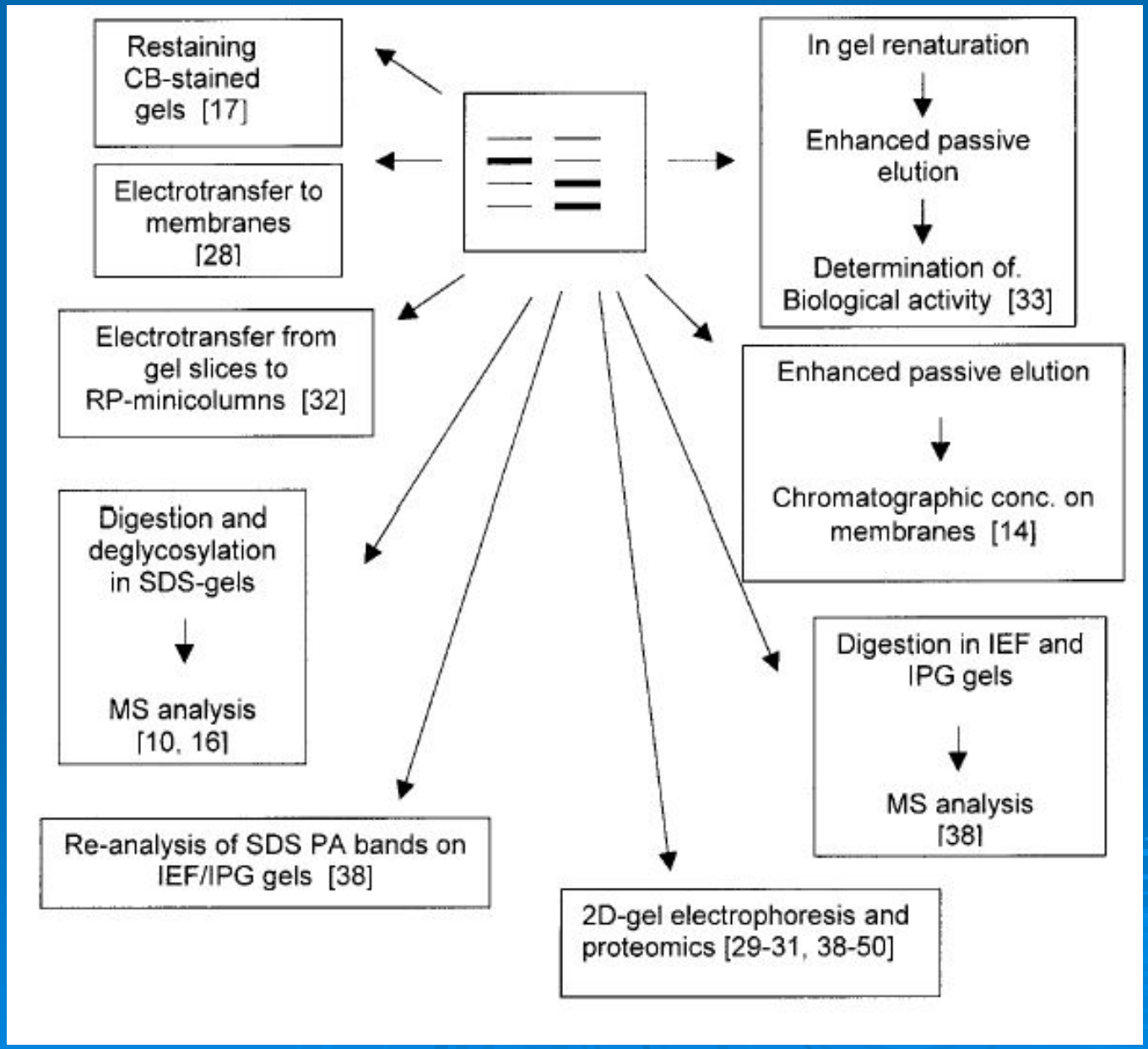
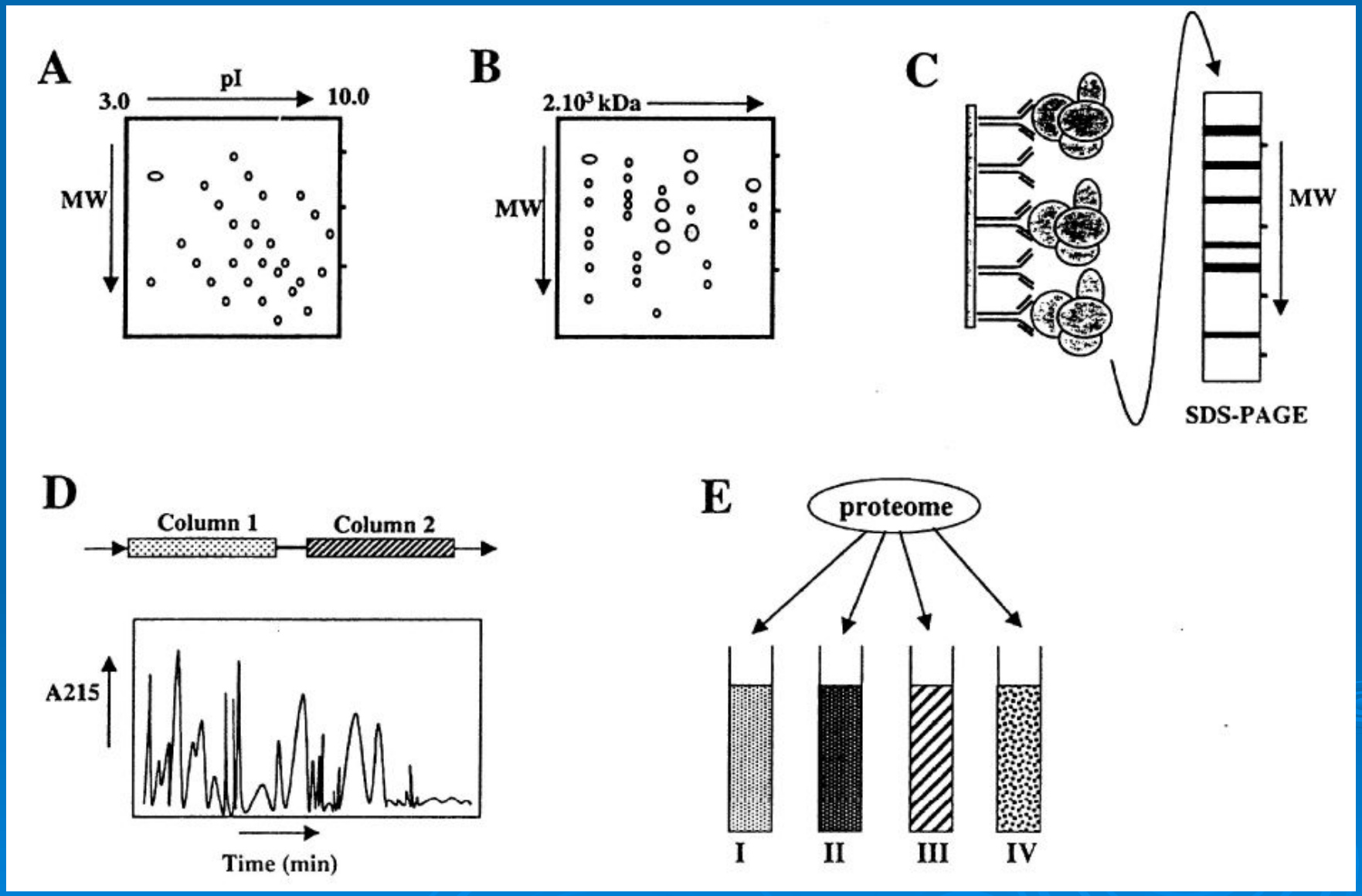
Table 9.1 A comparison of reagents for detecting and quantitating proteins in solution.

Assay	Detection Wavelengths (nm) †	Sensitivity and Effective Range	Mechanism of Action	Notes
NanoOrange protein quantitation assay (N-6666)	485/590	10 ng/mL to 10 µg/mL	Binds to detergent coating on proteins and hydrophobic regions of proteins; the unbound dye is nonfluorescent	• High sensitivity • Little protein-to-protein variation • Rapid and accurate assay with a simple procedure • Compatible with reducing agents
Bradford assay REF (Coomassie Brilliant Blue)	595	1 µg/mL to 1.5 mg/mL	Directly binds specific amino acids and protein tertiary structures; the dye's color changes from brown to blue	• High protein-to-protein variation • Not compatible with detergents • Rapid assay • Useful when accuracy is not crucial

BCA method (bicinchoninic acid)	562	0.5 µg/mL to 1.2 mg/mL	Cu^{2+} is reduced to Cu^{+} in the presence of proteins at high pH; the BCA reagent chelates Cu^{+} ions, forming purple-colored complexes	• Compatible with detergents, chaotropes and organic solvents • Not compatible with reducing agents • The sample must be read within 10 minutes
Lowry assay (biuret reagent plus Folin–Ciocalteu reagent)	750	1 µg/mL to 1.5 mg/mL	Cu^{2+} is reduced to Cu^{+} in the presence of proteins at high pH; the biuret reagent chelates the Cu^{+} ion, then the Folin–Ciocalteu reagent enhances the blue color	• Lengthy procedure with carefully timed steps • Not compatible with detergents or reducing agents
CBQCA protein quantitation assay (C-6667)	450/550	10 ng/mL to 150 µg/mL	Reacts with primary amine groups on proteins in the presence of cyanide or thiols; the unbound dye is nonfluorescent	• Sensitivity depends on the number of amines present • Not compatible with buffers containing amines or thiols • High sensitivity

Fluorescamine (F-2332)	390/475	0.3 µg/mL to 13 µg/mL	Reacts with primary amine groups on proteins; unbound dye is nonfluorescent	• Sensitivity depends on the number of amines present • Reagent is unstable • Not compatible with Tris or glycine buffers
OPA (*o*-phthaldialdehyde) (P-2331)	340/455	0.2 µg/mL to 25 µg/mL	Reacts with primary amine groups on proteins in the presence of β-mercaptoethanol; unbound dye is nonfluorescent	• Sensitivity depends on the number of amines present • Not compatible with Tris or glycine buffers • Low cost
UV absorption	205/280	10 µg/mL to 50 µg/mL or 50 µg/mL to 2 mg/mL	Peptide bond absorption Tryptophan and tyrosine absorption	• Sensitivity depends on number of aromatic amino acid residues present • Nondestructive • Low cost


Restaining CB-stained gels [17]
Electrotransfer to membranes [28]
Electrotransfer from gel slices to RP-minicolumns [32]
Digestion and deglycosylation in SDS-gels
MS analysis [10, 16]
Re-analysis of SDS PA bands on IEF/IPG gels [38]
2D-gel electrophoresis and proteomics [29-31, 38-50]
In gel renaturation
Enhanced passive elution
Determination of. Biological activity [33]
Enhanced passive elution
Chromatographic conc. on membranes [14]
Digestion in IEF and IPG gels
MS analysis [38]


A
pI
3.0
10.0
MW
B
2.10³ kDa
MW
C
MW
SDS-PAGE
D
Column 1
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A215
Time (min)
E
proteome
I
II
III
IV

Figure 6. Tank (wet) transfer system.

