Clin Exp Immunol 1998; **111**:564–573

The Italian quality control study for evaluation of CD4 cells in centres involved in the treatment of HIV-1 patients

F. PANDOLFI*†, C. ALARIO*, E. GIRARDI‡, L. RAVA'‡, G. IPPOLITO‡, A. KUNKL§, F. AIUTI* & THE OTHER MEMBERS OF THE ITALIAN CD4 QUALITY CONTROL GROUP¶ **Department Clinical Medicine, Chair of Clinical Immunology and Allergy, La Sapienza University, †Chair of Internal Medicine, Catholic University, and ‡Centro Riferimento AIDS, Spallanzani Hospital, Rome, and §Department of Immunology, S. Martino Hospital, University of Genoa, Genoa, Italy*

(Accepted for publication 6 November 1997)

SUMMARY

We report on the experience of establishing a national network for a quality control programme in evaluating CD4 cell counts in most Italian centres involved in the care of patients with HIV disease. The 68 centres were divided according to their geographical location into eight groups, and twice a year (tests A and B) they received three coded whole blood samples (two were replicates of the same sample) obtained from two informed HIV^+ patients, one with CD4 counts/mm^3 expected to be <200 and one with values >300. The medians of the determinations performed by the labs involved in each of the eight areas were taken as the 'true' values for each sample. Unsatisfactory performances for percentage of CD4 cells were identified as a CD4 analysis with residual values $\geq \pm 5\%$ and with deviates $\geq \pm 2$. For absolute numbers of CD4 cells, an unsatisfactory performance was defined as CD4 counts with residual $> \pm 100$ CD4 cells/mm^3 and with deviates $\geq \pm 2$. The residual value is the CD4 value reported by each lab minus the median value. The deviate is the residual divided by the modified interquartile range (IQR $\times 0{\cdot}75$). Most of the centres provided reliable results. However, some labs failed to provide satisfactory results for percentages (6·25% of the tested labs for test A and 6·17% for test B) or absolute numbers (16·25% test A and 12·34% test B). Only 3·7% of the labs gave unsatisfactory results in both tests. Four of the unsatisfactory results from the two tests gave an error in absolute numbers $> \pm 200$ CD4 cells/mm^3. Our data suggest that most Italian labs provide reliable results in evaluating the numbers of CD4 cells in HIV-1^+ samples, but the importance of running a quality control programme is highlighted by our experience with those centres which provide unsatisfactory data which may lead to incorrect classification of the patients or assessment of treatment.

Keywords HIV-1 CD4 quality control

INTRODUCTION

CD4 counts have been shown to be of great value, together with assessment of viral load, in the classification and management of patients with HIV-1 [1–7]. This marker is predictive of progression to AIDS. In addition, many treatment guidelines, including antiretroviral therapy and prophylaxis and treatment of opportunistic infections, rely on absolute CD4 counts [8–11].

As with most laboratory tests, evaluation of percentages and absolute numbers of CD4 cells is subject to significant variability [12–15]. Unsatisfactory counts may result in an incorrect classification of patients in relation to treatment [16]. In addition to diurnal variations, CD4 levels may vary according to the use of tobacco, caffeine, alcohol, exercise and stress [17]. Laboratory testing also introduces variability depending on the MoAbs, fluorochromes, instruments [18,19]. Procedures and guidelines have been established by the Centers for Disease Control (CDC) in order to standardize these technical problems [20].

¶F. Dammacco, F. Silvestris (Department of Biomedical Sciences, University of Bari); A. Facchini (Immunological Lab., University of Bologna); S. Del Giacco, V. Arangino, P. Manconi (Department of Internal Medicine, University of Cagliari); S. Romagnani, M. G. Giudizi, R. Biagiotti (Clinical Immunology, University of Florence); M. P. Terranova (Department of Immunology, S. Martino Hospital, University of Genova); M. Moroni, M. Galli (Infectious Disease, University of Milan), A. Lazzarin, L. Soldini (Infectious Disease, IRCCS H S. Raffaele, Milan); G. Semenzato, R. Zambello, R. Sancetta (Department of Clinical and Experimental Medicine, University of Padoa); V. Tiracchia (Clinical Immunology, La Sapienza University, Roma).

Correspondence: Professor F. Pandolfi, c/o Department of Allergy and Clinical Immunology, Viale dell' Universita' 37, 00185 Rome, Italy.

Since the determination of CD4 levels is particularly important in HIV disease, the Italian Ministry of Health has promoted a quality control (QC) of CD4 lymphocyte counts for all the clinical centres involved in trials coordinated by the Italian National Institute of Health. These centres deal with more than 90% of all Italian HIV-1 patients. In this study we report on the first year of our experience.

MATERIALS AND METHODS

Establishment of a national network for QC of CD4 counts in HIV-1 patients

We identified 68 Italian clinical centres which are currently involved in trials with antiviral drugs on patients with HIV-1 disease. The large majority of patients with HIV-1 disease living in Italy are referred to these centres. On the basis of their geographical location, we identified nine additional centres, with established experience in flow cytometry and involved in care of HIV patients, which were requested to coordinate the QC tests in their regions. Each of the nine reference centres (RC) had seven to nine affiliated clinical centres. Five RC were located in northern Italy (two in Milan, and one each in Bologna, Genoa and Padoa), two were in central Italy (Florence and Rome), one in southern Italy (Bari) and one in Sardinia (Cagliari). The Rome centre acted as the national coordinator.

Each RC contacted the affiliated clinical centres and conducted a preliminary survey of the techniques, instruments and reagents routinely used by the labs that routinely evaluate the percentages and absolute numbers of CD4 cells for their patients. The few labs using technical procedures different from those recommended by CDC guidelines [21] were required to modify their protocols and to adopt the CDC criteria. Different cytometers and brands of MoAbs were used by different laboratories to perform CD4 counts. Labs where the CD4 counts were routinely performed by more than one operator performed one separate test for each operator, thus giving a total number of performances above the number of tested labs. Labs were coded to maintain confidentiality of the results.

