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Internal quality assurance in a clinical virology laboratory. II. Internal quality control

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***Aims*—In April 1991 additional quality control procedures were introduced into the virology section of the Clinical Microbiology and Public Health Laboratory, Cambridge. Internal quality control (IQC) samples were gradually included in the serological assays performed in the laboratory and supplemented kit controls and standard sera.**

***Methods*—From April 1991 to December 1993, 2421 IQC procedures were carried out with reference sera.**

***Results*—The IQC samples were evaluated according to the Westgard rules. Violations were recorded in 60 of 1808 (3·3%) controls and were highest in the IQC samples of complement fixation tests (25/312 (8%) of controls submitted for complement fixation tests).**

***Conclusions*—The inclusion of IQC samples in the serological assays performed in the laboratory has highlighted batch to batch variation in commercial assays. The setting of acceptable limits for the IQC samples has increased confidence in the validity of assay results.**

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Quality control measures permit the assessment of accuracy and precision in assays performed in the diagnostic laboratory. Their inclusion is designed to increase the probability that assay results reported by the laboratory are valid and that clinicians may confidently use those results when making diagnostic or therapeutic decisions.

For many years internal quality control (IQC) procedures have played an essential part in monitoring the day to day performance of assays used in clinical biochemistry departments. To date, most diagnostic microbiology laboratories (including microbial serology) have relied on control material supplied as an integral part of commercial assays. Although kit controls can be used to determine whether a particular assay run is acceptable they are not capable of monitoring batch to batch variation.

Internal quality control operates by detecting errors which may be random or systematic. Random errors are difficult to eliminate but may be minimised through training and supervision. System based errors may be related to the sensitivity, specificity, reproducibility, or stability of an assay. Although the results of serological assays may be expressed in terms of positivity or negativity, most are expressed as antibody titres or ratios (for example, in enzyme linked immunosorbent assays (ELISAs) as the ratio of the optical density (OD) of the test sample to the OD of the cut off control) or unit values or concentrations (for example, when international, national or arbitrary standards are included). Therefore, most results are expressed as numerical values making them amenable to simple statistical analysis.

Internal quality control samples are included in the assays performed in the laboratory and, when the results lie within pre-determined limits, are used to validate the test results.[1] IQC samples are also useful to monitor the performance of equipment and the stability of reagents indirectly. IQC samples can be international, national or local standard sera or pools of serum samples, well characterised in previous assays, exhibiting values within clinically significant ranges. A sufficient volume of each control is required so that IQC samples can be included in each test run enabling intra- and inter-batch assay variation to be monitored continuously.

From April 1991 to December 1993, 2421 IQC samples (assay controls), in addition to and independent of kit controls and standard sera, were gradually included in 17 serological assays performed at the Clinical Microbiology and Public Health Laboratory, Cambridge.

Methods

Local sera, well characterised "in house" in previous assays, or international or national serum standards with values within clinically significant ranges were included in a number of assays performed in the laboratory (table 1). The results obtained with patients' serum samples in these assays were only considered valid if the control values lay within their expected ranges.

The mean or target value and the standard deviation (SD) of the proposed assay control were calculated from the results obtained after testing the sample on 20 separate occasions. The SD is a measure of the scatter of values around a mean and is used to set acceptable limits for results subsequently obtained with the assay control. Control serum samples exhibit a normal stochastic distribution of results, therefore 95·5% of the values should lie within

