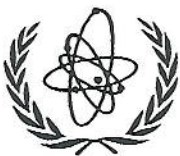# The External Quality Assurance Programme for use with the FAO/IAEA Rinderpest Competitive ELISA

Interim Report (EQAP/RP/1998A)

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THE FAO/IAEA EXTERNAL QUALITY ASSURANCE PROGRAMME FOR DISEASE DIAGNOSIS

THE EQAP FOR THE FAO/IAEA RINDERPEST COMPETITIVE ELISA; EQAP/RP/1998A.

SUMMARY

The External Quality Assurance Programme (EQAP) consists of three equally important items: the Questionnaire, the monitoring of the Internal Quality Control (IQC) data and the External Quality Control (EQC) test panel. The EQAP is conducted twice per year.

Twenty-eight laboratories participated in the fourth round of the FAO/IAEA rinderpest competitive ELISA, EQAP/RP/1998a. Of these 19 confirmed receipt of the EQA panel. The questionnaire, IQC and EQC results were returned by 16, 15 and 19 laboratories respectively and results are presented in this report.

Overall, results show that the majority of participating laboratories has an acceptable proficiency in conducting the rinderpest FAO/IAEA ELISA test. However, several laboratories still need to improve their IQC practices; i.e., they must concentrate on the monitoring and analyses of the IQC data and should regularly check the calibration of their ELISA equipment. With regard to the EQC test panel, 19 laboratories returned EQC results with an overall agreement of 100% for samples 1, 2 and 5 and of 95% for sample 3 and 4, giving an overall agreement of 98%. No sample had to be excluded. These EQC results are the best ever produced with the competitive Rinderpest ELISA.

The results of this round show that the EQAP is a valuable tool in the assessment of both the results obtained from and the proper functioning of the FAO/IAEA rinderpest ELISA. Furthermore, the EQAP can assist counterpart laboratories to establish and implement Quality Control/Quality Assurance (QC/QA) procedures for conducting the FAO/IAEA ELISA, and to advise on the implementation of similar QC/QA procedures in other laboratory activities.

Based on the results of the proficiency testing of the last 2 consecutive EQAP Rinderpest rounds 12 laboratories qualified as "provisionally recognized" and 2 laboratories qualified as "recognized".

1. INTRODUCTION

For any testing laboratory it is essential that assurance can be given that the test results produced are valid and reliable. It is also very important that results are comparable between different laboratories involved in similar assessments. Many diagnostic tests contain an element of subjectivity in their interpretation of results, and this renders both internal and external assurance difficult to operate. One of the distinct advantages of an ELISA-based system is the objectivity of reading the results and the ability to process data using a computer. Thus, it is possible to incorporate a high level of internal quality control for every ELISA test plate used. Indeed, Internal Quality Control is now a routine operation for most laboratories utilizing FAO/IAEA ELISA based testing systems [1].

Equally important is the determination whether a laboratory is giving the correct interpretation of the results even when the assay is shown to be functioning correctly. The procedures for establishing the assurance that the test results provided from a laboratory are reliable form the basis for an External Quality Assurance Programme (EQAP).

In 1990 an EQAP was carried out for the FAO/IAEA rinderpest indirect ELISA kit. These results have been published in detail [2]. In 1991, laboratories involved in the Pan African Rinderpest Campaign (PARC) switched over to a competitive ELISA rinderpest kit. In determining external quality assurance for the competitive ELISA, a test panel of 40 'unknown' sera was distributed among the

PARC laboratories. In 1992, 1993 and 1994, such test panels were sent out to a total of 20, 21 and 17 participating laboratories, respectively [3-5].

In September 1994, an FAO/IAEA consultants meeting was convened with the aim of extending and further improving the EQAP for veterinary laboratories in developing countries utilizing FAO/IAEA ELISA kits. The meeting focused on establishing procedures that would lead to "Recognition" of veterinary laboratories as competent in utilizing FAO/IAEA ELISA kits for specific diseases and tasks. The conclusions and recommendations of this meeting are contained in the report " Establishment of external quality assurance procedures for use with FAO/IAEA ELISA kits" [6].

This improved EQAP for veterinary laboratories is based on i) proof of the presence and use of Quality Assurance/Quality Control systems, ii) the continual satisfactory performance of processes and output, and iii) participation in external quality control test rounds. To obtain such proof, the EQAP consists of three critical elements as detailed below:

- *Survey Questionnaire:*

A questionnaire-based survey of individual laboratories is utilized to provide a regular system for monitoring the presence and use of the key quality elements. It is a mandatory requirement that all laboratories participating in the FAO/IAEA EQAP should complete and return such a questionnaire. The information gathered through the Questionnaire is updated at least once per year by the officer in charge[1] in the participating laboratory. The satisfactory presence of the relevant key elements is determined by the EQAP Coordinator in close collaboration with the appropriate Technical Officer of the Subprogramme in Animal Health and Production of the Joint FAO/IAEA Programme, and forms an essential part of the assessment of the participating laboratory.

- *Internal Quality Control (IQC):*

It is mandatory that laboratories fulfill the requirements for IQC as specified in the designated standard assay protocol. These include the use of appropriate reference standard control sera, the application of test acceptance criteria, the monitoring of test performance through the use of control charts, and the provision of relevant data for third party assessment. The IQC data are used to assess the repeatability and precision of the test conducted in that particular laboratory [7]. These data can be used by the test operator to detect trends and shifts in test performance, also [8].

- *External Quality Control (EQC):*

External Quality Control involves proficiency testing; i.e., inter-laboratory comparisons between two or more laboratories. For inter-laboratory proficiency testing, each laboratory conducts the designated test method on a defined panel of test samples, the EQC panel. Identical panels of test samples are dispatched to the participating laboratories for concurrent testing. The proficiency testing is conducted twice per year.

In February 1998 a "follow-up" consultants meeting entitled: "The FAO/IAEA External Quality Assurance Programme (EQAP) and Movement Towards a Generic Veterinary Diagnostic Testing Laboratory Accreditation Scheme" was convened to consider the design, impact and proposals for future implementation of the current FAO/IAEA EQAP for Animal Disease Diagnosis and make recommendations with regard to its central purposes and future direction. In addition, the Consultants considered the broader question of a generic QA "accreditation" scheme for veterinary diagnostic testing laboratories that could be made available through international, regional, or national organizations as

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[1] The officer responsible for the diagnosis and monitoring of rinderpest in an EQAP participating laboratory.

appropriate to the country of interest. This broader discussion was stimulated by the fact that few developed and no developing countries have nationally organized schemes to measure and recognize the QA systems and technical competence of veterinary diagnostic testing laboratories, but such a scheme is of vital importance to the quality of policy and decisions and actions taken on national animal health issues and the international trade of livestock and livestock commodities. It followed that, in the Subprogramme's role as a Collaborating Center to the Office International Epizooties (OIE, or World Animal Health Organization), it would be appropriate to consider the FAO/IAEA EQAP within the broader scope of an international scheme for veterinary diagnostic laboratory accreditation for two reasons: 1) to use information learned through the design and implementation of the FAO/IAEA EQAP to assist in the appropriate development of an international scheme and 2) to ensure that the FAO/IAEA EQAP objectives and procedures are in harmony with international QA guidelines as they develop in this area [9].

The objectives of the EQAP effort were and remain to a) develop reference data for the assessment of new FAO/IAEA diagnostic assay performance in the field, b) determine the user's general QA status and specify assay proficiency, c) enhance the user's QA awareness and culture, d) provide an organized and transparent mechanism to enhance the national and international credibility of the user's laboratory. In addition, the data developed through the FAO/IAEA EQAP can be used from a programmatic perspective as baseline data for a) the development of appropriate intervention strategies, b) monitoring project implementation, and c) evaluation of project impact during and after the project's conclusion.

It is recognized that the FAO/IAEA EQAP is programmatic in nature and is designed to assist counterpart laboratories to bridge the gap between what they have now and formal national or international recognition of Quality Management and technical competence.

The first round of the new EQAP for the FAO/IAEA rinderpest competitive ELISA (RP95a) started in October 1995. In total, 23 laboratories participated, all from the PARC programme. From the information collected from the Questionnaire, it was concluded that the routine monitoring of the IQC data by the test operator and the calibration of equipment needed more attention in most laboratories. The IQC analysis of the different laboratories showed that most laboratories produced reliable results. However, several laboratories needed to reduce the variation in IQC data, thus avoiding that the IQC data extend beyond the Upper and Lower Control Limits (UCL and LCL, respectively). There was an overall agreement on the EQC test panel results of 97%. Only 2 laboratories wrongly identified a positive test sample as negative. A comprehensive report on this round was distributed [10].

The second round of the EQAP for the FAO/IAEA rinderpest competitive ELISA (RP96a) started in August 1996 [11]. To assure confidentiality, a code number identified the participating laboratories.

The third round of the EQAP for the FAO/IAEA rinderpest competitive ELISA (RP97a) was started in July 1997 (RP97a). An overall agreement on the EQC test panel results of 93% was observed. Each laboratory received a **new code number** for this round [12].

Twenty-eight laboratories participated in the fourth round of the FAO/IAEA rinderpest competitive ELISA, EQAP/RP/1998a. Of these 19 confirmed receipt of the EQA panel. The questionnaire, IQC and EQC results were returned by 16, 15 and 19 laboratories respectively and results are presented in this report. Code numbers are the same as in the RP97a.

MATERIALS AND METHODS

Many parties are involved in the different steps, of which a round of the External Quality Assurance Programme consists of and great effort from each participant is needed to assure final success. An overview of the different steps and involvement for the Rinderpest EQAP is shown below in Fig. 1.
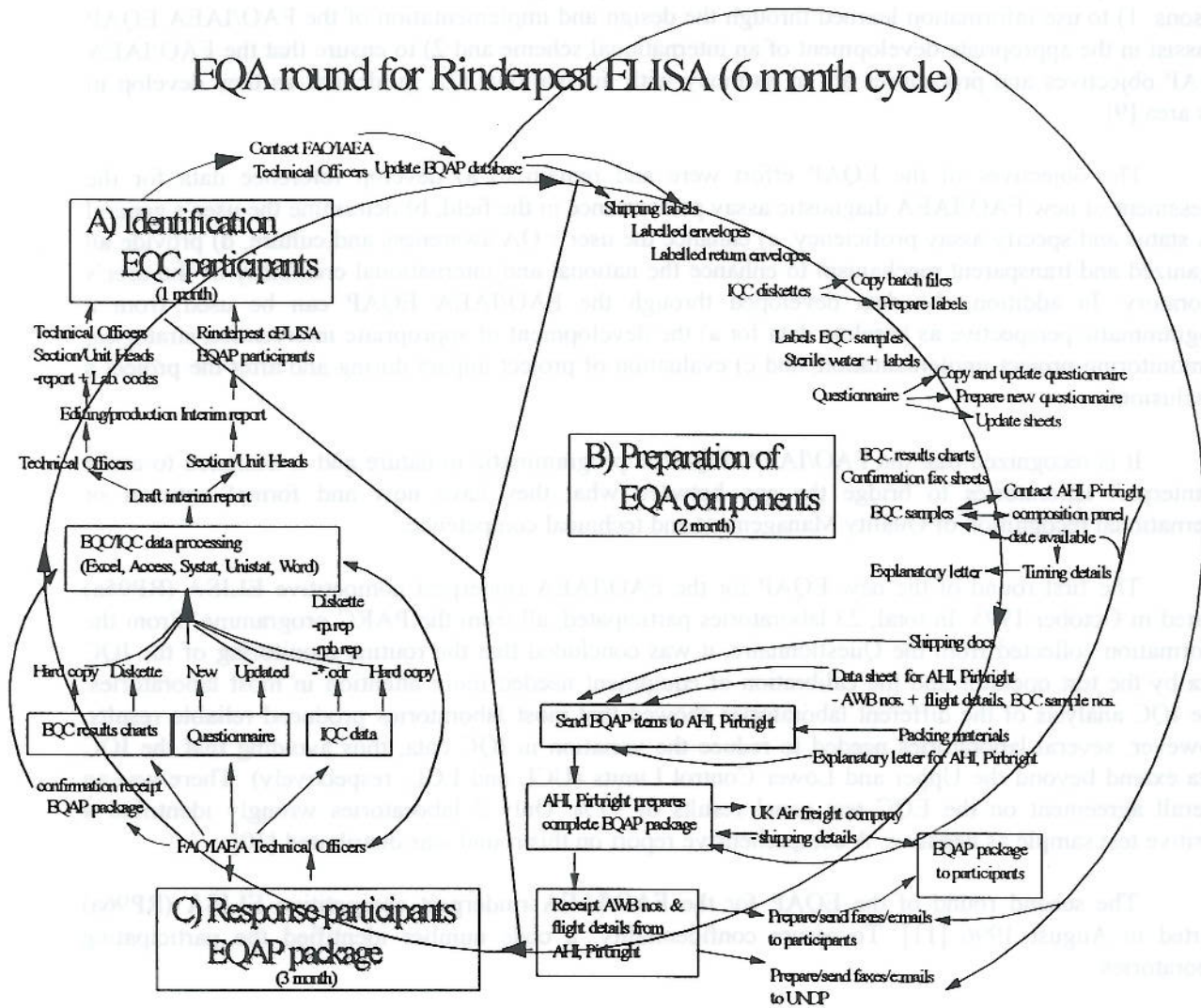

Fig. 1. Overview of the Rinderpest ELISA EQAP round.

A total of 28 laboratories participated in the fourth EQAP round for the FAO/IAEA rinderpest competitive ELISA. Of these 28 laboratories, 21 are located in Africa and 7 in the Middle East. Participants from Africa are part of the PARC programme and participants from the Middle East are part of the of the Middle East Rinderpest Eradication Programme (MEREP).

2.1. Questionnaire

Laboratories, which had already completed the Questionnaire during former EQAP rounds, received a copy of their completed Questionnaire and were asked to review and, if applicable, update the

information. Laboratories, which had not completed or returned the Questionnaire during former EQAP round were sent a new Questionnaire and were asked to complete and return this.

The Questionnaire consisted of the following 9 categories:

A : Administrative information
B : General information on other diagnostic activities performed in the laboratory
C : Laboratory facilities
D : Maintenance and calibration of equipment
E : Handling of test results
F : Monitoring of IQC data
G : Laboratory staff
H : Other quality assurance procedures within the laboratory
I : Availability, specifications and usage of computers

2.2. Internal Quality Control (IQC) Data

The IQC data provide valuable information on the test performance in an individual laboratory. The IQC data for the FAO/IAEA rinderpest competitive ELISA consists of the four replicates of the monoclonal antibody control (Cm), of the high positive control (C++), of the medium positive control (C+), and of two replicates of the negative control (C-) and the conjugate control (Cc). For IQC evaluation, the mean of the 4 values of the 4 wells is taken for the Cm, C++ and C+. The Cc and C- are tested in duplicate only, and the mean of the 2 values is taken.

Prior to incorporation into the competitive ELISA for rinderpest, the IQC samples were tested extensively under different circumstances by the WRL using the same ELISA. Given that the variation in optical density (OD) values and percentage inhibition (PI) values is normally distributed, ± 3 standard deviations (SD) were calculated and used to set the UCL (+ 3 SD) and the LCL (- 3 SD) of each IQC serum sample. These control limits are provided with the FACT SHEET of each new ELISA kit.

As part of the EQAP, the participating laboratories receive a diskette containing a 'batch' file ,which copies the 'instatqc' or 'eqstat.qc' file from the computer linked with the ELISA reader to the diskette. The 'instatqc' or 'eqstat.qc' file saves the IQC data of each ELISA plate read. This is applicable only for laboratories using the FAO/IAEA computer program RPEIA or EDI (ELISA Data Interchange).

If a laboratory was not using the EDI program to read and calculate the ELISA plates, the laboratory was requested to send printouts of the IQC data of the last 30-40 test plates in a table format.

For the IQC evaluation, the mean (± 2 SD) of the 4 values of the 4 wells per ELISA plate is taken for respectively the Cm, C++, C+, and the mean of the two values for the Cc and C- respectively.

2.3. External Quality Control Test Panel

The External Quality Control (EQC) test panels consisted of 5 freezes dried serum samples; 3 positive samples and 2 negative samples. The EQC test panels for this round were prepared and dispatched by Dr. John Anderson, Animal Health Institute (AHI), Pirbright, U.K. The serum samples were derived from experimentally immunized animals using rinderpest vaccine. All sera were undiluted and obtained from a single animal except for the sera used for Sample 2, which was a pooled positive sample of two immunized animals. The test samples, 1.0 ml serum per aliquot, were freeze-dried in one batch at AHI. The samples were tested prior to and after freeze-drying. Each test sample was subsequently labeled with a unique code number; hence each laboratory received uniquely coded unknown test panels.

The laboratories were requested to reconstitute the EQC samples on the day of testing using attached distilled water, and to treat and test these samples in a manner identical to that of their field

sera. The laboratories were requested to provide the EQC results in terms of positive or negative results for each sera and to submit the full computer print out of the test plate, thus including IQC data of the ELISA plate and the PI values for each EQC test sample.

2.4. Distribution

In July 1998 the EQC test panels were dispatched from AHI, U.K., principally by international courier to the UNDP offices in the different countries. All participating laboratories and their associated UNDP offices were notified by fax/telex/e:mail of the date of dispatch. The laboratories were urged to collect the EQC test panel from their respective UNDP Offices as soon as possible. It was not possible to ascertain the travel time of each test panel nor the condition it arrived in. However, since the sera were freeze-dried, it was not expected that the time or temperature experienced during shipment would add any unwanted variables.

To avoid loss or loss of track of EQA panels and enhance timely submission of results the following communication scheme was set up for future shipments[2]:
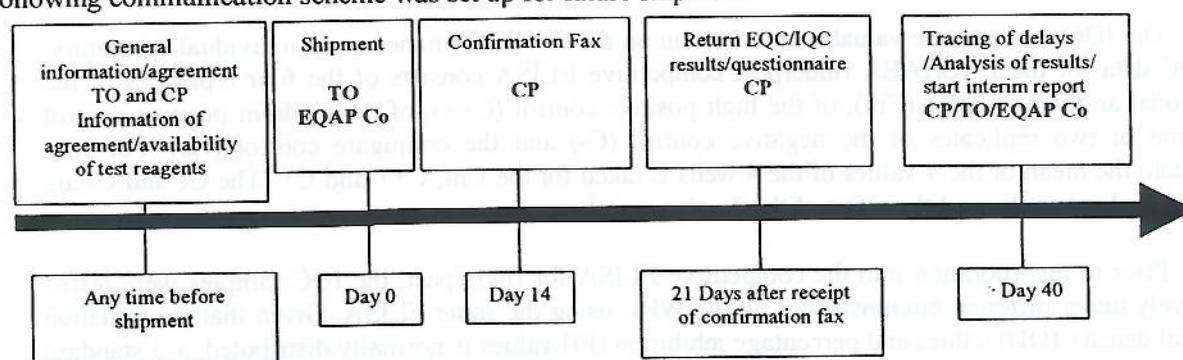
It is assumed that after a maximum of two weeks post-shipment the confirmation fax must be received by the TO. After receipt of the confirmation fax the laboratory is given three weeks produce and return results resulting in a maximum of 5 weeks from shipment of the EQA panel to the receipt of results.

Results from some laboratories were received very late by the EQA coordinator. These laboratories are requested to pay attention that their results are received on time during the next rounds to assure that they will be included in the report. In general, laboratories are requested to adhere more strictly to the deadline.

3. RESULTS

A total of 28 laboratories participated in this round. Nineteen laboratories (68%) confirmed receipt of the panel and of these 16 (84%), 15 (79%) and 19 (100%) laboratories sent questionnaire, IQC data and EQC results respectively. Six laboratories informed the EQAP Coordinator that they were not able to fulfill all requirements of the EQAP for various reasons. The main reasons being that a) the laboratory was still awaiting receipt of a new ELISA kit and/or b) had broken/missing equipment and/or c) the EQAP items got lost in the mail and/or d) there were customs clearance problems. An overview of the results received by the EQAP Coordinator is given in Table I.

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[2] CP = Counterpart; TO = Technical Officer; EQAP Co = EQAP Co-ordinator

Laboratory 6 supplied EQC results from the last round (RP97a)

TABLE I. OVERVIEW EQAP RESULTS OF THE FOURTH EQAP ROUND (EQAP/RP/1998A)

Lab.Code	Quest.	IQC	EQC	Lab.Code	Quest.	IQC	EQC
1	x	x	x	17			
2	x	x	x	18			
3				19	x		x**
4				20*			
5	x	x***	x	21			
6				22	x	x	x
7*				23	x	x	x
8			x	24	x	x	x
9	x	x***	x	25*			
10	x	x	x	26	x		x
11	x	x	x	27*			
12	x	x	x	28			
13			x	29	x	x	x
14	x	x	x	30	x	x	x
15		x	x	31	x	x	x
16			x	32			

IQC: Internal Quality Control data; EQC: External Quality Control data; Quest: Questionnaire
* Laboratory did not participate in this EQAP round, ** EQC data were from last round (RP97a), ***diskette did not contain sufficient or any IQC data

3.1. Questionnaire

Sixteen laboratories returned the completed and/or updated Questionnaire during this EQAP round (TABLE I.). The collected information categorized by subject per laboratory is attached in Attachment I. The information presented in *bold italic* format is new or updated since the last EQAP round. Accumulated and updated information of the questionnaire of the last four EQAP rounds of an overall number of 26 laboratories (laboratories 1, 2, 3, 4, 5, 9, 10, 11, 12, 13, 14, 15, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 and 31) is compiled in Attachment 1. No questionnaire information at all has been received from laboratories 6, 7, 8, 16 and 17.