QC tests for CD4

In this study we describe the first two identical tests (test A and test B) performed approximately 6–8 months apart by 69 labs (test A) and 63 labs (test B; these figures include the RC). Cagliari did not perform test B. The tests were conducted as follows. Each RC shipped three samples (or more in the case of multiple operators) to each of the geographically associated clinical centres. The three samples were obtained from two informed, consenting HIV-1^+ patients, one with expected CD4/mm^3 >300 cells and one with expected CD4/mm^3 between 100 and 200 cells. To allow the identification of possible regional bias, in test B each RC was also required to ship the samples to the geographically closest RC. Analysis of the samples by the closest RC did not reveal the presence of regional bias. All but one of the expected >300 samples were in the correct range (except for the Genoa sample in test B, with a median of 258). Of the expected <200 samples, three gave results between 200 and 250 (Cagliari and Rome in test A, and Bari in test B), one gave 267 (Padoa test A) and one 389 (Florence test A). Two of the three shipped samples were identical. Samples were coded, shipped by courier and reached the lab usually within the day in which the blood was drawn. Over 80% of the labs performed or fixed the samples within 24 h from drawing (mean 17 h, median 8 h). Twenty-six percent of the labs fixed the samples upon arrival and performed the analysis later. Each lab performed the evaluation of percentage and absolute numbers of CD4 cells in the three samples according with their routine protocols.

Statistical analysis

Since the samples shipped by one RC were different from those sent by another RC, the 'true' value for each sample was defined as the median of the determinations performed by the seven or more labs involved in the test and also including the values provided by the local RC. Two statistical parameters were evaluated for the analysis of results: residual value and deviate. Residual was calculated as the reported value minus the group median for the sample. Deviate was calculated as the residual divided by the modified interquartile range ($0{\cdot}75\times$ IQR).

Unsatisfactory performance for CD4 cell percentage was defined, according to the 1993 NIH Guidelines for flow cytometric immunophenotyping (version 1.0), as a CD4 analysis with a residual value $\geq \pm 5\%$ and with a deviate $\geq \pm 2$.

For absolute numbers of CD4 cells, an unsatisfactory level of performance was defined as CD4 count with a residual $> \pm 100$ CD4 cells/mm^3 and with a deviate $\geq \pm 2$.

Reproducibility of the results was measured by comparing the percentage and absolute counts in the duplicates. A spread $>5\%$ (for percentages) or >100 CD4/mm^3 indicated reproducibility problems.

The χ^2 test or the Fisher exact test when appropriate were used to compare proportions. The Mann–Whitney *U*-test was used to investigate the existence of a statistically significant difference in the median hours of delay between the time of the blood samples being drawn and the time of processing or fixation.

RESULTS

Test A

Results of the first test are summarized in Figs 1 and 2 (only one set of data is shown for duplicate samples). Figure 1 is related to the

Fig. 1. (See next page.) Test A: sample with expected >300 CD4/mm^3. Results obtained by individual laboratories in each area coordinated by a reference centre (BA, Bari; BO, Bologna; CA, Cagliari; FI, Florence; GE, Genoa; MI1 and 2, Milan 1 and Milan 2; PD, Padoa; RM, Rome). (a) Percentage of CD4 cells. The 0 line represents the 'true' value, calculated as the median of all the determinations performed in each area. Individual results are expressed as percentage residuals according to 1993 NIH Guidelines for flow cytometry. Residual values $\geq \pm 5\%$ and with deviates $\geq \pm 2$ are considered as unsatisfactory (in this case the deviate value is reported in the Figure). Median percentage values were: 19 (BA), 24 (BO), 23 (CA), 18 (FI), 20 (GE), 20 (MI1), 29 (MI2), 33 (PD), 39 (RM). (b) Absolute numbers of CD4 cells. The 0 line represents the 'true' value, calculated as the median of the determinations performed in each area. Individual results are expressed as residual values. An unsatisfactory level of performance was defined as CD4 counts with residual values $> \pm 100$ CD4 cells/mm^3 and with deviates ≥ 2 (in this case the deviate value is reported in the Figure). Median absolute values were: 331 (BA), 551 (BO), 481 (CA), 480 (FI), 608 (GE), 309 (MI1), 649 (MI2), 509 (PD), 729 (RM).

