The Use of Monoclonal Antibodies in Forensic Science

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Introduction

Although the advent of DNA profiling in forensic science is an enormous step forward for the identification of those involved in crime, it will still be necessary to identify the types of stains and on many occasions the red cell antigens and enzyme polymorphisms will have to be detected.

This is especially so when grouping semen stains in which there are few or no spermatozoa. In this situation it is first necessary to identify the presence of semen using biochemical markers which are present in the seminal plasma, followed by ABH grouping and the determination of other genetic markers which are present in the stain extract and also in the control samples obtained from suspects and victims.

The time taken for the analysis may also be an important factor when the police are in a hurry to eliminate suspects from their enquiries. In such cases, if a person cannot be eliminated by traditional grouping, there could be justification for the continued detention of the suspect pending DNA analysis.

The introduction of monoclonal antibodies (MCA) for detection purposes and grouping procedures was inevitable. MCAs have many theoretical advantages over their polyclonal counterparts, not least of which is their monospecificity. Other advantages include the supply of large volumes of reagent which should be available over long periods of time (theoretically forever) and the ability to pick specifities which have been hitherto unobtainable.

Many problems have been experienced with the use of MCAs in the absorption-inhibition and absorption-elution methods which have been used for many years in forensic science laboratories, and it has become apparent that the ELISA technique could be a considerable improvement.

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Edited by W.R. Mayr

In the UK, Fletcher and co-workers at the Central Research Establishment have pioneered the use of MCAs and ELISA in forensic science, but we are also aware that this work is also being pursued in many other countries.

At the Metropolitan Police Laboratory we have had approximately 18 months experience with the use of MCAs and ELISA for the detection of semen and also for use in the ABH, Lewis and MN blood group systems.

Identification of Semen

Two MCAs have been used in this context, both of which were obtained from the U.S.A. These detect proteins which are present in seminal plasma and are therefore unaffected by the presence or absence of spermatozoa.

Seminal Vescicle Antigen

The MCA which recognises this antigen is termed MHS-5 (Mouse anti-Human Semen-5) and is used for the detection of epitopes on small peptide fragments in the seminal plasma. Initially it was used in conjunction with a polyclonal mouse IgG-horse radish peroxidase (HRP) but cross reactions were observed with samples of saliva from both males and females.

Biotinylated MHS-5 with steptavidin-HRP as conjugate eliminated this cross-reactivity and specificity for semen of man and higher primates, was achieved.

p30

This is a protein produced in the prostate and has been used for some considerable time as a seminal plasma marker by immunoprecipitation with a polyclonal antibody produced in rabbits.

The testing system which we have used employs initially MCA to p30 followed by a polyclonal anti p30-alkaline phosphatase conjugate.

In general this method has worked well with no cross-reactivity being observed although a few tests gave inconclusive results. The method is not as quantitatively reproducible as that using MHS-5 and this could be as a result of the more complicated testing routine.

Neither anti-p30 nor MHS-5 were found to cross-react with vaginal secretion or any other body fluids but the system was not able to be used to determine the post-coital interval. This could be due to many factors.

Case-work trials are now underway at the MPFSL and it is expected to use them for routine testing in the near future. They will be the subject of future publications.

Now that we have reliable systems for detecting semen, it would be desirable to obtain MCAs which are specific for vaginal secretion, urine, faeces and sweat.

Grouping of blood and body fluids

A number of surveys have been carried out whereby MCAs have been compared with polyclonal antisera using the traditional inhibition and elution systems for blood and body fluid grouping.

In most of our initial testing we encountered problems with the red cells adhering to the plastic tubes and thus rendering the inhibition testing unworkable.

With the elution system the MCAs which were available to us provided problems with very strong antigen-antibody binding such that heat elution was prevented.

Subsequent testing using an ELISA method has shown an enormous increase in the reliability and sensitivity of the detection of the ABH antigens. There is still a problem with AB stains but it is felt that this is not insurmountable.

It is of interest to note that some of the MCAs will detect the ABH antigens in bloodstains far better than those in body fluid stains and the converse is also true. This is not surprising as the oligosaccharide structures are different one from the other and it may prove to be of considerable advantage when typing stains which are mixtures of blood and other body fluids.

The Lewis MCAs which we have been testing have shown some rather surprising results in that they appear to be detecting a straightforward difference between secretors and non-secretors rather than Le(a) and Le(b) types. Again this can be explained by the differences in the basic oligosaccharide structures and may be of considerable benefit in forensic science because we are able to distinguish between the Le(a-b-) secretors and non-secretors. However these particular MCAs do not provide very good results with the typing of whole cells and using other MACs which detect Le(a) and Le(b) could cause problems with reporting for court purposes.

Although there have been many publications on the use of a MCA for the typing of Gm(3) from dried blood we have not attempted to assess its value. This is for 2 reasons. Firstly this is not a good marker for discrimination purposes within a white caucasian population. Secondly, we use the presence of Gm(3) to confirm the absence of Gm(1) and Gm(2) such that we can report a Gm(-1,-2) phenotype. If we were to use a very sensitive method for the detection of Gm(3) by the use of a specific MCA, and still detect Gm(1) and Gm(2) by polyclonal antisera we would defeat the object of the exercise.

Discussion

It is becoming clear that there is a considerable advantage in using MCAs linked to an ELISA detection system:-

(1) Specificity of each MCA
(2) Large volumes of reagent
(3) Longer 'shelf-life' of the reagent

(4) Ease of staff training (ELISA is far less complex than elution or inhibition techniques)

(5) Objectivity of results

The conjugates are a vital part of the procedure and we feel that there could be some improvement with these particular reagents.

Evaluation of each MCA requires a good deal of work and the following testing protocol is suggested:-

1. Control blood and body fluid samples
2. Control stains of blood and other body fluids
3. Ageing experiments
4. Case-work evaluation
5. Determination of negative values

Nearly all of the forensic science laboratories have to use commercially obtained reagents and this poses some of the most difficult problems.

1. How reproducible are the results from batch to batch?
2. Can we obtain sufficient quantities?
3. Can we obtain the same reagents over long periods of time?
4. Once characterised can we rely on the company to keep the reagent consistent?
5. If there are additives, will the company tell us what they are?

When an individual laboratory obtains a MCA which they consider to be suitable for work in forensic science it would be of considerable value to provide this information to their colleagues, at home and abroad, as soon as possible. Also, if all of the forensic science laboratories will be using the same reagents it will be necessary to decide a testing protocol to which we could all adhere.

However there still remains the problem of how to disseminate the information and we suggest a sub-group of the haemogenetics society with a representative in each of the member countries who would be responsible for informing his colleagues.