

## ORIGINAL ARTICLE

## Validation of a simple, rapid, and economical technique for distinguishing type 1 and 2 fibres in fixed and frozen skeletal muscle

W M H Behan, D W Cossar, H A Madden, I C McKay

*J Clin Pathol* 2002;**55**:375–380

See end of article for authors' affiliations

Correspondence to: Professor W M H Behan, Department of Pathology, Western Infirmary, Dumbarton Road, Glasgow G11 6NT, Scotland, UK; [wmb1q@clinmed.gla.ac.uk](mailto:wmb1q@clinmed.gla.ac.uk)

Accepted for publication 24 October 2001

**Aims:** To produce a method of distinguishing between type 1 and 2 skeletal muscle fibres that would be more economical and reproducible than the standard ATPase method and be applicable to both fixed and frozen tissue. Because the ATPase method has been accepted as the basis for fibre identification for the past 50 years, the new method should not give significantly different results.

**Methods:** Isoforms of myosin correlate with isoforms of myofibrillar ATPase and an immunohistochemical (IHC) double labelling protocol was devised using monoclonal antibodies to fast and slow myosin. This required one tissue section rather than four. The results of the two methods were compared by means of morphometric analysis of skeletal muscle biopsies from 20 normal healthy volunteers.

**Results:** There were no significant differences ( $p = 0.57$ ) in the percentages of type 1 (46% using the IHC method v 48% using ATPase) or type 2 fibres (54% v 52%, respectively). The 2a and 2b subtypes were distinguished easily. Analysis of variance revealed that cross sectional area ( $\mu\text{m}^2$ ), diameter ( $\mu\text{m}$ ), form factor, and density of fibre staining (a measure of substrate—enzyme or protein) were all similar. The method worked equally well on fixed material.

**Conclusion:** An IHC method based on the fast and slow isoforms of myosin shows no significant differences in fibre type analysis from the standard ATPase method although it provides important advantages because it is applicable to fixed (including archival) material, is economical and reproducible, and yields a permanent preparation.

The gold standard of diagnosis in muscle disease is based on histological examination. The distribution and changes in type 1 and 2 fibres are key features in differentiating between primary myopathy and neurogenic atrophy, and in identifying specific vulnerability in various disorders.<sup>1, 2</sup> However, the main method used, which is based on the distinction between different isoforms of the myofibrillar enzyme, ATPase, was established approximately 50 years ago<sup>3–6</sup> and, although refined since then<sup>7–9</sup> has several disadvantages: it is critically dependent on pH, temperature, and time of incubation, it requires several sections, is time-consuming, can be applied only to frozen tissue, and provides temporary preparations.

However, isoforms of myosin provide an excellent means of identifying the same fibres,<sup>10–14</sup> and monoclonal antibodies to these are commercially available. An immunohistochemical (IHC) method, with all the advantages of ease, simplicity, rapidity, and economic use of tissue, together with major additional factors—the ability to be used on fixed tissue, the simultaneous identification of both fibre types and subtypes, and the production of permanent preparations—should provide a superior alternative.

“The gold standard of diagnosis in muscle disease is based on histological examination”

Therefore, we analysed skeletal muscle fibre type percentages and characteristics, including density of staining as an indication of substrate concentrations, in biopsy material from the vastus lateralis muscle of 20 healthy volunteers, using such a method with monoclonal antibodies to fast and slow myosin. A combined protocol was used because this meant that type 1, 2a, and 2b fibres could all be identified on the same section. The results were compared with the routine ATPase results on the same biopsies. It was assumed that approximately the same picture would be given by the two methods

but, because the ATPase technique has provided an enormous amount of diagnostic and research data, it was important to establish the degree of correlation. Our results indicated that there were no significant differences.

After validation of the IHC method on frozen tissue, we used it with similar success on formalin fixed, paraffin wax embedded biopsy and archival muscle samples.

## METHODS

## Tissue samples

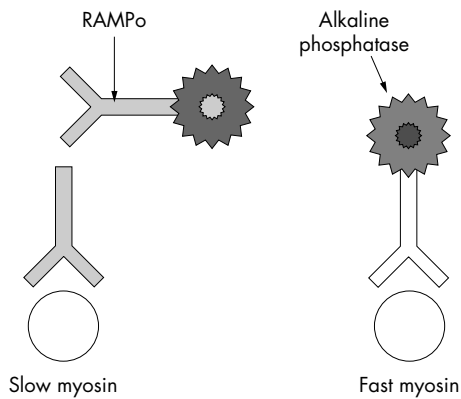
Needle muscle biopsies were obtained from the vastus lateralis of 20 normal healthy adults (16 men: age range, 16–58 years; mean age, 32 and four women: age range, 26–53 years; mean age, 36), frozen, and then processed as described previously.<sup>15</sup> Conditions conformed to an ethical protocol granted by the Western Infirmary, Glasgow, Scotland.

Other material consisted of biopsy samples from patients with AIDS, which were formalin fixed and paraffin wax embedded, and postmortem samples from patients with a variety of neuromuscular diseases.