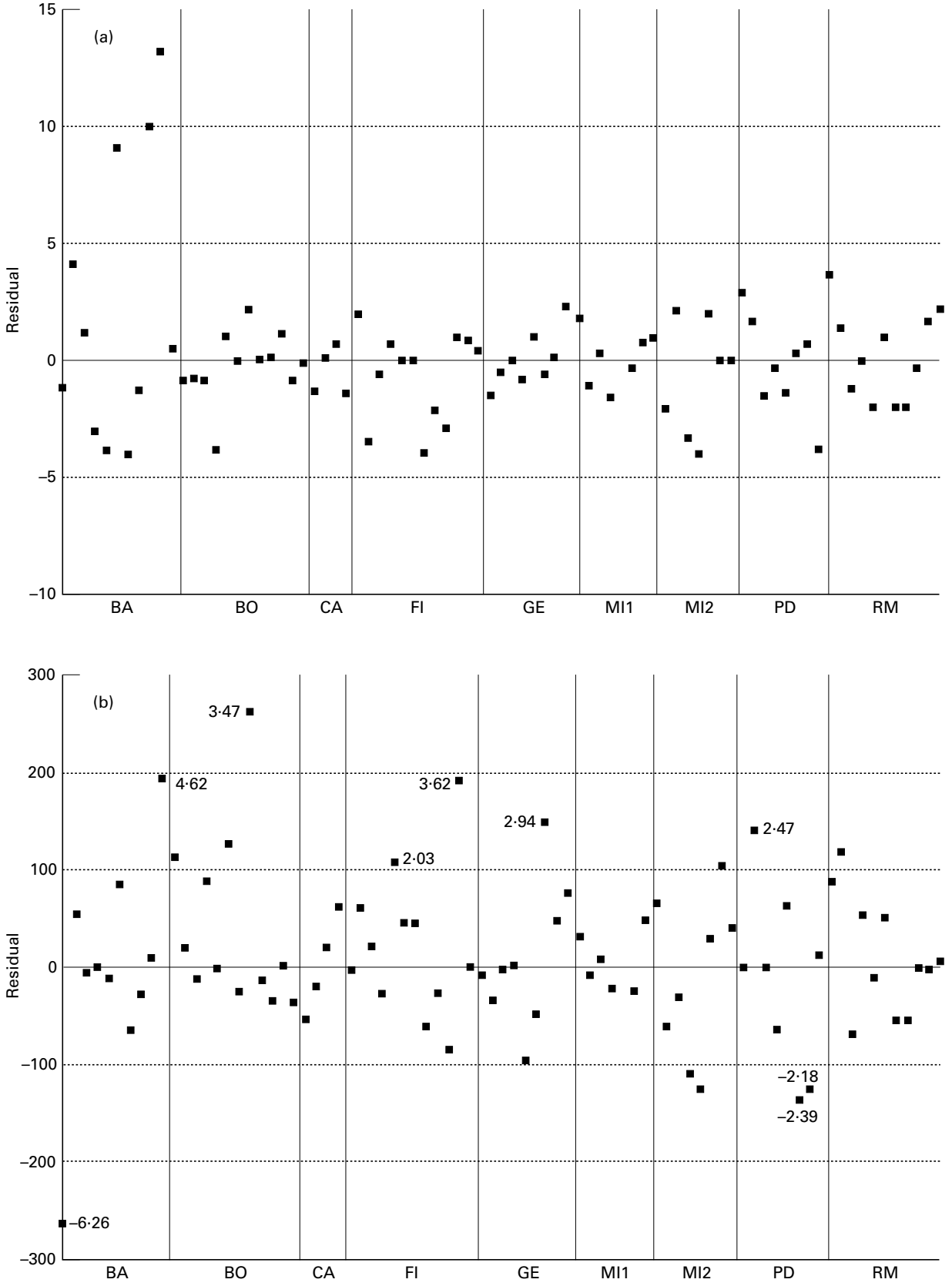

Fig. 1. See previous page for caption.

sample with > 300 CD4/mm^3. Figure 1a shows the determinations of percentages. All results were satisfactory. In fact, even if three results showed percentage residuals above the 5% limit, their deviates were < 2. Analysis of the absolute counts is shown in Fig. 1b. Ten results were above 100 CD4 cells/mm^3 and five below 100. Analysis of the deviates indicate that nine of the 15 results also had deviates > 2, thus resulting in an unsatisfactory performance.

In Fig. 2 are reported the data obtained with the sample with < 200 CD4 cells/mm^3. For the percentages of CD4 cells, five data showed residual values > 5% and all of them also had deviates > 2 (Fig. 2a). In the evaluation of absolute numbers, five labs gave residual values above or below 100, and all also had deviates > 2 (Fig. 2b).

The ability of the labs to give unsatisfactory results in identifying the duplicate samples was also evaluated. Three labs gave results diverging by > 5% and eight gave absolute numbers on the same duplicate samples with a difference > ± 100 cells/mm^3.

Test B

Results of the second test (performed 6–8 months after test A) are summarized in Figs 3 and 4. Figure 3 is related to the sample with > 300 CD4. Figure 3a shows the determinations of percentages. Two labs gave results above and four labs below 5% of residual values. Of these, two above and two below also had a deviate value > 2. Analysis of the absolute counts is shown in Fig. 3b. Eight labs were above a residual value of 100 and two below. Of these, six (above) and one (below) also had deviates > 2.

In Fig. 4 are reported the data obtained with the sample of < 200 CD4 cells. For the percentages of CD4 cells, five labs showed residual values > ± 5%, and of these four also had deviates > 2 (Fig. 4a). In the evaluation of CD4 absolute numbers, six labs gave residual values above or below 100, and all also had deviates > 2 (Fig. 4b).

The ability of the labs to give unsatisfactory results in identifying the duplicate samples in test B showed that four labs gave results diverging by > 5%, and two of them gave absolute numbers on the same duplicate samples with a difference > ± 100 cells/mm^3.

Analysis of the two tests (A and B) indicates that only three labs gave unsatisfactory results in both tests.

Effects of methods, materials or instrumentation on the results

We also evaluated if some technical aspects of the analysis could account for the unsatisfactory results observed. Recovery was calculated by 61 centres (89·7%) determining the expression of CD14 and CD45. Three labs used the Immunocount system [22] and four labs gave no information. Absolute numbers were calculated using the percentages of CD4 cells and the absolute numbers of lymphocytes determined by a separate cell counter by 62 labs. These centres used a wide variety of cell counters of different brands. Six labs provided absolute numbers directly using either the Immunocount system associated with an Ortho cytometer, which provide absolute counts (three labs) or an Ortho absolute cytometer without using the Immunocount (three labs), but evaluating the recovery by CD14/CD45. Data were obtained using anti-CD4 monoclonal reagents purchased from Becton Dickinson (36 labs), Ortho (15 labs), Coulter (six labs) or Dako (three labs) (these data were not available for all centres). Analysis was performed with cytometers manufactured by Becton Dickinson (35 labs), Ortho (23 labs) or Coulter (nine labs).

The mean delays between the time when blood samples were drawn and the time of processing or fixation were 16·16 h and 19·52 h for tests with satisfactory and unsatisfactory results, respectively (medians 7·25 h and 24·25 h). These differences were not statistically significant. The proportions of fixed blood samples were 22·8% and 16·7% among the satisfactory and unsatisfactory results, respectively. Among the blood samples with satisfactory results, 53·7% reached the laboratories at room temperature, while among those samples with unsatisfactory results, 48% were at room temperature. Both differences were not statistically significant. Unsatisfactory results were uncommon in samples fixed (only 13% of the labs fixing their samples gave unsatisfactory data, of a total of 26% who fixed the samples). Although our data argue in favour of early processing/fixation of the samples, our numbers are too small to allow definitive conclusions. These data suggest that the results were largely uninfluenced by the conditions of transport of the samples from the RC to the individual laboratory.

