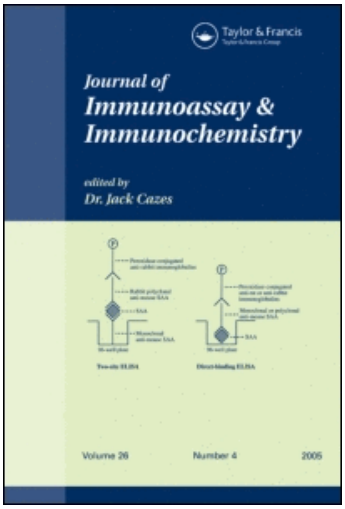

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Potential of Laser Immunoassay for Detection of HIV in Human Blood Serum and Urine

Anita Saxena[a]; K. B. Roy[b]; H. B. Bohidar[a]
[a] School of Physical Sciences, Jawaharlal Nehru University, New Delhi, India [b] Centre for Biotechnology, Jawaharlal Nehru University, New Delhi, India

Online publication date: 11 June 2003

To cite this Article Saxena, Anita , Roy, K. B. and Bohidar, H. B.(2003) 'Potential of Laser Immunoassay for Detection of HIV in Human Blood Serum and Urine', Journal of Immunoassay and Immunochemistry, 24: 4, 383 — 394
To link to this Article: DOI: 10.1081/IAS-120025776
URL: http://dx.doi.org/10.1081/IAS-120025776

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF IMMUNOASSAY & IMMUNOCHEMISTRY
Vol. 24, No. 4, pp. 383–394, 2003

Potential of Laser Immunoassay for Detection of HIV in Human Blood Serum and Urine

Anita Saxena,[1] K. B. Roy,[2] and H. B. Bohidar[1,*]

[1]School of Physical Sciences and [2]Centre for Biotechnology, Jawaharlal Nehru University, New Delhi, India

ABSTRACT

The potential of Light Scattering Immunoassay (LIA) for detection of HIV in human blood serum has been explored by monitoring the agglutination of antigen coated polystyrene particles by dynamic light scattering. Elisa tested human sera having HIV, TB, Filaria along with normal sera have been analyzed using two specific synthetic peptide antigen (SP1, SP2) and one nonspecific peptide antigen (NSP). Few paired human sera and urine samples and nonspecific (of nonHIV diseases) urine samples have also been tested using the same antigens to check the possibility of replacement of sera by urine.

Key Words: Dynamic light scattering; HIV; Agglutination; Specific and nonspecific antigens; Human blood serum and urine.

*Correspondence: H. B. Bohidar, School of Physical Sciences, Jawaharlal Nehru University, New Delhi-110067, India; Fax: +91-11-26198234: E-mail: bohi0700@mail.jnu.ac.in.

DOI: 10.1081/IAS-120025776 1532-1819 (Print); 1532-4230 (Online)

INTRODUCTION

Diagnostic assays for detecting HIV antibodies, which are quick and easy to perform outside a laboratory, are gaining importance. Such tests were developed in the late 1980s and gained popularity in early 1990, and termed as *Rapid Assay*. They have found wide usage in a number of testing situations like emergency rooms, physician's clinics, autopsy rooms, and blood banks. The latex agglutination test is one of such tests in which the formation of antigen–antibody agglutination complex is monitored by different probes. The laser immunoassay (LIA) technique suggested by Cohen and Benedek[1] probes the size of the agglutinated complex, and is under development in our lab for detection of infectious diseases. It was successfully performed on malaria samples[2–5] and found to be more simple to perform compared to ELISA, though as regards sensitivity, it was found superior. LIA is a physical technique in contrast to other commonly used diagnostic procedures like Western Blot, ELISA, radio-immunoassay, etc., that solely rely on chemical/or combinations of physical and chemical methods.

The sensitivity of laser immunoassay (LIA) owes its origin to the fact that the intensity of the light scattered, I from a particle of radius R, is proportional to R^6. Thus, a small change in the size of the agglutinate has a profound effect on the value of I. The advent of intensity and frequency-stabilized lasers, coupled with availability of highly sensitive and rapid electro-optic signal analysis capabilities, has increased the potential of this technique many fold. In this article, we report the detection of HIV in human sera and urine samples and address the specificity problem, and compare the results of LIA with that of ELISA in an effort to explicitly bring out the promise offered by this technique.

