Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Journal of Immunological Methods, 119 (1989) 153–187
Elsevier

JIM 05204

Review article

Immunoblotting and dot blotting

D.I. Stott

Department of Bacteriology and Immunology, University of Glasgow, Western Infirmary, Glasgow G11 6NT, Scotland, U.K.

(Received 3 February 1989, accepted 9 February 1989)

Key words: Immunoblotting; Dot blotting; Slot blotting; Dot immunobinding; Electrophoresis; Western Blotting; (Methods); (Review)

Introduction

A variety of methods has been available for many years for the analytical separation of mixtures of proteins into their component parts by electrophoresis in a gel, usually agarose or polyacrylamide. Popular techniques are: zonal electrophoresis in agarose gel or on cellulose acetate membranes; discontinuous electrophoresis in polyacrylamide gel (PAGE); SDS-polyacrylamide gel electrophoresis (SDS-PAGE); isoelectric focusing (IEF); and two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), which is capable of resolving complex mixtures of proteins containing hundreds or even thousands of components. All of these methods require some means of identifying particular proteins of interest. In some cases it is possible to do this simply on the basis of mobility, molecular weight (MW) (SDS-PAGE) or by using selective stains, e.g., for enzyme activity. These methods of identification are severely limited in applicability and, for this reason, antibodies have been used as highly specific probes for electrophoretically separated proteins ever since the invention of immunoelectrophoresis. Direct overlay of the gel with antibody (immunofixation) has also been used to identify antigens of interest but such methods suffer from the disadvantages of prolonged incubation times resulting in diffusion of the bands and consequent loss of resolution. Immunofixation is also primarily limited to agarose systems since antibody molecules cannot readily penetrate polyacrylamide gels owing to their small pore size.

Thus, the development of methods of transfer of separated proteins from gels to membranes, where they are readily accessible to high MW probes such as antibody molecules, opened up a new vista for electrophoretic analysis of proteins. The idea for this approach arose from the technique developed by Ed Southern for analysing DNA fragments separated on agarose gels by capillary transfer to a nitrocellulose membrane where they could easily be probed by RNA or cDNA (Southern, 1975). The method of transfer is termed 'blotting' since the pattern of bands on the nitrocellulose membrane is an exact replica of the pattern in the original gel and therefore the technique for analysis of DNA became known as the 'Southern blotting' method. By a somewhat dubious geographical analogy, analysis of RNA molecules by a similar technique became known as 'Northern blotting' and transfer of proteins to membranes is sometimes called 'Western blotting'. Since the latter name bears no relationship to the method or origins of the technique I shall use the

Correspondence to: D.I. Stott, Department of Bacteriology and Immunology, University of Glasgow, Western Infirmary, Glasgow G11 6NT, Scotland, U.K.

Abbreviations: BSA, bovine serum albumin; DBM, diazobenzyloxymethyl; DPT, diazophenylthioether; IEF, isoelectric focusing; NC, nitrocellulose; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulphate.

0022-1759/89/$03.50 © 1989 Elsevier Science Publishers B.V. (Biomedical Division)

more informative name of immunoblotting to describe the transfer of protein from a gel to a membrane where it is detected by antibody. Dot blotting, or dot immunobinding, is a variant of this in which antigens are detected in samples spotted directly on to a membrane without prior separation.

The first descriptions of immunoblotting were published independently in 1979 by Renart, Reiser and Stark and Towbin, Staehelin and Gordon. Renart, Reiser and Stark described a capillary transfer method in which the proteins become covalently bound to chemically activated paper. Specific antigens were identified by incubating the paper with antiserum followed by ^{125}I-labelled protein A and autoradiography. Towbin, Staehelin and Gordon transferred proteins electrophoretically from polyacrylamide gels on to nitrocellulose membranes and probed with two layers of antibody, the second layer being labelled with ^{125}I, fluorescein or horseradish peroxidase. Since these first reports, many adaptations of the immunoblotting technique have been developed and applied in an enormous variety of studies. It would be impossible to cover all the applications of immunoblotting in this review so I shall confine myself to a discussion of the basic technique and a selection of some of the uses to which it has been put that may be of particular interest. For earlier developments and applications the reader is referred to the excellent reviews by Gershoni and Palade (1983), Towbin and Gordon (1984), Beisiegel (1986), Bjerrum and Heegaard (1988a,b) and Gershoni (1988).

Procedure

Method of transfer

The simplest method of transferring protein from a gel to a membrane is by diffusion in which a membrane is placed on each side of the gel and immersed in buffer for 36–48 h, resulting in two replicate blots (Bowen et al., 1980; Lee et al., 1982). A somewhat faster method is by inducing a flow of solvent from the gel through the membrane by capillary attraction. This is achieved by placing a membrane on top of the gel followed by chromatography paper and a pile of paper towels on top of the membrane. Fluid is drawn by capillary attraction through the membrane into the paper towels and trapped in the membrane. Transfer is aided by placing a source of buffer in the form of wet chromatography paper under the gel, as in the original Southern technique (Southern, 1975), but this is not essential and acceptable transfer efficiencies can be obtained by omitting the buffer source and allowing the gel to dry to a thin layer during blotting. As in diffusion blotting, a membrane can be placed on both sides of the gel to obtain replica blots (Smith and Summers, 1980; Reinhart and Malamud, 1982). These methods work best with agarose gels, which have very large pores that do not hinder the movement of large protein molecules (Lanzillo et al., 1983; Elkon, 1984; Schibeci et al., 1986). Efficient transfer can be achieved in about 90 min to 2 h and acceptable results can be obtained in as little as 10 min with some sacrifice of efficiency (Koch et al., 1985).

With polyacrylamide gels, transfer is very inefficient by these methods although it can be improved by the use of composite polyacrylamide/agarose gels with cross-linking agents that can be cleaved after electrophoresis but before blotting to increase the pore size (Renart et al., 1979), or by the use of a vacuum to increase the flow of solvent through the membrane (Peferoen et al., 1982). Diffusion of the bands with loss of resolution due to the prolonged times required for transfer from polyacrylamide gels is a further disadvantage of diffusion and capillary blotting. Advantages are simplicity of operation and reduction of the danger of distorting or tearing the gel if blotting is performed on one side only, since it is not necessary to remove the gel from its supporting plate or plastic film. A method of electroblotting which eliminates this problem by supporting gels on nylon sheets, which are conductive when wet, has been reported by Nishizawa et al. (1985).

Electrophoretic transfer is normally preferred for polyacrylamide gels as it is faster and complete transfer can usually be obtained. A wet membrane is placed on one side of the gel, ensuring uniform contact over the whole area. The gel and membrane are then sandwiched between pads of foam rubber, scouring pads ('Scotch Brite') or chromatography paper, which must be saturated with the transfer buffer. Plastic grids are placed either

side of the sandwich which is inserted into a tank of buffer and a potential gradient applied across the gel, usually for about 2 h. Problems which may arise include: incomplete transfer due to a variety of causes, e.g., too short a transfer period, voltage too low, pH of the transfer buffer too low, proteins aggregated in the gel, absence of SDS in the gel, uneven transfer which may be due to inappropriate geometry of the electrodes or cassette, trapped air bubbles, high MW proteins moving more slowly than low MW proteins, proteins that do not bind or bind poorly to the membrane, etc.

Design of equipment

A variety of types of electrophoretic blotting apparatus have been used and several are available commercially. Most designs use platinum wire electrodes but careful attention must be paid to the spacing and geometry of wire electrodes as some arrangements may result in a non-uniform electric field which can give uneven transfer of proteins. Bittner et al. (1980) advocated a zig-zag array with a minimum spacing of 5.5 cm between parallel runs of wire. Gershoni et al. (1985) made a careful study of electrode design using computer models for a large number of electrode arrays and also experimental measurements of field distribution and uniformity of transfer of ^{125}I-labelled BSA. They concluded that asymmetric arrays result in non-uniform fields and uneven transfer of proteins. The most uniform fields and protein transfer were produced by symmetrical arrays consisting of four independent wires on each side. It was suggested that such an arrangement could be used to generate a field gradient with a higher potential difference between anode and cathode in the upper (high MW) region of the gel to counteract differences in the rate of transfer between large and small molecules from SDS gels, thereby permitting quantitative measurements.

A simpler way of generating a uniform field is to use plate electrodes covering the whole area of the gel. This would be prohibitively expensive using platinum, but electrode plates made of graphite (Gibson, 1981; Olmsted, 1981; Stott et al., 1985), or a platinum wire or conductive glass anode used in combination with a stainless steel cathode (McLellan and Ramshaw, 1981; Svoboda et al., 1985) have been used successfully. Stainless steel has the disadvantage of being chemically reactive but excellent results can be obtained with graphite, which is much cheaper than platinum wire.

In most systems the gel sandwich is placed in a plastic cassette, inserted vertically into the tank and fully immersed in buffer. There is usually a space between the sandwich and electrodes to allow free escape of the large quantities of hydrogen and oxygen released at the cathode and anode respectively. Where plate electrodes are used they may be placed in direct contact with the gel sandwich, thereby increasing the voltage gradient and efficiency of transfer, in which case the plastic cassette or grids have spacers glued to the surfaces in contact with the electrodes to allow free escape of gas (Stott et al., 1985). A cassette with circular holes may result in uneven transfer but this can be overcome by cutting vertical slots (Stott et al., 1985) or by constructing a cassette from a plastic frame strung with a net of silk or nylon thread (Gershoni, 1988). It is also important to eliminate air bubbles trapped between the membrane and gel or between paper and gel as these obstruct protein transfer. They can be seen through the wet membrane and removed by gentle stroking. Some types of equipment have been designed to reduce this problem by assembly of the sandwich under buffer in the tank (Shuttleworth, 1984; Stott et al., 1985).

Horizontal blotting systems are becoming increasingly popular and several are now commercially available. These dispense with a buffer tank altogether, the sole source of buffer being layers of chromatography paper saturated with buffer. The electrodes are plates made of graphite (Kyhse-Andersen, 1984; LKB NovaBlot, Sartorius Sartoblot, Biometra Fast-Blot), perforated steel sheets (Vaessen et al., 1981), or a platinum-coated titanium plate or wire mesh anode with a stainless steel cathode (Bio-Rad Trans-Blot, Hoefer Semi-Phor). Walker (1988) described a very simple, cheap, horizontal blotting apparatus suitable for teaching, constructed from two graphite plates.

Wet chromatography paper is placed on the horizontal anode plate and the gel sandwich is then positioned on the wet paper with the membrane facing the anode (for SDS gels) and covered with additional layers of paper soaked in buffer

followed by the cathode plate. This system has the advantage that the gel sandwich is set up in situ and it is easy to blot several gels simultaneously by placing them on top of each other. If multiple layers are blotted, proteins are prevented from passing through the membrane of one sandwich on to the next by interposing sheets of dialysis membrane between the layers. The system is economical in requiring relatively small quantities of buffer and no cooling is required owing to the low power consumption. A possible disadvantage is the danger that gas generated at the surfaces of the electrode plates will become trapped and prevent transfer of proteins but this does not appear to be a serious problem in practice.

Type of gel

In principle, immunoblotting can be performed using any type of gel used for protein electrophoresis. Agarose gels have a large pore size that does not restrict the movement of protein molecules and are very fragile. For these reasons they are best suited to capillary blotting. Polyacrylamide gels, with or without SDS, have small pore sizes that restrict the free movement of large molecules and therefore proteins do not elute efficiently by capillary blotting. Electrophoretic transfer is recommended for these gels. Two-dimensional gels can be treated in the same way as one-dimensional polyacrylamide gels. Isoelectric focusing gels should be pre-equilibrated with transfer buffer containing 1% SDS and 20% glycerol (to prevent swelling of the gel), omitting the methanol, before electrophoretic transfer since the focused proteins are at their isoelectric points and do not transfer efficiently without pre-equilibration (Stott and McLearie, 1986). If it is desired to maintain the proteins in their native conformation, the SDS may be omitted but it is then recommended that the pH of the transfer buffer be raised to pH 8.8.

Membranes

A variety of different kinds of membrane is now available for immunoblotting but nitrocellulose is still the most widely used. The physicochemical basis of binding of proteins to nitrocellulose is believed to be largely due to hydrophobic interactions. Ionic interactions are unlikely to be involved to a large extent since the nitro group is not charged and only small amounts of glucuronic acid are likely to be present in the cellulose. This is supported by the fact that binding can take place, and is even enhanced, at high salt concentrations. The hypothesis that hydrophobic bonding is a major factor is borne out by the observation that non-ionic detergents such as Triton X-100 or Nonidet P-40 can eluate up to 90% of bound protein (Gershoni and Palade, 1982; Kakita et al., 1982; Lin and Kasamatsu, 1983; Flanagan and Yost, 1984). This should be taken into account if it is found to be necessary to use washing buffers containing detergents in order to reduce non-specific binding. A theoretical treatment of the hydrophobic interactions of proteins with nitrocellulose membranes is considered by Van Oss et al. (1987). Hydrogen bonding with the nitro group also probably contributes to the binding energy.

Nitrocellulose with a pore size of 0.45 μm is used for most purposes but low MW polypeptides can pass through without binding. 0.1 μm membranes are recommended for these molecules since the higher matrix density results in more efficient trapping of low MW proteins (Burnette, 1981; Lin and Kasamatsu, 1983). When blotting a new protein(s) for the first time it is advisable to check the binding efficiency by placing a second membrane behind the first and staining to determine the amount of protein that has passed through the first membrane, especially if the proteins of interest are of low MW.

Fixation of proteins to the membrane can be used to prevent elution during washing and incubation steps. Acid/alcohol, glutaraldehyde, chemical cross-linking and ultraviolet irradiation have all been used (Gershoni and Palade, 1982; Kakita et al., 1982; Jahn et al., 1984; Cannon et al., 1985; Faye and Chrispeels, 1985) but the epitopes of many proteins are sensitive to such treatment and may no longer be detectable by antibody, so each antigen/antibody combination should be tested before being subjected to this type of treatment. A further disadvantage of nitrocellulose is its relatively low protein binding capacity, in the region of 80 $\mu g/cm^2$ (Gershoni and Palade, 1982). Membranes containing mixtures of nitrocellulose and cellulose acetate (e.g.,

the Millipore HAWP series) have even lower capacities so it is preferable to use the pure form.

Several other types of membrane are now commercially available, e.g., cationic nylon membranes such as Zetabind (AMF/CUNO, Meriden, U.S.A.) (also available under the name of Zeta Probe from Bio-Rad, South Richmond, CA, U.S.A.), Gene-Screen (NEN, Boston, MA, U.S.A.), Biodyne B (Pall Process Filtration, Portsmouth, U.K.). These membranes are extremely tough and do not crack when dry, unlike nitrocellulose. They also have a much higher protein binding capacity (up to 500 $\mu g/cm^2$), although this is highly concentration dependent (Gershoni and Palade, 1982), and proteins are not easily washed off as they bind with very high affinity. Unfortunately, cationic membranes also tend to bind proteins non-specifically resulting in high backgrounds, even after blocking; most of the commonly used protein stains (Coomassie blue, amido black, Ponceau S, etc.) also bind to the membrane, although alternative staining methods have been devised (see paragraph on stains, p. 165).

One of the earliest papers on immunoblotting (Renart et al., 1979) described the use of chemically activated (DBM) paper, containing diazo groups. DPT paper, which is easier to prepare, was subsequently used for immunoblotting by Reiser and Wardale (1981). These activated papers bind covalently to protein, eliminating the problem of loss of antigens during subsequent procedures but since the paper is unstable in the activated form they are rather inconvenient to use as activation must be performed immediately before loading in the tank. Resolution is poorer than that obtained using nitrocellulose or nylon membranes owing to the coarse texture of the paper. Cyanogen bromide-activated paper has been used for capillary blotting (Bhullar et al., 1981) and dot blotting (Newman et al., 1981) but not for electrophoretic blotting. Nylon-based membranes that covalently bind protein without requiring previous activation (Immunodyne I and II from Pall Process Filtration, U.K.) are now commercially available. The binding capacity of such membranes is similar to nitrocellulose (70–120 $\mu g/cm^2$). Tests carried out by Marlow and Handa (1987) and myself (unpublished) indicate that Immunodyne II is superior to nitrocellulose in resistance to elution by detergents and does not bind antibody non-specifically after blocking. These membranes appear to be promising candidates for immunoblotting and dot or slot blotting of proteins that bind weakly to nitrocellulose or are eluted from nitrocellulose by detergents. Unfortunately, the manufacturers refuse to reveal the nature of the chemistry of the binding reaction, although they do say that the active groups form a covalent link with primary amino groups on the protein.

Transfer buffers

Electrophoretic blotting on to nitrocellulose is normally performed in 25 mM Tris, 192 mM Glycine, pH 8.3, containing 20% methanol, as originally described by Towbin et al. (1979). The methanol prevents swelling of the gel during transfer and also enhances the binding of protein to the nitrocellulose membrane, although it does reduce the efficiency of elution of protein from the gel. Isopropanol has also been used for the same purpose (Clegg, 1982). For isoelectric focusing gels we use Tris-glycine buffer at pH 8.8 to ensure that it is well above the p*I* of the proteins (Stott and McLearie, 1986) (Table I). Buffers containing glycine should not normally be used with membranes that bind covalently to proteins since the binding sites on the membrane would be blocked by the primary amino group of the glycine. Other buffers such as 0.025 M sodium phosphate or 0.01 M sodium borate can be used instead (see Table I). If methanol is omitted the gel should be pre-swollen in the same buffer before electrophoretic transfer.

Blocking

If the antigens are to be detected by overlay with an antiserum, monoclonal antibody, or some other probe, it is essential to block the protein binding sites on the membrane to prevent non-specific binding of the probe. The blocking agent(s) and conditions used will depend on the type of membrane and sensitivity of the system under investigation. Examples of commonly used blocking agents are given in Table II. 3% bovine serum albumin is often used in combination with 10% serum although the latter contains approximately 3 mg/ml of albumin and the bovine serum albumin may not always be necessary. It is essential

TABLE I

ELECTROPHORETIC TRANSFER BUFFERS AND MEMBRANES

Gel	Membrane	Transfer buffer	Reference
SDS-PAGE	NC	25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3	Towbin et al. (1979)
		(1) 0.3 M Tris, 20% methanol, pH 10.4 (2) 25 mM Tris, 20% methanol, pH 10.4 (3) 40 mM 6-amino-*n*-hexanoic acid, 25 mM Tris, 20% methanol, pH 9.4	Kyhse-Anderson (1984) [a]
	Cationic nylon (Zetabind)	15.6 mM Tris, 120 mM glycine, pH 8.3, no methanol	Gershoni and Palade (1982)
	Cationic nylon (Zetabind)	41 mM Tris, 40 mM boric acid, pH 8.3	Gershoni and Palade (1982)
	DBM paper	25 mM sodium phosphate, pH 5.5 or 6.5	Bittner et al. (1980); Van Dongen et al. (1983)
		10 mM sodium borate, pH 9.2	Renart and Sandoval (1984)
	DPT paper	10 mM sodium borate, pH 9.2	Reiser and Wardale (1981); Renart and Sandoval (1984)
	Immunodyne I and II	25 mM sodium phosphate, pH 7.2 or 8.3	Pall process filtration instruction manual
PAGE in 8 M urea, low pH	NC	0.7% acetic acid	Towbin et al. (1979)
Native PAGE	DEAE paper (DE81)	7.5 mM Tris, 1.2 mM boric acid, pH 8.9	McLellan and Ramshaw (1981)
IEF gels	NC	25 mM Tris, 44 mM glycine, 20% methanol, pH 8.8	Stott and McLearie (1986)
	Immunodyne II	25 mM sodium phosphate, 20% methanol, pH 8.8	Stott (unpublished)

[a] This is a discontinuous system for semi-dry blotting in which filter papers saturated with buffer are layered in the following order: anode plate, buffer 1, buffer 2, membrane gel, buffer 3, cathode plate.

that the blocking proteins do not cross-react with the antibody probes and, if there is any possibility of this occurring, it is advisable if possible to use serum from the same species as the final antibody, e.g., if the first layer is a monoclonal antibody followed by labelled rabbit anti-mouse immunoglobulin, one would block with normal rabbit serum. BSA may be contaminated with bovine IgG and therefore may not be suitable when using protein A or an anti-IgG that could cross-react with bovine IgG. Powdered milk or casein give very good, low backgrounds, are cheap and readily available although they may inhibit specific antibody binding to some antigens (personal observation). While having the merit of being cheap, gelatin is not usually an effective blocking agent, although good results are said to have been obtained in some systems (Saravis, 1984).