No significant differences were observed in the unsatisfactory results according to the brand of MoAbs used to detect the CD4 molecule or the brand of cytofluorimeter used.

DISCUSSION

We have implemented a project to assess the quality of results in the enumeration of CD4 cells (both percentages and absolute numbers) in HIV-1$^+$ blood samples by over 60 Italian centres which are the large majority of those involved in antiviral trials of HIV-1$^+$ patients. Three coded samples (two of them identical) from two HIV$^+$ subjects (one with CD4 counts > 300 and one < 200/mm^3) were sent twice a year (tests A and B) to each lab in nine Italian regions. The labs provided the percentages and absolute CD4 values of the samples. Individual results were compared with the median of all the results of each region and unsatisfactory data were determined according to NIH guidelines for percentages (CD4 analysis with residual values ≥ ± 5% and with deviates ≥ 2) and, for absolute numbers, as those which gave residual values of ± 100 counts with deviates ≥ 2. Reproducibility was assessed by comparing the data provided for the two identical samples.

While acceptability criteria for percentages of CD4 cells were adopted from NIAID, residuals for absolute CD4 counts have been worked out by us. The criterion of ± 100/mm^3 residual for CD4 counts showed a number of unacceptable CD4 absolute counts similar to the number of unacceptable percentages in the samples with < 200 CD4/mm^3. On the other hand, a higher number of

Fig. 2. (See next page.) Test A: sample with expected < 200 CD4/mm^3. Results obtained by individual laboratories in each area coordinated by a reference centre. Results are summarized as reported in Fig. 1. (a) Percent of CD4 cells. Median percentage values were: 7 (BA), 9 (BO), 11 (CA), 21 (FI), 15 (GE), 8 (MI1), 11 (MI2), 19 (PD), 19 (RM). (b) Absolute numbers of CD4 cells. Median absolute values were: 156 (BA), 176 (BO), 211 (CA), 389 (FI), 170 (GE), 143 (MI1), 125 (MI2), 267 (PD), 220 (RM).

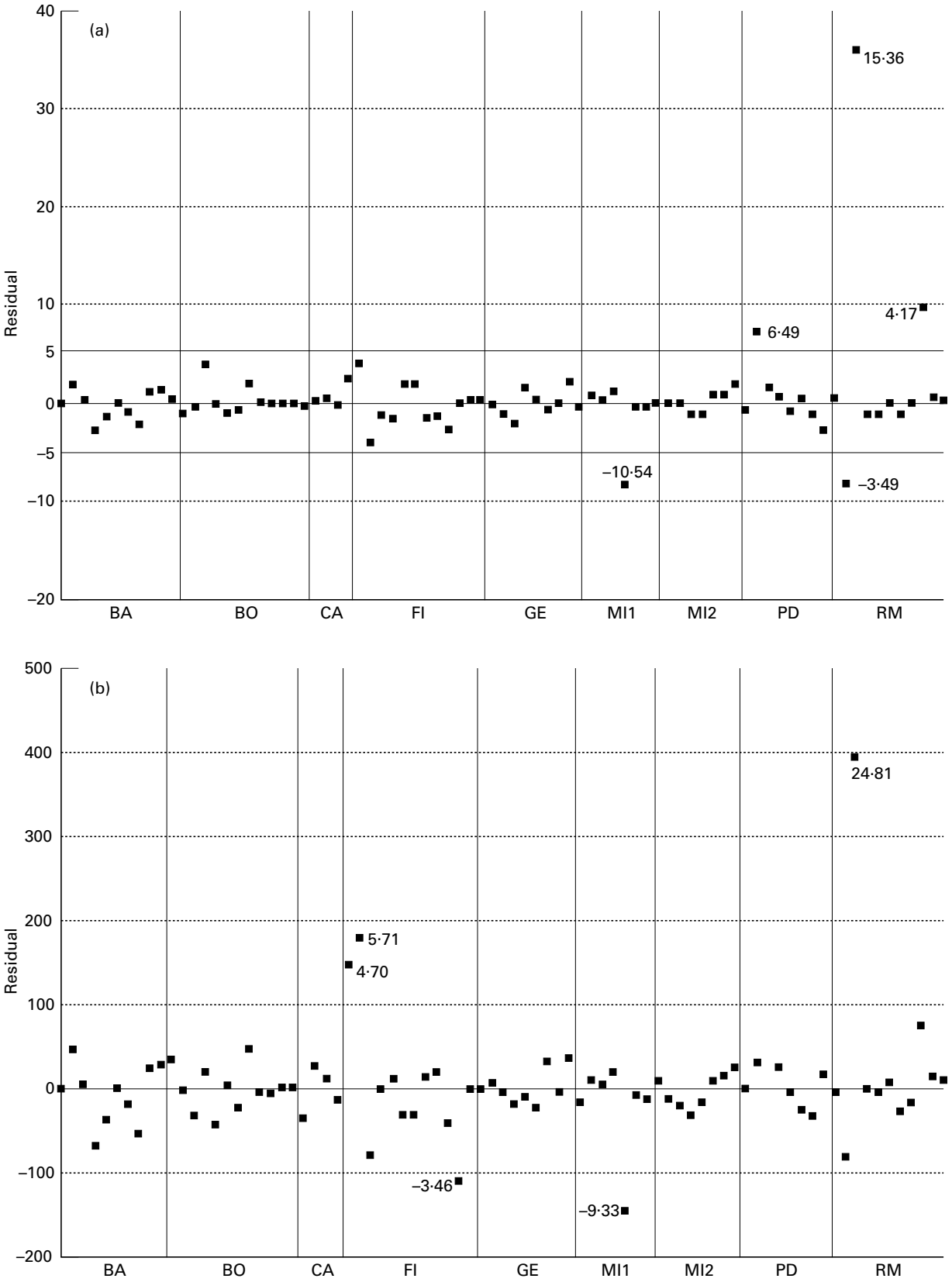

Fig. 2. See previous page for caption.

unacceptable absolute counts (compared with percentages) was detected in samples with > 300 CD4 cells. This suggests that the ± 100 cells/mm^3 limit might be too narrow an acceptable residual for samples with > 300/mm^3. However, this criterion was chosen considering a higher interlaboratory spread of CD4 counts compared with percentages, due to the combination of haematological with flow cytometric data.

