

What type of blood is present on the Shroud of Turin? Existing Data vs. To Be Determined

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Abstract

Initial characterization of the bloodstains on the Shroud of Turin indicates that blood components are present, findings that are consistent with the presence of real blood on the cloth. The bloodstains on the Shroud have been primarily evaluated from a chemical perspective, though much less so from the context of immunology, particularly in elucidating the species of origin. For example, while it is commonly reported that the blood is human, the immunological data for the type of blood present on the Shroud remains incomplete. To date, this issue is still scientifically unresolved. Furthermore, it is often misunderstood that previous studies have ruled out the possibility that blood from other animal types (cow, horse, chicken, etc.) might be present on the Shroud, when, in fact, this subject has never been addressed by experimentation. Indeed, with one exception (rabbit), which occurred serendipitously, no data exists regarding this issue. Here, the current knowledge of the types of blood that might exist on the Shroud of Turin is discussed, emphasizing results for which empirical data actually exists, the strength of such data, and estimations that remain to be determined.

Initial Characterization of Bloodstains: Blood is present

When the Shroud of Turin was scientifically examined almost forty years ago, one of the fundamental questions addressed was “Does real blood exist on the cloth?” Various chemical tests were performed by two major groups, those headed by Baima Bollone, an Italian scientist, and the scientists Heller and Adler, associated with the Shroud of Turin Research project, or STURP, the American research team, led by Jackson and Jumper. Baima Bollone extracted threads from the cloth for his studies whereas the STURP team relied on tape-lift samples taken from the Shroud’s surface. The bulk of Heller and Adler’s work was published in peer-reviewed scientific journals, while most of Baima-Bollone’s findings were reported in Shroud specialty journals, particularly *Sindon*, and also in various compendium-style books. John Heller would also describe his findings (and experiences) in a narrative style book. The conclusion of both parties was that the bloodstains on the Shroud were, in fact, composed of blood (1-14). Others have challenged these findings, particularly the noted microscopist, Walter McCrone, who believed the bloodstains were merely painted on, and thus solely the product of pigments (15-17), although the majority of the evidence would go against this point. Moreover, such arguments do not take into account the immunological data that has been reported for bloodstained regions, gathered by both Baima Bollone and Heller and Adler, which is the main subject of this paper.

What type of blood is it? Part I- Have human blood components been detected?

After initial, presumptive testing indicated that real blood was present on the cloth, the logical extension was to determine what type of blood might exist. Scientifically, there are two types of methods that allow the species of origin to be determined for bloodstains: immunological testing using antibodies directed against specific blood components and DNA testing, evaluating

expression of particular genes of interest. Whereas DNA testing is often at the forefront of modern scientific evaluation in such cases, DNA studies on the Shroud are particularly hampered by the possibility of contamination by individuals throughout its history. While it has been reported that human DNA has been found on the Shroud (18-20), such results are essentially meaningless in demonstrating that the blood is of human origin as similar data could exist by an individual touching the cloth or even standing over it (21-25). It is important to bear in mind that unlike other studies in which DNA is extracted from tissue or bone, the Shroud is an imprint (either authentic or manufactured) of the form of a human body with bloodstains that may have been transferred or applied. Given the communal nature of the object in its past and even more recent history, certain restrictions must be placed on any conclusions that may be drawn from the data. Indeed, for any of the DNA findings reported, there is no evidence that any of the DNA signals specifically come from blood cells (21). Relatedly, it is scientifically unknown if all of the bloodstains arrived on the Shroud at the same time and are from same individual, or even the same species (see below).

The results of immunological testing on Shroud samples are of greater strength than the DNA data, although not without caveats, particularly at the most critical stage (see below). Immunological tests were among the last studies that Heller and Adler would perform, at a time when the amount of sample left for testing was waning (4,11). Indeed, most of their effort was invested in chemical testing to first determine if the samples tested positive for blood. Baima Bollone would also perform immunological testing, including the ABO typing of bloodstains. These latter findings have been previously reviewed in detail (26), and are only briefly referred to below in the relevant context.