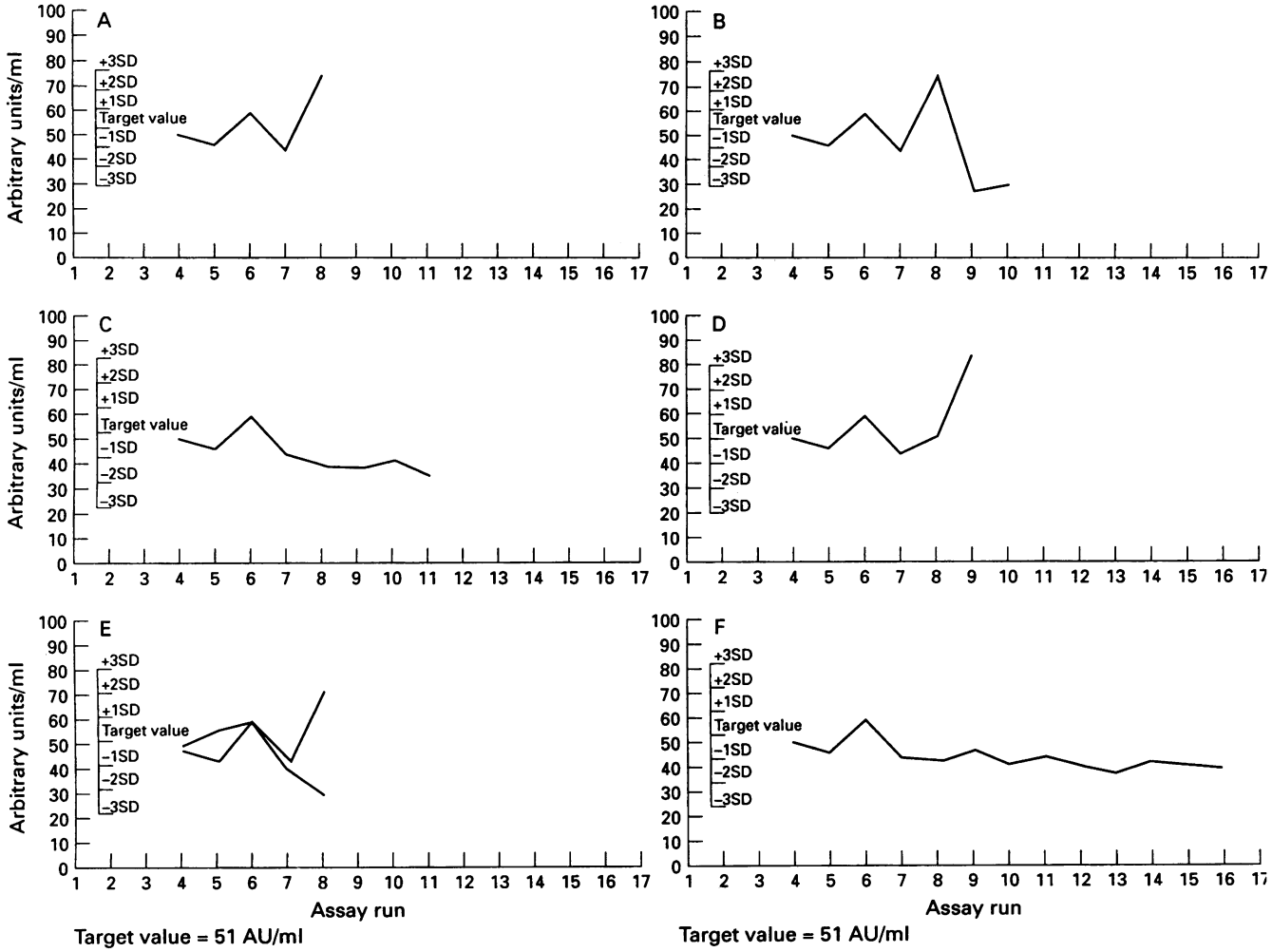

Figure 1 Violations of the Westgard rules. A–C: Warning rules (A: 1_{2SD}; B: 2_{2SD}; C: 4_{1SD}). D–F: Mandatory rules (D: 1_{3SD}; E: R_{4SD}; F: 10_x).

±2SD of the mean or target value and 99·7% within ±3SD.[2]

Shewhart plots[3] were drawn, with the target value, and the limit values of ±1SD, ±2SD and ±3SD delineated for each control used. Subsequent values obtained with the assay controls were plotted and Westgard rules were applied to determine the validity of each assay run. Westgard rules define specific performance limits and are designed to detect both random and systematic errors.[3] Three of the six commonly used Westgard rules are *warning rules* whose violation should trigger a review of test procedures, reagent performance and equipment calibration, and three are *mandatory rules* which, if broken, should result in the rejection of the results obtained with patients' serum samples in that assay. Figures 1A to 1C illustrate the three warning rules and figs 1D to 1F the three mandatory rules.

Warning rule 1_{2SD} (fig 1A) is violated if the control value exceeds the mean by ±2SD (an event likely to occur normally in less than 5% of cases). Warning rule 2_{2SD} (fig 1B) detects systematic errors and is violated when two consecutive control values exceed the target value on the same side of the mean by ±2SD. Warning rule 4_{1SD} (fig 1C) is violated if four consecutive control values exceed the same limit (mean +1SD or mean −1SD) and may indicate the need to perform instrument maintenance or reagent calibration.

