Commentary
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Immunology and art: Using antibody-based techniques to identify proteins and gums in artworks

Our diverse world cultural heritage encompasses a vast range of objects, from fine art to monumental cultural heritage sites, which represent not only artistic developments but also power, politics and commerce. They are combinations of materials and cultural influences that vary over time and according to style, taste, region and artist or workshop. The careful study of how an artwork is made is critical to its preservation: correct identification of materials can guide conservators in treatments but also can direct curators and conservators in display and storage strategies. This identification can also help reveal working practices of the artist, studio or guild: for example, the use of egg tempera, egg mixed with oil or only oil as a binding media in paintings marks a distinct alteration in working practice in Italy in the mid to late 15th century (Dunkerton 1996; Higgitt and White 2005). Furthermore, the identification can also give insight into the trade customs of a town or region and, in some cases, may facilitate authenticating, dating, and determining the regional provenance of an object. For example there are pigments that were only used over certain time periods or became commonly used only later in the 19th century: thus their presence, in conjunction with other supporting evidence, can aid in determining the likely date of origin for an artwork. Scientific examination is critical in providing this knowledge and is an essential component in modern technical studies of artworks and cultural heritage.

Throughout history a great variety of natural products have been used in art and one of the most common applications of organic materials has been as binding media, adhesives and varnishes[1]. Mixed with pigments they form the basis for paints (binding media), as adhesives they allow solid joints in furniture, and as varnishes they supply protective coatings for paintings, wood, and other decorative surfaces. Animal and plant materials used in art as binding media and adhesives include oils, waxes, resins, gums, mucilages and proteins. Eggs, milk, and animal glues (made from bones, skin, or fish bladders) composed primarily of the proteins ovalbumin, casein, and collagen — can not only be found in artworks but are also used to conserve art. Plant gums such as gum tragacanth[2], cherry gum, and gum arabic, known as polysaccharides, have been used mainly as binding media for water soluble paint.

Analysing the binding media and adhesives on artworks presents unique challenges. The natural materials, which can be used individually or in combination, are actually complex chemical mixtures whose composition can alter over time due to human intervention (e.g. conservation treatments), environmental conditions (heat, light, humidity, biodeterioration), and chemical interactions between the components of the mixture.In addition, the organic material can be encased in a complex solid matrix, such as pigments, and its concentration in that matrix tends to be very low. Furthermore, the original organic binder or adhesive may itself be a mixture (multiple protein sources or oil and protein mixture, for example) or may have become so via the migration of materials, for example between layers of paint during conservation treatment with adhesives or the application of coatings. And finally, that a sample of the artwork is required in order to identify proteins and gums dictates that the analytical techniques be

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[1]Natural products are also the source of organic materials used as pigments and dyes, for example cochineal is extracted from insects (*Dactylopius* spp.) (Cardon 2003; Phipps 2010), and early Indian yellow was derived by collecting the urine of cows who had consumed marigold flowers (Baer *et al*. 1986). These dyes and pigments are not the topic of this publication.

[2]Gum Tragacanth is the dried sap (a mixture of polysaccharides) obtained from Middle Eastern legumes of the genus *Astragalus*, including *A. adscendens*, *A. gummifer*, and *A. tragacanthus*.

both sensitive and specific. One of the main concerns of art-focused scientific analysis is how to obtain information either without a sample or by taking the smallest possible representative sample.

Protein and gum analytical methods are a complex subject. Only a brief, simplified discussion can be mentioned in this paper and further details can be found in the literature (Schultz *et al.* 2009, ref 14–25). Nowadays, commonly used methods for protein and gum analysis in works of art are micro-chemical (e.g. staining methods, spot tests) and instrumental methods (e.g. Fourier transform infrared spectroscopy (FTIR), gas chromatography mass spectrometry (GC-MS), high performance liquid chromatography (HPLC)). Micro-chemical tests used in conservation can lead to the sorting of organic materials into classes such as proteins, oils, and carbohydrates, for example. (Schramm 1995) FTIR can also characterize organic materials into classes, and is generally a more reliable technique than micro-chemical tests. In addition FTIR can identify waxes and in some cases can further characterize certain organic materials into sub-classes (e.g. diterpenoid and triterpenoid resins). However, if the concentration of the organic material is low and if there are IR-active inorganic compounds present (such as calcium carbonate, for example), the IR signal from the organic component can be masked. Other analytical techniques (e.g. GC-MS and HPLC) can lead to a more specific identification of the organic material (collagen, linseed oil, beeswax etc.). Mass spectrometric techniques (proteomics) have recently demonstrated some success with protein identification in artworks but currently are not common techniques in conservation laboratories. However, all of these analytical methods are associated with an expert level of knowledge and skills and require relatively expensive equipment.