Nonionic detergents have been used as blocking agents and have the advantage of being both cheap and readily available (Batteiger et al., 1982; Blake et al., 1984; Daneels et al., 1985) (Table III). Blotted proteins can be stained after blocking, which is more difficult with protein blockers, al-

TABLE II

BLOCKING BUFFERS

The following blocking agents are dissolved in phosphate-buffered saline, pH 7.2–7.4, or Tris saline, pH 7.4, unless otherwise stated.

Membrane	Blocking agent	Reference
NC	Bovine serum albumin, 2–3%	Towbin et al. (1979); Gershoni and Palade (1982)
	Animal serum, 10%	Hawkes (1983); Ahmed et al. (1985); Stott et al. (1986)
	Haemoglobin, 1–5%	Gershoni and Palade (1982); Winter (1982)
	Ovalbumin, 1–5%	Hanff et al. (1982); Carnow et al. (1985)
	Casein, 1–2% or saturated	Ramirez et al. (1983); Dresel and Schettler (1984); Mandrell and Zollinger (1984)
	Gelatin, 0.1–3%	Gorelick et al. (1983); Lin and Kasamatsu (1983); Bradbury et al. (1984); Saravis (1984); Faye and Chrispeels (1985)
	Milk, 5% (w/v)	Johnson et al. (1984); Miskimins et al. (1985) (DNA probe)
	Polyvinylpyrrolidone, 2%	Bartles and Hubbard (1984)
	Bovine serum albumin (0.2%)/ Ficoll (0.2%)/polyvinyl-pyrrolidone (0.2%) in borate-buffered saline	Stott and McLearie (1986) (DNA probe)
	Tween 20, 0.05–0.5%	Batteiger et al. (1982); Blake et al. (1984); Daneels et al. (1986)
	Ethanolamine (10%) followed by BSA (3%)	Kay et al. (1983)
Cationic nylon [a]	Haemoglobin, 1%	Gershoni and Palade (1982)
	BSA, 10%	Gershoni and Palade (1982)
	Haemoglobin (1%)/gelatin (0.1%)	Gorelick et al. (1983)
CNBr paper	BSA (0.1%)/glycine (1%)/ ethanolamine (10%)	Bhullar et al. (1981)
DBM and DPT paper	Gelatin (0.25%)/ethanolamine (10%) in 0.1 M Tris-HCl pH 9.0	Renart et al. (1979); Reiser and Wardale (1981)
Immunodyne I and II	Casein (0.5%)	Pall process filtration instruction manual

[a] High temperatures and prolonged incubation, e.g., 50–60 °C overnight, are normally recommended.

though not always impossible (see section on stains). Unfortunately they have the severe disadvantage of causing loss of protein by elution from the membrane; losses can be as high as 80–90% in the case of Nonidet P-40 and Triton X-100 (Schneider, 1980; Farrah et al., 1981; Lin and Kasamatsu, 1983). Tween 20 is claimed to give good results with some proteins (Batteiger et al., 1982) but Hoffman and Jump (1986) found that some immunoglobulins and several other proteins were dissociated from nitrocellulose by 0.05% Tween 20, as much as 97% being lost in some cases. Moreover, artefactual binding of monoclonal antibodies to protein bands unrelated to their specificity has been observed after blocking with Tween 20 (Wedege and Svenneby, 1986; Bird et al., 1988). The use of nonionic detergents in blocking and washing solutions should therefore be avoided unless severe background problems are encountered, in which case the effect of detergents on binding of the protein under investigation should be tested. One way of avoiding this prob-

TABLE III

CHROMOGENIC AND CHEMILUMINESCENT SUBSTRATES USED FOR DETECTION OF ENZYME-CONJUGATED PROBES

Enzyme	Substrate	Reference
Acid phosphatase	Naphthol AS-MX phosphate/ fast violet B salt	Brower et al. (1985)
Alkaline phosphatase	Nitro blue tetrazolium/ 5-bromo-4- chloroindolyl phosphate	Leary et al. (1983); Blake et al. (1984)
	Naphthol AS-MX phosphate/ fast red	O'Connor and Ashman (1982)
	TR salt	Gershoni et al. (1985a)
	β-naphthyl phosphate/ fast blue B	Turner (1983)
Horseradish peroxidase	4-chloro-1-naphthol/H_2O_2	Hawkes et al. (1982); Dresel and Schettler (1984); Faye and Chrispeels (1985)
	Diaminobenzidine/H_2O_2 [a]	de Blas and Cherwinski (1983); Gershoni et al. (1985a)
	Aminoethylcarbazole/ H_2O_2	Clegg (1982)
	o-dianisidine/H_2O_2	Towbin et al. (1979)
	Luminol/luciferin/4-methyl-umbelliferone/H_2O_2	Laing (1986)
	Luminol/4-iodophenol/H_2O_2	Schneppenheim and Rautenberg (1987)
β-Galactosidase	5-bromo-4-chloroindolyl-β-D-galactopyranoside	Amersham International catalogue
	Naphthol AS-BI-β-D-galacto-pyranoside	Ibid.

[a] The diaminobenzidine reaction is enhanced by the addition of cobalt and nickel salts (de Blas and Cherwinski, 1983). A silver enhancement kit is also available (Amersham International plc).

lem is to use a filter that binds proteins covalently (see section on membranes).

Stringent blocking conditions are often advocated for positively charged nylon membranes, which can give high non-specific backgrounds, e.g., incubation at 50–60 °C for prolonged periods, but 0.5% casein at ambient temperature or 37 °C for 30 min to 1 h gives low backgrounds with Biodyne A and B and Immunodyne I or II (Pall Process Filtration protocol; and Stott, unpublished observations). For detection of anti-DNA autoantibodies by reverse immunoblotting (see section on reverse immunoblotting, p. 173) we block and wash nitrocellulose membranes with 0.2% bovine serum albumin, 0.2% Ficoll and 0.2% polyvinyl pyrrolidone in borate-buffered saline (Stott and McLearie, 1986). This gives excellent clear backgrounds for both single-stranded and double-stranded DNA and would presumably be suitable for detection of any DNA-binding protein.

Primary probes

Protein molecules blotted on to a membrane are most commonly probed with a specific antibody, either polyclonal or monoclonal. Polyclonal antibodies may be in the form of antiserum or affinity purified antibody. The latter may be preferable if problems are encountered with high backgrounds. Monoclonal antibodies may be in

the form of serum, ascites fluid or tissue culture fluid. Any probe that binds specifically to the protein under investigation can be used in place of antibody, e.g., lectins for detection of glycoproteins, DNA or RNA for nucleic acid binding proteins, hormones for detection of hormone receptors, etc. The primary probe may be labelled directly (see section on detection systems, p. 162) or a second layer may be used.

The primary antibody or other reagent is diluted in blocking solution to prevent non-specific adsorption to the membrane, the optimal dilution, incubation time and temperature being determined for the system under investigation (see section on dot and slot blotting, p. 167). The membrane is placed in the bottom of a plastic dish, which should be slightly larger than the membrane, and sufficient overlay solution, containing the probe, added to just cover it; 0.1–0.13 ml/cm^2 is sufficient. Incubation is performed on a rocking machine (not a shaker) at 15–60 cycles/min, usually for 1 h at 37°C, 2 h at ambient temperature or overnight at 4°C. Longer incubations may sometimes be necessary, e.g., for IgE antibodies. If the probe is in short supply, it can be applied to a narrow strip of chromatography paper or cellulose acetate which is then laid over a narrow strip of the blotted membrane, protein side uppermost in a shallow trough cut in a perspex block (5 mm deep). The block is covered with a glass plate to prevent evaporation and incubated as above without rocking. Washing and probing with a second layer antibody are performed in deeper troughs (10 mm deep) cut in a separate block. A large number of membrane strips can be probed with different antibodies in this way. A micromethod for probing antigens on very small strips of nitrocellulose (2–3 mm wide) has been described by Nghiem (1988) who used the technique to screen hybridoma supernatants taken directly from multiwell tissue culture plates. The strips are placed in 35 mm diameter wells in culture plates with 50–500 μl culture fluid and incubated on a rotating wheel or shaker. Washing and incubation with a second probe are performed in the same way.

Whatever the nature of the primary probe, unbound probe is rinsed off with buffer and the membrane thoroughly washed in the same buffer, normally without a blocking agent. This is carried out on a rocker or shaken with 4–6 changes of ice-cold buffer, approximately 10 min per change. If high non-specific binding of the probe is a problem it may be necessary to add protein or a nonionic detergent to the wash buffer. The same protein as used in the blocking buffer may be added, usually at a lower concentration, e.g., 0.5% BSA. Alternatively, the wash buffer can include Triton X-100 or Tween 20 at concentration from 0.05% to 1%, but elution of proteins from the membrane may be an undesirable side-effect, especially at higher concentrations, as described above (see section on blocking, p. 157). This may be avoided by the use of a membrane that binds proteins covalently (see section on membranes, p. 156).

Secondary probes

If the primary probe has not been labelled, a secondary, labelled probe is applied in the same way as described above. This may be anti-immunoglobulin specific for the species of antibody used in the first layer, or protein A (from *Staphylococcus aureus*). If a labelled anti-immunoglobulin is used it is essential to ensure that it does not cross-react with components of the blocking buffer or the antigens under investigation (see section on blocking, p. 157). Protein A reacts with many species of IgG and therefore cannot be used if serum is included in the blocking buffer. Protein A does not bind equally well to all species, classes and subclasses of IgG, e.g., it binds well to human IgG1, -2 and -4, mouse IgG2a and -2b, rabbit, rat, guinea pig and pig IgG, but poorly or not all all to human IgG3, mouse IgG1 and -3, chicken, sheep and goat IgG and IgM, IgA, IgD and IgE of any species (Johnstone and Thorpe, 1982; Kronvall et al., 1970). An alternative probe is protein G from *Streptococcus*, which has a broader specificity than protein A (Björck and Kronvall, 1984). The advantages of such double layer techniques are: (1) the same labelled secondary probe can be used for a large number of primary antibodies of different specificities, as well as suitable negative controls, without the necessity of purifying and labelling each one; (2) a second layer antibody enhances the signal since more than one molecule of anti-Ig can bind to the

primary antibody, resulting in increased sensitivity; (3) it avoids modifications of the primary antibody due to radiolabelling, conjugation, etc., which may lead to non-specific binding.

Denaturation of the proteins

Immunoblotting is most commonly used to analyse proteins separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) after reduction by 2-mercaptoethanol or dithiothreitol and heating at 100 °C. The sample is also sometimes alkylated to prevent reoxidation of the thiol groups (Stott and Feinstein, 1973). SDS binds tightly to proteins conferring a negative charge on them and, unless it is removed before probing with antibody, it will inhibit antibody binding. Some antigenic determinants may be irreversibly denatured by such treatment. Furthermore, reduction may also result in loss of epitopes due to unfolding and separation of polypeptide chains, e.g., Tovey et al. (1987) observed partial loss of antigenicity in several proteins, and complete loss in at least one antigen, separated under denaturing conditions. Urea in the gel or methanol in the transfer buffer may cause similar effects.

The simplest way of avoiding such problems is to use non-denaturing gels, e.g., PAGE in the absence of SDS, or isoelectric focusing (Reinhart and Malamud, 1982). Bjerrum (1988) used agarose gels containing non-ionic detergents (Triton X-100, Tween 20 or Berol) for immunoblotting of membrane proteins. The inhibitory effect of non-ionic detergents on the binding of proteins to nitrocellulose was circumvented by overlaying the gels with a layer of agarose before blotting, a technique that could also be applied to polyacrylamide gels. Frequently, however, it is essential to use a denaturing gel. Many antibodies still recognise protein antigens after blotting from denaturing gels, possibly because SDS is stripped off the protein during transfer allowing renaturation to occur and because some epitopes are not conformation dependent. Monoclonal antibodies are particularly susceptible to loss of binding activity due to antigen denaturation since they normally recognise only one epitope. If a problem is suspected, attempts can be made to renature the protein on the membrane by incubating in buffer containing 4 M urea (Bowen et al., 1980; Lee et al., 1982) or a nonionic detergent such as Nonidet P-40 etc. (Petit et al., 1982; Frey and Afting, 1983; Hjerten, 1983; Islam et al., 1983; Bradbury and Thompson, 1984; Mandrell and Zollinger, 1984; Thorpe et al., 1984). Wedege et al. (1988) tested 14 different detergents for their ability to restore the antigenic conformation of meningococcal membrane proteins and concluded that ionic and zwitterionic detergents with an alkyl chain length of at least ten carbon atoms is required. Effectiveness of renaturation was proportional to chain length.

Muilerman et al. (1982) and Van der Meer et al. (1982) stained denatured enzyme, which had lost enzymatic activity, by overlaying with antibody plus native enzyme. Complexes were formed between membrane bound, denatured enzyme, antibody and free native enzyme, which were then stained for enzyme activity This is essentially a variant on using enzyme conjugated antibodies.

Detection methods

(1) Radioisotopes. Radioisotopes are still the most popular method of labelling antibodies or other probes and, of these, ^{125}I is the most widely used for labelling proteins. ^{125}I-labelled antibodies against mouse, rat, rabbit and human immunoglobulins are commercially available (Amersham International U.K.) and can readily be prepared in the laboratory by a variety of labelling methods, e.g., the chloramine-T reaction (Hunter, 1973), the Iodogen method (Fraker and Speck, 1978), the Bolton and Hunter method (Bolton and Hunter, 1973) etc. Other isotopes can also be used. For maximum sensitivity, ^{131}I may be preferred as it produces a much denser image than ^{125}I due to greater efficiency of trapping of the β radiation by X ray film, compared with the γ radiation emitted by ^{125}I. The shorter half life of ^{131}I is a disadvantage as it cannot be stored for long periods. Other isotopes such as ^{35}S and ^{14}C have also been used (Frey and Afting, 1983) but are less efficient for autoradiography; nucleic acids are labelled with ^{32}P, either by incorporation of $^{32}PO_4$ into cells or by nick translation (Maniatis et al., 1982).

Fluorography, in which the image is enhanced by means of an intensifying screen or fluor, is widely used for the detection of high energy β and γ emitters. A plastic screen coated with a fluor is placed on the opposite side of the membrane to

the X ray film and the sandwich firmly pressed together in a cassette or between glass plates in a black plastic bag at $-70\,^{\circ}C$. β particles or γ rays that are not trapped in the emulsion pass through the film to the screen and excite the fluor, which emits photons. These activate the silver halide crystals in the emulsion resulting in a greatly enhanced image, 8–10-fold for ^{32}P and 30–40-fold for ^{125}I (Swanstrom and Shank, 1978). For low energy β emitters such as ^{3}H, ^{14}C or ^{35}S, the membrane can be impregnated with diphenyl oxazole (PPO) (Southern, 1975; Erickson et al., 1982; Fisher et al., 1982) or one of the commercial fluorography solutions such as EN^{3}Hance (NEN, Boston, MA, U.S.A.) (Burnette, 1981) or Amplify (Amersham International, Amersham, U.K.).

Precise alignment of the X ray film with the membrane can be important in order to identify the tracks if some tracks do not contain detectable bands and can also be used for precise localisation of a radioactive band with respect to a stained band on the membrane. A simple way of ensuring exact alignment is to mark two corners of the membrane and the centre of the opposite side with spots of radioactive ink. This can easily be prepared by the addition of a small amount of any ^{14}C-labelled compound to ordinary fountain pen ink or india ink to about 40–80 kBq/ml. The advantages of using radioisotopes are high sensitivity, the ability to obtain several exposures from the same blot for optimal sensitivity and resolution and the ability to quantitate the image by scanning densitometry. The disadvantages are: precautions must be taken during handling and disposal of radioisotopes and the exposure times can be very long, from a few hours to 2–3 weeks.

(2) Enzyme-conjugated probes. Enzyme-conjugated antibodies can be used to detect antigens if the membrane is incubated with a chromogenic substrate yielding an insoluble product that precipitates at the site of production and remains bound to the membrane. The systems in use are directly appropriated from histological staining techniques and are thoroughly tried and tested. Horseradish peroxidase- or alkaline phosphatase-conjugated antibodies are available commercially or may be prepared by coupling with glutaraldehyde (Avrameas, 1969). Some inactivation of the enzyme and/or antibody may take place and the product should always be tested carefully. Considerable enhancement of the signal can be achieved by overlaying with anti-Ig followed by peroxidase-anti-peroxidase (PAP) complexes (Frazer and Wisdom, 1985). Suitable substrates for these and other enzymes are listed in Table III. Peroxidase activity is inhibited by azides and therefore sodium azide should not be included in wash buffers, although it is possible to add it to overlay solutions provided it is washed out before addition of substrate, since inhibition is reversible.

The advantages of enzyme-conjugated antibodies are ease of handling and storage (no problem of decay if stored in aliquots at $-20\,^{\circ}C$ or $-70\,^{\circ}C$) and rapid development of the colour (minutes instead of days). Sensitivity can be in the range 0.1– 10 ng of antigen per band depending upon the system. This is poorer (10–100 $\times$ higher minimum concentrations) than autoradiography or fluorography, although it is possible to achieve similar levels of sensitivity in some cases, e.g., with the PAP technique. There are several other disadvantages which should be considered. Firstly, some substrates, e.g., 3,3-diaminobenzidine and *o*-dianisidine, may be carcinogenic (4-chloro-1-naphthol is thought to be non-carcinogenic and also gives lower backgrounds without loss of sensitivity (Hawkes et al., 1982). Secondly, once the colour has developed it is not possible to intensify or reduce it to improve the image. Thirdly, the colour fades on drying and, in the case of horseradish peroxidase, in light. In the former case, contrast can be regained simply by wetting the membrane; loss of the image due to bleaching can be prevented by storing in the dark. Finally, non-specific binding of enzyme-conjugated antibodies has been observed in some situations (Tovey et al., 1987). These were presumably due to non-specific interactions of the enzyme with certain proteins since they were not detected when affinity purified radiolabelled antibody was used.

An alternative to chromogenic substrates is to develop peroxidase-conjugated antibodies with luminol in the presence of an enhancer such as 4-iodophenol or firefly luciferin and 4-methylumbelliferone (Laing, 1986; Hauber and Geiger, 1987; Schneppenheim and Rautenberg, 1987). The luminol is oxidised to a chemiluminescent product with emission of photons which are detected by

exposing the membrane to X ray film and developing as for autoradiography. Exposure times are extremely short (from 1 s to 10 min) and multiple exposures can be made from the same blot, which is not possible with chromogenic substrates. The luciferin/methylumbelliferone method is approximately three times more sensitive than chloronaphthol but sensitivity with iodophenol is about 100 × that of the colour reaction and appears to be similar to radioactivity. A direct comparison of the two methods has not yet been made. A photodetection device for use in immunoblots and dot blots has been described by Leong et al. (1988). Chemiluminescence has not been fully exploited yet but, since it combines the speed and safety of enzyme systems with the sensitivity of radioisotopes, it would seem to have great potential.