Power supply/Air condition

Of 26 laboratories 16 (62%) reported problems with the power supply. Six laboratories have both types of power problems, namely "power cuts" and voltage "irregularities", 4 laboratories reported only power cuts and 5 laboratories indicated only voltage irregularities. Seven laboratories do not have any power problem. Regarding the length of period of power problems 7 laboratories have power cuts of less than 12 hours and 13 reported irregular periods. Asked about the frequency of power problems 2 laboratories reported weekly and 15 irregular periods of power problems. Fourteen laboratories use a stabilizer, 3 use a stabilizer but only for selected equipment (e.g. refrigerator). Seventeen laboratories have access to an emergency power supply for their refrigerator and freezer. Sixteen laboratories have access to a generator in case of a power failure. Eight do not have a generator. Sixteen laboratories have an air-condition (8 do not have air-condition). The average temperature was 25.52 °C with a variation of Min. 15°C to Max. 42°C.

Pipettes, Tips and ELISA readers

Most laboratories use pipettes from Biohit Proline® (15 labs) or Titertek (10 labs) followed by Finnpipette (8 labs), Gilsson Pipettman (6 labs) and Socorex (1 lab) and tips from Biohit Proline® (12 labs) and Micronics (9 labs). Less frequently tips from Finntips, Conetreff, Volac 200 and Costar are in use. Twenty-three and 21 laboratories use 5-50ul single and multichannel pipettes respectively. Eighteen

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and 20 laboratories use 50-250ul single and multichannel pipettes respectively. Eight and 5 laboratories use 250-1000ul single and multichannel pipettes respectively .

The Multiskan Plus Mark II is the most commonly used ELISA reader (15 labs), followed by the BDSL Immunoscan Plus (9 labs) and Multiskan MCC/340 (4 labs).

Handling of test results

With regard to plate reading and calculation of ELISA results, 23 laboratories use the EDI programme: Ten laboratories are using EDI version 2.11., 9 laboratories are using RPEIA version 1.03 , 4 laboratories use ED 2.2., 3 laboratories are using RPEIA version 1.01., 2 laboratories use Procomm and 2 laboratories calculate results manually. Some laboratories indicate to use RPEIA and EDI together.

Twenty laboratories have a computerized system SID (17 labs), Panacea (6 labs), EPI-info (2 labs), Access (2) or a spreadsheet programme[3], to link the test results with other details of the field samples.

The majority of the laboratories (13) use the IQC data to determine whether the ELISA plate readings are 'within' limits and can be accepted. Two laboratories indicated that the IQC data are monitored using the 'instatqc' programme. Eight laboratories reported that they do not undertake any IQC monitoring.

Sample storage

All laboratories (26 labs) store serum samples at -20°C, in most cases using Cryopreservation vials (13 labs), Vacutainers (7 labs), Nalgene storage system (7 labs), Micronics (7 labs), Serum storage plates (3 labs) or others e.g. 10 ml tubes (1 lab). Twelve laboratories have access to -80°C freezers and 7 to Liquid Nitrogen facilities. Nineteen laboratories reported keeping a serum bank ranging from 500 to 45.000 samples with an average of 11.328 samples.

Computer/Data Processing

Nineteen laboratories reported that a computer is used for reading of ELISA plates and/or storage of data. For the first time more Pentiums (9 laboratories) than 486 processor-equipped-computers (6 laboratories) are in use. Three laboratories use 386 and 1 laboratory uses a 286 CPU computer. Hopefully this trend will continue.

Water quality and equipment calibration

Twenty-two laboratories use distilled water. Nineteen laboratories have access to deionized and 11 laboratories to bi-distilled water. Nineteen laboratories reported that filters and cartridges are changed in the following pattern: once per year (2 laboratories), twice per year (6 laboratories), every three months (5 laboratories), every month (1 laboratory), three times per month (1 laboratory). Six laboratories reported that cartridges and filters are changed following the manufacturers recommendations (1 lab), conductivity control (1) or "when needed" (4 labs).

Twenty-two laboratories reported that no equipment calibration (ELISA reader and pipettes) procedures are carried out. Two laboratories undertake calibration procedures following the manual. One laboratory checks the accuracy of its ELISA reader by comparing OD readings with another ELISA reader.

3.2.   Internal Quality Control Data

Fifteen laboratories: 1, 2, 5*, 9*, 10, 11, 12, 14, 15, 22, 23, 24, 29, 30 and 31 returned IQC data but information only from 13 laboratories could be evaluated. Evaluation from some laboratories

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[3]   SID (Sero-monitoring Information Database), Panacea and EPI-info are epidemiological computer programs

* IQC data could no be evaluated due to lack of information e.g. diskette empty, only one printout

e.g. laboratory 14, 30 and 31 show that the assay is well within limits. Intra- and interassay variation is well under control and also the statistical parameter show a good degree of consistency. IQC results from the majority of laboratories e.g. laboratories 2, 12, 15, 22, 23, 24 and 29 show that there are still some outlayers and further adjustment and consistency is required to maintain the assay under control. Finally there is a group of laboratories e.g. laboratory 10 and 11, which apparently needs urgently substantial adjustment in the performance of the ELISA because almost all data fall outside the upper or lower control limits. In these cases it is obvious that the assay is not under control and must be adjusted as soon as possible. There is a general trend indicating low OD values for the Cm. These values are often very close or below the lower control limit (OD < 0.4). Possible reasons for this may the use of old and/or not properly stored reagents or if encountered in a freshly supplied assay wrong dilution/concentration of reagents. The producer has been informed about these findings.

3.3. External Quality Control Test Panel

EQC results of 19 laboratories have been analyzed and are presented in this report. Table II shows the qualitative results per laboratory; i.e., the determination whether a serum sample is considered to be negative or positive in the assay.

TABLE II. QUALITATIVE RESULTS OF THE EQC TEST PANEL PER LABORATORY
(50% cut-off)

Lab. Code	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	Pos.	Pos.	Pos.	Neg.	Neg.
2	Pos.	Pos.	Pos.	Neg.	Neg.
5	Pos.	Pos.	Pos.	Neg.	Neg.
8	Pos.	Pos.	Pos.	Neg.	Neg.
9	Pos.	Pos.	Pos.	Neg.	Neg.
10	Pos.	Pos.	Pos.	Neg.	Neg.
11	Pos.	Pos.	Pos.	Neg.	Neg.
12	Pos.	Pos.	Pos.	Neg.	Neg.
13	Pos.	Pos.	Pos.	Neg.	Neg.
14	Pos.	Pos.	Pos.	Neg.	Neg.
15	Pos.	Pos.	Pos.	Neg.	Neg.
16	Pos.	Pos.	Pos.	Neg.	Neg.
22	Pos.	Pos.	Pos.	Neg.	Neg.
23	Pos.	Pos.	Pos.	Neg.	Neg.
24	Pos.	Pos.	Pos.	Pos.	Neg.
26	Pos.	Pos.	Pos.	Neg.	Neg.
29	Pos.	Pos.	Neg.	Neg.	Neg.
30	Pos.	Pos.	Pos.	Neg.	Neg.
31	Pos.	Pos.	Pos.	Neg.	Neg.
Total No. of Labs	Agreement	Agreement	Agreement	Agreement	Agreement
20	100%	100%	95%	95%	100%

TABLE III. QUANTITATIVE RESULTS OF THE EQC TEST PANEL PER LABORATORY (PI)

Lab. Code	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	87	75	69	29	5
2	82	66	65	33	34
5	83	70	59	27	20
8	82	69	54	33	13
9	89	69	56	21	20
10	85	81	74	48	30
11	89	72	69	36	14
12	87	63	56	35	13
13	75	61	53	25	31
14	81	76	68	42	25
15	93	81	72	41	26
16	90	79	66	38	12
22	75	71	56	45	10
23	88	73	65	35	38
24	96	88	84	61	29
26	90	78	68	45	27
29	74	61	47	15	5
30	88	71	55	32	35
31	88	80	71	40	37

Table III shows the quantitative data; i.e., the percentage inhibition (PI) values for the EQC test samples as determined and submitted by the laboratories.

Table IV shows the summary statistics of all laboratories. The EQC test panel results as submitted by the participants show for samples 1, 2 and 5 100 % agreement for each sample. Ninety-five percent of agreement was achieved for samples 3 and 4. This is the best result achieved with EQC samples up to date.

TABLE IV. SUMMARY STATISTICS OF THE EQC TEST SAMPLES.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean	85	73	64	36	22
Standard Error	1	2	2	2	2
Median	87	72	65	35	25
Standard Deviation	6	7	9	10	11
Sample Variance	37	53	84	109	116
Range	22	27	37	46	33
Minimum	74	61	47	15	5
Maximum	96	88	84	61	38
Count	19	19	19	19	19
Coef. Variation (%)	7	10	14	29	48

Figures 3a-e show the frequency distributions for the EQC test results. The results of the individual laboratories are presented by their respective laboratory code number in each column. These histograms provide a visual reference for each laboratory's position within the distribution of all results. The horizontal line shows the cut-off value (50%).
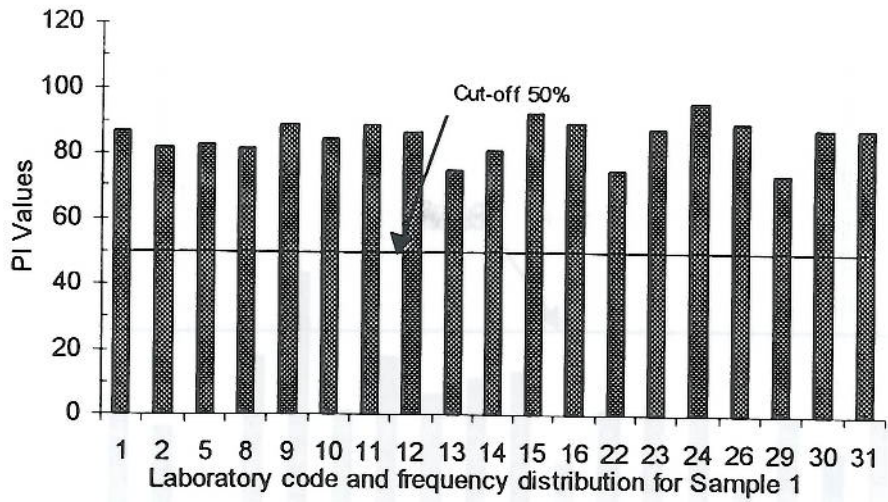
10

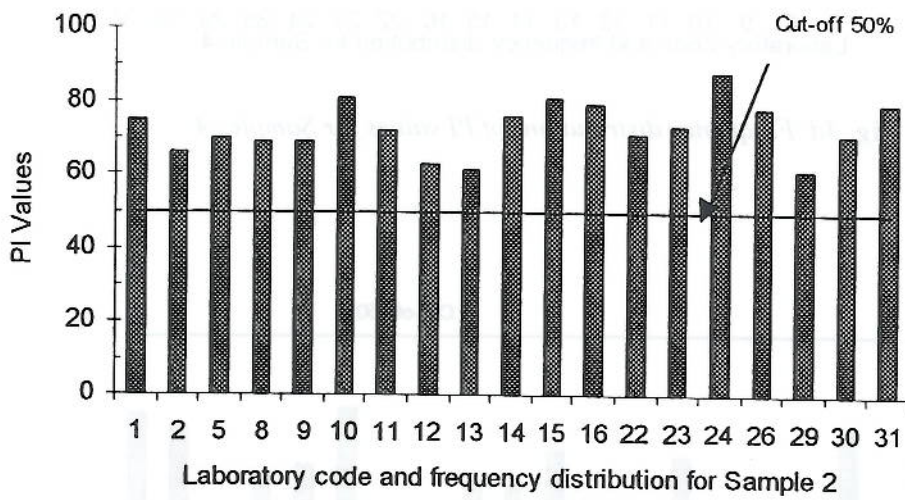*Fig. 3a. Frequency distribution of PI values for Samples 1.*

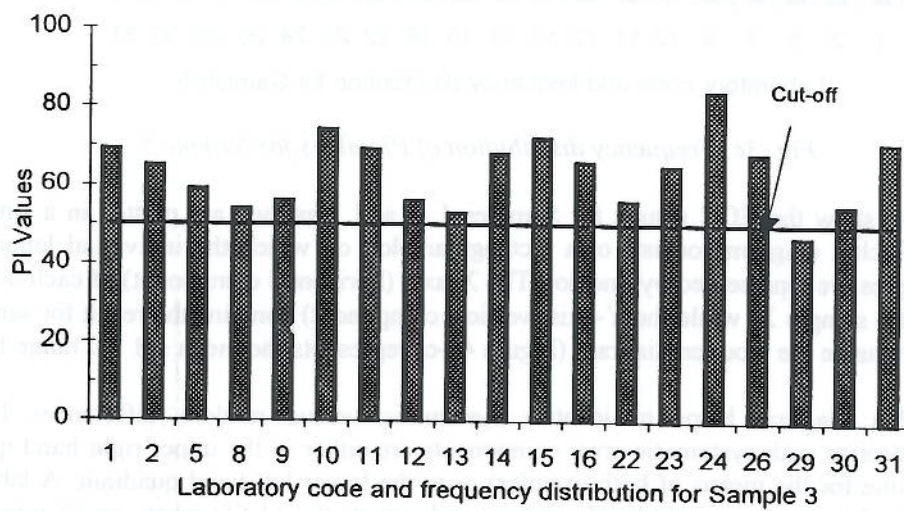*Fig. 3b. Frequency distribution of PI values for Samples 2.*

Fig. 3c. Frequency distributions of PI values for Samples 3.
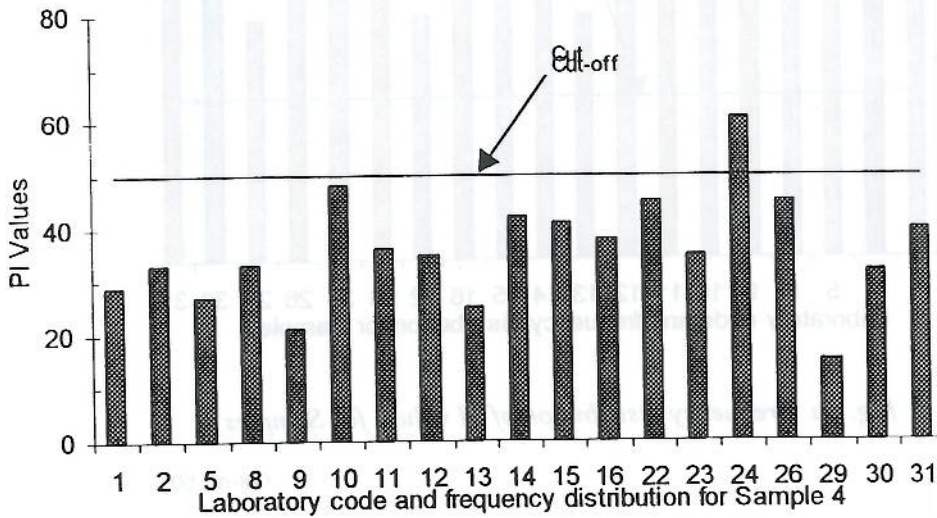
11

Fig. 3d. Frequency distributions of PI values for Samples 4.
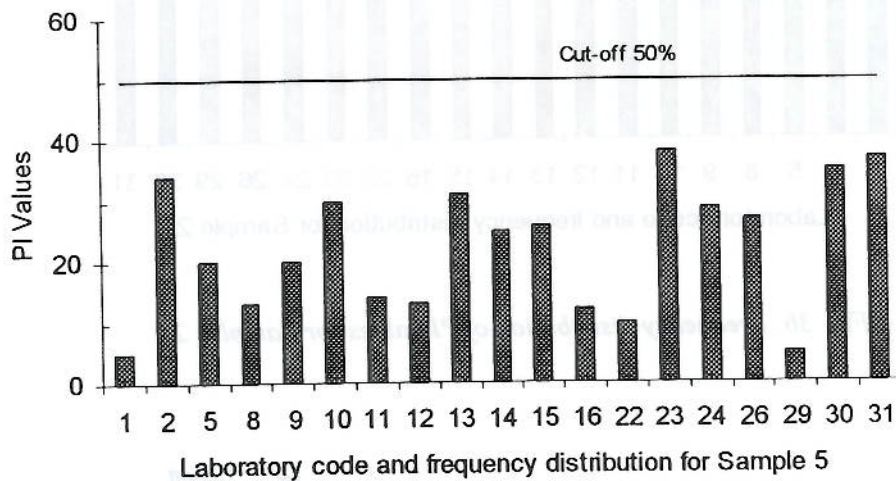

Fig. 3e. Frequency distribution of PI values for Sample 5.

Figures 4a-c show the EQC results for Samples 1, 2 and 3 as they are plotted in a simplified Youden diagram. Such a diagram consists of a rectangular plot, on which the individual laboratory's results for two samples are represented by one dot. The X-axis (horizontal component) of each dot is the laboratory's result for sample X, while the Y-axis (vertical component) contains the result for sample Y. The small rectangle inside the Youden diagram (Figure 4a-c) represents the mean ± 1 SD range for both samples.

The Youden diagram helps to identify systematic versus random differences between laboratories. Laboratories with systematic error components are either in the upper right hand quadrant (as formed by the line for the means of both samples) or in the lower left-hand quadrant. A laboratory with results positioned in the upper right hand quadrant and outside the +1 SD range, could indicate that the laboratories values for both positive samples are too high, possibly due to an increased level of diagnostic sensitivity of the assay in that laboratory. A laboratory positioned in the lower left quadrant of
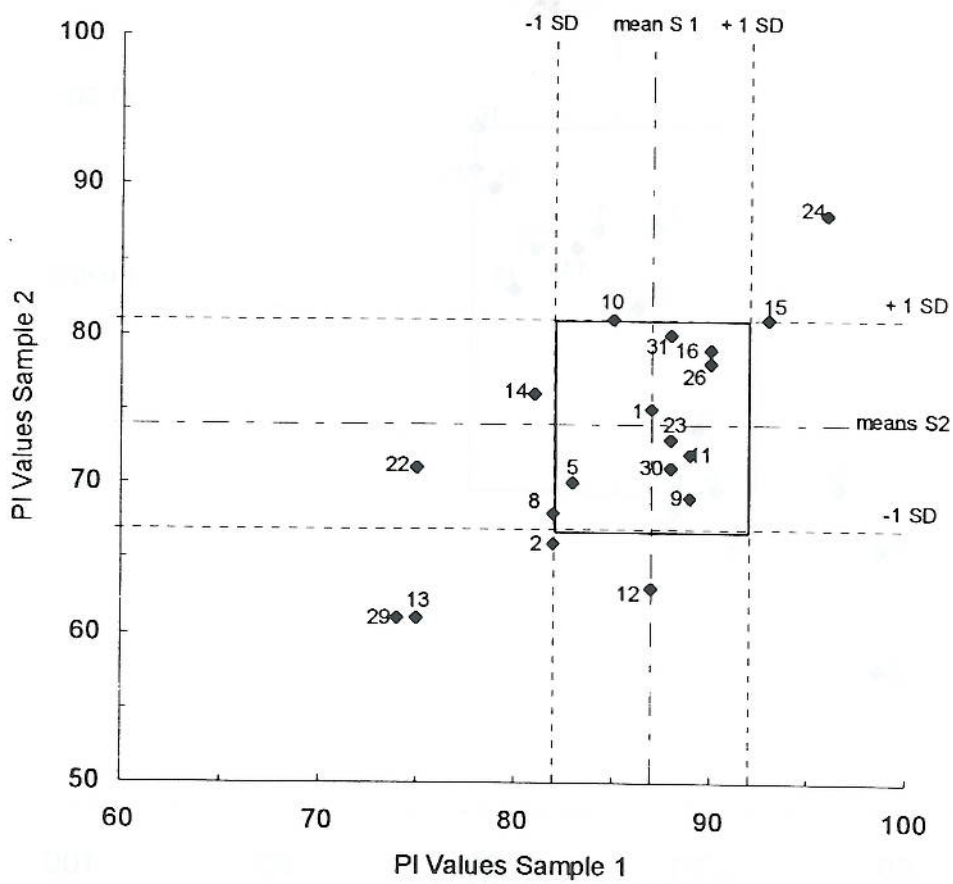
12

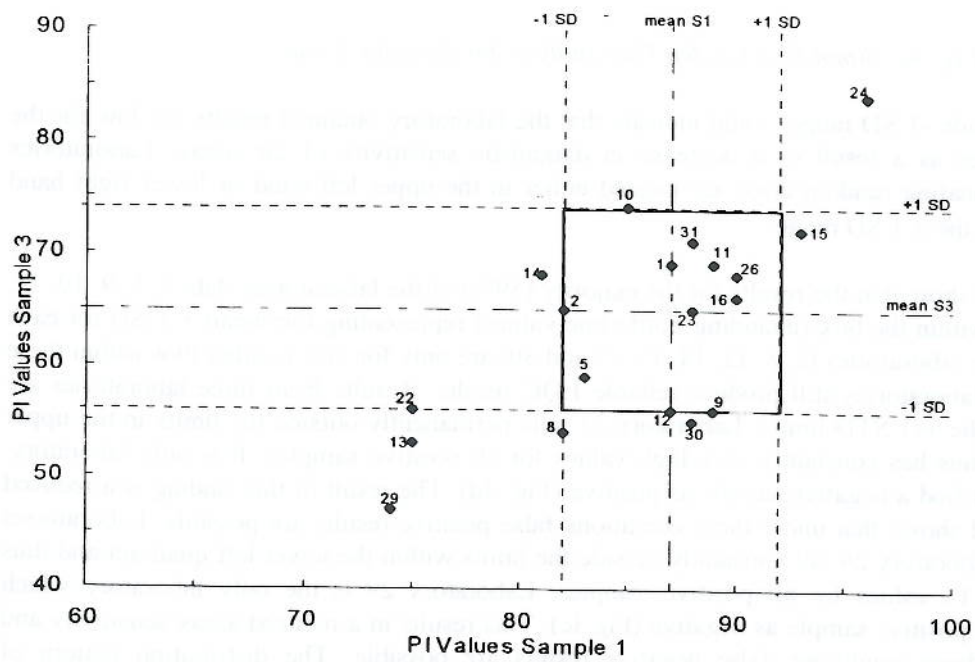Fig. 4a. Simplified Youden Plot analysis for Samples 1 and 2.