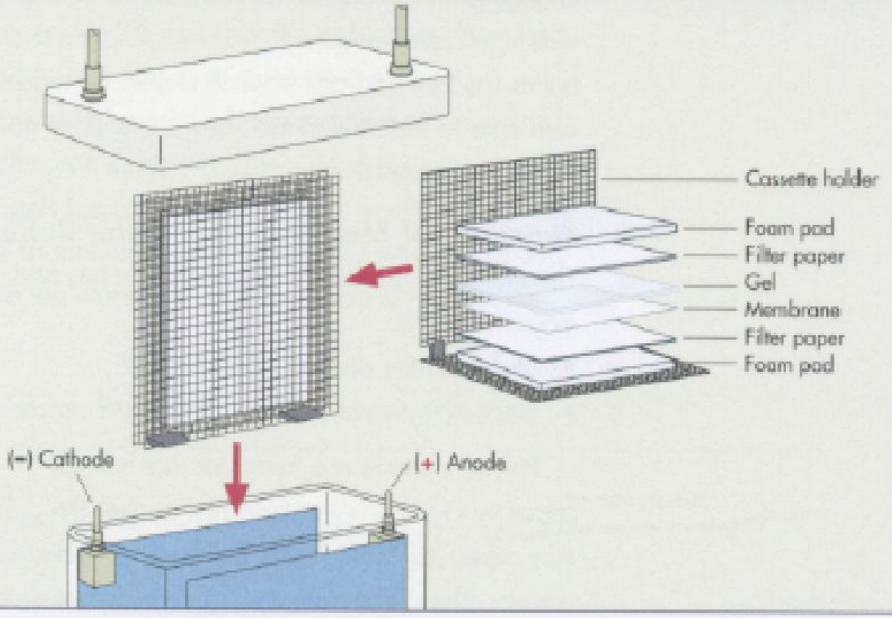
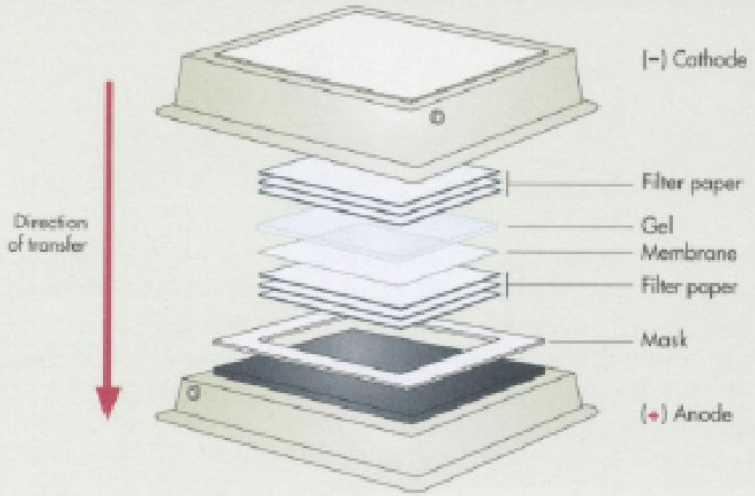
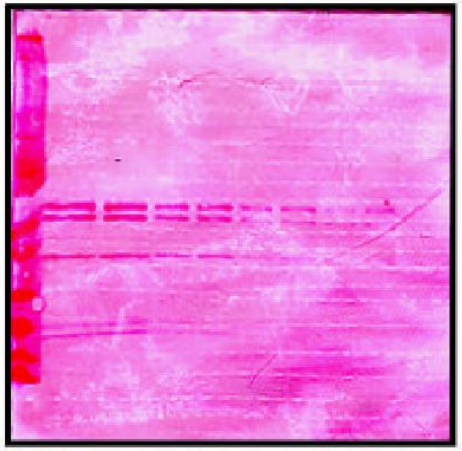


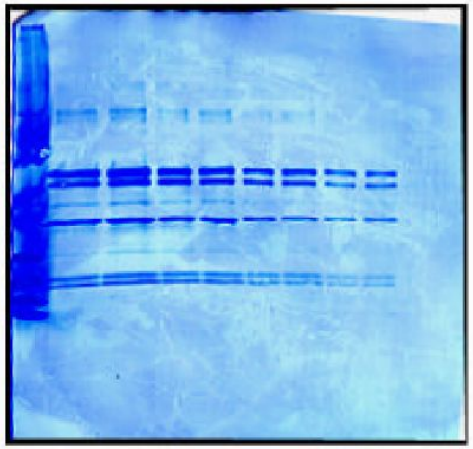
Figure 7. Semi-dry transfer system.



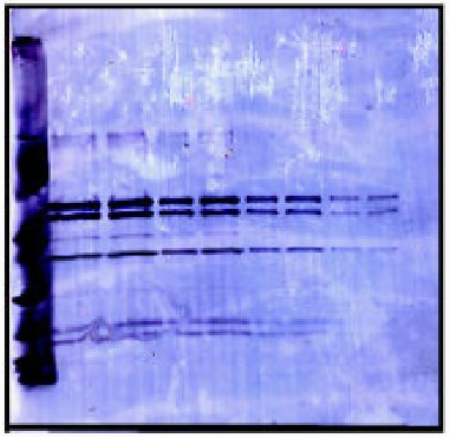
Ponceau S



CBB G-250



Amido Black



CBB R-250

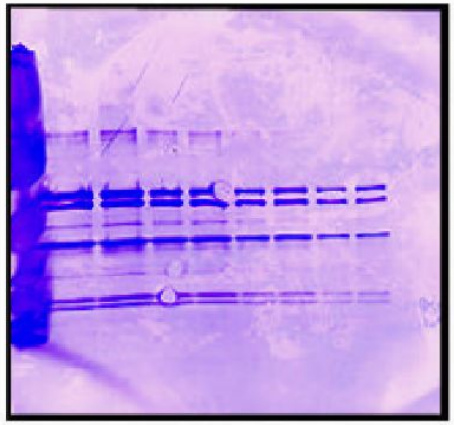


Figure 10. Calf liver proteins are visualized after electroblotting to Immobilon-P membranes: (A) Transillumination, (B) Coomassie Brilliant Blue, (C) Ponceau-S red, (D) Amido black and (E) CPTS total protein stains. Left to right, molecular weight standards and 12.2 µg, 6.1 µg, 3.1 µg of the lysate per lane.

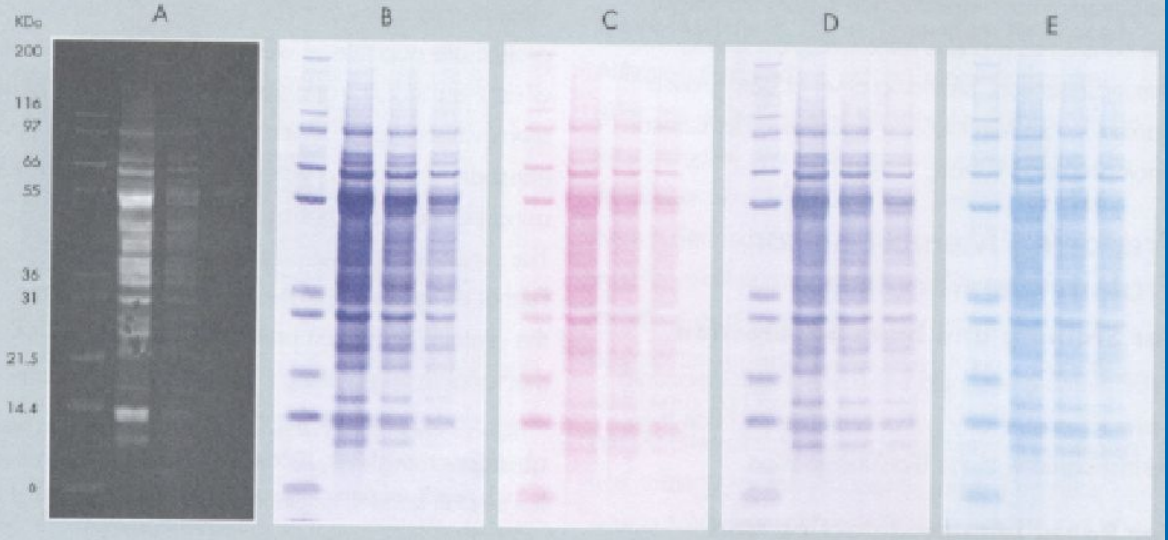


Table 5. *Common stains used in western blotting and their attributes.*

	Detection Reagent	Approximate Sensitivity (protein per spot)	Reference
Reversible	Ponceau-S red	5 µg	Dunn *et al.*, 1999
	Fast green FC	5 µg	Dunn *et al.*, 1999
	CPTS	1 µg	Bickar *et al.*, 1992
	Sypro Ruby	1–2 ng	Haugland, 2002
	Sypro Rose	1–2 ng	Haugland, 2002
Irreversible	Amido black 10B	1 µg	Dunn *et al.*, 1999
	Coomassie Brilliant Blue R-250	500 ng	Dunn *et al.*, 1999
	India ink	100 ng	Dunn *et al.*, 1999
	Colloidal gold	4 ng	Dunn *et al.*, 1999

Protein blotting membranes - recommended applications

	Hybond-C pure	Hybond ECL	Hybond-P	Hybond-C extra
Western Blotting Detection System				
Radioactive	✔✔✔	✔✔	✔✔	✔✔
Enhanced chemiluminescence	✔✔	✔✔✔	✔✔✔	✔
Chemifluorescence	✔	✔	✔✔✔	✘
Chromogenic	✔✔✔	✔✔	✔✔	✔
Colloidal gold	✔✔✔	✔✔	✔✔	✔
Specialty Labelling				
ECL Western blotting kit	✔✔	✔✔✔	✔✔	✔
ECL Plus Western blotting kit	✔	✔✔	✔✔✔	✔
ECF Western blotting kit	✔	✔	✔✔✔	✘
Glycoprotein detection kit	✔✔	✔✔	✔✔✔	✔
Expression screening	✔	✔	✔	✔✔✔
Reprobing	✔	✔	✔✔✔	✔✔

✔✔✔	Highly recommended
✔✔	Recommended
✔	Can be used
✘	Not recommended

Hybond Hybridization Membranes

Amersham Biosciences Hybond™ protein membranes are renowned for their excellent reproducibility, optimisation for various applications and careful packaging to maximise shelf life.

Hybond-C pure

- 100% pure unsupported nitrocellulose
- Excellent signal:noise across all labelling and detection systems—radioactive, non-radioactive or chromogenic

Hybond ECL

- 100% pure unsupported nitrocellulose
- Validated for use with ECL™ Western blotting system

Hybond-P, PVDF

- Ideal for reprobing
- Hydrophobic polyvinylidene difluoride (PVDF) membrane optimised for protein transfer
- Higher physical strength for significant handling advantages over unsupported nitrocellulose
- Particularly recommended for use with the ECL Plus™ and ECF™ Western blotting and ECL glycoprotein systems

Hybond-C extra

- High physical strength, supported mixed-ester nitrocellulose
- Protein binding capacity 80 - 100 µg/cm^2
- Highly recommended for expression screening with its low background, high signal:noise and handling ease

Table 1. *Comparison of PVDF and nitrocellulose membrane attributes and applications*

Attributes/Applications	Nitrocellulose	PVDF
Physical strength	Poor	Good
Protein binding capacity	80 – 100 µg/cm²	100 – 300 µg/cm²
Solvent resistance	No	Yes
Western transfer	Yes	Yes
Total protein stain	Colloidal gold Ponceau-S red Amido black India ink Sypro® blot stains	Colloidal gold Ponceau-S red Amido black India ink Coomassie™ Blue dye
Detection	Chromogenic Chemiluminescent Fluorescent Radioactive	Chromogenic Chemiluminescent Fluorescent Chemifluorescent Radioactive
Double-blotting method	No	Yes
Rapid immunodetection	No	Yes
Western reprobing	Yes	Yes
Edman sequencing	No	Yes
Amino acid analysis	Yes	Yes
Binding in the presence of SDS	Poor	Good
On-membrane digestion for mass spectrometry	No	Yes
Direct MALDI-TOF MS analysis	No	Yes
Data can be archived	No	Yes

Table 3. Protein blotting membranes – recommended applications

Application	Hybond-P	Hybond ECL	Hybond-C extra
Western blotting			
Detection method			
ECL	*highly recommended*	*highly recommended*	*suitable*
ECL Plus	*highly recommended*	*recommended*	*suitable*
chromogenic	*recommended*	*highly recommended*	*suitable*
colloidal gold	*recommended*	*highly recommended*	*not recommended*
ECF	*highly recommended*	*suitable*	*not recommended*
radioactive	*suitable*	*highly recommended*	*recommended*
Reprobing Westerns	highly recommended	not recommended	suitable
Glycoprotein	highly recommended	recommended	suitable
Expression screening	suitable	not recommended	highly recommended