EXPERIMENTAL

Polystyrene beads of average diameter of 90 nm (Sigma, USA) were used as carrier particles. Two specific synthetic peptide antigens (SP1 and SP2) for HIV and one nonspecific random peptide antigens (NSP) were used for the assay. The selection of HIV specific antigens are based on a previous ELISA study on a series of synthetic peptides, derived from immunodominant epitopes of GP120 and GP41 of AIDS virus. The antigens were coated on 1% (v/v) polystyrene beads in bicarbonate buffer and incubated overnight at room temperature. Then it was centrifuged (at 20,000 rpm) to remove uncoated antigen. Blood serum

was mixed with coated polystyrene beads in 1:10 ratio and measurement were taken after 2 h of mixing.

The light scattering apparatus consisted of a laboratory goniometer with two arms. One of these arms housed the excitation source, which was a solid state frequency doubled Nd:YAG laser radiating at a wavelength of 532 nm. These experiments were carried out at a fixed scattering angle of 90°. The other arm of the goniometer had the photo multiplier tube mounted onto it enabling angle dependent detection of scattered light. All experiments were done at room temperature ($25 \pm 2^{\circ}$C). Glass capillary cells were used to minimize the sample volume (20 μL).

Scattered light from the sample solutions was detected by a photomultiplier tube, and the photocurrent was suitably amplified and digitized before it was fed to a 1024 channel digital correlator (Brookhaven Instruments Inc., USA, model BI—9000AT). The whole scattering apparatus was placed on a vibration isolation table (Newport Corp., USA). In all the experiments, the difference between the measured and calculated baseline was not allowed to go beyond ±0.1%. The data that showed excessive baseline difference was rejected. All the data exhibited a single, but broad, particle size distribution. In LIA experiments, the intensity auto-correlation function of the scattered light is measured, and in literature this method is generally referred to as dynamic light scattering (DLS) technique, which is briefly described below.

A LIA or DLS experiment measures the time correlation function $g_2(t)$ of the scattered intensity $I(t)$ at a given q defined by:

$$g_2(t) = \frac{\langle I(t')I(t'+t)\rangle}{\langle I(t')\rangle^2} \tag{1}$$

which is related to the scattered field auto correlation function, $g_1(t)$ by the Siegert relation[6]:

$$g_2(t) = A + B|g_1(t)|^2 \tag{2}$$

where A defines the baseline of the correlation function as:

$$|g_2(t)|_{t\to\infty} = A \tag{3}$$

and B is the spatial coherence factor. The ratio (B/A) is the signal modulation, and better data quality demands $(B/A) \geq 50\%$.

For solutions containing particles undergoing Brownian motion (i.e., polymer or colloidal solutions), the field autocorrelation function, $g_1(t)$ is given as:

$$g_1(t) = \sum_i A_i \exp(-\Gamma_i t) \tag{4}$$

where Γ_i is the relaxation frequency, which characterizes various relaxation modes that include relaxations due to the translational diffusion, rotational diffusion, and bending modes, etc. The relative mode strength (amplitude) of the i-th relaxation mode is A_i. For the present case, center of mass diffusion is the dominant process and Γ_i has been identified as $\Gamma_i = D_i\, q^2$, where the translational diffusion coefficient of the i-th particle is D_i and the scattering vector $q = (4\pi n/\lambda)\sin(\theta/2)$, where n is the refractive index of the solution, θ is the scattering angle, and λ is the wavelength of light source in medium. The expression for $g_1(t)$ remains valid for polydisperse samples and for situations where the relaxation frequency distribution has several peaks. In the present situation, the sample solutions were highly polydisperse. Fitting of double or multi-exponential relaxations did not yield acceptable chi-square values. On the other hand, CONTIN provided excellent data with acceptable statistical accuracy. This analysis yielded the normalized variance of Γ, which gave the effective polydispersity, P values. Again, P and $\overline{D}$ are related to the particle concentration, N_i and molecular weight, M_i of the i-th scattering particle as[6]:

$$\overline{D} = \frac{\sum_i D_i N_i M_i^2}{\sum_i N_i M_i^2} \tag{5}$$

Consequently, polydispersity P can be defined as:

$$P = \frac{\left\langle (D - \overline{D})^2 \right\rangle}{(\overline{D})^2} \tag{6}$$