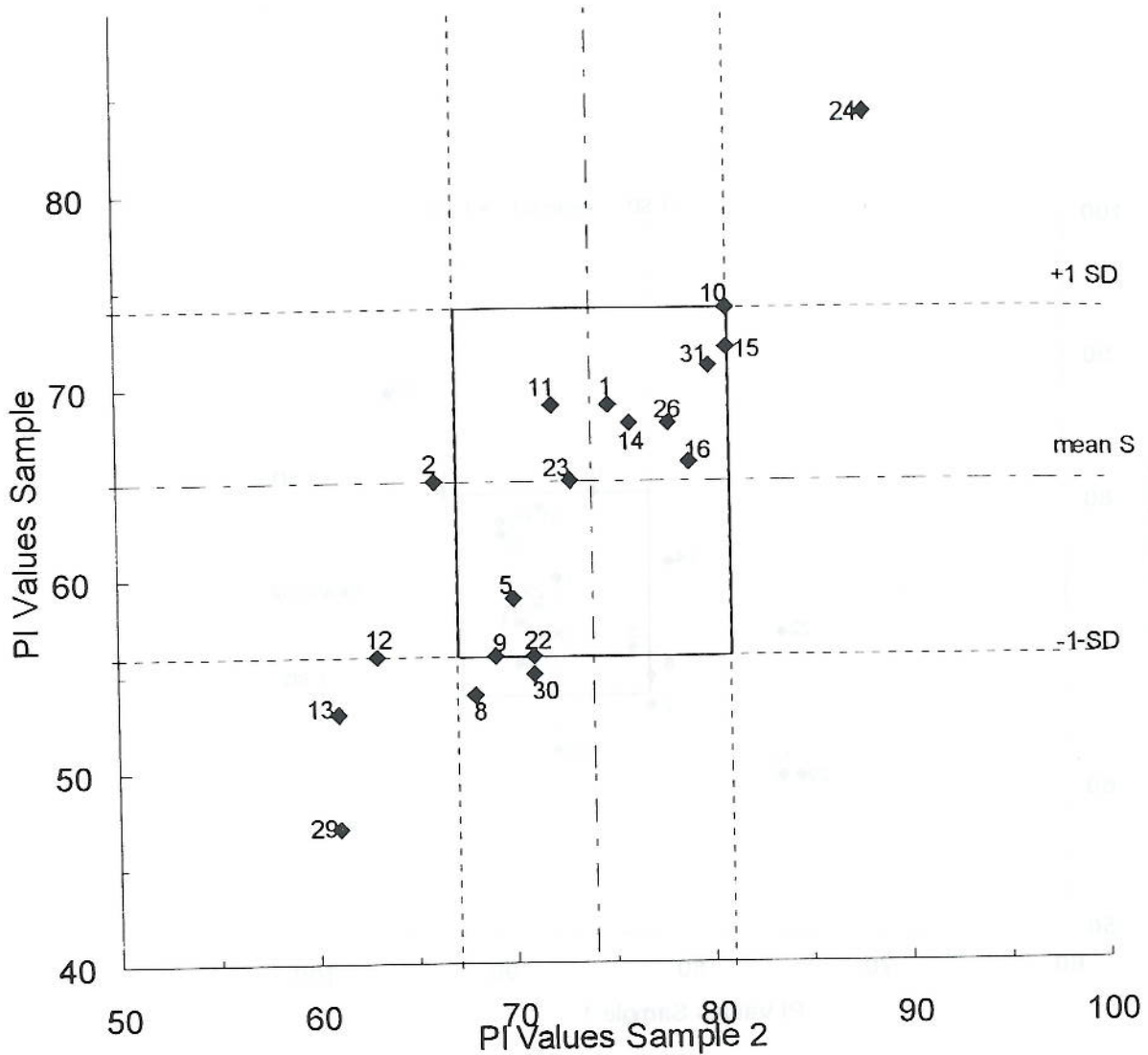Fig. 4b. Simplified Youden Plot analysis for Samples 1 and 3.

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Fig. 4c. Simplified Youden Plot analysis for Samples 2 and 3.

the diagram and outside -1 SD range, could indicate that the laboratory obtained results too low for the both positive samples as a result of a decrease in diagnostic sensitivity of the assay. Laboratories reporting results indicating random error are located either in the upper left hand or lower right hand quadrant and outside the ± 1 SD range.

Figures 4a-c show that the results for the majority (59%) of the laboratories (lab. 1, 5, 9, 10, 11, 16, 23, 26, 31) fall within the box (including borderline values) representing the mean ± 1 SD for each of the samples. Some laboratories (2, 8, 12, 14, 15, 22 and 30) are only for one Youden Plot within these limits. All of these laboratories still produce reliable EQC results. Results from three laboratories are completely outside the +-1 STD limits: Laboratory 24 falls permanently outside the limits in the upper right quadrant and thus has constantly very high values for all positive samples. It is only laboratory, which wrongly identified a negative sample as positive (Fig. 3d). The result of this finding is a reduced assay specificity and shows that under these conditions false positive results are possible. Laboratories 13 and even more laboratory 29 fall constantly outside the limits within the lower left quadrant and thus has constantly low PI values for all positive samples. Laboratory 29 is the only laboratory which wrongly identified a positive sample as negative (Fig.3c). This results in a reduced assay sensitivity and shows that under these conditions false negative results are possible. The distribution pattern of laboratories indicates mainly problems of systematic errors (outside upper right or outside lower left) suggesting that laboratories 24, 29 and 13 should critically examine possible reasons for this type of

errors e.g. water quality, wrong/old/dirty filter etc.. The analysis of the Youden Plots does not indicate any sign of random error. Comparing the overall results from the Youden plot analysis with the RP96ac(55%) and RP97a (35%) this round achieved the best results (59%) where most laboratories fall within the 1 STD limit.

4. DISCUSSION AND CONCLUSIONS

Quality Control/Quality Assurance procedures are essential to testing laboratories as they provide confidence in test results, as well as informing test operators of unacceptable trends in assay performance. The assurance that the test results produced are reliable is not only of importance to the test operator or owner of the animal, but for all outside interested parties. To achieve this, the Animal Production and Health Subprogramme of the Joint FAO/IAEA Division has initiated an External Quality Assurance Programme.

The return of 19 (68%) EQC test panel results was still acceptable, although the objective is to have 100% return. The EQC test panel was distributed using a courier service.

4.1. Questionnaire

A compiled summary of the information given in the questionnaire is reported in Attachment 1.

QA/QC practices

It can be concluded that several laboratories still need to improve their IQC practices; i.e., they must pay attention to the monitoring and analyses of the IQC data and should regularly check the calibration of their ELISA equipment. Guidelines have been developed to assist counterparts in checking the calibration of pipettes and ELISA readers, and a TECDOC entitled: "Internal Quality Control (IQC) of Competitive Enzyme Linked Immunosorbent Assay for Measurement of Antibodies against Rinderpest and Peste des Petits Ruminants Viruses." has been prepared by the subprogramme of the Joint FAO/IAEA for the routine monitoring of IQC data using Control Charts [8].

Handling of test results, EDI

In general results indicate that there is an acute need to install and use the latest version of EDI. e.g. EDI 2.2. EDI 2.3.. These versions store IQC data on a separate eqstat.qc files, which ease identification, retrieve and manipulation of data considerably. From the information supplied only 4 laboratories use EDI version 2.2.. Eight laboratories (laboratory 2, 5, 12, 13, 15, 18, 27 and 30) indicate not to monitor their IQC data. The Technical Officer will assure the distribution, installation and use of the latest version of EDI.

Power supply and temperature fluctuations

In general results indicate that the situation for the power supply has improved e.g. there are less power problems encountered. At the same time there is a trend indicating that more laboratories are better equipped with alternative power supplies e.g. they are able to switch over to an emergency power net or have access to a generator. But there is still a critical situation encountered in 6 laboratories (2, 11, 12, 20, 21 and 23) because they indicate to have power cuts but have no access to an emergency power supply nor to a generator. This condition has a direct impact on the laboratories with air-condition. Temperature fluctuations from 15 °C - 42 °C may be a reason for inconsistency of results and improvement in this area is necessary.

Equipment calibration

The majority of the laboratories (22 labs) informed that they do not calibrate ELISA equipment (pipettes and reader). This is a very critical since pipetting errors may be the reason for all kind of variation in an assay. All ELISA equipment (reader, pipettes etc.) should be checked and, if necessary calibrated following the procedures as outlined by the producer or the respective protocol or manual. The ELISA reader should be checked with a Standard Absorbance plate at least during each visit of the Technical Officer. A document entitled "The Laboratory Wizard – A practical loose-leaf edition guide for all who want to share and update ordinary information reported from technical staff of diagnostic laboratories world-wide" [17] is available to assist in laboratory calibration and maintenance procedures.

Nineteen laboratories change filters and cartridges, but only 2 laboratories do so after a control (e.g. conductivity control or manufacturer's recommendation). A more uniform and controlled approach towards criteria and frequency in change of filters and cartridges is necessary. The quality of water must be checked on a more consistent basis to eliminate this possibility of assay variation.

Nineteen laboratories reported to use a computer for the reading of ELISA plates and storage of data. For the first time more Pentiums (9 laboratories) than 486 processor-equipped-computers (6 laboratories) are in use. Hopefully this trend will continue. Some laboratories did not supply any information on the availability of computers and it is not clear how they produce and store the data.

It can be concluded that several laboratories still need to improve their IQC practices; i.e., they must pay attention to the monitoring and analyses of the IQC data and should regularly check the calibration of their ELISA equipment. Guidelines have been developed to assist counterparts in checking the calibration of pipettes, ELISA readers and other laboratory equipment [13].

4.2. IQC Data

The analyses of IQC data of the individual laboratories are reported in Attachment II.

As not all laboratories were using the same ELISA kit batch and it was not known by the EQAP coordinator when a laboratory started to work with a new kit, the Upper and Lower Control Limits as shown in the Control Charts in this report are average Upper and Lower limits. Furthermore, any additional information on a specific ELISA plate was also not known by the coordinator; e.g., identification of the test operator, date of testing, and the batch number of the ELISA kit per ELISA plate. This information is very important and necessary for a correct IQC evaluation and should be written on the Control Chart. For instance, if an empty ELISA plate is run several times to test whether a system is functioning, such plates should be properly identified on the Control Charts.

As part of establishing Quality Control/Quality Assurance procedures within a laboratory, the test operators should maintain Control Charts themselves [8]. For the EQAP rounds in future, the laboratories will be asked to submit copies of such Control Charts with all relevant information of the last ± 40 plates for external assessment.

Obviously the test operator should aim to minimize both the 'within plate' and the 'between plates' variation. Furthermore it must be emphasized again that, in the possible event of the value of an Internal Quality Control sample, especially the OD value of the C++, falling outside the UCL and LCL, and the assay still giving a 'correct' positive or negative value to the test samples, the results of that assay should be considered questionable. The assay must be carefully examined in this situation and the cause for the failure to obtain controls within the limits, determined and eliminated.

The latest EDI version should be installed in the computer as soon as possible and older versions (e.g. EDI 2.1., RPEIA) should be deleted. EDI will during installation overwrite any present older EDI version and will also create a new subdirectory 'eqstat.qc' for the automatic storage of IQC data. The existing subdirectory 'Instatqc' or 'Eqstat.qc' and its file(s) will remain unchanged.

In general, the laboratories are producing reliable results as the majority of the IQC results are within the UCL and LCL with acceptable variation. However, some laboratories should take notice of the "within plate" and "between plate" variation in their IQC results and should initiate measures to reduce that variation. The most likely causes for variation of the IQC data are:

i) Water quality

Data from the Questionnaire shows that the majority of the laboratories are using distilled and deionized water. The frequency of cleaning or replacing filters and columns varies from laboratory to laboratory, depending on the type of distiller/deionizer used. The test operator should ensure that the filter/columns are changed as advised in the manufacturer's documentation. If the test operator still suspects water quality to be a problem, it is suggested that an alternative (if available) water source is utilized for the ELISA and results then compared.

ii) The test operator
Where the test is performed by more than one operator, it is almost inevitable that greater variation in results will occur. As long as test operators obtain good test results, there is no problem. However, as part of Quality Assurance, the laboratory should aim for high repeatability and precision. Therefore, it is suggested that test operators carefully compare their results with respect to IQC data and identify any differences. In this way, possible variations in the technique of performing the ELISA may be highlighted and necessary steps taken to decrease the variation.

iii) Pipetting precision
This is an important factor in variation, particularly where small volumes are being pipetted. Often it is the major cause of the differences in variation observed between test operators.

As explained in detail in the ELISA manual, the assay data expressed in OD and PI values for the Cm and the assay data expressed in PI values for the C++, C+, C- and Cc, are used to determine whether or not the test has performed within acceptable limits of variability, and therefore whether or not the test data may be accepted for any given ELISA plate.

While it is likely that, if the value of a control falls just outside the Upper and Lower Control limit, the assay will still give a correct positive or negative value to the test sera, the results as such are questionable. The assay must be examined in this situation and the cause for the failure to obtain controls within the limits determined and corrected. It is not acceptable to carry on testing sera with controls consistently falling outside the limits. Something is clearly wrong and it must be investigated and resolved.

4.3. The EQC Test Panel

With regard to the EQC test panel, 19 laboratories returned EQC results with an overall agreement of 100% for samples 1, 2 and 5 and of 95% for sample 3 and 4, giving an overall agreement of 98%. No sample had to be excluded. These EQC results are the best ever produced with the competitive Rinderpest ELISA.

Overall, the results of this EQAP for the FAO/IAEA rinderpest competitive ELISA show that each participating laboratory had a high proficiency for conducting the assay.

4.4. EQAP/RP/ 1997a and 1998a.

Provisional recognition
Since participation and submission of correct results of the proficiency testing for at least two consecutive rounds is defined as a key element for the EQA programme 12 laboratories qualified for the status "Provisionally Recognized Laboratory".
These laboratories are: 1, 5, 8, 9, 10, 12, 13, 14, 15, 24, 26 and 31.

Recognition
Two laboratories have supplied all information (questionnaire, IQC and EQC) as required during the last two rounds and have qualified for the status "recognition".
These laboratories are: 23 and 30

The recognized laboratories will receive an FAO/IAEA recognition document and this information will be forwarded to OIE and FAO.

Future changes in "recognition" status and focus of EQA programme.
During an IAEA consultants' meeting entitled " The FAO/IAEA External Quality Assurance Programme (EQAP) and Movement Towards a Generic Veterinary Diagnostic Testing Laboratory Accreditation Scheme" and subsequent discussions it was agreed that the category "Provisionally recognized" will disappear. Nevertheless in this report the category "Provisionally Recognized Laboratory" is still used for internal purpose. The category "recognition" will remain. It is emphasized that in order to achieve recognition a laboratory must fulfill and submit all components (Questionnaire, IQC and EQC data) of the EQA programme.

Quality management and documentation is an essential component of the EQA programme. Special attention will be given to calibrating procedures of laboratory equipment (ELISA reader, pipettes, pH meters, temperature measurement of freezers and refrigerators) and the self-monitoring of internal quality controls is encouraged (IQC data) [8, 13].

5. RECOMMENDATIONS

1)   Following the conclusions and recommendations of a recent consultants meeting entitled: "The FAO/IAEA External Quality Assurance Programme (EQAP) and Movement Towards a Generic Veterinary Diagnostic Testing Laboratory Accreditation Scheme" the three pillars of the FAO/IAEA EQA programme will remain IQC, EQC and information supplied through a questionnaire, but the focus will be on Quality Management and documentation of specific laboratory activities through Standard Operating Procedures (SOPs). It is understood that participation in the EQA Programme will assist in creating a quality management working environment, which will assist participants - especially from developing countries, who do not count with a national accreditation body - to bridge the gap between what they have now and formal national or international recognition of Quality Management and technical competence.

2)   Understanding of the principles of assay validation still widely differs. The basis for any EQA participation is a correctly validated assay. The paper from R. Jacobson "Validation of Serological Assays for Diagnosis of Infectious Diseases" is recommended as a guideline to assist the continuing process of assay validation [11].

3)   The Questionnaire is considered an essential component of the EQAP! It is urged that the laboratory officers complete the Questionnaire as accurately as possible. The information gathered with the Questionnaire will require regular updating by the laboratory officer in charge, in close collaboration with the test operator, and should be done at least once a year. In some cases the information provided by the laboratory might need some further clarification. This need will be determined by the EQAP Coordinator on an individual basis during future EQAP rounds. Focus will be on information about Quality Management and documentation of specific activities through Standard Operating Procedures (SOPs).

4)   The maintenance and calibration of ELISA equipment needs improvement in most laboratories. Specific guidelines have been prepared and will be distributed. Additionally ELISA Standard Absorbance plates will be distributed to measure the accuracy of ELISA readers.

5)   The implementation of a routine monitoring of the IQC data by the participating laboratories is a major objective of the EQAP. For that purpose a TECDOC entitled: "Internal Quality Control (IQC) of Competitive Enzyme Linked Immunosorbent Assay for Measurement of Antibodies against Rinderpest and Peste des Petits Ruminants Viruses." has been prepared and will be distributed.

6)   For the continued success of the programme, it is of vital importance that participating laboratories keep to the time limits set by the EQAP Coordinator regarding confirmation of receipt of the EQC test panel and the returning of results. If a laboratory foresees problems in keeping to the time limits, it is the responsibility of the laboratory to contact and inform their FAO/IAEA Technical Officer or the EQAP Coordinator immediately.

7)   The target of the EQAP is 100% participation by laboratories including the return of questionnaire, IQC, and EQC data. This involves extensive communication between the counterparts, their FAO/IAEA Technical Officer, and the EQAP Coordinator. As the EQAP becomes more of a routine for all involved, it is expected that a higher percentage of returned results could be achieved. To avoid wasting time tracing lost results/EQAP materials, it is recommended that a courier service be used where possible.

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- Attachment I -

Questionnaire Summary

Questionnaire summary of results

Sixteen laboratories returned the completed and/or updated Questionnaire during this EQAP round (TABLE I.). The collected information categorized by subject per laboratory is attached in Attachment I. The information presented in *bold italic* format is new or updated since the last EQAP round. Accumulated and updated information of the questionnaire of the last four EQAP rounds of an overall number of 26 laboratories (laboratories 1, 2, 3, 4, 5, 9, 10, 11, 12, 13, 14, 15, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 and 31) is compiled in Attachment 1. No questionnaire information at all has been received from laboratories 6, 7, 8, 16 and 17.

Power supply/Air condition

Of 26 laboratories 16 (62%) reported problems with the power supply. Six laboratories have both types of power problems, namely "power cuts" and voltage "irregularities", 4 laboratories reported only power cuts and 5 laboratories indicated only voltage irregularities. Seven laboratories do not have any power problem. Regarding the length of period of power problems 7 laboratories have power cuts of less than 12 hours and 13 reported irregular periods. Asked about the frequency of power problems 2 laboratories reported weekly and 15 irregular periods of power problems. Fourteen laboratories use a stabilizer, 3 use a stabilizer but only for selected equipment (e.g. refrigerator). Seventeen laboratories have access to an emergency power supply for their refrigerator and freezer. Sixteen laboratories have access to a generator in case of a power failure. Eight do not have a generator. Sixteen laboratories have an air-condition (8 do not have air-condition). The average temperature was 25.52 °C with a variation of Min. 15°C to Max. 42°C.