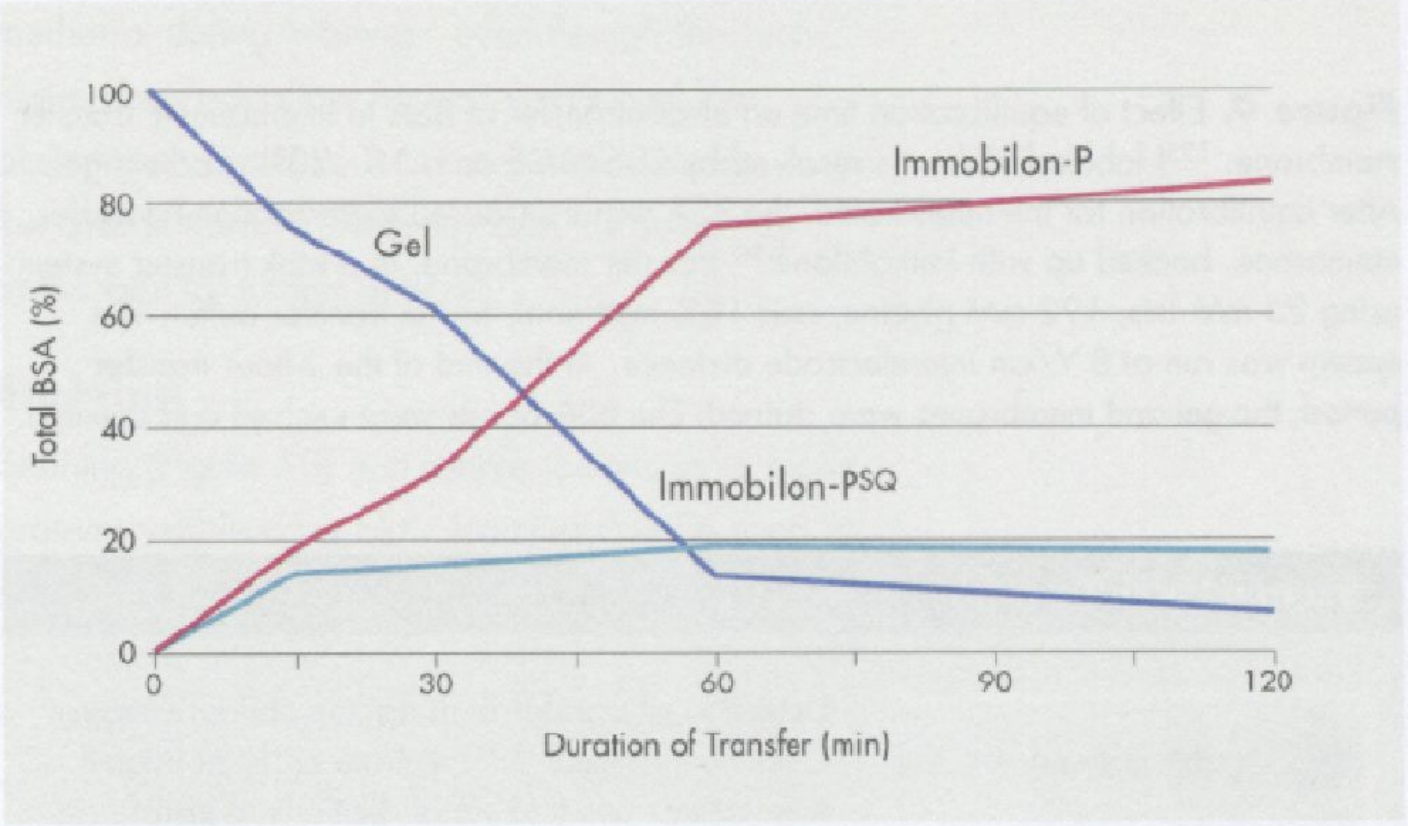
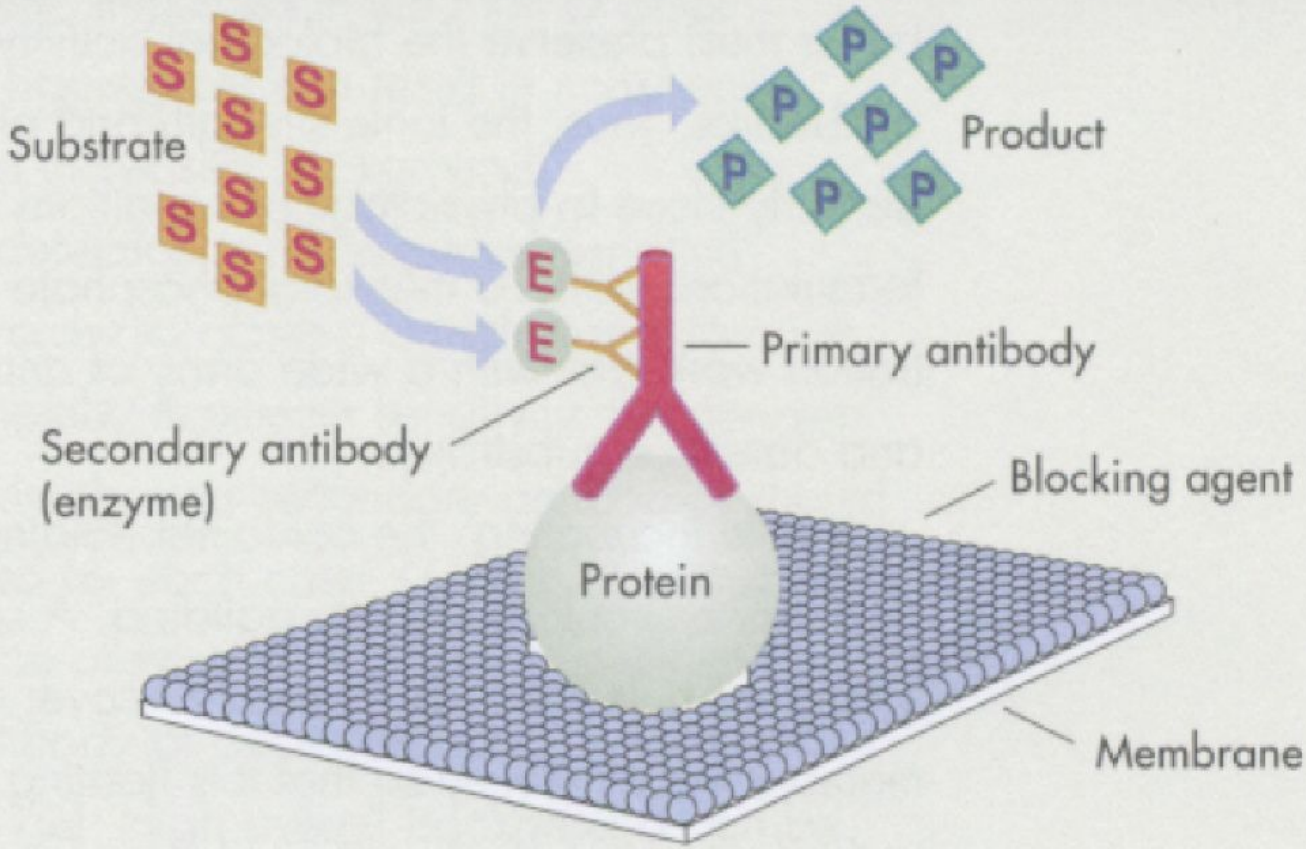


Figure 8. Electrotransfer of BSA. 25 picomoles of ^{125}I-labeled BSA were resolved by SDS-PAGE on a 10–20% gradient gel. After equilibration for 5 minutes, the BSA was transferred to Immobilon-P transfer membrane, backed up with Immobilon-P^{SQ} transfer membrane, in a tank transfer system using 25 mM Tris, 192 mM glycine, and 10% methanol, as the transfer buffer. The system was run at 8 V/cm interelectrode distance. At 15, 30, 60, and 120 minutes, a gel/membrane cassette was removed and stained. The BSA bands were excised and counted.

Figure 11. Membrane-based immunodetection.



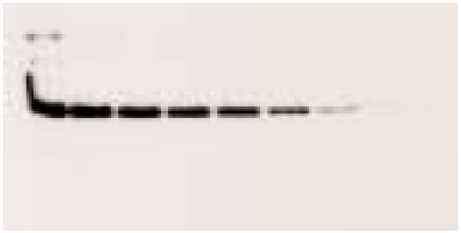


Fig 28. Detection of actin using ECL Western Blotting System. Ten-fold serial dilutions of actin (from 100 ng to 195 pg) were separated by electrophoresis and proteins were transferred to Hybond-P membrane. Primary antibody was mouse anti-actin (product code N350). Detection was performed using ECL detection reagents and Hyperfilm ECL.

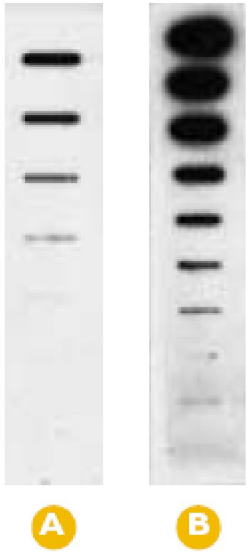


Fig 27. Target: mouse IgG slot blotted onto PVDF (Hybond-P, RPN2020F); loadings: doubling dilutions starting at 5ng; detection: 1:2500 dilution of anti-mouse Ig HRP conjugate (NA 931) using (A) ECL detection reagents (RPN2106) and (B) ECL Plus detection reagents (RPN2132); exposure: on Hyperfilm ECL (RPN2103) for 5 minutes.

Table 3. Protein blotting membranes – recommended applications

Application	Hybond-P	Hybond ECL	Hybond-C extra
Western blotting			
Detection method			
ECL	*highly recommended*	*highly recommended*	*suitable*
ECL Plus	*highly recommended*	*recommended*	*suitable*
chromogenic	*recommended*	*highly recommended*	*suitable*
colloidal gold	*recommended*	*highly recommended*	*not recommended*
ECF	*highly recommended*	*suitable*	*not recommended*
radioactive	*suitable*	*highly recommended*	*recommended*
Reprobing Westerns	highly recommended	not recommended	suitable
Glycoprotein	highly recommended	recommended	suitable
Expression screening	suitable	not recommended	highly recommended

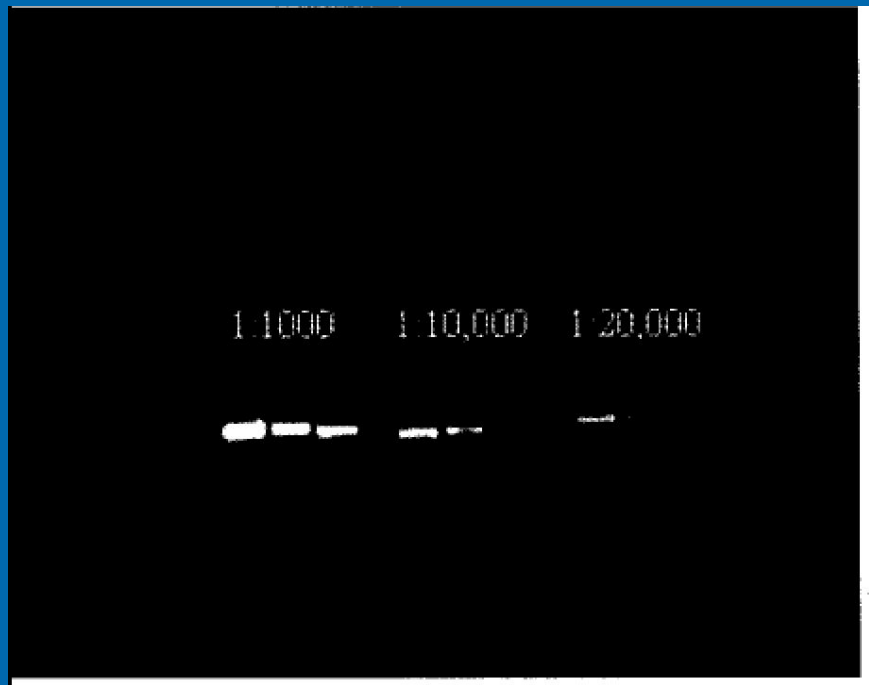


Fig. 1 *The effect of anti-chaperonin peroxidase conjugate on signal intensity. Chaperonin loaded in triplicate at 40, 20 and 10 ng/well (left to right), blotted to nitrocellulose and treated with antibody conjugate as noted. The 1:1000 gave enhanced signal and good intensity at the lower chaperonin concentration; however, background on film was relatively high (not shown). The middle dilution of 1:10,000 proved to be a good concentration for both cooled CCD camera and film detection.*