Further details of this discussion can be found elsewhere.[6]

In dilute solutions, the concentration dependence of the translational average diffusion coefficient can be expressed adequately by a first order expansion:

$$\overline{D} = \overline{D}_o(1 + k_D C) \tag{7}$$

where $\overline{D}_o$ is the z-average diffusion coefficient at infinite dilution, and k_D is the diffusion second virial coefficient. According to the Einstein relation, the $\overline{D}_o$ is inversely proportional to the translational frictional coefficient, f_t at infinite dilution by the relation:

$$\overline{D}_o = \frac{k_B T}{f_t} \tag{8}$$

where k_B is the Boltzmann constant and T is absolute temperature. The value of f_t obtained via Eq. (8) can be used for a direct estimation of

hydrodynamic radius, R, of the agglutinates, provided they have a spherical shape using the relation $f_t = 6\pi\eta R$ as per the Stokes law

$$D = \frac{k_B T}{6\pi\eta R} \tag{9}$$

where η is the solvent viscosity at temperature T. The measurement involves basically two steps: (i) measurement of the size of the coated latex and (ii) measurement of size of the agglutinates. These measurements were carried out under various experimental conditions for sera and urine samples.

RESULTS AND DISCUSSIONS

Initially, LIA was performed on a set of 90 human sera samples of which 40 were from HIV patients, 20 from TB patients, 20 from Filaria patients, and 10 were from healthy individuals. The LIA experiments were performed at two sera dilutions, 1:5 and 1:100, to see the effect of dilution. The results are presented in Table 1 and Figs. 1–2. Using a cut-off value (mean of normal sera readings ±1 standard deviation (SD)), only 53% of LIA data tailed with ELISA data both for SP1 and SP2 at 1:5 sera dilutions, while 65% with SP1 and only 22% with SP2 tallied with ELISA at 1:100 sera dilutions. These results were unsatisfactory in comparison to data with malarial sera studied previously.[1–5] We then looked at variance of the data using different cut-off standard values. These are the mean of normal sera readings against specific antigen (1136 nm for SP1 and 705 nm for SP2 at 1:5 dilution, 140 nm for SP1 and 132 nm for SP2 at 1:100 dilution), mean of normal sera readings against nonspecific antigen (240 nm at 1:5 dilution and 98 nm at 1:100 dilution), and mean of HIV/TB/Filaria sera readings against nonspecific antigen (906 nm for HIV, 462 nm for TB, 410 nm for Filaria at 1:5 dilution, and 98 nm for HIV, 100 nm for TB, 97 nm for Filaria at 1:100 dilution). The sero-positivity is also calculated by comparing each sera readings with SP1, SP2, and NSP. A sample is treated as positive if its value is greater than the value with nonspecific antigen (Table 1). The maximum detectability (84.5%) of HIV sera were with cut-off based on normal sera readings with nonspecific antigen.

Performance of an immunoassay is defined by its sensitivity (detection of true positive sera, i.e., HIV and specificity (detection of true negative sera). The observed specificity of this immunoassay (detection of TB and Filaria) is given in Table 1 with all the three cut-off values, and it was found that best results were obtained with the first cut-off values.

Table 1. Percent sero-positivity of different diseases by comparing size values of Ag–Ab complex of different sera with different cut-off values.

Sera	Antigen	Cut-off value = mean of normal sera values with specific antigen (%)	Cut-off value = mean of normal sera values with nonspecific antigen (%)	Cut-off value = mean of HIV sera values with nonspecific antigen (%)	Comparison of each serum value of SP1 and SP2 with respective nonspecific value (%)
			At 1:5 dilution		
HIV	SP1	53	84.5	57.5	82.5
	SP2	52.5	84.5	47.5	55
TB	SP1	90	50	65	80
	SP2	90	35	65	95
FIL	SP1	85	45	41.2	65
	SP2	85	31	35.3	80
			At 1:100 dilution		
HIV	SP1	65	84.5	85	80
	SP2	22	55	57.5	40
TB	SP1	90	66.6	57.1	70
	SP2	100	80.9	76.2	90
FIL	SP1	81.5	44	35.3	62.5
	SP2	87.5	68.8	64.7	83.5