Pipettes, Tips and ELISA readers

Most laboratories use pipettes from Biohit Proline® (15 labs) or Titertek (10 labs) followed by Finnpipette (8 labs), Gilsson Pipettman (6 labs) and Socorex (1 lab) and tips from Biohit Proline® (12 labs) and Micronics (9 labs). Less frequently tips from Finntips, Conetreff, Volac 200 and Costar are in use. Twenty-three and 21 laboratories use 5-50ul single and multichannel pipettes respectively. Eighteen and 20 laboratories use 50-250ul single and multichannel pipettes respectively. Eight and 5 laboratories use 250-1000ul single and multichannel pipettes respectively .

The Multiskan Plus Mark II is the most commonly used ELISA reader (15 labs), followed by the BDSL Immunoscan Plus (9 labs) and Multiskan MCC/340 (4 labs).

Handling of test results

With regard to plate reading and calculation of ELISA results, 23 laboratories use the EDI programme: Ten laboratories are using EDI version 2.11., 9 laboratories are using RPEIA version 1.03 , 4 laboratories use ED 2.2., 3 laboratories are using RPEIA version 1.01., 2 laboratories use Procomm and 2 laboratories calculate results manually. Some laboratories indicate to use RPEIA and EDI together.

Twenty laboratories have a computerized system SID (17 labs), Panacea (6 labs), EPI-info (2 labs), Access (2) or a spreadsheet programme[1], to link the test results with other details of the field samples.

The majority of the laboratories (13) use the IQC data to determine whether the ELISA plate readings are 'within' limits and can be accepted. Two laboratories indicated that the IQC data are monitored using the 'instatqc' programme. Eight laboratories reported that they do not undertake any IQC monitoring.

Sample storage

All laboratories (26 labs) store serum samples at -20°C, in most cases using Cryopreservation vials (13 labs), Vacutainers (7 labs), Nalgene storage system (7 labs), Micronics (7 labs), Serum storage plates (3 labs) or others e.g. 10 ml tubes (1 lab). Twelve laboratories have access to -80°C freezers and 7 to Liquid Nitrogen facilities. Nineteen laboratories reported keeping a serum bank ranging from 500 to 45.000 samples with an average of 11.328 samples.

Computer/Data Processing

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[1]   SID (Sero-monitoring Information Database), Panacea and EPI-info are epidemiological computer programs

Nineteen laboratories reported that a computer is used for reading of ELISA plates and/or storage of data. For the first time more Pentiums (9 laboratories) than 486 processor-equipped-computers (6 laboratories) are in use. Three laboratories use 386 and 1 laboratory uses a 286 CPU computer. Hopefully this trend will continue.

Water quality and equipment calibration

Twenty-two laboratories use distilled water. Nineteen laboratories have access to deionized and 11 laboratories to bi-distilled water. Nineteen laboratories reported that filters and cartridges are changed in the following pattern: once per year (2 laboratories), twice per year (6 laboratories), every three months (5 laboratories), every month (1 laboratory), three times per month (1 laboratory). Six laboratories reported that cartridges and filters are changed following the manufacturers recommendations (1 lab), conductivity control (1) or "when needed" (4 labs).

Twenty-two laboratories reported that no equipment calibration (ELISA reader and pipettes) procedures are carried out. Two laboratories undertake calibration procedures following the manual. One laboratory checks the accuracy of its ELISA reader by comparing OD readings with another ELISA reader.

Conclusions and recommendations

QA/QC practices

It can be concluded that several laboratories still need to improve their IQC practices; i.e., they must pay attention to the monitoring and analyses of the IQC data and should regularly check the calibration of their ELISA equipment. Guidelines have been developed to assist counterparts in checking the calibration of pipettes and ELISA readers, and a TECDOC entitled: "Internal Quality Control (IQC) of Competitive Enzyme Linked Immunosorbent Assay for Measurement of Antibodies against Rinderpest and Peste des Petits Ruminants Viruses." has been prepared by the subprogramme of the Joint FAO/IAEA for the routine monitoring of IQC data using Control Charts.

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Nineteen laboratories reported to use a computer for the reading of ELISA plates and storage of data. For the first time more Pentiums (9 laboratories) than 486 processor-equipped-computers (6 laboratories) are in use. Hopefully this trend will continue. Some laboratories did not supply any information on the availability of computers and it is not clear how they produce and store the data.

SUMMARY EQAP/RP/1998A QUESTIONNAIRE
Pipettes, Tips and Readers
(February 1999)

Lab no.	Pipettes: Biohit Proline	Finn pipette	Titer-tek	Gilson Pipetteman	Pipettes: Others	Tips: Micro-nics	Tips: Biohit Proline	Tips: Others	Single 5-50ul	Single 50-250ul	Single 250-1000ul	Multi 5-50ul	Multi 50-250ul	Multi 250-1000ul	Multiskan MCC/340	Multiskan Plus Mark II	BDSL Imm.skan Plus
1	x		x			x			x	x	x	x	x		x	x	x
2	x						x		x	x	x	x	x				x
3			x				x		x	x	x	x	x				x
4	x							Finntips	x	x	0.5-10ul	x	x	50-200		x	
5	x		x						x	x		x	x			x	x
6	x								x	x		x	x				
7	excluded																
8																	
9	x						x		x	x	x	x			x		x
10	x						x		x	x		x					x
11	x		x				x		x			x					x
12		x	x	x		?		Conetreff	x	x	x	x	x	x		Multiskan +?	
13	?								x	x		x	x			Titertek	
14		x	x	–				Titertek/Finn tips	x	x		x	x				
15	x	x		x	Socorex		x	300-1000ul	x			x	50-200		organon Teknika reader 280 S		
16															Multiscan Plus Lab System		
17																	
18	x	x							x			x	x			x	
19	x		x			x	x		x	x		x	x			x	
20	Jeneon Seal, Volac, BDSL, Biohit Proline						x		x	x		x	x			x	x
21	x	x				x	x	Volac 200	x	x	x	x	x	x	MCC		x
22		x					x		2	x		2	x			x	
23	x	x		x		x	x		x			x	x			x	
24	x		x				x		x	x		x	x			x	
25			x			x			x	x		x	x			x	
26	x					x	x		x		x	x	x	x	x	x	x
27	?					x			x	x	x	x	x	x		x	x
28			x	x		x			x		x	x	x	x		x	
29	x	x	x			x	x		x	x		x	x	x		x	
30	x	x	x	x			x	Costar	x	x	x	x	x			x	
31	x	x					x		10-100			x	x			x	x

Note: **bold** indicates update

Attachment I

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SUMMARY EQAP/RP/1998A QUESTIONNAIRE
Power supply
(February 1999)

Lab no.	Length of period with supply problems: <12 hrs	>12 hrs	irregular	Frequency of periods with power problems: weekly	monthly	irregular	Type of power problems: no power	voltage	Stabilizer used	Access to emergency power supply*	Access to generator	Airco in lab.	Temp. In lab °C
1	x					x		x	yes	yes	yes	no	24
2			x			x	x	x	yes	no	no	no	22
3	x					x	x	x	yes	yes	yes	yes	26
4			x			x	x	x	no	yes	yes	no	17-25
5													
6													
7													
8	no problems								no	yes	yes		22-30
9			x	x			x	x	yes	yes	yes	no	22
10			x			x	x	x	no **	no	no	yes	33
11			x			x	x		?	no	no	yes	22-30
12			x			x	x		?	?	?	yes	30-42
13	no problems							x	yes	yes	no	yes	25
14			x				x		yes	yes	yes	yes	25
15	no problems												
16													
17									yes	yes	yes	yes	25-26
18	no problems		x			x	x		not all eq.	yes	yes	no	30
19	yes		x	x			x	x	yes	no	no	no	15-25
20			x			x	x	x	no	no	no	yes	15-42
21			x			x			yes	yes	yes	yes	28
22	no problems					x	x		yes	no	no	yes	23
23			x			x	x		yes	yes	yes	yes	27
24	no problems					x	x		no	no	sometimes	yes	25
25			x			x	x	x	yes	yes	yes	no	16-20
26	x			x					not all eq.	yes	yes	yes	29
27	x		x			x			yes	yes	yes	yes	20-22
28	x					x			not all eq.	yes	yes	yes	25-30
29			x			x	x	x	yes	yes	yes	yes	24
30	x					x		x		yes	yes	no	
31	no problems									yes	yes	no	

* only for refrigerator and deepfreezer

** will be installed soon

Note: **bold** indicates update

Attachment I

SUMMARY EQAP/RP/1998A
QUESTIONNAIRE
Handling of test results
(February 1999)

Lab no.	Using EDI yes/no	If yes, which EDI program				Linkage test-results with other details of source sample			IQC monitoring	
		RPEIA 1.01	RPEIA 1.03	EDI 2.11	EDI 2.2	computerized	other	yes/no	s; Instat	Automeas. of IQC
1	yes		x			yes, SID		yes		
2	yes				x	yes, SID, Excel		no		
3	no, manually and spreadsheets					no				
4	yes			x		SID: at present Problems, EPI INFO		yes		
5	yes		x			SID 3		yes		
6								no		
7										
8										
9	yes		x			yes, SID		yes		
10	yes			x		yes, Access, EPI-info			yes	
11	not yet					not yet				
12	yes			x		no	manual	no		
13	yes		x			yes, Panacea		no		
14	no									
15	yes	?	?	?		Procomm, SID 3, Excel		no		
16										
17										
18	yes		x			SID 3		no		
19	yes				x	**SID3**	**manual**	**yes**		
20	yes		x	x		SID 3, Access		yes		
21	yes		x	x		no	EPI-info*			
22	yes			x		yes, SID/Panacea		yes		
23	yes			x		SID 3		yes		
24	yes		x			yes, SID 3, Panacea 2		yes		
25	yes	x	x	not yet		yes, SID 2		yes		
26	yes		x			yes, SID/Panacea 2		yes		
27	yes	x	x			no	manual	no		
28	yes					yes, SID		yes		
29	yes				x	yes, Panacea			yes	
30	yes	x	x			yes, SID/Panacea/Procom		no		
31	yes				x	yes, SID3		yes	no	

Note bold indicates update

Attachment I

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SUMMARY EQAP/RP/1998A QUESTIONNAIRE
Sample storage
(February 1999)

Lab. No.	Cryo-preservation	Serum storage, using:					Stored at: -20	Access to:		Establishing Serum bank yes/no
		Cryopres.- & Nalgene sys.	Micronics	Serum storage plates	Vacutainers	Others		-80	Liquid nitrogen	
1	x	x					x	yes	no	yes, 20.000
2	x						x	yes, limited space	yes,limited space	yes, 800
3	x				x		x	yes	no	yes, 45000
4	x		x				x	no	bo	yes, 4000
5	x		x				x	no		yes, 13000
6										
7										
8		x					x	no	no	yes, 15000
9							x	yes,limited space	no	yes, 1550
10	x		x		x		x	no	no	yes, 2500
11			x		x		x	no	no	yes, 7100
12	x						x	yes	yes	?
13	x			x	x		x	yes	no	yes, 22800
14		x		x			x	no	no	yes, 7000
15		x		x			x			
16										
17										no
18	x		x				x	no	no	yes,15000
19	x		x		x		x	no	yes	yes, 500
20					x		x	no	yes	no
21	no		x				x	no	no	
22			x				x	x	no	yes
23	x						x	no	no	yes, 4000
24	x	x	x				x	no	no	no
25					x		x	yes	yes	yes, 20000
26	x		x		x		x	no	no	yes, 13000
27		x	x				x	?	?	?
28				x	5ml	10ml tubes	x	yes	no	yes, 7000
29							x	yes	no	yes, 17000
30	x	x						yes	yes	no
31	x						x	yes	yes	

Note : bold indicates update

Attachment I

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SUMMARY EQAP/RP/1998A QUESTIONNAIRE

Computers Data processing

(February 1999)

Lab. No.	Computer available for reading ELISA plates	Computer available for data storage
1	Pentium	
2		
3		
4	386	Pentium, 600 MB
5	486, 36 MB	
6		
7		
8		
9	286,386	Pentium
10	551,648 MB	173,302 MB
11	Pentium, 2.1 GB	
12		
13		
14		
15		
16		
17		
18	486, 30 MB	
19	**Pentium, 538 MB**	Pentium, 538 MB
20	**Pentium, 538 MB**	
21	CPU 286	CPU 486
22	Pentium	
23	Pentium, 491 KB?	
24	CPU 486, 1.2 MB ?	5,1 MB
25	?	
26	386	?
27	CPU 486/Pentium	CPU 486/Pentium
28	CPU 486, ?	
29	**Pentium/50MB**	idem
30	CPU 486	CPU 386
31	Pentium/2GB	32MB

Note : **bold** indicates update

Attachment 1

SUMMARY EQAP/RP/1998A
QUESTIONNAIRE
Water quality and Equipment calibration
(February 1999)

Lab. No.	Water Quality			Changing of filters/cartridges		Equipment* calibration	
	Deionised	Distilled	Bi-distilled	yes/no	Frequency	yes/no	If yes, how
1	x	x	x	yes	once per month	yes	according to manual
2		x	x	no		no	
3	x	x		yes	if needed	no	ELISA reader as per manual (?)
4	x		x	yes	every 3 months	yes	
5	x	x	x	yes	if needed	no	
6							
7							
8				yes	conductivity control	no	
9	x	x		yes	every 3 months	ELISA reader	OD reading compared in other reader
10	x		x	no	every 3 months	not yet	
11	x	x		no		no	
12	x	x		no		no	
13	x	x	x	yes	3 x per month	no	
14	x	x	x	yes	6 moths	no	
15	x	x	x	yes	6 months		
16							
17				yes	3 months	no	
18	x			yes	If needed	no	
19	x	x		yes	every 3 months	no	
20	x		x	no		no	
21			x		3 months	no	
22	x	x		yes	6 months	no	
23	x	x		yes	once per year	no	
24	x	x			annually	no	
25	x	x	x	yes	when necessary	no	
26	x	x		?		no	
27				yes	twice a year	no	
28	x		x	yes	twice a year	no	
29	x	x		yes	manufacturer's recom.	no	new ELISA reader (Sep. 95)
30	x	x		yes	6 months	no	
31	x	x		yes			

* ELISA reader and Pipettes

rp98aque.xls

Note : **bold** indicate update

Attachment I

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- Attachment II -

IQC Control Charts

EVALUATION OF THE INTERNAL QUALITY CONTROL (IQC) DATA

Fifteen laboratories: 1, 2, 5*, 9*, 10, 11, 12, 14, 15, 22, 23, 24, 29, 30 and 31 returned IQC data but information only from 13 laboratories could be evaluated. Evaluation from some laboratories e.g. laboratory 14, 30 and 31 show that the assay is well within limits. Intra- and interassay variation is well under control and also the statistical parameter show a good degree of consistency. IQC results from the majority of laboratories e.g. laboratories 2, 12, 15, 22, 23, 24 and 29 show that there are still some outlayers and further adjustment and consistency is required to maintain the assay under control. Finally there is a group of laboratories e.g. laboratory 10 and 11, which apparently needs urgently substantial adjustment in the performance of the ELISA because almost all data fall outside the upper or lower control limits. In these cases it is obvious that the assay is not under control and must be adjusted as soon as possible. There is a general trend indicating low OD values for the Cm. These values are often very close or below the lower control limit (OD < 0.4). Possible reasons for this may the use of old and/or not properly stored reagents or if encountered in a freshly supplied assay wrong dilution/concentration of reagents. The producer has been informed about these findings.

In this interim report, the following control charts of the IQC data have been plotted and are presented :

i)   a chart with the OD values for the Cm (mean of the OD values of the four wells of each Control Sample, with an error bar of ± 2 standard deviations) per ELISA plate,

ii) a chart with the PI values for the C++ and C+ (mean of the PI values of the four wells for each Control Sample, with an error bar of ± 2 standard deviations) per ELISA plate, and

iii) a chart with the PI values of the C- and Cc (mean of the PI values of the two wells for each Control sample)

As not all laboratories were using the same ELISA kit batch and it was not known by the EQAP coordinator when a laboratory started to work with a new kit, the Upper and Lower Control Limits as shown in the Control Charts in this report are average Upper and Lower limits. Furthermore, any additional information on a specific ELISA plate was also not known by the coordinator; e.g., identification of the test operator, date of testing, and the batch number of the ELISA kit per ELISA plate. This information is very important and necessary for a correct IQC evaluation and should be written on the Control Chart. For instance, if an empty ELISA plate is run several times to test whether a system is functioning, such plates should be properly identified on the Control Charts.

As part of establishing Quality Control/Quality Assurance procedures within a laboratory, the test operators should maintain Control Charts themselves [8]. For the EQAP rounds in future, the laboratories will be asked to submit copies of such Control Charts with all relevant information of the last ± 40 plates for external assessment.

The values of the IQC data presented on the Control charts in this EQAP report represent the mean values of the 4 wells per IQC sample ± 2 Standard Deviations. The mean value is indicated with a small circle/square/star, and the ± 2 SD range is given as a vertical error bar. The Standard Deviation or the length of the error bars is an indication of the 'within plate' variation. If the error bars are long, it means that the Standard Deviation of the mean of the four wells is high; there is a high variation between the 4 wells of one IQC sample, and therefore the 'within plate' variation is high. The 'between plates' variation represents the variation between the mean values of each IQC sample of different ELISA plates (the Coefficient of Variation (%) can be used as an indicator and is given together with other basic statistical parameters per IQC sample). Additionally a linear regression trendline, which

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* IQC data could no be evaluated due to lack of information e.g. diskette empty, only one printout

permits to show on one view as whereto the test is drifting is plotted for the mean values as shown in Fig. 2 below.

Obviously the test operator should aim to minimize both the 'within plate' and the 'between plates' variation. Furthermore it must be emphasized again that, in the possible event of the value of an Internal Quality Control sample, especially the OD value of the C++, falling outside the UCL and LCL, and the assay still giving a 'correct' positive or negative value to the test samples, the results of that assay should be considered questionable. The assay must be carefully examined in this situation and the cause for the failure to obtain controls within the limits, determined and eliminated.

Apart from the Control charts, the basic statistical parameters of the mean values of each IQC sample are presented in tables per laboratory.

The latest EDI version should be installed in the computer as soon as possible and older versions (e.g. EDI 2.1., RPEIA) should be deleted. EDI will during installation overwrite any present older EDI version and will also create a new subdirectory 'eqstat.qc' for the automatic storage of IQC data. The existing subdirectory 'Instatqc' and its file(s) will remain unchanged.
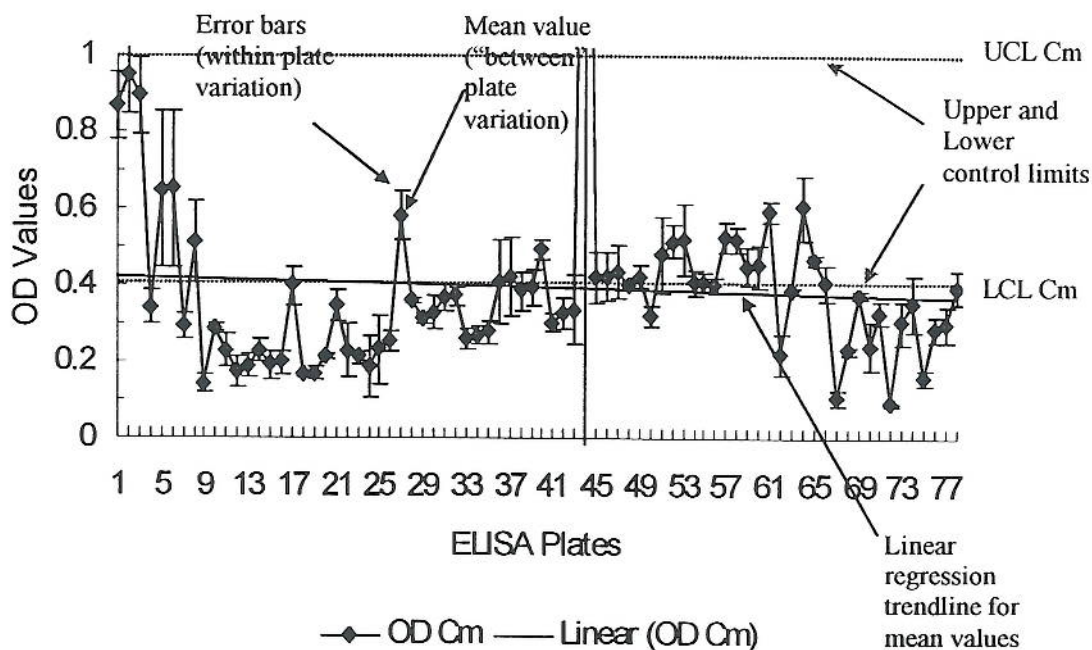
Fig.2. Example for IQC control chart.

The following upper and lower control limits are presented in the graphs:

		UCL	LCL
OD	Cm	1.0	0.4
PI	C++	100	80
PI	C+	81	55
PI	C-	25	-25
PI	Cc	105	95

Evaluation of IQC data of Laboratory 1

General observation and summary:
This laboratory has submitted all EQAP components. It identified correctly all EQC samples and had performed well in the last round.