## ATPase protocol

The standard ATPase method was used at pH values of 9.4, 4.6, and 4.3 on the frozen tissue samples, together with a negative control.<sup>15</sup> For each case, two 10  $\mu\text{m}$  serial sections were used, so that a total of eight was required. The ATPase preparations were done as part of the usual routine series of muscle stains<sup>15</sup> (haematoxylin and eosin, modified Gomori trichrome, oil red O, periodic acid Schiff with and without diastase; and histochemistry for the demonstration of succinic and

**Abbreviations:** IHC, immunohistochemistry; NRS, normal rabbit serum; TBS, Tris buffered saline



**Figure 1** Diagram of the staining protocol.

nicotinamide dehydrogenases, cytochrome oxidase, acid phosphatase, and the identification of deficiencies in myophosphorylase, adenylate deaminase, and phosphofructokinase).

### IHC protocol

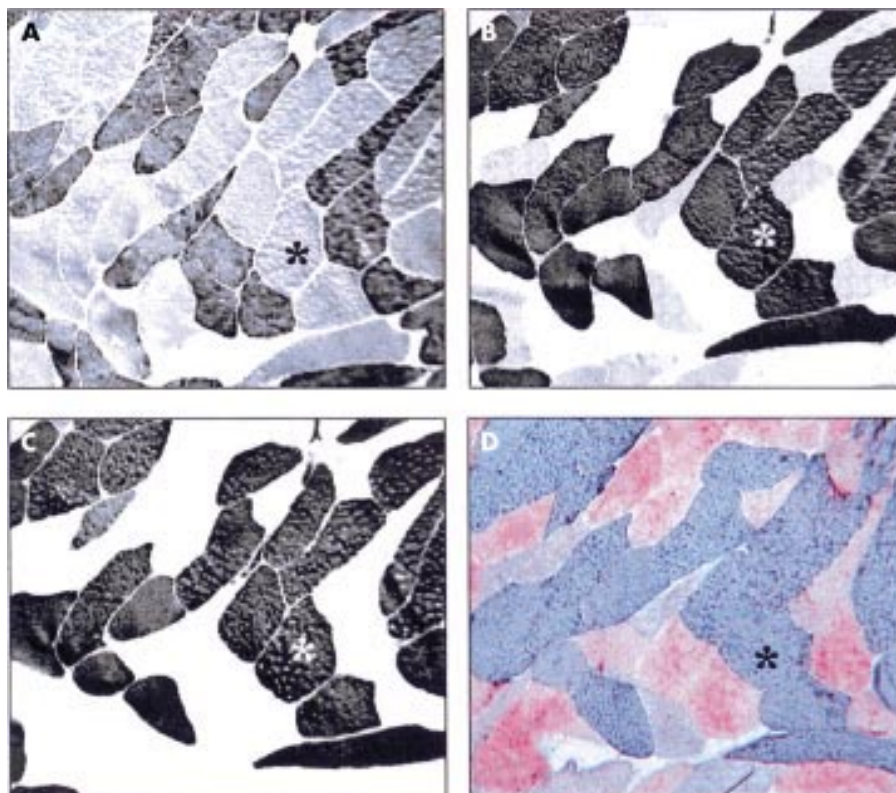
Two 10 µm sections from each case were placed on one slide. The control consisted of two sections of normal unrelated skeletal muscle, incorporated into each run of up to 10 cases. Commercial antibodies to fast and slow isoforms of myosin were used, each with a different visualisation system, so that specific identification of each fibre type on the same section was feasible. In brief, after fixation in acetone the antibody to slow myosin was applied, followed by a peroxidase conjugated rabbit antimouse antibody; the result was visualised as black type 1 fibres using the commercial Vector SG substrate kit (Vector Laboratories, Peterborough, UK). The alkaline phosphatase conjugated antibody to fast myosin was then applied;

red type 2 fibres were visualised using the commercial Vector red substrate kit. The full details of the simple standard protocol<sup>16</sup> (fig 1), used widely in routine pathology laboratories and which we modified slightly, are as follows.

Sections were incubated with 20% normal rabbit serum (NRS) in Tris buffered saline pH 7.6 (TBS) for 10 minutes, after which the excess serum was drained off and the sections incubated in monoclonal antibody to slow myosin (Sigma-Aldrich, Poole, Dorset, UK) diluted 1/2000 in 4% NRS for 30 minutes, followed by three washes in TBS. The sections were then incubated in peroxidase conjugated rabbit antimouse antibody (Dako Ltd, Ely, Cambridgeshire, UK), diluted 1/50 in 4% NRS, for 60 minutes, and then washed three times in TBS. Vector SG peroxidase substrate solution (Vector Laboratories) was then applied, controlling the reaction by microscopic examination over five to 15 minutes. The sections were washed in running tap water, incubated in TBS for five minutes, and then in 20% NRS for 10 minutes. Excess serum was drained off before incubation in alkaline phosphatase conjugated monoclonal antibody to fast myosin (Sigma-Aldrich), diluted 1/50 in 4% NRS, for 60 minutes. After washing as above, the sections were incubated in Vector red alkaline phosphatase substrate solution (Vector Laboratories) for 10–20 minutes, controlling the reaction by microscopic examination. The final wash was in running tap water, after which the sections were dehydrated through graded alcohols, cleared in xylene, and mounted in synthetic medium.