Methods that reply on amino acid concentration for the identification of proteins such as GC-MS and HPLC are compromised if there is biological contamination or if there is a complex mixture of proteins present. The small sample size that is usually available for analyses, with a trend to decrease it even further, can be another factor for these techniques. While FTIR requires only a very small amount of material for analysis (typically a barely visible scraping or chip smaller than a period in this text.), the information that can be obtained is limited. GC-MS and HPLC analysis require larger sample sizes than FTIR[3], which can be prohibitive for some artworks. In summary, the commonly used methods for the characterization and identification of organic materials such as staining methods, chromatographic and spectroscopic techniques can be limited in unfavorable cases by sample size, sample mixture and degradation as well as by the influence of micro-organisms. Immunological or antibody-based techniques provide an alternative approach to the identification of proteins and gums.

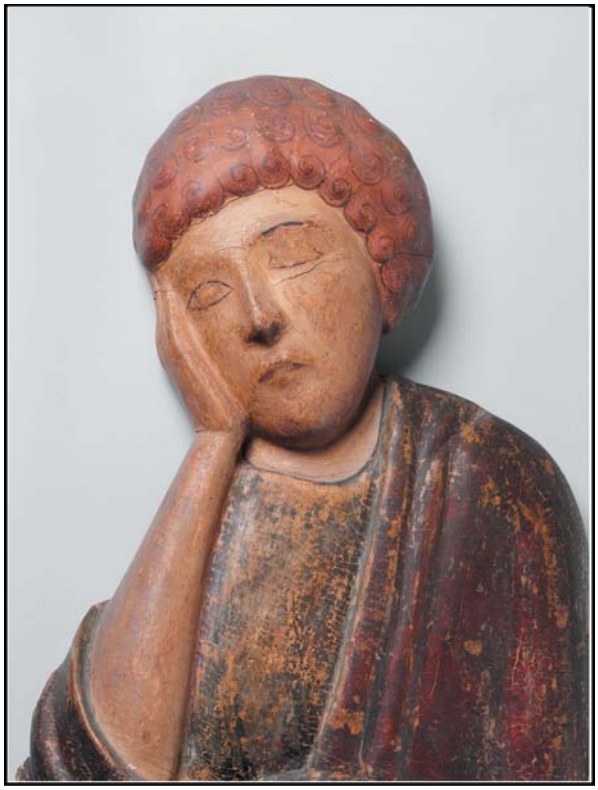
The specificity and sensitivity of immunological techniques has driven investigations in archaeology and conservation research periodically over the last thirty years. (e.g. Acenzi *et al.* 1985; Hyland *et al.* 1990; Loy 1993; Cattaneo *et al.* 1995; Scott *et al.* 1996; Hodgins and Hegdes 2000) However, typically the proteins or gums are found in low concentration in an inorganic matrix (archaeological bone or paint, for example) and the relatively large sample size required was a significant deterrent for applications to artworks. In the last decade, there has been resurgence in the application of immunological techniques to artworks primarily because of the commercial availability of antibodies to proteins from species of interest, increased specificity and sensitivity as well as the overall decrease in cost. Recent research has successfully demonstrated the use of immunological methods in the field of conservation science for the identification of proteins and gums in artworks. (Hodgins 1999; Heginbotham *et al.* 2004; Mazurek 2006, Scott *et al.* 2009) In particular, the enzyme-linked immunosorbent assay technique (ELISA) offers great potential, since it requires minimal sample handling, is highly sensitive and specific, and so far appears less affected than other methods by protein and gum degradation or contamination. Once the technique is established, ELISA is reasonably straightforward and relatively inexpensive and does not require sophisticated instrumentation. But the major benefit of ELISA is its ability to screen simultaneously for several closely related binding media in a single sample and to specifically identify the components of the mixture.

