Detection of the presence of antibodies against *Toxoplasma gondii* in human colostrum by Raman spectroscopy and principal component analysis

Cuauhtemoc Araujo-Andrade
Juan L. Pichardo-Molina
Centro de Investigaciones en Óptica A. C,
Loma del Bosque No.115
Col. Lomas del Campestre
C.P: 37150, León, Guanajuato, México
E-mail: jpichardo@cio.mx

Gloria Barbosa-Sabanero
Universidad de Guanajuato
Instituto de Investigaciones Médicas
Facultad de Medicina
20 de Enero 929, Col. Obregón
C.P. 37320. León, Guanajuato, México

Claudio Frausto-Reyes
Centro de Investigaciones en Óptica A. C,
Loma del Bosque No.115
Col. Lomas del Campestre
C.P: 37150, León, Guanajuato, México

Angelica Torres-López
Instituto de Investigaciones Médicas
Facultad de Medicina
20 de Enero 929, Col. Obregón
C.P. 37320. León, Guanajuato, México

Abstract. More than 60 million people in the United States and 23 million people in Mexico probably are infected with the *Toxoplasma* parasite, but very few have symptoms because the immune system usually keeps the parasite from causing illness. However, for people whose immune system is compromised, the consequences can be fatal. Toxoplasmosis is detected indirectly by different serological tests, where the sample requires a previous preparation. We analyze the feasibility to use Raman spectroscopy and principal component analysis (PCA) as an alternative method to detect the presence or absence of antibodies IgG (immunoglobulin G), IgM (immunoglobulin M), and IgA (immunoglobulin A), against *Toxoplasma gondii*, in a simple and fast way, in samples of human colostrum from a group of volunteers who were in contact with the parasite and others who were not in contact with the parasite. [DOI: 10.1117/1.2749740]

Keywords: antibodies; Raman spectroscopy; principal component analysis; colostrum; toxoplasmosis; data processing.

Paper 06366R received Dec. 13, 2006; revised manuscript received Feb. 7, 2007; accepted for publication Feb. 7, 2007; published online Jun. 21, 2007.

1 Introduction

Toxoplasma gondii is a protozoan parasite that infects up to a third of the world's population. Infection is mainly acquired by the ingestion of contaminated food or water containing oocysts shed by cats. Primary infection is usually subclinical but in some patients cervical lymphadenopathy or ocular disease can be presented. When the infection is acquired during pregnancy, it may cause severe damage to the fetus. In immunocompromised patients, reactivation of latent disease can cause life-threatening encephalitis.[1–4]

Toxoplamosis detection is made using a serological test, such as the Sabin-Feldman dye test,[5] immunofluorescent antibody test,[6] enzyme-linked immunosorbent assay (ELISA),[7] immunoglobulin G (IgG) avidity test[8–10] and agglutination and differential agglutination test.[11] The principal objective of these tests is to determine the immune condition of the patient. These methods require chemical reagents and special preparation of the sample which increases the overall test time. Particularly, the detection of antibodies associated to an antigen (*Toxoplasma gondii*), in biological fluids like sanguineous serum or in our case human colostrum, is often the only form of diagnosing the infection. Antibodies are one of the principal modes of defense of our body against antigens and are divided[12] into five classes: IgG, IgM, IgA, IgD, and IgE.

In the last decade, it has been demonstrated that using optical spectroscopy combined with multivariate analysis, it is possible to detect and quantify analytes in serum blood and human tissue.[13] Raman spectroscopy is a technique that promises to enable rapid *in vivo* characterization of tissue and bodily fluids compared with those methods currently being used as a diagnostic test.[14–18] The major advantages of Raman spectroscopy are high sensitivity to subtle molecular changes, minimal sample preparation, and noninvasive monitoring.

Furthermore, the spatial resolution of Raman microspectroscopy in the low micrometer scale and its ability to probe samples under *in vivo* conditions enable new insights into living single cells without the need for fixatives, markers, or stains. Raman spectroscopy has been used to characterize normal tissue, benignant and malignant tumors,[19,20] protein characterization, and antibodies in aqueous solutions.[21–28]

This paper shows the promising application of Raman spectroscopy and principal component analysis (PCA), to detect and identify antibodies against *T. gondii* in colostrum samples of pregnant women.

