United Kingdom scheme for external quality assessment in virology. Part II. Specimen distribution, performance assessment, and analyses of participants' methods in detection of rubella antibody, hepatitis B markers, general virus serology, virus identification, and electron microscopy

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SUMMARY Methods for the preparation and pre-distribution testing of specimens for external quality assessment in virology have been defined and criteria for allocation of scores for participants' reports on each category of specimen have been established. Specimens for detection of rubella antibody or markers of hepatitis B infection consist of human serum samples, which are distributed after detailed assessment of the expected results. In testing for rubella antibody or hepatitis B surface antigen (HBsAg) the scores given for reports of positive, equivocal, or negative depend on the specimen's content of antibody or HBsAg as established in the external quality assessment laboratory. For general virus serology two serum samples must be tested against a designated antigen by the complement fixation method; the score allocated for each participant's results depends on the ratio of the two titres he records, which is then compared with a target value derived from the results of a panel of participating laboratories. In virus identification and electron microscopy specimens are prepared from cultures or from clinical samples, and scores depend on the accuracy of identification. The pre-distribution tests necessary to establish the virus content and stability of these specimens have been defined, and media suitable for transporting specimens for virus culture, fluorescent antibody staining, or electron microscopy have been developed. A participant's overall success rate for each specimen is judged from the mean score (maximum 2) calculated from the scores of all participants examining the specimen. Mean scores were highest for detection of rubella antibody or HBsAg (from 1.67 to 1.96) and lowest for specimens containing certain small enteric viruses distributed for electron microscopy (0.82 to 1.12). Participants' reports on the methods used for each specimen have been analysed. Current changes and developments in methods have been recorded, and attempts have been made to relate the use of various techniques and test kits to successes or failures with various types of specimen.

The general method of operation of the United Kingdom scheme for external quality assessment (EQA) in five categories of viral diagnostic work is described in the accompanying paper (p 534). Each

of these categories or distribution types—that is, rubella antibody detection, hepatitis B surface antigen detection, general virus serology, virus identification, and electron microscopy—presents individual problems in EQA. The methods of specimen preparation for each distribution type are described, as are the criteria used for allocation of scores for each category of specimen. Examples are

presented of the information obtained by analyses of participants' reports on the methods used.

Methods and results

RUBELLA ANTIBODY DETECTION

Laboratories in the UK often need to determine the immune status of girls and women of child bearing age in relation to the need for and the success of rubella vaccination, and the EQA laboratory therefore distributes serum specimens to enable participants to monitor their success in this test.

Participants are asked to report on each sample and to give details of the method(s) used; the distribution summary prepared by the EQA laboratory records the overall results and the success rates associated with use of various techniques.

Specimens for monitoring assay of rubella IgM antibody have also been distributed to those participants who, in answer to a questionnaire, have specifically requested them. About half of those taking part in the rubella test request specimens for monitoring IgM. Performance in rubella IgM assay has not so far been scored, but this test is being constituted as a new distribution type with which scores and performance ratings will be associated.

Source and preparation of serum samples

Pooled human sera of known rubella antibody content donated by participating laboratories are often used to prepare EQA specimens, and plasma donations obtained from blood transfusion laboratories are also an important source. Single donations, if available, are preferable to pooled samples because of the greater ease of exclusion of undesired non-specific effects and of known markers of hepatitis B infection. All human sera are checked for HBsAg by radioimmunoassay. Supplies of human sera negative for rubella antibody are limited, and fetal bovine serum has occasionally been used as a substitute. Human samples, however, provide a more realistic exercise and are used whenever possible. Plasma donations to be used as EQA specimens are clotted by addition of 0.5 ml of 40% aqueous calcium chloride to each 100 ml plasma. The plasma is incubated at 37°C until the clot forms, then frozen at -20°C, and the clot is removed after thawing. Thrombin may be used to assist clotting of plasmas that have been stored for long periods. Sera are distributed without lyophilisation and are preserved by addition of sodium azide at 0.08% final concentration.

Antibody assays before distribution (IgG)

Serum samples are titrated by a radial haemolysis method¹ in parallel with a reference serum of known

potency, preferably the British Standard 69/60 or an equivalent accurately calibrated against it. Twofold dilutions of the test serum and the standard are tested in agarose gels containing rubella haemagglutinin and sheep red blood cells. Zones of haemolysis are measured, and the means of triplicate observations are plotted against the log of serum dilutions. Reference serum and test serum should give parallel straight lines, and the unitage of the test serum can then be derived from comparison with the controls.