ELISA procedures developed for biotechnology must be adapted to take into account the challenges presented by samples from artworks: an ELISA procedure developed in a medical laboratory to detect a specific disease marker would not give optimal results for detection of ovalbumin in, for example, a paint

[3]The sample size for techniques such as GC-MS or HPLC can be as low as tens of micrograms for a sample containing only the organic material of interest however sometimes the analysis requires significantly more material due to the low concentration of intact organic material of interest in an inorganic matrix or natural ageing processes which can alter the chemical structure of the protein.

sample from a polychrome sculpture. The state of degradation of the antigen, interference from pigments and other impurities, biodeterioration, and the efficiency of the extraction method can all impact the ELISA response (i.e. antibody-antigen complexation, interference with the reporting system, etc.). Therefore, ELISA analyses for art samples is not quantitative and can only definitively confirm the presence of those proteins or gums that were part of the assay screening. Challenges such as sample extraction from an inorganic matrix, low organic concentration in the sample and unknown degradation state of the proteins and gums all diminish the quantitative nature of ELISA. In addition unknown concentrations of soluble cations from pigments can also effect the antigen conformation, antigen-antibody complexation and even the reporting system. When two materials are shown to be present by ELISA, generally the relative intensity of their colorimetric response cannot be construed as an indication of concentration. In case of multiple proteins in a single sample, ELISA results only prove that two or more proteins are present but not in which concentration. It is advised not to relate OD value intensities with protein quantitation when dealing with art samples. However, in the cases presented here a comparison of intensities (semi quantitative) is possible since the layers/parts analyzed are from the same sample and were tested on the same plate and in the same experiment.

During the past three years the Department of Scientific Research at the Metropolitan Museum of Art has been adapting immunological methods with assistance of Columbia University[4], in particular ELISA, with the aim of developing a broad method for reliable and reproducible assays that will allow screening for the most common proteins and gums used in artworks from a single sample in a single experiment. Colorimetric indirect ELISA assay methodologies were optimized for art samples using polyclonal primary antibodies in order to increase the likelihood of antigen detection. After the parameters for the ELISA protocol and materials were determined, the technique was extensively tested and evaluated on samples from replicas before it was applied to artworks.

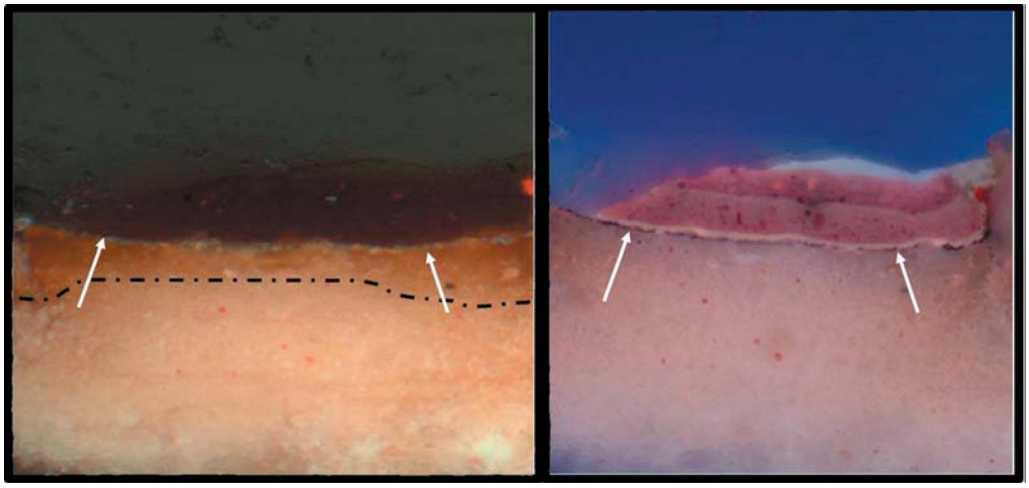


Figures 1. *Saint John* (with detail). Italy, late 13th century. Red pine covered with canvas, gesso, and tin foil and painted with oil glazes; height 571/2 in. (146.1 cm). The Metropolitan Museum of Art, The Cloisters Collection, 1925 (25.120.215).

[4]Initially Dr. David Scicchitano, Department of Biology, New York University facilitated the project.