Mandatory rule 1_{3SD} (fig 1D) applies when

Table 1 IQC material used for assay controls

Assay	*Control sera*
HIV 1+2 antibody ELISA	HIV 1 low positive (DMR)*
HIV 1+2 antibody ELISA	HIV 2 low positive (DMR)
HAV total antibody ELISA	Pool of positive sera
HAV IgM ELISA	Pool of positive sera
HBsAg ELISA	Serum containing 1 IU/ml
HBsAg ELISA	Serum containing 100 IU/ml
HBsAg ELISA	Serum containing 1000 IU/ml
anti-HBs ELISA	Serum containing 10 mIU/ml
anti-HBs ELISA	Serum containing 120 mIU/ml
anti-HBs ELISA	Serum containingg 600 mIU/ml
anti-HBc ELISA	Pool of positive sera
HCV antibody ELISA	Positive control serum (DMR)
Rubella SRH	15 IU/ml serum (DMR)
Rubella latex agglutination	250 IU/ml serum
T gondii latex agglutination	Antibody positive serum (blood donation)**
Paul-Bunnell antibody	Pool of positive sera
L pneumophila RMAT	Pool of positive sera
HSV IgG ELISA	Antibody positive serum (blood donation)
CMV IgG ELISA	Antibody positive serum (blood donation)
VZV IgG ELISA	Antibody positive serum (blood donation)
Respiratory CFT	Blood donation A
Respiratory CFT	Blood donation B

* DMR: Division of Microbiological Reagents, Central Public Health Laboratory, London.
** Regional Blood Transfusion Centre, Cambridge.
All sera not designated DMR or blood donation were local "in-house" sera.
CMV = cytomegalovirus; CFT = complement fixation test; HAV = hepatitis A virus.

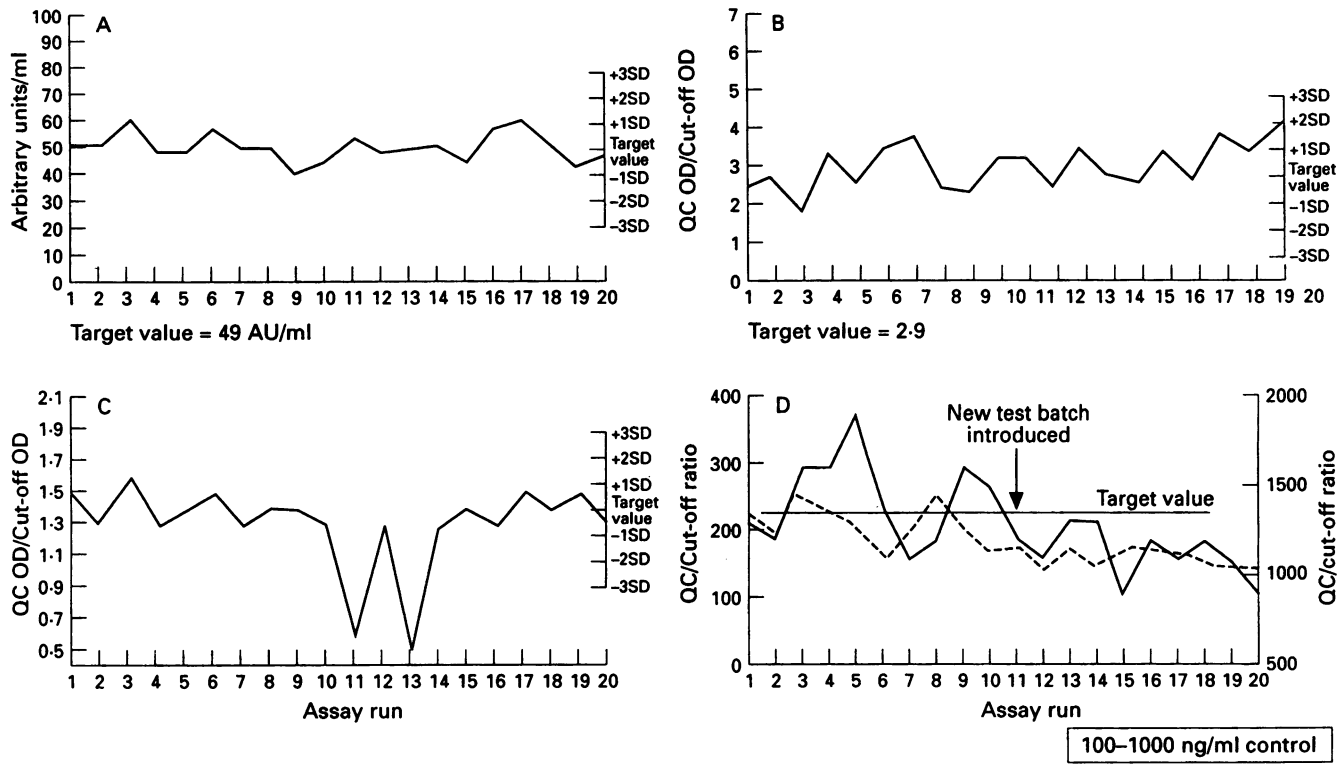

Figure 2 Shewhart plot of A: VZV IgG ELISA IQC (violations of the Westgard rules not detected); B: HIV antibody ELISA IQC (violations of the Westgard rules not detected); C: HCV antibody ELISA IQC (violations of the 1_{3SD} rule detected); D: HBsAg ELISA IQC (violation of the 10_x rule detected).