EQA specimens are prepared at the required antibody content by dilution with negative serum or, if this is not available, with fetal bovine serum. Pilot batches of the specimens are assayed by radial haemolysis in agarose gel against standard sera including a cut off control serum containing antibody at 15 IU/ml prepared by the Public Health Laboratory Service. Control gels (without haemagglutinin) are also used. Specimens are also titrated by haemagglutination inhibition and when necessary tested by other methods likely to be used by an appreciable number of participants, such as enzyme linked immunosorbent assay (ELISA). When suitable pilot specimens have been assayed and found satisfactory the main batches are prepared and again tested. Repeated assays to establish antibody content accurately are particularly important for samples with antibody concentrations <50 IU/ml, and the pre-distribution tests are ideally done by more than one member of staff on different days so that the element of within laboratory variability is included. It must also be established that low titre specimens will remain stable for at least one week at ambient temperature. After distribution, sets of specimens are returned to the EQA laboratory by post (postal return specimens) and these are stored at 4°C immediately on receipt and tested as soon as possible to check for any deterioration in the mail. The antibody content of serum samples that are not lyophilised but preserved with sodium azide has appeared stable when held for two to six days under postal conditions or at 4°C for four to five weeks, but some deterioration of low titre specimens has been detected after longer periods. Specimens are normally prepared within 7-10 days before distribution. Each distribution consists of three to five specimens, among which are usually at least one negative or weakly positive specimen. All serum specimens distributed are accompanied by a warning of potential infectivity.

Reporting and scoring for EQA of rubella antibody assay

The forms accompanying the specimens allow the participants to select one of three reports by ticking

Table 1 *Scoring for five categories of specimens containing rubella antibody (IgG)*

Category	Antibody content	Report	Recommendation to vaccinate or specimen sent to reference laboratory	Score allocated
1	>50 IU/ml	Positive, immune	Not relevant	2
		Low/equivocal, immunity doubtful	" "	0
		Negative, non-immune	" "	0
2	25–50 IU/ml	Positive, immune	" "	2
		Low/equivocal, immunity doubtful	" "	1
		Negative, non-immune	" "	0
3	15–25 IU/ml	Positive, immune	" "	2
		Low/equivocal, immunity doubtful	" "	2
		Negative, non-immune	" "	0
4	<15 IU/ml	Positive, immune (used ELISA)	" "	1
		Positive, immune (ELISA not used)	" "	0
		Low/equivocal, immunity doubtful	Recommended or referred	2
		Low-equivocal, immunity doubtful	Not recommended or referred	1
		Negative, non-immune	Not relevant	2
5	Not detected	Positive, immune	Not relevant	–1
		Low/equivocal, immunity doubtful	Recommended or referred	1
		Low/equivocal, immunity doubtful	Not recommended or referred	0
		Negative, non-immune	Not relevant	2

ELISA = enzyme linked immunosorbent assay.

the appropriate box: positive, patient immune; or low/equivocal, immunity doubtful; or negative, patient non-immune. The questions "would you have recommended rubella vaccination?" (yes/no) and "would you have sent the specimen to a reference laboratory?" (yes/no) must also be answered, and information on the method(s) used is also requested.

The present (1983) practice within the Public Health Laboratory Service is that rubella antibody concentrations of 15 IU/ml are taken to indicate immunity, and vaccination is usually recommended for individuals with values less than this.¹ The scores allocated for EQA in rubella antibody detection are based on this practice. Depending on the pre-distribution antibody assays specimens are allocated to one of five categories for purposes of scoring.

- 1 Positive—antibody assay >50 IU/ml
- 2 Positive (weak)—antibody assay about 25–50 IU/ml
- 3 Positive (very weak)—antibody assay about 15–25 IU/ml

4 Antibody present below 15 IU/ml—antibody assay <15 IU/ml

5 Negative, no antibody detected—antibody not measurable

In each category the score allocated depends on the report indicated. In the case of negative specimens or those containing antibody at concentrations <15 IU/ml, the participant's responses to the questions on vaccination advice or referral of the specimen to a specialist laboratory and the method he has used may also be taken into account (Table 1). Table 2 shows that the average scores achieved by all participants examining specimens for rubella antibody were high for positive specimens containing antibody at ≥50 IU/ml but that success rates were lower for negative and weakly positive specimens.

Analyses of methods used for rubella antibody testing
Most laboratories reported using radial haemolysis, haemagglutination inhibition, or ELISA singly or in combination, and Table 3 shows that during 1982–3

Table 2 *Mean scores calculated for all participants examining specimens for rubella antibody or hepatitis B surface antigen 1982–3**

Specimen categories	Mean scores per specimen
Rubella	1 1.96, 1.95, 1.91, 1.91, 1.96, 1.86, 1.94, 1.94
Rubella	2 1.87, 1.82
Rubella	3 1.79
Rubella	4 1.68
Rubella	5 1.90, 1.90, 1.92, 1.85, 1.93, 1.89
Hepatitis	1 1.81, 1.87, 1.87, 1.83, 1.83
Hepatitis	2 1.67
Hepatitis	4 1.88, 1.91, 1.86
Hepatitis	5 1.86, 1.78

*The maximum score per specimen is 2.

RPHA = reverse passage haemagglutination method.