The ELISA method proved invaluable in the study of *Saint John*, an Italian polychrome statue from a late thirteenth-century Crucifixion group at The Cloisters (Figure 1). The figure was carved from red pine that was covered with canvas and gesso[5] and painted. The garments are gilded with tin foil, or tin leaf, and glazed with oil-resin coatings. In polychrome sculptures the gesso layer is a foundation layer, usually composed of chalk or gypsum combined with animal glue (collagen). (Cennini 1960, pp. 67–71) If the gilding[6] is burnished, the leaf would have been applied either on an additional bole layer[7] or directly on the gesso with animal glue or egg white (ovalbumin), which is called ground-gilding. (Nadolny 2006) As part of his research on Italian medieval sculpture to be included in a forthcoming catalogue of the collection (Castelnuovo-Tedesco and Soultanian 2010), Jack Soultanian, conservator in the Department of Objects Conservation, wanted to determine the nature of the adhesive saturation of the gesso layer he observed just beneath the gilding on the *Saint John*, which is typical of ground-gilding.

A sample containing all the layers of gesso, paint, gilding, and glazes was taken from the sculpture. Examination of a mounted cross section of the sample under the microscope showed a slight saturation of the gesso immediately beneath the metal leaf layer (figure 2), confirming that an adhesive may have been used for the application of the leaf. Attenuated total reflectance (ATR)-FTIR microspectroscopy of the cross section could verify the presence of protein in the gesso and in the upper saturated area but could not provide specific information about its identity. It did, however, exclude an oil or a resin as a binding adhesive. Indirect ELISA testing for collagen, ovalbumin, casein, and gum was proposed. ELISA can easily identify mixtures of binding media but only gives information on the protein and gum content of the whole sample. Because the gilding adhesive did not form a distinct layer that could be clearly separated from the gesso or the gilding layer, the sample for analysis was a mixture of the two materials. A scraping from the gesso layer immediately below the gesso saturated with adhesive was also sampled

