Point-of-care International Normalised Ratios: UK NEQAS experience demonstrates necessity for proficiency testing of three different monitors

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Summary
External quality assessment (EQA) or proficiency testing is widely considered to be necessary for International Normalised Ratio (INR) determinations performed in conventional laboratory settings. There is increasing use of near-patient-test (NPT) or point-of-care (POC) INR devices and it is not known whether EQA is also necessary for these monitors. We report here on six years experience of proficiency testing for POC monitors used by health care professionals. Three devices were used by >10 centres who participated in the programme, the CoaguChek (CUC), the CUC-S and the TAS or Rapidpoint Coag. Not all users of the same type of monitor obtained the same INR result when analysing the same plasma sample. For the three monitors the CV of results in different centres was 11–14%. The variation between results in different centres could relate to inappropriately handled proficiency testing material, inaccuracies in the calibration of the system by the manufacturer or deterioration during transport/storage of the test strips. In each survey 10–11% of centres using POC monitors obtained INR results which were >15% different from those in other centres using the same monitors. For hospital laboratories using conventional INR techniques this figure was 12%. The relationship between INR results obtained by users of the Rapidpoint Coag or TAS monitor and results obtained by conventional techniques was not constant over the period of study. During one period INRs with TAS were 13.7% greater than with conventional methods. For the remaining three time periods results were similar. Our data suggest that the variation between INR results determined with three POC monitors show similar variation to that observed in hospital laboratories using conventional methods. Based on our data we recommend that users of these POC monitors participate regularly in an independent external proficiency testing programme.

Keywords
INR, NPT/POC, proficiency testing

Introduction
Oral anticoagulants are widely used in the treatment and prevention of thromboembolic disease. Optimal oral anticoagulant therapy requires regular monitoring to ensure an appropriate balance between anti-thrombotic effects and unwanted haemorrhagic side effects. The test of choice in this respect is the prothrombin time (PT) expressed as International Normalised Ratio (INR). The INR system takes account of variability in the responsiveness to the defect induced by coumarin anticoagulants (1–2), although difficulties remain (3) for example in relation to Quicks and Owrens versions of the PT (4, 5).

The INR system has undoubtedly contributed to improvement in oral anticoagulant control (6) and its use minimises the problems encountered when reagent sensitivity is not taken into account (7–9). There have been reports of important discrepancies between INRs obtained with different thromboplastins (10), indicating the importance of vigilance through external quality assessment and proficiency testing. The problems arising from large scale laboratory monitoring of oral anticoagulants have led to the development of a number of near-patient-test (NPT) or point-of-care (POC) monitors. The INR system has been validated for NPT instruments in expert centres (11, 12) and for patient self-testing and self-management (13). External quality assessment (EQA) is considered to be necessary for laboratory based INR/PT tests in Europe (9, 14–16), in North America (17, 18) and elsewhere. It is not known whether EQA is also required for INRs determined using POC monitors, although guideline documents have recommended this (19, 20). In the present study we report on external quality assessment of INRs determined...
with NPT/POC monitors used by health care professionals, as part of the UK National External Quality Assessment Scheme (NEQAS) programme over a six-year period.

Methods

UK NEQAS for PT/INR

Two different UK NEQAS programmes were available for INR determination during the period of study. Centres performing INR determinations by techniques which are calibrated for anticoagulated (citrated) plasma participate in the main UK NEQAS (Blood Coagulation) programme and are provided only with lyophilised plasmas. This includes conventional laboratory techniques for INR determination and NPT instruments calibrated for use with citrated plasma. Centres employing techniques which are calibrated only for testing native (non-anticoagulated) whole blood are registered with a separate POC/NPT programme. Despite the fact that these devices are not calibrated for use with citrated plasma, some monitors are capable of producing a result with plasma and EQA can be offered. These centres are provided with lyophilised plasmas prepared in exactly the same way as the main programme but are also provided with distilled water and diluent containing calcium chloride. The details of sample preparation and testing procedures are given below.

Plasma preparation

Plasma was collected from individual patients receiving warfarin therapy as follows; approximately 600 ml was collected over 45 minutes (min) into citrate phosphate dextrose anticoagulant using a Haemovetnic Ultralight instrument, centrifuged twice at 2,500 g at 4°C for 30 min and stored in bulk at −55°C for up to 12 weeks. The plasma was then thawed and buffered with 17 mM Hydroxymethylpiperazine-ethane sulphonic acid and lyophilised in 0.5-ml aliquots for six days. These lyophilised samples were then stored at −20°C prior to dispatch by post. All plasmas were single donations. This procedure was employed for preparation of plasmas used in both NEQAS programmes. The effect of this processing on INR determination has been described (10).

NPT NEQAS programme

The UK NEQAS NPT programme was established in March 1996 (21). Each participating centre normally received two different plasma samples in surveys conducted every three months during the period of study. Data from 1996–2002 are included in this report. Participants were provided with lyophilised plasma, a sealed vial containing 0.5 ml of distilled water (dil A) and a sealed vial containing 0.5 ml of a diluent containing calcium chloride (dil B). An instruction sheet invites centres to add the complete contents of dil A to the lyophilised plasma using a disposable plastic pasteur pipette provided. After 5 min the entire contents of dil B are added in the same way, mixed and tested immediately.