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

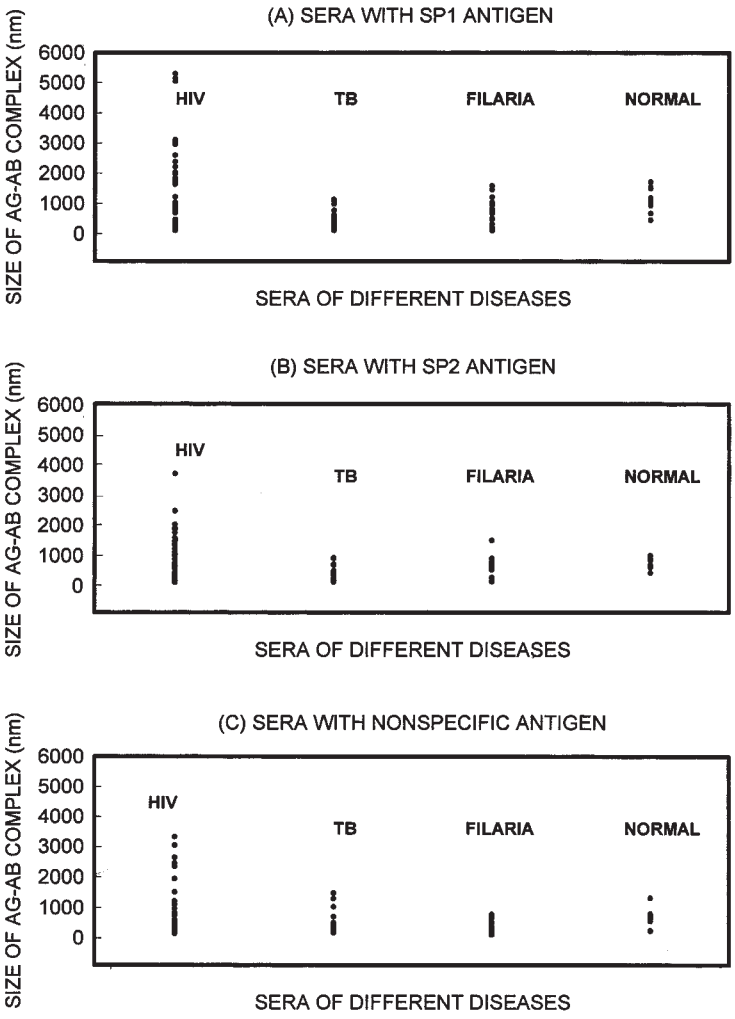

Figure 1. 1:5 Serum dilution.

Our data shows that few normal sera at both dilutions were giving higher agglutinate size values than expected, and few sera samples showed visible precipitation within an hour, even with nonspecific antigen where agglutination was not supposed to occur, and surprisingly, that too with uncoated latex.

The higher values of normal sera and nonaids sera, even with nonspecific (NSP) antigen, may be due to the presence of some sticky