IQC data:
IQC data from 6 plates were submitted as a 3 KB Rpbrep.qc file. One plate was totally outside limits and deleted. The remaining 5 plates are displayed below. The laboratory should use the latest version of EDI to ease organization and analysis of IQC data. More data are needed (>40 plates) to get a realistic picture of the assay performance. The OD values for the Cm from 3 out of 5 plates are below the lower control limit. Intraassay variation is acceptable. The assay comes back on track but needs further observation.
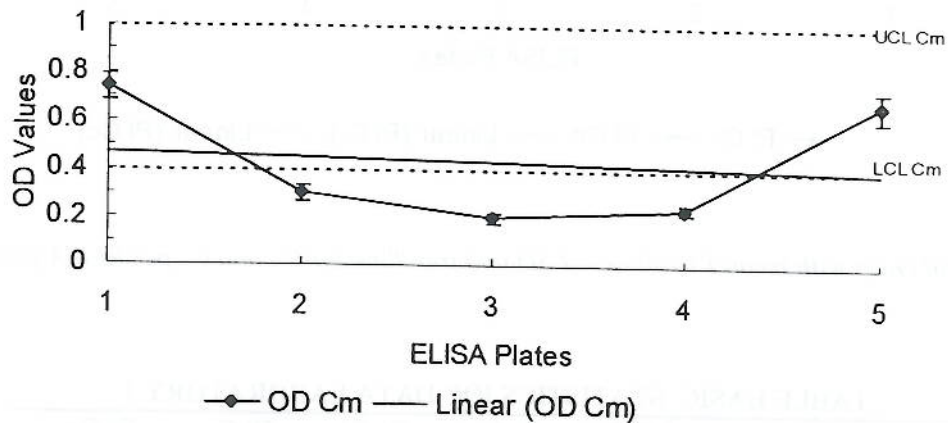
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
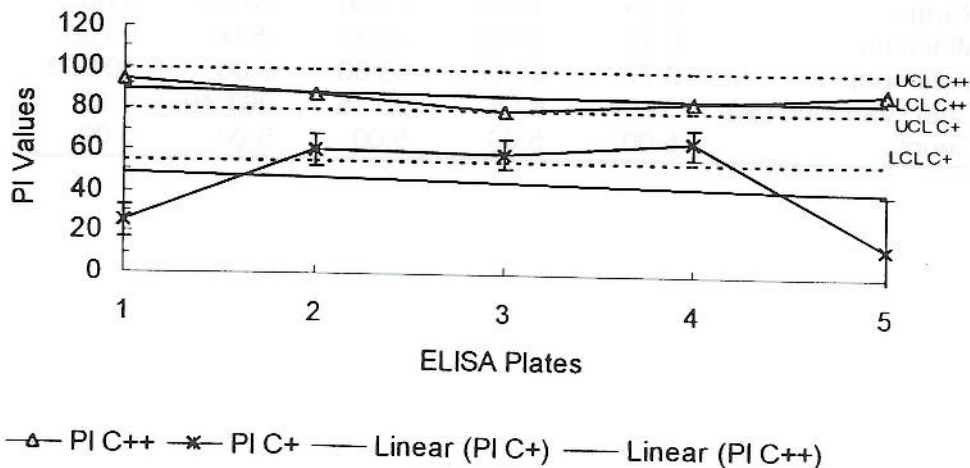
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate
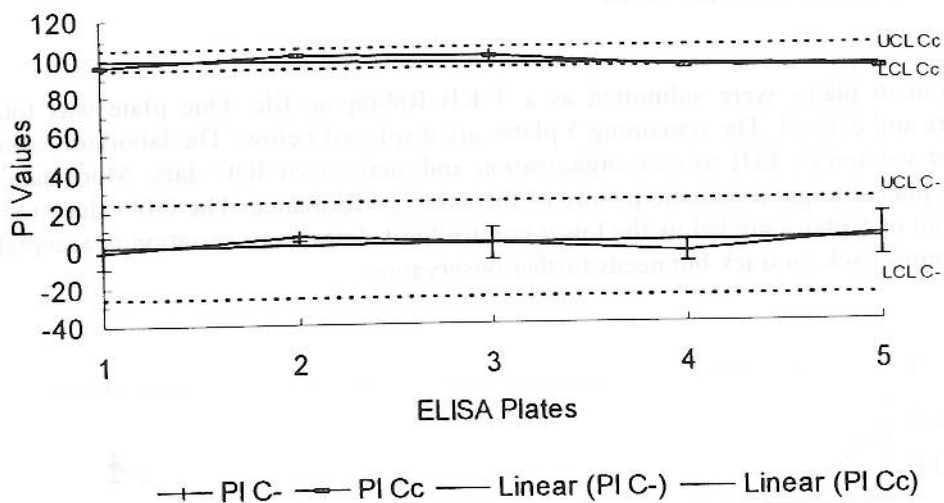
Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE BASIC STATISTICS IQC DATA LABORATORY 1

	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.43	86.75	44.30	1.70	96.80
Standard Error	0.05	1.17	4.97	1.44	1.07
Median	0.30	86.00	56.00	1.50	96.00
Standard Deviation	0.24	5.25	22.22	4.55	3.39
Sample Variance	0.06	27.57	493.59	20.68	11.51
Range	0.59	18.00	71.00	14.00	8.00
Minimum	0.18	77.00	-3.00	-5.00	93.00
Maximum	0.77	95.00	68.00	9.00	101.00
Coef. Variation (%)	54.97	6.05	50.15	267.49	3.50
Count	5.00	5.00	5.00	5.00	5.00

Evaluation of IQC data of Laboratory 2

General observation and summary:
This laboratory had submitted only EQC data during the last round.
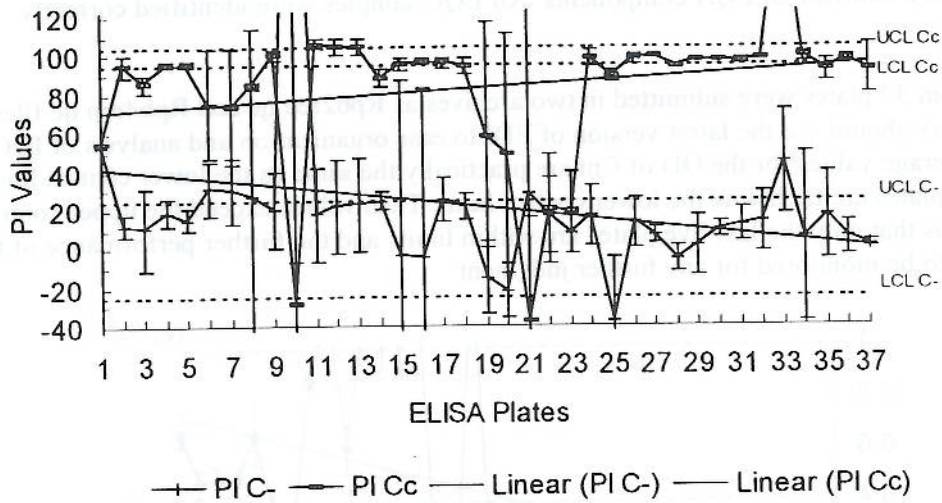
IQC data:
IQC data from 37 plates were submitted in two archives as Rpb2rep.qc and Rpb4rep.qc files. The laboratory should use the latest version of EDI to ease organization and analysis of IQC data. The average values for the OD of Cm are within limits, but a number of values plates is below the lower limit and there is a considerable interassay variation. The trendline shows a continuos decrease. Interassay variation must be brought under control.
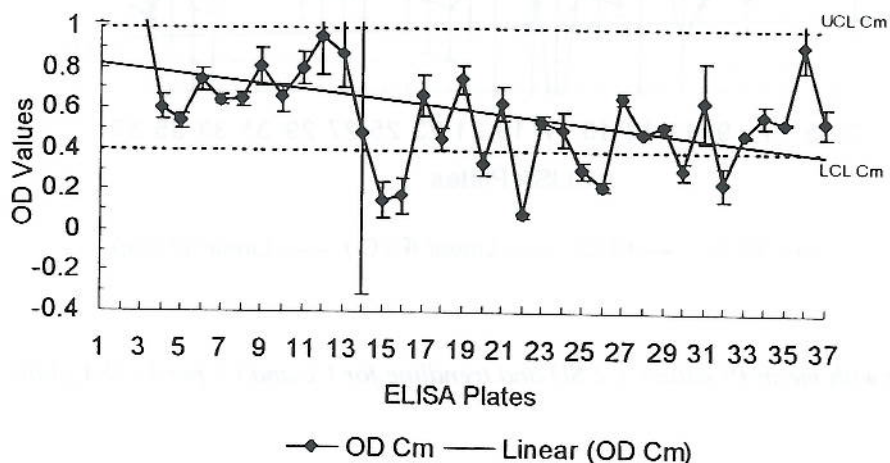
. Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
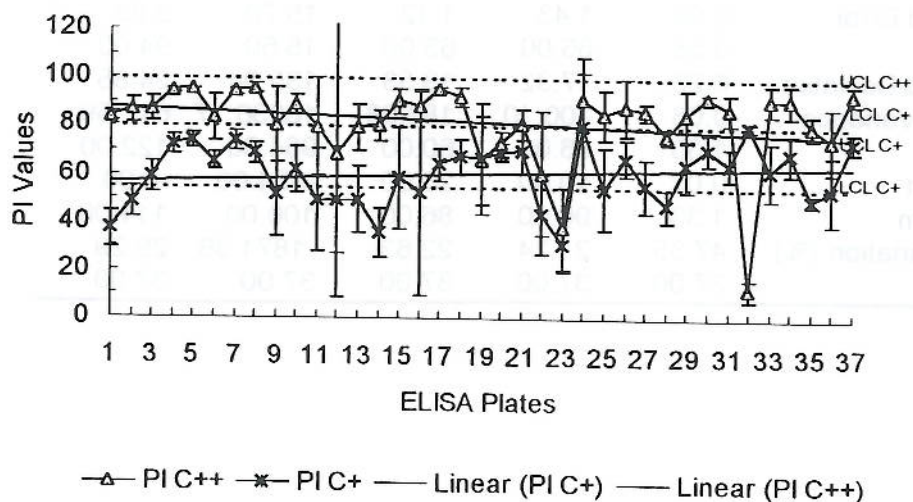
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate
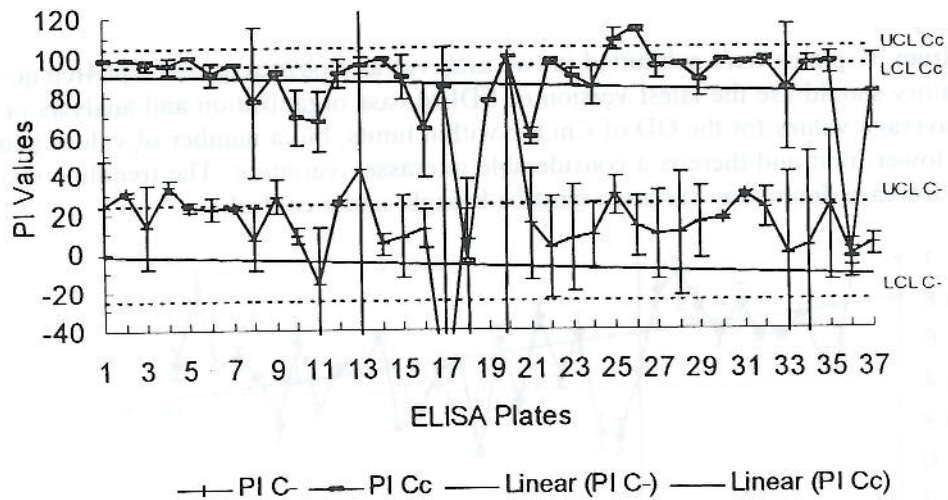
Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE BASIC STATISTICS IQC DATA LABORATORY 2

	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.59	81.54	59.79	-7.22	84.84
Standard Error	0.03	1.43	1.12	15.70	2.89
Median	0.58	86.00	63.00	15.50	94.00
Standard Deviation	0.28	17.32	13.53	135.04	24.85
Sample Variance	0.08	300.10	183.06	18236.17	617.59
Range	1.30	86.00	60.00	954.00	122.00
Minimum	0.02	10.00	26.00	-854.00	-8.00
Maximum	1.33	96.00	86.00	100.00	114.00
Coef. Variation (%)	47.35	21.24	22.63	-1871.36	29.29
Count	37.00	37.00	37.00	37.00	37.00

Evaluation of IQC data of Laboratory 5.

General observation and summary:
This laboratory had submitted all EQA data. All EQC samples were identified correctly.

IQC data:
The file did not contain any IQC data.

Evaluation of IQC data of Laboratory 9.

General observation and summary:
This laboratory had submitted all EQA data. All EQC samples were identified correctly.

IQC data:
The file did contain IQC data from 3 plates only, which could not be used for analysis.

# Evaluation of IQC data of Laboratory 10

General observation and summary:
This laboratory returned all EQA components. All EQC samples were identified correctly.

IQC data:
IQC data from 37 plates were submitted in two archives as Rpb2rep.qc and Rpb4rep.qc files. The laboratory should use the latest version of EDI to ease organization and analysis of IQC data. The average values for the OD of Cm are practically the same as the lower control limit. The first 20 plates are far below the lower control limit. Then values exceed the upper control limit. It seems that only the last five plates are within limits and the further performance of the assay needs to be monitored for any further judgment.
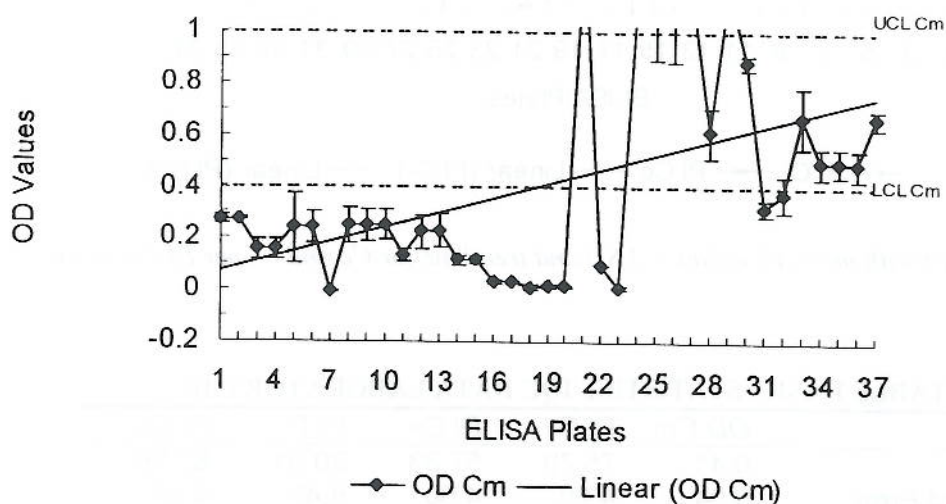
. Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
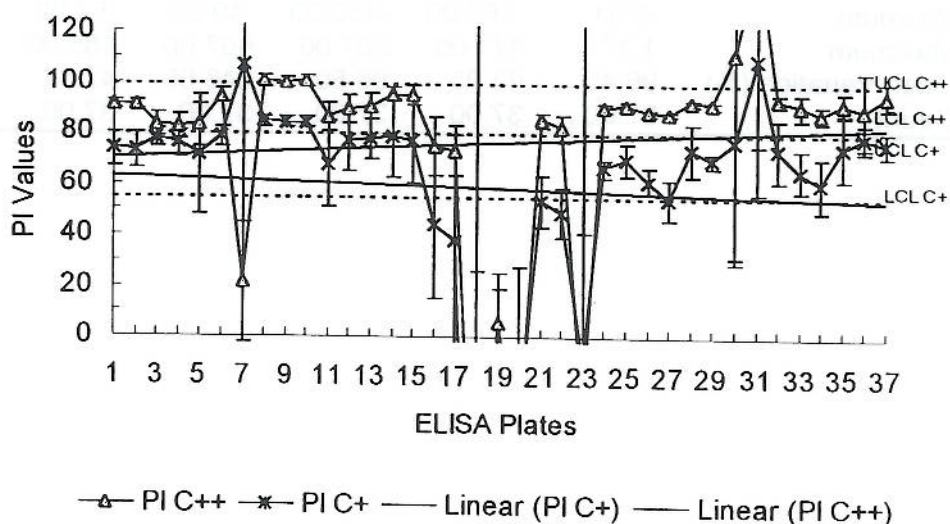
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate

Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE BASIC STATISTICS IQC DATA LABORATORY 10

|	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.41	75.79	57.83	20.91	82.58
Standard Error	0.04	4.00	4.60	8.42	4.57
Median	0.26	90.00	70.00	13.00	96.00
Standard Deviation	0.39	48.46	55.81	72.47	39.34
Sample Variance	0.15	2348.77	3114.40	5251.73	1547.78
Range	1.38	333.00	607.00	656.00	242.00
Minimum	-0.01	-162.00	-400.00	-49.00	-57.00
Maximum	1.37	171.00	207.00	607.00	185.00
Coef. Variation (%)	96.45	63.95	96.50	346.65	47.64
Count	37.00	37.00	37.00	37.00	37.00

**Evaluation of IQC data of Laboratory 11.**

General observation and summary:
This laboratory returned all EQA components. All EQC samples were identified correctly.

IQC data:
IQC data from 18 plates were submitted. The operator should check whether the latest version of EDI is in use. The average values for the OD of Cm and C++ are too low. The operator should carefully check whether the controls are placed in the right wells on the plate. It seems that they were mixed up. The values in the basic statistics do not make sense. The assay is not under control.
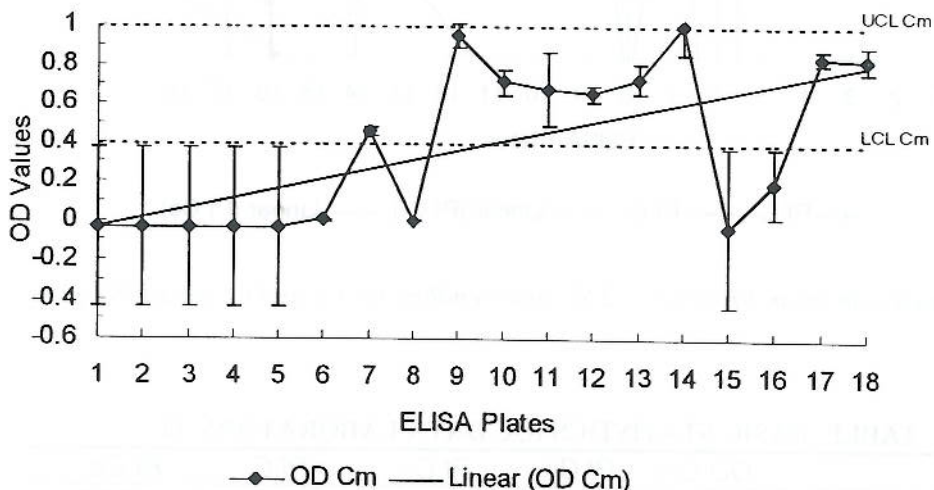
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
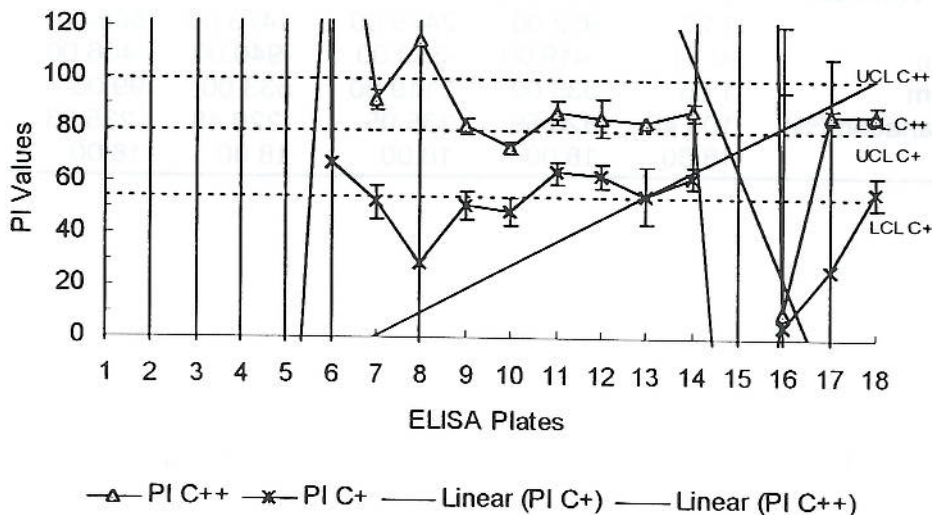
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate
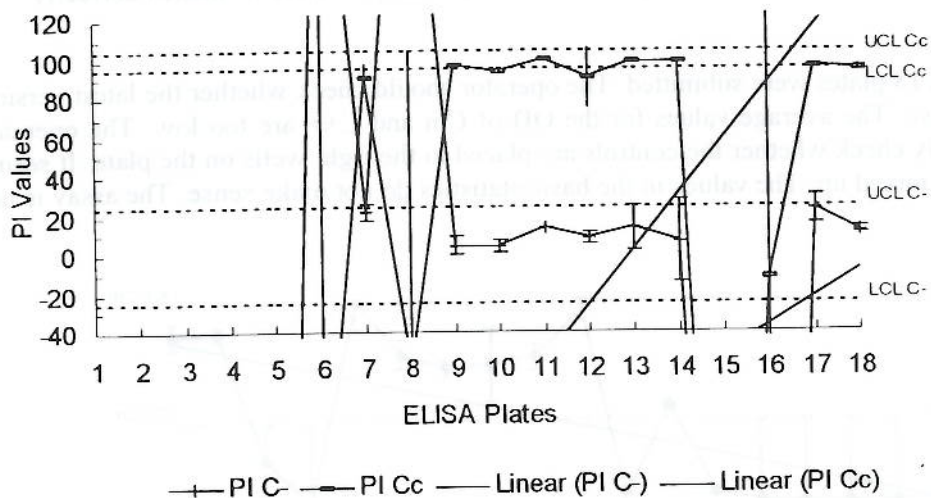
Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE. BASIC STATISTICS IQC DATA LABORATORY 11

|	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.38	22.94	299.61	-127.78	-97.06
Standard Error	0.05	22.35	83.00	46.95	38.13
Median	0.36	83.00	53.50	4.00	56.00
Standard Deviation	0.42	189.66	704.25	281.72	228.79
Sample Variance	0.17	35969.29	495965.00	79365.38	52345.77
Range	1.27	952.00	2479.00	1473.00	505.00
Minimum	-0.21	-419.00	-360.00	-940.00	-406.00
Maximum	1.06	533.00	2119.00	533.00	99.00
Coef. Variation (%)	109.40	826.59	235.05	-220.48	-235.73
Count	18.00	18.00	18.00	18.00	18.00

Evaluation of IQC data of Laboratory 12.