Immunological testing relies on the use of specific molecular tools termed antibodies which exist as part of our own immune system, important in defense against various pathogens. For the purposes of research, such antibodies may be intentionally produced in animal species, directed against the molecule(s) of interest, which in this case were the specific blood components albumin and immunoglobulin. The production of such antibodies is illustrated in Figure 1. The scientific principles involved here are the same as when an individual receives a particular vaccine and is immunized against smallpox, flu, etc. The immune system is stimulated to produce antibodies against the injected protein, thus providing protection in the future. In this case, rabbits are immunized with human albumin or immunoglobulin, and

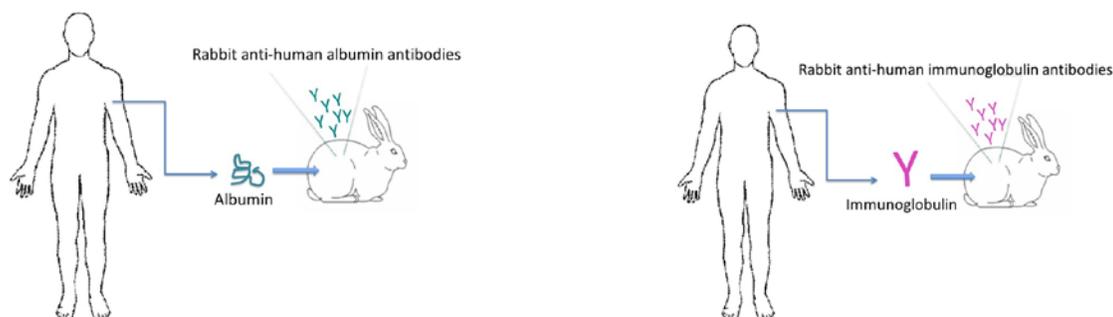


Figure 1. Production of antibodies in rabbits generated against human blood components (albumin and immunoglobulin). Antibodies such as these were used in the species characterization of the Shroud of Turin bloodstains. See text for details.

the antibodies are subsequently purified and used in experiments to detect the presence of those proteins in blood samples (Figure 1). These particular molecules were chosen as together they comprise approximately 80% of blood serum proteins: albumin 60%, and immunoglobulin 20%. Both Baima Bollone and Heller & Adler evaluated the presence of immunoglobulin, whereas Heller & Adler also tested immunologically for albumin. Surprisingly, unlike the chemical data, none of the results from either group has ever been presented and published in a peer-reviewed scientific journal, with the exception of a brief mention of having positive immunochemical results (6). Yet, it is the results of the studies involving these two proteins that the vast majority of evidence for the conclusion that the Shroud blood is of human origin is based on. It bears repeated emphasis that Adler would, in fact, not conclude that the evidence demonstrated the presence of human blood (6,11), whereas Baima-Bollone would do so unequivocally (3,5,8), although with much less confirmation (see below).

Cross-reactivity and controls: Human blood origin cannot be definitely concluded

In experimental studies involving antibodies, two major things have to be considered: cross-reactivity and controls. The interpretation and validity of the results is only as strong as these two factors; without proper attention, ambiguity begins to creep in and confound the results. Cross-reactivity refers to the fact that even though antibodies are produced against a particular type of protein (human albumin, for example), these antibodies may also react with albumin from other species, or even different proteins. This has to be experimentally verified. To illustrate this point, Figure 2 shows a screenshot taken from a modern day scientific catalog featuring the types of antibodies that were used by Heller and Adler as well as Baima Bollone in their studies of the Shroud of Turin bloodstains. An anti-human albumin antibody listing is shown, but all of the same caveats hold for antibodies directed against human immunoglobulin as well. Note that under the Reactivity heading numerous other species are predicted to also



Figure 2. Catalog screen shot illustrating the cross-reactivity of rabbit antibodies made against human albumin with albumin of other species. Antibodies such as these (polyclonal) were used in the characterization of the Shroud of Turin bloodstains. The full spec sheet may be found at: <http://www.abcam.com>.