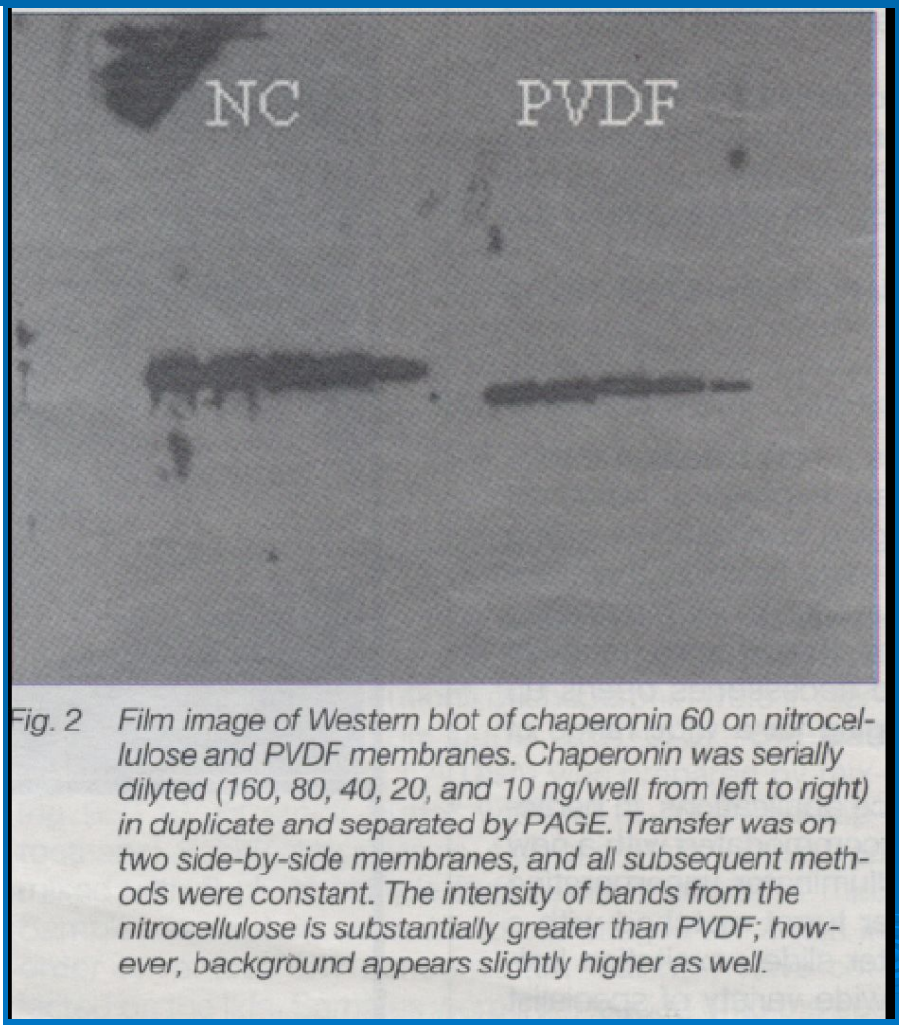


Fig. 2 *Film image of Western blot of chaperonin 60 on nitrocellulose and PVDF membranes. Chaperonin was serially dilyted (160, 80, 40, 20, and 10 ng/well from left to right) in duplicate and separated by PAGE. Transfer was on two side-by-side membranes, and all subsequent methods were constant. The intensity of bands from the nitrocellulose is substantially greater than PVDF; however, background appears slightly higher as well.*

Table 2.8. Comparison of Blocking Reagents

Blocking Reagent	Membrane Compatibility	Recommended Concentration	Notes
Gelatin	Nitrocellulose	1–3%	Requires heat to solubilize
Non-fat dry milk, BLOTTO, Blotting Grade Blocker	Nitrocellulose, PVDF, Nylon	0.5–5%	PVDF and nylon require higher concentrations of non-fat dry milk than nitrocellulose
BSA	Nitrocellulose, PVDF, Nylon	1–5%	PVDF and nylon require higher concentrations of BSA than nitrocellulose
Tween-20	Nitrocellulose	0.05%–0.3%	May strip some proteins from the blot

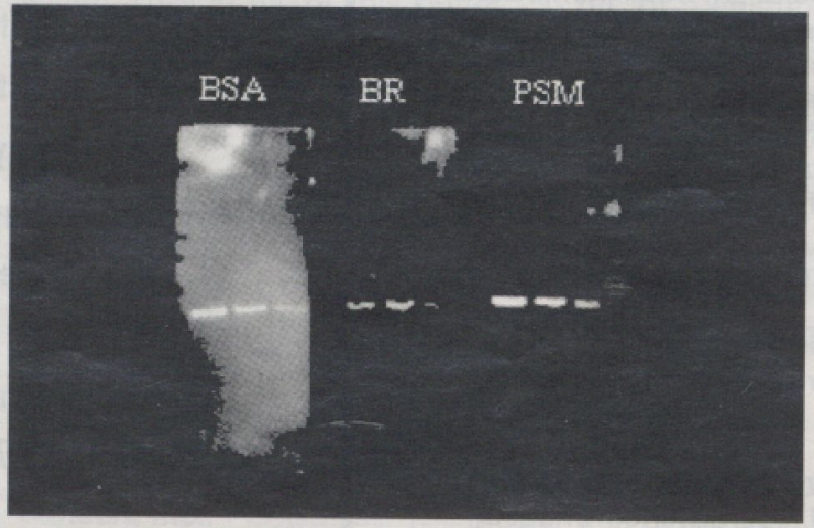


Fig. 3 Comparison of chaperonin Western blots blocked with BSA, blocking reagent and powdered skimmed milk. Chaperonin was loaded in triplicate at 40, 20 and 10 ng/well, separated and transferred to nitrocellulose. The membrane was cut in thirds and blocked with 3% BSA, 1x blocking reagent and 3% powdered skimmed milk (store brand). Powdered skimmed milk generated the strongest signals while significant background is seen with BSA blocking.

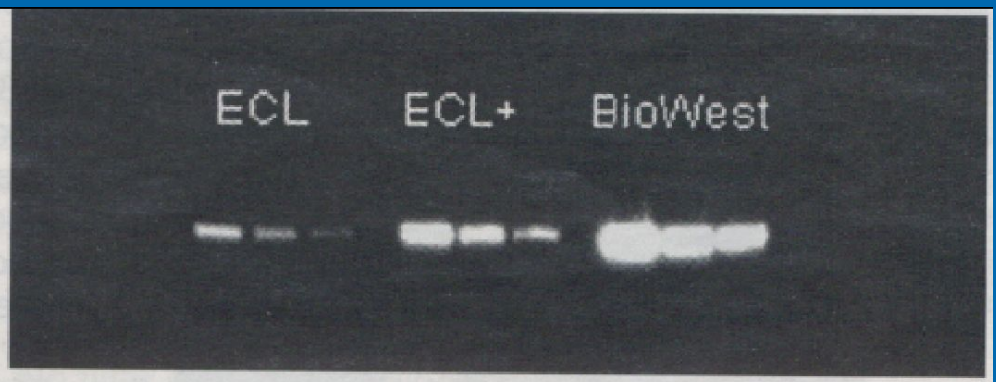


Fig. 4 Comparison of BioWest, ECL and ECL Plus chemiluminescent peroxidase substrates. Three concentrations of chaperonin 60 (40, 20 and 10 ng/well) were loaded in triplicate on a 4–20% polyacrylamide gel, separated and transferred to nitrocellulose. Prior to the addition of substrate, the membrane was cut into thirds and each of the chemiluminescent substrates was applied separately. The BioWest substrate generated a signal 3.5 times greater than ECL Plus and 14 times greater than ECL. This membrane was subsequently imaged for 150 min to assess the duration of the signal.

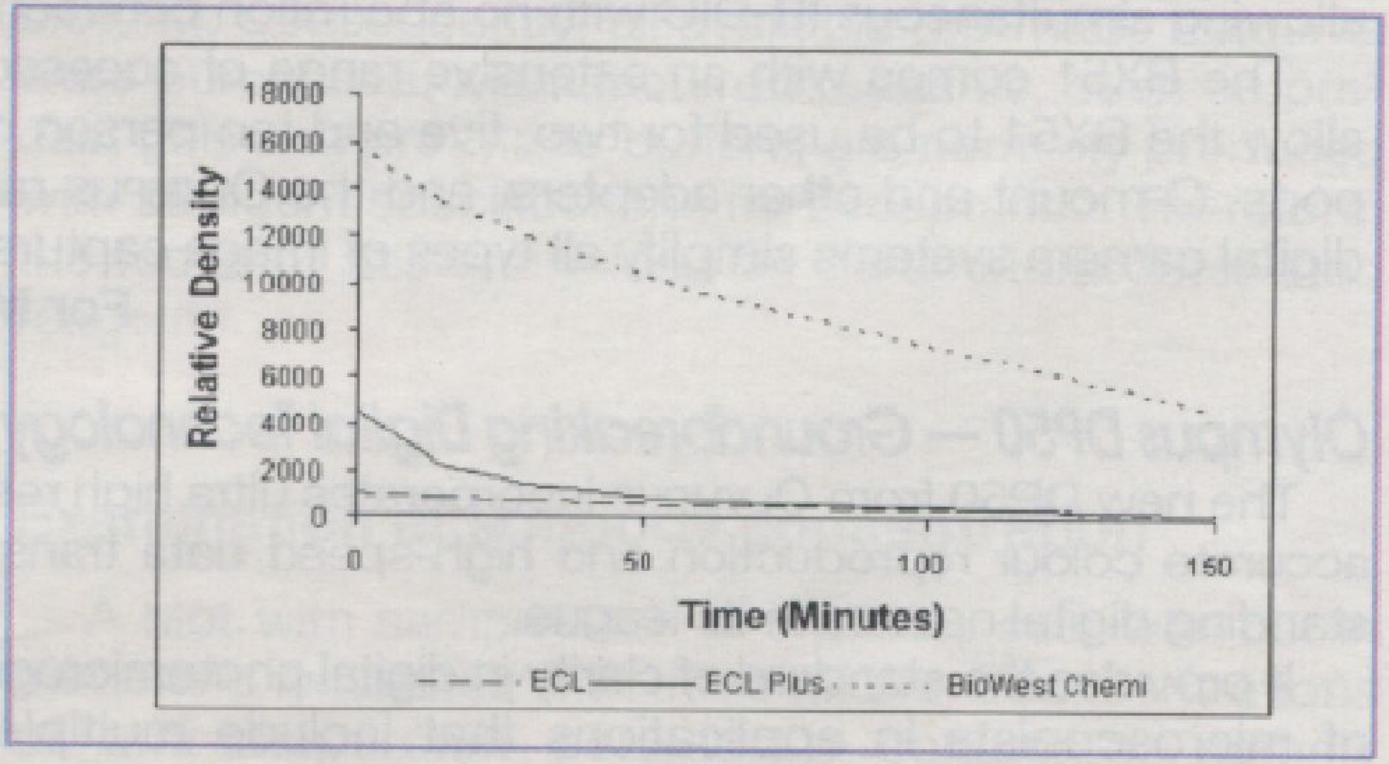
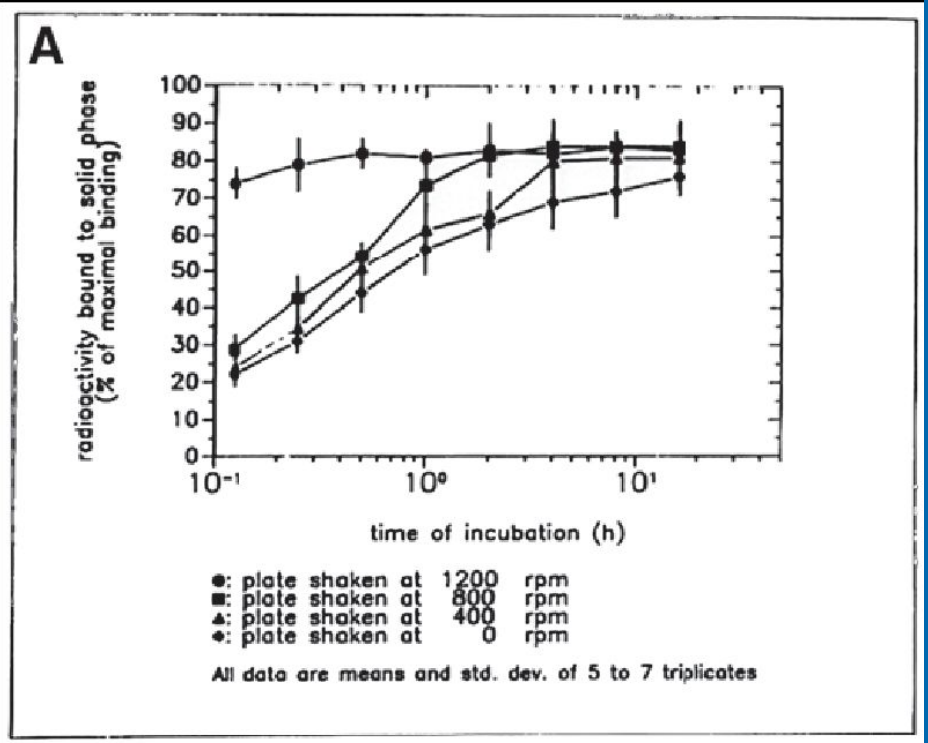
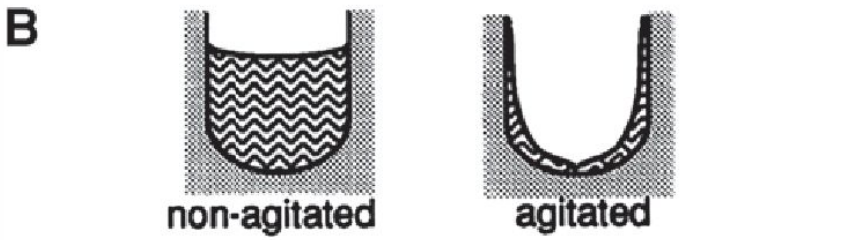


Fig. 5 Time course analysis of band intensities for ECL, ECL Plus and BioWest substrates. Parallel blots of chaperonin 60 were imaged at 0, 15, 30, 60 and 150 min and then measured for relative density. Initial intensity of the BioWest substrate was 3.4 times greater than ECL Plus and 14 times greater than ECL. In addition, the signal duration of BioWest greatly exceeded the other substrates.