The antibodies used were: (1) for type 1 fibres, monoclonal antimyosin (skeletal, slow; clone NOQ7.5.4D; Sigma-Aldrich). (2) For type 2 fibres, monoclonal antimyosin (skeletal, fast; alkaline phosphatase conjugate; clone MY-32; Sigma-Aldrich).

On completion, type 1 fibres are black whereas the type 2 fibres are pink. Type 2a and 2b subtypes can be distinguished because 2b are completely pink whereas 2a are intermediate between black and pink, appearing as a granular, dark, pinkish grey (fig 2).



**Figure 2** Comparison of frozen sections stained for myofibrillar ATPase at (A) pH 9.4, (B) pH 4.6, and (C) pH 4.3 and (D) by immunohistochemistry for slow (dark grey) and pink (fast) myosin. Type 1 fibres are dark grey whereas type 2a and 2b fibres are distinguishable as pale greyish pink and pink, respectively. Original magnification, ×100.

**Table 1** Comparison of percentages of type 1 and 2 fibres in ATPase and immunohistochemistry (IHC) methods

Sex	Fibre type	ATPase at pH 9.4		IHC	
		No. of fibres identified	% Of total	No. of fibres identified	% Of total
Male (16)	Type 1	577	45	557	44
	Type 2	703	55	723	56
Female (4)	Type 1	186	58	176	55
	Type 2	133	42	144	45
Males and females (20)	Type 1	763	48	733	46
	Type 2	836	52	867	54

The above method also works very well on formalin fixed, paraffin wax embedded tissue, following the usual preliminary steps—that is, sections taken to water and endogenous peroxidase removal with 0.3% hydrogen peroxide for 10 minutes. Antigen retrieval is necessary and this was achieved by five minutes at full pressure in a microwaveable pressure cooker (1 mM EDTA), followed by a 20 minute cool down period, one wash in water, and then trypsin digestion (0.1% trypsin/calcium chloride) for one minute. The method continues as above.

### MORPHOMETRIC ANALYSIS

The SCION package (SCION Corporation, Maryland, USA) running on Microsoft Windows 95 and downloaded from the website <http://www.scioncorp.com> was used. It is capable of the wide range of image processing and analysis functions required for fibre analysis.<sup>17–20</sup> Linear and geometric measurements including diameter, length, area, and perimeter of fibres can be made. Up to 500 measurements can be stored at one time in the results window. Spatial calibration is supported by this program so it can provide real world area and length measurements (such as  $\mu\text{m}$ ). A semiautomatic analysis routine is followed because this allows the observer to reject tissue artefacts.<sup>21</sup>

Tissue sections were viewed using the  $\times 10$  objective and visualised on the monitor. Several still images for each case were imported into the image analysis package, SCION Image for Windows, and morphometric data on muscle fibres retrieved by means of a semiautomated routine using the SCION software.

The following parameters were recorded:

- (1) Area: area of fibre cross section.
- (2) Mean density: average grey value within a selection.
- (3) X-Y centre: centre of the best fitting ellipse.
- (4) Modal density: most frequently occurring grey value within a selection.
- (5) Perimeter/length: length around the outside of a polygon selection.
- (6) Ellipse major axis: length of the major axis of the best fitting ellipse.
- (7) Ellipse minor axis: length of the minor axis of the best fitting ellipse.
- (8) Integrated density: sum of the grey values in a selection, with background subtracted.
- (9) Min/Max: minimum and maximum grey values within a selection.

Eighty fibres were counted at random on each section examined. Using the ATPase method, the 80 fibres were counted on the pH 9.4 preparation as type 1 and 2 and then a further 80 on the pH 4.6 section were analysed as type 2a or 2b. Using the IHC method, the 80 fibres were counted and classified on one preparation as type 1, 2a, or 2b.

### Statistical methods

The staining methods were compared by analysis of variance to see whether they had different influences on estimates of the morphometric variables, such as cross sectional area and minor axis of the fibre cross sections.

To test whether the two staining methods gave different estimates of the proportions of type 1 and type 2 fibres, the  $\chi^2$  test was used. A separate  $\chi^2$  was calculated for each individual and the verdict was based on summation of the  $\chi^2$  components and degrees of freedom.

### RESULTS

The 20 biopsies from the normal healthy controls yielded suitable samples. The morphometric and densitometric measurements had distributions sufficiently close to normal to allow the use of parametric statistical analysis.