(3) Fluorochrome-labelled antibodies. Fluorochrome-conjugated antibodies can be applied to detect blotted antigens, the most commonly used reagent being fluorescein isothiocyanate (Towbin et al., 1979; Mirande et al., 1982; Ohashi et al., 1982; Piechulla and Küntzel, 1983), although rhodamine has also been used (Weihing, 1983). The bands are detected and photographed by viewing under ultraviolet light which causes them to fluoresce green (fluorescein) or red (rhodamine), allowing for the possibility of double labelling as described above. Although extensively employed in immunohistology, fluorochromes are not popular for immunoblotting despite the absence of any need for special handling precautions compared with radioisotopes and their lack of carcinogenic properties. The reasons are probably their lower

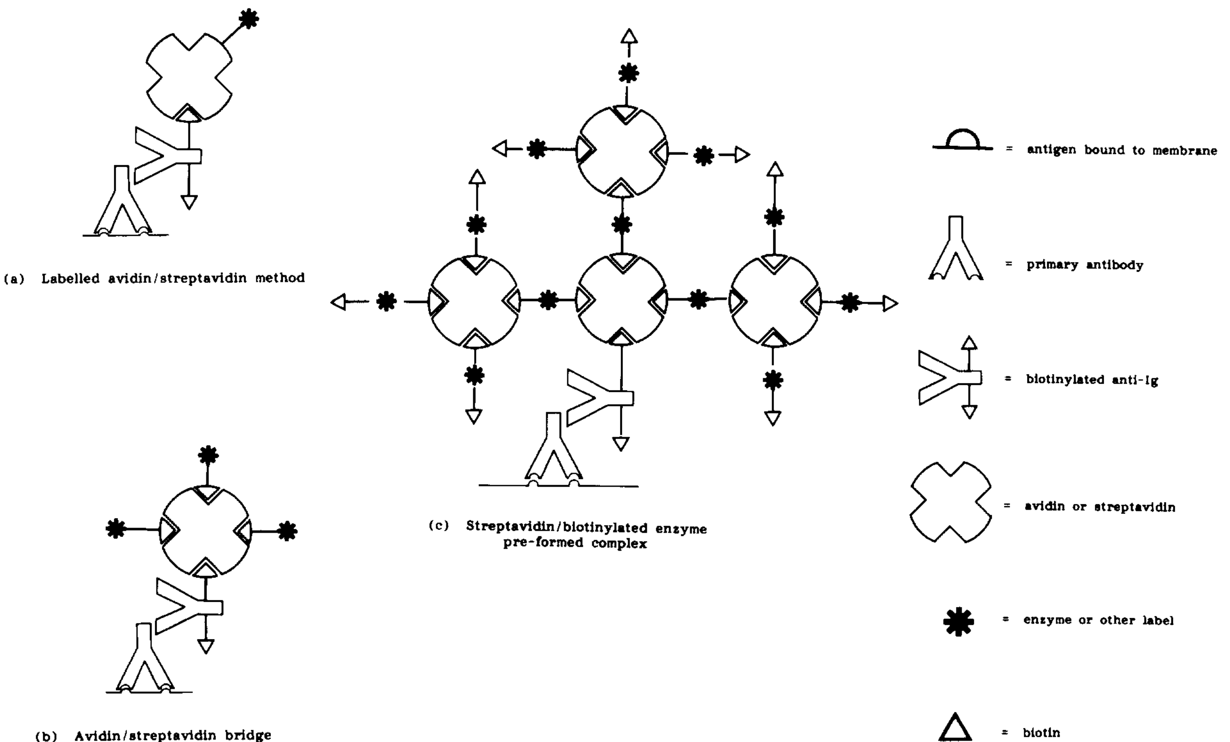

Fig. 1. Detection of antigen bound to nitrocellulose by three methods using the high affinity of biotin for avidin or streptavidin. In all three methods the primary antibody/antigen complex bound to nitrocellulose is overlayed with biotinylated anti-Ig (secondary probe). The complexes are then detected with either: (a) labelled avidin or streptavidin; (b) unlabelled avidin or streptavidin followed by labelled biotin which binds to the free binding sites on the avidin or streptavidin (the bridge method); (c) a pre-formed complex of avidin or streptavidin with labelled biotin. The label can be any of those described in systems (1)–(4) although horseradish peroxidase is the most commonly used.

sensitivity (Towbin et al., 1979), especially rhodamine, and their tendency to fade after prolonged exposure to light.

(4) Gold-labelled antibodies. Anti-Ig labelled with colloidal gold has also been used for immunoblotting (Brada and Roth, 1984; Hsu, 1984) and gold-labelled anti-mouse, anti-rabbit IgG and protein A are commercially available (Janssen Life Sciences Products, U.K., Sera-lab, U.K. and Bio-Cell Research Laboratories, U.K.). A range of particle sizes from 5 to 40 nm is available but only the larger sizes (15–40 nm) are suitable for staining blots. Pink bands are produced which can be enhanced by developing with a silver stain if higher sensitivity is required. As little as 50 pg of antigen can be detected by this method (Moeremans et al., 1983, 1984) but the technique has not yet been widely used for immunoblotting.

(5) Biotin/streptavidin. In this method, instead of conjugating an enzyme or fluorochrome directly to anti-Ig, the very high affinity of biotin for multiple binding sites on the proteins avidin and streptavidin is exploited. The system may be used in three different ways (Fig. 1). In the first, the blot is overlayed with antibody followed by biotinylated anti-Ig, then labelled streptavidin or avidin which binds tightly to the anti-Ig. Alternatively, unlabelled streptavidin may be allowed to bind to the biotinylated anti-Ig followed by biotinylated enzyme so that the streptavidin forms a bridge between the two. The third system resembles the PAP system (see (2) above) in that a complex of streptavidin and biotinylated enzyme is allowed to bind to the biotinylated anti-Ig. A variety of reagents are commercially available for each of these procedures (Amersham International U.K.). Streptavidin is said to give better results than avidin as it is not charged at physiological pH and gives lower backgrounds. It can also be used in combination with lectins as, unlike avidin, it does not contain carbohydrate. Since avidin and streptavidin are multivalent there is an enhancement effect if the bridge system or the complex are used and sensitivity is claimed by the manufacturers to be higher than for standard antibody-enzyme conjugates. The technique is very flexible as it can be used in conjunction with any of the detection systems described in (1)–(4) above. To date, it has not been used extensively, possibly due to initial problems with background staining using avidin, but the streptavidin system appears to have potential for immunoblotting.

(6) Multiple probing. Blots can be probed with two or more different antibodies in several ways. Multiple blots can be made by replacing the membrane at intervals during blotting and probing each with a different antibody (McLellan and Ramshaw, 1981), but the blots will not be exact replicas. Alternatively, they can be probed sequentially, the preceding probe being eluted before addition of the next (Legocki and Verma, 1981; Reiser and Wardale, 1981; Symington et al., 1981; Anderson et al., 1982; Erickson et al., 1982; Gullick and Lindstrom, 1982), but the elution buffer, which is usually of low pH or contains SDS, urea or 2-mercaptoethanol, may remove the antigen from the membrane as well as the probe. If different detection systems are used for each antibody, the same membrane can be probed for different antigens without intermediate elution steps (Neumann et al., 1985). By applying antibodies conjugated to different enzymes and developing with different substrates, double or even triple colour staining is possible, allowing the possibility of distinguishing between different antigens or isotypes of the same substance in the same mixture of proteins (Geysen, 1984; Lee et al., 1988). Lee et al. were able to distinguish between three different forms of interferon on the same blot by overlaying with antibodies of different species, specific for each of the different forms of interferon, followed by horseradish peroxidase- or alkaline phosphatase-conjugated anti-Ig specific for each species of primary antibody. The colour was then developed using two or even three different substrates, resulting in different coloured bands. However, for most purposes it is adequate to run the same sample in several tracks or, preferably, a single wide slot and cut the membrane into strips, each of which can then be overlayed separately.

(7) General protein stains. When blotting for the first time with a new apparatus, a novel membrane or a different type of protein preparation, it is advisable to check the efficiency of transfer by staining both the membrane and the gel with a non-specific protein stain after blotting. It is also useful to stain the membrane for total proteins after detection of specific antigens by one of the

above methods. Nitrocellulose can be stained with one of the standard protein stains such as 1% amido black 10B (also known as amido schwarz or naphthalene black) in 7% acetic acid. Destaining is accomplished with 30% ethanol/5% acetic acid/65% H_2O or 25% isopropanol/10% acetic acid/65% H_2O (Gershoni and Palade, 1982). Other stains that have been used are aniline blue black (Bowen et al., 1980), Ponceau S (Muilerman et al., 1982) and fast green (Reinhart and Malamud, 1982). Coomassie brilliant blue R binds to nitrocellulose and therefore gives high background staining. Unfortunately, these anionic dyes are not suitable for positively charged nylon membranes such as Zetabind as they bind strongly to the membrane. Staining with colloidal gold is very sensitive (Brada and Roth, 1984; Hsu, 1984; Surek and Latzko, 1984; Rohringer and Holden, 1985; Daneels et al., 1986) and can be further enhanced by combination with the silver stain (Moeremans et al., 1985). It is pH sensitive, depending upon the p*I* of the protein, but most proteins stain at low pH (circa 3.5). Staining positively charged nylon membranes results in high backgrounds but Merril and Pratt (1986) have developed a silver stain suitable for both nitrocellulose and nylon.

Protein bands on nitrocellulose or cationic membranes can also be identified by iodination in situ with chloramine-T/KI followed by the formation of a purple complex between the bound iodine and starch (Kumar et al., 1985). A related technique, originally devised for the detection of small peptides on paper (Rydon and Smith, 1952), involves chlorination of the polypeptide and detection of the bands with starch/KI solution. Proteins and polypeptides bound to nitrocellulose can be detected by soaking the membrane in 50% ethanol/H_2O for a few seconds before chlorination (Stott, unpublished observations). This method will identify any molecule containing a peptide bond (tyrosine is not involved in the reaction). It is therefore useful for staining small polypeptides that do not bind conventional protein stains. Alternatively, blotted proteins can be biotinylated in situ (LaRochelle and Froehner, 1986) and stained by one of the avidin or streptavidin staining techniques described in (5) above. A kit for this purpose is available from Bio-Rad (U.S.A.).

A very simple and sensitive general protein stain for nitrocellulose makes use of the binding of colloidal carbon (India ink) to proteins in the presence of a nonionic detergent such as Triton X-100 or Tween 20 (Hancock and Tsang, 1983). Pelikan fount india ink gives the best results and we have found that it is even possible to stain the membrane by this method after blocking and fluorography or autoradiography. Although the background stains grey due to the blocking proteins bound to the membrane, the tracks are readily discernible making it possible to identify the precise location of a particular band on the autoradiograph.

The gel can also be stained before transfer, the stained proteins being blotted on to the membrane in the normal way. The ethanol/acetic acid fixatives normally used in staining denature the proteins, however, resulting in the loss of conformational epitopes. The presence of a dye complexed with antigen may also alter the structure of some epitopes preventing recognition by the relevant antibody.

MW standards labelled with a radioisotope, fluorescence (Law and Lingwood, 1985) or biotin (Della-Penna et al., 1986) can be applied to the gel before electrophoresis and blotted on to the membrane. Iodination of MW marker proteins, in our experience, results in heavy labelling of one or two components and very weak labelling of the rest. It is therefore necessary to label the proteins separately and mix them in equal proportions with respect to radioactivity. Biotin-conjugated standards (available from Bio-Rad, U.S.A.) are detected by one of the methods described in (5) above.

Quantitation

The amount of antigen present in a band can be quantified in several ways but for the results to be meaningful it is important to ensure that transfer from the gel to the membrane is reproducible and, preferably, complete. If transfer is incomplete high MW proteins may transfer less efficiently than the low MW proteins. One should also carry out a preliminary experiment to determine whether the antigens are retained completely by the membrane. This can easily be tested by placing a second membrane behind the first

and staining both. If retention is incomplete, it may be improved by using a membrane of smaller pore size, e.g., 0.1 μm, or by reducing the voltage. Serially diluted standards should be included, preferably of known concentration, although arbitrary units can be used if the absolute concentration is unknown.

The simplest method of measuring the amount of antigen present in a band on a blot is to use a radiolabelled probe and scan the autoradiograph or fluorograph using a transmission densitometer. Some densitometers give a direct readout of area under each peak, although the background level should be chosen carefully and the reading may be inaccurate if the background is variable. Alternatively, the area may be measured manually with a planimeter or simply by tracing the peaks on good quality tracing paper, cutting them out and weighing. Peak area can then be plotted against concentration using the standards. A linear relationship between image density and antigen concentration has been obtained in this way for bacterial initiation factors (Howe and Hershey, 1981), yeast cytochromes (Vaessen et al., 1981) and rabbit antibodies (Batteiger et al., 1982).

Coloured bands on a membrane can also be quantified, either by reflectance densitometry (Guengerich et al., 1982; Towbin et al., 1982; Rordorf et al., 1983) or, preferably, by rendering the membrane transparent by soaking in an organic solvent such as xylene, toluene, liquid paraffin or microscope immersion oil (Ramirez et al., 1983; Nakamura et al., 1985; Stott et al., 1985; Palfreyman et al., 1988) and scanning with a transmission densitometer. Alternatively, bands can be excised, solubilised and read spectrophotometrically (Gershoni, 1988). Uhl and Newton (1988) obtained a linear relationship between the quantity of IL-1 (from 10 to 1000 ng) and horseradish peroxidase activity by cutting out the bands and incubating with *o*-phenylenediamine. The soluble product was measured spectrophotometrically. A radioimmunoassay capable of measuring protein in the range 20–150 ng has also been developed (Dennis-Sykes et al., 1985). One of the advantages of measuring protein concentration from immunoblots is that the separation process removes interfering substances, e.g., cross-reacting antigens or enzyme inhibitors, from the antigen to be measured. It also makes it possible to separate different forms of the same antigen, e.g., isotypes or precursor molecules. If these problems do not arise, antigen concentration can be measured quickly and simply by dot or slot blotting.

Dot and slot blotting

These procedures are essentially identical and can be used either as a qualitative method for rapidly screening a large number of samples for the presence of antigen or antibody activity (Glenney et al., 1983; Littauer et al., 1986), or as a quantitative technique for determination of antigen concentration. Samples (0.5–5 μl, usually as serial dilutions) are applied as a spot or rectangular slot to a strip or sheet of nitrocellulose and allowed to dry. The membrane is then blocked, overlayed with antibody and developed by one of the detection systems described above. Larger sample volumes can be adsorbed to the membrane using a vacuum manifold containing multiple filtration chambers with small holes or slots. Some models contain as many as 96 chambers so that samples from a microtitre plate can easily be assayed, providing a useful method for screening hybridoma supernatants and determination of immunoglobulin class and subclass (Bennett and Yeoman, 1983; Sternberg and Jeppesen, 1983; Beyer, 1984; Hawkes, 1986). A single sheet of nitrocellulose is clamped under the wells, which seal against the membrane to prevent leakage between samples. The chambers are then filled with antigen solution which is sucked through the membrane under vacuum, the antigen being retained on the membrane. If different antibodies are to be tested, the whole blocking and overlay procedure can be performed in the apparatus. If the same antibody is to be used for each sample, the membrane is removed from the apparatus, blocked and overlayed with antibody, etc., in a dish.

In its simplest form, as a qualitative assay, the technique is useful for assessing the various parameters affecting the quality of immunoblotting after transfer of antigen to the membrane, e.g., optimal blocking conditions, antibody dilution, washing buffers, etc. By applying serial dilutions of the antigen the sensitivity of a given detection system can be determined. By applying

a series of standard antigen dilutions to the membrane and scanning, the concentration of antigen can be determined (Jahn et al., 1984; Vertosick and Kelly, 1987; Palfreyman et al., 1988).

Special applications

The number of applications of immunoblotting and dot blotting is limited only by the imagination and new developments are appearing all the time. In the space available it is impossible, therefore, to review all the areas in which these techniques have been applied. Many applications have been discussed in earlier reviews (Gershoni and Palade, 1983; Towbin and Gordon, 1984; Beisiegel, 1986; Bjerrum and Heegaard, 1988b; Gershoni, 1988) and I shall limit myself to a few special applications that may be of interest to the reader, including some recent, novel approaches.

(1) Affinity purification of antibodies
Small quantities of antibody (approximately 2–10 μg) can be affinity purified by immunoblotting and subsequent elution of the bound antibody, which has been used for immunocytochemical staining (Olmsted, 1981, 1988; Allis et al., 1982). This can be a viable alternative if pure antigen is not available for affinity chromatography and it is difficult or too time consuming to raise monoclonal antibodies, or if there are good reasons for preferring to use a polyclonal antibody preparation. The antigen extract is applied to the full width of a gel, electrophoresed, blotted, blocked and overlayed with the antiserum in the normal way. A nitrocellulose strip containing the antigen-antibody complex is then cut out and the antibody eluted with a low pH buffer or chaotropic agent. Elution of antibody still bound to the antigen does not appear to be a problem but, if this should cause difficulties, one of the matrices that binds the antigen covalently can be used. Specific antibodies can also be adsorbed out of an antiserum and the supernatant used as a negative control (Cox et al., 1983). An interesting variation on this theme is to label the antibody with fluorescein isothiocyanate while still bound to the membrane followed by elution of the labelled antibody (Talian et al., 1983). This has the advantage of protecting the antigen binding site during the reaction since it is still bound to antigen. Antibodies against *Plasmodium* antigens have also been purified by elution from recombinant expression proteins in lysed bacterial plaques bound to nitrocellulose. The eluted antibodies were used to detect native parasite antigens by immunoblotting (Lyon and Weber, 1988).

(2) Allergens
Allergens are frequently components of complex natural materials or organisms such as pollens, house dust mite, animal fur, yeasts, fungi, etc., and are therefore difficult to identify and purify. IgE antibodies also require highly sensitive assays. Immunoblotting is ideally suited to this kind of problem and has considerable potential for identification, purification and investigation of the structure and properties of allergens. Identification of IgE antibodies against defined allergens by dot blotting (Derer et al., 1984; Singh and Knox, 1985) could well prove to be a useful technique in the clinical immunology laboratory for diagnosis of hypersensitivity reactions. Several

TABLE IV

IMMUNOBLOTTING AND DOT BLOTTING STUDIES OF ALLERGENS AND IgE ANTIBODIES

Allergen	References
Grass pollens	Baldo et al. (1982); Peltre et al. (1982); Sutton et al. (1982); Ford et al., (1985, 1986); Mecheri et al. (1985); Singh and Know (1985); Haas et al. (1986); Alterman et al. (1987)
Tree pollens	Ipsen and Larsen (1988); Lauzurica et al. (1988)
House dust mite	Krilis et al. (1984); Tovey and Baldo (1984, 1985)
Storage mite	Johansson et al. (1988)
Cereal proteins and gliadins	Vainio (1986); Freedman et al. (1988)
Soybean proteins	Burks et al. (1988)
Insect venoms	Einarsson (1987); Hoffman (1987)
Alternaria allergens	Kroutil and Bush (1987)

immunoblotting studies have been published (Table IV) but, in view of the enormous variety and complexity of allergens, there is clearly much more to be done. Ipsen and Larsen (1988) compared immunoblotting with crossed radioimmunoelectrophoresis for analysis of the specificity of IgE antibodies against three pollens and concluded that, although immunoblotting is likely to prove extremely useful, binding of IgE antibodies is sensitive to denaturing and reducing conditions. This must clearly be taken into account in attempting to analyse allergen by immunoblotting and it would be advisable to perform the analysis using both denaturing and non-denaturing conditions, at least until the optimal conditions have been determined.

(3) Antigens recognised by T lymphocytes

Most applications of immunoblotting involve analysis of antigens using antibodies or some other molecular probe such as a lectin. An immunoblotting assay for antigens recognised by T cells has been developed by Lamb et al. (1988), based on a technique originally used to identify the mitogenic components of PHA (Laurent et al., 1985). Antigens are separated by SDS-PAGE and blotted on to nitrocellulose in the normal way. The membrane is then cut into narrow strips parallel with the top and bottom edges. The strips are sterilised and either added directly to the wells of a tissue culture plate or dissolved in dimethyl sulphoxide and precipitated as fine particles before addition to the plate. Peripheral blood lymphocytes or a T cell clone are then cultured with the extract and cell proliferation assayed by incorporation of [^{3}H]thymidine. The position of an antigen on the gel can thus be identified and its MW determined from its ability to induce a T cell response. The technique has been used to analyse T cell antigens of influenza virus, mycobacteria and house dust mites (Young and Lamb, 1986; Lamb and Young, 1987; O'Hehir et al., 1987). It has also been used to analyse a recombinant mycobacterial antigen and fusion proteins containing different regions of the same antigen separated by gel electrophoresis (Lamb et al., 1987).

(4) Autoantigens and autoantibodies

Immunoblotting is a powerful technique for analysis and characterisation of autoantigens and autoantibodies in autoimmune diseases and animal models of autoimmune diseases, e.g., the complex nuclear protein antigens and RNP particles to which autoantibodies are found in systemic lupus erythematosus, mixed connective tissue disease and Sjögren's syndrome, mitochondrial antigens in primary biliary cirrhosis, thyroid antigens in the autoimmune thyroid diseases, etc. (reviewed by Elkon et al., 1987; and see Table V). Before the development of immunoblotting such antigens could only be studied by conventional immunochemical techniques such as immunodiffusion, fluorescence microscopy, etc., which did not allow separation of the different antigenic and non-antigenic components often present in association with each other.