Table 2.10. Comparison of Detection Reagent Systems

	Horseradish Peroxidase	Alkaline Phosphatase	Gold
Sensitivity	500 pg	100 pg (Immun-Blot) 10 pg (Immun-*Star*) 5 pg (Amplified AP)	100 pg 10 pg (Enhanced)
Substrates	4CN – purple DAB – brown	BCIP/NBT – purple CDP-*Star* – emits light	none
Comparative cost	least expensive	more expensive most expensive (Immun-*Star*)	more expensive
Stability of stored blots	poor	good	good
Restrictions	azide, endogenous peroxidase activities	endogenous phosphatase activities	none





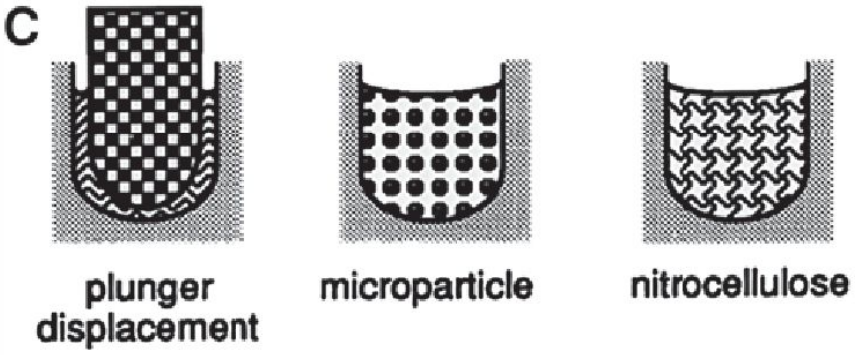


Fig. 1. The diffusion dependence of solid-phase immunoassay and methods used to reduce its influence. **(A)** The effect of vortexing (shaking) microtiters wells on establishment of equilibrium (from ref. ***13***). **(B)** Illustration of the physical effect of vortexing microtiter wells (rotary agitation) on the distribution of the fluid phase relative to the solid phase. The fluid phase is depicted by wavy lines. **(C)** Alternative methods of confining the reaction volume to within close proximity to the solid phase bearing the immobilized reactant.

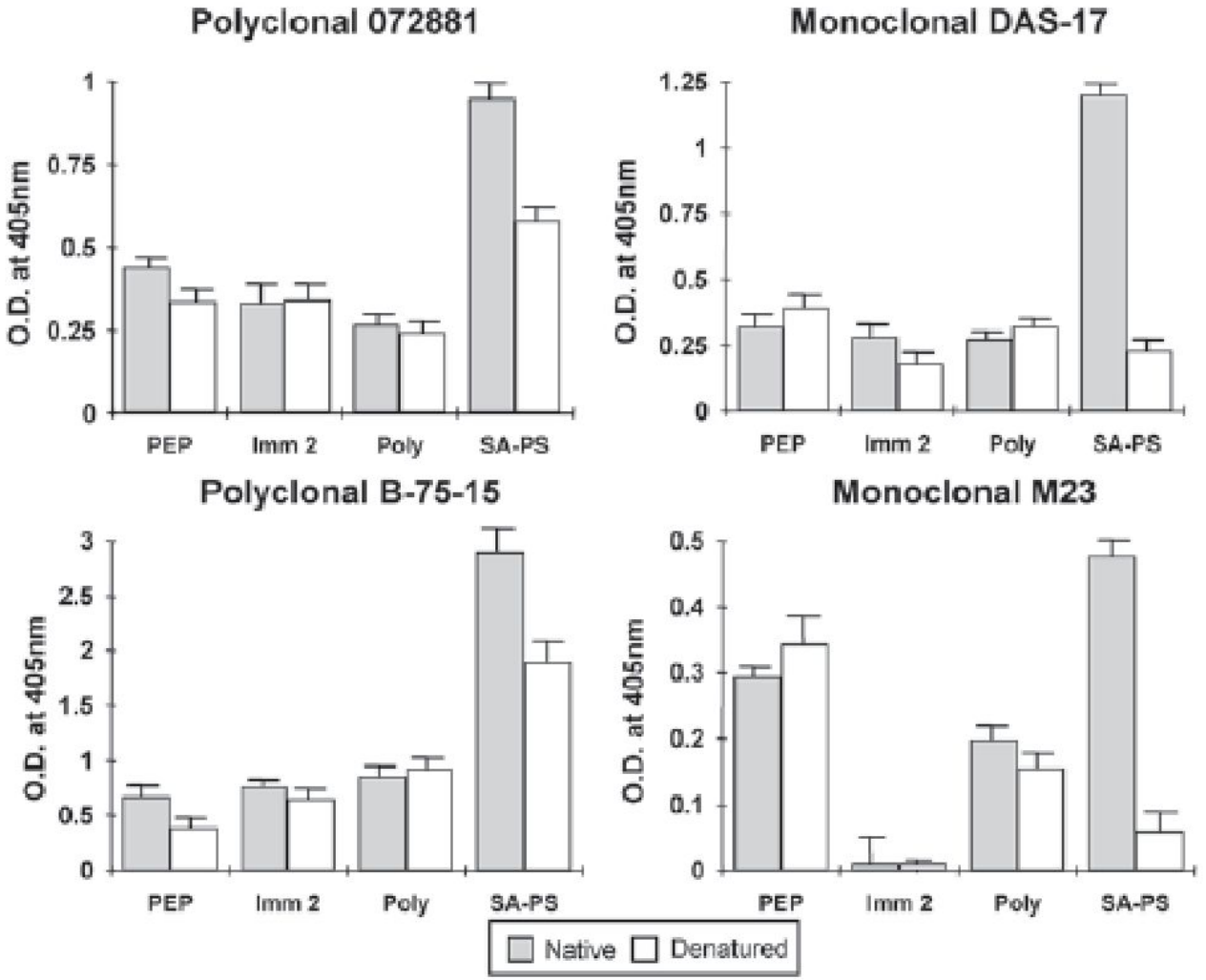


Fig. 5. The specificity of two pAbs and two mAbs for bovine IgG1 immobilized on various surfaces by adsorption or via a streptavidin–biotin linkage (SA-PS) with or without prior denaturation in 6 *M* guanidine-HCl. Histograms were constructed using OD_{405} values calculated from triplicate log-log titration plots. From Butler et al. *(16)*.

Table 1
Characteristics of the More Commonly Used Solid Phases

Solid phase	Bonding force	Relative surface area	Performance characteristics
Plastic labware			
Polystyrene	Hydrophobic	Modest	
Polystyrene-irradiated	Hydrophobic, hydrophilic, and covalent	Modest	Low background, reproducible
Surface-functionalized	Hydrophobic and polystyrene	Modest	Readily adapted to automation
Bead			
Polystyrene (PS) beads	Hydrophobic	Moderate	Yield assays with broad dynamic ranges
Derivatized PS beads	Covalent, hydrophobic, and hydrophilic	High	Less convenient to use than labware; more difficult to automate
Beaded agarose and derivatives	Hydrophilic and covalent	High	Minimal protein denaturation; high background and difficult to automate
Microparticles	Hydrophobic and covalent	Very high	"Solution-phase performance" owing to colloidal nature; wide dynamic range; magnetized variants make them automatable
Membranes			
Nitrocellulose (NC)	Hydrophobic and hydrophilic	Very high	Desorption and background problems hinder their use in quantitative assays
Nylon	Hydrophobic	Very high	Serious background problems reduce signal:noise ratio
Charge-modified nylon	Hydrophilic, covalent, and hydrophobic	Very high	Problems similar to nylon but perhaps less denaturation and less desorption
Functionalized nitrocellulose	Hydrophobic, covalent, and hydrophilic	Very high	Similar to NC but less desorption
PVDF (Immobilon P)	Hydrophobic	Very high	Very high and stable binding; may be best for immunoblotting

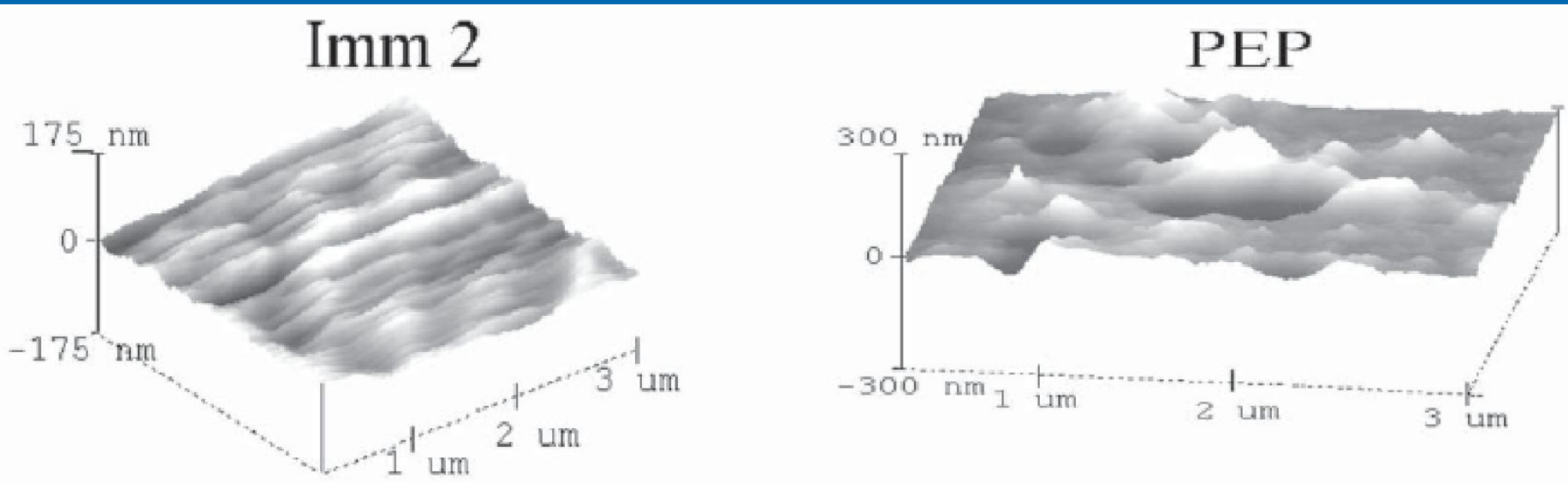


Fig. 8. The surface topography of PEP (right) and Imm 2 (left). Images are for 3 μm squares obtained by atomic force microscopy in the Tapping Mode. Note that the maximum surface height depicted (Z axis) is 175 nm for Imm 2 but 300 nm for PEP. From Butler et al. *(4)*.

Table 2
Adsorption-Induced Conformational Change

Protein	Phenomenon	Authors
Albumin	Conformational change after adsorption on glass	Bull, 1956 *(32)*
IgG	Concentration-dependent allosteric conformers after adsorption on polystyrene	Oreskes and Singer, 1961 *(38)*
IgG	Molecule unfolding and changes in antigenicity when adsorbed on polystyrene	Kochwa et al., 1967 *(39)*
IgG	Thermodynamic evidence for conformational change	Nyilas et al., 1974 *(40)*
Monoclonal Ab	Altered specificity after adsorption	Kennel, 1982 *(41)*
Tryptophan synthase	Altered enzymic and antigenic activity after adsorption	Friquet et al., 1984 *(42)*
Lactic dehydrogenase	Conformational alteration after dehydrogenase adsorption on polystyrene	Holland and Katchalski-Katzir, 1986 *(43)*
Monoclonal Ab	Loss of activity after adsorption on polystyrene	Suter and Butler, 1986 *(44)*
IgG, IgA	Loss of antigenicity after adsorption to polystyrene	Dierks et al., 1986 *(12)*
Ferritin	Cluster formation on silica wafers	Nygren, 1988 *(19)*
Antifluorescein	Functional monoclonal antifluorescein adsorbed on polystyrene is clustered	Butler et al., 1992 *(7)*
Antifluorescein	Adsorbed MAbs lose 90% of their activity on polystyrene	Butler et al., 1993 *(15)*
Antitheophylline	MAb adsorbed on polystyrene loses 90% of its activity	Plant et al., 1991 *(37)*
Antiferritin	Adsorbed functional antiferritin is clustered on the surface of polystyrene	Davis et al., 1994 *(8)*
Bovine IgG1	Antigenicity of IgG1 or Gu-HCl denatured IgG1 is similar and much less than IgG1 immobilized through a streptavidin linkage	Butler et al., 1977 *(16)*
Bovine IgG1	Superficial layer of IgG1 adsorbed in multilayers is most antigenic	Butler et al., 1977 *(16)*
Myoglobin	Adsorption of myoglobin effects reactivity of conformation-specific monoclonal antibody	Darst et al., 1988 *(45)*