It can be seen from table 1 that there was no significant difference ( $p = 0.57$ ) between the overall percentages of type 1 and 2 fibres identified by the two different methods. For the group as a whole, the results were: type 1, 48% using the ATPase protocol *v* 46% using IHC and for type 2, 52% *v* 54%, respectively. When the groups were analysed as males and females, there were sex differences—men tended to have more type 2 fibres than women (55% and 56% *v* 42% and 45%, respectively) and women more type 1 fibres than men (58% and 55% *v* 45% and 44%, respectively), but again the results were similar for the ATPase and IHC methods.

Direct comparison of type 2 subtype percentages in the two methods was not possible. Both 2a and 2b fibres could be counted easily on the IHC sections. The subtypes could not, of course, be distinguished using ATPase at pH 9.4, but when the ATPase preparations at pH 4.6 and 4.3 were used, an overestimate of type 1 fibres always resulted. However, it can be seen from table 2 that the IHC subtype results we obtained are comparable to accepted values for vasti muscles.<sup>1–2 7 17 22 23</sup>

**Table 2** Percentage analysis of 2a and 2b fibre subtypes in male and female individuals using immunohistochemistry

Sex of case	Subtype	No. of fibres identified	% Of all fibre types
Males	Type 2a	360	28
	Type 2b	363	28
Females	Type 2a	60	19
	Type 2b	84	26

**Table 3** Comparison of diameter and cross sectional area of type 1 and 2 muscle fibres in ATPase and immunohistochemistry (IHC) methods

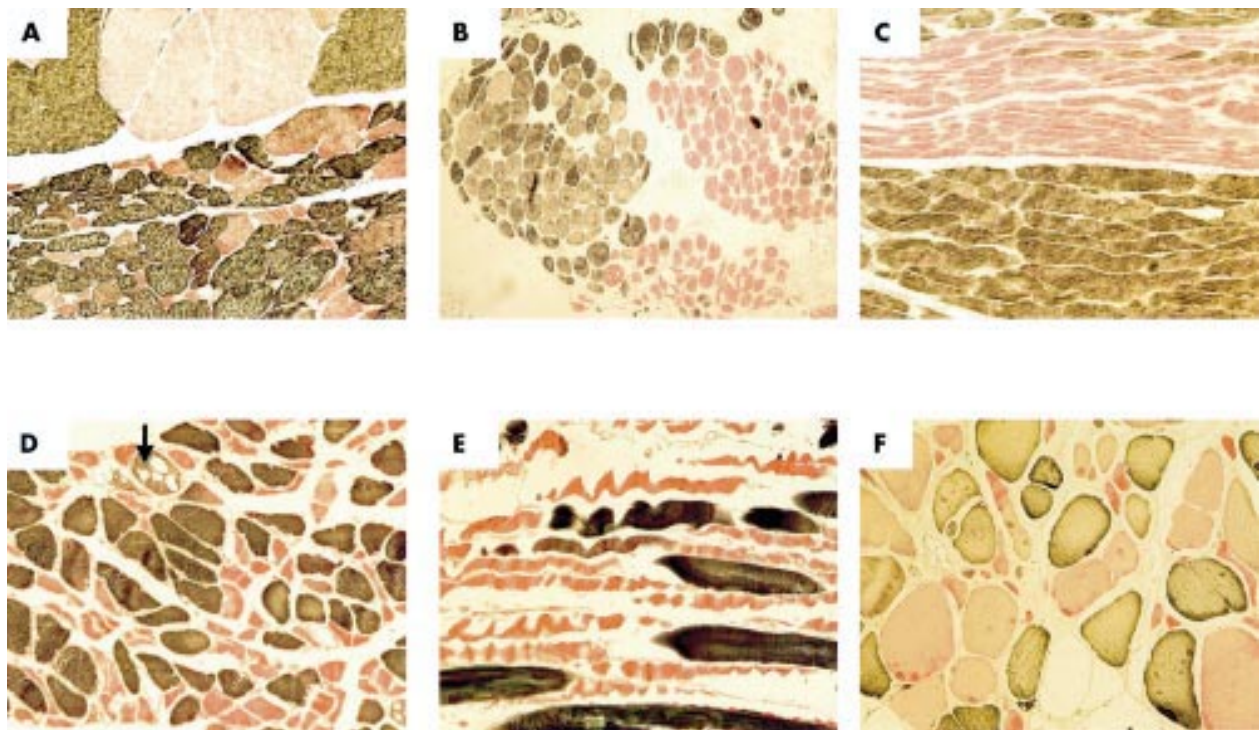
Staining method	Type 1	Type 2 (2a and 2b)	Type 2a	Type 2b	All
IHC					
No. of fibres measured	733		420	447	1600
Mean minor axis/ $\mu\text{m}$	64.8		61.0	65.5	64.0
SD of minor axis/ $\mu\text{m}$	11.3		12.6	13.0	12.3
Mean area/ $\mu\text{m}^2$	4923		4616	5227	4928
SD of area/ $\mu\text{m}^2$	1444		1581	1692	1568
ATPase at pH 9.4					
No. of fibres measured	763	83.6			1599
Mean minor axis/ $\mu\text{m}$	65.4	65.8			65.6
SD of minor axis/ $\mu\text{m}$	11.8	12.0			11.9
Mean area/ $\mu\text{m}^2$	5007	5264			5141
SD of area/ $\mu\text{m}^2$	1504	1520			1517

Comparative analysis of cross sectional area ( $\mu\text{m}^2$ ) and diameter was carried out (table 3). Cross sectional area is regarded as being sensitive to the angle at which a fibre is cut and therefore the minor axis length (diameter), which is relatively insensitive to this variation, is used also. No significant differences were found. The IHC method made the fibres appear slightly smaller than the ATPase method, the minor axis being on average 2% smaller and the area 4% smaller. These differences were much less than the differences between individuals and they are caused by mild shrinkage occurring during the IHC method.

