Advanced ImmunoChemical Inc.
Providing for the Advancement of Medical Science

ASSAY NOTES
Product Information

Courtesy of HyTest, Ltd.

Chimeric cardiac troponin I antibodies

The problems associated with heterophile antibodies have been recognized for a number of decades. Estimates of the prevalence of heterophile antibod- ies vary considerably but as far as the general population is concerned, it is considered to be at least 10% (1).

Heterophile antibodies arise when people are exposed to different animals or products derived from animals. These antibodies are typically human anti-mouse (HAMA), anti-rabbit, anti-goat, anti-sheep, anti-cow, anti-pig, anti-rat or anti-horse. As far as immunodiagnostics is concerned, the problem is most commonly associated with HAMA due to the fact that most diagnostics assays use mouse derived antibodies.

HAMA might cause false positive or negative results

In diagnostic tests, HAMA might cause false positive and sometimes false negative results as well (2-3). False positive results are caused by the cross-linking of assay antibodies by HAMA, whereas false negative results can be obtained when the HAMA binds to assay antibodies and in doing so blocks the binding of the antigen (see Figure 1).

False positive results can cause delays in making a correct diagnosis and indeed even in unnecessary hospital admissions if the test is used for the diagnosis of life-threatening conditions such as acute myocardial infarction (4). A study of subjects investigated with cardiac Troponin I due to suspected myocardial infarction found that HAMA caused false positives in 5.5% of subjects with raised cTnI and 14% of subjects with raised cTnI and normal creatine kinase (5).

CLINICAL UTILITY

- **Early marker of acute myocardial infarction**
- **Prevent heterophile antibody effect**

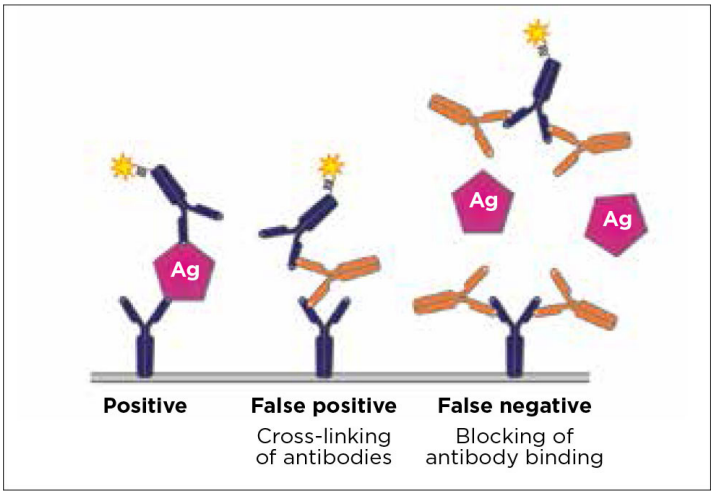

Figure 1. Human anti-mouse antibodies (HAMA) can cause both false positive and false negative results in immunoassays.

In order to overcome the problems caused by HAMA, most assay manufacturers take additional measures during the assay development. The most frequent procedure involves suppressing the non-specific binding with blocking reagents, which vary from normal mouse IgG to more refined formulations. Whilst in most cases these measures are adequate, they do result in additional costs in terms of both material and labor. The effect of HAMA can also be suppressed with the use of Fab fragments. However, this also adds a manufacturing step and increases the costs for each assay.

Chimeric antibodies help to avoid the HAMA effect

A powerful tool to solve the issue with HAMA in diagnostics tests is the use of chimeric or fully humanized antibodies. Troponin assays are particularly susceptible to HAMA due to low cut-off value requirements and because the levels of cTnI even in the plasma of AMI patients are very low. Two of our anti-cardiac troponin I (cTnI) antibodies have been converted to chimeric proteins by changing the antibody constant regions from mouse to human derived sequences. The chimeric cTnI antibodies Rec19C7 and Rec16A11 consist of the original mouse derived variable regions that are responsible for antigen specificity and human derived constant regions of IgG1 isotype (see Figure 2).

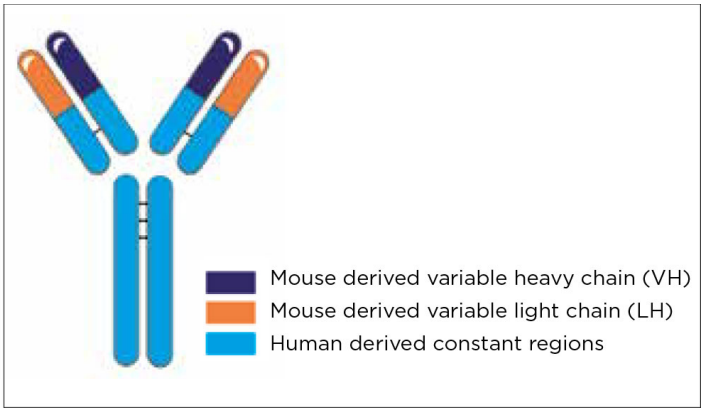

Figure 2. Schematic illustration of chimeric cTnI antibodies.

