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Inadequacies in Serological ABO Typing of Ancient Artifacts: The Shroud of Turin as a Case Example

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Abstract

Serological determination of ABO blood type is extremely valuable in evaluation of fresh blood but has major drawbacks in the analysis of aged objects. The Shroud of Turin is a full-size linen cloth bearing the ventral and dorsal images of man containing wounds consistent with scourging and crucifixion. Controversy exists regarding the authenticity and age of the artifact; it has been proposed to be the two-thousand-year-old burial cloth of Jesus, or alternatively, a clever, medieval hoax. During the last public exhibition in 2015, approximately 2 million visitors came to view the cloth for just a few minutes at short distance. It is widely reported that the Shroud bloodstains are blood type AB, based on previous findings that were never published in a peer-reviewed format. Here, the shortfalls of using serological testing for ABO determination of ancient objects are discussed using the Shroud of Turin as a case example. It is determined that the AB designation for the Shroud bloodstains is inconclusive and should be considered as type unknown pending further testing.

Keywords: ABO; Blood Typing; Immunology; Turin Shroud; Serology

Abbreviations: RBCs: Red Blood Cells; DNA: Deoxyribo Nucleic Acid.

Introduction

The ABO blood grouping system was discovered by Landsteiner in 1901, which became the foundation for modern day blood transfusion and transplantation [1,2]. This system is based on the central immunological principle of self- versus non-self-recognition. Red blood cells (RBCs) express two major blood group antigens on their surfaces, A and B, either alone or together comprising the blood types A, B, and AB, respectively. Persons who express neither A nor B are designed as type O, derived from the word ohne, which means "without". The system is complementary in that the serum of individuals contains antibodies against the particular A and B antigens they lack. For example, the serum of O individuals contains both anti-A and anti-B, whereas the serum of type AB persons has neither (Table 1).

Blood Type	RBC antigen	Serum antibodies
A	A	Anti-B
B	B	Anti-A
AB	AB	-
O	O	Anti-A, Anti-B

Table 1: The ABO Blood Groups.

Determination of blood type may be done serologically by either evaluation of the antigens that are expressed on RBCs, referred to as forward typing, or by testing for the presence

of anti-blood group antibodies in serum, termed reverse typing. In blood transfusions or organ transplantation, both forward and reverse typing are performed as they cross-check and complement each other, with a clearly predictable result (Table 1).

In modern day forensic analysis, DNA methodology has become the technique of choice for blood type determination as it circumvents many of the problems that can arise with serological methods, especially in older samples (see below). Unlike serological testing, DNA analysis evaluates the presence or absence of the genes encoding the enzymes responsible for construction of blood group molecules. With fresh bloodstains, screening for the presence or absence of such genes has become relatively routine within the past several decades [3,4]. With older samples this can be more challenging, although ABO typing techniques have been adapted for use with severely degraded DNA [5].

In serological studies of blood types using ancient mummies, it was found that variations in forward typing techniques gave inaccurate and conflicting results for half of the 14 samples evaluated [6].

Relatedly, serological analysis of Jewish skeletal remains that were 1600-2000 years old, gave a disproportionate number of results that were AB (51%), a much higher value than would be expected based on modern statistics and heredity [7,8]. As recently noted by Gosh, serological methods are not suitable for older blood samples as contamination with other organisms is likely to confound the results [9] (Table 2). Indeed, many different types of organisms express both A and B blood group antigens, including bacteria and fungi [9-13].

Fresh Blood		Aged Blood	
Blood Type	Expected Result	Blood Type	Expected Result
A	A	A	A,AB
B	B	B	B,AB
AB	AB	AB	AB
O	O	O	A,B,AB

Table 2: Forward Typing Methods in Fresh Vs Aged Samples.