Our data suggest that most Italian labs provide reliable results in evaluating the numbers of CD4 cells in HIV-1$^+$ samples, but the importance of running a quality control programme is highlighted by our experience with those centres which provide unsatisfactory data. Several labs failed to yield satisfactory results for percentages (6·25% of the tested labs for test A and 6·17% for test B) or absolute numbers (16·25% test A and 12·34% test B). Only 3·7% of the labs gave unsatisfactory results in both tests. Reproducibility was incorrectly performed by 9·8% and 3·7% of the labs in the two tests, respectively. Four of the unsatisfactory results from the two tests gave an error in the absolute numbers > ± 200 CD4 cells/mm^3. Labs submitting unacceptable data can be studied longitudinally in regular quality control programmes with the aim of encouraging them to correct the problems and achieve a better performance with the next sample.

We identified the labs with unsatisfactory results, and each RC organized an additional test shortly after test A or B. This included analysis of an HIV$^+$ blood sample at the RC and at the lab that provided unsatisfactory results. If the labs failed this additional test, they were invited to perform the test again at the RC to discuss possible technical problems. In addition, we also investigated the set up performance and stability of flow cytometers over a 10-day period (A. Kunkl *et al*. in preparation). We used the FITC Combo Kit (provided by Flow Cytometry Standards Corp., San Juan, PR), which includes a QCWindows FITC reference standard and a mixture of FITC quantitative and blank standards (Quantum 26 FITC microbeads) with Quick Call software [23]. Seventeen percent of the tested labs had performance parameters outside the acceptable range and their instruments required calibration. Some of these labs indeed gave unsatisfactory results in test A or B. Others reasons for poor performance were occasionally identified as unsatisfactory results obtained with the cell counters or as delayed processing of the samples. This occurred, for instance, in the Bari region in test A, where two labs analysed the samples with considerable delay (> 48 h) due to problems in the shipment. Since the deviate is used as a second conclusive criterion for definition of lab performance with the aim of protecting a lab when the spread and variation is unusually large, this probably accounts for the high residual (with deviates < 2) observed in Fig. 1a in the Bari coordinated region.

We have considered the use of stabilized blood samples in our protocol, which would permit the use of a truly national distribution. At the time of our study, the only available such reagent was Ortho Absolute Control. However, while this stabilized blood properly works on both Ortho and Becton Dickinson cytometers, some problems have been experienced with Coulter cytometers. These are possibly related to the use of Coulter Q-Prep lysing solution, which may alter the forward scatter of the stabilized cells (P. Salvini, Ortho Diagnostic Systems, Milan, personal communication). Since we also wanted to include in our survey labs equipped with Coulter instruments, we decided not to use the stabilized samples. In addition, our aim was the evaluation of CD4 cells in samples from HIV$^+$ donors, and we choose to use HIV$^+$ rather than normal samples. In the attempt to enforce a truly national distribution, we performed one additional test (test D, data not shown), consisting of the shipment of the same HIV$^+$ blood sample from the national centre in Rome to all the peripheral centres. With this test, however, we experienced considerable variability in the time the samples reached some labs far away from Rome, reinforcing the validity of the regional approach we used in tests A and B.

The importance of running a quality control programme is evident for every laboratory investigation. In particular, evaluation of CD4 cells is remarkably relevant in trials for determining the effects of anti-retroviral treatment since, apart for the determination of viral burden, several conclusions on the efficacy of the drugs under test are established on the basis of modifications of CD4 cell counts during treatment. Therefore, a precise evaluation of CD4 counts is needed. Quality control programmes are performed at both the intra- and interlaboratory levels, but reports in the international literature on the experience of interlaboratory tests at a national level are limited [14,24–27]. This study provides the experience of a national network.

Our data show that unsatisfactory performances in the tests were unrelated to the usage, in the individual labs, of different commercial anti-CD4 MoAbs or cytometers, suggesting that unacceptable results were the effect of analysis performed by the individual laboratories. No significant association was observed in the overall data between the conditions of transport of the samples (delay in reaching the laboratory, arrival at room temperature instead of at 4°C) and poor performance. However, our data, although the sample is too small to reach significance, suggest that better performances were provided by labs who fixed or analysed the samples in the same day of blood drawing. This indicates the importance of the logistical framework of quality control protocols, considering that rapid shipment of HIV-infected blood samples is not generally available. We have addressed this issue by designing a national quality control programme articulated on several RC acting at a regional level. Our data reinforce

Fig. 3. (See p570.) Test B: sample with expected > 300 CD4/mm^3. Results obtained by individual laboratories in each area coordinated by a reference centre. Results are summarized as reported in Fig. 1. (a) Percent of CD4 cells. Median percentage values were: 36 (BA), 22 (BO), 19 (FI), 21 (GE), 14 (MI1), 30 (MI2), 27 (PD), 30 (RM). (b) Absolute numbers of CD4 cells. Median absolute values were: 624 (BA), 346 (BO), 408 (FI), 258 (GE), 323 (MI1), 416 (MI2), 370 (PD), 673 (RM).

Fig. 4. (See p571.) Test B: sample with expected < 200 CD4/mm^3. Results obtained by individual laboratories in each area coordinated by a reference centre. Results are summarized as reported in Fig. 1. (a) Percent of CD4 cells. Median percentage values were: 17 (BA), 22 (BO), 5 (FI), 16 (GE), 10 (MI1), 9 (MI2), 8 (PD), 7 (RM). (b) Absolute numbers of CD4 cells. Median absolute values were: 224 (BA), 177 (BO), 36 (FI), 60 (GE), 104 (MI1), 103 (MI2), 154 (PD), 125 (RM).