Results

Isotype switch has no effect on antibody performance

The subclass of native 19C7 antibody is IgG2b, whereas that of the chimeric RecChim19C7 is IgG1. In order to determine if the isotype switch has any impact we compared the performance of the native and chimeric 19C7 in several antibody combinations (see Figure 3). The performance of the two 19C7 forms proved to be very similar and differences in most cases are within 10%, which corresponds to inter-assay CV.

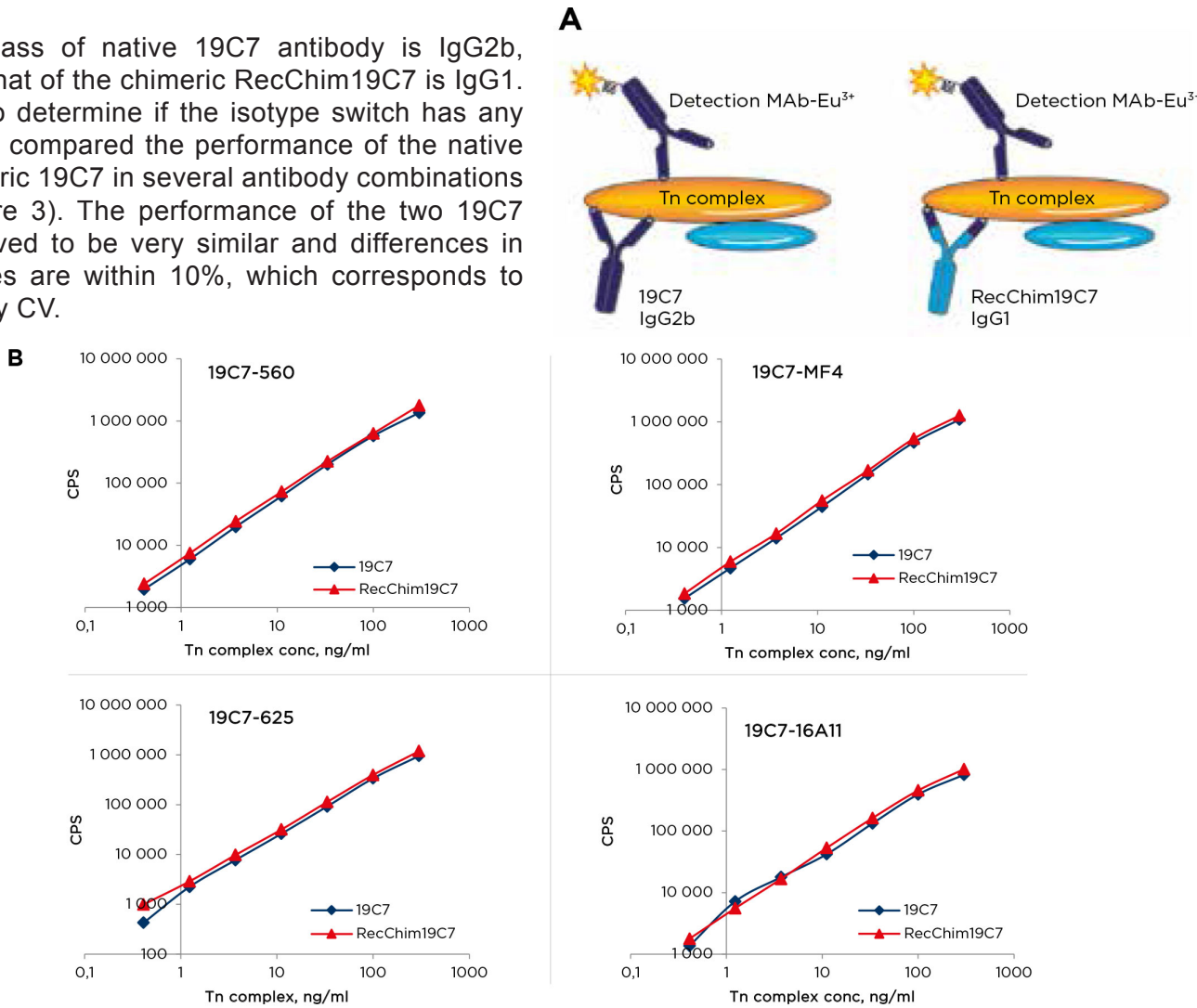

Figure 3. Isotype switch has no effect on performance of antibody 19C7. *A) Schematic illustration of assay setup. Native (left) or chimeric (right) 19C7 was used as the capture antibody, native anti-cTnI (560, MF4, 625 or 560) labeled with Eu3+ as the detection antibody and troponin complex as the antigen. B) The performance of chimeric RecChim19C7 (sub class IgG1) was compared to native 19C7 (sub class 1gG2b) in four different sandwich immunoassays as indicated in the picture.*