The autoantibodies themselves can also be characterised by their specificity, class, subclass and idiotype without the necessity of purifying the antigens for analysis. Class and subclass of antibodies specific for each component of a complex mixture of antigens can be identified by overlaying the blotted antigens with autoimmune serum followed by class or subclass specific monoclonal anti-Ig and a third layer of labelled anti-mouse Ig (Elkon et al., 1987). Idiotypes have been identified on rheumatoid factors (Chen et al., 1984; Fong et al., 1986), anti-DNA antibodies (Halpern et al., 1984) and cryoglobulins (Elkon et al., 1987).

(5) Cell adhesion proteins

Molecules that bind to cell surfaces can be detected on blots by cell adherence. Hayman et al. (1982) developed a method for detecting cell adhesion proteins in human plasma by overlaying blots with rat kidney cells. After washing and staining the cells were found to bind to two bands: fibronectin and a 70 kDa protein (vitronectin) (reviewed by Hayman and Ruoslahti, 1988). This approach may have possibilities for the analysis of other molecules bound by cell surface receptors.

(6) Crossed immunoelectrophoresis and rocket immunoelectrophoresis

Most immunoblotting applications require prior electrophoresis of the proteins in polyacrylamide or agarose gel without precipitation by antibody. It is also possible to blot precipitated proteins from immunodiffusion gels if the complexes are

TABLE V

AUTOANTIGENS AND AUTOANTIBODIES ANALYSED BY IMMUNOBLOTTING AND DOT BLOTTING

Autoantigen	References
Breast cancer	Ronai and Sulitzeanu (1986)
Brain cell membrane proteins	Narendran and Hoffman (1988)
Bullous pemphigoid	Labib et al. (1986)
Eye muscle	Ahmann et al. (1987)
Fat cells	Weetman et al. (1987a)
Histones	Costa and Monier (1986a,b); Costa et al. (1986); Konstantinov (1986)
Microsomal and membrane proteins (liver, kidney, stomach)	Dow et al. (1985); Frazer et al. (1987); Kenna et al. (1987); Kyriatsoulis et al. (1987)
Mitochondrial antigens	Penner et al. (1986); Manns et al. (1987); Mendel-Hartvig et al. (1987); Al-Hussami et al. (1988); Fusconi et al. (1988); Kyriatsoulis et al. (1988)
Muscle proteins	Koga et al. (1987)
Myelin basic protein and M2 antigen	Lebar and Lees (1985); Bansal et al. (1987)
Nuclear antigens	Guldner et al. (1983, 1986); Habets et al. (1983, 1985a,b); Ahmed et al. (1985); De Rooij et al. (1985); Elkon and Jankowski (1985); Navarro et al. (1986); Pettersson et al. (1986); Williams et al. (1986); Kimura et al. (1987); Westgeest et al. (1987);
Retinal S-antigen	Fling et al. (1988); Knospe et al. (1988)
Sperm antigens	Naaby-Hansen and Bjerrum (1985); Hald et al. (1987)
Thyroid antigens	Kotani et al. (1986); Bako et al. (1987); Weetman et al. (1987b)

first dissociated by low or high pH. Bütikofer et al. (1985) transferred the complex pattern of arcs from crossed immunoelectrophoresis gels of human serum and erythrocyte membrane proteins to nitrocellulose by dissociation of the complexes at pH 2.5 and identified individual proteins by probing with monoclonal antibodies. Levasseur et al. (1988) used a pH 11 buffer to dissociate immune complexes after both crossed and rocket immunoelectrophoresis and were able to measure as little as 0.3 ng of inter-α-trypsin inhibitor in unconcentrated urine by rocket immunoelectrophoresis using the peroxidase-anti-peroxidase detection system.

(7) Epitope mapping

The epitopes of various proteins have been mapped and, in some cases, correlated with bio-

TABLE VI

EPITOPE MAPPING OF PROTEINS BY IMMUNOBLOTTING AND DOT BLOTTING

Protein	References
α_2-macroglobulin	Van Leuven et al. (1986)
CAD protein	Carrey and Hardie (1986)
Complement C9	Luzio and Jackson (1988)
Cytochrome c oxidase	Jarausch and Kadenbach (1985)
Fibronectin	Vartio et al. (1982); Dziadek et al. (1983)
Herpes simplex virus	Cohen et al. (1986)
Histone H1	Costa et al. (1986)
Mitochondrial membrane protein	Mendel-Hartvig and Nelson (1983)
Myelin basic protein	Sheng et al. (1988)
Newcastle disease virus	Samson (1986)
Spectrin	Yurchenco et al. (1982)
SV40 capsid protein	Reiser and Wardale (1981)

TABLE VII

CHARACTERISATION OF INFECTIOUS ORGANISMS AND ANTIBODIES AGAINST THEM BY IMMUNOBLOTTING AND DOT BLOTTING

	References
Bacteria	
Borrelia	Grodzicki and Steere (1988)
Brucella	Delia et al. (1987)
Campylobacter	Dunn et al. (1987)
Chlamydia	King et al. (1985); Cevenini et al. (1986); Caldwell et al. (1987); Patel et al. (1988);
Clostridium	Heard et al. (1986); Rautenberg et al. (1986)
Gonococcus	Swanson and Barrera (1983); Hadfield and Glynn (1984);
Haemophilus	Schalla et al. (1986)
Leptospira	Chapman et al. (1987); Kelson et al. (1988)
Lipopolysaccharide (*E. coli* etc.)	De Jongh Leuvenink et al. (1985); Sturm et al. (1984)
Meningococcus	Coll et al. (1986); Wedege and Froholm (1986); Wedege et al. (1988)
Micropolyspora	Aznar et al. (1988)
Mycobacteria	Andersen et al. (1986); Coates et al. (1986); Milner et al. (1987); Van Vooren et al. (1988)
Mycoplasma	Kenny and Cartwright (1984); Kibe et al. (1985); Jacobs et al. (1986); Andersen et al. (1987); Sasaki et al. (1987); Young and Ross (1987)
Rickettsia	Tamura et al. (1985)
Streptococcus	Ogier et al. (1984); Aitchison et al. (1987)
Treponema	Hensel et al. (1985)
Yersinia	Stahlberg et al. (1987)
Yeasts	
Candida	Burnie et al. (1985); Matthews and Burnie (1988)
Viruses	
BK virus	Christie et al. (1988)
Bluetongue virus	Adkison et al. (1987)
Bursal disease virus	Fahey et al. (1985)
Cauliflower mosaic virus	Burger and Du Plessis (1983)
Coronaviruses	Battaglia et al. (1987)
Cytomegalovirus	Landini et al. (1985); Mirolo et al. (1986); Jankowski and Styczynski (1987); Porath et al. (1987); Shimokawa et al. (1987)
Epstein-Barr virus	Lin et al. (1985)
Hepatitis virus	Pillot and Petit (1984); Talbot et al. (1984)
Herpes viruses	Lehtinen (1985); Lehtinen et al. (1985); Snowden and Halliburton (1985); Heberling and Kalter (1987); Okazaki et al. (1987); McKendall et al. (1988)
HIV	Blumberg et al. (1987); Chiodi et al. (1987); Knuver et al. (1987); Thorpe et al. (1987); Blomberg and Klasse (1988); Grimaldi et al. (1988); Heberling et al. (1988); Tersmette et al. (1988)
HTLV	Ulstrup et al. (1986); Yamaguchi et al. (1988)
Japanese encephalitis virus	Srivastava et al. (1987)
Parvovirus	Porter et al. (1984)
Pseudorabies virus	Todd et al. (1987)
Rabies virus	Heberling et al. (1987)
Respiratory syncytial virus	Routledge et al. (1987)
Retroviruses	Oda et al. (1986)
Rotaviruses	Reynolds and Hughes (1985)
SV40	Dietrich (1985)
T-cell Lymphotropic virus	Biberfeld et al. (1985); Fang et al. (1986); Ohta et al. (1986)

(*Table VII continued on next page*)

(Table VII continued)

	References
Protozoa	
Entamoeba	Gandhi (1986)
Leishmania	Pappas et al. (1983)
Pneumocystis	Graves et al. (1986); Walzer and Linke (1987); Walzer et al. (1987)
Toxoplasma	Partanen et al. (1983); Brooks et al (1985); Herbrink et al. (1987)
Trypanosoma	Wong et al. (1986)
Metazoa	
Nematodes	Lucius et al. (1983); Boyce et al. (1988)
Tapeworms	Sorice et al. (1985); Lightowlers et al. (1986); Gottstein et al. (1987)

logical activity by immunoblotting and probing with monoclonal antibodies. Vartio et al. (1982) and Dziadek et al. (1983) mapped fibronectin in this way and correlated biological activity with particular regions of the molecule by inhibition with the same monoclonal antibodies as were used to map the epitopes. Carrey and Hardie (1986) mapped the phosphorylation sites on a multifunctional protein (CAD) by blotting polypeptides generated by limiting proteolysis and direct digestion of the bands on nitrocellulose with trypsin. Luzio and Jackson (1988) defined epitopes on complement component C9 (which is involved in the generation of the membrane attack complex) by chemical and enzymic cleavage combined with immunoblotting. Other proteins mapped by immunoblotting are listed in Table VI.

(8) Immunisation

An individual protein can be separated from a complex mixture and identified by immunoblotting with a polyspecific antiserum. The isolated protein can then be used to raise a specific antiserum or monoclonal antibody by cutting out the band containing the antigen, which is then chopped finely and injected subcutaneously or intraperitoneally. Alternatively, the antigen can be eluted with a nonionic detergent or dimethyl sulphoxide, or the nitrocellulose can be dissolved in acetone or methanol and the protein extracted or injected as a slurry (Anderson, 1985; Knudsen, 1985; Parekh et al., 1985). Using this approach, Larsson and Nilsson (1988) succeeded in obtaining an immune response to bovine serum albumin by intrasplenic immunisation of mice with as little as 70 ng of protein blotted on to a membrane. Larger amounts ($\geqslant 0.8$ μg) stimulated an immune response after intraperitoneal injection. This could be envisaged as a 'boot-strap' method of purifying a protein since the blotted protein could be used to produce an antibody which, in turn, could then be used to affinity purify the same protein.

(9) Infectious organisms

The characterisation of infectious agents by serological typing is a very old established technique in the clinical and research laboratory as is also, conversely, the identification of antibodies against such organisms for diagnosis. The advent of immunoblotting and dot blotting has lent a new dimension to this field with a veritable explosion of data on the identification and characterisation of microorganisms and studies on the immune response against them using these techniques. It is hoped that immunoblotting will lead to simple methods for the precise identification of infectious organisms that are difficult to type by conventional means; dot blotting may lead to the development of simple, precise 'dipstick' type assays for rapid diagnosis in the field and the clinical laboratory. Many earlier reports have been reviewed by Towbin and Gordon (1984) and Beisiegel (1986) but a selection of recent papers is listed in Table VII.

(10) Microsequencing

Vandekerckhove et al. (1985) and Aebersold et al. (1986) used immunoblotting as a micropreparative procedure in order to determine the amino acid sequence of polypeptides blotted on to glass fibre paper.

(11) Nucleic acid binding proteins

Proteins that bind to defined sequences of DNA or RNA can also be studied by protein blotting, which provides a promising way of identifying putative gene regulatory proteins. Nucleic acid binding proteins from HeLa cells, viruses, oocytes, *Drosophila* and heat shock proteins have been analysed by blotting and overlay with labelled DNA, RNA or histones (Bowen et al., 1980; Baumann and Hand, 1982; Aubertin et al., 1983; Richter and Smith, 1983; Gabor and Bennett, 1984; Rozier and Mache, 1984; Wolff et al., 1985). Miskimins et al. (1985) used a DNA probe to identify nuclear proteins that bind to the promoter region of the transferrin receptor gene.

(12) Spectrotype analysis of antibodies by reverse immunoblotting

Isoelectric focusing (IEF) in thin layer gels was originally devised by Awdeh et al. (1968) for the analysis of antibody spectrotypes. The antibody secreted by each clone of plasma cells focuses into a unique pattern of closely spaced bands – the clonotype (due to microheterogeneity). The spectrotype of antibody in a polyclonal response consists of the sum of the clonotypes of all the responding clones (Williamson et al., 1973). Thus, it is possible to determine the number of responding clones and changes in the behaviour of individual clones during an immune response from the IEF spectrotype of the antibodies. In the original method anti-hapten antibodies were identified after focusing in polyacrylamide gel by overlay with radioactive hapten (Williamson, 1973) but a disadvantage of the technique is that high MW antigens penetrate the gel very slowly. Early attempts to circumvent this problem involved precipitation of the focused antibodies with sodium sulphate and cross-linking with glutaraldehyde or dimethylsuberimidate to prevent diffusion during the lengthy incubation times required (Keck et al., 1973; Williamson, 1973). Autoantibodies against thyroglobulin (Nye and Roitt, 1980) and DNA (Yoshida et al., 1985) have been characterised in this way.

Isoelectric focusing in agarose gel facilitates penetration by macromolecules and overlaying the gel with anti-Ig followed by staining of the precipitated proteins has been used to identify paraproteins in the sera and urine of patients with multiple myeloma (Sinclair et al., 1983, 1984a, 1986a), chronic lymphocytic leukaemia (Sinclair et al., 1984b, 1986b) and the normal population (Sinclair et al., 1986c). The method can also be used to quantitate monoclonal immunoglobulin present in serum (Sinclair et al., 1986d). The greater sensitivity of immunoblotting has been exploited for the detection of paraproteins in patients with B cell neoplasia (Blangarin et al., 1984; Thompson and Keir, 1984; Heys et al., 1986; Graziani and Righetti, 1987; Norden et al., 1987; Schipper et al., 1988) and to study oligoclonal banding patterns in cerebrospinal fluid (Moyle et al., 1984; Nespolo et al., 1987).

Specific antibodies against high MW antigens can also be spectrotyped by focusing, blotting and overlay with labelled antigen. We have coined the phrase 'reverse immunoblotting' for this technique since conventional immunoblotting, in which antigen is analysed by probing with antibody, has been inverted in this method. Stott and McLearie (1986) developed a reverse immunoblotting method for spectrotype analysis of autoantibodies against thyroglobulin, ssDNA and dsDNA (labelled with ^{32}P by nick translation followed by S1 nuclease digestion). 6 M urea is incorporated into the gel when focusing anti-DNA antibodies to dissociate complexes with DNA. The technique was used to study the autoimmune response in human and animal models of autoimmune thyroiditis (Stott et al., 1986, 1988a), systemic lupus erythematosus (Stott et al., 1986, 1988b) and allergic uveoretinitis (Forrester et al., in press). Grimaldi et al. (1988) used a similar method to identify antibodies of restricted heterogeneity against gp120 and p24 HIV antigens in AIDS patients. Focusing in polyacrylamide restricts the spectrotype to IgG antibodies whereas IgA and IgM will focus in agarose gels, although better spectrotypes result if the latter are subjected to mild reduction to dissociate them into subunits (Sinclair et al., 1983). Schibeci et al. (1986) used capillary blotting and overlay with ^{125}I-labelled tetanus toxoid to detect antibodies focussed in agarose gels. An alternative approach used by Knisley and Rodkey (1986) is to blot the antibodies on to nitrocellulose sheets coated with antigen. By incubating with class, subclass or allotype

specific anti-Ig, information about the nature of the antibody was obtained.

Conclusions

The principle advantage of blotting proteins on to a membrane support lies in the exposure of the molecules to the external environment rendering them available for probing with antibodies or other ligands, instead of remaining inaccessible inside the gel. From this simple fact, a prodigious variety of applications has developed, exploiting the combination of high resolution one- and two-dimensional electrophoretic techniques with immunoblotting to analyse complex mixtures of proteins, inter- and intramolecular structure and its relationship to biological activity, the complex antigenic architecture of both pathogenic and non-pathogenic organisms, etc. Fingerprinting, viz., the use of immunoblot patterns produced by a patient's antibodies to identify an infectious microorganism, parasite or autoantigens may eventually lead to new methods of diagnosis and monitoring of diseases, in addition to a better understanding of the structural organisation and interrelationships of infectious organisms and autoantigens and of the regulation of the immune response against them. Dot blotting, combined with the reliability and precision of monoclonal antibodies and rapid detection techniques, may lead to the development of simple, rapid diagnostic tests which can be used in the clinical laboratory and possibly in the field.

IEF-reverse immunoblotting makes it possible to dissect out the humoral immune response and study the behaviour of individual clones of plasma cells secreting antibody against antigens from infectious microorganisms and in autoimmune disease.

Technical developments may include the development of better membrane materials, including those which bind molecules covalently, and possibly membranes which form reversible covalent bonds so that antigens can be easily eluted after blotting for purification and structural studies. Further developments are also likely to take place in improving the speed and sensitivity of detection systems such as the chemiluminescent technique.

No doubt there will, in the future, be many technical innovations and new applications of immunoblotting and dot blotting other than those discussed here, but I shall refrain from the temptation to guess what these might be for, as Medawar (1965) said: "It is impossible to predict new ideas ... for to predict an idea is to have an idea and if we have an idea it can no longer be the subject of a prediction."

Acknowledgements

I wish to thank the Scottish Hospitals Endowments Research Trust and the Scottish Home and Health Department for their support for some of the work described in this review.

I would also like to thank Mrs. Anne McIlveen for her assistance with the secretarial work and Dr. H. Towbin for critically reading the manuscript. Any errors and omissions are entirely my own.

References

Adkinson, M.A., Stott, J.L. and Osburn, B.I. (1987) Identification of bluetongue virus protein-specific antibody responses in sheep by immunoblotting. Am. J. Vet. Res. 48, 1194.

Aebersold, R.H., Teplow, D.B., Hood, L.E. and Kent, S.B.H. (1986) Electroblotting on to activated glass. High efficiency preparation of proteins from analytical sodium dodecyl sulphate-polyacrylamide gels for direct sequence analysis. J. Biol. Chem. 261, 4229.

Ahmann, A., Baker, J.R., Weetman, A.P., Wartofsky, L., Nutman, T.B. and Burman, K.D. (1987) Antibodies to porcine eye muscle in patients with Graves' ophthalmopathy: identification of serum immunoglobulins directed against unique determinants by immunoblotting and enzyme-linked immunosorbent assay. J. Clin. Endocrinol. Metab. 64, 454.

Ahmed, C.A., Stott, D.I. and Campbell, A.M. (1985) Analysis of Nuclear and Cytoplasmic Protein Antigens in Systemic Rheumatic Disease. In: H. Peeters (Ed.), Protides of the Biological Fluids, Proc. 33rd Colloq., 1985. Pergamon Press, Oxford, p. 205.

Aitchison, E.J., Lambert, P.A., Smith, E.G. and Farrell, I.D. (1987) Serodiagnosis of *Streptococcus faecalis* endocarditis by immunoblotting of surface protein antigens. J. Clin. Microbiol. 25, 211.

Al-Hussami, O., Godinot, C., Monier, J.M., Trepo, C. and Monier, J.C. (1988) Use of immunoblotting to characterise the mitochondrial antigens recognised by anti-mitochondrial autoantibodies. J. Immunol. Methods 113, 61.

Allis, C.D., Ziegler, Y.S., Garvosky, M.A. and Olmsted, J.B. (1982) A conserved histone variant enriched in nucleoli of mammalian cells. Cell 31, 131.

Alterman, L., Bird, C., Callus, M., Ford, A. and Thorpe, R. (1987) The use of monoclonal antibodies raised against a human IgE myeloma paraprotein for the study of allergen extracts and sera from allergic patients. Clin. Exp. Immunol. 67, 617.

Andersen, H., Birkelund, S., Christiansen, G. and Freundt, E.A. (1987) Electrophoretic analysis of proteins from Mycoplasma hominis strains detected by SDS-PAGE, two-dimensional gel electrophoresis and immunoblotting. J. Gen. Microbiol. 133, 181.

Anderson, A.B. Yuan, Z.L., Haslov, K., Vergmann, B. and Bennedsen, J. (1986) Interspecies reactivity of five monoclonal antibodies to *Mycobacterium tuberculosis* as examined by immunoblotting and enzyme-linked immunosorbent assay. J. Clin. Microbiol. 23, 446.

Anderson, P.J. (1985) The recovery of nitrocellulose-bound protein. Anal. Biochem. 148, 105.