Results

The Shroud of Turin is an approximately fourteen-foot-long cloth with the faint image of a man containing bloodstains consistent with scourging and crucifixion. Carbon-14 dating tests performed in 1988 assigned the Shroud a proposed date 1260-1390 [14] although such results have recently been questioned, both in terms of data homogeneity and whole cloth representation [15-17]. The bloodstains contain authentic blood components including hemoglobin, albumin, and immunoglobulin, although the species of origin remains to be demonstrated [18-20]. In the early 80's, Baima Bollone reported a designation of AB in a Shroud-specialty journal, resulting from experiments using the mixed agglutinin (forward typing) method [21]. In this technique, multivalent antibodies specific for a particular blood group antigen are added to the sample; unbound antibodies are removed by washing, and fresh RBCs of the same type as the antibody are added. For a positive result, a type of clumping, or agglutination or RBCs is observed (Figure 1, left-hand side). In aged archaeological samples, this method is typically problematic, in typing of fresh blood (left), antibodies bind to red blood cell antigens and after washing, new red blood cells of the identical type are added, forming a type of sandwich (agglutinin), which is visible under a microscope. With aged blood, antibodies may recognize similar antigens present on bacteria (shown) and fungi, resulting in a false positive result (Figure 1, right-hand side), (Table 2), a limitation acknowledged by the original researchers, [21] (Table 2). Note that antigens, antibodies, and red blood cells are not drawn to scale in this figure.

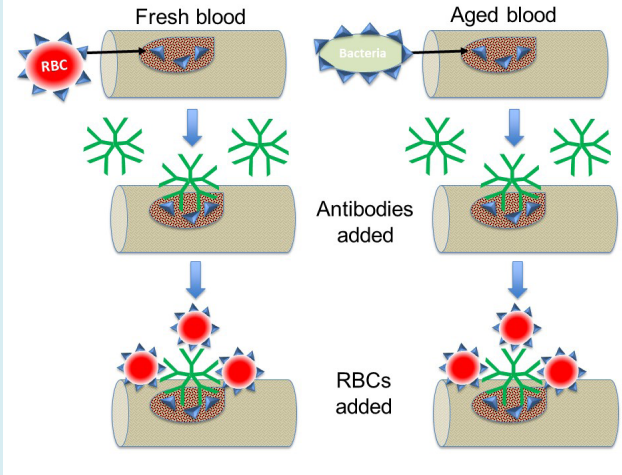

Figure 1: Mixed Agglutinin Method for Forward Typing of Fresh Vs Aged Samples.

As a control in these studies, white fibers adjacent to the bloodstain were included, which tested negative [21], indicating that contamination was not widespread. It could be argued, however, that contamination would be greatest in the most nutrient rich areas of the cloth, i.e., the bloodstains. Regarding this issue, a microscopic examination of bloodstained threads taken from the Shroud showed heavy contamination by both bacteria and fungi; threads chosen from other areas, less so [22].

In a follow up study, a different method (immuno

chemistry) involved peroxidase-labeled antibodies. These studies yielded similar results as before, type AB [22]; however, this experimental design suffers from the same limitations as the original studies, false positives resulting from contamination cannot be excluded [9].

To cross-check the forward typing results, a reverse typing method (Lattes technique) was used to evaluate the presence or absence of anti-A and anti-B antibodies (Table 1). Unfortunately, this technique is even more problematic with aged samples than forward typing, as it is extremely unlikely that antibodies remain functionally stable over time [9]. Typically, reverse typing is never used in studies of ancient material because the most likely result is AB, regardless of the original (true) blood type (Table 3). Not unexpectedly, this was the reported result [21]. Note that even if the designation of AB for Shroud fibers was correct, this method cannot distinguish between a true AB result and a negative finding with another blood type due to antibody instability and loss of function (Figure 2). With any blood type other than AB, verification is possible, but since the serum of AB individuals does not contain anti-A or anti-B antibodies to begin with (Table 1), the contention is exceptionally circular. Therefore, these findings are not useful for cross-checking or confirmation of blood typing of older samples [9].

Fresh Blood		Aged Blood	
Blood Type	Expected Result	Blood Type	Expected Result
A	A	A	AB
B	B	B	AB
AB	AB	AB	AB
O	O	O	AB

Table 3: Reversing Typing Methods in Fresh Vs Aged Samples.

