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Letter to the Editor

Detection of Cystic Fibrosis Protein by Electrofocusing

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We have previously described an assay for the detection of individuals homozygous or heterozygous for cystic fibrosis (CF), which employs analytical electrofocusing in thin layer polyacrylamide gels to examine sera for a cystic fibrosis protein (CFP) and other protein markers (bands B, C, D) that we feel are diagnostic for CF (8, 9). In the letter by Smith *et al.* (4) and an abstract of work presented by Thomas *et al.* (5), two attempts at reproducing our assay are described. Both groups of investigators claim to have analyzed CF homozygote and normal control sera using electrofocusing methods exactly analogous to those published (8, 9) with the obvious exception that neither group used the electrofocusing apparatus used in our method (2, 6, 8, 9). Smith *et al.* (4) used an LKB Multiphor and Thomas *et al.* (5) used a Brinkman electrofocusing apparatus. Neither group of investigators could detect *Consistent* differences between the stained serum protein patterns obtained (*e.g.*, CFP) for CF and normal control samples, although Thomas did note in his presentation that some CF samples contained protein bands near pH 8.5 that were not found in the majority of the normal control samples tested. Partial confirmation of our claims of a protein unique to CF with a pI near 8.5 and a molecular weight of 1,000–10,000 is evident, however, from the report of Altland *et al.* (1). These investigators were able to demonstrate a small molecular weight protein unique to sera from CF homozygotes and heterozygotes by performing a second step electrophoresis utilizing the serum protein fraction which focuses between pH 7 and 9. Curiously enough neither Smith *et al.* (4) nor Thomas *et al.* (5) could reproduce either Altland's findings (1) or ours (8, 9).

Detection of CFP using our method involves rather exacting requirements for the collection of serum samples and their subsequent analysis by electrofocusing. Minor variations in the methods described for sample collection and storage may cause degradation and loss of CFP and this by itself could lead to erroneous results since many CF samples would appear to be CFP negative, making it impossible to observe a consistent difference between a group of CF samples and normal control samples by electrofocusing. It is of interest to note that the same methods of sample collection and storage that we use (8) also result in CF homozygote and heterozygote carrier samples which are positive for CF ciliary dyskinesia factor (CDF) (3) as detected by a modified rabbit tracheal bioassay (10, 11), whereas normal healthy control sera are CDF negative.

From the results presented by Thomas *et al.* (4) and Figure 1 of the letter of Smith *et al.* (5), it is obvious that neither group has reproduced the protein banding patterns presented in either of our previous reports (8, 9). The following points are evident upon comparing their figure (5) to those previously published (8). (*1*) The pH gradients are not the same, (*2*) the location of the sample pads relative to the anode is not the same and, (*3*) the protein banding patterns are totally different. We question the validity of the techniques used, claims of having reproduced our method for detecting CFP, or supposed improvements designed to enhance detection of CFP, since Smith *et al.* (4) and Thomas *et al.* (5) could not even reproduce our protein banding patterns. It is quite obvious from their figure that the pH region of 8.3 to 9 is poorly resolved and overloaded. CFP is a thin, light blue band (when stained), which can easily be obscured by other protein bands focusing near it, unless the composition of the pH gradient is exact and the resolution is great enough to separate the bands between pH 8 to 9. The length of the gel used in the LKB Multiphor is about half that used in our apparatus. This difference in gel length would reduce the separation between bands about 2-fold and thereby greatly impair the resolution required to detect CFP.

Several technical details concerning our method of electrofocusing were given in our previous report (8); however, minor facets of the method which we felt were obvious from reports cited (2, 6) are indicated below in the hope that other investigators will be able to reproduce the patterns shown previously (8) and thereby localize and detect CFP. Duplication of the protein patterns we obtain is essential for the detection of CFP. (*1*) We use the Awdeh-Williamson (2, 6) apparatus and associated methodology. The apparatus does not have a cooling block and has carbon electrodes which are thoroughly cleaned with distilled water after each run. The plates used are coated with gelatin before making a gel (6). (*2*) The cathode solution consists of a 5% solution of TEMED (*N,N,N′-N′*-tetramethylenediamine) v/v in deionized, double-distilled water ($DDDH_2O$). The anode solution consists of a 1:30 dilution of 85% (w/v) reagent grade phosphoric acid in $DDDH_2O$. NaOH and H_2SO_4 can not replace TEMED and H_3PO_4 as changes in the electrode solutions will markedly affect the protein patterns obtained, regardless of whether the LKB Multiphor or our apparatus is used. (*3*) The sample pads (Whatman, 3MM) are placed with their lower edge 1 cm from the anode and are left on the gel undisturbed throughout the entire duration of the run (18–24 hr). We feel that prolonged exposure of the sample to the acidic pH generated at the anode and 4 M urea are favorable for the demonstration of CFP. (*4*) The pH gradient described (8) must be duplicated to obtain the protein patterns shown. The Ampholine composition used, was determined, as was our pH 3–8 gradient described elsewhere (7), specifically to enhance the separation of serum proteins focusing in particular pH regions while retaining conditions elsewhere (i.e., pH near the anode) favorable for the detection of these proteins and for the dissolution of complexes or alteration of precursors, that may be liberating the proteins of interest during electrofocusing.

The use of 4 M urea, the duration of electrofocusing, voltage used, electrofocusing solutions, location of the sample pads, volume of serum analyzed, and the apparatus used, all have important favorable or adverse effects on the demonstration of CFP and each of these parameters of our method have been investigated carefully over the past 4 years. We hope that the details of our method given above and in previous reports (8, 9) will

allow duplication of our assay for detection of cystic fibrosis homozygotes and heterozygote carriers by other investigators. Although the Awdeh-Williamson apparatus (2, 6) (which should be used to duplicate our method) is simple to construct and very inexpensive (less than $25 per box), we can appreciate the fact that a commercially produced apparatus such as the LKB Multiphor is more readily accessible to other investigators. Accordingly, we are currently exploring the use of the LKB Multiphor for the detection of CFP and comparing the results to those obtained with our apparatus. When our research is completed, the findings will be made available to interested investigators. Preliminary results indicate that minor but significant alterations in our method are necessary for the detection of CFP using the LKB Multiphor.

Note Added in Proof. Since the original copy of this letter was submitted for publication we have completed studies investigating the use of the LKB Multiphor for the detection of CFP. The complete methodology and results obtained will appear in: Wilson, G. B., Arnaud, P., Monsher, M. T., and Fudenberg, H. H.: An improved method for the detection of cystic fibrosis protein in serum using the LKB Multiphor electrofocusing apparatus. Pediat. Res. (Submitted for publication.)

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