Chimeric antibodies prevent the HAMA effect in *in vitro* diagnostic assays

The performance of different combinations of chimeric and native antibodies was tested using HAMA containing serum samples that were obtained from acute myocardial patients in order to verify that the chimeric antibodies are not sensitive to the HAMA effect. HAMA concentrations in serum samples ranged from 807 ng/ml to 6220 ng/ml (see Figure 4). The highest HAMA containing serum gave a very high background signal with all combinations that were based on the use of native antibodies.

Meanwhile, the lower HAMA containing serum samples gave a lower but significant background signal. At the same time, all assays in which the chimeric antibodies were used gave either no or just a negligible background signal with all of the tested HAMA-samples.

The results of this test clearly show that the HAMA effect can be prevented with the use of chimeric antibodies which makes them a potential new tool to overcome this common problem.

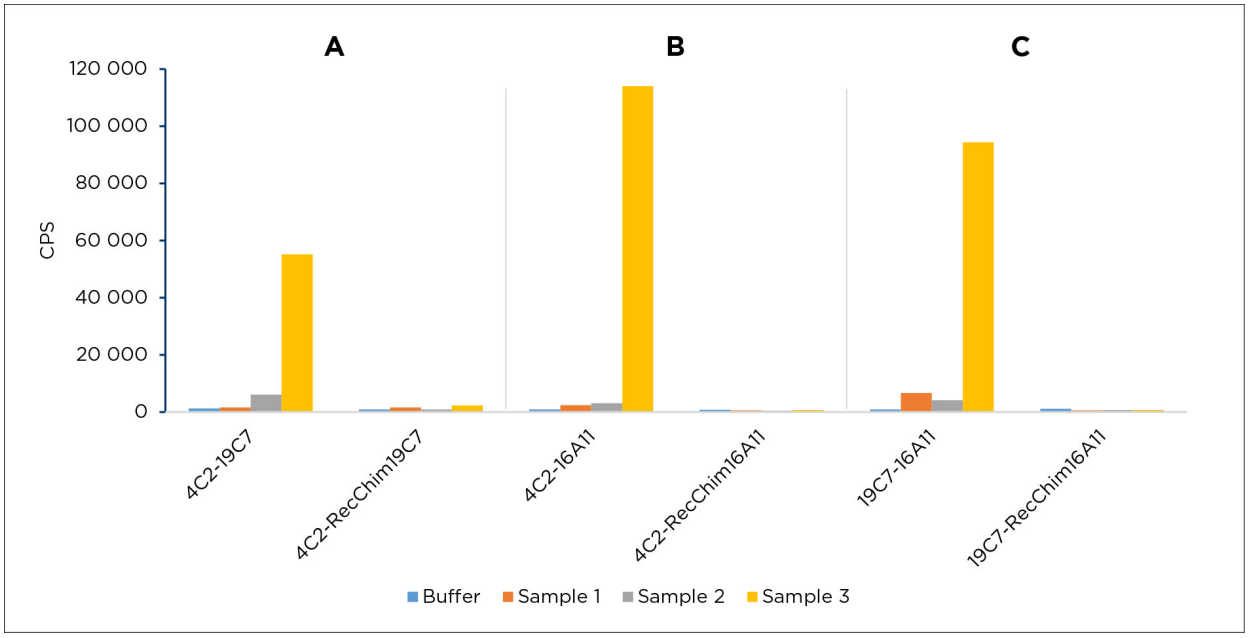

Figure 4. Chimeric antibodies mitigate the HAMA effect. *The performance of chimeric and native 19C7 and 16A11 in the presence of HAMA was tested with three serum samples with varying HAMA concentrations: 807 ng/ml in Sample 1, 1388 ng/ml in Sample 2 and 6220 ng/ml in Sample 3. As a control, buffer without serum was used. The following immunoassay comparison were tested: 4C2—Mab19C7 and 4C2— RecChim19C7 (A), 4C2—16A11 and 4C2—RecChim16A11 (B) and 19C7—16A11 and 19C7—RecChim16A11 (C).*

References

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Ordering Information:

MONOCLONAL ANTIBODIES

Product Name	Cat #	MAb	Subclass	Remarks
Recombinant chimeric anti-cTnI	RC2-TIc	RecChim19c7	IgG1	EIA
		RecChim16A11	IgG1	EIA

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