General observation and summary:
This laboratory returned all EQA components. All EQC samples were identified correctly.
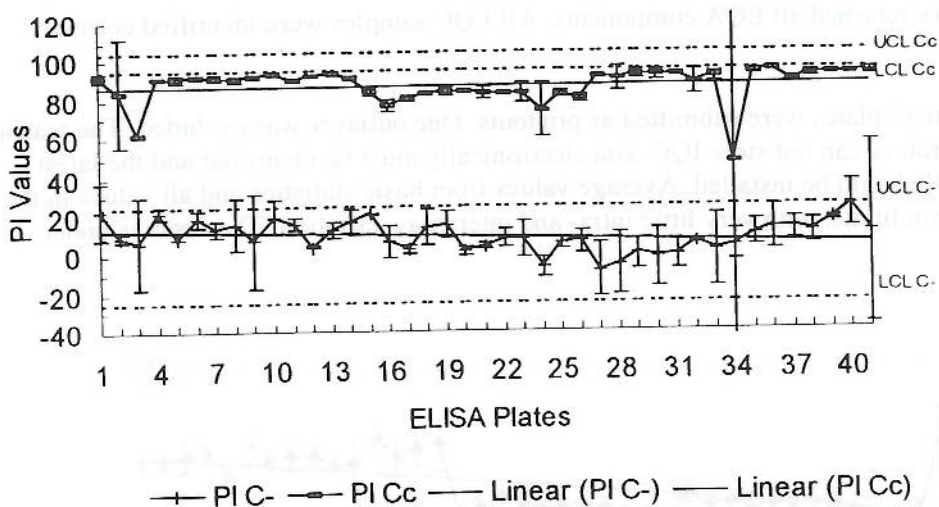
IQC data:
IQC data from 7 plates were submitted. The operator should check whether the latest version of EDI is in use. The average values for the OD of Cm and C++ are a bit too high. More plates needed to be analyzed to come to a final conclusion. From the little data supplied it looks that the assay is on the right track.
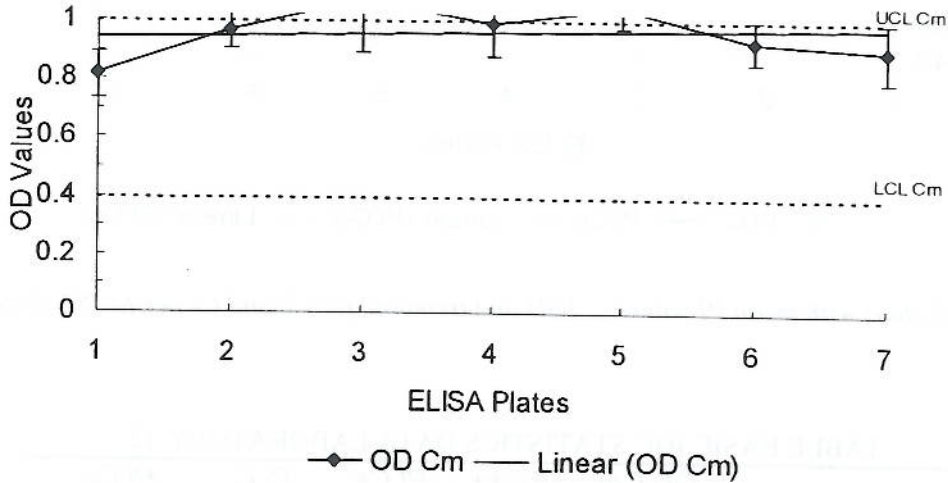
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
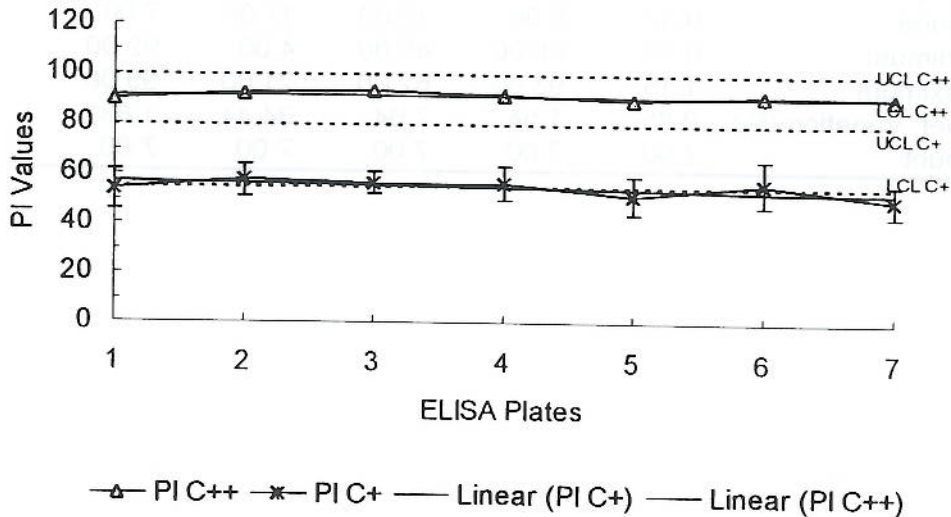
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate
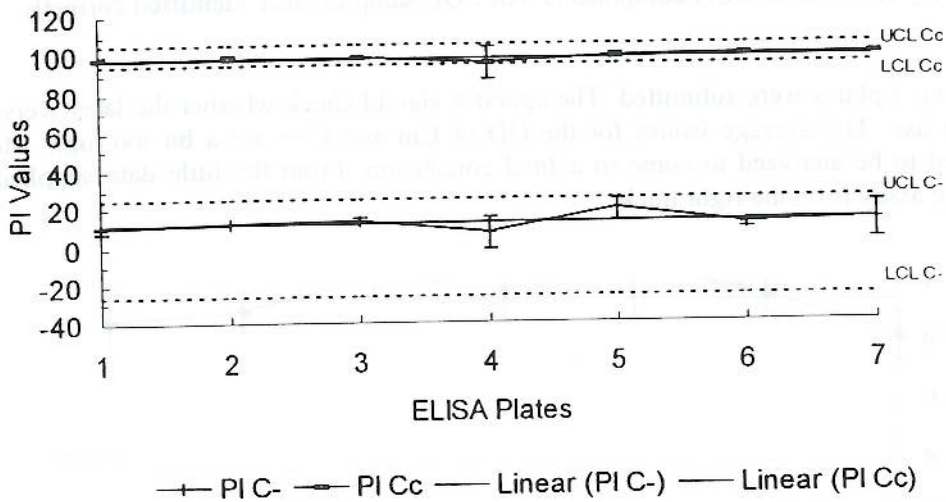
Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE BASIC IQC STATISTICS DATA LABORATORY 12

|	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.96	91.14	54.39	12.00	98.00
Standard Error	0.02	0.27	0.79	1.10	0.48
Median	0.96	91.00	55.00	11.50	98.00
Standard Deviation	0.09	1.41	4.16	4.13	1.80
Sample Variance	0.01	1.98	17.28	17.08	3.23
Range	0.37	5.00	17.00	17.00	7.00
Minimum	0.78	88.00	45.00	4.00	92.00
Maximum	1.15	93.00	62.00	21.00	99.00
Coef. Variation (%)	9.85	1.54	7.64	34.44	1.83
Count	7.00	7.00	7.00	7.00	7.00

Evaluation of IQC data of Laboratory 14.

General observation and summary:
This laboratory returned all EQA components. All EQC samples were identified correctly.

IQC data:
IQC data from 42 plates were submitted as printouts. One outlayer was excluded. The reason why this laboratory can not store IQC data electronically must be identified and the latest version of EDI should be installed. Average values from basic statistics and all values in the graph are within limits, with very little intra- and interassay variation. The assay is under control.
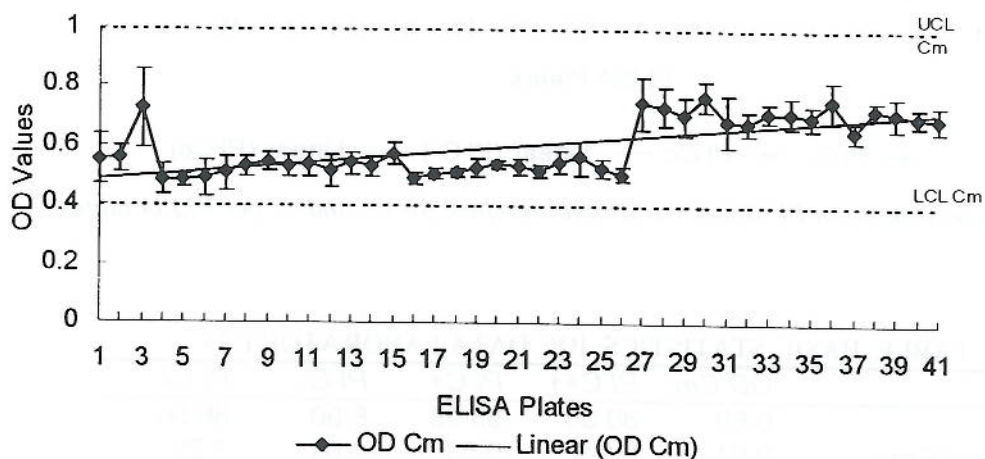
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
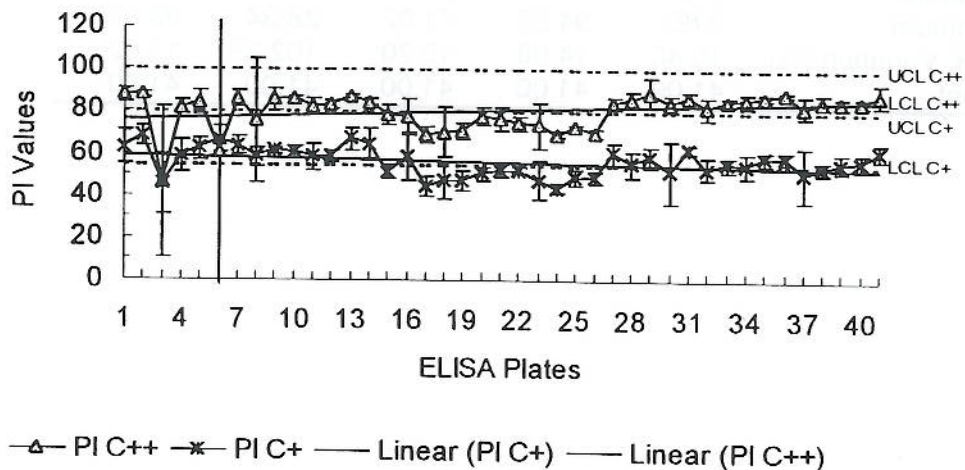
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate

Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE. BASIC STATISTICS IQC DATA LABORATORY 14

	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.60	80.05	55.98	8.90	86.56
Standard Error	0.01	0.94	0.53	1.01	1.29
Median	0.55	83.88	56.34	8.62	90.95
Standard Deviation	0.10	11.99	6.83	9.11	11.70
Sample Variance	0.01	143.80	46.65	83.06	136.91
Range	0.35	116.15	32.16	41.83	92.19
Minimum	0.46	-21.78	38.87	-13.50	1.68
Maximum	0.80	94.37	71.02	28.34	93.87
Coef. Variation (%)	16.40	14.98	12.20	102.39	13.52
Count	41.00	41.00	41.00	41.00	41.00

Evaluation of IQC data of Laboratory 15.

General observation and summary:
This laboratory returned EQC and IQC data. All EQC samples were identified correctly.
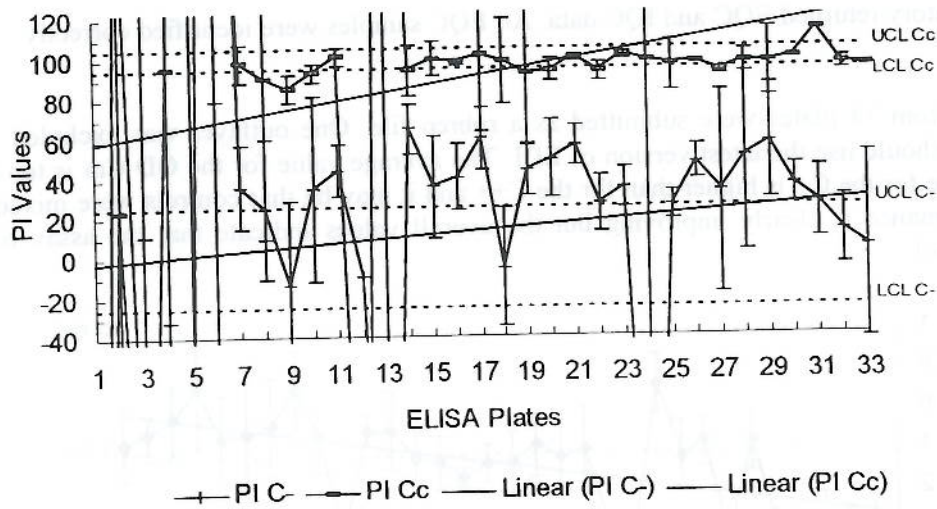
IQC data:
IQC data from 34 plates were submitted as a rpbrep file. One outlayer was excluded. The laboratory should use the latest version of EDI. The average value for the OD Cm is too low. The average for the C+ is higher than for the C++ and it may be that controls were mixed up. The performance is clearly improving but the overall values indicate that the assay is not under control.
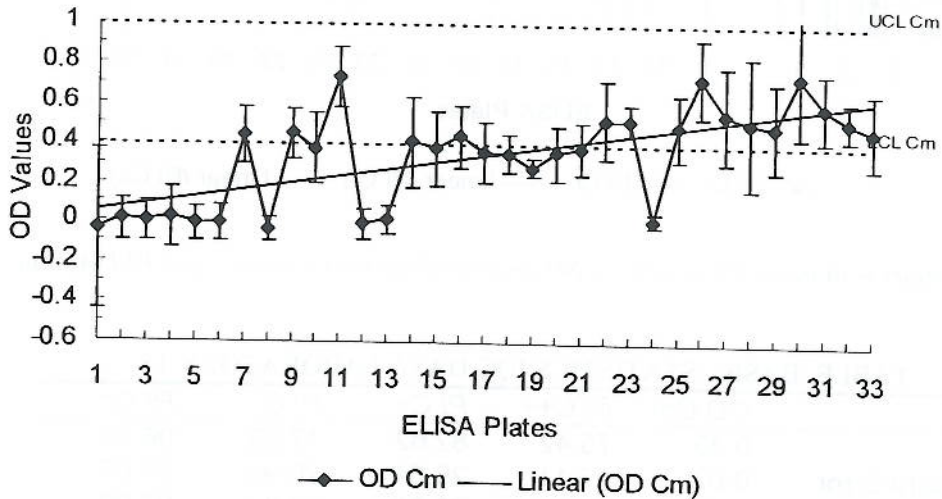
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
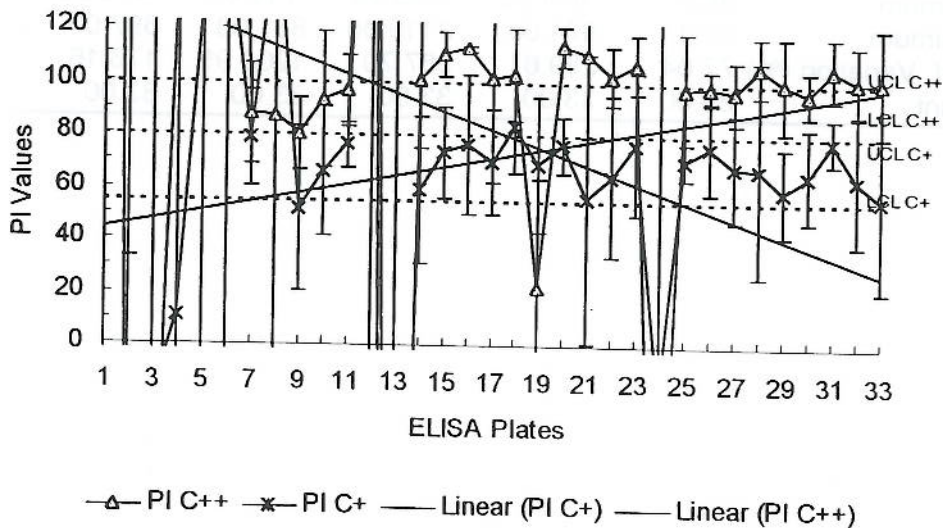
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate

Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE. BASIC STATISTICS IQC DATA LABORATORY 15

|	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.33	75.42	82.62	17.23	96.45
Standard Error	0.02	17.11	26.54	26.95	21.07
Median	0.38	98.00	70.00	33.00	97.00
Standard Deviation	0.26	195.80	303.79	217.27	169.90
Sample Variance	0.07	38337.08	92288.76	47206.93	28867.47
Range	1.14	1785.00	2885.00	1597.00	1103.00
Minimum	-0.21	-811.00	-766.00	-716.00	-406.00
Maximum	0.93	974.00	2119.00	881.00	697.00
Coef. Variation (%)	77.04	259.61	367.70	1260.95	176.16
Count	33.00	33.00	33.00	33.00	33.00

Evaluation of IQC data of Laboratory 22.

General observation and summary:
This laboratory returned all EQA components. All EQC samples were identified correctly.