The form factor was also measured because it gives an indication of the number of fibres cut obliquely rather than transversely and therefore validates the above measurements.<sup>18</sup> It is

defined as  $4\pi A/p^2$ , where A is the cross sectional area and p is the perimeter. A circle has a value of 1, whereas a straight line has a value of 0. Most normal muscle cells cut in true cross section give a form factor of 0.80 (range, 0.75–0.85). This was the case in both sets of sections.

Density of fibre staining was compared in the two methods, as a measure of substrate (enzyme or protein) concentration. Again, the values were comparable, with male type 2 fibres staining more than male type 1 and female type 1 and 2 being approximately equal on both protocols. The IHC sections could be used to analyse type 2 subtypes: this revealed that type 2b was more abundant than 2a in both sexes. The other parameters examined (X-Y centre, modal density, integrated density) also showed good correlation, and the fact that there were two colours made analysis easier.



**Figure 3** (A) Neurogenic atrophy. A group of normal sized fibres is seen in the upper part of the section with a severely atrophied group below. The latter includes both type 1 and 2 fibres, suggesting denervation before the establishment of reinnervation (original magnification,  $\times 400$ ). (B,C) Neurogenic atrophy (transverse section and longitudinal section, respectively) with reinnervation. Adjacent fascicles are composed almost entirely of one type or the other (original magnification,  $\times 100$ ). (D) Inflammatory myopathy. Normal mosaic pattern of type 1 and 2 fibres with severe selective atrophy of type 2. Architectural disorganisation (arrow) can be seen in a type 1 fibre (original magnification,  $\times 200$ ). (E) Severe wasting of type 2 fibres (original magnification,  $\times 200$ ). (F) Gross variation in size of both type 1 and 2 fibres in a case of muscular dystrophy. Distinction between the two types is clear (original magnification,  $\times 400$ ).

The results given here show that there is excellent agreement between the traditional ATPase and the novel immunohistochemical method we propose. On archival and postmortem tissue the unique advantage of the new technique became apparent, with a clear and unambiguous distinction between the fibre types. Figure 3 illustrates this in a variety of conditions. In fig 3A, a case of neurogenic atrophy, fibres are of normal size in the upper half of the section but grouped and grossly atrophied in the lower part. Both type 1 and 2 fibres are seen in the atrophied area, helping to distinguish "primary" denervation from one occurring after reinnervation. In contrast, fig 3B/C is also from a case of neurogenic atrophy but does show evidence of reinnervation, with two fascicles composed almost entirely of one type or the other. In each fascicle, focal fibre atrophy is seen, indicating continuing denervation.

Figure 3D is from a case of polymyositis. The mosaic pattern of fibre types is preserved but there is obviously selective atrophy of type 2 fibres. Severe damage to myosin is detectable in a type 1 fibre. Muscle wasting associated with chronic obstructive airways disease is shown to result mainly from atrophy of type 2 fibres in fig 3E. Figure 3F illustrates the features in a case of muscular dystrophy. Although fibre type distinction can be blurred on the ATPase stain, it is clear on the IHC method, where gross variation in both fibre types, with occasional split ones, is detectable.

## DISCUSSION

It is well established that human skeletal muscle consists of two main fibre types, divided according to metabolic and physiological features. Changes in the normal mosaic pattern of type 1 and 2 fibres—whether they show a proportionate increase or decrease, are grouped or not, hypertrophied or atrophied—are key features in the diagnosis of myopathies.<sup>1,2</sup> Fibre type changes in association with major cardiovascular risk factors (insulin resistance, hypertension, and obesity), chronic heart failure, and respiratory failure are also of current interest,<sup>24–27</sup> as is their relation to physical activity<sup>28</sup> and the selection and training of young athletes.<sup>29–31</sup>

The myosin protein and the myosin ATPase enzyme are associated intimately both anatomically and functionally because myosin is the protein upon which ATPase acts to convert chemical energy to mechanical energy. Just as there are several isoforms of ATPase, allowing fibre type delineation, there are distinct isoforms of myosin heavy chains—at least seven have been identified in normal skeletal muscle, correlating with phenotypic differences in the contractile, metabolic, and size properties of fibres.<sup>14</sup> Numerous investigations using histochemical, immunohistochemical, biochemical, and electrophoretic methods on normal skeletal muscle, including individual fibres, have confirmed that there is a strong correlation between ATPase activity and myosin heavy chain type.<sup>10–14, 32</sup> Indeed, because many metabolic properties are only loosely coupled with the molecular properties of myofibrillar ATPases, and correlate better with myosin heavy chain isoforms, it has been stated categorically that: "...myosin heavy chain isoforms appear to be the best choice for fibre delineation".<sup>13</sup>