react with this particular antibody (mouse, sheep, goat, etc.), even though this antibody was generated using only human albumin. This is because albumin from other species contain certain regions that are similar with human albumin, which this antibody will recognize. Thus, in using such antibodies one cannot be absolutely certain that reactivity is restricted to the particular species used to make them. This has to be experimentally verified for each particular antibody and under the specific binding and washing conditions used within each individual laboratory. The author can personally attest to this having performed numerous analyses involving many different types of antibodies (27). In their studies on albumin, Heller & Adler screened their antibody for reactivity against albumin from the following species as controls: human (positive control) and chimpanzee, baboon, cow, pig, and horse (negative controls). Two of the negative controls tested positive (chimpanzee and baboon), with Adler

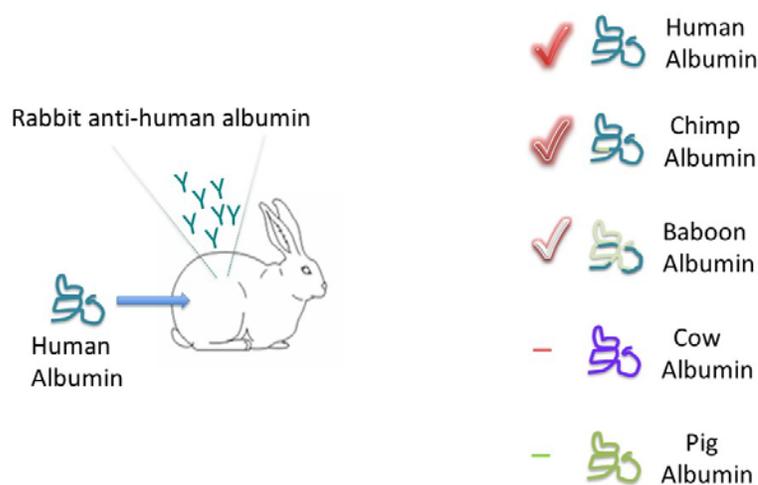


Figure 3. Cross-reactivity of anti-human specific antibodies used in Shroud studies. Antibodies raised against protein of one species may also react with the same protein from other species. See text for details.

noting that chimpanzee antigen reacted almost as strongly as human (4,6,9,11). Based on these results alone, before any Shroud samples were tested, these findings show that a definitive conclusion could not be reached. Thus, when Shroud samples tested positive in their antibody test, technically it could be equally argued that the blood on the Shroud is characterized as chimpanzee blood. This is why Adler reported that “we were only willing to say it was a primate’s blood”, (11). This is the scientifically correct conclusion. Anything else, including arguments that incorporate mention of the image of cloth being that of a man, etc., extends the data beyond what it demonstrates. Interpretation of the immunological data must be concluded separately from any influence of the image on the cloth. Anything else compromises the scientific integrity of the results.

As noted previously, albumin was one of two blood proteins that were studied. In studies on immunoglobulin, the same caveats mentioned above apply here as well. Baima Bollone’s studies do not mention cross-reactive testing (3,5,7,8). This is essential. It is as important, if not more important, as the testing on the sample itself. Adler’s studies on immunoglobulin are much less

detailed than those on albumin, but even if one assumes that such controls might have been performed, the same stipulations mentioned above apply. Indeed, it is known that such “anti-human” immunoglobulin antibodies cross-react with immunoglobulin from other species.

