

# The conundrum of antibodies of undetermined significance (AUS) masking clinically significant alloantibodies: what is your laboratory protocol?

Clameo Chimfombo  
SydPath Pathology (St Vincent Hospital Sydney)  
Moree Hospital, NSW  
Australia

# Antibodies of Undetermined Significance ( AUS)

- Some clinically significant alloantibodies can weaken and evanesce overtime, thus making them difficult to detect.
- 18% of reported alloantibodies are antibodies of undetermined significance (AUS) also known as non-specific antibodies and are generally unexplained agglutination reactions.
- Current Australia and New Zealand Society of Blood Transfusion (ANZBST) guidelines for transfusion and immunohaematology laboratory practice (1<sup>st</sup> ed, November 2016) section 2.7.7 states  
“ Using a variety of different techniques – for example, enzyme- treated cells, polyethylene glycol (PEG)-IAT, prewarmed reagents or neutralisation – may assist in confirming the presence of Rh antibodies, antibodies weakly reactive by IAT or suspected mixture of antibodies ”  
p15.



# AIM

- Discuss a protocol recently published in a peer reviewed article and the ramifications of investigating AUS.
- Learn from peers their respective protocols and discuss their practicality and challenges.



# Science

## Ficin-Treated Red Cells Help Identify Clinically Significant Alloantibodies Masked as Reactions of Undetermined Specificity in Gel Microtubes

*Ben C. Hill, MD,<sup>1</sup> Courtney A. Hanna, MD,<sup>1</sup> Jill Adamski, MD, PhD,<sup>2</sup> Huy P. Pham, MD, MPH,<sup>1</sup> Marisa B. Marques, MD,<sup>1</sup> Lance A. Williams III, MD<sup>1\*</sup>*

*Laboratory Medicine 48:1:24-28*

DOI: 10.1093/labmed/lmw062

### ABSTRACT

Non-specific antibodies or antibodies of undetermined significance (AUS) often pose problems for a blood bank technologist and physician. It is well known that antibodies can weaken and evanesce over time, thus eluding detection by routine blood bank techniques. Special enhancement techniques exist (eg, ficin treatment); however, they are often underutilized due to concerns over expense. Ficin is known to enhance reactivity caused by antibodies in the ABO, Rh, Kidd, Lewis, I, and P blood group systems, while destroying reactivity of antibodies in the Duffy, and MNS blood group systems. Herein, we discuss our

protocol for using ficin treatment to determine the specificity of antibodies that would otherwise be classified as AUS. Of the 97 AUS specimens that were treated with ficin, we were able to identify 25 new alloantibodies that would have otherwise been missed without ficin treatment. Thus, we believe our protocol enhances transfusion safety, while minimizing additional workload and cost.

**Keywords:** antibodies of undetermined significance, AUS, gel, ficin, evanesce, alloantibodies

# Index Case

- Patient with history of multiple transfusion.



