THE USEFULNESS OF Km(3) TYPING IN BLOODSTAINS

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Known positive and negative controls should always be simultaneously tested when bloodstain grouping is carried out. In a multiallelic system such as Gm/Km it is also wise, if negative results are to be reported, to select a suitable antithetical marker as a control. One can then be sure that a negative result for a particular factor really is negative and not just that insufficient material is present.

When grouping bloodstains in the Gm system it is common to use Gm(4) and, or Gm(10) as a positive marker for a Gm(1-,2-) phenotype.
In the Km system approximately 87 % of caucasian and 50 % negro populations are negative for Km(1) and although guidelines to the reliability of a negative result, such as condition, age and colour of the stain can be employed, this is not an infallible judgement. Far better is the use of the antithetical Km(3) marker, so long as the antigens and, or antisera are of equal and compatible quality.
This investigation was carried out to assess these qualities in commercially available anti-Km(3) and anti-D (Km 3), with reference to blood stain grouping.

Method

The grouping was performed on microtitre plates.
The factors Km(1) and (3) were tested using dilutions of the stain extract and of serum samples.
A total of 280 samples were tested in parallel on stains and sera. Of these bloodstains a representative sample

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of phenotypes was selected (a total of 12 stains) and stored under different conditions to test the relative stability and reliability of the two antigens (fig. 1) over a period of 7 weeks.

Results & Discussion

Although a slightly lower level of Km(1-) was observed, 85 % compared to 87 %, this survey was in reasonable agreement with other frequencies for a German population (fig. 2).
The occurence of the (1+3-)phenotype was extremely rare, only 1 % of the total. One sample was negative for both factors and this could possibly be due to a low level of light chains or immunoglobulins in the serum. No discrepancies were found between the sera and bloodstains, although the high frequency of Km (3+) could suggest nonspecific inhibition. A possible source of error could have been a lack of reaction between the antiserum and indicator cells, but this was eliminated by the use of controls throughout the experiments.

From the sample stored under different conditions the following results were observed:

Most stains retained their activity for the duration of this experiment.

Most stains showed no reduction of activity for either Km(1) or Km (3) although dilutions up to 1/10 of the stain extract were used.

One sample which was typed as (1+3+) gave varying reactions for Km(1) at 56° C and at 37° C in the humid chamber. This could be mistaken for a (1-3+)result.

After incubation at 37° C in the humid chamber for 7 weeks, 3 samples gave unreadable results because the indicator cells did not form a button in the well.

In these experiments Km(1) and (3) showed approximately equivalent levels of activity (fig. 3).

Summary

Km(3) can reliably be used as a positive marker for a Km(1-)phenotype.

In casework it must be borne in mind that results can be affected and sometimes changed under extreme storage conditions but this also applies to most other systems.

The phenotype Km(1+3-) is rare but when possible it is wise to include this as a control as well as the normal antiserum controls.

The titration of the Km(3) was low and was therefore relatively expensive to use.

When stain extracts of Km(1+3+)phenotypes were titrated they appeared to show approximately equivalent strengths. In practice, so long as a titration series is made on stain extracts no mistake in typing should occur.

References:

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2. Davie M.J. (1979). ABO, Gm and Km grouping of bloodstains on microtitre plates. J. Forensic Sci. Soc. 19: 59-64.

Storage Conditions

Room Temperature

37°C

Moist Chamber 37°C

56°C

Fig. 1: Experimental storage conditions for testing stability of Km(1) and Km(3)

Km(1,3) Microtitre

N=280

Km	N	%
1-3+	237	84,6
1+3+	39	13,9
1+3-	3	1,1
1-3-	1	0,4

Fig. 2: Distribution of Km(1) and Km(3) phenotypes in Münster

Titration of stain extract

Km1+3+	1	3
1/5	–	–
1/10	–	–
1/15	–	–
1/20	+	–
1/25	2	1
control	4	4

Fig. 3: comparison of relative intensities of Km(1) and Km(3) in stains