Other immunological studies related to the human origin of blood on the Shroud

In 1985, Baima Bollone reported the reactivity of Shroud bloodstain samples with antibodies directed against the MNS blood group antigens (28). While the initial rationale of such studies was to help further define the possible ethnicity of the type of blood present, it was overlooked that the MNS system distinguishes between primate and human blood. The S antigen is exclusive to the human species (29). Baima Bollone reported that reactivity with antibodies directed against M and N antigens (shared by both humans and related primates) showed “intense binding”, whereas anti-S reactivity exhibited “fairly good binding” (28). It is difficult to know precisely what conclusions should be drawn from these data. As in previous studies, there was no inclusion (or mention) of cross-reactivity controls. Thus, unfortunately, the significance of these findings and the strength of the results is unknown. These results exist in a very brief report published in the Shroud specialty journal *Sindon*, and are typically unmentioned when citing evidence for the human origin of the blood. Actually, the first citation of the relationship between the MNS blood system and the origin of species of Shroud bloodstains was not until 2012 (26), well after these findings were first reported. Typing studies involving the ABO blood group system have also been performed on Shroud bloodstains by the same investigators, concluding a blood type of AB (5,8). These studies have been extensively reviewed elsewhere (26), and will not be expanded upon here except to say that ABO typing studies cannot effectively distinguish human blood from that of other species (26,27). Moreover, as the ABO antigens are oligosaccharides, they are shared by a number of organisms, including bacteria, which could result in false positives during testing, a point which Adler emphasized (9,11).

What type of blood is it? Part 2-Have animal blood components been detected? Has anyone ever looked?

A natural companion question when considering the above studies is to ask if anyone has ever looked to see if other types of blood (animal) might exist on the Shroud. In other words, one could directly test for human blood, but has the accompanying study ever been done, that is to directly test for animal blood? The answer is a decisive no, such studies have never been performed. Moreover, it may also seem surprising to some that the existing data do not rule out the possibility that animal blood might be present, in fact they do not even directly address it. Previous studies only considered the possibility that human blood might exist on the Shroud, not any alternative. Even in the studies of Heller and Adler where the reactivity of their antibody with albumin from various species was examined (Figure 3), such tests only measure the specificity of their particular antibody (anti-human); this is an entirely separate issue from examining the Shroud bloodstains for blood from different species. To do this, one would need to use a panel of antibodies, each one directed against the particular species of interest (Figure 4). Furthermore, even if previous tests had established that human blood did,

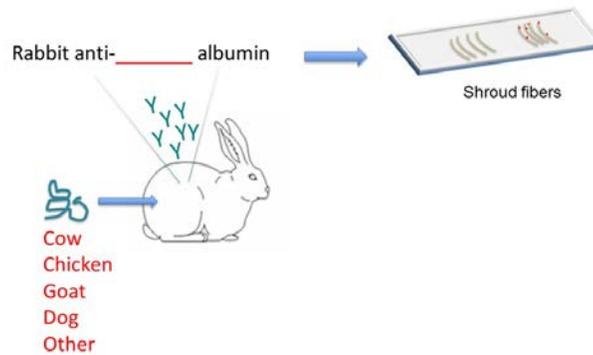


Figure 4. Immunological tests to determine if blood from animal species exists on the Shroud. A panel of different antibodies, each created against albumin from selected species would need to be tested individually with Shroud samples. Shroud fibers from non-bloodstain areas on the left-hand side of the microscope slide would serve as negative controls to ensure the specificity of reactivity with bloodstain fibers. See text for details.

in fact, exist on the Shroud, these findings are mutually exclusive from the possibility that blood from other species might be present. There is no empirical data at the current time that addresses the possibility that blood from other species might be found on the Shroud except for one finding, which occurred serendipitously. In evaluating the presence of human immunoglobulin in Shroud samples (3,7), Baima Bollone used an indirect antibody detection system, in which a labeled secondary antibody is used to detect an unlabeled primary antibody, forming a type of sandwich (Figure 5); also, see (26) for more detail. One important control,