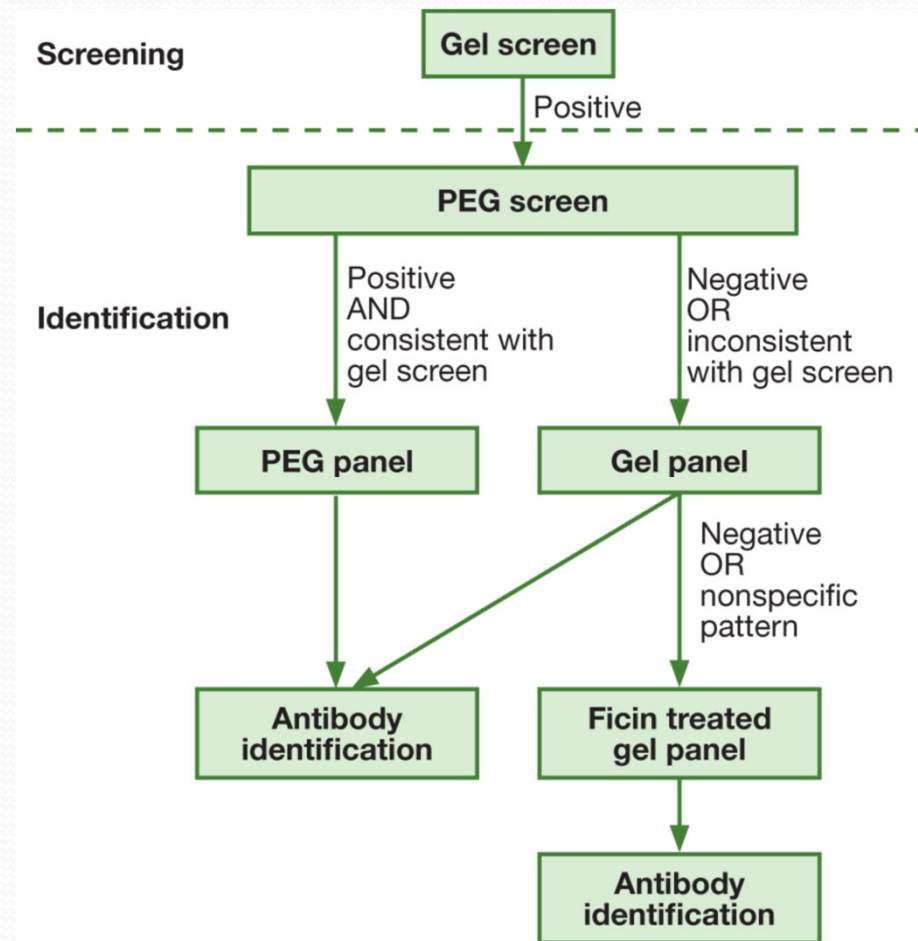
- AUS identified (previously identified 18 months earlier)



- Ficin- treated reagent applied to patient plasma (ficin is a proteolytic enzyme which enhances reactivity in ABO, Rh, Kidd, Lewis, P an I antigens while destroying Duffy and MSN antigens)



- Two clinically significant antibodies; **anti- C** and **anti- e** identified (patient phenotypically negative for these antigens)




From: Ficin-Treated Red Cells Help Identify Clinically Significant Alloantibodies Masked as Reactions of Undetermined Specificity in Gel Microtubes

Lab Med. 2017;48(1):24-28. doi:10.1093/labmed/lmw062

Lab Med | © American Society for Clinical Pathology, 2016. All rights reserved.



- 
- Of the 97 AUS investigated with ficin-treated red cells, 25 new alloantibodies were identified with 22 being clinically significant.
  - Concluded: Ficin-treated red cells help identify clinically significant alloantibodies masked as reactions of undetermined specificity in gel microtubes.



## Summary of New Alloantibodies Requiring Ficin for Identification

Antibody Specificity	Antibodies Identified (All Methods), No.	Antibodies Requiring Ficin-Treated Cells for Identification, No. (%)
D	61	8 (13.1%)
C	22	2 (9.1%)
E	51	10 (19.6%)
e	2	1 (50.0%)
Jk <sup>a</sup>	11	1 (9.1%)
Le <sup>a</sup>	7	2 (28.6%)
Le <sup>b</sup>	1	1 (100%)
<b>Total</b>	<b>155</b>	<b>25 (16.1%)</b>