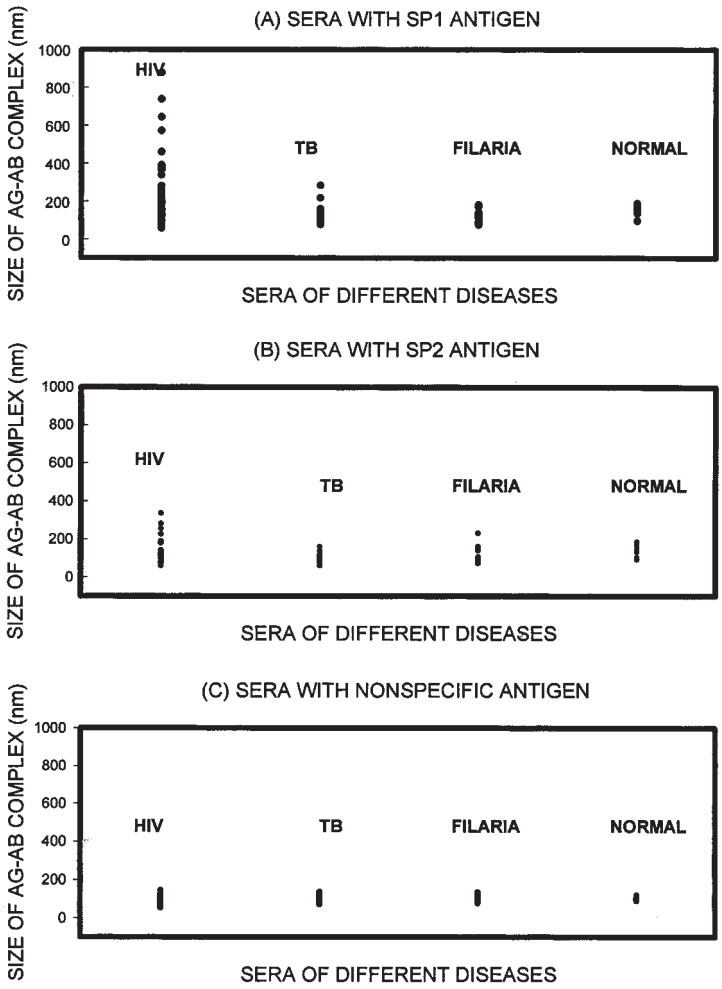

Figure 2. 1:100 Serum dilution.

proteins in the sera, which adhered to the antigen coated polystyrene beads causing a nonspecific coagulation. There are at least three principal types of interactions occurring in our system.

1. Antigen–antibody complex formation or agglutination—this is the specific interaction, which should be the only interaction present.

2. Van der Waals attractions between sticky proteins and the antigen coated latex beads, which cause the nonspecific coagulation.
3. Coulombic repulsive interactions between the beads determined by the surface charge on the beads coated with antigen and/or adhering proteins, which prevented agglutination and gave low readings for HIV samples resulting in false negative results.

The third factor is dependent on pH and ionic strengths, and we tried to suppress this effect by adding salt and detergent, which marginally improved the situation. It seemed the major problem was the nonspecific agglutination, which if removed, will lower the cutoff value, and, thus, the false negative results may fall on the positive side. Light scattering technique is sensitive to sizes of the particles; the nonspecific complexes made significant contributions and artificially gave high size values. Nonspecific agglutination of latex particles by sera from normal individuals is not uncommon,[7] thus though latex agglutination test is sensitive, it results in a fairly high number of false positive cases.[8]

Replacement of Sera by Urine Samples

Few studies were made on paired sera and urine samples to see whether sera can be replaced by urine, keeping in mind the various advantages of using urine. Namely, (a) it is free of disease virus, (b) it can be obtained in large quantities, and (c) it can be stored at 4°C, while sera requires 70°C for storage.

Results show (Fig. 3) that urine samples give the same results, both for specific and nonspecific, i.e., the size of antigen–antibody (Ag–Ab) complex was almost the same. Thus, urine can be used in place of sera and, also, there is a clear distinction between specific and nonspecific antigens. Size values are in the range 70–110 nm for nonspecific antigen and in the range 120–230 nm for specific antigen. Urine samples of non-HIV diseases (Filaria, TB, and pregnancy), as well as healthy controls showed a high degree of sensitivity (Fig. 4A, B). Size values were in the range 40–120 nm, both with specific and nonspecific antigens, showing that in nonHIV samples there is no specific Ag–Ab complex formation.

CONCLUSION

Preliminary investigations have shown that specificity of the assay is better than the sensitivity (Table 1) using the first cut-off value, and SP1

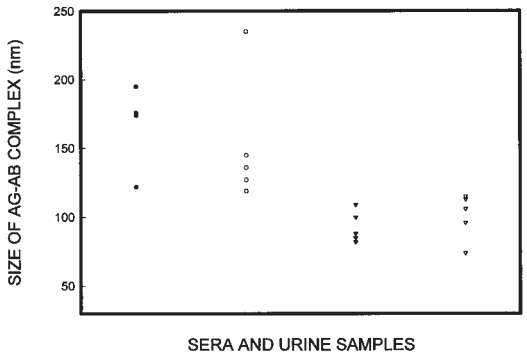

Figure 3. Comparison of the size of Ag–Ab complex formed by paired sera and urine samples with specific and nonspecific antigens.