the control value exceeds the target value by ±3SD; the assay run is then regarded as out of control. Mandatory rule R_{4SD} (fig 1E) is a range rule within a run and is only applied when the control is tested in duplicate. The rule is violated when the difference in SD between the duplicates exceeds 4 SD. The 10_x (mandatory) rule (fig 1F) detects systematic error and is violated when the last 10 consecutive control values are on the same side of the mean or target value.

In the IQC scheme reported here the R_{4SD} range rule was not applied as the assay controls were tested singly.

Results

Examples of Shewhart plots illustrating the performance of the assay controls, used in the varicella zoster virus (VZV) IgG antibody ELISA, HIV 1+2 antibody ELISA, hepatitis C virus (HCV) antibody ELISA, and hepatitis B virus surface antigen (HBsAg) ELISA over 20 consecutive assay runs, are shown in figs 2A to 2D, respectively.

No violations of the Westgard rules were found with the assay controls in the VZV IgG ELISA or the HIV 1+2 antibody ELISA during the period illustrated in figs 2A and 2B. In the HCV antibody ELISA the 1_{3SD} rule was violated on two occasions triggering the rejection of those particular assay runs (fig 2C). A reduction in the sensitivity of the HBsAg ELISA after the introduction of a new batch of reagents was indicated by violation of the 10_x rule (fig 2D).

A total of 2421 assay controls were included in the serological assays performed between 1991 and 1993. The Westgard rules were applied to the results obtained with 1808 assay controls included in 891 assays. A total of 29 (1·6%) violations of the 1_{3SD} rule and 31 (1·71%) violations of the 10_x rule were detected (table 2). On 18 occasions, both the 1_{3SD} and the 10_x rules were violated at the same time. Table 3 illustrates the results obtained with the HIV 1 IQC sample in a HIV 1+2 (combined) antibody ELISA when a new batch of assay reagents was introduced. The increased sensitivity of the new batch changed the expected mean or target value of the IQC sample from 2·8 (OD of the sample:OD of the cut off value) to 4·4. Therefore, if the target value and the performance limits had not been recalculated, the results of two assay runs, performed with

Table 2 Assay controls and violations of the Westgard rules, 1_{3SD} and 10_x

Assay control	Number tested	Rule Violation	
		1_{3SD}	10_x
HBsAg (1 IU/ml)	278	8	7
(100 IU/ml)	318	3	7
(1000 IU/ml)	317	1	5
Anti-HBs (10 mIU/ml)	22	1	0
(120 mIU/ml)	22	0	0
(500 mIU/ml)	22	0	0
Anti-HBc	25	0	0
Anti-HCV	94	3	1
Anti-HIV 1+2 a	278	4	4
Anti-HIV 1+2 b	278	6	6
VZV IgG	108	2	1
CMV IgG	23	0	0
HSV IgG	23	1	0
Total	1808	29 (1·60%)	31 (1·71%)

Table 3 Violation of the Westgard rule 1_{3SD}, caused by introducing a new batch of assay reagents: HIV 1 control in the HIV 1+2 antibody ELISA

Assay run number	Ratio of OD sample to OD cut off	Assay batch	Control parameter			Acceptability	
			Mean	SD	Acceptable range	If control parameters recalculated	If control parameters NOT recalculated
			2·8*	0·95	0·05–5·6		
1	2·6	A				Yes	Yes
2	1·6	A				Yes	Yes
3	4·2	A				Yes	Yes
4	1·8	A				Yes	Yes
5	2·7	A				Yes	Yes
			4·4**	0·83	1·9–6·9		
6	5·2	B				Yes	Yes
7	3·5	B				Yes	Yes
8	5·0	B				Yes	Yes
9	3·3	B				Yes	Yes
10	4·6	B				Yes	Yes
11	3·5	B				Yes	Yes
12	3·7	B				Yes	Yes
13	4·0	B				Yes	Yes
14	4·2	B				Yes	Yes
15	4·6	B				Yes	Yes
16	5·7	B				Yes	No***
17	3·7	B				Yes	Yes
18	4·7	B				Yes	Yes
19	6·0	B				Yes	No***
20	3·9	B				Yes	Yes
			2·8	0·95	0·05–5·6		
21	3·3	A				Yes	Yes
22	4·1	A				Yes	Yes
23	3·7	A				Yes	Yes
24	4·1	A				Yes	Yes
25	3·6	A				Yes	Yes
26	3·2	A				Yes	Yes
27	1·7	A				Yes	Yes

* Calculated from the results from 20 assay runs with batch A.
** Control parameters recalculated using results obtained with batch B.
*** >3SD above the mean.

the new batch of reagents, would have been rejected through violations of the 1_{3SD} rule.