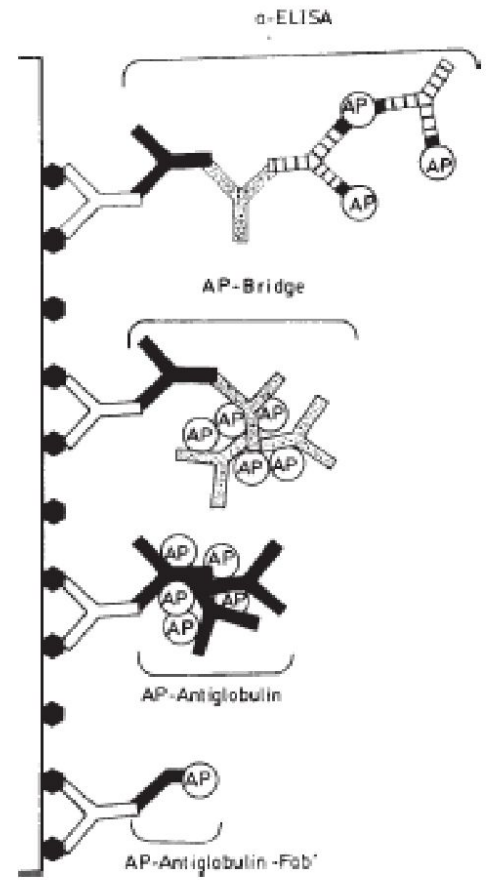


Fig. 11. Comparison of various detection systems used in ELISAs. AP = alkaline phosphatase, hexagon = solid-phase antigen, open antibody = primary antibody; solid antibody = isotype-specific antiglobulin; dotted antibody = tertiary antiglobulin in one case conjugated with AP; hatched antibody = anti-AP. Conjugates depicted in middle two examples are one-step glutaraldehyde conjugates. a-ELISA = a highly amplified ELISA based on use of a soluble enzyme-antibody immune complex. From Koertge and Butler *(17)*.

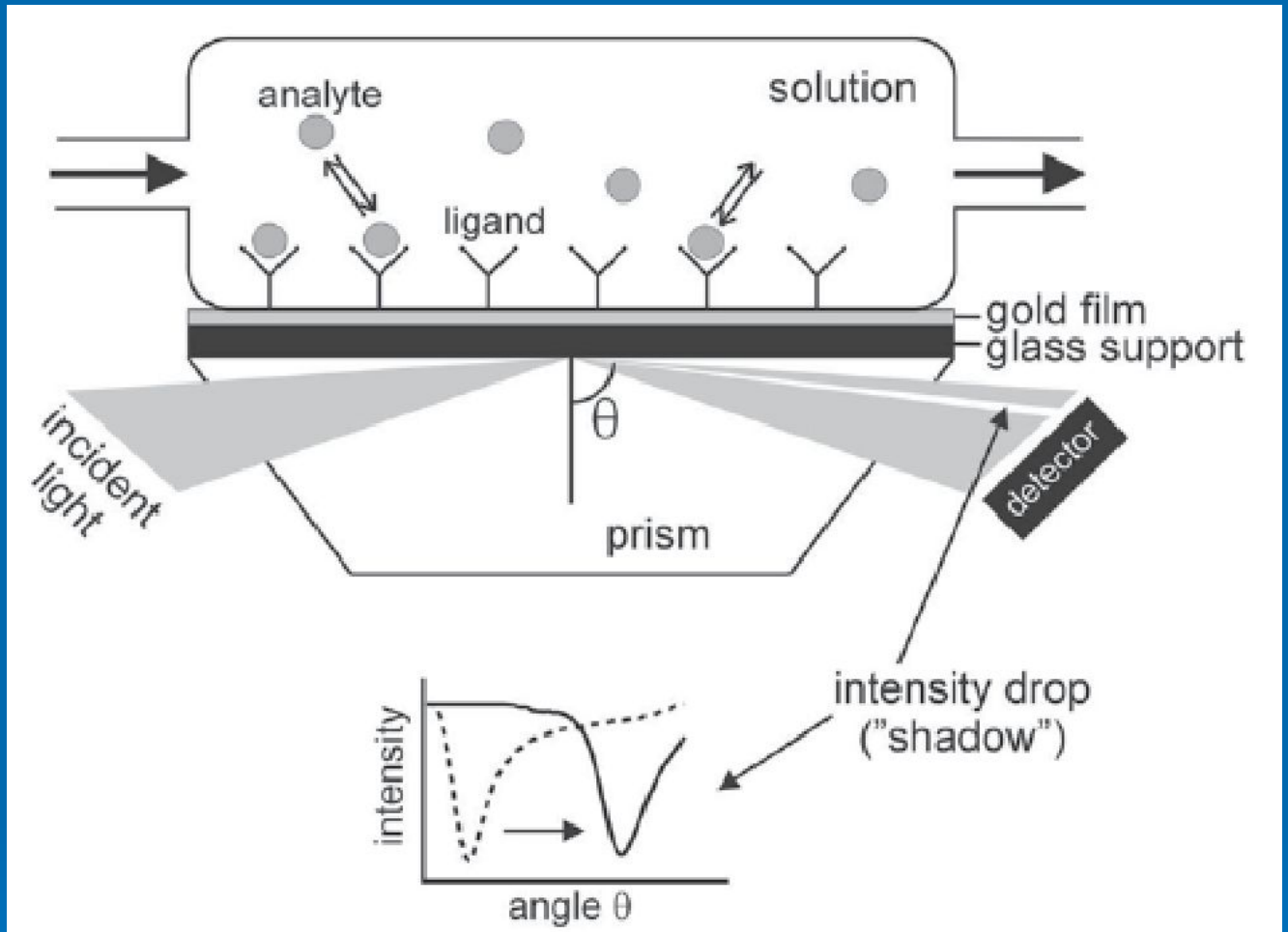


Fig. 1. Schematic view of a surface plasmon resonance (SPR) detector as utilized in a Biacore system. SPR arises when light is totally internally reflected from a metal-coated interface between two media of different refractive index (a glass prism and solution). If the incident light is focused on the surface in a wedge, the drop in intensity at the resonance angle appears as a "shadow" in the reflected light wedge, which is detected by a position-sensitive diode array detector. When an interaction between an immobilized ligand (e.g., an antibody, Y) and an analyte in solution (filled circles) occurs, the "shadow" is shifted on the detector, i.e., the angle θ changes.

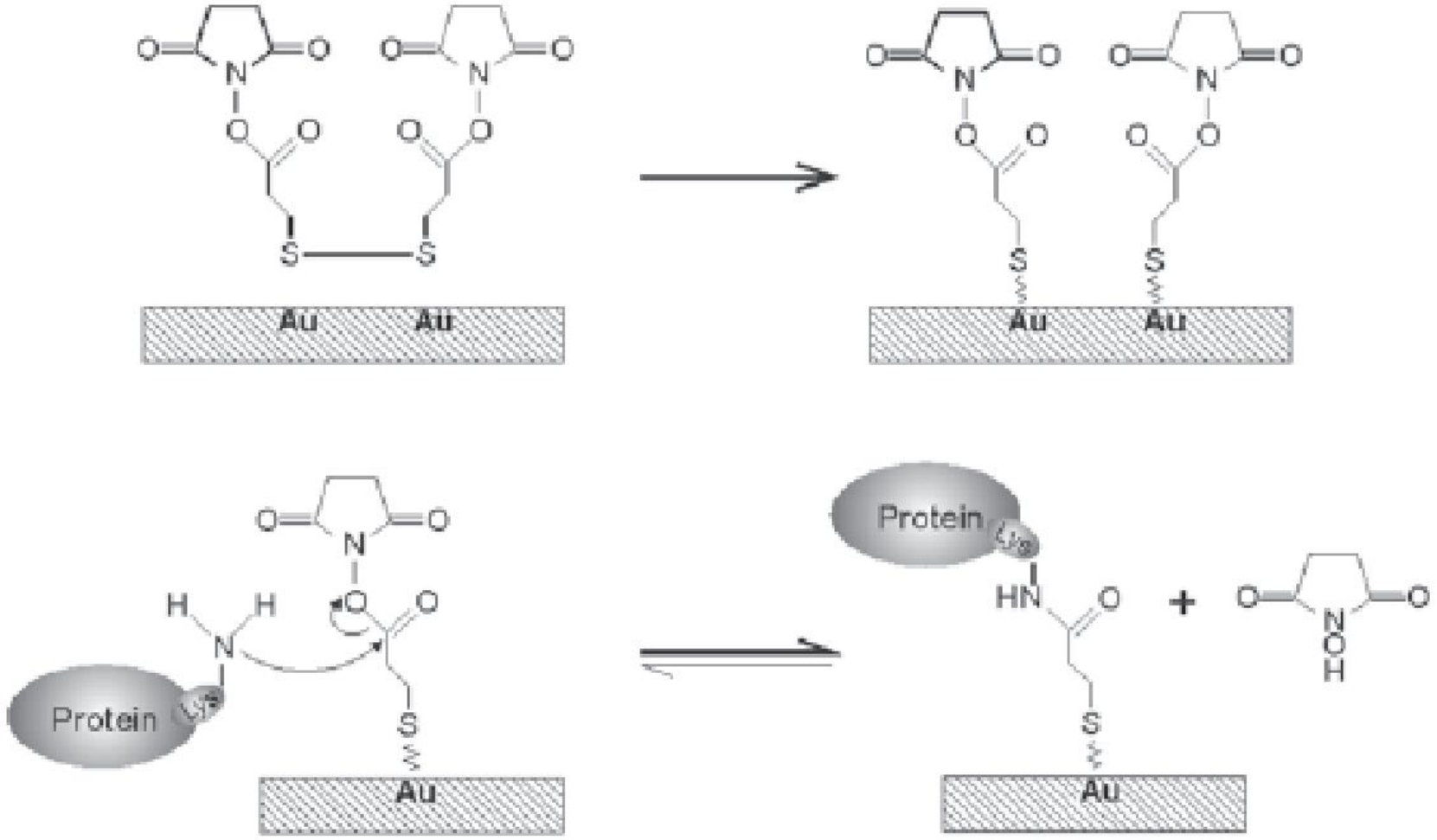


Fig. 2. Immobilization of proteins to a gold surface using 3,3'-dithiodipropionic acid-di(N-succinimidylester) (DSP).

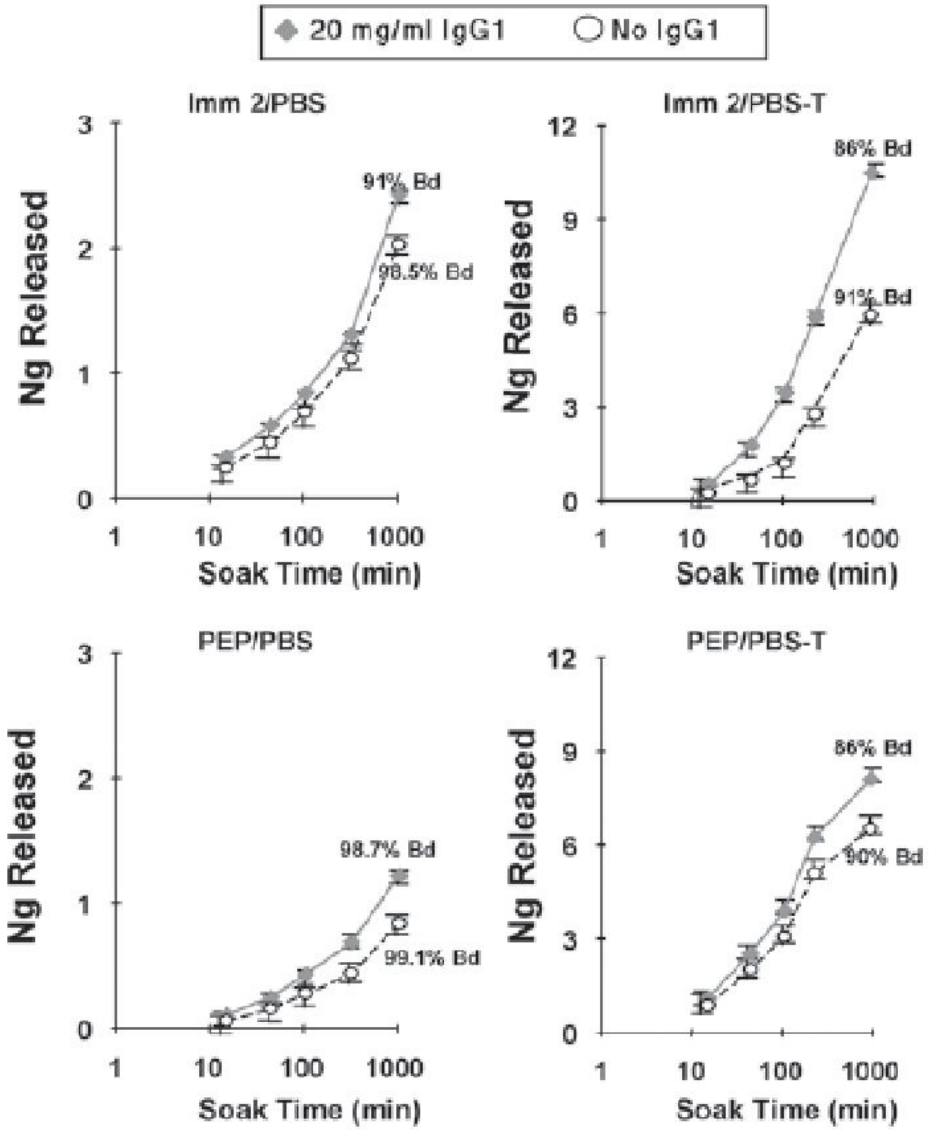


Fig. 2. Effect of protein and detergent on desorption of bovine IgG1. Data are expressed as the nanograms of IgG1 released over time in the presence or absence of excess ligand (20 µg/mL IgG1) in PBS and in the presence of a non-ionic detergent Tween-20 (PBS-T). The percentage value given is the amount remaining bound at the conclusion of the study (16 h). From Butler et al. *(4)*.