Anderson, N.L., Nance, S.L., Pearson, T.W. and Anderson, N.G. (1982) Specific antiserum staining of 2-dimensional electrophoretic patterns of human plasma proteins immobilised on nitrocellulose. Electrophoresis 3, 135.

Aubertin, A.M., Tondre, L., Lopez, C., Obert, G. and Kirn, A. (1983) Sodium dodecyl sulphate-mediated transfer of electrophoretically separated DNA-binding proteins. Anal. Biochem. 131, 127.

Avrameas, A. (1969) Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. Immunochemistry 6, 43.

Awdeh, Z.L., Williamson, A.R. and Askonas, B.A. (1968) Isoelectric focusing in polyacrylamide gel and its application to immunoglobulins. Nature 219, 66.

Aznar, C., Andre, P.M., Deunff, J. and Robert, R. (1988) Investigation of human immune response to *Micropolyspora faeni* antigens by enzyme-linked immunoelectrodiffusion assay and immunoblotting. J. Clin. Microbiol. 26, 443.

Bako, G., Islam, M.N., Vertes, M., Farid, N.R. and Leovey, A. (1987) Dot immunobinding assay for antibodies to TSH receptor. Radiobiol. Radiother. 28, 595.

Baldo, B.A., Sutton, R. and Wrigley, C.W. (1982) Grass allergens with particular reference to cereals. Prog. Allergy 30, 1.

Bansal, G., Martenson, R.E., Leveille, P. and Campagnoni, A.T. (1987) Characterization of a novel monoclonal anti-myelin basic protein antibody: use in immunoblotting and immunohistochemical studies. J. Neuroimmunol. 15, 279.

Bartles, J.R. and Hubbard, A.L. (1984) ^{125}I-wheat germ agglutinin blotting: Increased sensitivity with polyvinylpyrrolidone quenching and periodate oxidation/reductive phenylamination. Anal. Biochem. 140, 284.

Battaglia, M., Passarani, N., Di Matteo, A. and Gerna, G. (1987) Human enteric coronarivuses: further characterization and immunoblotting of viral proteins. J. Infect. Dis. 155, 140.

Batteiger, B., Newhall, W.J. and Jones, R.B. (1982) The use of Tween 20 as a blocking agent in the immunological detection of proteins transferred to nitrocellulose membranes. J. Immunol. Methods 55, 297.

Baumann, E.A. and Hand, R. (1982) Phosphorylation and dephosphorylation alter the structure of D2 hybrid T antigen. J. Virol. 44, 78.

Beisiegel, U. (1986) Protein Blotting. Electrophoresis 7, 1.

Bennett, F.C. and Yeoman, L.C. (1983) An improved procedure for the 'dot immunobinding' analysis of hybridoma supernatants. J. Immunol. Methods 61, 201.

Beyer, C.F. (1984) A 'dot-immunobinding assay' on nitrocellulose membrane for the determination of the immunoglobulin class of mouse monoclonal antibodies. J. Immunol. Methods. 67, 79.

Bhullar, B.S., Hewitt, J. and Candido, E.P.M. (1981) The large high mobility group proteins of rainbow trout are localised predominantly in the nucleus and nucleoli of a cultured trout cell line. J. Biol. Chem. 256, 8801.

Biberfeld, G., Bredberg-Raden, U., Bottiger, B., Biberfeld, P., Morfeldt-Mansson, L., Suni, J., Vaheri, A., Saxinger, C. and Gallo, R. (1985) Antibodies to human T lymphotropic virus type III demonstrated by a dot immunobinding assay. Scand. J. Immunol. 21, 289.

Bird, C.R., Gearing, A.J.H. and Thorpe, R. (1988) The use of Tween-20 alone as a blocking agent for immunoblotting can cause artefactual results. J. Immunol. Methods 106, 175.

Bittner, M., Kupferer, P. and Morris, C.F. (1980) Electrophoretic transfer of proteins and nucleic acids from slab gels to diazobenzyloxymethyl cellulose or nitrocellulose sheets. Analyt. Biochem. 102, 459.

Bjerrum, O.J. (1988) Native immunoblotting of membrane proteins separated in presence of non-ionic detergent. In: O.J. Bjerrum and N.H.H. Heegaard (Eds.), Handbook of Immunoblotting of Proteins, Vol. II, Experimental and Clinical Applications. CRC Press, Boca Raton, FL.

Bjerrum, O.J. and Heegaard, N.H.H. (1988a) CRC Handbook of Immunoblotting of Proteins, Vol. I, Technical Descriptions. CRC Press, London.

Bjerrum, O.J. and Heegaard, N.H.H. (1988b) CRC Handbook of Immunoblotting of Proteins, Vol. II. Experimental and Clinical Applications. CRC Press, London.

Bjorck, L. and Kronvall, G. (1984) Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. J. Immunol. 133, 969.

Blake, M.S., Johnston, K.H., Russell-Jones, G.J. and Gotschlich, E.C. (1984) A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. Anal. Biochem. 136, 175.

Blangarin, P., Deviller, P., Kindbeiter, K. and Madjar, J.J. (1984) Gamma heavy chain disease studied by two-dimensional electrophoresis and immuno-blotting techniques. Clin. Chem. 30, 2021.

Blomberg, J. and Klasse, P.J. (1988) Specificities and sensitivities of three systems for determination of antibodies to human immunodeficiency virus by electrophoretic immunoblotting. J. Clin. Microbiol. 26, 106.

Blumberg, R.S., Hartshorn, K.L., Ardman, B., Kaplan, J.C., Paradis, T., Vogt, M., Hirsch, M.S. and Schooley, R.T.

(1987) Dot immunobinding assay for detection of human immunodeficiency virus-associated antigens. J. Clin. Microbiol. 25, 1989.

Bolton, A.E. and Hunter, W.M. (1973) The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. Biochem. J. 133, 529.

Bonfa, E., Llovet, R. and Elkon, K. (1988) Immunoblot analysis of IgG subclasses of multiple lupus autoantibodies. J. Immunol. 140, 2231.

Bowen, B., Steinberg, J., Laemmli, U.K. and Weintraub, H. (1980) The detection of DNA-binding proteins by protein blotting. Nucl. Acids Res. 8, 1.

Boyce, W.M., Branstetter, B.A. and Kazacos, K.R. (1988) Comparative analysis of larval excretory-secretory antigens of *Baylisascaris procyonis, Toxocara canis* and *Ascaris suum* by Western blotting and enzyme immunoassay. Int. J. Parasitol. 18, 109.

Brada, D. and Roth, J. (1984) Golden blot - detection of polyclonal and monoclonal antibodies bound to antigens on nitrocellulose by protein A-gold complexes. Anal. Biochem. 142, 79.

Bradbury, J.M. and Thompson, R.J. (1984) Photoaffinity labelling of central nervous system myelin. Evidence for an endogenous type I cyclic AMP-dependent kinase phosphorylating the larger subunit of 2′,3′-cyclic nucleotide 3′-phosphodiesterase. Biochem. J. 221, 361.

Bradley, W.C., Mills, S.D., Preston, M.A., Barton, L.J. and Penner, J.L. (1984) Detection of lipopolysaccharides in polyacrylamide gels by transfer to nitrocellulose followed by immunoautoradiography with antibody and ^{125}I-protein A: LPS blotting. Anal. Biochem. 137, 129.

Brooks, R.G., Sharma, S.D. and Remington, J.S. (1985) Detection of *Toxoplasma gondii* antigens by a dot-immunobinding technique. J. Clin. Microbiol. 21, 113.

Brower, M.S., Brakel, C.L. and Garry, K. (1985) Immunodetection with streptavidin-acid phosphatase complex on Western blots. Anal. Biochem. 147, 382.

Burger, J.G. and Du Plessis, D. (1983) Detection of partially proteolysed cauliflower mosaic virus coat protein in infected leaf tissue by Western blotting. J. Virol. Methods 7, 11.

Burks, Jr., A.W., Brooks, J.R. and Sampson, H.A. (1988) Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. J. Allergy Clin. Immunol. 81, 1135.

Burnette, W.N. (1981) Western blotting: electrophoretic transfer of proteins from sodium dodecyl sulphate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. 112, 195.

Burnie, J. and Matthews, R.C. (1987) Immunoblot analysis: a new method for fingerprinting hospital pathogens. J. Immunol. Methods. 100, 41.

Burnie, J.P., Matthews, R.C., Fox, A. and Tabaqchali, S. (1985) Use of immunoblotting to identify antigenic differences between the yeast and mycelial phases of *Candida albicans*. J. Clin. Pathol. 38, 701.

Butikofer, P., Frenkel, E.J. and Ott, P. (1985) Crossed immunoblotting: identification of proteins after crossed immunoelectrophoresis and electrotransfer to nitrocellulose membranes. J. Immunol. Methods 84, 65.

Caldwell, H.D., Stewart, S., Johnson, S. and Taylor, H. (1987) Tear and serum antibody response to *Chlamydia trachomatis* antigens during acute chlamydial conjunctivitis in monkeys as determined by immunoblotting. Infect. Immun. 55, 93.

Carnow, T.B., Manthorpe, M., Davis, G.E. and Varon, S. (1985) Localised survival of ciliary ganglionic neurons identifies neuronotrophic factor bands on nitrocellulose blots. J. Neurosci. 5, 1965.

Carrey, E.A. and Hardie, D.G. (1986) Mapping of radiolabelled peptides derived from proteolysis of polypeptides bound to nitrocellulose after Western blotting. Analyt. Biochem. 158, 431.

Cevenini, R., Rumpianesi, F., Sambri, V. and La Placa, M. (1986). Antigenic specificity of serological response in *Chlamydia trachomatis* urethritis detected by immunoblotting. J. Clin. Pathol. 39, 325.

Chapman, A.J., Adler, B. and Faine, S. (1987) Genus-specific antigens in *Leptospira* revealed by immunoblotting. Zentralbl. Bakteriol. Mikrobiol. Hyg. 264, 279.

Chen, P.P., Fong, S., Normansell, D., Houghten, R.A., Karras, J.G., Vaughan, J.H., Lerner, R.A. and Carson, D.A. (1984) Anti-hypervariable region antibody induced by a defined peptide: An approach for studying the structural correlates of idiotypes. Proc. Natl. Acad. Sci. U.S.A. 81, 1784.

Chiodi, F., Bredberg-Raden, U., Biberfeld, G., Bottiger, B., Albert, J., Asjo, B., Fenyo, E.M. and Norrby, E. (1987) Radioimmunoprecipitation and Western blotting with sera of human immunodeficiency virus infected patients: a comparative study. AIDS Res. Hum. Retroviruses 3, 165.

Christie, K.E., Flaegstad, T. and Traavik, T. (1988) Characterization of BK virus-specific antibodies in human sera by Western immunoblotting: use of a zwitterionic detergent for restoring the antibody-binding capacity of electroblotted proteins. J. Med. Virol. 24, 183.

Clegg, J.C.S. (1982) Glycoprotein detection in nitrocellulose transfers of electrophoretically separated protein mixtures using concanavalin A and peroxidase: application to Arenavirus and Flavivirus proteins. Anal. Biochem. 127, 389.

Coates, S.R., Hansen, D., Schecter, G., Slutkin, G., Hopewell, P., Affronti, L. and Echenberg, D.F. (1986) Identification of *Mycobacterium tuberculosis* antigens in Seibert fractions by immunoblotting. J. Clin. Microbiol. 24, 126.

Cohen, G.H., Isola, V.J., Kuhns, J., Berman, P.W. and Eisenberg, R.J. (1986) Localization of discontinuous epitopes of herpes simplex virus glycoprotein D: use of a nondenaturing ('native' gel) system of polyacrylamide gel electrophoresis coupled with Western blotting. J. Virol. 60, 157.

Coll, P., Borche, L., Ausina, V., Mirelis, B. and Prats, G. (1986) Dot-immunobinding assay with a monoclonal antibody for detection of group B meningococcal antigen. Eur. J. Clin. Microbiol. 5, 44.

Costa, O. and Monier, J.C. (1986a) Antihistone antibodies detected by ELISA and immunoblotting in systemic lupus

erythematosus and rheumatoid arthritis. J. Rheumatol. 13, 722.

Costa, O. and Monier, J.C. (1986b) Antihistone antibodies detected by micro-ELISA and immunoblotting in mice with lupus-like syndrome (MRL/1, MRL/n, PN, and NZB strains). Clin. Immunol. Immunopathol. 40, 276.

Costa, O., Tchouatcha-Tchouassom, J.C., Roux, B. and Monier, J.C. (1986) Anti-H1 histone antibodies in systemic lupus erythematosus: epitope localization after immunoblotting of chymotrypsin-digested H1. Clin. Exp. Immunol. 63, 608.

Cox, J.V., Schenk, E.A. and Olmsted, J.B. (1983) Human anticentromere antibodies: distribution, characterisation of antigens and effect on microtubule organisation. Cell 35, 331.

Daneels, G., Moeremans, M., DeRaeymaeker, M. and De Mey, J. (1986) Sequential immunostaining (gold/silver) and complete protein staining (AuroDye) on Western blots. J. Immunol. Methods 89, 89.

Dao, M.L. (1985) An improved method of antigen detection on nitrocellulose: in situ staining of alkaline phosphatase conjugated antibody. J. Immunol. Methods. 82, 225.

De Blas, A.L. and Cherwinski, H.M. (1983) Detection of antigens on nitrocellulose paper immunoblots with monoclonal antibodies. Anal. Biochem. 133, 214.

De Jongh-Leuvenink, J., Vreede, R.W., Marcelis, J.H., De Vos, M. and Verhoef, J. (1985) Detection of antibodies against lipopolysaccharides of *Escherichia coli* and *Salmonella* R and S strains by immunoblotting. Infect. Immun. 50, 716.

Delia, S., Vullo, V., Mastroianni, C.M., Contini, C., Massetti, A.P., Cignarella, L. and Sorice, F. (1987) Use of dot immunobinding assay (DIB) for the rapid diagnosis of human brucellosis. J. Infect. 14, 88.

Della-Penna, D., Christoffersen, R.E. and Bennett, A.B. (1986) Biotinylated proteins as molecular weight standards on Western blots. Anal. Biochem. 152, 329.

Dennis-Sykes, C.A., Miller, W.J. and McAleer, W.J. (1985) A quantitative Western Blot method for protein measurement. J. Biol. Stand. 13, 309.

Derer, M.M., Miescher, S., Johansson, B., Frost, H. and Gordon, J. (1984) Application of the dot immunoblotting assay to allergy diagnosis. J. Allergy Clin. Immunol. 74, 85.

De Rooij, D.J., Habets, W.J., Stapel, S., Van de Putte, L.B. and Van Venrooij, W.J. (1985) Clinical significance of antibodies against nuclear antigens as determined by immunoblotting. Scand. J. Rheumatol. 56 (suppl.), 93.

Dietrich, J.B. (1985) Use of purified antipeptide sera of selected specificity for the detection of the simian virus 40 large T antigen by Western blotting. Biosci. Rep. 5, 137.

Dow, C.A., De Aizpurua, H.J., Pedersen, J.S., Ungar, B. and Toh, B.H. (1985) 65–70 KD protein identified by immunoblotting as the presumptive gastric microsomal autoantigen in pernicious anaemia. Clin. Exp. Immunol. 62, 732.

Dresel, H.A. and Schettler, G. (1984) Characterisation and visualisation of the low density lipoprotein receptor by ligand blotting using anti-low density lipoprotein enzyme linked immunosorbent assay (ELISA). Electrophoresis 5, 372.

Dunn, B.E., Blaser, M.J. and Snyder, E.L. (1987) Two-dimensional gel electrophoresis and immunoblotting of *Campylobacter* outer membrane proteins. Infect. Immun. 55, 1564.

Dziadek, M., Richter, H., Schachner, M. and Timpl, R. (1983) Monoclonal antibodies used as probes for the structural organisation of the central region of fibronectin. FEBS Lett. 155, 321.

Einarsson, R. (1987) Monitoring of antibodies in patients on immunotherapy with insect venoms by immunoblotting. Int. Arch. Allergy Appl. Immunol. 83, 217.

Elkon, K.B. (1984) Isoelectric focusing of human IgA and secretory proteins using thin layer agarose gels and nitrocellulose capillary blotting. J. Immunol. Methods 66, 313.

Elkon, K.B. and Jankowski, P.W. (1985) Fine specificities of autoantibodies directed against the Ro, La, Sm, RNP, and J-1 proteins defined by two-dimensional gel electrophoresis and immunoblotting. J. Immunol. 134, 3819.

Elkon, K.B., Bonfa, E., Li-Chu, J. and Parnassa, A.P. (1987) Characterization of autoantigen and autoantibodies by immunoblotting. Electrophoresis 8, 445.

Erickson, P.F., Minier, L.N. and Lasher, R.S. (1982) Quantitative electrophoretic transfer of polypeptides from SDS polyacrylamide gels to nitrocellulose sheets: a method for their re-use in immunoautoradiographic detection of antigens. J. Immunol. Methods 51, 241.

Fahey, K.J., O'Donnell, I.J. and Azad, A.A. (1985) Characterization by Western blotting of the immunogens of infectious bursal disease virus. J. Gen. Virol. 66, 1479.

Fang, C.T., Darr, F., Kleinman, S., Wehling, R.H. and Dodd, R.Y. (1986) Relative specificity of enzyme-linked immunosorbent assays for antibodies to human T-cell lymphotrophic virus, type III, and their relationship to Western blotting. Transfusion 26, 208.

Farrah, S.R., Shah, D.O. and Ingram, L.O. (1981) Effects of chaotropic and antichaotropic agents on elution of poliovirus adsorbed on membrane filters. Proc. Natl. Acad. Sci. U.S.A. 78, 1229.

Faye, L. and Chrispeels, M.J. (1985) Characterisation of N-linked oligosaccharides by affinoblotting with concanavalin A-peroxidase and treatment of the blots with glycosidases. Anal. Biochem. 149, 218.

Fisher, P.A., Berrios, M. and Blobel, G. (1982) Isolation and characterisation of a proteinaceous subnuclear fraction composed of nuclear matrix, peripheral lamina and nuclear pore complexes from embryos of *Drosophila melanogaster*. J. Cell Biol. 92, 674.

Flanagan, S.D. and Yost, B. (1984) Calmodulin-binding proteins: Visualisation by ^{125}I-calmodulin overlay on blots quenched with Tween 20 or bovine serum albumin and poly (ethylene oxide). Anal. Biochem. 140, 510.

Fling, S.P., Knospe, V. and Gregerson, D.S. (1988) Preparation of overlapping peptides of bovine retinal S-antigen and their localisation by immunoblotting with peptide-specific antibodies. Curr. Eye Res. 7, 191.

Fong, S., Chen, P.P., Gilbertson, T.A., Weber, J.R., Fox, R.I. and Carson, D.A. (1986) Expression of three cross-reactive idiotypes on rheumatoid factor autoantibodies from pa-

tients with autoimmune diseases and seropositive adults. J. Immunol. 137, 122.

Ford, S.A., Tovey, E.R. and Baldo, B.A. (1985) Identification of orchard grass (*Dactylis glomerata*) pollen allergens following electrophoretic transfer to nitrocellulose. Int. Arch. Allergy Appl. Immunol. 78, 15.

Ford, S.A., Baldo, B.A., Geraci, D. and Bass, D. (1986) Identification of *Parietaria judaica* pollen allergens. Int. Arch. Allergy Appl. Immunol. 79, 120.

Fraker, P.J. and Speck, J.C. (1978) Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril. Biochem. Biophys. Res. Commun. 80, 849.

Frazer, H.E. and Wisdom, G.B. (1985) Detection of autoantigens by immunoblotting using a peroxidase-anti-peroxidase complex. J. Immunol. Methods 80, 221.

Frazer, I.H., Jordan, T.W., Collins, E.C., Andrews, P. and Mackay, I.R. (1987) Antibody to liver membrane antigens in chronic active hepatitis. IV. Exclusion of specific reactivity to polypeptides and glycolipids by immunoblotting. Hepatology 7, 4.

Freedman, A.R., Galfrè, G., Gal, E., Ellis, H.J. and Ciclitira, P.J. (1988) Western immunoblotting of cereal proteins with monoclonal antibodies to wheat gliadin to investigate coeliac disease. Int. Arch. Allergy Appl. Immunol. 85, 346.