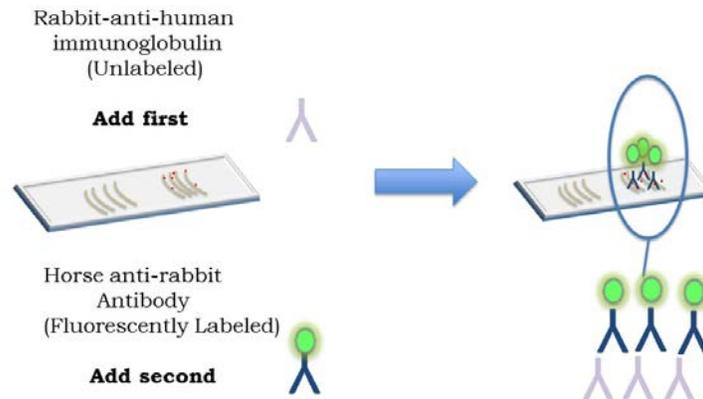


Figure 5. Indirect antibody assay. In this method, an unlabeled primary antibody is added to the sample, followed by a fluorescently-labeled secondary antibody to detect the presence of the primary antibody. See text for details.

which was included in these studies is the addition of only the labeled secondary antibody to the sample, (without the primary antibody). This helps to ensure that the observed fluorescence is dependent upon the primary antibody being present, and does not result from merely nonspecific binding of the secondary antibody to the sample. In Baima Bollone's studies, these

control groups were negative. Because the secondary antibody that was used is directed against rabbit antibody (immunoglobulin), these data rule out the possibility that rabbit blood components are present in the sample. This was not the intent of the study, nor commented on in the original paper, but is a fortuitous outcome of the experimental design. To date, this result remains the single piece of data regarding the possibility that animal blood components might exist on the Shroud. Whether or not blood from other species (cow, horse, goat, chicken, sheep, cat, etc.) might be present is unknown, it remains to be investigated.

Further Considerations and Discussion

In the initial studies of the Shroud, it is understandable that investigators would first want to focus on trying to establish if the bloodstains were, in fact, composed of blood, and then try to determine what species the blood might originate from. As discussed in this report, the bloodstains of the Shroud have been studied primarily from a chemical perspective, much less so from the context of immunology. It cannot be said with scientific certainty that the blood on the Shroud is of human origin. Such previous immunological tests would need to be repeated using more recently developed antibodies with a restricted and well-defined specificity.

Given the mysterious nature of the Shroud's origin and whereabouts during history, it is important to consider all possibilities, to rule things out as well as trying to rule them in. Even if human blood is found to be present, the question would still remain open if blood from any other species might exist on the cloth. Indeed, it has been suggested that the bloodstains may have been touched up at some point during the Shroud's indefinite 600-2,000 year history. Rigorous science would approach the problem from both angles. One strategy has been attempted, but the interpretation of the results is hampered because of the restricted specificity of the antibodies that were available. The second approach has never been undertaken. Both are important as this is a key, fundamental question concerning the characterization of the Shroud bloodstains. In testing the blood on the Shroud, it is important to maintain objectivity and to separate the findings in the laboratory from the image on the cloth. Any discussion about the likelihood of a forger using blood from apes, goats, chickens, or any other untested species is purely anecdotal. From an immunological perspective, it is a relatively straightforward question. Either the results are definitive or they are not.

Two important points should be mentioned regarding the methodology used in the previous studies. First, the production of antibodies has improved vastly since some forty years ago. Indeed, an entire new technology, the creation of monoclonal antibodies, has revolutionized the field of immunochemistry (26,31). Second, the readout systems for antibody reactivity have also greatly improved, becoming much less subjective and easier to interpret than those in the past. In the studies performed by Baima Bollone and Heller and Adler, reactivity was scored by a visual examination through a microscope and assigning the relative intensity of fluorescently-labeled antibodies that were bound using a relative scale. Such results are mainly qualitative and relatively subjective; the data is typically converted into a type of ranking scale for binding, such as poor (-), good (++), very good (+++), and strong (++++). In the more modern technology that currently exists, results may be more objectively and accurately evaluated by allowing computer analysis to determine the extent of antibody reactivity. Moreover, such methods are far more sensitive than those used in previous years, allowing greater resolution with smaller amounts of material. Additionally, there are alternative approaches that are especially helpful when the amount of material is limiting. For example, in a conventional

approach, to test for positivity of blood types from 5 different species, 5 different samples are required, one for each type (Figure 6). However, using sequential strategy, multiple species can