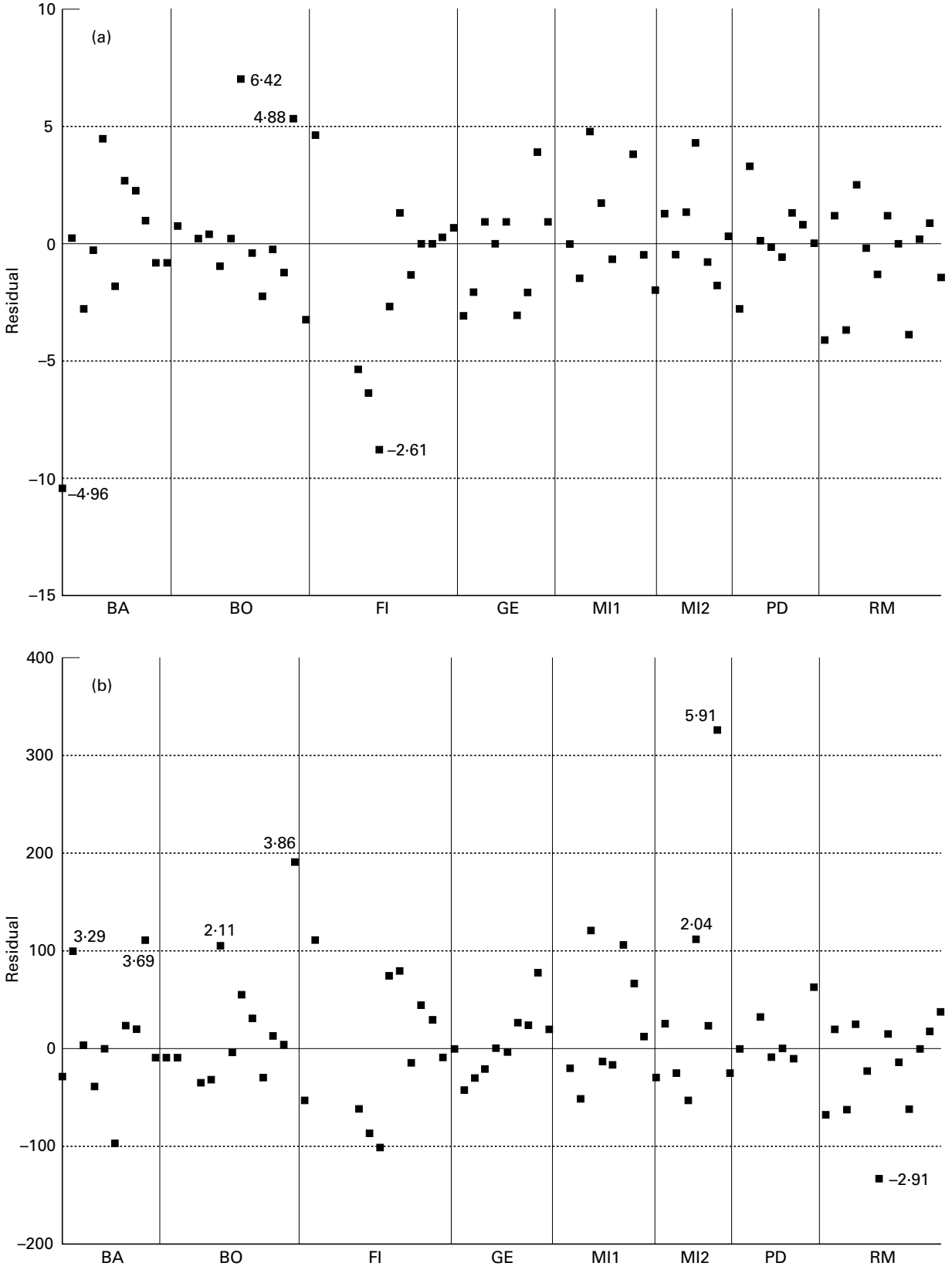

Fig. 3. See previous page for caption.