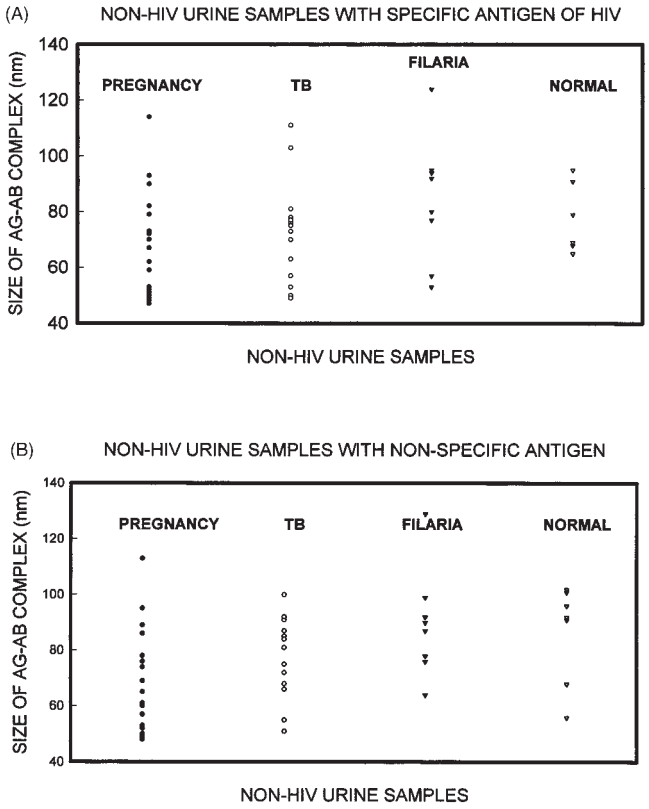

Figure 4. NonHIV urine samples with specific and nonspecific antigen of HIV.

Table 2. Comparison of size of Ag–Ab complex formed by paired sera and urine samples with specific and nonspecific antigens.

	Specific antigen		Nonspecific antigen	
Serum no.	Sera (nm)	Urine (nm)	Sera (nm)	Urine (nm)
1	122	119	88	74
2	176	136	109	113
3	122	127	100	115
4	195	235	85	106
5	174	145	82	96

is found to be more specific than SP2. Had the nonspecific agglutination not been there, definitely our results would have been better. Steps are underway to improve the sensitivity and specificity of LIA by breaking the nonspecific agglutination, using an ultra-sonicator[9] we have designed and developed for the purpose. The representative results obtained from sera paired urine samples certainly show promise, which calls for more in depth investigations (Table 2).

ACKNOWLEDGMENTS

We are thankful to Prof. S. K. Kar for material help, and acknowledge financial support from the Department of Science and Technology, Government of India.

REFERENCES

1. Cohen, R.J.; Benedek, G.B. Immunoassay by light scattering spectroscopy. Immunochemistry **1975**, *12*, 963–966.
2. Bhakat, P.; Roy, A.; Roy, K.B.; Saxena, A.; Bohidar, H.B. Laser light scattering immunoassay for malaria. J. Immunoassay **1999**, *20* (3), 103–114.
3. Bhakat, P.; Roy, A.; Roy, K.B.; Saxena, A.; Bohidar, H.B. Laser light scattering immunoassay for malaria: an alternative to ELISA. Laser News **1999**, *10* (2), 17–21.
4. Antony, T.; Saxena, A.; Roy, K.B.; Bohidar, H.B. Laser light scattering immunoassay—an improved data analysis by Contin method. J. Biochem. & Biophys. Meth. **1998**, *38*, 75–85.

5. Antony, T.; Saxena, A.; Roy, K.B.; Bohidar, H.B. Laser light scattering in the detection of immune complexes. Laser News **1996**, *7*, 11–13.
6. Bohidar, H.B. Characterization of polyelectrolytes by dynamic laser light scattering. In *Handbook of Polyelectrolytes and Applications*; Tripathy, S., Kumar, J., Nalwa, H.S., Eds.; American Scientific Publishers: CA, 2002; Vol. 2, 117–144.
7. Ash, K.O. Reference intervals (normal ranges): a challenge to laboratories. Am. J. Med. Technol. **1980**, *46*, 505–511.
8. *User Comparison of Quantitative Clinical Laboratory Methods Using Patient Samples: Proposed Guideline*; Order code EP9-P. National Committee for Clinical Laboratory Standards: USA, 1985; Vol. 6 (1).
9. Sobanski, M.A.; Ellis, R.W.; Hastings, J.G.M. Rotavirus detection using ultrasound enhanced latex agglutination and turbidimetry. J. Immunoassay **2000**, *21* (4), 315–325.

Received May 2, 2003
Accepted July 14, 2003
Manuscript 3100