IQC data:
IQC data from 40 plates (March-October 98) were submitted and are displayed below. The mean OD value for the Cm is a bit too low (0.39) and the linear regression trendline shows a constant downward move. The activity of the monoclonal antibody is going off. One should use a new vial and test whether the OD values return within limits.
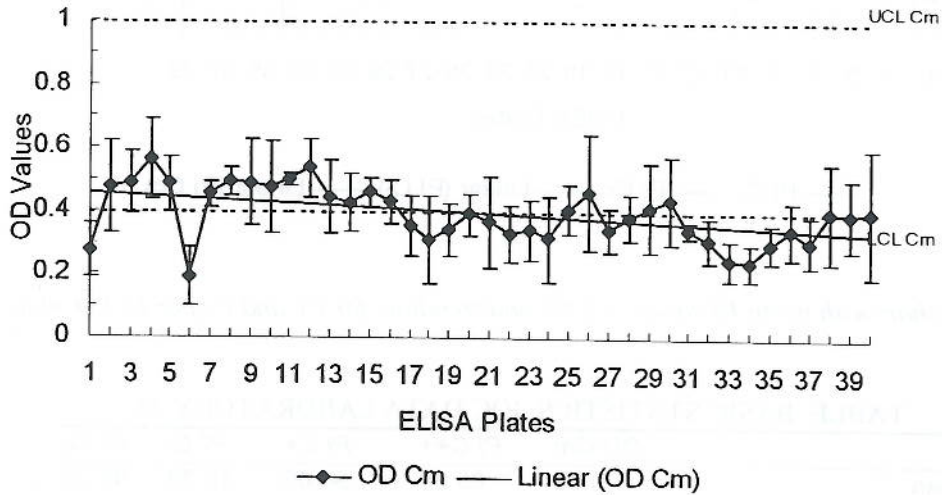
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
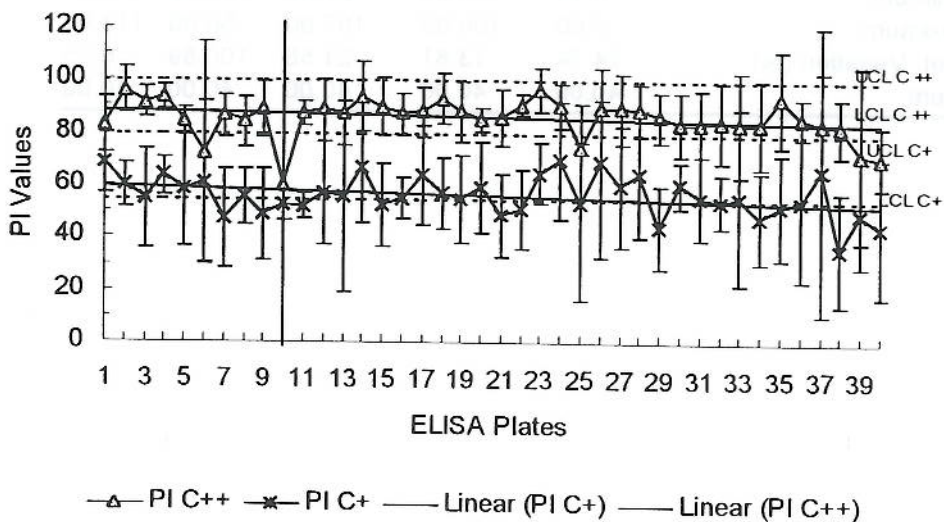
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate
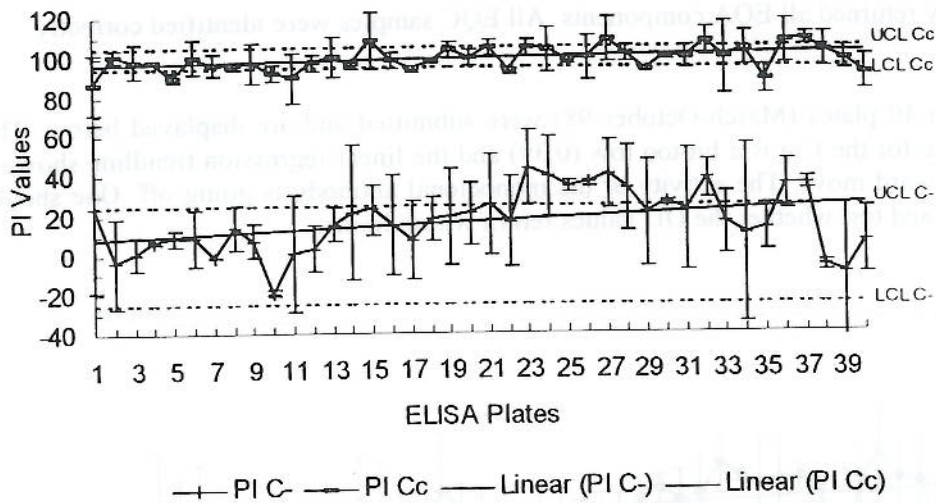
Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE BASIC STATISTICS IQC DATA LABORATORY 22

	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.39	85.68	56.06	16.33	98.29
Standard Error	0.01	0.94	0.96	1.84	0.69
Median	0.38	87.00	56.00	14.00	98.00
Standard Deviation	0.10	11.83	12.10	16.47	6.14
Sample Variance	0.01	140.06	146.36	271.29	37.70
Range	0.47	126.00	81.00	73.00	32.00
Minimum	0.14	-20.00	26.00	-23.00	81.00
Maximum	0.60	106.00	107.00	50.00	113.00
Coef. Variation (%)	24.74	13.81	21.58	100.89	6.25
Count	40.00	40.00	40.00	40.00	40.00

## Evaluation of IQC data of Laboratory 23.

General observation and summary:
This laboratory returned all EQA components. All EQC samples were identified correctly.
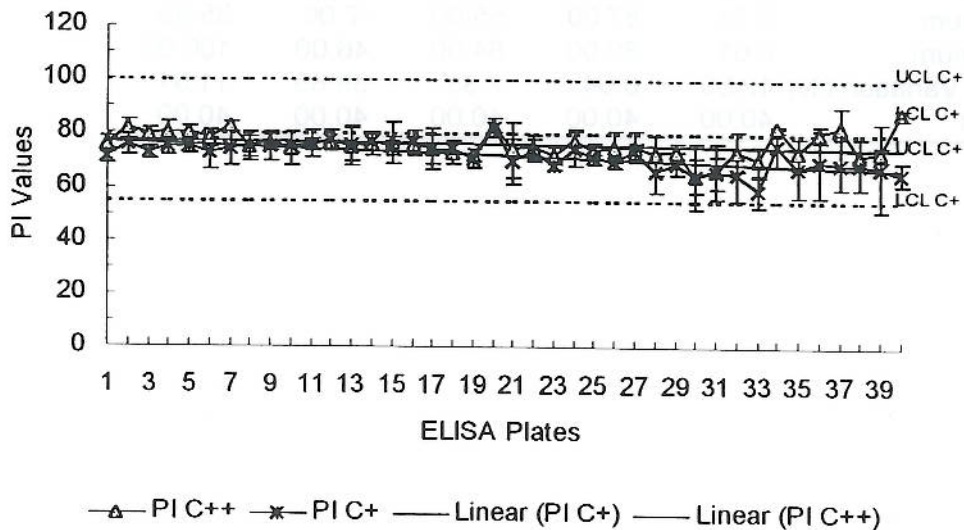
IQC data:
The diskette with the IQC data contained several folders. The most recent 40 plates were taken for IQC analysis and are displayed below. These are the same values as given in the Rp1997a. For this reason the comment in this report is the same as in the 1997a report.
The linear regression trendline and the basic statistical data indicate that there is no clear distinction between the PI values of the C++ and C+. A new batch of C++ control serum may solve this problem. The average value for the C++ is too low. The OD average value for the Cm is still within limits but is coming too close to the lower control limit. The assay is still under control but action needs to be taken.
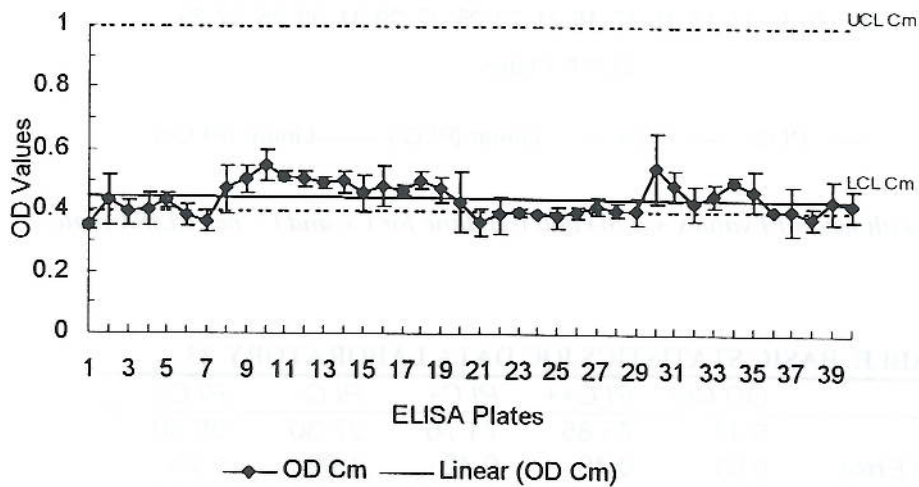
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate

Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate
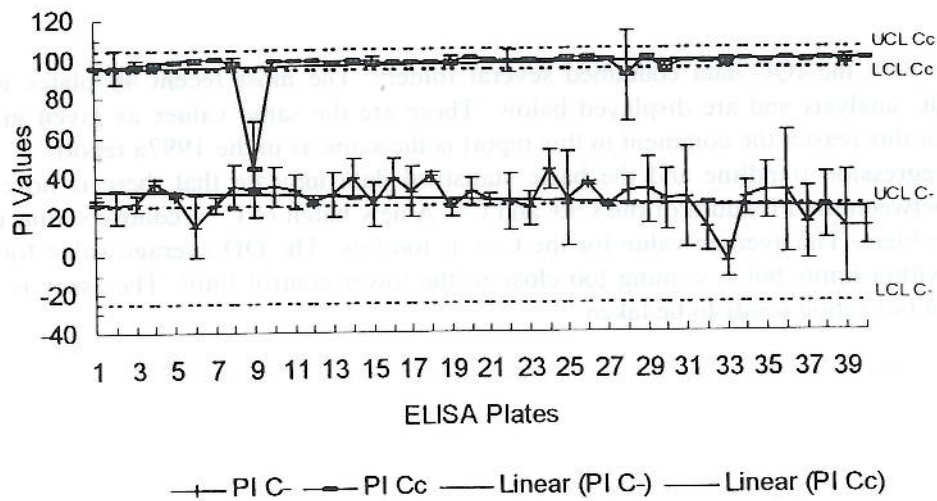
Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE. BASIC STATISTICS IQC DATA LABORATORY 23

|	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.44	75.85	71.76	27.30	95.90
Standard Error	0.01	0.40	0.42	1.09	1.23
Median	0.43	75.00	73.00	28.00	98.00
Standard Deviation	0.06	5.03	5.27	9.79	11.03
Sample Variance	0.003	25.34	27.80	95.78	121.74
Range	0.26	32.00	29.00	53.00	65.00
Minimum	0.34	57.00	55.00	-7.00	35.00
Maximum	0.61	89.00	84.00	46.00	100.00
Coef. Variation (%)	12.59	6.64	7.35	35.85	11.51
Count	40.00	40.00	40.00	40.00	40.00

Evaluation of IQC data of Laboratory 24.

General observation and summary:
This laboratory returned all EQA components. All EQC samples, but sample 4 were identified correctly. Sample 4 was identified as positive (cut-off 50%).
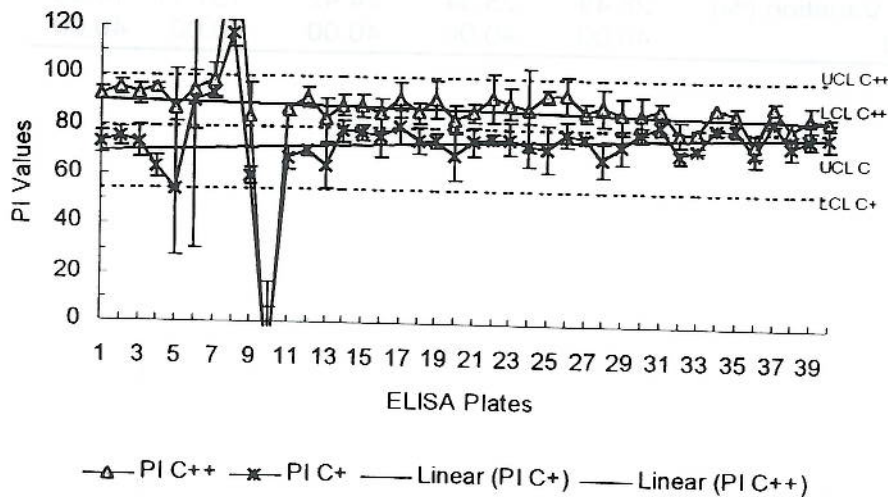
IQC data:
The most recent 40 plates were taken for IQC analysis and are displayed below. These are the same values as given in the Rp1997a. For this reason the comment in this report is the same as in the 1997a report.
The OD average value for the Cm is coming below the lower control limit (0.39) and there is a considerable interassay variation in the first 12 plates with an improving trend. Intrassay variation is somewhat high in all plates. PI values for C++ and C+ are converging towards 80% making a clear distinction between strong and moderate positive difficult. The assay is under control but action needs to be taken.
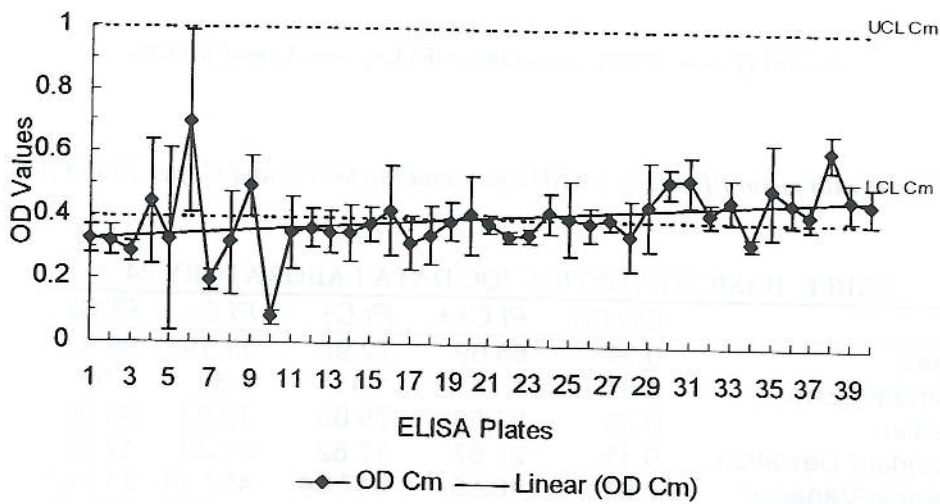
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate

Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate
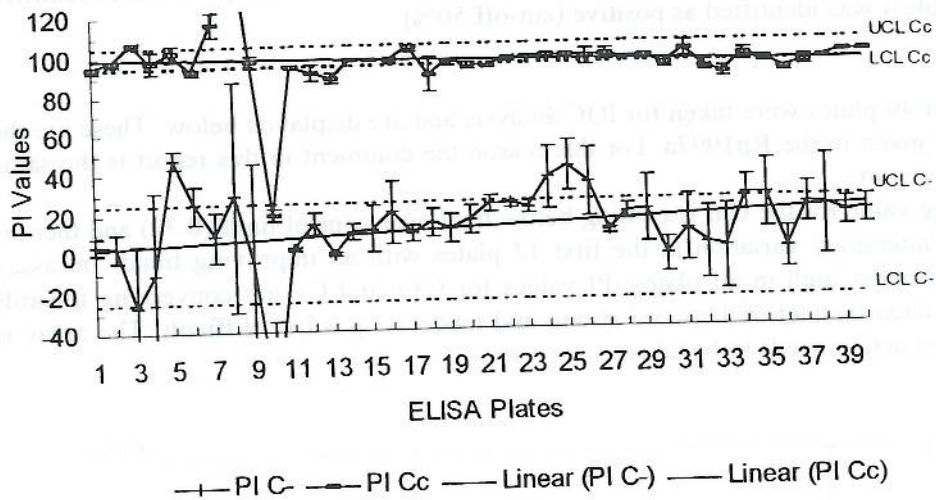
Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE. BASIC STATISTICS IQC DATA LABORATORY 24

|	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.39	86.69	72.98	11.19	96.03
Standard Error	0.01	1.74	1.41	2.39	1.99
Median	0.39	87.50	75.00	13.00	96.00
Standard Deviation	0.11	21.97	17.82	21.38	17.82
Sample Variance	0.01	482.57	317.64	457.14	317.67
Range	0.79	211.00	149.00	143.00	154.00
Minimum	0.07	-48.00	-29.00	-94.00	18.00
Maximum	0.86	163.00	120.00	49.00	172.00
Coef. Variation (%)	28.49	25.34	24.42	191.11	18.56
Count	40.00	40.00	40.00	40.00	40.00

# Evaluation of IQC data of Laboratory 29.

General observation and summary:
This laboratory returned all EQA components. All EQC samples, but sample 3 were identified correctly. Sample 3 was identified as negative (cut-off 50%).
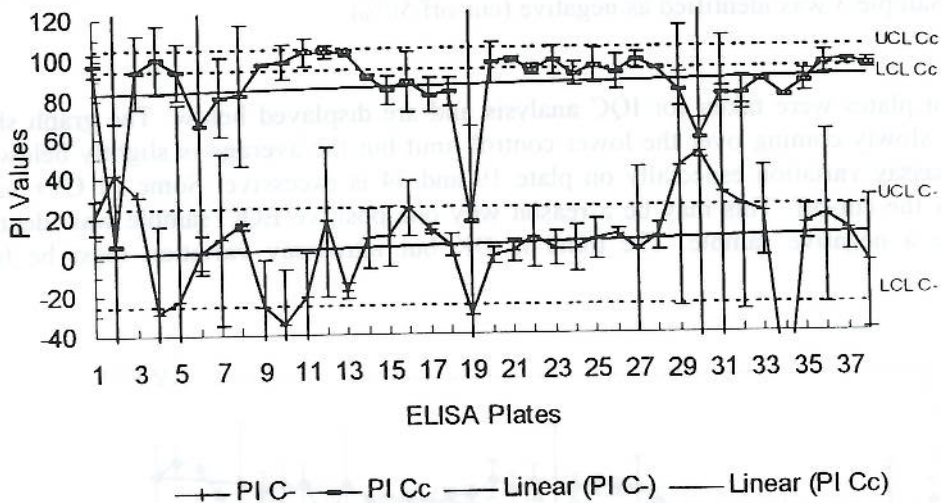
IQC data:
Thirty-eight plates were taken for IQC analysis and are displayed below. The graph shows OD values slowly coming over the lower control limit but the average is slightly below this limit. Intraassay variation especially on plate 19 and 34 is excessive. Some PI C++ values drop below the cut-off. This may be a reason why one positive EQC sample was identified wrongly as a negative sample. The trend is OK but intraassay variation must be better controlled.
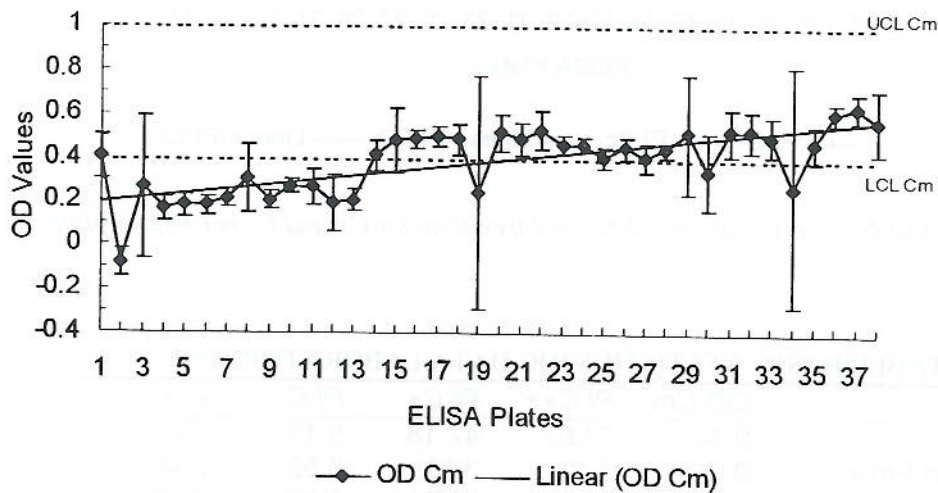
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
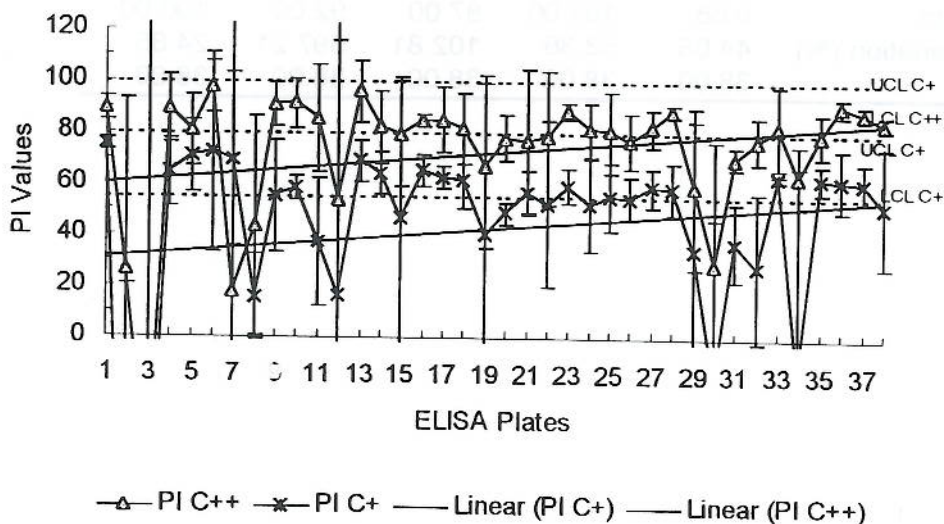
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate

Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE BASIC STATISTICS IQC DATA LABORATORY 29

|	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.38	71.87	42.18	5.13	85.89
Standard Error	0.01	3.11	3.52	3.52	2.45
Median	0.44	82.00	58.00	7.00	93.00
Standard Deviation	0.17	38.35	43.37	30.65	21.34
Sample Variance	0.03	1470.55	1881.01	939.18	455.51
Range	0.81	343.00	261.00	200.00	122.00
Minimum	-0.12	-240.00	-174.00	-108.00	-16.00
Maximum	0.68	103.00	87.00	92.00	106.00
Coef. Variation (%)	44.08	53.36	102.81	597.21	24.85
Count	38.00	38.00	38.00	38.00	38.00

## Evaluation of IQC data of Laboratory 30.

General observation and summary:
This laboratory returned all EQA components. All EQC samples were identified correctly.