Table 3 Proportions of participants testing individual external quality assessment specimens for rubella antibody and returning the report equivocal, analysed according to the technique used (1982 and 1983)

Rubella antibody content	Date	Users of radial haemolysis (alone)	Users of haemagglutination inhibition (alone)	Users of ELISA (alone)
Negative	Jan 1982	2/71, 2/71	1/29, 1/28	0/8, 1/8
	June 1982	1/72	0/22	0/3
	Feb 1983	2/67	0/16	0/14
	July 1983	0/68, 1/68 (Range 0-3%)	0/14, 1/14 (Range 0-7%)	0/21, 0/19 (Range 0%)
Positive*	Jan 1982	0/87, 0/87, 0/85	0/30, 1/30, 0/31	0/8, 0/8, 0/8
	June 1982	0/83, 0/84, 5/69†	1/19, 1/22, 8/20†	0/6, 0/6, 0/6†
	Feb 1983	0/91, 1/86	1/16, 3/15	0/13, 0/14
	July 1983	7/86, 0/90, 0/88 (Range 0-8%)	4/14, 1/14, 1/14 (Range 0-40%)	1/20, 0/20, 0/20 (Range 0-5%)

*Includes rubella specimen categories 1, 2, and 3 (see Table 2).

†The antibody content of this specimen was 25 IU/ml.

the use of ELISA (alone) increased, use of haemagglutination inhibition (alone) decreased, and the use of radial haemolysis (alone) remained fairly constant. Equivocal reports were returned by a higher proportion of users of haemagglutination inhibition than users of radial haemolysis or ELISA, particularly in the case of specimens containing low concentrations of antibody. These and other results obtained with EQA specimens containing low concentrations of rubella antibody have emphasised the unsatisfactory nature of the haemagglutination inhibition test for detecting susceptible subjects requiring vaccination.

Participants are also asked to record the quantitative results of their tests when appropriate. If a significant proportion of users of a particular technique or commercial kit report quantitative values for any individual specimen, the relevant figures are recorded in the distribution summary, which enables participants to compare their own result with the mean and range recorded by other laboratories. Fourteen users of the ELISA kit Rubazyme G (Abbott Laboratories) reported that a specimen containing rubella antibody at 11 IU/ml gave indices ranging from 0.55 to 1.79 (mean 1.16). In this test the cut off value recommended as indicating immunity to rubella is an index of 1.0 (about 12 IU/ml). The participants' consensus mean therefore agreed well with the expected value, but participants were asked to note the wide scatter of indices around the mean and were reminded that testing and reporting of such borderline samples requires special consideration and meticulous use of controls. In another instance a specimen contained about 90 IU of rubella antibody per ml, and 26 users of the haemagglutination inhibition test reported titres that ranged from 1/8 to 1/64 (median 1/32). Such a wide range of titres is not uncommon for the haemagglutination inhibition method and users have been encouraged to calibrate their tests in terms of IU/ml and to use appropriate control samples.

DETECTION OF HEPATITIS B SURFACE ANTIGEN (HBsAg)

Many clinical laboratories not specialising in work on hepatitis need a rapid, simple test for detecting HBsAg in patients' sera, such as the reverse passive haemagglutination method (RPHA). Tests based on this method are not highly sensitive, but in the UK the proportion of samples positive for HBsAg at a level not detectable by RPHA is low and these tests are therefore often used in hospital practice. Blood transfusion and other laboratories that test blood products need a highly sensitive test for detecting HBsAg in donated blood and they therefore generally use radioimmunoassay. In addition, large diagnostic laboratories sometimes act as specialist centres for hepatitis and use a range of techniques both for HBsAg and for other hepatitis B markers. The EQA scheme attempts to meet the needs of all these varied participants. The serum specimens distributed for EQA may be tested by whatever method(s) the participant normally uses, although scoring is based on results obtained by RPHA techniques. Specimens containing HBsAg at concentrations detectable only by the more sensitive radioimmunoassay or ELISA are occasionally included in the distributions, but up to 1983 they have not been scored. All participants are asked to test for HBsAg; those who wish to use the specimens to monitor their tests for other hepatitis B markers may also do so, although no scores are given for this. Results of all tests and the methods that have been used are reviewed in the distribution summary.

Source and preparation of samples

Suitable human plasma samples are generally obtained from blood transfusion laboratories and are processed by methods similar to those described for rubella EQA. Serum samples negative for HBsAg are generally in shorter supply than positive samples because the latter are rejected for transfu-

Table 4 Scores allocated for various reports in external quality assessment of detection of HBsAg

Category of specimen	Report	Acceptable evidence of confirmation given or statement made of referral to specialist laboratory	Score allocated
1 Positive, RPHA titre $\geq 1/32$	Positive	Referral or confirmation	2
	Positive	No referral or confirmation	1
	Equivocal	Referral	1
	Equivocal	No referral	0
	Negative	Not relevant	-1
2 Positive, RPHA titre 1/8 or 1/16	Positive	Referral or confirmation	2
	Positive	No referral or confirmation	1
	Equivocal	Referral	2
	Equivocal	No referral	1
	Negative	Not relevant	0
4 Negative, RPHA non-specific agglutination	Positive	Referral	0
	Positive	No referral	-1
	Equivocal	Referral	2
	Equivocal	No referral	1
	Negative	Not relevant	2
5 Negative	Positive	Referral	0
	Positive	No referral	-1
	Equivocal	Referral	1
	Equivocal	No referral	0
	Negative	Not relevant	2

RPHA = reverse passage haemagglutination method.

sion. Samples potentially suitable as EQA specimens are tested for HBsAg and antibody to HBsAg by RPHA and radioimmunoassay, for antibody to HBc antigen by ELISA and radioimmunoassay, and for HBe antigen and antibody by ELISA and radioimmunoassay. The sensitivity of the methods used is monitored by use of appropriate controls, where possible calibrated against standards such as the British Standard for HBsAg. Five samples are usually sent out at each distribution, among which are included at least one negative serum and also samples positive for hepatitis B markers other than HBsAg. If necessary, HBsAg positive samples may be diluted with a negative serum to obtain suitable specimens of low potency. Preparation of pilot and final batches of specimens is as for rubella specimens. All sera are handled under the required conditions of safety and are posted under the appropriate "hazard of infection" labels; samples positive for e antigen are occasionally distributed. Sera are not lyophilised but are preserved with sodium azide 0.08%.