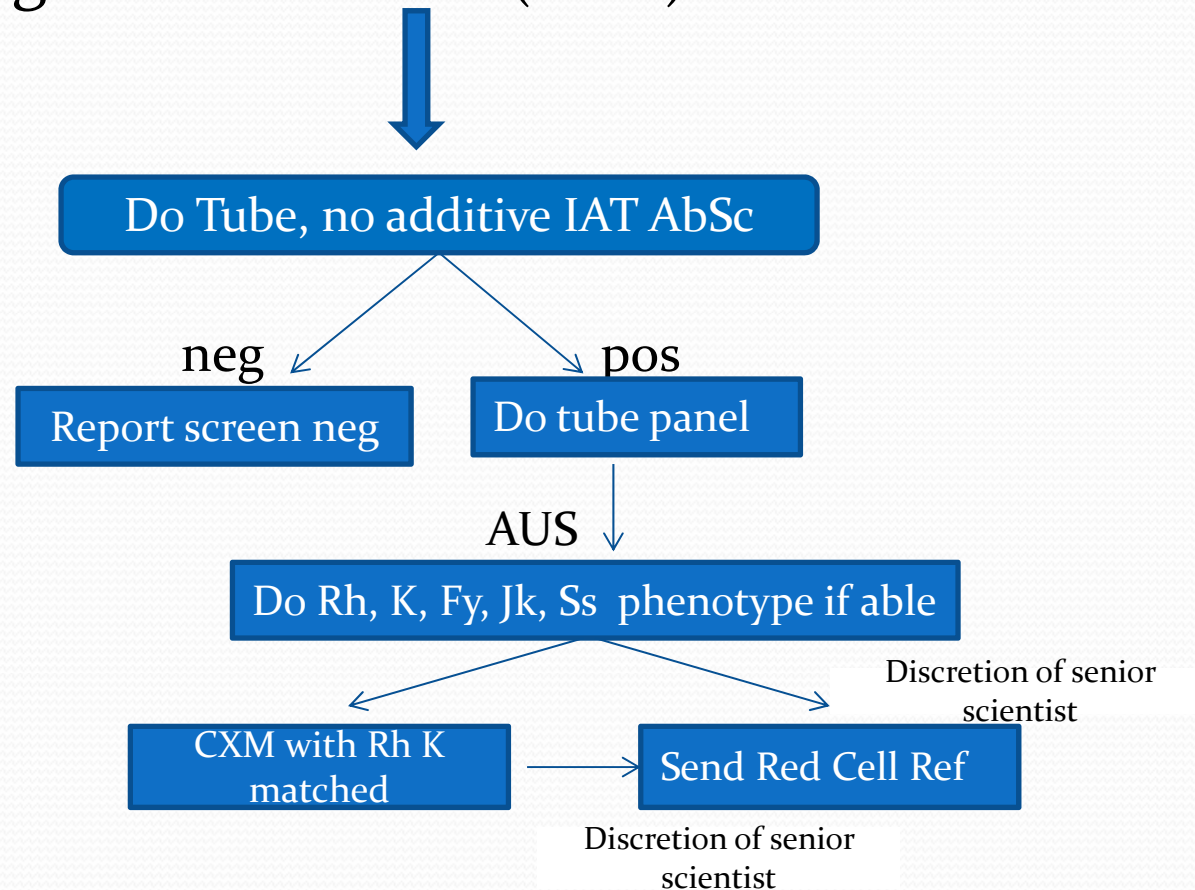


# Discussion

- Should AUS only be reported after utilising different techniques for example enzyme-treated cells as was done in index case?
- What are the reasonable approaches in establishing a similar protocol noting that 75 of the 97 of AUS workup still showed AUS?
- Let's share protocols aiming to improve current practises.

# Our protocol

- Screening CAT screen (AUS)





# Acknowledgements

- Ms Rosemary Marando, Senior Scientist, Transfusion, SydPath, St Vincent's Hospital Sydney





# References

- Australia and New Zealand Society of Blood Transfusion ( ANZSBT) Guidelines for transfusion and immunohaematology laboratory practise. ANZSBT 1<sup>ST</sup> Edition, November 2016
- Hill, B.C., Hanna, C.A., Adamski, J., Pham, H.P., Marques, M.B. and Williams III, L.A., 2017. Ficin-Treated Red Cells Help Identify Clinically Significant Alloantibodies Masked as Reactions of Undetermined Specificity in Gel Microtubes. *Laboratory medicine*, 48(1), pp.24-28.
- Liu, C. and Grossman, B.J., 2013. Antibody of undetermined specificity: frequency, laboratory features, and natural history. *Transfusion*, 53(5), pp.931-938.
- Tormey, C.A. and Hendrickson, J.E., 2013. Antibodies of undetermined significance: nuisance or near miss?. *Transfusion*, 53(5), pp.926-928.