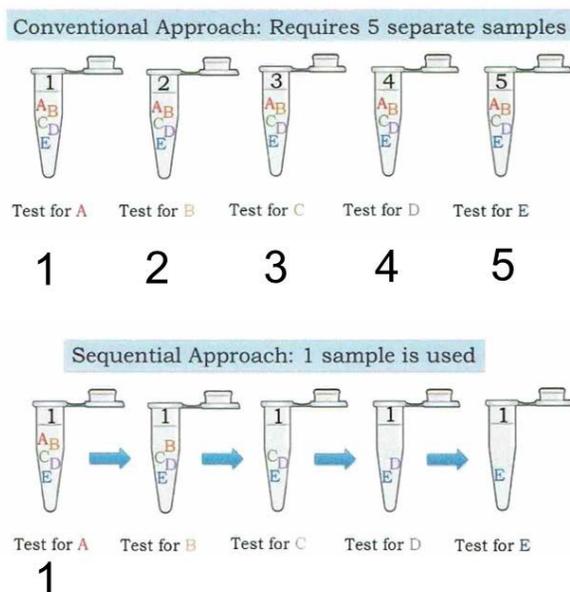


Figure 6. Sequential versus Conventional Approach of Antibody Evaluation. The sequential method uses far less sample (only a single sample is required), yet provides similar information as the conventional approach using one sample for each antibody tested. See text for details.

be evaluated with just a single sample (Figure 6). In this scheme, the sample is first reacted with antibody specific for one species (species A); unreacted material is then tested for the second species (species B), and so forth. This approach has been successfully used in multiple types of immunological analysis where the amount of sample is particularly limiting.

Another methodology to consider in evaluating the companion question is the use of molecular biology (DNA) analysis to evaluate for the presence of blood from other species. In this case, contamination from various persons handling the Shroud is not an issue as DNA sequencing is specific and sensitive enough to distinguish particular genes of interest from various species. Next generation sequencing would be particularly useful in this approach (32).

Finally, on a personal note, I had always been casually interested in the Shroud since seeing the STURP photographs in an issue of National Geographic in the magazine rack at a grocery store where I worked during college in the early 1980s. In preparation for a possible trip to Turin in 2010, I began to study the Shroud in more detail, particularly the original scientific articles whenever available. Having a research background in immunology, the studies on the bloodstains naturally caught my interest. Here, the reader is invited to (re)consider the often-repeated refrain that the blood on the Shroud has been shown to be human, commonly reported in most Shroud-related publications, interviews, websites, media outlets, etc. This question should be particularly discussed in the context of immunology, where such studies have their experimental basis. The reader is encouraged to show the original data to any card-carrying immunologist with antibody experience and mention two phrases, cross-reactivity and controls.

Then ask if the data conclusively show that the blood is human, and whether or not the companion question regarding the presence of animal blood has been addressed. Regarding the first, it bears repeating again that Adler refrained from drawing the scientific conclusion that the blood on the Shroud is of human origin. This is despite the fact that he may have in his own personal belief, thought that it most likely was (similar to my opinion). A professional scientist realizes that when one fails to maintain objectivity this can lead down a difficult path. The data is the data. It cannot be emphasized enough that one should not allow the image on the cloth to influence the interpretation of the results on the scientific analysis of the blood. Relatedly, one should set aside emotion and objectively evaluate where our current knowledge lies. The scientific reality is that the immunological data does not conclusively demonstrate that the blood is of human origin. The other point is that alternative possibilities have never been addressed. Given the enigmatic nature of the artifact it is important to consider both. In the end, it is a matter of what level of scientific rigor one wishes the science of the Shroud to maintain and how accurately the known information is to be conveyed.

Summary

In summary, the current paper has evaluated the previous findings on the bloodstains of the Shroud of Turin, with particular emphasis on the species of origin in the context of immunology. The current knowledge of the types of blood that might exist on the Shroud is discussed regarding the strength of existing data and related suppositions that remain to be experimentally determined.

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