Reporting and scoring for HBsAg

The form accompanying the specimens allows participants to select one of the three reports—that is, HBsAg positive, equivocal, or negative. The form states that the specimen, if positive, should be regarded as having come from a newly detected case of antigenaemia. To achieve a full score of 2 marks for a positive specimen the participant must indicate how he has confirmed the results—for example, by use of two different methods for HBsAg both giving a positive result, by neutralisation of a positive RPHA, ELISA, or radioimmunoassay test using an

anti-HBs serum, by demonstration of a positive result from a test for a second marker of hepatitis B infection, or in the case of a laboratory without facilities for specialised work on hepatitis by a statement that his normal procedure would be to send the serum to a specialist centre for confirmation of the result.

Five categories of specimen have been established for purposes of scoring, and the appropriate category of each specimen is determined by the EQA laboratory's results.

- 1 HBsAg positive, RPHA titre (Hepatest, Wellcome) $\geq 1/32$
- 2 HBsAg positive, RPHA titre 1/8–1/16 (weak)
- 3 HBsAg positive, but RPHA tests negative (titre $< 1/8$)
- 4 HBsAg negative, but RPHA tests gave non-specific agglutination
- 5 HBsAg negative

Table 4 indicates the allocation of scores for various reports on samples in categories 1, 2, 4, and 5; up to 1983 no scores have been allocated for category 3. Table 2 lists the mean scores achieved by participants in 1982–3 for individual specimens in the four categories indicated.

Analyses of methods used for markers of hepatitis B infection

For HBsAg, the method most commonly specified was RPHA. The titres obtained by the participants varied widely with a single specimen—for example, specimen 813 (July 1983) was titrated by 52 users of the Hepatest kit—and the titres varied from 1/8 to 1/512 (median 1/64). Attempts have been made to correlate such variations with specific batches of

Table 5 Reports by users of RPHA, ELISA, or radioimmunoassay (RIA) on the HBsAg status of specimens containing low concentrations of this antigen

Specimen	Method	Result reported			Total users
		Positive	Equivocal	Negative	
No 753 British standard HBsAg 20 units/ml*	RPHA	18	8	64	90
	ELISA	40	0	1	41
	RIA	41	0	1	42
No 814 <20 British units HBsAg per ml†	RPHA	2	0	82	84
	ELISA	44	0	4	48
	RIA	38	0	0	38

*Distributed December 1982.

†Distributed July 1983.

RPHA = reverse passage haemagglutination method.

ELISA = enzyme linked immunosorbent assay.

named kits. The large number of different batches in use at any one time has made it difficult to obtain meaningful figures on batch variations, but the numbers of users of named kits or individual reagents and the results obtained with each have been quoted in the distribution summaries. Results obtained with specimens containing low concentrations of HBsAg reflect the comparative sensitivities of the RPHA, ELISA, and radioimmunoassay techniques (Table 5). The British Standard for HBsAg, diluted to contain 20 units/ml was distributed to all participants in December 1982. As expected, most users of RPHA tests did not detect antigen at this concentration whereas most users of three different ELISA kits and all users of six different radioimmunoassay tests did. Some participants may report on tests for other markers of hepatitis B infection. The approximate numbers of participants reporting on other markers (July 1983) were anti-HBs 24; anti-HBc 24; HBe and anti-HBe 37. For anti-HBs participants generally used either radioimmunoassay or ELISA tests, and ELISA was the commonest method for anti-HBc and for HBe antigen and antibody. Tabulations in the distribution summary of participants' methods and results for these markers are intended to make the users aware of the potential of different tests and to help them identify any points of difficulty. In a recent distribution 14 of 15 users of an ELISA kit for detection of hepatitis B antigen obtained positive results with a certain specimen whereas all of 11 users of a second kit reported negative results with the same specimen.

GENERAL VIRUS SEROLOGY (COMPLEMENT FIXATION TESTS)

The range of antigens surveyed includes influenza A and B, adenovirus group, respiratory syncytial virus, measles, mumps, herpes simplex, varicella zoster, cytomegalovirus, *Chlamydia* group, *Mycoplasma pneumoniae*, *Coxiella burnetii*. The sources and general treatment of sera are as for rubella and hepatitis

B. Samples containing high titres of antibody against most of these antigens, however, are generally only obtainable by pooling small volumes of human convalescent sera donated by participant laboratories.