IQC data:
Fourteen plates are displayed below. All values are within limits and consistent. Intra- and interassay variation is minimal. The assay is full under control.
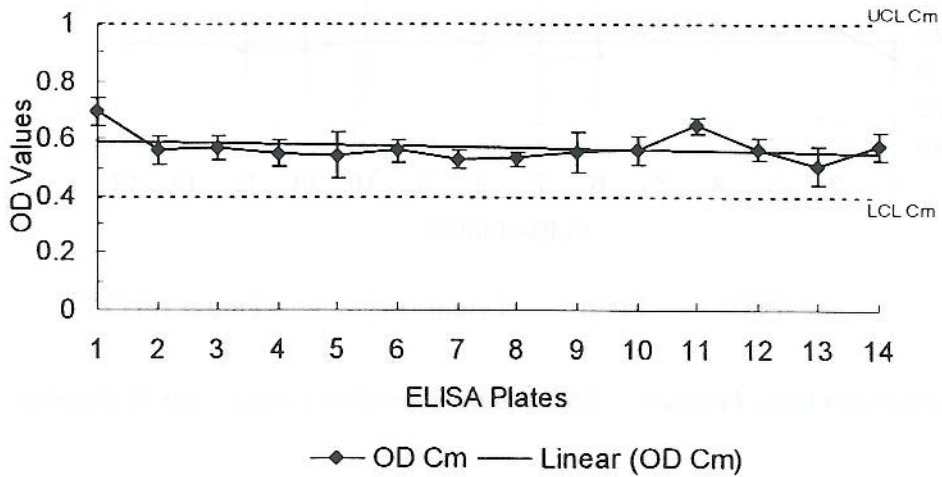
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate
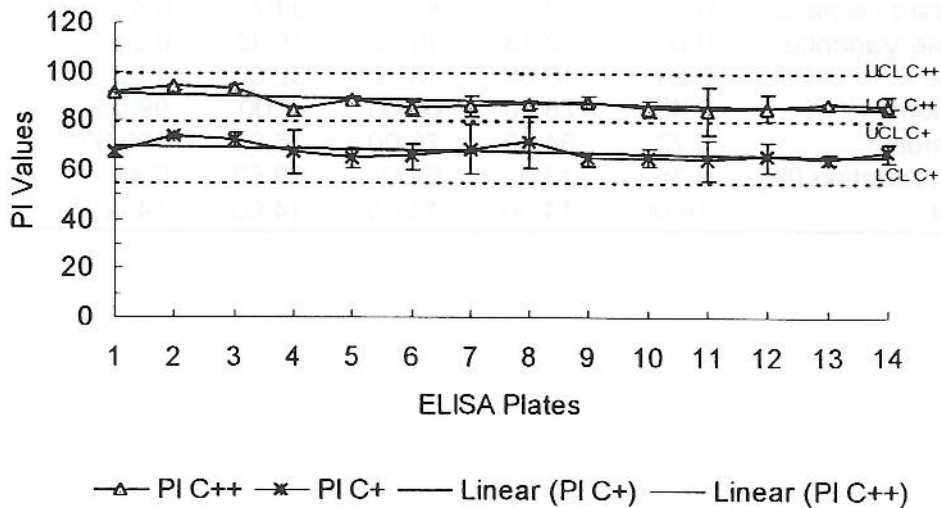
Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate
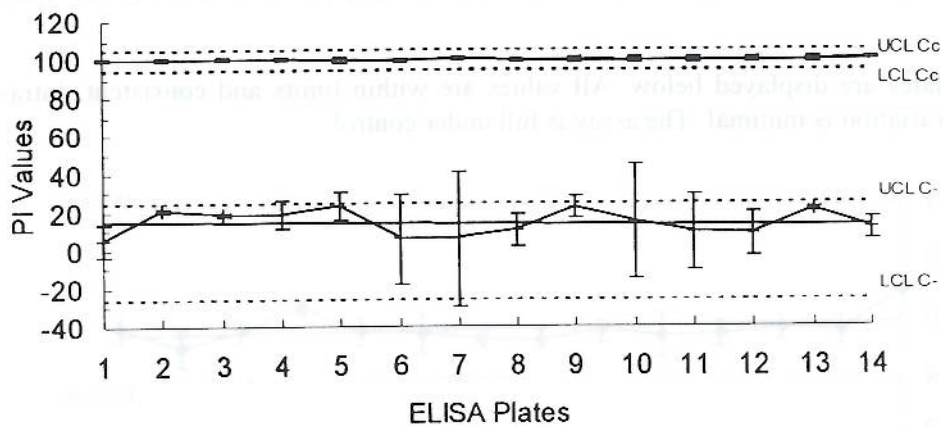
Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE BASIC STATISTICS IQC DATA LABORATORY 30

|	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.57	87.45	67.23	13.64	99.64
Standard Error	0.01	0.47	0.54	1.64	0.09
Median	0.56	87.00	66.00	16.00	100.00
Standard Deviation	0.05	3.53	4.03	8.67	0.49
Sample Variance	0.003	12.43	16.25	75.13	0.24
Range	0.24	16.00	16.00	32.00	1.00
Minimum	0.48	78.00	60.00	-7.00	99.00
Maximum	0.72	94.00	76.00	25.00	100.00
Coef. Variation (%)	9.15	4.03	6.00	63.53	0.49
Count	14.00	14.00	14.00	14.00	14.00

Evaluation of IQC data of Laboratory 31.

General observation and summary:
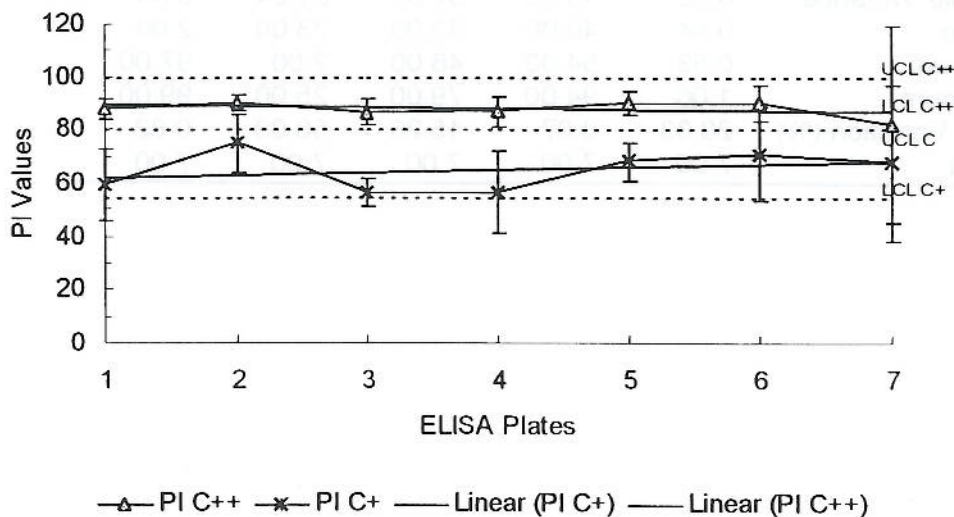This laboratory returned all EQA components. All EQC samples were identified correctly.
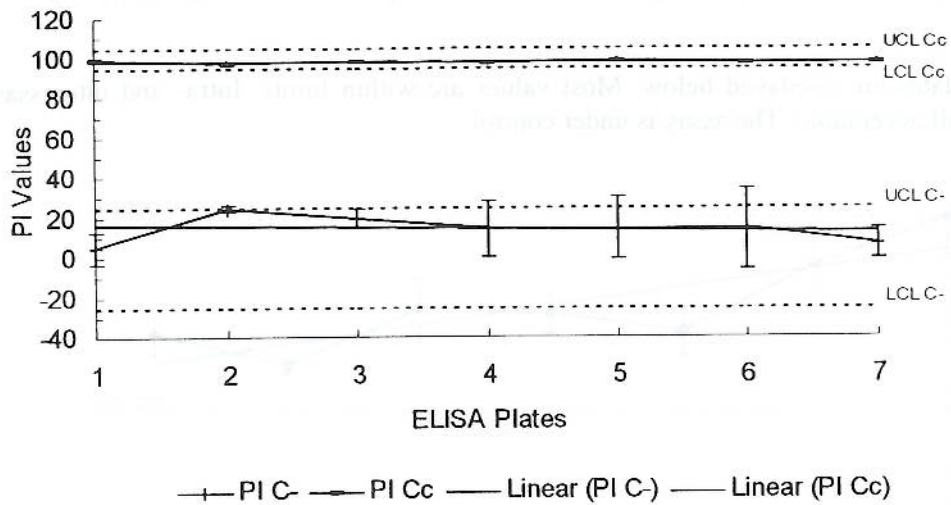
IQC data:
Only seven plates are displayed below. Most values are within limits. Intra- and interassay variation is still acceptable. The assay is under control.
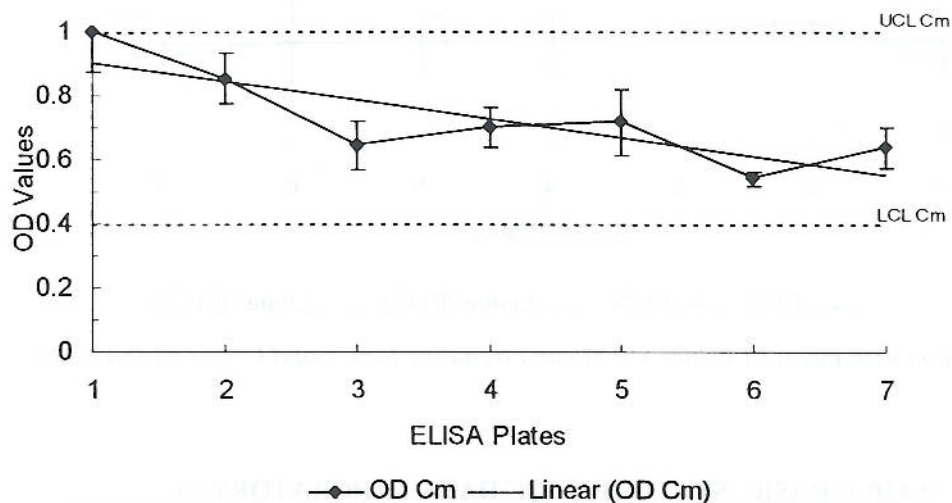
Control chart with mean OD values ± 2 SD and linear trendline for Cm per ELISA plate

Control chart with mean PI values ± 2 SD and trendlines for C++ and C+ per ELISA plate

Control chart with mean PI values ± 2 SD and trendline for Cc and C- per ELISA plate

TABLE BASIC STATISTICS IQC DATA LABORATORY 31

	OD Cm	PI C++	PI C+	PI C-	PI Cc
Mean	0.73	87.54	64.71	14.00	97.79
Standard Error	0.03	1.33	1.87	2.10	0.21
Median	0.68	88.50	65.00	13.50	98.00
Standard Deviation	0.15	7.06	9.88	7.84	0.80
Sample Variance	0.02	49.89	97.54	61.54	0.64
Range	0.54	40.00	33.00	23.00	2.00
Minimum	0.53	54.00	46.00	2.00	97.00
Maximum	1.06	94.00	79.00	25.00	99.00
Coef. Variation (%)	20.33	8.07	15.26	56.03	0.82
Count	7.00	7.00	7.00	7.00	7.00

- Attachment III -

Accumulated Data for Determination of "Recognition"

Accumulated Data for Determination of "Recognition" or "Provisional Recognition"

The criteria for "recognition" and "provisional recognition" are outlined in the Joint FAO/IAEA document, 1994 entitled "Establishment of external quality assurance procedures fur use with the FAO/IAEA ELISA kits" [6]:

1.   Recognized laboratory

Criteria: the laboratory that successfully fulfilled all of the requirements of the EQAP for the designated assay including passing the most recent proficiency tests

2.   Provisionally Recognized Laboratory

Criteria: a newly participating laboratory has successfully fulfilled all of the requirements of the EQAP for the designated assay including passing its first proficiency test

or

the laboratory has successfully passed the last two or more proficiency tests but has not fulfilled other requirements of EQAP

or

a recognized laboratory has failed the most recent proficiency test

Recognition will be withdrawn if a laboratory fails to meet the necessary EQAP requirements for the designated assay. Laboratories not fulfilling the requirements of EQAP will not be granted recognition.

Criteria applied

In Attachment III the submission of information (Questionnaire, IQC and EQC) is shown first as a quantitative (registration of any result or information submitted) and then as a qualitative information (Analysis/resume of questionnaire (emphasis on quality management e.g. calibration of equipment etc.), IQC data (mean values for Cm, C++, C+, C- and Cc within limits) and EQC data (correct identification of samples and location within Youden plot analysis)

For final evaluation purpose the qualitative information has been reduced to "ok" or "not ok" for each of the three categories.

An "ok" under the category questionnaire means that the laboratory has submitted updated and relevant information about the laboratory infrastructure, facilities, staff, equipment and quality assurance procedures.

An "ok" under the category IQC data means that the mean data of the IQC fall within the lower and upper control limits. Inter- and intraassay variation and the background colour are acceptable.

An "ok" under the category EQC data means that when a common cut-off (e.g. 50%) is applied EQC samples were identified correctly as positive or negative.

Twenty-eight laboratories have participated in the Rp98a. Out of these 16 have sent new or updated questionnaires, 15 have supplied information of their IQC data and 19 have submitted EQC results for this round.

Provisional recognition

Since participation and submission of correct results of the proficiency testing for at least two consecutive rounds is defined as a key element for the EQA programme 12 laboratories qualified for the status "Provisionally Recognized Laboratory".

These laboratories are: 1, 5, 8, 9, 10, 12, 13, 14, 15, 24, 26 and 31.

Recognition

Two laboratories have supplied all information (questionnaire, IQC and EQC) as required during the last two rounds and have qualified for the status "recognition".

These laboratories are: 23 and 30

The recognized laboratories will receive an FAO/IAEA recognition document and this information will be forwarded to OIE and FAO.

Future changes in "recognition" status and focus of EQA programme

During an IAEA consultants' meeting entitled " The FAO/IAEA External Quality Assurance Programme (EQAP) and Movement Towards a Generic Veterinary Diagnostic Testing Laboratory Accreditation Scheme" and subsequent discussions it was agreed that the category "Provisionally recognized" will disappear. Nevertheless in this report the category "Provisionally Recognized Laboratory" is still used for internal purpose. The category "recognition" will remain. It is emphasised that in order to achieve recognition a laboratory must fulfil and submit all components (Questionnaire, IQC and EQC data) of the EQA programme.

Quality management and documentation is an essential component of the EQA programme. Special attention will be given to calibrating procedures of laboratory equipment (ELISA reader, pipettes, pH meters, temperature measurement of freezers and refrigerators) and the self-monitoring of internal quality controls is encouraged (IQC data) [4, 17].

EQAP Rinderpest
Accumulated data

Adm. Info	Quantitative Info				Qualitative Info				Result
	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	Provisional/ Recognition
95a									
96a	5	x			ok		ok	upper right, outside	
97a	1	x	x	x	ok	too low, not ok	ok	central inside	PR
98a	1	x	x	x	ok	only 5 plates, not ok	ok	central inside	PR
	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a									
96a	3			x			ok	upper right, inside	
97a	2								
98a	2	x		x	not ok	ok	ok	1 border, 2 slightly oustside	
	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a									
96a	28	x	x	x	ok		ok	central inside	
97a	3		x	x			ok	central inside	PR
98a									
	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a									
96a	26								
97a	4	x	x	x	ok		ok	central inside	
98a									
	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a									
96a	30		x	x	ok		ok		
97a	5	x	x	x	ok		ok	outside	
98a	5	x	x	x	not ok	no iqc data on diskette	ok	inside, lower left	PR
	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a									
96a	29			x					
97a	6						not ok	central left outside	
98a									
	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a									
96a									
97a	7								
98a									

Attachment III

EQAP Rinderpest
Accumulated data

Adm. Info	Quantitative Info			Qualitative Info				Result
Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	Provisional/ Recognition
95a								
96a 25			x			ok	central left, slightly outside	PR
97a 8			x			ok	upper right, outside	PR
98a 8			x			ok	lower left, slightly out, 1 border	
Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a								
96a 31	x	x	x	ok		ok	central inside	PR
97a 9	x	x	x	ok	ok	ok	central left, bordeline inside	PR
98a 9	x	x	x	ok	not ok	ok	inside low	
Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a								
96a 27	x	x	x	ok	ok	not ok	central left, slightly outside	R
97a 10	x	x	x	ok	ok, C++, C+ slightly low	ok	central inside	PR
98a 10	x	x	x	ok	not ok	ok	inside upper border	
Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a 22								
96a 11						ok	central inside	
97a 11	x	x	x	ok	not ok			
98a								
Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a 8								
96a 12	x	x	x	ok	ok	ok	central inside	PR
97a 12	x	x	x	not ok	only 7 plates	ok	lower left, slightly outside, 1 bord	PR
98a								
Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a 13			x			ok	central left, slighly oustide	PR
96a 13			x			ok	central inside	PR
97a 13			x			ok	lower left, outside border	PR
98a								
Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a 11						ok	lower central, outside	
96a 14								
97a 14	x	x	x	ok	ok	ok	2 slightly outside, left, 1 inside	PR
98a								

EQAP Rinderpest
Accumulated data

Adm. Info	Quantitative Info			Qualitative Info				Result
Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	Provisional/ Recognition
15	x		x	ok		ok	central lower left, slightly outside	
15		x	x		not ok	ok	1 border, 2 outside upper right	PR
Lab code	**Quest.**	**IQC**	**EQC**	**Quest.**	**IQC**	**EQC**	**Youden**	
23		x	x		ok	ok	central upper left, outside	
16								
16			x			ok	inside central	
Lab code	**Quest.**	**IQC**	**EQC**	**Quest.**	**IQC**	**EQC**	**Youden**	
17								
17								
Lab code	**Quest.**	**IQC**	**EQC**	**Quest.**	**IQC**	**EQC**	**Youden**	
19	x	x						
18	x	x	x	ok	ok	ok	lower left, outside	PR
Lab code	**Quest.**	**IQC**	**EQC**	**Quest.**	**IQC**	**EQC**	**Youden**	
21	x			ok				
19								
19	x		x* from 97	ok				
Lab code	**Quest.**	**IQC**	**EQC**	**Quest.**	**IQC**	**EQC**	**Youden**	
20	x		x	ok		ok	lower central, outside	
20	x	x	x	ok	ok	ok	lower left, slightly outside	R
Lab code	**Quest.**	**IQC**	**EQC**	**Quest.**	**IQC**	**EQC**	**Youden**	
15	x		x	ok		ok	upper right, slightly out	
21								

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EQAP Rinderpest
Accumulated data

Adm. Info		Quantitative Info			Qualitative Info				Result
	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	Provisional/ Recognition
95a									
96a	14	x	x	x	ok	ok	ok	central inside	PR
97a	22	x	x		ok	ok, Cm slightly too low	ok	1 border, 2 outside lower left	
98a	22	x	x	x	ok	not ok, OD Cm too low	ok		
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
96a	23	x	x	x	ok	C++ low, C- high, not OK	ok	upper right, slightly outside	R
97a	23	x	x	x	ok	ok, C++ very close to C+	ok	central inside	
98a									
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a	9	x	x	x	ok	ok	ok	central inside	PR
96a	24	x	x	x	ok	ok	ok	central inside	R
97a	24	x	x	x	ok	ok, C++ very close to C+	not ok	extremely outside lower left	PR
98a									
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a	12	x	x	x	ok	ok	ok	central inside	PR
96a	25	x	x	x	ok	Cm low, C-high, not ok	ok	upper right, slightly outside	R
97a									
98a									
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a	7	x	x	x	ok	ok	ok	upper right, slightly out	PR
96a	26	x	x	x	ok	low OD, but mean still ok	ok	central inside	R
97a	26	x	x	x	ok		ok	central inside	PR
98a									
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a	6	x	x	x	ok	ok	ok	lower left, outside	PR
96a	27								
97a									
98a									
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
95a	4	x	x	x	ok	ok	ok	upper right, outside	PR
96a	28	x	x	x	ok	ok	ok	central inside	
97a									
98a									

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EQAP Rinderpest
Accumulated data

Adm. Info		Quantitative Info			Qualitative Info				Result
	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	Provisional/ Recognition
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
96a	2	x		x	ok		ok	central inside	
97a	29	x	x	x	ok		ok	upper right, slightly outside	PR
98a	29	x	x	x	ok	ok	not ok	extremely oustide lower left	
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
96a	1	x	x	x	ok	ok	ok	upper right, slightly out	PR
97a	30	x	x	x	ok	ok	ok	upper right, slightly outside	R
98a	30	x	x	x	ok	ok	ok	1 inside, 2 slightly low outside	R
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
96a									
97a									
98a	31	x	x	x	ok	ok, but need more data	ok	inside	PR
95a	Lab code	Quest.	IQC	EQC	Quest.	IQC	EQC	Youden	
96a									
97a									
98a	32								

96a EQC sample 2 was excluded from evaluation
97a EQC sample 2 was excluded from evaluation

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Attachment III