Frey, J. and Afting, E.G. (1983) Isolation and renaturation of α_2-macroglobulin receptor from diploid human fibroblasts. Biochem. J. 214, 629.

Fusconi, M., Ghadiminejad, I., Bianchi, F.B., Baum, H., Bottazzo, G.F. and Pisi, E. (1988) Heterogeneity of antimitochondrial antibodies with the M2-M4 pattern by immunofluorescence as assessed by Western immunoblotting and enzyme linked immunosorbent assay. Gut 29, 440.

Gabor, G. and Bennett, R.M. (1984) Biotin-labelled DNA: A novel approach for the recognition of a DNA binding site on cell membranes. Biochem. Biophys. Res. Commun. 122, 1034.

Gandhi, B.M. (1986) A dot immunobinding assay (DIA) on nitrocellulose membrane for the serological detection of antibodies to *Entamoeba histolytica*. Trans. R. Soc. Trop. Med. Hyg. 80, 883.

Gershoni, J.M. (1988) Protein Blotting: A Manual. In: D. Glick (Ed.), Methods of Biochemical Analysis, Vol. 33. John Wiley, New York, p. 1.

Gershoni, J.M. and Palade, G.E. (1982) Electrophoretic transfer of proteins from sodium dodecyl sulphate polyacrylamide gels to a positively charged membrane filter. Anal. Biochem. 124, 396.

Gershoni, J.M. and Palade, G.E. (1983) Protein blotting: principles and applications. Analyt. Biochem. 131, 1.

Gershoni, J.M., Bayer, E.A. and Wilcheck, M. (1985a) Blot analyses of glycoconjugates: enzyme-hydrazide - a novel reagent for the detection of aldehydes. Anal. Biochem. 146, 59.

Gershoni, J.M., Davis, F.E. and Palade, G.E. (1985b) Protein blotting in uniform or gradient electric fields. Analyt. Biochem. 144, 32.

Geysen, J., DeLoof, A. and Vandesande, F. (1984) How to perform subsequent or 'double' immunostaining of two different antigens on a single nitrocellulose blot within one day with an immunoperoxidase technique. Electrophoresis 5, 129.

Gibson, W. (1981) Protease-facilitated transfer of high molecular weight proteins during electrotransfer to nitrocellulose. Anal. Biochem. 118, 1.

Glenney, J.R., Glenney, P. and Weber, K. (1983) Mapping the fodrin molecule with monoclonal antibodies. A general approach for rod-like multidomain proteins. J. Mol. Biol. 167, 275.

Glynn, P., Gilbert, H., Newcombe, J. and Cuzner, M.L. (1982) Rapid analysis of immunoglobulin isoelectric focusing patterns with cellulose nitrate sheets and immunoperoxidase staining. J. Immunol. Methods 51, 251.

Gorelick, F.S., Cohn, J.A., Freedman, S.D., Delahunt, N.G., Gershoni, J.M. and Jamieson, J.D. (1983) Calmodulin-stimulated protein kinase activity from rat pancreas. J. Cell Biol. 97, 1294.

Gottstein, B., Zini, D. and Schantz, P.M. (1987) Species-specific immunodiagnosis of *Taenia solium* cysticercosis by ELISA and immunoblotting. Trop. Med. Parasitol. 38, 299.

Graves, D.C., McNabb, S.J., Worley, M.A., Downs, T.D. and Ivey, M.H. (1986) Analyses of rat *Pneumocystis carinii* antigens recognized by human and rat antibodies by using Western immunoblotting. Infect. Immun. 54, 96.

Graziani, M.S. and Righetti, G. (1987) Immunoblotting for detecting Bence Jones proteinuria. Clin. Chem. 33, 1079.

Grimaldi, L.M.E., Roos, R.P., Devare, S.G., Robey, W.G., Casey, J.M., Gurney, M.E., Apatoff, B.R. and Lazzarin, A. (1988) Restricted heterogeneity of antibody to gp120 and p24 in AIDS. J. Immunol. 141, 114.

Grodzicki, R.L. and Steere, A.C. (1988) Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigen preparations for diagnosing early Lyme disease. J. Infect. Dis. 157, 790.

Guengerich, F.P., Wang, P. and Davidson, N.K. (1982) Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits and humans using immunochemical staining coupled with sodium dodecyl sulphate polyacrylamide gel electrophoresis. Biochemistry 21, 1698.

Guldner, H.H., Lakomek, H.J. and Bautz, F.A. (1986) Anti-(U1)RNP and anti-Sm autoantibody profiles in patients with systemic rheumatic diseases: differential detection of immunoglobulin G and M by immunoblotting. Clin. Immunol. Immunopathol. 40, 532.

Gullick, W.J. and Lindstrom, J.M. (1982) Structural similarities between acetyl choline receptors from fish electric organs and mammalian muscle. Biochemistry 21, 4563.

Habets, W.J., De Rooij, D.J., Hoet, M.H., Van de Putte, L.B. and Van Venrooij, W.J. (1985) Quantitation of anti-RNP and anti-Sm antibodies in MCTD and SLE patients by immunoblotting. Clin. Exp. Immunol. 59, 457.

Habets, W.J., De Rooij, D.J., Salden, M.H., Verhagen, A.P., Van Eekelen, C.A., Van de Putte, L.B. and Van Venrooij, W.J. (1983) Antibodies against distinct nuclear matrix proteins are characteristic for mixed connective tissue disease. Clin. Exp. Immunol. 54, 265.

Habets, W.J., Hoet, M.H., Van de Pas, J. and Van Venrooij, W.J. (1985) Characterisation of nuclear and cytoplasmic autoimmune antigens. In: H. Peeters (Ed.), Protides of the Biological Fluids, Vol. 33. Pergamon, Oxford, p. 199.

Hadfield, S.G. and Glynn, A.A. (1984) Application of imunoblotting to the differentiation of specific antibodies in gonorrhoea. Immunology 51, 615.

Hald, J., Naaby Hansen, S., Egense, J., Hjort, T. and Bjerrum, O.J. (1987) Autoantibodies against spermatozoal antigens detected by immunoblotting and agglutination. A longitudinal study of vasectomized males. J. Reprod. Immunol. 10, 15.

Halpern, R., Schiffenbauer, J., Solomon, G. and Diamond, B. (1984) Detection of masked anti-DNA antibodies in lupus sera by a monoclonal anti-idiotype. J. Immunol. 133, 1852.

Hancock, K. and Tsang, V.C.W. (1983) India ink staining of proteins on nitrocellulose paper. Analyt. Biochem. 133, 157.

Hanff, P.A., Fehniger, T.E., Miller, J.N. and Lovett, M.A. (1982) Humoral immune response in human syphillis to polypeptides of *Treponema pallidum*. J. Immunol. 129, 1287.

Hass, H., Becker, W.M., Maasch, H.J. and Schlaak, M. (1986) Analysis of allergen components in grass pollen extracts using immunoblotting. Int. Arch. Allergy Appl. Immunol. 79, 434.

Hauber, R., Geiger, R. (1987) A new, very sensitive, bioluminescence-enhanced detection system for protein blotting. Ultrasensitive detection systems for protein blotting and DNA hybridization, I. J. Clin. Chem. Clin. Biochem. 25, 511.

Hawkes, R. (1983) Identification of concanavalin A-binding proteins after sodium dodecyl sulphate gel electrophoresis and protein blotting. Anal. Biochem. 123, 143.

Hawkes, R. (1986) The dot immunobinding assay. In: J.J. Langone and H. Van Vunakis (Eds.), Methods in Enzymology, Vol. 121, Immunochemical Techniques Part I, Academic Press, London, pp. 484–491.

Hawkes, R., Niday, E. and Gordon, J. (1982) A dot-immunobinding assay for monoclonal and other antibodies. Anal. Biochem. 119, 142.

Hayman, E.G. and Ruoslahti, E. (1988) Cell attachment to blotted proteins. In: O.J. Bjerrum and N.H.H. Heegaard (Eds.), CRC Handbook of Immunoblotting of Proteins, Vol. II, Experimental and Clinical Applications. CRC Press, London, p. 81.

Hayman, E.G., Engvall, E., A'Hearn, E., Barnes, D., Pierschbacher, M. and Ruoslahti, E. (1982) Cell attachment on replicas of polyacrylamide gels reveals two adhesive plasma proteins. J. Cell Biol. 95, 20.

Heard, S.R., Rasburn, B., Matthews, R.C. and Tabaqchali, S. (1986) Immunoblotting to demonstrate antigenic and immunogenic differences among nine standard strains of *Clostridium difficile*. J. Clin. Microbiol. 24, 384.

Heberling, R.L. and Kalter, S.S. (1987) A dot-immunobinding assay on nitrocellulose with psoralen inactivated Herpes virus simiae (B virus). Lab. Anim. Sci. 37, 304.

Heberling, R.L., Kalter, S.S., Smith, J.S. and Hildebrand, D.G. (1987) Serodiagnosis of rabies by dot immunobinding assay. J. Clin. Microbiol. 25, 1262.

Heberling, R.L., Kalter, S.S., Marx, P.A., Lowry, J.K. and Rodriguez, A.R. (1988) Dot immunobinding assay compared with enzyme-linked immunosorbent assay for rapid and specific detection of retrovirus antibody induced by human or simian acquired immunodeficiency syndrome. J. Clin. Microbiol. 26, 765.

Hensel, U., Wellensiek, H.J. and Bhakdi, S. (1985) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoblotting as a serological tool in the diagnosis of syphilitic infections. J. Clin. Microbiol. 21, 82.

Herbrink, P., Van Loon, A.M., Rotmans, J.P., Van Knapen, F. and Van Dijk, W.C. (1987) Interlaboratory evaluation of indirect enzyme-linked immunosorbent assay, antibody capture enzyme-linked immunosorbent assay, and immunoblotting for detection of immunoglobulin M antibodies to *Toxoplasma gondii*. J. Clin. Microbiol. 25, 100.

Heys, A.D., Norden, A.G., Fulcher, L.M. and Flynn, F.V. (1986) Bence Jones protein detection: a rapid immunoblotting technique for routine use on unconcentrated urine. Ann. Clin. Biochem. 23, 571.

Hjerten, S. (1983) A study on the renaturation of membrane proteins after solubilisation in SDS or following polyacrylamide gel electrophoresis in SDS, with special reference to a phosphatase from *Acholeplasma laidlawii*. Biochim. Biophys. Acta 736, 130.

Hoffman, D.R. (1987) Allergens in *Hymenoptera* venom. XVIII. Immunoblotting studies of venom allergens. J. Allergy Clin. Immunol. 80, 307.

Hoffman, W.L. and Jump, A.A. (1986) Tween 20 removes antibodies and other proteins from nitrocellulose. J. Immunol. Methods 94, 191.

Howe, J.G. and Hershey, J.W.B. (1981) A sensitive immunoblotting method for measuring protein synthesis initiation factor levels in lysates of *Escherichia coli*. J. Biol. Chem. 256, 12, 836.

Hsu, Y.-H. (1984) Immunogold for detection of antigen on nitrocellulose paper. Anal. Biochem. 142, 221.

Hunter, W.M. (1973) Radioimmunoassay. In: D.M. Weir (Ed.), Handbook of Experimental Immunology, 2nd ed. Blackwell Scientific Publications, Oxford, p. 17.1.

Ipsen, H. and Larsen, J.N. (1988) Detection of antigen-specific IgE antibodies in sera from allergic patients by SDS-PAGE immunoblotting and crossed radioimmunoelectrophoresis. In: O.J. Bjerrum and N.H.H. Heegaard (Eds.), CRC Handbook of Immunoblotting of Proteins, Vol. II. Experimental and Clinical Applications. CRC Press, London, p. 159.

Islam, M.N., Briones-Urbina, R., Bako, G. and Farid, N.R. (1983) Both TSH and thyroid-stimulating antibody of Graves' disease bind to an M_r 197000 holoreceptor. Endocrinology 113, 436.

Jacobs, E., Bennewitz, A. and Bredt, W. (1986) Reaction pattern of human anti-*Mycoplasma pneumoniae* antibodies in enzyme-linked immunosorbent assays and immunoblotting. J. Clin. Microbiol. 23, 517.

Jahn, R., Schiebler, W. and Greengard, P. (1984) A quantitative dot-immunobinding assay for proteins using nitrocellulose membrane filters. Proc. Natl. Acad. Sci. U.S.A. 81, 1684.

Jankowski, M. and Styczynski, P. (1987) Comparison of cyto-

megalovirus antibodies by means of CF, ELISA and immunoblotting techniques. Arch. Immunol. Ther. Exp. 35, 313.

Jarausch, J. and Kadenbach, B. (1985) Structure of the cytochrome *c* oxidase complex of rat liver. 2. Topological orientation of polypeptides in the membrane as studied by proteolytic digestion and immunoblotting. Eur. J. Biochem. 146, 219.

Johansson, E., Van Hage Hamsten, M. and Johansson, S.G. (1988) Demonstration of allergen components in the storage mite *Lepidoglyphus* destructor by an immunoblotting technique. Int. Arch. Allergy Appl. Immunol. 85, 8.

Johnson, D.A., Gautsch, J.W., Sportsman, J.R. and Elder, J.H. (1984) Improved technique utilising nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Technol. 1, 3.

Johnson, T.K., Yuen, K.C.L., Denell, R.E. and Consigli, R.A. (1983) Efficient transfer of proteins from acetic acid-urea and isoelectric focusing gels to nitrocellulose membrane filters with retention of protein antigenicity. Analyt. Biochem. 133, 126.

Johnstone, A. and Thorpe, R. (1982) Immunochemistry in Practice. Blackwell, Oxford, p. 210.

Kakita, K., O'Connell, K. and Permutt, M.A. (1982) Immunodetection of insulin after transfer from gels to nitrocellulose filters. A method of analysis in tissue extracts. Diabetes 31, 648.

Kay, M.M.B., Goodman, S.R., Sorensen, K., Whitfield, C.F., Wong, P., Zaki, L. and Rudloff, V. (1983) Senescent cell antigen is immunologically related to band 3. Proc. Natl. Acad. Sci. U.S.A. 80, 1631.

Keck, K., Grossberg, A.L. and Pressman, D. (1973) Specific characterization of isoelectrofocused immunoglobulins in polyacrylamide gel by reaction with ^{125}I-labelled protein antigens or antibodies. Eur. J. Immunol. 3, 99.

Kelson, J.S., Adler, B., Chapman, A.J. and Faine, S. (1988) Identification of leptospiral flagellar antigens by gel electrophoresis and immunoblotting. J. Med. Microbiol. 26, 47.

Kenna, J.G., Neuberger, J. and Williams, R. (1987) Identification by immunoblotting of three halothane-induced liver microsomal polypeptide antigens recognized by antibodies in sera from patients with halothane-associated hepatitis. J. Pharmacol. Exp. Ther. 242, 733.

Kenny, G.E. and Cartwright, F.D. (1984) Immunoblotting for determination of the antigenic specificities of antibodies to the *Mycoplasmatales*. Isr. J. Med. Sci. 20, 908.

Kibe, M.K., Bidwell, D.E., Turp, P. and Smith, G.R. (1985) Demonstration of cross-reactive antigens in F38 and related mycoplasmas by enzyme-linked immunosorbent assay (ELISA) and immunoblotting. J. Hyg. 95, 95.

Kimura, M., Mohri, H., Shimada, K. and Andoh, T. (1987) Antibodies to extractable nuclear antigens in graft-vs-host F1 mice as determined by immunoblotting: absence of anti-Sm antibody. J. Med. Sci. Biol. 40, 83.

King, S.M., Otter, T. and Witman, G.B. (1985) Characterization of monoclonal antibodies against chlamydomonas flagellar dyneins by high-resolution protein blotting. Proc. Natl. Acad. Sci. U.S.A., 82, 4717.

Knisley, K.A. and Rodkey, L.S. (1986) Affinity immunoblotting. High resolution isoelectric focusing analysis of antibody clonotype distribution. J. Immunol. Methods 95, 79.

Knospe, V., Fling, S.P. and Gregerson, D.S. (1988) Assignment of several epitopes to cyanogen bromide peptides of bovine retinal S-antigen by immunoblotting with peptide-specific antibodies. Curr. Eye Res. 7, 181.

Knudsen, K.A. (1985) Proteins transferred to nitrocellulose for use as immunogens. Anal. Biochem. 147, 285.

Knuver-Hopf, J., Mohr, H., Caspari, G., Beyer, J. and Schmitt, H. (1987) Colour stability of new anti-HIV immunogold blotting versus conventional immunoblotting techniques. J. Immunol. Methods 100, 281.

Koch, C., Skjodt, K. and Laursen, I. (1985) A simple immunoblotting method after separation of proteins in agarose gel. J. Immunol. Methods 84, 271.

Koga, K., Abe, S., Hashimoto, H. and Yamaguchi, M. (1987) Western blotting method for detecting antibodies against human muscle contractile proteins in myositis. J. Immunol. Methods 105, 15.

Konstantinov, K., Russanova, V. and Russeva, V. (1986) Antibodies to histones and disease activity in systemic lupus erythematosus: a comparative study with an enzyme-linked immunosorbent assay and immunoblotting. Arch. Dermatol. Res. 278, 410.

Kotani, T., Umeki, K., Matsunaga, S., Kato, E. and Ohtaki, S. (1986) Detection of autoantibodies to thyroid peroxidase in autoimmune thyroid diseases by micro-ELISA and immunoblotting. J. Clin. Endocrinol. Metab. 62, 928.

Krilis, S., Baldo, B.A., Sutton, R. and Basten, A. (1984) Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. I. Demonstration of multiple allergens by immunochemical and biological analysis. J. Allergy Clin. Immunol. 74, 132.

Kronvall, G., Seal, U.S., Finstad, J. and Williams, R.C. (1970) Phylogenetic insight into evolution of mammalian Fc fragment of γG globulin using staphylococcal protein A. J. Immunol. 104, 140.

Kroutil, L.A. and Bush, R.K. (1987) Detection of *Alternaria* allergens by Western blotting. J. Allergy Clin. Immunol. 80, 170.

Kumar, B.V., Lakshuri, M.V. and Atkinson, J.P. (1985) Fast and efficient method for estimation of proteins. Biochem. Biophys. Res. Commun. 131, 883.

Kyhse-Andersen, J. (1984) Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. J. Biochem. Biophys. Methods 10, 203.

Kyriatsoulis, A., Manns, M., Gerken, G., Lohse, A.W., Ballhausen, W., Reske, K. and Meyer zum Buschenfelde, K.H. (1987) Distinction between natural and pathological autoantibodies by immunoblotting and densitometric subtraction: liver-kidney microsomal antibody (LKM) positive sera identify multiple antigens in human liver tissue. Clin. Exp. Immunol. 70, 53.

Kyriatsoulis, A., Manns, M., Roth, U., Gerken, G., Lohse, A., Wollensak, S., Reske, K. and Meyer zum Buschenfelde, K.H. (1988) Strategy for the characterization of autoanti-

gens in autoimmune diseases. Investigation of the target antigens of antimitochondrial antibodies by radioimmunoassay, immunoblotting, monoclonal antibodies and affinity chromatography. J. Immunol. Methods 109, 113.

Labib, R.S., Anhalt, G.J., Patel, H.P., Mutasim, D.F. and Diaz, L.A. (1986) Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. J. Immunol. 136, 1231.

Laing, P. (1986) Luminescent visualisation of antigens on blots. J. Immunol. Methods 92, 161.

Lamb, J.R. and Young, D.B. (1987) A novel approach to the identification of T-cell epitopes in *Mycobacterium tuberculosis* using human T-lymphocyte clones. Immunology 60, 1.

Lamb, J.R., Ivanyi, J., Rees, A.D.M., Rothbard, J.B., Howland, K., Young, R.A. and Young, D.B. (1987) Mapping T cell epitopes using recombinant antigens and synthetic peptides. EMBO J. 6, 1245.

Lamb, J.R., O'Hehir, R.E. and Young, D.B. (1988) The use of nitrocellulose immunoblots for the analysis of antigen recognition by T lymphocytes. J. Immunol. Methods 110, 1.

Landini, M.P., Re, M.C., Mirolo, G., Baldassarri, B. and La Placa, M. (1985) Human immune response to cytomegalovirus structural polypeptides studied by immunoblotting. J. Med. Virol. 17, 303.