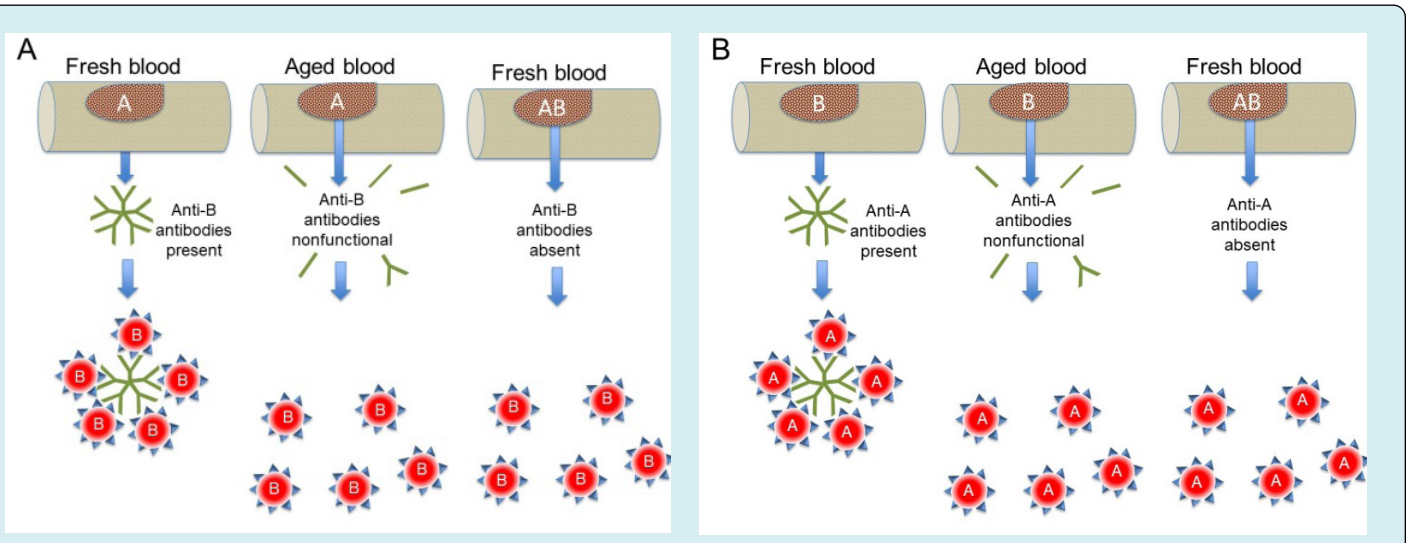

Figure 2: Mixed Agglutinin Method for Forward Typing of Fresh Vs Aged Samples. In (A), fresh and aged blood samples that are type A are evaluated using the Lattes method. A visible reaction is not observed with aged blood as antibodies have become nonfunctional over time (compare left and middle); note that this result is the same as a person having AB blood (right). (B) Similar to (A), except that an example is shown using red blood cells of the B type. Note that antigens, antibodies, and red blood cells are not drawn to scale in this figure.

Discussion

Serological blood typing of ancient artifacts is particularly challenging when little to no background information exists regarding the genotype of the sample in question. With articles such as skeletal remains or relatively well-preserved mummies, environmental or familial information may be available to help corroborate blood type findings. For objects like the Shroud, where bloodstains are relatively solitary in terms of typing evidence, reliability on the data is essential for confidence in the results.

As each artifact is accompanied by unique and special circumstances of preservation, storage, and exposure to environmental conditions, the limitations of techniques must be determined on an individual basis. Thus, what may work for one object does not guarantee success with another. Ideally, having results from both serological and molecular biology studies on the same object to cross-check and verify one other is ideal, although this is not always possible. As use of serological methods for blood typing of older materials is frequently unreliable, the strictest caution must be used in the interpretation of the results, especially those for which contamination has already been established. In consideration of the current serological data that exists, the blood type designation for the Shroud bloodstains is best described as inconclusive and should be considered type unknown pending further testing.

Conclusions

The current perspective has evaluated the existing blood typing data for the bloodstains on the Shroud of Turin. As discussed, unreliability in serological evaluation of aged materials limits the conclusions that may be drawn from such results. After modern review and evaluation, the designation for the Shroud of Turin bloodstains should be listed as inconclusive, type unknown.

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