Assessment of specimens, reporting, and scoring

In early attempts to develop EQA in general virus serology acute and convalescent pairs of sera were simulated and the results were scored according to whether or not the participants detected a significant rise in titre. It proved difficult to provide enough satisfactory pairs of sera, however, and the system currently used entails distribution of two or four sera (A and B, X and Y) stated not to represent clinical pairs, with a request that their titres be reported against 1 to 4 specified antigens. The expected results are established before distribution by repeated titrations done in the EQA laboratory, preferably by more than one member of staff on different days and using various batches of antigen and sources of complement. The geometric mean titres of each serum against the selected antigens are determined, and the ratios of these titres B:A (or Y:X) are calculated. For each of the selected antigens the titre of antibody in serum B may be higher than A, or lower, or the same, but this does not affect the suitability of the sera for issue. Sera A and B may sometimes be duplicates.

Participants are scored for the result obtained by testing two sera against one designated antigen. The score allocated depends on the ratio between the two serum titres that they report and how close this value is to the target, derived from a reference panel as described below. The ratio (reciprocal titre of serum B (or Y): reciprocal titre of serum A (or X)) is also described less precisely in terms of the number of twofold dilution steps difference in titre between the two sera. For a result in which the titre of serum A is 1/8 and the titre of serum B is 1/64, the titre of B is three twofold dilution steps higher than A, and the ratio B:A is 8. For tests of this kind

Table 6 Scores allocated for various reports on the titres of sera A and B against a specified antigen, when the target ratio B:A, as determined by reference participant laboratories, is 8

Report	Ratio B:A	Score
B is lower than A (by any amount)	≤ 0.5	-1
B is the same as A	1	0
B is one twofold dilutions higher than A	2	1
B is 2 " dilutions " " A	4	2
B is 3 " " " " A (Target)	8	2
B is 4 " " " " A	16	2
B is 5 " " " " A	32	1
B is 6 " " " " A	64	0
B is ≥ 7 " " " " A	≥ 128	-1

the scores allocated depend not only on the EQA laboratory's findings but also on the results of a panel of (usually 15) participating laboratories who return their reports in the usual way. On receipt of each panel member's results the ratios (reciprocal titre of serum B (or Y): reciprocal titre of serum A (or X)) reported for each of the test antigens are tabulated. Then for each antigen the median value for the panel's ratios is selected and this median is the target which determines the scoring for that test.

With the results from the reference panels for the current and previous distributions a precision factor can be calculated; this gives a measure of the standard deviation of the reference panel's ratios and allows calculation of an acceptable range of results around the target ratio.² In practice the acceptable range of results usually includes ratios twofold less than and twofold more than the target. If the target ratio of titres B:A is 8—that is, the titre of B is three twofold dilutions higher than A—then the calculated acceptable range of results either side of the target will generally include the ratio B:A = 4 (titre of B is two twofold dilutions higher than A) and the ratio B:A = 16 (titre of B is four twofold dilutions higher than A). Participants whose reports fall within the acceptable range for ratios are given a score of 2, and lower scores are given for more widely divergent ratios (Table 6). Only exceptionally have the actual titres recorded by the participants been taken into account when scores are allocated.

With this scoring system mean scores for individual antigens tested against two sera (specimen/assessments) calculated from all participating laboratories have varied in 1982 from 1.52 to 1.79 (eight specimen/assessments) and in 1983 from 1.73 to 1.92 (seven specimen/assessments). Information on the methods used by participants has not been regularly requested, but a major survey in 1980 indicated that many variations of complement fixation technique were in use. Antibody concentration determined by methods other than complement fixation are not at present scored.

VIRUS IDENTIFICATION

Source and preparation of specimens

As for other EQA specimens the intention is to simulate clinical material in such a way that specimens can be assimilated into the routine work of the laboratory without being given excessive attention. The over-riding need for stability and reproducibility of specimens, however, conflicts to some extent with realistic simulation of clinical material, and specimens normally consist of tissue culture grown virus in transport medium, distributed with an appropriate clinical history. Although about 85% of participants receive the specimens within two days of posting, a few, usually outside the UK, receive them after six to seven days, and so stability must be ensured for up to one week. Because of the need to establish its purity, stability, and sterility before distribution, the virus used must be at least partly adapted to growth in tissue culture. Every specimen must contain an adequate minimum titre of virus and the average virus content will therefore often be higher than in a clinical specimen. For these reasons viruses in EQA specimens are probably easier to identify than those in specimens from patients. About 20 different viruses have so far been used, including those listed in Table 7.