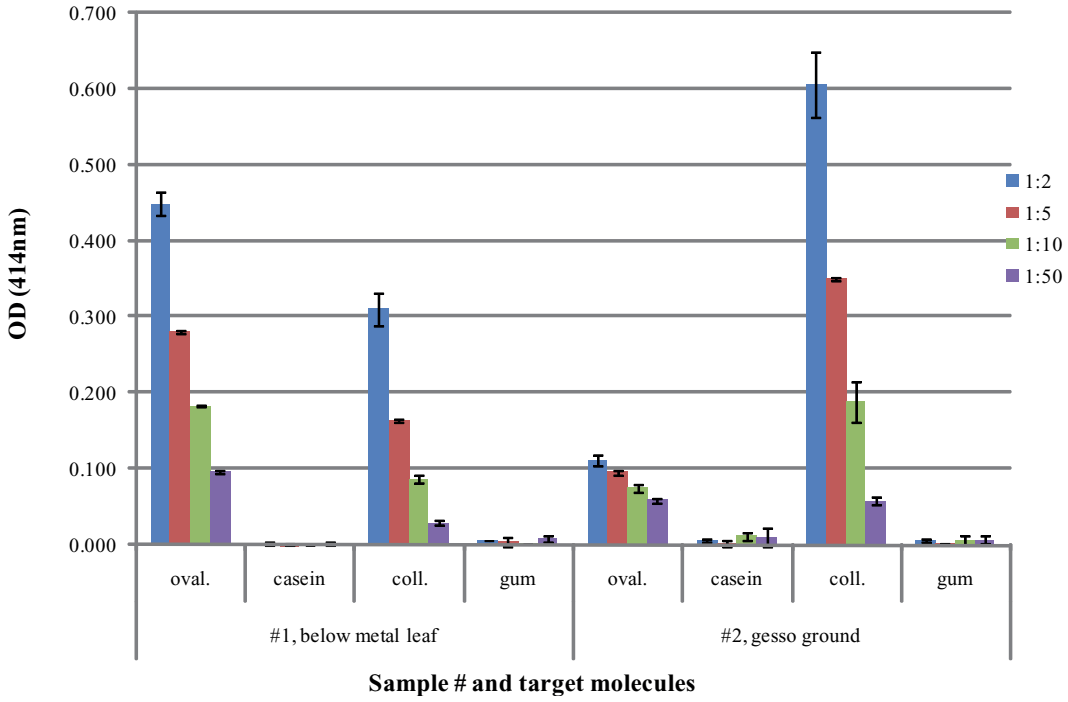


Figures 2. Cross section of a sample of layers of gesso, paint, gilding, and glazes on *Saint John* in normal (left) and ultraviolet (right) illumination. In normal illumination a slight saturation of the gesso immediately beneath the metal leaf layer (indicated by the white arrows) is visible (out-lined by the black dotted line), indicating that an adhesive was used for the application of the leaf. Above the thick gesso layer is a very thin layer of tin leaf (indicated by white arrows in both photographs) that is barely visible under normal illumination but can be clearly seen as a thin, dark layer under ultraviolet illumination. Various oil-resin coatings lie on top of the tin leaf. Original magnification 200x.

[5]In Western European art, gesso is traditionally chalk with an animal glue binder. It is the layer between the support (canvas or wood) and the gilding or paint layers for many paintings and sculptures. It is usually applied in many thin layers often with sanding in order to provide an even, absorbent surface for painting or gilding.

[6]"Gilding" is the practice to embellish an object with gold leaf or to try to make it look like gold or precious metals using less expensive metal leaf. Besides gold, silver and tin metal foils have also been used for gilding, in combination with colored glazes, in order to achieve a rich, opulent effect.

[7]Bole is a mixture of clay and animal glue and can occur in many colors including black, yellow and red-brown. It is polished smooth before the application of the metal leaf. The animal glue in the bole layer can be reactivated with water in order to achieve a very smooth gilding effect called "water gilding".



Figures 3. ELISA result for the identification of the binding medium on *Saint John* (figure 1). On the left is the result for a scraping of the gesso layer and the gilding adhesive immediately below the metal leaf. On the right is the result for a scraping of only the gesso layer beneath those two layers. In order to confirm the presence of the antigen, four dilutions were prepared from each sample, one for each anti-body: ovalbumin (egg), casein (milk), collagen (animal glue), and polysaccharides (gums). Both ovalbumin and collagen are clearly present in the first sample, while only collagen is present in the second. Thus the binder for the gesso is animal glue and the adhesive for the gilding is egg.

for comparison, so that the binder of the gesso could be distinguished, if appropriate, from the gilding adhesive. The ELISA analysis clearly showed that collagen (animal glue) is the binder for the gesso and that ovalbumin (egg) is the adhesive for the gilding (figure 3).