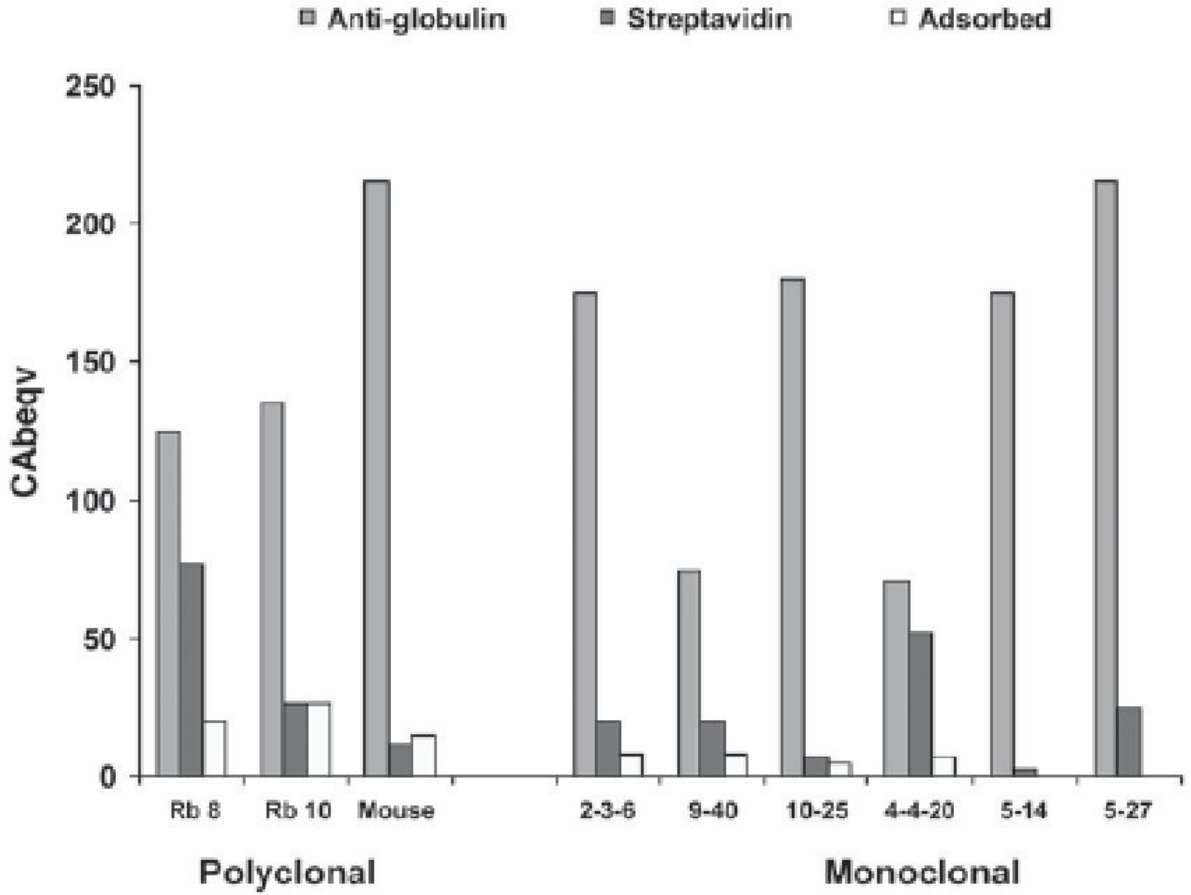


Fig. 4. The proportion of functional capture antibody equivalent (Cabeqv) after immobilization of capture antibodies (CAbs) specific for fluorescein, by different methods. The three CAbs on the left are polyclonals whereas the six CAbs on the right-hand side are monoclonals. CAbeqv is the equivalent of an antibody with two functional sites. Because the standard for this assay was an adsorbed Cab with only one functional site, a value of "200 percent functional" means that 100% of the antibodies are functional with two binding sites. The immobilization procedures included use of a primary antiglobulin, a streptavidin–biotin linkage *(44)*, and direct adsorption. Equal amount of immobilized CAbs were established using iodinated CAbs. From Butler et al. *(15)*.

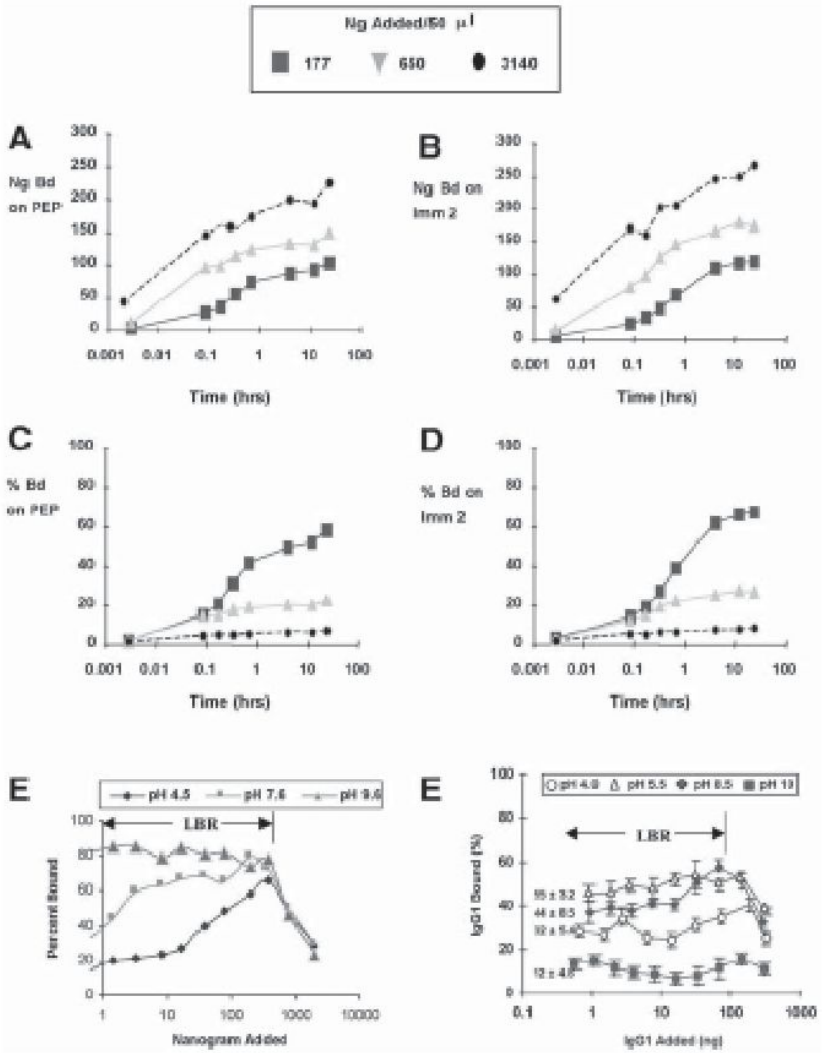


Fig. 3. Characteristics of adsorption of IgG on hydrophobic surfaces. Kinetics of adsorption of human IgG on polydimethylsiloxane elaster (PEP) (A,C) and Immulon 2 (Imm 2) (B,D). Adsorption was studied at three different concentrations, the lowest (177 ng/well) approximates the upper limits of the LBR. Data are presented both in terms of nanograms bound (A, B) and proportion adsorbed (C, D). From Butler et al. *(4)* (E) The influence of pH on the adsorption of rabbit IgG on Imm 2 expressed as percentage bound. From Butler et al. *(15)*. Only at alkaline pH is a region of constant percent adsorption, i.e., an LBR, observed. All IgGs studied to date behave similarly. (F) The effect of pH on the adsorption of bovine IgG1 (pI = 5.5) on PEP, expressed as percentage adsorption. The mean percentage bound in the LBR ± SD is given to the left of plots. Note that adsorption is best at the pI of IgG1. From Butler et al. *(4)*.

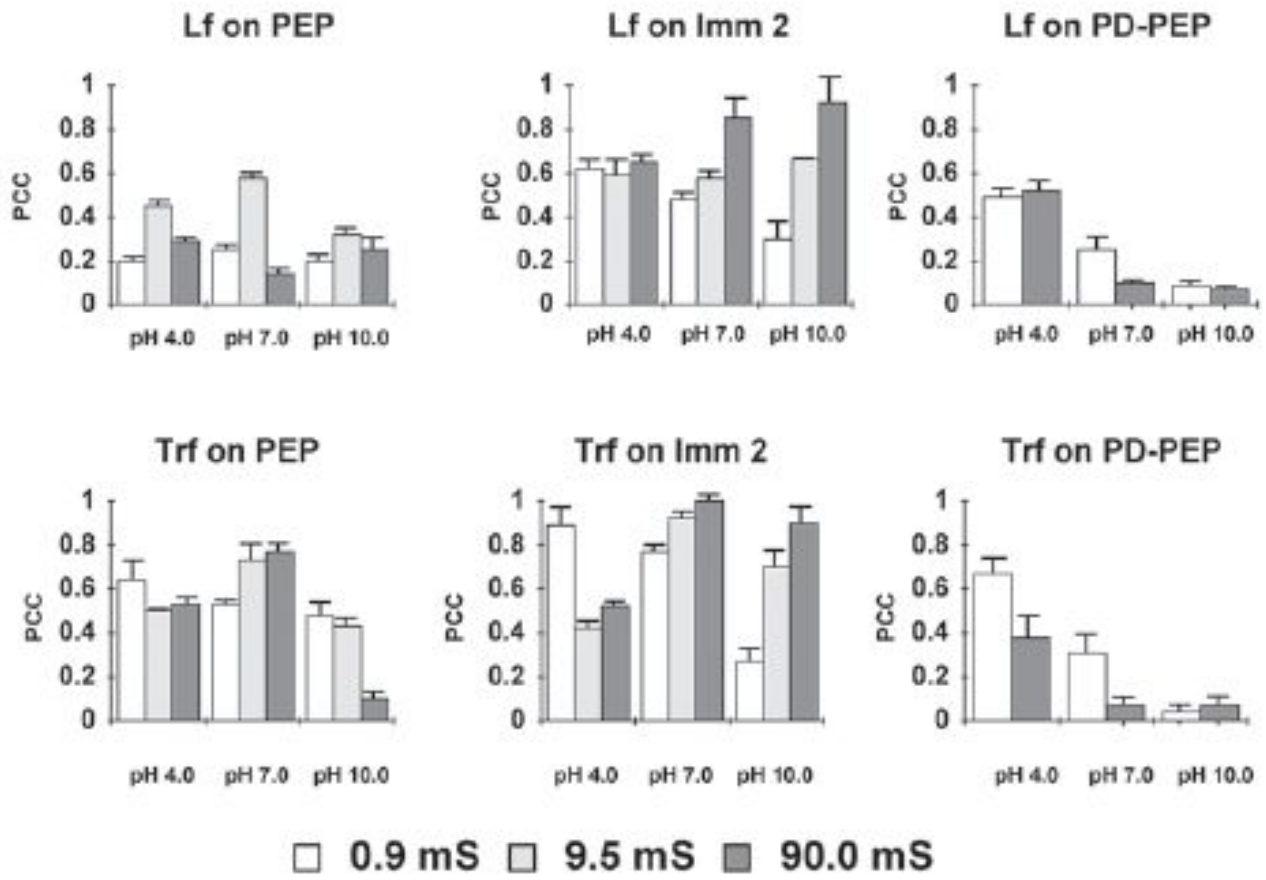


Fig. 7. Summary of the protein capture capacity (PCC) of Imm 2 and PEP for lactoferrin (Lf) (p*I* = 7.9) and transferrin (Trf) (p*I* = 5.3) at various pHs and ionic strengths indicated in milli Siemens (mS). Error bars depict standard deviations. PD-PEP = plasma discharge treated PEP. From Butler et al. *(4)*.