Address all correspondence to Juan Pichardo, Optical Engineering, CIO, Loma del Bosque 115, Col. Lomas del Campestre-Leon, Guanajuato 37150 Mexico; Tel: 52-477-441-42-00; Fax: 52-477-441-4209; E-mail: jpichardo@cio.mx

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Table 1 Negative samples (NS) and positive samples (PS) to antibodies anti-*T. gondii* of human colostrum.

Sample	IgG	IgM	IgA
NS1	−	−	−
NS2	−	−	−
NS3	−	−	−
NS4	−	−	−
NS5	−	−	−
PS6	+	−	−
PS7	+	−	−
PS8	+	−	−
PS9	−	+	−
PS10	−	+	−
PS11	−	+	−

2 Materials and Methods

This study was carried out with more than 600 pregnant women; the volunteers answer a questionnaire before they were selected to participate in this study. We selected 208 women for the ELISA test to detect and identify antibodies against *T. gondii*. However, calostrum samples were obtained from only 39 volunteers, and 11 women gave a positive result. The volunteers selected were from the central region of Mexico and had similar socioeconomic and ethnic life styles. The mean age of the group was of 21.2±1.3 yr. Written consent was obtained from volunteers, and a study was conducted according to the Declaration of Helsinki.

2.1 *Colostrum Samples*

Colostrum samples were obtained and collected from the department of gynecology and obstetrics of the General Regional Hospital and the Infantile Maternal Hospital, León, Gto., Mexico. To make the ELISA test and the Raman measurements of the colostrum, the samples were centrifuged to 2050 rpm for 20 min at 4 °C, to separate the lipid and cellular structure.

Each sample was tested by the indirect ELISA at the Institute of Medical Research of the University of Guanajuato. For this study six positive and five negative samples were selected. From these six positive samples, three were positive to IgG only, and three were positive to IgM, as shown in Table 1.

2.2 *Raman Spectroscopy*

Raman spectra (RS) were obtained using a Raman system with a back scattering geometry. In this system, linearly polarized radiation of 514.5 nm from a 2.6 W water-cooled argon laser (Spectra Physics, Stabilite 2017) was used as an excitation source. The laser light was focused on the sample with a 40× microscope objective. RS were recorded with a monochromator (Jobin Yvon, HR 460) equipped with an air-cooled CCD (256 × 1024 pixels), and a 1200 grooves/mm grating. The Grams software (version 3.04) was used to acquire the spectra. To reject Rayleigh emission light and plasma frequencies of the laser a holographic Super Notch-Plus filter (Kaiser Optical Systems, HSPF-31453) and an interference filter (Melles Griot, 03 FIL 204) were used, respectively. The Raman system was calibrated using the 520 cm^{-1} Raman line of a silicon wafer; Fig. 1 shows a sketch of the experimental system.

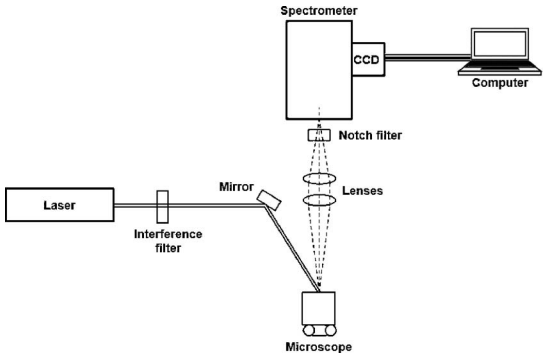

Fig. 1 Schematic diagram of the experimental setup of Raman spectroscopy.

To collect the RS, a drop of each sample was put onto an aluminum substrate, the solid residues were examined after the sample dried, and then a zone of the sample was focused in the microscope. Multiple spectra were obtained on the solid residues of each sample by moving the substrate on an *X-Y* stage. A total of 165 RS were obtained from the 11 samples of colostrum, where 75 spectra correspond to negative samples and 90 spectra correspond to positive samples to *T. gondii*. To collect the RS, each sample was irradiated with a laser power of 18 mW using 10 s of acquisition time.

2.3 *Data Preprocessing*

The baseline was corrected from RS to eliminate the fluorescence contribution of each spectrum, and smoothed using the adjacent averaging method using 10 points for the averaging and normalized applying the maximum normalization transformation. Finally, PCA algorithms were performed on RS to locate groupings that differentiate positive samples from the negative samples.

