instead of a peroxidase conjugate. We then used double labelling⁵ to show that swine anti-HPL (peroxidase indirect conjugate) did not block all the sites recognised by the rabbit anti-HPL (visualised by the alkaline phosphatase conjugate).

Similar double labelling experiments with anti-immunoglobulins were difficult to interpret because both antisera stained the stroma. However, our impression was that blocking was incomplete. Even if blocking had been successful and both antisera recognised the same determinant, the specificity of one of the antisera would still require to be shown by loss of activity after absorption with IgG.

PAP method

Both the indirect and PAP methods are sensitive, reliable, and convenient. We use the indirect method because it produces excellent results, and satisfactory commercial peroxidase conjugates are available. Using the PAP technique we have failed to show a consistent increase in sensitivity; an extra reagent is involved so the number of variables is increased; it takes 45-60 minutes longer and is more expensive.

There is no theoretical reason why the PAP method should increase specificity. All second and subsequent antibodies are merely disclosing solutions, attaching only to the immunoglobulin of the first antibody, and should have no antihuman activity. The second antibody cannot (and should not) distinguish between the specific and the contaminating antibodies which cause so-called 'background staining'. If sensitivity is increased it must be to all the first antibodies.

Previous comparisons of the PAP and indirect methods have not used the same second antibodies. A valid comparison would be a quantitative experiment comparing the PAP method with a conjugate made with the same second (unlabelled) antibody in similar antibody concentrations.

Trypsinisation

We regularly use antisera to many different antigens, including CEA, alphafetoprotein, placental and pituitary hormones, Hb_sAg, and epithelial membrane antigen, at dilutions of 1:50 to 1:2000 and are able to demonstrate them satisfactorily without enzyme pre-digestion.⁶ Improvement in staining for immunoglobulin after tryp-

sinisation has been reported by several groups⁷ (references in MacIver and Mepham letter) but its use may cause false-negative results and increased collagen staining as well as loss of sections.⁸ The question whether collagen staining is due to the presence of immunoglobulin or some other antigen remains to be resolved. Not all antisera stain collagen. It is possible that an anti 'collagen' is a common contaminating antibody.

Non-immune serum

Dr MacIver and Mr Mepham still include incubation with non-immune serum in their schedule to avoid 'background staining'. We have not found that leaving out this reagent impairs our results, and we use several non-affinity purified antisera which do not stain stroma or tissues in which the antigen is absent. Although very low levels of non-specific protein binding can be detected by RIA, the immunoperoxidase method is too insensitive to show this binding. We do not recognise the concept of 'background' or 'non-specific' staining and think that such staining is specific and is generally the result of the presence of contaminating antibodies to irrelevant antigens. Only absorption controls can elucidate this problem.

CONCLUSION

Whether the indirect or PAP method is preferred or whether trypsinisation is included or omitted remain matters for investigation and debate. In spite of the problems of antigen purity and availability, absorption is the most reliable control at the present time. Although we prefer affinity purified antisera, we use several that are not so treated but produce good clean results, and all the techniques we use are within the capacity of a routine department. In immunocytochemistry, as in histopathology in general, there is a need for quality control; the validation of positive results is as important as their achievement.

E HEYDERMAN

A BULMAN

AR GIBBONS

Department of Morbid Anatomy,
St Thomas's Hospital Medical School,
London SE1 7EH

References

- Walker RA. Demonstration of carcinoembryonic antigen in human breast carcinomas by the immunoperoxidase technique. *J Clin Pathol* 1980;33:356-60.
- Goldman M. *Fluorescent antibody methods*. New York and London: Academic Press, 1968;155-60.
- Ormerod MG. Antigenic determinants of carcinoembryonic antigen. *Scand J Immunol* 1978;8:suppl 8, 433-8.
- Kohler G, Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495-7.
- Mason DY, Sammons RE. Alkaline phosphatase and peroxidase for double immunoenzymatic labelling of cellular constituents. *J Clin Pathol* 1978;31:454-60.
- Heyderman E. The immunoperoxidase method in histopathology: applications, methods and controls. *J Clin Pathol* 1979;32:971-8.
- Huang S-N, Minassian H, More JD. Application of immunofluorescent staining on paraffin sections improved by trypsin digestion. *Lab Invest* 1976;35:383-90.
- Darmady EM, MacIver AG. *Renal Pathology*. London-Boston: Butterworths, 1980;516.

Discrepancies in the weight of plastic vials

When using single-pan analytical balances, discrepancies of several milligrammes in the weight of plastic objects have been observed. Occasionally a gradual drift in the apparent weight was found to occur.

We found that weighing discrepancies were worst with plastic vials or plastic material when they were handled with latex or vinyl disposable gloves. Even minimal handling of the vial, such as that involved in unscrewing and replacing the cap while wearing gloves, was found to lead to errors of the order of 1-5 mg. This is illustrated in Table 1.