A histochemical reaction for ATPase, carried out with preincubation at different pH values, forms the basis on which fibres are classified as type 1 (slow, oxidative), 2a (fast, oxidative/glycolytic), and 2b (fast, glycolytic),<sup>1–8, 15</sup> and normal values were established 30 years ago.<sup>6, 7, 15, 22, 23, 33–35</sup> The method, however, has several disadvantages, including difficulty in achieving reproducibility because of the critical dependence on pH, temperature, and incubation time, in addition to the fact that the preparation fades and, of course, the problem that it will not work on fixed tissue because of enzyme degradation.

Our aim was to provide a simple and rapid IHC protocol, based on the identification of slow and fast myosin, as an alternative. We used commercially available materials. It would have been ideal to have each monoclonal antibody directly conjugated but unfortunately only one—alkaline phosphatase conjugated monoclonal antibody to fast myosin—could be obtained. If a peroxidase conjugated monoclonal antibody to slow myosin had also been available, we could have used a sequential direct method, instead of a combination of direct and indirect techniques. No doubt this modification will become available in the near future. However, the technique worked very well. The indirect method was performed first to avoid any detection of the conjugated antibody by the antimouse secondary layer. There was no advantage to using a biotinylated antibody in the first layer because no increase in sensitivity was needed. The Vector SG substrate gives a black end product, which incorporates the company's own enhancing effect and provides a highly effective contrast to the other (red) fibres much better than the usual brown colour produced when diaminobenzene is used.

"Changes in the normal mosaic pattern of type 1 and 2 fibres—whether they show a proportionate increase or decrease, are grouped or not, hypertrophied or atrophied—are key features in the diagnosis of myopathies"

The most important advantage of the IHC protocol is probably that it can be used in the diagnostic distinction of fibre types in paraffin wax embedded muscle tissue. Its other advantages include the ability to study both fibre types on the same preparation, economy of use, and the production of permanent and colourful preparations so that image analysis is easy. Economy of tissue use is important now that needle biopsies are becoming routine and micromethods for all parameters, including gene analysis, are available. An immunofluorescent technique was also considered but this would offer only a temporary preparation, which is unsuitable for morphometry, and examination is tedious. A few previous attempts at IHC protocols, using monoclonal antibodies to various neural<sup>36–38</sup> and muscle<sup>39, 40</sup> antigens have been made. Two groups used antibodies to myosin but a single labelling technique was used, which could not identify type 2 subtypes.<sup>24, 40, 41</sup> None is as satisfactory as the one described here.

The superiority of an IHC method that can be applied to paraffin wax embedded muscle has been stressed recently by workers using antibodies to dystrophin and the dystrophin associated glycoproteins in the diagnosis of muscular dystrophy.<sup>42</sup> They pointed out that such a method obviates all the problems of freezing, handling, safety issues, and the storage of frozen material, in addition to allowing satisfactory analysis of archival tissue.

Our results indicated no significant differences in type 1 or 2 proportions and a clear distinction between type 2a and 2b with similar morphometry (cross sectional area, minor axis length, and form factor measurements). The minor axis was, on average, 2% smaller using IHC than with the ATPase method, and the cross sectional area was 4% smaller, probably because of a small amount of shrinkage occurring during the IHC method, but these differences were less than the differences between individuals. They make no significant difference to the interpretation of myopathy and are simply a minor factor to be considered in the learning curve of the method, as stated previously.<sup>42</sup> The density of fibre staining was comparable, indicating a correlation between enzyme (ATPase) and protein (myosin) concentrations.

Myosin isoforms are regulated developmentally in the same way as myofibrillar ATPases. Using our IHC method, we could not distinguish between type 1 and the minor type 2C subtype,

### Take home messages

- We have developed an immunohistochemical method based on the fast and slow isoforms of myosin, which shows no significant differences in fibre type analysis from the standard ATPase method
- This method provides important advantages over the ATPase method because it is applicable to fixed (including archival) material, it is economical and reproducible, and it yields a permanent preparation

which forms approximately 2–3% of normal fibres and is considered to be a precursor of type 1. These fibres, however, are important only in the context of regeneration, when other features will be present. The IHC method will score in single fibre analysis when there may be hybrid expression of both myofibrillar ATPase and myosin heavy chain isoforms, but the former is very difficult to evaluate by staining intensity.<sup>12</sup>

As can be seen from the illustrations, the colour combination made analysis easy in the pathological diagnosis of both fixed and frozen tissue.

We conclude that this novel method offers a great improvement over the standard ATPase method.