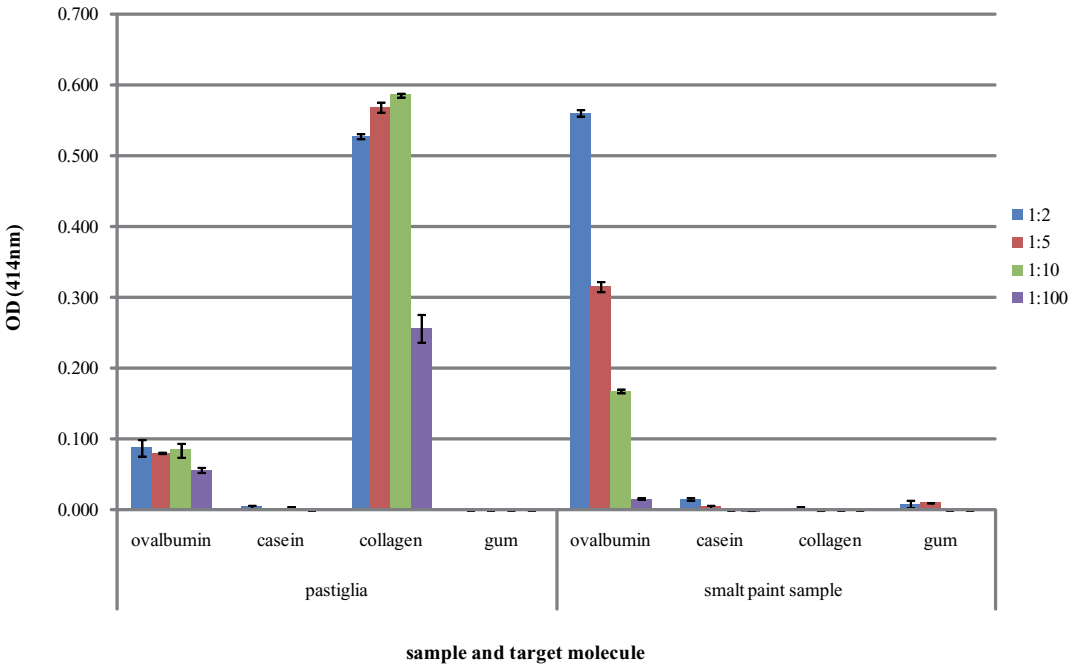
The indirect ELISA method was also applied to the examination of the Nur al-Din Room (figure 4), a winter reception room from an upper-class Syrian house with inscriptions dating it to 1707. The room's woodwork is ornamented with a technique called '*ajami*, using raised designs made of gesso (pastiglia[8]), mainly in floral patterns or poetic inscriptions in Arabic, decorated with gold and tin leaf, paint and glazes. The literature suggests that animal glue and egg, as well as gums, may have been used as binding media for the pastiglia and the different paints (Matthews 1997; Baumeister *et al.* 2010). The possibility that mixtures of proteins and gums may be present makes these materials ideal candidates for ELISA analysis. Two different samples were taken, one from the pastiglia underlying paint layers on a ceiling beam and the other, a blue smalt paint sample, from a protected area on the tenon[9] of a cornice section (figure 5). The samples were tested for ovalbumin, casein, collagen, and gum (figure 6). The result for the pastiglia shows the presence of two different proteins: collagen is the main binding medium, yet traces of ovalbumin were also detected. That the binding medium was a mixture of proteins cannot be excluded, but it is more likely that the ovalbumin migrated or penetrated from other paint layers or was introduced during a former consolidation or restoration. The result for the protected smalt paint clearly shows that ovalbumin is the only binding medium present (which means that the ovalbumin in the pastiglia is likely to have come from the paint layers above). The results are significant because they clearly demonstrate that different binding media were selected for different pigments and different types of paint. (Schultz, *et al* 2010).

[8]Pastiglia is a type of raised decoration found in paintings, sculpture and room decoration that is made by the careful application of thick gesso in intricate designs.

[9]A tenon is half of a physical joint in a wooden object called a "mortise and tenon" joint. The tenon portion is a tongue of wood that will fit inside a corresponding opening (mortise) in another piece of wood.

Figures 4. Nur al-Din Room. Damascus, Syria; dated ah 1119/ ad 1707 in an inscription anddecorated with wood, marble, stucco, glass, mother-of-pearl, ceramics, tile, stone, iron, colours, and gold; height 22 ft. 1/2 in., width 16 ft. 81/2 in. (6.72 × 5.1 m), distance from inside front entrance to back wall 26 ft. 43/4 in. (8 m). The Metropolitan Museum of Art, Gift of The Hagop Kevorkian Fund, 1970 (1970.170).

Figures 5. To test for ovalbumin, casein, collagen, and gum, samples were taken in the Nur al-Din Room (Fig. 1) from areas of the '*ajami*' (pastiglia near smalt paint) on a ceiling beam (left) and from a patch of smalt paint from a protected area on a cornice section (right).



Figures 6. ELISA result for the identification of the binding medium in the pastiglia and smalt paint from the Nur al-Din Room (figure 3). From each sample, four dilutions of the extraction were prepared for each antibody: ovalbumin (egg), casein (milk), collagen (animal glue), and polysaccharides (gums). Both collagen and ovalbumin are clearly present in the pastiglia sample, while only ovalbumin is present in the smalt paint. This result suggests that the ovalbumin traces found in the pastiglia are a result of migration or penetration from the smalt layer above. Thus, the binder for the pastiglia is animal glue (collagen) and the binder for the smalt paint is egg (ovalbumin).

Antibody-based techniques are clearly poised to take a significant and complementary role in the analysis of artworks in the near future. Already the ELISA technique has proven to be an effective and useful analytical tool, its greatest asset being the unambiguous identification and differentiation of proteins and gums, even in mixtures, in a single experiment on a relatively small sample. In the Department of Scientific Research ELISA is being used to analyze a broad range of art samples in order to ensure the reliability of the technique, and ongoing research designed to ameliorate limitations such as sample extraction, pigment interference, and degradation state will continue to improve our ability to apply the ELISA technique to artworks and interpret the results.

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