Lanzillo, J.J., Stevens, J., Tumas, J. and Fanburg, B.L. (1983) Avidin biotin amplified immunoperoxidase staining of angiotensin-1-converting enzyme transferred to nitrocellulose after agarose isoelectric focusing. Electrophoresis 4, 313.

LaRochelle, W.J. and Froehner, S.C. (1986) Immunochemical detection of proteins biotinylated on nitrocellulose replicas. J. Immunol. Methods 92, 65.

Larsson, A. and Nilsson, B.O. (1988) Immunisation with nanogram quantities of nitrocellulose-bound antigen, electroblotted from sodium dodecyl sulphate-polyacrylamide gels. Scand. J. Immunol. 27, 305.

Laurent, P., Beal, B., Gaspar, A. and Guilloux, L. (1985) Blotting methods applied to investigations of the mitogenic activity of phytohaemagglutinin. J. Immunol. Methods 82, 181.

Lauzurica, P., Gurbindo, C., Maruri, N., Galocha, B., Diaz, R., Gonzalez, J., Garcia, R. and Lahoz, C. (1988) Olive (*Olea europea*) pollen allergens – I. Immunochemical characterisation by immunoblotting, CRIE and immunodetection by a monoclonal antibody. Mol. Immunol. 24, 329.

Law, H. and Lingwood, C.A. (1985) Use of fluorescent standards in protein transfer and immunoblotting. Accurate estimation of the molecular weight of immunoreactive species. Anal. Biochem. 149, 404.

Leary, J.J., Brigati, D.J. and Ward, D.C. (1983) Rapid and sensitive colorimetric method for visualising biotin-labelled DNA probes hybridised to DNA or RNA immobilised on nitrocellulose: Bio-blots. Proc. Natl. Acad. Sci. U.S.A. 80, 4045.

Lebar, R. and Lees, M.B. (1985) Dot immunobinding as a tool for the study of the CNS myelin antigen, M2. J. Neuroimmunol. 8, 43.

Lee, C.-Y., Huang, Y.-S., Hu, P.-C., Gomel, V. and Menge, A.C. (1982). Analysis of sperm antigens by sodium dodecyl sulphate gel/protein blot radioimmunobinding method. Anal. Biochem. 123, 14.

Lee, N., Zhang, S.-Q. and Testa, D. (1988) A rapid multicolour Western blot. J. Immunol. Methods 106, 27.

Legocki, R.P. and Verna, D.P.S. (1981) Multiple immunoreplica technique: screening for specific proteins with a series of different antibodies using one polyacrylamide gel. Anal. Biochem. 111, 385.

Lehtinen, M. (1985) Immunoblotting and ELISA analysis of HSV-1 and HSV-2-specified polypeptides by using an immunoblocking method. Ann. Clin. Res. 17, 66.

Lehtinen, M., Koivisto, V., Lehtinen, T., Paavonen, J., Leinikki, P. (1985) Immunoblotting and enzyme-linked immunosorbent assay analysis of serological responses in patient infected with herpes simplex virus types 1 and 2. Intervirology 24, 18.

Leong, M.M., Fox, G.R. and Hayward, J.S. (1988) A photodetection device for luminol-based immunodot and Western blotting assays. Anal. Biochem. 168, 107.

Levasseur, S., Sesboue, R., Salier, J.P., Diarra-Mehrpour, M., Bourguignon, J. and Martin, J.P. (1988) Improvement of the sensitivity of mono- and bi-dimensional immunoelectrophoretic techniques by transfer on to nitrocellulose. J. Immunol. Methods 114, 121.

Lightowlers, M.W., Rickard, M.D. and Mitchell, G.F. (1986) Immunization against *Taenia taeniaeformis* in mice: identification of oncospheral antigens in polyacrylamide gels by Western blotting and enzyme immunoassay. Int. J. Parasitol. 16, 297.

Lin, J.C., Choi, E.I. and Pagano, J.S. (1985) Qualitative and quantitative analyses of Epstein-Barr virus early antigen diffuse component by Western blotting enzyme-linked immunosorbent assay with a monoclonal antibody. J. Virol. 53, 793.

Lin, W. and Kasamatsu, H. (1983) On the electrotransfer of polypeptides from gels to nitrocellulose membranes. Analyt. Biochem. 128, 302.

Littauer, U.Z., Giveon, D., Thierauf, M., Ginzburg, I. and Ponstingl, H. (1986) Common and distinct tubulin binding sites for microtubule-associated proteins. Proc. Natl. Acad. Sci. U.S.A. 83, 7162.

Lucius, R., Rauterberg, E.W. and Diesfeld, H.J. (1983) Identification of immunogenic proteins of *Dipetalonema viteae (Filarioidea)* by the Western blotting technique. Tropenmed. Parasitol. 34, 133.

Luzio, J.P. and Jackson, P. (1988) Epitope mapping of proteins. In: O.J. Bjerrum and N.H.H. Heegaard (Eds.), CRC Handbook of Immunoblotting of Proteins, Vol. II, Experimental and Clinical Applications. CRC Press, London, p. 25.

Lyon, J.A. and Weber, J.L. (1988) Preparation and use of monospecific antibodies selected using recombinant expression proteins adsorbed to nitrocellulose. In: O.J. Bjerrum and N.H.H. Heegaard (Eds.), CRC Handbook of Immunoblotting of Proteins. CRC Press, London, p. 95.

McKendall, R.R., Pettit, M. and Woo, W. (1988) The poly-

specific immunoglobulin response to HSV-1 viral proteins: determination of immunogenic proteins and relative antibody titres to individual polypeptides by immunoblotting. J. Med. Microbiol. 25, 49.

McLellan, T. and Ramshaw, J.A.M. (1981) Serial electrophoretic transfers: a technique for the identification of numerous enzymes from single polyacrylamide gels. Biochem. Genet. 19, 647.

Mandrell, R.E. and Zollinger, W.D. (1984) Use of a zwitterionic detergent for the restoration of the antibody-binding capacity of electroblotted meningococcal outer membrane proteins. J. Immunol. Methods 67, 1.

Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular cloning, a laboratory manual. Cold Spring Harbor Lab., Cold Spring Harbor, NY, pp. 108–114.

Manns, M., Gerken, G., Kyriatsoulis, A., Trautwein, C., Reske, K. and Meyer zum Buschenfelde, K.H. (1987) Two different subtypes of antimitochondrial antibodies are associated with primary biliary cirrhosis: identification and characterization by radioimmunoassay and immunoblotting. Hepatology 7, 893.

Marlow, S.J. and Handa, A.K. (1987) Immuno slot-blot assay using a membrane which covalently binds protein. J. Immunol. Methods 101, 133.

Matthews, R. and Burnie, J. (1988) Diagnosis of systemic candidiasis by an enzyme-linked dot immunobinding assay for a circulating immunodominant 47-kilodalton antigen. J. Clin. Microbiol. 26, 459.

Mecheri, S., Peltre, G. and David, B. (1985) Purification and characterization of a major allergen from *Dactylis glomerata* pollen: the Ag Dg 1. Int. Arch. Allergy Appl. Immunol. 78, 283.

Medawar, P. (1965) A biological retrospect. Nature 207, 1327.

Mendel-Hartvig, I. and Nelson, B.D. (1983) Studies on beef heart ubiquinol-cytochrome *C* reductase. Topological studies on the core proteins using proteolytic digestion and immunoreplication. J. Bioenerg. Biomembranes 15, 22.

Mendel-Hartvig, I., Frostell, A. and Totterman, T.H. (1987) Primary biliary cirrhosis: antigenic specificity of IgM-type mitochondrial antibodies analyzed by immunoblotting and ELISA. Int. Arch. Allergy Appl. Immunol. 83, 265.

Merril, C.R. and Pratt, M.E. (1986) A silver stain for the rapid quantitative detection of proteins or nucleic acids on membranes or thin-layer plates. Anal. Biochem. 156, 96.

Milner, A.R., Lepper, A.W., Symonds, W.N. and Gruner, E. (1987) Analysis by ELISA and Western blotting of antibody reactivities in cattle infected with *Mycobacterium paratuberculosis* after absorption of serum with *M. phlei.* Res. Vet. Sci. 42, 140.

Mirande, M., Kellermann, O. and Wallen, J.-P. (1982) Macromolecular complexes from sheep and rabbit containing seven aminoacyl-tRNA synthetases. II. Structural characterisation of the polypeptide components and immunological identification of the methionyl-tRNA synthetase subunit. J. Biol. Chem. 257, 11049.

Miribel, L. and Arnaud, P. (1988) Electrotransfer of proteins following polyacrylamide gel electrophoresis. Nitrocellulose versus nylon membranes. J. Immunol. Methods 107, 253.

Mirolo, G., Landini, M.P., Baldassarri, B., Re, M.C., Lazzarotto, T. and La Placa, M. (1986) Relationship between antibody titres against human cytomegalovirus detected by enzyme immunoassay and titres to individual viral polypeptides studied by immunoblotting. Microbiologica 9, 373.

Miskimins, W.K., Roberts, M.P., McClelland, A. and Ruddle, F.H. (1985) Use of a protein blotting procedure and a specific DNA probe to identify nuclear proteins that recognise the promoter region of the transferrin receptor gene. Proc. Natl. Acad. Sci. U.S.A. 82, 6741.

Moeremans, M., Daneels, G., Van Dijck, A., Langanger, G. and De Mey, J. (1984) Sensitive visualisation of antigen-antibody reactions in dot and blot immune overlay assays with immunogold and immunogold/silver staining. J. Immunol. Methods 74, 353.

Moeremans, M., Daneels, G. and De Mey, J. (1985) Sensitive colloidal metal (gold and silver) staining of protein blots on nitrocellulose membranes. Analyt. Biochem. 145, 315.

Moyle, S., Keir, G. and Thompson, E.J. (1984) Viral immunoblotting: a sensitive method for detecting viralspecific oligoclonal bands in unconcentrated cerebrospinal fluid. Biosci. Rep. 4, 505.

Muilerman, H.G., Ter Hart, H.G.J. and Van Dijk, W. (1982) Specific detection of inactive enzyme protein after polyacrylamide gel electrophoresis by a new enzyme immunoassay method using unspecific antiserum and partially purified active enzyme: application to rat liver phosphodiesterase I. Anal. Biochem. 120, 46.

Naaby-Hansen, S. and Bjerrum, O.J. (1985) Auto- and iso-antigens of human spermatozoa detected by immunoblotting with human sera after SDS-PAGE. J. Reprod. Immunol. 7, 41.

Nakamura, K., Tanaka, T., Kuwahara, A. and Takeo, K. (1985) Microassay for proteins on nitrocellulose filter using protein dye-staining procedure. Anal. Biochem. 148, 311.

Navarro, E., Bach, M., Duran, N., Puigdomenech, P. and Palau, J. (1986) A multiple sample immunoblotting system (MSIS) for the intrinsic detection of antinuclear autoantibodies. J. Immunol. Methods 91, 75.

Navendran, A. and Hoffman, S.A. (1988) Identification of autoantibody reactive integral brain membrane antigens. A two-dimensional analysis. J. Immunol. Methods. 114, 227.

Nespolo, A., Bianchi, G., Salmaggi, A. and Cerrato, D. (1987) Immunoblotting on polyvinyldifluoride improves detection of oligoclonal IgG bands in CSF. Clin. Chem. 33, 1669.

Neumann, D., Gershoni, J.M., Fridkin, M. and Fuchs, S. (1985) Antibodies to synthetic peptides as probes for the binding site on the α subunit of the acetylcholine receptor. Proc. Natl. Acad. Sci. U.S.A. 82, 3490.

Newman, P.J., Kahn, R.A. and Hines, A. (1981) Detection and characterisation of monoclonal antibodies to platelet membrane proteins. J. Cell Biol. 90, 249.

Nghiem, H.-O. (1988) Miniaturization of the immunoblot technique. Rapid screening for the detection of monoclonal and polyclonal antibodies. J. Immunol. Methods 111, 141.

Nishizawa, H., Murakami, A., Hayashi, N., Iida, M. and Abe, Y. (1985) Fabric reinforced polyacrylamide gels for electroblotting. Electrophoresis 6, 349.

Norden, A.G., Fulcher, L.M. and Heys, A.D. (1987) Rapid typing of serum paraproteins by immunoblotting without antigen-excess artifacts. Clin. Chem. 33, 1433.

Nye, L. and Roitt, I.M. (1980) Isoelectric focusing of human antibodies directed against a high molecular weight antigen. J. Immunol. Methods 35, 97.

O'Conner, C.G. and Ashman, L.K. (1982) Application of the nitrocellulose transfer technique and alkaline phosphatase-conjugated anti-immunoglobulin for determination of the specificity of monoclonal antibodies to protein mixtures. J. Immunol. Methods 54, 267.

Oda, T., Hatsushika, M., Watanabe, S. Ikeda, S., Sumii, H., Arakaki, Y., Nakamura, T., Tsutsui, K., Seki, S. and Akiyama, K. (1986) Immunoelectron microscopic and immunoblotting analyses of a retrovirus produced in a human lymphoblastoid cell line with a monoclonal antibody. Cell Mol. Biol. 32, 343.

Ogier, J.A., Klein, J.P., Sommer, P. and Frank, R.M. (1984) Identification and preliminary characterization of saliva-interacting surface antigens of Streptococcus mutants by immunoblotting, ligand blotting, and immunoprecipitation. Infect. Immun. 45, 107.

Ohashi, K., Mikawa, T. and Maruyama, K. (1982) Localisation of Z-protein in isolated Z-disk sheets of chicken leg muscle. J. Cell Biol. 95, 85.

O'Hehir, R.E., Young, D.B., Kay, A.B. and Lamb, J.R. (1987) Cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite). A comparison of T and B cell antigen recognition. Immunology 62, 635.

Ohta, M., Ohta, K., Mori, F., Nishitani, H. and Saida, T. (1986) Sera from patients with multiple sclerosis react with human T cell lymphotropic virus-I *gag* proteins but not *env* proteins – Western blotting analysis. J. Immunol. 137, 3440.

Okazaki, K., Chiya, S., Ideguchi, S. and Kimura, S. (1987) Typing of herpes simplex virus types 1 and 2 by immunoblotting analysis using polyclonal antisera to herpes simplex virus glycoproteins. J. Virol. Methods 18, 169.

Olmsted, J.B. (1981) Affinity purification of antibodies from diazotised paper blots of heterogeneous protein samples. J. Biol. Chem. 256, 11955.

Olmsted, J.B. (1988) Affinity purification of antibodies from blots. In: O.J. Bjerrum and N.H.H. Heegaard (Eds.), CRC Handbook of Immunoblotting of Proteins, Vol. II, Experimental and Clinical Applications. CRC Press, London, p. 87.

Palfreyman, J.W., Vigrow, A., Button, D. and Glancy, H. (1988) Simple method for scanning immunoblots. J. Immunol. Methods 109, 199.

Pappas, M.G., Hajkowski, R. and Hockmeyer, W.T. (1983) Dot enzyme-linked immunosorbent assay (Dot-ELISA): A micro technique for the rapid diagnosis of visceral leishmaniasis. J. Immunol. Methods 64, 205.

Parekh, B.S., Mehta, H.B., West, M.D. and Montelaro, R.C. (1985) Preparative elution of proteins from nitrocellulose membranes after separation by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Anal. Biochem. 148, 87.

Partanen, P., Turunen, H.J., Paasivuo, R., Forsblom, E., Suni, J. and Leinikki, P.O. (1983) Identification of antigenic components of Toxoplasma gondii by an immunoblotting technique. FEBS Lett. 158, 252.

Patel, J.D., Joseph, J.M. and Falkler, W.A. (1988). Direct detection of *Chlamydia trachomatis* in clinical specimens by a dot immunobinding technique using monoclonal antibody. J. Immunol. Methods 108, 279.

Peferoen, M., Huybrechts, R. and De Loof, A. (1982) Vacuum blotting: a new simple and efficient transfer of proteins from sodium dodecyl sulphate-polyacrylamide gels to nitrocellulose. FEBS Lett. 145, 369.

Peltre, G., Lapeyre, J. and David, B. (1982) Heterogeneity of grass pollen allergens (*Dactylis glomerata*) recognised by IgE antibodies in human patients' sera by a new nitrocellulose immunoprint technique. Immunol. Lett. 5, 127.

Penner, E., Goldenberg, H., Meryn, S. and Gordon, J. (1986) A dot-immunobinding assay for antimitochondrial antibodies. Hepatology 6, 381.

Petit, C., Suron, M.E., Gilbert, M. and Theze, J. (1982) Direct detection of idiotypic determinants on blotted monoclonal antibodies. Ann. Immunol. (Inst. Pasteur) 133D, 77.

Pettersson, I., Wang, G., Smith, E.I., Wigzell, H., Hedfors, E., Horn, J. and Sharp, G.C. (1986) The use of immunoblotting and immunoprecipitation of (U) small nuclear ribonucleoproteins in the analysis of sera of patients with mixed connective tissue disease and systemic lupus erythematosus. A cross-sectional, longitudinal study. Arthritis Rheum. 29, 986.

Piechulla, B. and Kuntzel, H. (1983) Mitochondrial polypeptide elongation factor EF-Tu of *Saccharomyces cerevisiae*. Functional and structural homologies to *Escherichia coli* EF-Tu. Eur. J. Biochem. 132, 235.

Pillot, J. and Petit, M.A. (1984) Immunochemical structure of the hepatitis B surface antigen vaccine I. Treatment of immobilized HBsAg by dissociation agents with or without enzymatic digestion and identification of polypeptides by protein blotting. Mol. Immunol. 21, 53.

Porath, A., Hanuka, N., Keynan, A. and Sarov, I. (1987) Virus-specific serum IgG, IgM and IgA antibodies in cytomegalovirus mononucleosis patients as determined by immunoblotting technique. J. Med. Virol. 22, 223.

Porter, D.D., Porter, H.G., Larsen, A.E. and Hadlow, W.J. (1984) Immunoenzyme Western blotting analysis of antibody specificity in Aleutian disease of mink, a parvovirus infection. J. Virol. 52, 745.

Ramirez, P., Bonilla, J.A., Moreno, E. and Leon, P. (1983) Electrophoretic transfer of viral proteins to nitrocellulose sheets and detection with peroxidase-bound lectins and protein A. J. Immunol. Methods 62, 15.

Rautenberg, P., Stender, F. and Ullmann, U. (1986) Detection of Clostridium difficile Toxin A by immunoblotting. Zentralbl. Bakteriol. Mikrobiol. Hyg. 261, 29.

Reinhart, M.P. and Malamud, D. (1982) Protein transfer from isoelectric focusing gels: the native blot. Anal. Biochem. 123, 229.

Reiser, J. and Wardale, J. (1981) Immunological detection of

specific proteins in total cell extracts by fractionation in gels and transfer to diazophenylthioether paper. Eur. J. Biochem. 114, 569.

Renart, J. and Sandoval, I.V. (1984) Western blots. In: S.P. Colowick, N.O. Kaplan and W.B. Jakoby (Eds.), Methods in Enzymology, Vol. 104, Enzyme purification and related techniques, part C. Academic Press, Orlando, FL, p. 455.

Renart, J., Reiser, J. and Stark, G.R. (1979) Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera – method for studying antibody specificity and antigen structure. Proc. Natl. Acad. Sci. U.S.A. 76, 3116.

Reynolds, D. and Hughes, J.H. (1985) Comparison of the rotazyme assay with an avidin-biotin-amplified dot-immunobinding assay for detecting rotaviruses. J. Infect. Dis. 152, 647.

Richter, J.D. and Smith, L.D. (1983) Developmentally regulated RNA binding proteins during oogenesis in *Xenopus laevis*. J. Biol. Chem. 258, 4864.

Rohringer, R. and Holden, D.W. (1985) Protein blotting: Detection of proteins with colloidal gold and of glycoproteins and lectins with biotin-conjugated and enzyme probes. Analyt. Biochem. 144, 118.

Ronai, Z. and Sulitzeanu, D. (1986) (Auto)antibodies in human breast cancer sera against antigens associated with breast cancer cells, detected by immunoblotting. J. Natl. Cancer Inst. 77, 1203.

Rordorf, C., Gambke, C. and Gordon, J. (1983) A multidot immunobinding assay for autoimmunity and the demonstration of novel antibodies against retroviral antigens in the sera of MRL mice. J. Immunol. Methods 59, 105.