Whenever possible the strains used will have had few passages in tissue culture. A sufficient quantity of virus is grown and stored at -70°C while samples are checked for purity, stability, and bacterial and fungal sterility. Several transport media have been studied and two have been found satisfactory for different purposes. Enteroviruses and adenoviruses retain adequate infectivity if stored harvests from infected tissue cultures are thawed and diluted for distribution in a HEPES buffered albumin containing medium, HH.³ This method is not satisfactory for less stable viruses, but influenza A, parainfluenza 3, respiratory syncytial virus, and cytomegalovirus have been successfully distributed as intact infected cultured cells suspended in a modified version of a medium (GTM) used for transport of cell cultures (J Dugdale, personal communication): gelatin (7.5%

Table 7 Mean scores achieved by all participants examining specimens distributed for virus identification and electron microscopy in 1982 and 1983

Type of distribution	Stated origin of specimen	Specimen contents	Mean score*
Virus identification	Respiratory	Influenza A	1.61
		Influenza B	1.40
		Parainfluenza virus type 3	1.43
		Respiratory syncytial virus	1.47
		Adenovirus type 2	1.37
		Cytomegalovirus	1.29
		Negative	1.63, 1.81
		Poliovirus type 3	1.66
		Echovirus type 17	1.69
		Coxsackievirus type B2	1.89
Electron microscopy	Faecal	Coxsackievirus type B4	1.75
		Negative	1.73
		Adenovirus	1.70
		Rotavirus	1.64
		Negative	1.54
		Calicivirus	0.82
		Parvovirus	1.00
		Astrovirus	1.12 (1982)
			0.96 (1983)
			1.70
	Skin scraping	Orf virus	

*The maximum score per specimen is two.

in distilled water) 100 ml, tissue culture medium 199 10-fold concentrate (Gibco) 10 ml, 4.4% sodium bicarbonate 2 ml, fetal calf serum 10 ml, and antibiotics. Infected cells in this medium are suitable for virus identification by culture or by immunofluorescence. Cell monolayers infected with the selected virus are trypsinised from their support, washed, and suspended in GTM + 10% dimethyl sulphoxide, and the suspension is frozen in 1 ml amounts in liquid nitrogen. On thawing, cells are washed free of dimethyl sulphoxide, counted, and suspended in GTM. Infectivity titrations may be done on such suspensions, and, as with the more stable viruses frozen in unmodified tissue culture maintenance medium, samples of the frozen virus stocks are used to check stability, purity, and sterility before the final specimens are prepared.

Stability at ambient temperature is tested in the selected transport medium. A suitable dilution of the stored virus is prepared in HH or GTM and replicate samples are kept in the dark at room temperature. One is titrated immediately and, if appropriate, examined by immunofluorescence, and further samples are titrated and examined after 4, 7, and 10 days, or at longer intervals. Table 8 shows the results of stability tests on four viruses distributed for EQA as infected cells suspended in GTM. Respiratory syncytial virus and cytomegalovirus were grown in human embryo lung fibroblasts and influenza A and parainfluenza 3 in secondary cultures of baboon kidney. It is clear that infectivity and antigenicity were adequately maintained for at least a week.

Neutralisation tests are used to confirm the identity of the virus. Tests are done in tissue culture using low ($10^2 \times$ median tissue culture dose) and

high (about $10^4 \times$ median tissue culture dose) doses of virus with suitable dilutions of the homologous standard antiserum. The low dose test verifies identity and the high dose test allows breakthrough of any possible contaminant virus that might be present in small amounts.⁴ Cross tests are also done using those antisera likely to be used by participants in the course of identification of the specimen—for example, pools of neutralising antisera against echoviruses, pooled adenovirus antisera, antisera against haemadsorbing viruses—to ensure that no unexpected reactions occur. If reagents are available for identification of the virus by immunofluorescence—for example, influenza virus, respiratory syncytial virus—the infected cells from which the specimen will be prepared are dried on slides and stained with homologous and all relevant heterologous reagents.

For distribution a dilution of the stored virus is selected which is expected to show a titre of about $10^2 \times$ median tissue culture dose/ml after seven days in transport medium at ambient temperature. Mixtures of infected and uninfected cells are used where necessary to simulate realistic specimens. If the infectivity of the virus is not appreciably impaired by freezing and thawing, the specimens are prepared from the frozen stock before posting and then refrozen at -70°C until required; for less stable viruses, usually distributed as infected cells in GTM, the specimens are prepared from the frozen stock immediately before packing and posting.

Reporting and scoring for virus identification

Participants are asked to record the date of receipt of specimens, the report they would have made to the clinician with its date, and to give information on

Table 8 *Stability of several viruses prepared as infected tissue culture cells suspended in gelatin transport medium and maintained at ambient temperature (about 20°C)*

Virus	Virus titre* and intensity of immunofluorescence† after storage for the indicated number of days					
	0	3 or 4	5 or 6	7 or 8	10	21
Respiratory syncytial virus	4.4 ++++	4.0 ++++	3.5 ++++			1.75 ++
Cytomegalovirus	4.0 ND	2.5 ND		2.75 ND	2.5 ND	
Influenza A	3.5 +++		2.5 +++	2.5 +++		
Parainfluenza 3	5.5 ++++	6.5 ND		6.5 ++++		

*log₁₀ 50% infectious doses for tissue culture.

†Arbitrary scale for assessment of intensity of fluorescence, 0 to ++++.

ND = not done.

their identification method(s). Scores are based only on the report and the general principles of scoring are the same for all specimens.

2 marks: fully correct identification—for example, echovirus type 17, influenza A. If reagents for full identification are not readily available, a less exact identification may be given 2 marks—for example, probable cytomegalovirus. The report adenovirus untyped is given 2 marks in the case of an adenovirus of high serotype number, but serotype identification is required for adenoviruses of low serotype number and for enteroviruses against which type specific antisera are available.