### ACKNOWLEDGEMENTS

We are grateful to P Cameron and I Downie for additional help with the histochemical preparations, to Dr C Smith for supplying extra fixed material, and Professor W R Lee for his advice with regard to the image analyser. This work was supported by the Connell and Barclay Trusts of Glasgow University

.....

### Authors' affiliations

**W M H Behan, D W Cossar, H A Madden**, Department of Pathology, University of Glasgow, Western Infirmary, Glasgow G11 6NT, Glasgow, Scotland, UK

**I C McKay**, Department of Immunology, University of Glasgow

### REFERENCES

- 1 **Banker BQ**, Engel AG. Basic reactions of muscle. In: *Myology*, Vol. 1, 2nd ed. Engel AG, Franzini-Armstrong C, eds. New York: McGraw Hill, 1994:832–88.
- 2 **Cullen MJ**, Johnson MA, Mastaglia FM. Pathological reactions of skeletal muscle. In: *Skeletal muscle pathology*. 2nd ed. Mastaglia FM, Lord Walton of Detchant, eds. Edinburgh: Churchill Livingstone, 1992:123–84.
- 3 **Padykula HA**, Herman E. The specificity of the histochemical method for adenosine triphosphatase. *J Histochem Cytochem* 1955;**3**:170–95.
- 4 **Padykula HA**, Herman E. Factors affecting the activity of adenosine triphosphatase and other phosphates as measured by histochemical techniques. *J Histochem Cytochem* 1955;**3**:161–9.
- 5 **Brooke MH**, Kaiser KK. Some comments on the histochemical characterisation of muscle adenosine triphosphates. *J Histochem Cytochem* 1961;**17**:431–2.
- 6 **Engel WK**. The essentiality of histo- and cytochemical studies in the investigation of neuromuscular disease. *Neurology* 1962;**12**:778–84.
- 7 **Brooke MH**, Kaiser KK. Muscle fiber types: how many and what kind? *Arch Neurol* 1970;**23**:369–77.
- 8 **Brooke MH**, Kaiser KK. Three "myosin adenosine triphosphatase" systems: the nature of their pH lability and sulphhydryl dependence. *J Histochem Cytochem* 1970;**18**:670–2.
- 9 **Round JM**, Matthews Y, Jones DA. A quick, simple and reliable method for ATPase in human muscle preparations. *Histochem J* 1980;**12**:707–10.
- 10 **Perrie WT**, Bumford SJ. Electrophoretic separation of myosin isoenzymes. Implications for the histochemical demonstration of fibre types in biopsy specimens of human skeletal muscle. *J Neurol Sci* 1986;**73**:89–96.
- 11 **Staron RS**, Johnson P. Myosin polymorphism and differential expression in adult human skeletal muscle. *Comp Biochem Physiol B Biochem Mol Biol* 1993;**106**:463–75.
- 12 **Pereira JAS**, Wessels A, Nijtmans L, et al. New method for the characterisation of single human skeletal muscle fibres demonstrates a relation between mATPase and MyHC expression in pure and hybrid fibre types. *J Muscle Res Cell Motil* 1995;**16**:21–34.
- 13 **Pette D**, Peuker H, Staron RS. The impact of biochemical methods for single fibre muscle analysis. *Acta Physiol Scand* 1999;**166**:261–77.
- 14 **Rivero JL**, Talmadge RJ, Edgerton VR. Interrelationships of myofibrillar ATPase activity and metabolic properties of myosin heavy chain-based fibre types in rat skeletal muscle. *Histochem Cell Biol* 1999;**111**:277–87.
- 15 **Dubowitz V**. Histological and histochemical stains and reactions. In: *Muscle biopsy—a practical approach*, 2nd ed. London: Bailliere Tindall, 1985:19–40.
- 16 **Beesley JE**. Multiple immunolabelling techniques. In: *Immunocytochemistry—a practical approach*. Beesley JE, ed. Oxford: Oxford University Press, 1993:103–25.
- 17 **Engel AG**. Quantitative morphological studies of muscle. In: *Myology*, 2nd ed. Engel AG, Franzini-Armstrong C, eds. New York: McGraw Hill, 1994:1018–45.
- 18 **Song SK**, Shimada N, Anderson PJ. Orthogonal diameters in the analysis of muscle fibre size and form. *Nature* 1963;**200**:1220–1.
- 19 **Aherne W**. A method of determining the cross sectional area of muscle fibres. *J Neurol Sci* 1968;**7**:519–28.
- 20 **Mize RR**, Holdefer RN, Nabors LD. Quantitative immunocytochemistry using an image analyser. I Hardware evaluation, image processing and data analysis. *J Neurosci Methods* 1988;**26**:1–24.
- 21 **Slavin G**, Sowter C, Ward P, et al. Measurements of striated muscle fibre diameters using interactive computer aided microscopy. *J Clin Pathol* 1982;**35**:1268–71.
- 22 **Brooke MH**, Engel WK. The histographic analysis of human muscle biopsies with regard to fiber types. 1. Adult male and female. *Neurology* 1969;**19**:221–33.