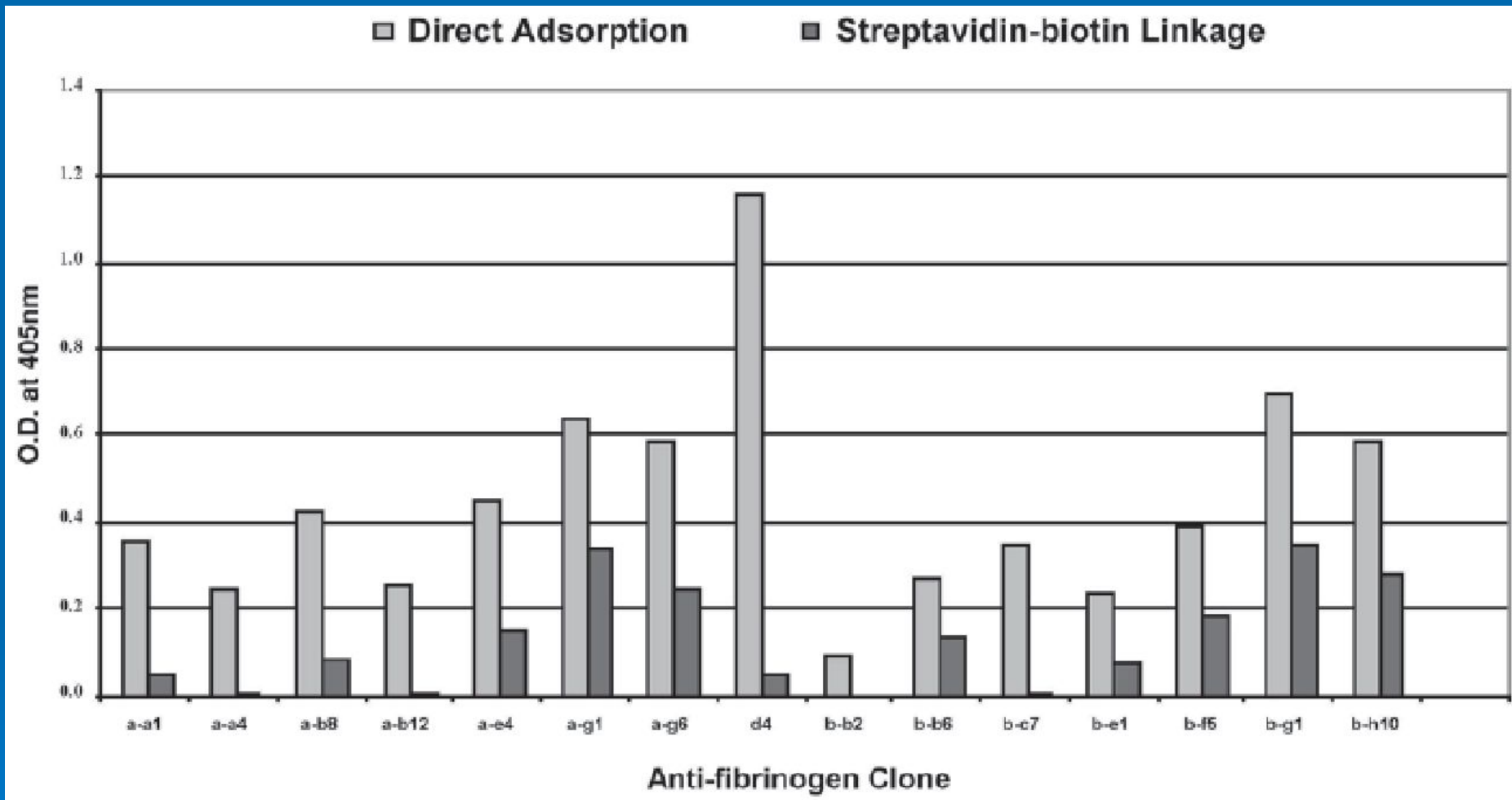


Fig. 9. The specificity of monophage for fibrinogen adsorbed on polystyrene versus for fibrinogen immobilized using a streptavidin–biotin linkage. The phage were cloned from an antibody phage display library on adsorbed fibrinogen (Sun and Butler, unpublished).

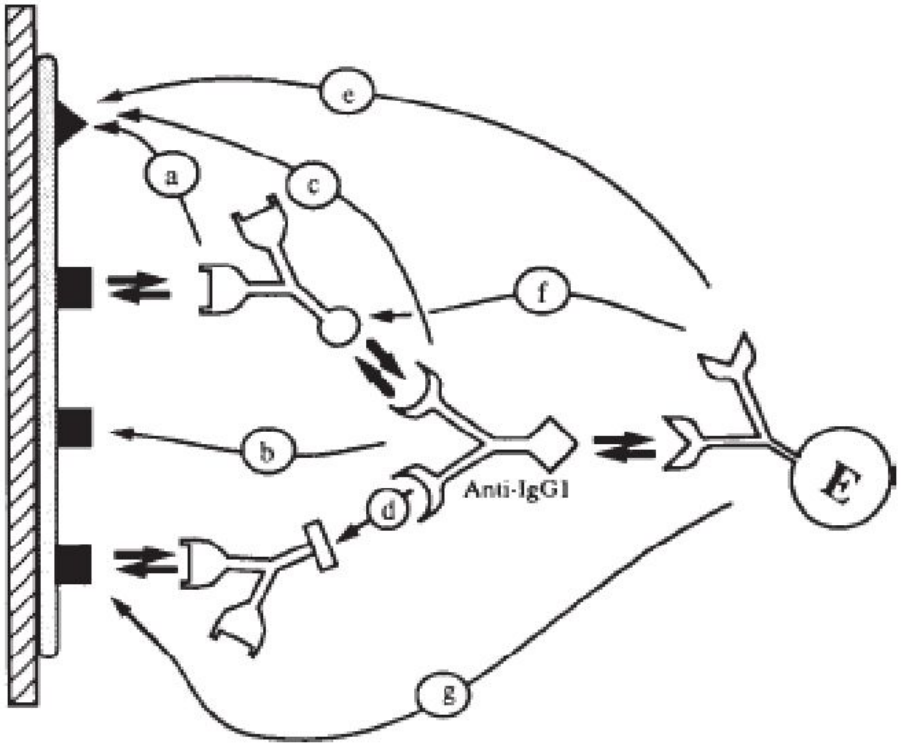


Fig. 10. Short-circuiting produces nonspecific binding (NSB) in ELISA. Antibodies are depicted as bivalent and as Y-shaped molecules. The shape of their Fabs and Fcs are designated to depict their paratope and epitope specificities. The solid, unidirectional arrows with arabic letters depict the various short circuits possible. Double, reciprocal arrows depict the desired or normal reaction pathway. The various short circuits illustrated are as follows: (a) primary antibody (analyte) cross-reacts with a second undesirable solid-phase epitope, (b) secondary antibody (part of detection system) recognizes the primary antigen on the solid phase, (c) secondary antibody recognizes the second undesirable epitope on the solid phase, (d) secondary antibody cross reacts with a primary antibody of a different isotype than that for which the assay was designed to measure, (e) the tertiary antibody (or reactant that carries the reported enzyme) recognizes an undesirable solid phase epitope; (f) the tertiary reactant recognizes and binds the primary antibody (analyte) and thus bypasses the secondary antibody, (g) the tertiary reactant binds to the solid phase itself. The latter situation could theoretically occur with any of the reactants. E = enzyme-conjugated antibody.

Table 1
Comparison of Host Expression Systems for the Production of Recombinant Proteins for Use in Diagnostic Assays

Host organism	Ease of use	Defined system	PTM	Background interference	Solubility issues	High[a] expression	Cost
E. coli	Yes	Yes	No	Frequent	Yes	No	Low
Yeast	No	No	Yes[b]	Rare	No	No	Low
Insect	Yes	Yes	Yes[b]	Rare	No	Yes	High
Mammalian	No	Yes	Yes	Rare	No	Variable	High
None (cell-based system)	Yes	No	No	Frequent	No	Yes	High

[a]High level expression can never be guaranteed but frequently is determined by the structure, modification, and synthetic pathway of the protein in each particular system.

[b]Although insect cells carry out glycosylation, there are subtle differences from mammalian cells that can affect the antigenicity of some proteins.

Abbreviation: PTM, post-translational modification.

Table 1
Overview of the Available Sensor Chips for the Biacore Systems as Distributed by Biacore[a]

Type	Surface characteristics	General applications
Sensor Chip CM5	CM–dextran	Standard surface, suitable for most applications
Sensor Chip SA	CM–dextran + streptavidin	Capture of biotinylated ligands
Sensor Chip NTA	CM–dextran + nitrilotriacetic acid (NTA)	Capture of poly His-tagged proteins via chelated nickel ions
Sensor Chip HPA	Thioalkane-covered gold surface	Creation of lipid monolayers from liposomes
Pioneer Chip B1	CM–dextran with lower degree of carboxymethylation	Lower immobilization capacity, reduces nonspecific binding
Pioneer Chip C1	Carboxylated surface without dextran matrix	If dextran matrix interferes with the interaction being studied; for binding particles (e.g., cells) too large to enter the dextran matrix
Pioneer Chip F1	CM–dextran with shorter dextran matrix	Lower immobilization capacity
Pioneer Chip J1	Plain gold surface	Build your own sensor surface inside the instrument!
Pioneer Chip L1	CM–dextran + lipophilic groups	Direct capture of liposomes
SIA Kit Au	Plain gold surface	Build your own sensor surface outside the instrument!

[a]Note that specific surfaces could be produced on most of the available sensor chips. For further details, *see* Note 1.

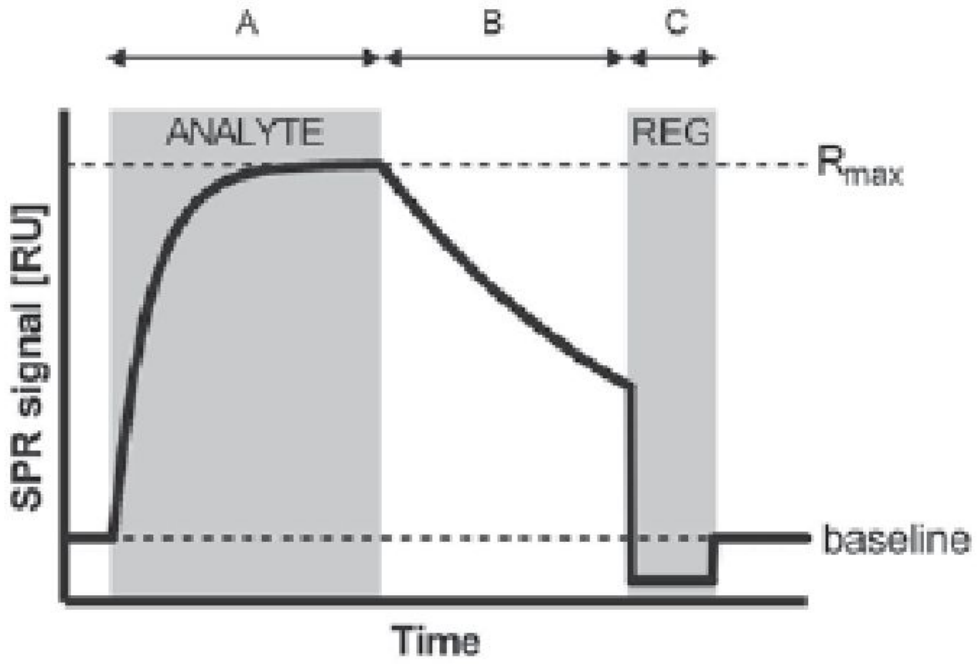


Fig. 2. Basic parts of a sensorgram. A typical sensorgram consists of three phases. (A) In the *association phase*, the analyte is injected over the immobilized ligand on the surface (ANALYTE, gray bar). With increasing interaction of analyte and ligand, an increasing response is detected [displayed in response units (RU)]. The maximal binding is specified as R_{max}. (B) The injection of the analyte is stopped by switching the system back to buffer (*dissociation phase*). In many cases the dissociation of the analyte is not complete after a reasonably long time. (C) Therefore an injection with an appropriate regeneration solution (REG, gray bar) is performed. After this *regeneration phase*, the baseline response level should be reached. If this is not the case, the regeneration can be repeated with either the same or another solution, keeping in mind the maintenance of the biological activity of the surfaces.