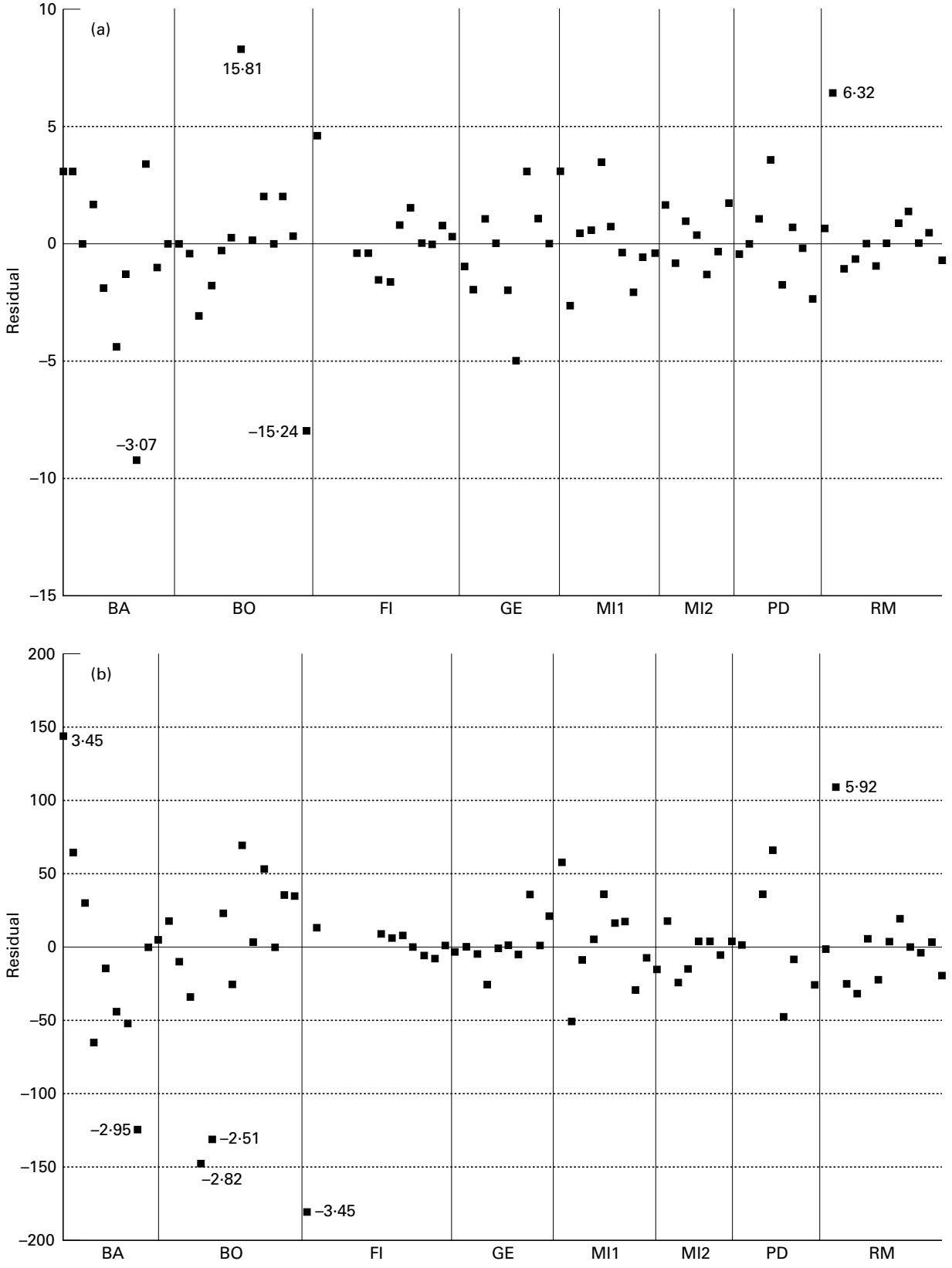

Fig. 4. See p569 for caption.

the importance of running a quality control programme for CD4 cells in centres dealing with the treatment of HIV patients, and the need for a careful performance of cytofluorimetric analysis.

ACKNOWLEDGMENTS

This work was supported by a contribution from the Istituto Superiore di Sanita', Ministero Sanita', AIDS grants nos 880/01 and 9403–03. List of participating laboratories coordinated by the nine RC: Aceti A., Università di Sassari; Airò P., Spedali Civili di Brescia; Albertazzi D., Arcispedale S. Maria Nuova, Reggio Emilia; Balestrieri A., University of di Cagliari; Bitti P., Ospedale S. Francesco, Nuoro; Bonara P., Ospedale Policlinico di Milano; Bonavia V. M., Ospedale Grande degli Infermi, Viterbo; Boni P., Policlinico S. Orsola, Bologna; Bosco O., Dipartimento di Medicina Interna, DIMI, Genova; Brando B., Ospedale Niguarda, Milano; Campanella A., Ospedale Galliera, Genova; Cherchi G. B., Ospedale A. Segni, Ozzieri (SS); Cherchi G. B., Ospedale SS. Annunziata, Sassari; Contu L., University of di Cagliari; Cordiali Fei P., Istituto S. Gallicano, Roma; Corrias L., Ospedale S. Martino, Oristano; Cosentini E., Immunoematologia, Università 'Federico II', Napoli; Coser P., Ospedale Bolzano; D'Arena G., Ospedale S. Giovanni Rotondo (FG); D'Elia D., Azienda U.S.L. 4, Prato; D'Erasmo F., Ospedale Di Venere, Carbonara (BA); De Felici A. P., Ospedale Spallanzani, Roma; De Rosa C., Ospedale 'Miulli', Acquaviva delle Fonti (BA); de Paoli P., Centro di Riferimento Oncologico, Aviano (Pordenone); Di Loreto M., Ospedale 'Giovanni XXIII', Bari; Fei C., Ospedale Civile di Sondrio; Fiaccadori F., Università di Parma; Francisci D., Università di Perugia; Gasponi A., Ospedale Bellaria, Bologna; Gerna G., Policlinico S. Matteo, Pavia; Giacometti A., Ospedale Umberto I, Ancona; Guarnori I., Ospedale di Lecco; Guerra G., Arcispedale S. Anna, Ferrara; Lombardi S., Ospedale Pediatrico Apuano, Massa Carrara; Maglino E., Ospedale Niguarda, Milano; Malacrida V., Presidio Ospedaliero di Busto Arsizio; Mancini E., Azienda USL Frosinone; Maroni P., Ospedale di Circolo di Varese; Martini D., Ospedale Amedeo di Savoia, Torino; Materazzi M., Azienda Ospedaliera Santa Maria, Terni; Miconi V., U.L.S.S. 6 Vicenza; Migali E., Presidio Ospedaliero, Arezzo; Moroni G. A., Ospedale S. Paolo, Milano; Ongaro G., Azienda Ospedale-Università Padova; Pasotto S., PMIP, Milano; Petrelli E., Azienda Ospedaliera S. Salvatore, Pesaro; Piccaluga A., Università di Cagliari; Piu G., Ospedale G. Brotzu, Cagliari; Quirino A., Ospedale Nuovo Pellegrini, Napoli; Ortolani D., Ospedale Borgo Roma, Verona; Recchia O., Ospedale S. Giovanni Addolorata, Roma; Rescaldani R., Ospedale S. Gerardo de' Tintori, Monza (MI); Romano M. R., Ospedale Civile S. Andrea, La Spezia; Roncaglia R., Policlinico Modena; Rossi E., Ospedale L. Sacco, Milano; Rottoli P., Università di Siena; Rumi C., Università Cattolica, Roma; Salvaterra T., Azienda Ospedaliera Carlo Poma, Mantova; Santoro A., Ospedale Di Summa, Brindisi; Scarso L., Istituto G. Gaslini, Genova; Schiattone M. L., Ospedale Maggiore, Bologna; Troncossi C., Ospedale per gli Infermi, Faenza; Tumietto F., Istituto di Malattie Infettive, Bologna; Tzantzoglou S., Policlinico Umberto I, Roma; Vendittis A., Ospedale S. Anna, Como; Viale P. L., Azienda USL di Piacenza.