- 23 **Johnson MA**, Polgar J, Weightman D, et al. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci* 1973;**18**:111–29.
- 24 **Bassett DR**, Jr. Skeletal muscle characteristics: relationships to cardiovascular risk factors. *Med Sci Sports Exerc* 1994;**26**:957–66.
- 25 **Marin P**, Andersson B, Krolkiewski M, et al. Muscle fiber composition and capillary density in women and men with NIDDM. *Diabetes Care* 1994;**17**:382–6.
- 26 **Poole-Wilson PA**, Ferrari R. Role of skeletal muscle in the syndrome of chronic heart failure. *J Mol Cell Cardiol* 1996;**28**:2275–85.
- 27 **Satta A**, Migliori GB, Spanevello A, et al. Fibre types in skeletal muscles of chronic obstructive pulmonary disease patients related to respiratory function and exercise tolerance. *Eur Respir J* 1997;**10**:2853–60.
- 28 **Glenmark B**. Skeletal muscle fibre types, physical performance, physical activity and attitude to physical activity in women and men. A follow-up from age 16 to 27. *Acta Physiol Scand Suppl* 1994;**623**:1–47.
- 29 **Schantz PG**. Plasticity of human skeletal muscle with special reference to effects of physical training on enzyme levels of the NADH shuttles and phenotypic expression of slow and fast myofibrillar proteins. *Acta Physiol Scand Suppl* 1986;**558**:1–62.
- 30 **Ricoy JR**, Encinas AR, Cabello A, et al. Histochemical study of the vastus lateralis muscle fibre types of athletes. *J Physiol Biochem* 1998;**54**:41–7.
- 31 **Jostarndt-Fogel K**, Puntchart A, Hoppeler H, et al. Fibre-type specific expression of fast and slow essential myosin light chain mRNAs in trained human skeletal muscles. *Acta Physiol Scand* 1998;**164**:299–308.
- 32 **Pette D**, Staron RS. Molecular basis of the phenotypic characteristics of mammalian muscle fibres. *Ciba Found Symp* 1988;**138**:22–34.
- 33 **Brooke MH**, Engel WK. The histographic analysis of human muscle biopsies with regard to fibre types IV Children's biopsies. *Neurology* 1969;**19**:591–605.
- 34 **Aherne W**, Ayyar DR, Clark PA, et al. Muscle fibre size in normal infants, children and adolescents—an autopsy study. *J Neurol Sci* 1971;**14**:171–82.
- 35 **Polgar J**, Johnson MA, Weightman D, et al. Data on fibre size in thirty six human muscles—an autopsy study. *J Neurol Sci* 1973;**19**:307–18.
- 36 **Dodson A**, Garson J, Burke M, et al. Monoclonal antibody that detects human type 1 fibres in routinely fixed wax embedded sections. *J Clin Pathol* 1987;**40**:172–4.
- 37 **Nakamura T**, Kawahara H, Myashita H, et al. Cross reactive identification of types 1 and 2c fibres in human skeletal muscle with monoclonal anti-neurofilament (200 kD) antibody. *Histochemistry* 1987;**87**:39–45.
- 38 **Oldfors A**, Seidal T. Immunohistochemical demonstration of different muscle fibre types in paraffin sections. *Histopathology* 1989;**15**:420–3.
- 39 **Rojiani AM**, Cho ES. Neuropathologic applications of immunohistochemical fiber typing in the non-neoplastic muscle biopsy. *Mod Pathol* 1998;**11**:334–8.
- 40 **Havenith MG**, Visser R, Schrijvers-van Scendel JMC, et al. Muscle fibre typing in routinely processed skeletal muscle with monoclonal antibodies. *Histochemistry* 1990;**93**:497–9.
- 41 **Matsumoto N**, Nakamura T, Yasui Y, et al. Immunohistochemical differentiation of fiber types in human skeletal muscle using monoclonal antibodies to slow and fast isoforms of troponin 1 subunit. *Biotech Histochem* 1997;**4**:191–7.
- 42 **Sheriffs IN**, Rampling D, Smith VV. Paraffin wax embedded muscle is suitable for the diagnosis of muscular dystrophy. *J Clin Pathol* 2001;**54**:517–20.



## Validation of a simple, rapid, and economical technique for distinguishing type 1 and 2 fibres in fixed and frozen skeletal muscle

W M H Behan, D W Cossar, H A Madden, et al.

*J Clin Pathol* 2002 55: 375-380

doi:

---

Updated information and services can be found at:

<http://jcp.bmj.com/content/55/5/375.full.html>

---

*These include:*

### References

This article cites 34 articles, 11 of which can be accessed free at:

<http://jcp.bmj.com/content/55/5/375.full.html#ref-list-1>

Article cited in:

<http://jcp.bmj.com/content/55/5/375.full.html#related-urls>

### Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

---

### Topic Collections

Articles on similar topics can be found in the following collections

[Clinical diagnostic tests](#) (637 articles)

[Immunology \(including allergy\)](#) (1279 articles)

---

### Notes

---

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>