2.4 *PCA*

PCA is a multivariate technique acting in an unsupervised manner and is used to analyze inherent structure of the data. PCA reduces dimensionality of the data set by finding an alternative set of coordinates: principal components (PCs). PCs are linear combinations of original variables, orthogonal to each other and designed in such a way that each one successively accounts for the maximum variability of the data set. When PC scores are plotted, they reveal relationships between the samples. A PC score plot provides insight into how much variance is explained by each PC, and how many PCs should be kept to achieve an acceptable correct classification.[26]

Data analysis was performed using The Unscrambler version 8.0 (Camo AS, Oslo, Norway).

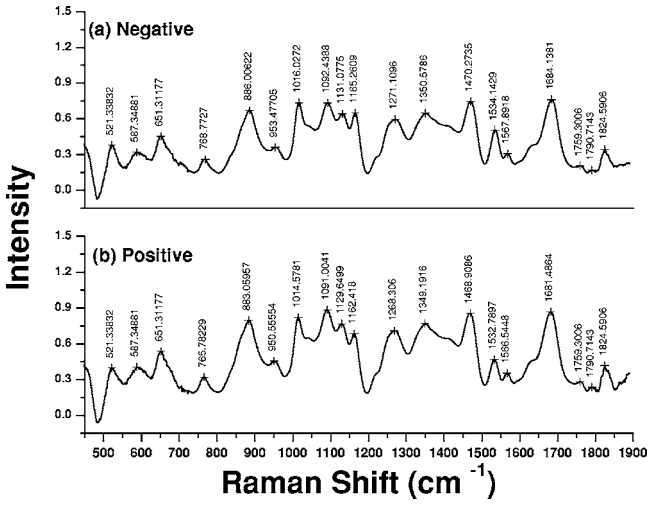

Fig. 2 Mean RS of (a) negative and (b) positive samples of human colostrum in the range from 450 to 1900 cm^{-1}.

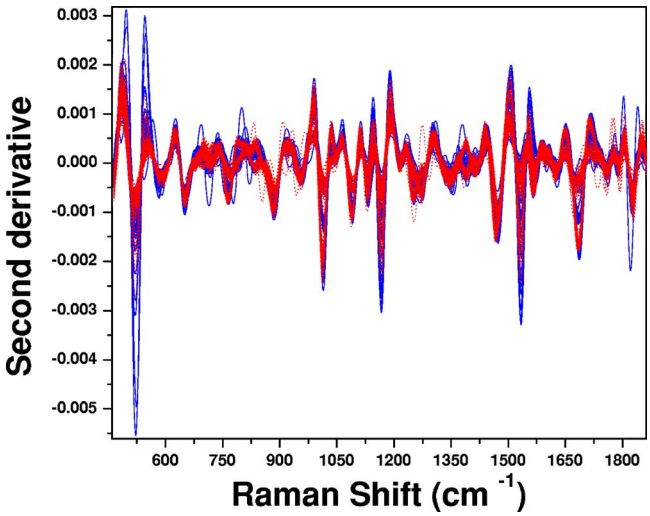

Fig. 3 Second derivative of RS of the positive and negative samples of human colostrum: dotted line, positive samples, and solid line, negative samples.

3 Experimental Results

RS and the second derivative of anti-*T. gondii* were plotted for their visual inspection. The positive and negative samples look similar, and for this reason mean spectra of each group were obtained to identify the differences more clearly. Figure 2 shows the mean spectra of negative and positive groups of human colostrum, respectively, in the range of 450 to 1900 cm^{-1}. The principal difference we observed between them is that the RC of negative samples show a shift of 2 to 3 cm^{-1} in some peaks such as 886, 1016, and 1684 cm^{-1} with respect to the corresponding bands of positive samples, while peaks centered at 521, 587, 651, 1759, 1790, and 1824 cm^{-1} did not show any shift between positive and negative samples.

The visual inspection of mean RS did not show any remarkable differences between positive and negative spectra, thus, PCA was performed and applied to our data. However, PCA was unable to reveal a clear discrimination between the spectra of positive and negative samples using the original RS.

Because discrimination between positive and negative samples was impossible by visual inspection and using PCA on the original RS of the colostrum samples, PCA analysis in a cross-validation method was used on the second derivatives of the RS of the samples to obtain the discrimination of the samples.

The second derivatives of each mean spectra were obtained based on the Savitzki-Golay algorithm using a polynomial function of second order with five data-point windows (see Fig. 3).