1 mark: identification of correct virus group—for example, myxovirus; identification of the wrong type within the correct group, where this misidentification would generally not have serious clinical or epidemiological consequences—for example, Coxsackievirus B5 instead of B2.

Zero: no virus found when a virus is present; unidentified agent reported; partial identification or misidentification of moderately serious degree—for example, Coxsackievirus for echovirus; mumps virus for parainfluenza 2 virus; haemadsorbing agent for influenza A.

-1 mark: virus of wrong group names—for example, adenovirus for enterovirus; more than one virus named, where reporting of the additional virus(es) would have serious clinical or epidemiological consequences.

Specimens not containing virus are also regularly distributed. In this case 2 marks are given for the correct report, zero for reporting an unidentified agent, and -1 for reporting a named virus.

The summary of each distribution gives full details of allocation of scores for each specimen, and also attempts to relate the methods used to success or otherwise in identification and the time taken by the participants to issue reports. The mean scores achieved by all participants examining specimens for virus identification in 1982-3 are given in Table 7.

Methods used for virus identification

Reports on their methods have generally been received from about 80 laboratories. A high proportion (94-99%) used neutralisation tests for identification of echoviruses, Coxsackie B viruses, or polioviruses, and 91-99% of the users reported correct results. Neutralisation was less often and successfully used for adenovirus: 49 of 78 laboratories used neutralisation for adenovirus 2 (63%), 43 of whom (88%) reported the correct type.

The proportion of participants using immunofluorescence for identifying respiratory viruses varied for different viruses: adenovirus (group) 14%, influenza B 49%, influenza A 57%, parainfluenza virus type 3 54%, respiratory syncytial virus 72%. The EQA samples sometimes contained large numbers of infected cells and could therefore be regarded as easy specimens; the success rates for identification by immunofluorescence varied from 91% (adenovirus) to 100% (respiratory syncytial virus and influenza B). Users of immunofluorescence, however, sometimes made errors in identification (one report of parainfluenza 3 for adenovirus, two reports of respiratory syncytial virus for parainfluenza 3). A number of participants used immunofluorescence for identifying viruses isolated in tissue culture: respiratory syncytial virus was distributed in November 1982 and of 57 users of immunofluorescence, 13 identified the virus in the original specimen, 25 in tissue cultures, and 19 in both; a similar practice was reported for influenza A in May 1983. Users of immunofluorescence generally issued their reports quickly: six users reported respiratory syncytial virus correctly on the day of receipt of the specimen and another six reported within two days of receipt. For influenza A 17 laboratories reported correctly within two days of receipt of the specimen, 16 of whom used immunofluorescence. False positive results were sometimes associated with use of immunofluores-

cence: in November 1982 a "respiratory" specimen containing no virus was reported positive by seven participants, two of whom had used immunofluorescence, and in May 1983 a similar negative specimen generated two false positives (parainfluenza 1 and respiratory syncytial virus), both from users of immunofluorescence.

ELECTRON MICROSCOPY

Source and preparation of specimens

This distribution type has attracted about 60 participants—that is, considerably fewer than for other categories of virological specimens. About 12 different viruses have been distributed including those listed in Table 7. The specimens are intended to simulate clinical material submitted for diagnostic electron microscopy, and a number of the specimens are also seen as having possible value for participants' self education. Of eight specimens distributed in 1982–3 seven were of faecal origin and one was a skin scraping. In previous years specimens have included tissue culture fluids said to have been inoculated with a respiratory specimen (coronavirus), simulated smears from a skin lesion (herpes virus), and a suspension prepared from infected tissue (papillomavirus). The material used has sometimes originated from patients, but the volumes required for a single distribution are large and animal sources have been used when the morphology of human and animal viruses are the same. Bovine rotavirus in bovine faeces and bovine papilloma virus in bovine tissues were used to simulate human specimens. Alternatively, viruses have been grown in tissue culture and then, if appropriate, added to extracts of normal faeces.

Before distribution the material is examined by at least one experienced electron microscopist on several occasions to verify the identity of the specimen and to exclude the presence of unintended viruses in quantities near the limit of detection. Tests of stability are done to ensure that the virus retains identifiable morphology for at least seven days at ambient temperatures. Stability has not been a problem when using faecal viruses, adenoviruses, or papillomavirus, but coronavirus from tissue cultures and herpes simplex virus dried on slides required stabilisation before distribution. In each case the incorporation of 2.5% sorbitol in the virus suspension allowed good preservation of morphology for up to seven days at ambient temperature.