Of the various attempts to overcome this problem, including spraying the inside of the balance with antistatic fluid, using earthed forceps to handle the vials, placing earthed conducting foil over the base of the balance immediately below the balance pan, and using an antistatic gun to remove the charge on the vials, only the last was found to give a measure of success (Table 1). The discrepancies in weight between columns I and III, particularly in the case of the more sensitive five-place balance—the Sartorius

Table 1

Plastic vial No.	I (g)	II (g)	Δ mg (II-I)	III (g)	Δ mg (III-I)
<i>Mettler N.31 balance</i>					
1	7.2422	7.2460	+ 3.8	7.2422	0.0
2	7.2615	7.2648	+ 3.3	7.2615	0.0
3	7.2115	7.2149	+ 3.5	7.2115	0.0
4	7.2851	7.2878	+ 2.7	7.2850	- 0.1
5	7.2387	7.2413	+ 2.6	7.2386	- 0.1
6	7.2700	7.2714	+ 1.4	7.2700	0.0
<i>Sartorius model 2414 balance</i>					
1	7.24242	7.24482	+ 2.40	7.24237	- 0.05
2	7.26211	7.26440	+ 2.29	7.26186	- 0.25
3	7.21223	7.21504	+ 2.81	7.21226	+ 0.03
4	7.28541	7.28957	+ 4.13	7.28512	- 0.29
5	7.23936	7.24153	+ 2.17	7.23893	- 0.43
6	7.29654	7.29873	+ 2.19	7.29654	0.00

Plastic vials (static free) were weighed without the use of gloves (column I). For each weighing the balances were allowed to come to an observed steady state before the weighing was noted. The caps of the vials were unscrewed and replaced with a gloved hand and the new weight was noted (column II). The effect of the static charge induced is shown in the third column (Δ mg II-I). With the vial sitting on the balance pan, the charge was removed using an antistatic gun held close to the vial, and the vials were reweighed, with the results shown in column III.

The differences between the original weights and those after the antistatic treatment are given (mg) in the last column, Δ mg (III-I).

Table 2

Glass vial No.	I (g)	II (g)	Δ mg (II-I)	III (g)	Δ mg (III-I)
<i>Mettler N.31 balance</i>					
1	15.7212	15.7212	0.0	15.7212	0.0
2	15.9221	15.9221	0.0	15.9221	0.0
3	15.6668	15.6664	- 0.4	15.6666	- 0.2
4	15.6740	15.6737	- 0.3	15.6738	- 0.2
5	17.7422	15.7422	0.0	15.7422	0.0
6	15.7883	15.7883	0.0	15.7883	0.0
<i>Sartorius model 2414 balance</i>					
1	15.72195	15.72195	0.0	15.72204	+ 0.09
2	15.92325	15.92279	- 0.46	15.92285	- 0.40
3	15.66892	15.66844	- 0.48	15.66850	- 0.42
4	15.67582	15.67532	- 0.52	15.67540	- 0.44
5	15.74452	15.74408	- 0.44	15.74417	- 0.35
6	15.78937	15.78915	- 0.22	15.78919	- 0.18

These results were obtained for glass vials. Column I represents initial weight; column II the weight after removing and recapping with the use of gloves; and column III the weight after antistatic treatment. For procedure and other details, see footnote to Table 1.

model 2414, may be accounted for by general errors in weighing.^{1,2} The balances were not housed in a proper balance room, and no account was taken of changes in humidity or temperature, although these would have been minimal because the time taken for the procedure for each vial occupied a few minutes, and all equipment was equilibrated at room temperature for at least 30 minutes beforehand. Table 2 shows the effect on glass vials; for the reasons given above it is considered insignificant.

We thought it important to draw attention to this source of error in what, for most biochemistry laboratories, is a

major determinant of analytical accuracy—the analytical balance.

Although it is possible that the magnitude of this error is related to atmospheric humidity, we have not noted any marked effect of dry days in comparison with very wet days. The influence of atmospheric humidity on the error in weighing plastic objects might well merit further investigation.

A FLECK
S CAINE
M MERRYWEATHER
Biochemistry Department,
Western Infirmary,
Glasgow G11 6NT

References

- ¹ IR Hainsworth, E Simpson. A survey of weighing performance in clinical biochemistry laboratories. *Clin Chim Acta* 1978;**84**:233.
- ² PRW Baker, DA Pantony, BT Saunderson. Electro balance survey—elemental user forum of the A.D. microchemical methods group. *Proc Anal Div Chem Soc* 1978;**15**:136.

Antiphagocytic effect of *Pseudomonas aeruginosa* exopolysaccharide

Infection with the form of *Pseudomonas aeruginosa* that produces large amounts of exopolysaccharide slime occurs occasionally in patients with underlying chronic debilitating diseases.¹ This mucoid colony type of the organism, when present, is frequently associated with the respiratory tract,² particularly in cystic fibrosis.³ Moreover, the incidence of these slime-producing organisms has been related to the severity of this disease.⁴ When the respiratory tract of these patients becomes colonised by the mucoid organisms, eradication is extremely difficult, which suggests that the slime-producing bacteria escape the host defence mechanisms.

We have studied the effects of cultured alveolar macrophages collected from two species, the rat and the guinea-pig,⁵ upon mucoid and non-mucoid types of *Ps. aeruginosa*. The mucoid form was isolated repeatedly from the sputum of a patient with chronic bronchitis, as was the associated non-mucoid form (from the same patient in an earlier stage of the disease). After short-term exposure of the non-slime-producing bacteria to the cultures of alveolar macrophages established on glass coverslips, clear evidence of phagocytosis was obtained. Giemsa-stained preparations showed that nearly all the macrophages contained intracellular bacteria after 2 hours, and in some instances more than 20 organisms had been phagocytosed. When slime-producing organisms were substituted, a complete absence of phagocytosis was observed. A cell-free suspension of the slime was prepared as reported by Schwarzmann and Boring⁶ and sterilised by passage through a membrane filter. The addition of a 1 in 4 dilution of this slime suspension to macrophage cultures exposed to non-mucoid organisms partly inhibited phagocytosis, which readily occurred in the absence of the exopolysaccharide. It appears, therefore, that