The first or second derivatives are common transformations of continuous function data and are often applied in spectroscopy; derivatives are useful to emphasize small differences in spectra. Some local information is lost in the differentiation, but the "peakedness" is supposed to be amplified and this trade-off is often considered advantageous.

The PCA analysis shows that the optimum number of PCs that explain the maximum variances of the data set is five. PC1 explains 16%, PC2 explains 7%, PC3 explains 5%, PC4 explains 4%, and PC5 explains 3%.

Figures 4(a) and 4(b) show the scatter plots of PC2 versus PC1 and PC3 versus PC2, respectively. In these score plots, negative and positive samples are divided by the solid line; the line highlights the discrimination between both groups of samples given by PCA. Also, the plots of scores of PC4 versus PC2 and PC5 versus PC2 were created and good separability between positive and negative groups in both plots was observed. And, for example, PC1 versus PC3 and PC4 versus PC5 do not show good separation between positive and negative samples.

From these results, it was found that PC2 is the factor that explains the differences associated with the absence or presence of antibodies anti-*T. gondii* in the colostrum samples, causing a well-defined separation between samples in the 2-D score plots, as is shown in Figs. 4(a) and 4(b).

It has been reported that loading vectors plotted as a function of the original variables exhibit peaks related to the maximum difference between samples.[29]

To investigate this, the loading vector for the PC2 component was plotted as a function of Raman shift. This new spectra shows the presence of the antibodies IgG, IgM, and IgA against *T. gondii* (see Fig. 5). The peaks or bands with maximum amplitude centered at 1119, 1172, 1195, 1513, 1542, and 1558 cm^{-1} are related to the position of the vibrational modes of the molecules, and are related to antibodies that have the maximum contribution to the spectra. The band centered in 1119 cm^{-1} has a tentative frequency assignment associate to $\nu(C\text{-}N)$,[31] at 1172 cm^{-1} to Tyr+Phe,[31,32] the band centered at 1558 cm^{-1} to protein retinal C=C—C=O,[32] the band centered at 1195 cm^{-1} to aromatic amino acids,[30] the band centered at 1513 cm^{-1} to lycopene,[32] and the band centered at 1542 cm^{-1} to Trp.[30] PCA shows that local differences between positive and negative samples are given by the preceding six bands. However, it is not possible to conclude whether these changes resulted only from changes in concentration or

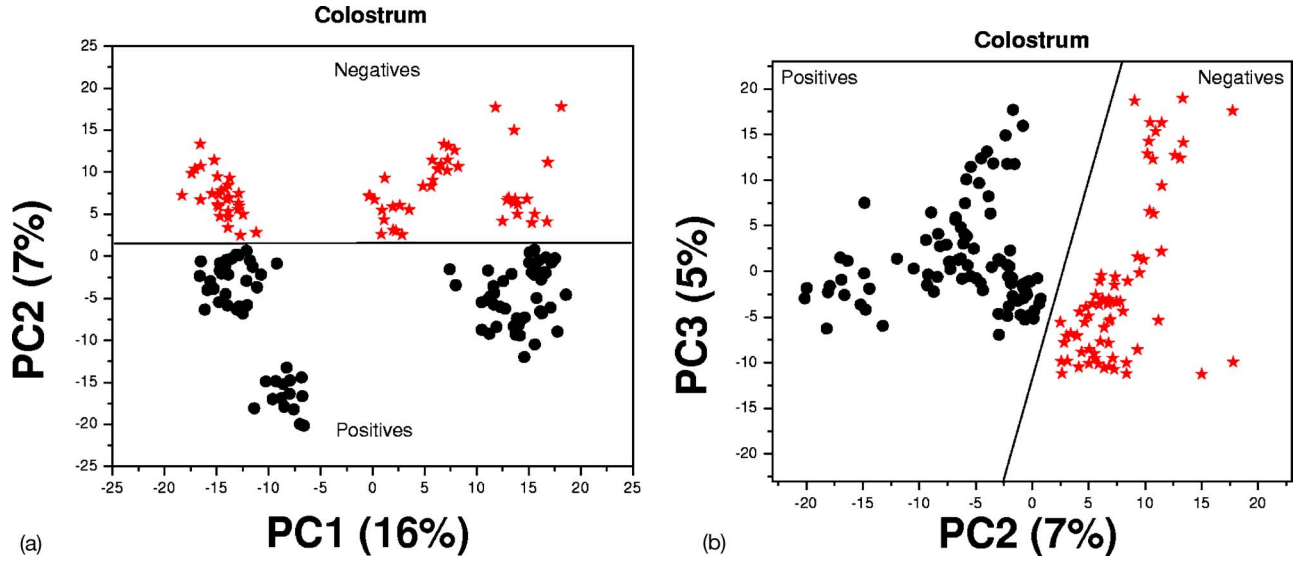

Fig. 4 Two-dimensional score plots of the second derivative of RS of human colostrum: (a) PC1 versus PC2 and (b) PC3 versus PC2.