Scoring for electron microscopy

Scores are allocated according to the accuracy of identification by a system similar to that described for virus identification. The mean scores achieved by participants examining specimens distributed for

electron microscopy in 1982–3 are shown in Table 7. In the case of viruses commonly encountered in clinical practice—for example, adenovirus and rotavirus—or those with distinct morphology—for example, orf virus—mean scores up to 1.7 were comparable to those achieved in virus identification. A number of specimens, however, contained viruses that are not commonly reported, such as parvovirus, calicivirus, astrovirus, and human respiratory coronavirus, and in these cases mean scores were much lower: 1.00 for parvovirus, for which the expected identification was small round virus, and 0.82 for calicivirus, for which either of the identifications calicivirus or small round virus of rough or structured appearance were given 2 marks. A faecal suspension containing astrovirus was distributed in 1982 and 1983; the specimens were both from the same patient, one having been stored for eight months and the other for 20 months. The material distributed on both occasions was judged by the organisers before and after distribution to have retained typical morphology, although the proportion of particles showing the characteristic star shaped form was probably slightly lower in 1983 than in 1982. The number of participants correctly identifying the virus was similar in both years. Fourteen laboratories reported astrovirus on both occasions; five who reported small round viruses in the first year reported astroviruses in the second, but seven who reported astroviruses in the first year reported only small round viruses in the second. Of a further eight laboratories who reported small round viruses in the first year, seven gave a similar report the next year and one reported no virus. It seemed therefore that the circulation of a specimen known to be positive for a virus whose morphological features required careful study for their recognition had not produced any clear improvement in terms of the proportion of participating laboratories correctly identifying the virus.

Methods and quantitative results in electron microscopy

Participants in electron microscopy have been asked to record whether they have concentrated the specimen before examining it and, if so, by what method, and also to report the stain used and its pH and concentration. The method of concentration most commonly used was high speed centrifugation (defined as $\geq 10\,000\text{ g}$) sometimes preceded by clarification at lower speeds, and a few participants chose methods using Lyphogel (Hawkesley and Son Ltd, Lancing, Sussex) or polyethylene glycol. The data collected have been recorded in the distribution summary, but because of the small numbers and varied skills of the users of different methods, no con-

clusions have been drawn about the relative merits of different techniques. Participants have also been asked to report the diameters of the particles they identify. These measurements have been recorded in the distribution summaries and microscopists have been asked to check how far their own results differ from the consensus value. These checks may in some cases indicate a need for further calibration of the microscope.

Discussion

By establishing routines of testing specimens for EQA in virology before distribution it has been possible to ensure adequate stability without lyophilisation. Markers of hepatitis B infection and rubella antibody (including IgM) in serum have proved stable for the required periods of about two weeks at 4°C (time taken for specimen preparation) and one week at ambient temperature (representing time in the post). Some specimens for virus identification and electron microscopy, however, have required special techniques for their preparation and preservation. A method has been developed for distribution of infected cells suitable for virus culture or staining with fluorescent antibody, and another for improving the morphological preservation of certain viruses distributed for electron microscopy.

EQA specimens are expected to reflect the nature of clinical material received for diagnosis, although specimens of special interest or educational value may represent a larger proportion of the total than would occur in practice. In EQA of detection of rubella antibody or hepatitis B markers at least one specimen giving borderline or non-specific reactions has been included in most distributions, and in virus identification, electron microscopy, and detection of HBsAg the proportion of negative specimens has been lower than would occur in practice. The inclusion of difficult and unusual specimens may mean that performance ratings sometimes underestimate participants' performance in routine practice. In some laboratories, however, this tendency may be negated by extra attention given to EQA specimens. The comment that the specimens are too difficult is made only infrequently, and the educational elements of the scheme seem to be appreciated even though their benefits are not always easy to document.

The general level of participants' performance with individual specimens is indicated by calculating the overall mean score for each specimen (Tables 2 and 7, and section on general virus serology). The mean scores per specimen are higher in rubella serology and detection of HBsAg than in virus identification or electron microscopy. This finding

corresponds with the observation that laboratories' performance ratings in the latter two distribution types are more widely scattered than in the former (p 534). For electron microscopy in particular it has been customary to emphasise the educational role of EQA. Difficult specimens have often been included for their instructive value alongside other specimens representing the general nature of material submitted for diagnosis by electron microscopy, and the mean scores reflect this practice (Table 7). Despite the educational intent, however, the rate of correct reporting of astrovirus was no higher in 1983 than for a similar specimen distributed in 1982.

The reports on participants' methods which are presented in each distribution summary are an indication of trends in current practice for each type of specimen. Year to year comparison of these reports reflects the increasing use of fluorescent antibody, ELISA, and radioimmunoassay. In screening for rubella antibody (IgG), however, the radial haemolysis method performs well and has lost little ground to ELISA tests, except perhaps for specimens containing small amounts of antibody. For HBsAg, RPHA tests remain widely used despite their comparative lack of sensitivity and occasional difficulties in interpreting results (Table 5). The scoring system used for HBsAg (Table 4) has been devised in such a way that participants using RPHA tests have not been penalised for their inability to detect low concentrations of antigen, and it may be debated whether this method of scoring is still justified. This and other aspects of scoring may need to be adjusted from time to time to reflect current practice.

Clinical virology is rapidly changing and corresponding changes in the system for EQA will also be required. An additional distribution type, detection of rubella IgM antibody, is now being formally set up, specimens having been successfully sent out on an informal basis in 1982 and 1983. Analysis of the success rates of various techniques used for rubella IgM, as reported in the distribution summary, has proved informative. A number of future developments can be envisaged, such as scoring tests for detection of hepatitis Be antigen and antibody, detection of IgM antibody to hepatitis A virus, and detection of antibody to cytomegalovirus by methods in addition to complement fixation. A progressive and flexible system will be necessary to allow such changes.

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