Routledge, E.G., Willcocks, M.M., Morgan, L., Samson, A.C., Scott, R. and Toms, G.L. (1987) Heterogeneity of the respiratory syncytial virus 22K protein revealed by Western blotting with monoclonal antibodies (published erratum appears in J. Gen. Virol. 1987, 68, 2272). J. Gen. Virol. 68, 1209.

Rozier, C. and Mache, R. (1984) Binding of 16S rRNA to chloroplast 30S ribosomal proteins blotted on nitrocellulose. Nucleic Acids Res. 12, 7293.

Samson, A.C. (1986) Anomalous behaviour of Newcastle disease virus haemagglutinin-neuraminidase protein in Western blotting analysis of monoclonal antibody binding sites. J. Gen. Virol. 67, 1199.

Saravis, C.A. (1984) Improved blocking of nonspecific antibody binding sites on nitrocellulose membranes. Electrophoresis 5, 54.

Sasaki, T., Bonissol, C., Stoiljkovic, B. and Ito, K. (1987) Demonstration of cross-reactive antibodies to mycoplasmas in human sera by ELISA and immunoblotting. Microbiol. Immunol. 31, 639.

Schalla, W.O., Sanders, L.L., Schmid, G.P., Tam, M.R. and Morse, S.A. (1986) Use of dot-immunobinding and immunofluorescence assays to investigate clinically suspected cases of chancroid, J. Infect. Dis. 153, 879.

Schibeci, A., Wade, A.W., Depew, W.T. and Szewczuk, M.R. (1986) Analysis of serum antibody repertoires by isoelectric focusing and capillary blotting onto nitrocellulose paper. J. Immunol. Methods 89, 201.

Schipper, H.I., Bertram, G. and Kaboth, U. (1988) Microheterogeneity of paraproteins. I. Diagnostic value of isoelectric focusing followed by immunoblotting. Clin. Chim. Acta 171, 271.

Schneider, Z. (1980) Aliphatic alcohols improve the adsorptive performance of cellulose nitrate membranes – application in chromatography and enzyme assays. Anal. Biochem. 108, 96.

Schneppenheim, R. and Rautenberg, P. (1987) A luminescence Western blot with enhanced sensitivity for antibodies to human immunodeficiency virus. Eur. J. Clin. Microbiol. 6, 49.

Sheng, H.Z., Martenson, R.E., Carnegie, P.R. and Bernard, C.C.A. (1988) Electroimmunoblotting of small peptides separated on urea-dodecyl sulphate (SUDS) gels. Application to myelin basic protein. J. Immunol. Methods 107, 13.

Shimokawa, K., Murayama, T., Ishida, S. and Furukawa, T. (1987) Analysis by immunoblotting of human cytomegalovirus antibody in sera of renal transport recipient. J. Med. Virol. 21, 339.

Shuttleworth, A.D. (1984) An improved electrophoretic transfer (electroblotting) apparatus. Electrophoresis 5, 178.

Sinclair, D., Kumararatne, D.S., Forrester, J.B., Lamont, A. and Stott, D.I. (1983) The application of isoelectric focusing to routine screening for paraproteinaemia. J. Immunol. Methods 64, 147.

Sinclair, D., Kumararatne, D.S. and Stott, D.I. (1984a). Detection and identification of serum monoclonal immunoglobulin by immunoisoelectric focusing. Limits of sensitivity and use during relapse of multiple myeloma. J. Clin. Pathol. 37, 255.

Sinclair, D., Dagg, J.H., Mowat, A.McI., Parrott, D.M.V. and Stott, D.I. (1984b) Serum paraproteins in chronic lymphocytic leukaemia. J. Clin. Pathol. 37, 463.

Sinclair, D., Dagg, J.H., Smith, J.G. and Stott, D.I. (1986a) The incidence and possible relevance of Bence-Jones protein in the sera of patients with multiple myeloma. Br. J. Haematol. 62, 689.

Sinclair, D., Dagg, J.H., Dewar, A.E., Mowat, A.McI., Parrott, D.M.V., Stockdill, G. and Stott, D.I. (1986b) The incidence, clonal origin and secretory nature of serum paraproteins in chronic lymphocytic leukaemia. Br. J. Haematol. 64, 725.

Sinclair, D., Sheehan, T., Parrott, D.M.V. and Stott, D.I. (1986c). The incidence of monoclonal gammopathy in a population over 45 years old determined by immunoisoelectric focusing. Br. J. Haematol. 64, 745.

Sinclair, D., Parrott, D.M.V. and Stott, D.I. (1986d) Quantitation of monoclonal immunoglobulins by immunoisoelectric focusing and its application for monitoring secretory B cell neoplasia. J. Immunol. Methods 90, 247.

Singh, M.B. and Knox, R.B. (1985) Grass pollen allergens: antigenic relationships detected using monoclonal antibodies and dot blotting immunoassay. Int. Arch. Allergy Appl. Immunol. 78, 300.

Smith, G.E. and Summers, M.D. (1980) The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl-paper. Anal. Biochem. 109, 123.

Snowden, B.W. and Halliburton, I.W. (1985) Identification of

cross-reacting glycoproteins of four herpesviruses by Western blotting. J. Gen. Virol. 66, 2039.

Sorice, F., Vullo, V., Contini, C., Mastroianni, C.M., Massetti, A.P., Monaco, M.T. and Delia, S. (1985) Use of dot immunobinding assay for the rapid diagnosis of human hydatidosis. Boll. Ist. Sieroter. 64, 414.

Southern, E.M. (1975) J. Mol. Biol. 98, 503.

Srivastava, A.K., Aira, Y., Mori, C., Kobayashi, Y. and Igarashi, A. (1987) Antigenicity of Japanese encephalitis virus envelope glycoprotein V3 (E) and its cyanogen bromide cleaved fragments examined by monoclonal antibodies and Western blotting. Arch. Virol. 96, 97.

Stahlberg, T.H., Granfors, K., Pekkola-Heino, K., Soppi, E. and Toivanen, A. (1987) Immunoblotting analysis of human IgM, IgG, and IgA response to chromosomally coded antigens of *Yersinia enterocolitica* 0 : 3. Acta Pathol. Microbiol. Immunol. Scand. 95, 71.

Sternberg, J. and Jeppesen, P. (1983) Dot-blotting – a novel screening assay for antibodies in hybridoma cultures. J. Immunol. Methods 64, 39.

Stott, D.I. and Feinstein, A. (1973) Biosynthesis and assembly of IgM. Free thiol groups present on the intracellular subunits. Eur. J. Immunol. 3, 229.

Stott, D.I. and McLearie, J. (1986) Isoelectric focusing and reverse immunoblotting of autoantibodies against high molecular weight antigens. Immunol. Invest. 15, 113.

Stott, D.I., McLearie, J. and Marsden, H.S. (1985) A gel transfer tank for immunoblotting and its application for analysis of nuclear protein antigens. Analyt. Biochem. 149, 454.

Stott, D.I., McLearie, J. and Neilson, L. (1986) Analysis of the clonal origins of autoantibodies against thyroglobulin and DNA in autoimmune thyroiditis and systemic lupus erythematosus. Clin. Exp. Immunol. 65, 520.

Stott, D.I., Hassman, R., Neilson, L. and McGregor, A.M. (1988) Analysis of the spectrotypes of autoantibodies against thyroglobulin in two rat models of autoimmune thyroiditis. Clin. Exp. Immunol. 73, 269.

Stott, D.I., Merino, J., Schurmans, S. and Lambert, P.-H. (1988) Expression of anti-DNA clonotypes and the role of helper T-lymphocytes during the autoimmune response in mice tolerant to alloantigens. Autoimmunity 1, 253.

Sturm, S., Fortnagel, P. and Timmis, K.N. (1984) Immunoblotting procedure for the analysis of electrophoretically fractionated bacterial lipopolysaccharide. Arch. Microbiol. 140, 198.

Surek, B. and Latzko, E. (1984) Visualisation of antigenic proteins blotted on to nitrocellulose using the immunogold staining (IGS) method. Biochem. Biophys. Res. Commun. 121, 284.

Sutton, R., Wrigley, C.W. and Baldo, B.A. (1982) Detection of IgE and IgG binding proteins after electrophoretic transfer from polyacrylamide gels. J. Immunol. Methods 52, 183.

Svoboda, M., Meuris, S., Robyn, C. and Christophe, J. (1985) Rapid electrotransfer of proteins from polyacrylamide gel to nitrocellulose membrane using surface-conductive glass as anode. Anal. Biochem. 151, 16.

Swanson, J. and Barrera, O. (1983) Immunological characteristics of gonococcal outer membrane protein II assessed by immunoprecipitation, immunoblotting, and coagglutination. J. Exp. Med. 157, 1405.

Swanstrom, R. and Shank, P.R. (1978) X-ray intensifying screens greatly enhance the detection by autoradiography of the radioactive isotopes ^{32}P and ^{125}I. Analyt. Biochem. 86, 184.

Symington, J., Green, M. and Brackmann, K. (1981) Immunoautoradiographic detection of proteins after electrophoretic transfer from gels to diazo-paper: Analysis of adenovirus encoded proteins. Proc. Natl. Acad. Sci. U.S.A. 78, 177.

Talbot, P.J., Knobler, R.L. and Buchmeier, M.J. (1984) Western and dot immunoblotting analysis of viral antigens and antibodies: application to murine hepatitis virus. J. Immunol. Methods 73, 177.

Talian, J.C., Olmsted, J.B. and Goldman, R.D. (1983) A rapid procedure for preparing fluoroscein-labelled specific antibodies from whole antiserum – its use in analysing cytoskeletal architecture. J. Cell Biol. 97, 1277.

Tamura, A., Ohashi, N., Urakami, H., Takahashi, K. and Oyanagi, M. (1985) Analysis of polypeptide composition and antigenic components of *Rickettsia tsutsugamushi* by polyacrylamide gel electrophoresis and immunoblotting. Infect. Immun. 48, 671.

Tersmette, M., Lelie, P.N., Van der Poel, C.L., Wester, M.R., De Goede, R.E., Lange, J.M., Miedema, F. and Huisman, J.G. (1988) Confirmation of HIV seropositivity: comparison of a novel radioimmunoprecipitation assay to immunoblotting and virus culture. J. Med. Virol. 24, 109.

Thompson, E.J. and Keir, G. (1984) Improved detection of oligoclonal and Bence-Jones proteins by kappa/lambda immunoblotting. Clin. Chim. Acta 143, 329.

Thorpe, R., Bird, C.R. and Spitz, M. (1984) Immunoblotting with monoclonal antibodies: loss of immunoreactivity with human immunoglobulins arises from polypeptides chain separation. J. Immunol. Methods 73, 259.

Thorpe, R., Brasher, M.D., Bird, C.R., Garrett, A.J., Jacobs, J.P., Minor, P.D. and Schild, G.C. (1987) An improved immunoblotting procedure for the detection of antibodies against HIV. J. Virol. Methods 16, 87.

Todd, D., Hull, J. and McNair, J. (1987) Antigenically important proteins of Aujeszky's disease (pseudorabies) virus identified by immunoblotting. Arch. Virol. 96, 215.

Tovey, E.R. and Baldo, B.A. (1984) Standardization of allergens. Quantitative definition of house dust mite extracts following electroblotting and detection of components with antibody and lectin probes. Int. Arch. Allergy Appl. Immunol. 75, 332.

Tovey, E.R. and Baldo, B.A. (1985) Detection of house dust mite allergens and frequency of IgE binding following electroblotting and enzyme immunoassay. Int. Arch. Allergy Appl. Immunol. 76, 82.

Tovey, E.R. and Baldo, B.A. (1987) Comparison of semi-dry and conventional tank buffer electrotransfer of proteins from polyacrylamide gels to nitrocellulose membranes. Electrophoresis 8, 384.

Tovey, E.R., Ford, S.A. and Baldo, B.A. (1987) Protein blot-

ting on nitrocellulose: some important aspects of the resolution and detection of antigens in complex extracts. J. Biochem. Biophys. Methods 14, 1.

Towbin, H. and Gordon, J. (1984) Immunoblotting and dot immunobinding - current status and outlook. J. Immunol. Methods 72, 313.

Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. 76, 4350.

Towbin, H., Ramjoue, H.-P., Kuster, H., Liverani, D. and Gordon, J. (1982) Monoclonal antibodies against eukaryotic ribosomes. Use to characterise a ribosomal protein not previously identified and antigenically related to the acidic phosphoproteins P1/P2. J. Biol. Chem. 257, 709.

Turner, B.M. (1983) The use of alkaline-phosphatase-conjugated second antibody for the visualisation of electrophoretically separated proteins recognised by monoclonal antibodies. J. Immunol. Methods 63, 1.

Uhl, J. and Newton, R.C. (1988) Quantitation of related proteins by Western blot analysis. J. Immunol. Methods 110, 79.

Ulstrup, J.C., Skaug, K., Figenschau, K.J., Orstavik, I., Bruun, J.N. and Petersen, G. (1986) Sensitivity of Western blotting (compared with ELISA and immunofluorescence) during seroconversion after HTLV-III infection. Lancet i, 1151.

Vaessen, R.T.M.J., Kreike, J. and Groot, G.S.P. (1981) Protein transfer to nitrocellulose filters. A simple method for quantitation of single proteins in complex mixtures. FEBS Lett. 124, 193.

Vainio, E. (1986) Immunoblotting analysis of antigliadin antibodies in the sera of patients with dermatitis herpetiformis and gluten-sensitive enteropathy. Int. Arch. Allergy Appl. Immunol. 80, 157.

Vandekerckhove, J., Bauw, G., Puype, M., Van Damme, J. and Van Montagu, M. (1985) Protein blotting on Polybrene-coated glass fibre sheets. A basis for acid hydrolysis and gas-phase sequencing of picomole quantities of protein previously separated on sodium dodecyl sulphate/polyacrylamide gel. Eur. J. Biochem. 152, 9.

Van der Meer, J., Dorssers, L. and Zabel, P. (1983) Antibody-linked polymerase assay on protein blots: a novel method for identifying polymerases following SDS-polyacrylamide gel electrophoresis. EMBO J. 2, 233.

Van Dongen, W.M.A.M., Moorman, A.F.M. and Destree, O.H.J. (1983) The accumulation of the maternal pool of histone HIA during oogenesis in *Xenopus laevis*. Cell Differ. 12, 257.

Van Leuven, F., Marynen, P., Cassiman, J.J., Van den Berghe, H. (1986) Reversed dot-blotting in hybridoma screening and epitope mapping. A model study with human α_2-macroglobulin to select complex-specific monoclonal antibodies. J. Immunol. Methods 90, 125.

Van Oss, C.J., Good, R.J. and Chaudhury, M.K. (1987) Mechanism of DNA (Southern) and protein (Western) blotting on cellulose nitrate and other membranes. J. Chromatogr. 391, 53.

Van Vooren, J.P., Turneer, M., Yernault, J.C., De Bruyn, J., Burton, E., Legros, F. and Farber, C.M. (1988) A multidot immunobinding assay for the serodiagnosis of tuberculosis. Comparison with an enzyme-linked immunosorbent assay. J. Immunol. Methods 113, 45.

Vartdal, F. and Vandvik, B. (1983) Multiple sclerosis: subclasses of intrathecally synthesised IgG and measles and varicella zoster virus IgG antibodies. Clin. Exp. Immunol. 54, 641.

Vartio, T., Zardi, L., Balza, E., Towbin, H. and Vaheri, A. (1982) Monoclonal antibodies in analysis of cathepsin G-digested proteolytic fragments of human plasma fibronectin. J. Immunol. Methods 55, 309.

Vertosick, Jr., F.T. and Kelly, R.H. (1987) Antibodies to native and denatured DNA. Quantitation using an immunoslot-blot technique. J. Immunol. Methods 102, 15.

Walker, J.H. (1988) Inexpensive equipment for polyacrylamide slab gel electrophoresis and flat bed electroblotting. Biochem. Soc. Trans. 16, 762.

Walzer, P.D. and Linke, M.J. (1987) A comparison of the antigenic characteristics of rat and human Pneumocystis carinii by immunoblotting. J. Immunol. 138, 2257.

Walzer, P.D., Stanforth, D., Linke, M.J. and Cushion, M.T. (1987) *Pneumocystis carinii*: immunoblotting and immunofluorescent analyses of serum antibodies during experimental rat infection and recovery. Exp. Parasitol. 63, 319.

Wedege, E. and Froholm, L.O. (1986) Human antibody response to a group B serotype 2a meningococcal vaccine determined by immunoblotting. Infect. Immun. 51, 571.

Wedege, E. and Svenneby, G. (1986) Effects of the blocking agents bovine serum albumin and Tween 20 in different buffers on immunoblotting of brain proteins and marker proteins. J. Immunol. Methods 88, 233.

Wedege, E., Bryn, K. and Froholm, L.O. (1988) Restoration of antibody binding to blotted meningococcal outer membrane proteins using various detergents. J. Immunol. Methods 113, 51.

Weetman, A.P., Nutman, T.B., Baker, Jr. J.R. and Burman, K.D. (1987a) Demonstration by immunoblotting of heterogeneity in the autoantibody response directed against fat cells in Graves' disease. FEBS Lett. 211, 69.

Weetman, A.P., Nutman, T.B., Burman, K.D., Baker, Jr., J.R. and Volkman, D.J. (1987b) Heterogeneity of thyroid autoantigens identified by immunoblotting. Clin. Immunol. Immunopathol. 43, 333.

Weihing, R.R. (1983) Purification of a HeLa cell high molecular weight actin binding protein and its identification in HeLa cell plasma membrane ghosts and intact HeLa cells. Biochemistry 22, 1839.

Westgeest, A.A., Van den Brink, H.G., De Jong, J., Swaak, A.J. and Smeenk, R.J. (1987) Antinuclear antibodies in patients with systemic lupus erythematosus: a comparison of counterimmunoelectrophoresis and immunoblotting. Rheumatol. Int. 7, 77.

Williams, D.G., Stocks, M.R., Charles, P.J. and Maini, R.N. (1986) Antibodies to La, Jo-1, nRNP and Sm detected by multi-track immunoblotting using a novel filter holder: a comparative study with counterimmunoelectrophoresis and

immunodiffusion using sera from patients with systemic lupus erythematosus and Sjogren's syndrome. J. Immunol. Methods 91, 65.

Williamson, A.R. (1973) Isoelectric focusing of immunoglobulins. In: D.M. Weir (Ed.), Handbook of Experimental Immunology, 2nd edn. Blackwell, Oxford, p. 8.1.

Williamson, A.R., Salaman, M.R. and Kreth, H.W. (1973) Microheterogeneity and allomorphism of proteins. Ann. N.Y. Acad. Sci. 209, 210.

Winter, J. (1982) Shiverer peripheral myelin contains P_2. Nature 298, 471.

Wolff, P., Gilz, R., Schumacher, J. and Riesner, D. (1985) Complexes of viroids with histones and other proteins. Nucleic Acids Res. 13, 355.

Wong, R.C., Hudson, L. and Hindmarsh, P.J. (1986) Immune precipitation and immunoblotting for the detection of Trypanosoma cruzi antigens. Trans. R. Soc. Trop. Med. Hyg. 80, 275.

Yamaguchi, K., Matutes, E., Kiyokawa, T., Nishimura, Y., Ishii, T., Takatsuki, K. and Catovsky, D. (1988) Comparison of immunoperoxidase staining with indirect immunofluorescence, ELISA, and Western blotting assays for detecting anti-HTLV-I antibodies in systemic lupus erythematosus. J. Clin. Pathol. 41, 57.

Yoshida, H., Yoshida, M., Izui, S. and Lambert, P.-H. (1985) Distinct clonotypes of anti-DNA antibodies in mice with lupus nephritis. J. Clin. Invest. 76, 685.

Young, D.B. and Lamb, J.R. (1986) T-lymphocytes respond to solid phase antigen: a novel approach to the molecular analysis of cellular immunity. Immunology 59, 167.

Young, T.F. and Ross, R.F. (1987) Assessment of antibody response of swine infected with *Mycoplasma hyopneumoniae* by immunoblotting. Am. J. Vet. Res. 48, 651.

Yurchenco, P.D., Speicher, D.W., Morrow, J.S., Knowles, W.J. and Marchesi, V.T. (1982) Monoclonal antibodies as probes of domain structure of the spectrin a subunit. J. Biol. Chem. 357, 9102.