from changes of molecular structure. To verify this hypothesis additional studies are required.

4 Summary

Raman spectroscopy and multivariate analysis PCA were used to make the detection of anti-*T. gondii* antibodies in colostrum samples of a group of pregnant women. In the original RS of colostrum samples, it was impossible to differentiate the positive and negative samples using visual inspection and PCA. To highlight the differences between spectra of positive and negative samples, PCA was conducted on the second derivatives of RS.

The 2-D scatter plots of the transformed RS of colostrum samples, PC2 versus PC1 and PC3 versus PC2, show a well-defined separation between negative and positive samples.

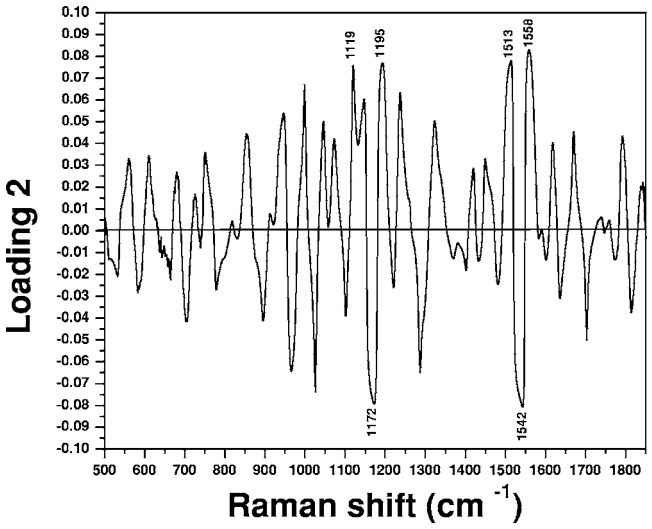

Fig. 5 One-dimensional loading plot of PC2; the peaks with highest amplitude show the positions of the principal differences between positive and negative samples.

This separation is mainly attributed to the frequency shifts of some bands and peaks that could be identified in the 1-D loading plot of PC2. These spectral variations are associated to the presence or absence of antibodies IgG, IgM, and IgA against *T. gondii* in colostrum samples.

In addition it is shown that the second derivative transformation is a powerful tool for the analysis of RS when the information in the original RS does not discriminate between groups of samples.

According to the results obtained with PCA, the main differences between positive and negative samples of colostrum correspond to the zone of about 900 to 1600 cm^{-1}, where tyrosine, phenylalanine, protein retinal, aromatic amino acids, and lycopene are presented.

5 Conclusions

The results presented here show that Raman spectroscopy and multivariate methods are suitable techniques for the analysis of biological fluids with the aim of clinical diagnosis of toxoplasmosis disease. In this paper, colostrum samples with IgG, IgM, and IgA antibodies including the IgG, IgM, and IgA anti-*T. gondii* were studied using Raman spectroscopy, which was capable of detecting the slight structural and chemical differences between the antibodies anti-*T. gondii*. It was also demonstrated that data preprocessing (for the second derivative) prior to PCA was a useful method to extract "hidden" information from spectroscopic data when the original spectra are not substantially different for visual inspection or analysis.

Finally, by means of PCA analysis of transformed RS, it was possible to discriminate between groups of positive and negative samples, and identify that the second score factor was responsible for the discrimination between samples.

Acknowledgments

The authors wish to thank Consejo Nacional de Ciencia y Tecnología (CONACYT) and Consejo Nacional de Ciencia y Tecnología (CONCyTEG) for financial support under Grant No. 0604k117090 Anexo1 and Grant No. 07–04–K662–080

Anexo2. We want to thank the editor and the referees for their valuable comments to improve this work. Also, we thank Martin Olmos for his technical assistance.

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