In the remaining 613 assays the Westgard rules were not applicable as the results were expressed either as an antibody titre or as positive or negative. In the *Legionella pneumophila* RMAT (57 assays), *Toxoplasma gondii* latex agglutination test (174 assays), hepatitis A virus total antibody ELISA (70 assays), hepatitis A virus IgM antibody ELISA (37 assays), and the Paul–Bunnell assay (57 assays), the results obtained with the assay controls were within the expected ranges (data not shown). In the rubella virus antibody assays, single radial haemolysis (79 assays) and the latex agglutination assay (113 assays) the controls were outside their expected ranges in one of 79 (1·26%) and four of 113 (3·53%) assays, respectively (data not shown). In the complement fixation tests two sera were included in 26 assay runs and were tested with influenza A, influenza B, adenovirus, *Chlamydia* sp, *Coxiella burnetii*, and *Mycoplasma pneumoniae* antigens. The results obtained with these controls show that 25 of 312 (8·0%) procedures were outside their expected ranges (table 4).

Discussion

The inclusion of IQCs with the ability to detect random and systematic errors permits the day to day monitoring of assay performance independently of kit controls and increases the probability that results obtained with clinical samples are valid.

The combination of Westgard rules used[14] and their warning or mandatory functions should be governed by the need to reject true errors but not to reject valid test results. If all test results were rejected when the assay control

Table 4 Results obtained with assay controls in the complement fixation tests

Control	Antigen	Titre	Acceptable range	Number tested	Number outside acceptable range	Per cent
A	Influenza A	8	<8–16	26	0	0·0
	Influenza B	<8	<8–8	26	1	3·8
	Chlamydia	16	8–32	26	5	19·2
	C Burnetti	<8	<8–8	26	0	0·0
	Adenovirus	32	16–64	26	5	19·2
	M pneumoniae	<8	<8–8	26	1	3·8
Subtotal				156	12	7·7
B	Influenza A	16	8–32	26	8	30·8
	Influenza B	8	<8–16	26	0	0·0
	Chlamydia	<8	<8–8	26	3	11·5
	C burnetti	<8	<8–8	26	0	0·0
	Adenovirus	<8	<8–8	26	1	3·8
	M pneumoniae	8	<8–16	26	1	3·8
Subtotal				156	13	8·3
Total				312	25	8·0

value was greater than the mean ± 2SD, then failure to allow for appropriate control values (for example, changing between batches of reagents) would result in a proportion of assay runs being falsely rejected.

Data obtained with the assay controls can also be used to set acceptable limits in assays in which anonymous samples are tested as part of an internal quality assessment scheme (IQAS) (see Part I). This can be accomplished by calculating the coefficient of variation (CV) from the mean and SD of the assay control. The CV can then be used to determine the range of acceptable values when a sample is repeated. This is particularly useful in assays where numerical or unit values are obtained. A discrepancy would be recorded if the difference between the two values was greater than the CV.

The CV should be calcuated from an assay control with a concentration of antigen or antibody that is close to that of the test sample, as the CV will change at different concentrations. In the anti-HBs assay the intermediate control had a CV of 15·2% and a discrepancy would be recorded if there was a greater than 15·2% difference between the results of the named and anonymous sample, whereas the high control had a CV of 13·5%.

Internal quality control operates by detecting errors. Random errors are impossible to eliminate but may be minimised through training and adherence to standard operating procedures. System based errors may be related to the sensitivity or specificity of an assay, the use of non-standard methods, poor equipment calibration, or the instability of reagents.

Although errors will inevitably occur, they can be significantly reduced through the introduction of IQC as an integral part of a comprehensive IQAS. Training must be continually updated with regular meetings to discuss quality control failures and successes, changes in standard operating procedures, the introduction of new assays, and the operation of new equipment. Publication of results within the laboratory and encouragement of staff to discuss technical problems and possible solutions can significantly improve the overall performance. Problems and inconsistencies in laboratory procedures can be identified through the IQAS and the use of IQC will increase the confidence in test results.

In conclusion, IQC procedures were performed as part of a comprehensive IQAS where some specimens received in the laboratory were resubmitted for testing anonymously. The conduct of and the results achieved with the IQAS are presented in Part I.

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