REFERENCES

1 Polk F, Fox R, Brookmeyer R *et al*. Predictors of the acquired immunodeficiency syndrome developing in a cohort of seropositive homosexual men. New Eng J Med 1987; **316**:61–66.

2 Moss AR, Bacchetti P, Osmond D *et al*. Seropositivity for HIV and the development of AIDS or AIDS related condition: three year follow up of the San Francisco General Hospital cohort. Br Med J (Clin Res Ed) 1988; **296**:745–50.

3 Stites DP, Moss AR, Bacchetti P *et al*. Lymphocyte subset analysis to predict progression to AIDS in a cohort of homosexual men in San Francisco. Clin Immunol Immunopathol 1989; **52**:96–103.

4 Taylor JM, Fahey JL, Detels R, Giorgi JV. CD4 percentage, CD4 number, and CD4:CD8 ratio in HIV infection: which to choose and how to use. J Acquir Immune Defic Syndr 1989; **2**:114–24.

5 Teitel JM, Freedman JJ, Garvey MB, Kardish M. Two-year evaluation of clinical and laboratory variables of immune function in 117 hemophiliacs seropositive or seronegative for HIV-1. Am J Hematol 1989; **32**:262–72.

6 Fahey JL, Taylor JM, Detels R, Hofmann B, Melmed R, Nishanian P, Giorgi JV. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. N Engl J Med 1990; **322**:166–72.

7 Tsoukas CM, Bernard NF. Markers predicting progression of human immunodeficiency virus-related disease. Clin Microbiol Rev 1994; **7**:14–28.

8 Centers for Disease Control. Guidelines for prophylaxis against *Pneumocystis carinii* pneumonia for persons infected with HIV. MMWR; 1989; **38** (Suppl. 5):1–9.

9 Nicholson J, Jones B, Echenberg D, Spira T, McDouglas J. Phenotypic distribution of T cells of patients who have subsequently developed AIDS. Clin Immunol Immunopathol 1987; **43**:82–87.

10 Masur H, Ognibene FP, Yarchoan R *et al*. CD4 counts as predictors of opportunistic pneumonias in human immunodeficiency virus (HIV) infection. Ann Intern Med 1989; **111**:223–31.

11 Polis MA, Masur H. Predicting the progression to AIDS. Am J Med 1990; **89**:701–5.

12 Mandigo K, Raboud JM, Haley L, Montaner JS. Variability in leukocyte subset measurements among five laboratories in Vancouver. Clin Invest Med 1995; **18**:349–56.

13 Schwartz A, Fernandez RE. Development of clinical standards for flow cytometry. Ann N Y Acad Sci 1993; **677**:28–39.

14 Brando B, Sommaruga E. Nationwide quality control trial on lymphocyte immunophenotyping and flow cytometer performance in Italy. Cytometry 1993; **14**:294–306.

15 Peddecord K, Benenson A, Hofherr L, Francis D, Garfein R, Cross G, Schalla W. Variability of reporting and lack of adherence to consensus guidelines in human T-lymphocyte immunophenotyping reports: results of a case series. J AIDS 1993; **6**:823–30.

16 Hoover DR, Graham NM, Chen B, Taylor JM, Phair J, Zhou SY, Munoz A. Effect of $CD4^+$ cell count measurement variability on staging HIV-1 infection. J Acquir Immune Defic Syndr 1992; **5**:794–802.

17 Raboud J, Haley L, Montaner J, Murphy C, Januszewska M, Schechter M. Quantification of the variation due to laboratory and physiologic sources in CD4 lymphocyte counts of clinically stable HIV-infected individuals. J AIDS 1995; **10** (Suppl. 2):S67–73.

18 Gelman R, Cheng SC, Kidd P, Waxdal M, Kagan J. Assessment of the effects of instrumentation, monoclonal antibody, and fluorochrome on flow cytometric immunophenotyping: a report based on 2 years of the NIAID DAIDS flow cytometry quality assessment program. Clin Immunol Immunopathol 1993; **66**:150–62.

19 Simson E, Groner W. Variability in absolute lymphocyte counts obtained by automated cell counters. Cytometry 1995; **22**:26–34.

20 Nicholson J. Immunophenotyping specimens from HIV-infected persons: laboratory guidelines from the Centers for Disease Control and Prevention. Cytometry 1994; **18**:55–59.

21 Centers for Disease Control. Revised guidelines for the performance of $CD4^+$ T-cell determinations in persons with HIV infections. MMWR 1994; **43**:1–21.

22 Mercolino T, Connelly M, Meyer E, Knight M, Parker J, Stelzer G, De Chirico G. Immunologic differentiation of absolute lymphocyte count with an integrated flow cytometric system: an new concept for absolute T-cell subset determination. Cytometry 1995; **22**:48–59.

23 Vogt R, Cross G, Henderson L, Phillips D. Model system evaluating fluorescein-labeled microbeads as internal standards to calibrate fluorescence intensity of flow cytometers. Cytometry 1989; **10**: 294–302.

24 Giorgi JV, Cheng HL, Margolick JB *et al*. Quality control in the flow

cytometric measurement of T-lymphocyte subsets: the multicenter AIDS cohort study experience. The Multicenter AIDS Cohort Study Group. Clin Immunol Immunopathol 1990; **55**:173–86.

25 Homburger H, Rosenstock W, Paxton H, Paton M, Landay A. Assessment of interlaboratory variability of immuno-phenotyping. Ann NY Acad Sci 1993; **677:** 43–49.

26 Kagan J, Gelman R, Waxdal M, Kidd P. NIAID Division of AIDS flow cytometry quality assessment program. Ann N Y Acad Sci 1993; **677:** 51–52.

27 Vesely R, Barths J, Vanlangendock F, Hannet I, Strauss K. Initial results of central European immunophenotyping quality control program (CEQUAL). Cytometry 1996; **26**:108–12.