Main NEQAS programme

The main UK NEQAS (Blood Coagulation) was established more than 25 years ago. During the period reported here each centre was provided with one or two plasma samples for INR determination in each of six surveys per annum. Participants receive only test plasmas and instructions to reconstitute with 0.5 ml of distilled water, which is not provided with the sample package, and which must be obtained locally. Participants are invited to determine INR using the technique in local use between 5 min and 30 min after reconstitution. When calcium chloride is not present within the thromboplastin reagent this is obtained locally.

Performance analysis

In the NPT programme INR results for each individual sample are grouped according to the monitor in local use, and the median of results in different centres is calculated. When there are at least 10 users of a monitor, results from each individual centre are compared to the median of results obtained by centres using the same technique. Individual results are considered to be within consensus if less than 15% from the peer group median. Results more than 15% from the median are considered to be out of consensus. If this occurs in three consecutive surveys, the centre is considered to be persistently outwith consensus.

Comparison of citrated plasma and whole blood

Venous blood was collected from 20 warfarinised subjects, and INRs were determined immediately using the CUC-S monitor. The remaining blood was simultaneously anticoagulated with 1/10 volume 0.109 M trisodium citrate, which was centrifuged at 2,000 g for 10 min. The citrated plasma from these samples was stored deep frozen at −55°C. This was later thawed and recalculated by addition of an equal volume of 12.5 mM calcium chloride and immediately used for INR determination using the same CUC-S monitor and test strip lot number as for the whole blood samples from these subjects.

Effect of calcium concentration on INR results with CoaguCheck (CUC)

The effect of different calcium chloride concentrations on INRs as determined using CUC was studied using lyophilised samples from 13 different patients and test strips requiring only 10 µl of sample (ministrips). Each plasma was allowed to reconstitute with 0.5 ml distilled water for 5 min and this was mixed with an equal volume of diluent containing calcium chloride at 25 mM, 16.7 mM or 12.5 mM. In each case INRs were determined using CUC immediately after addition of calcium chloride. All tests were performed with the same CUC device and test strip lot number.

Stability of samples after re-calcification

Participants who employed the CUC and CUC-S systems were required to add diluent containing calcium chloride to the test plasma prior to analysis and were requested to initiate testing immediately after mixing. To assess what would happen if this instruction was disregarded, delay between addition of calcium to plasma and INR measurement was assessed by determination of INR using the CUC at timed intervals after recalification. Four different samples were analysed using standard volume strips (35-µl test sample required) and three using ministrips. Each sample was analysed immediately after re-calcification and re-tested leaving a delay of 2.5, 5, 7.5 and 10 min between addition of calcium and application of the sample to the test strip.
NPT instruments
As of May 2002 there were 276 participants in the NPT programme of whom 69 used the CUC monitor and 207 used the CUC-S (both Roche Diagnostics, Lewes, UK). These monitors were used by a number of different types of operator as follows; nurse – 68%; laboratory scientists – 11%; pharmacists – 10%; others – 11%. Participation is not restricted to the UK and centres from other countries are included.

CUC data from 1998 – 2002 are included, during which mini-strips were used. All tests performed with diluent containing 12.5 mM calcium chloride except where specified in the text. Since the CUC and CUC-S are not calibrated for testing anticoagulated plasma results obtained could not be directly compared to conventional methods.

The Thrombolytic Assessment System (TAS) or Rapid Point Coag (Bayer Diagnostics, Newbury Berks, UK) was used by 23 different centres, with up to 20 participants returning results in each survey. These centres all employed test strips calibrated for citrated whole blood or plasma (PT-One) during the study period of 1996 – 2002. Since this test system was calibrated for anticoagulated plasma, results could be compared directly to the results obtained on the same citrated plasmas when they were analysed by conventional techniques in other centres. For this reason TAS results were included in the main programme which had been established for conventional methods, rather than the NPT/POC programme.

Thirteen of the TAS users were hospital laboratories, seven were GPs, two were in coronary care units and one was in a pharmacy.

Results

Effect of calcium concentration on INR results with CUC
The results of INRs determined with CUC ministrips was significantly influenced by calcium concentration. The mean INR of 13 different plasmas was 4.21 if diluent containing 25 mM calcium chloride was used, compared to significantly lower mean INRs of 3.42 and 3.13 when 16.7 and 12.5 mM calcium chloride was employed, respectively (analysis of variance, p< 0.01). The same plasma samples had also been analysed by participants of the main UK NEQAS scheme (conventional INR methods) who obtained a mean INR of 2.76, which was significantly (ANOVA, p< 0.01) lower than CUC results obtained with 25 mM and 16.7 mM calcium chloride but not with 12.5 mM calcium chloride.

Comparison of whole blood and recalcified citrated plasma
The mean CUC-S INR of 20 warfarinised patients was 2.53 (range 1.4 to 3.5) for whole blood samples compared to 2.66 for recalcified plasma samples from the same patients (r = 0.82). This small difference was not statistically significant.

Stability of samples after re-calcification
The mean INR of seven samples analysed immediately after addition of calcium was 3.16, compared to a mean INR of 3.14 if samples were analysed 2.5 min later. There was a small reduction to mean 3.04 and 3.03 if samples were analysed after 5 or 7.5 min. These reductions were not statistically significant. One sample clotted between 7.5 and 10 min.

CUC results – Ministrips
A total of 21 samples were analysed by between 62 and 114 centres using CUC and ministrips during 1998 – 2002. All of these samples had also been analysed by participants in the main UK NEQAS programme using conventional laboratory methods. There was no significant difference between the mean of INR results with CUC ministrips (3.01) and the mean obtained with conventional thromboplastins in hospital laboratories using conventional methods (2.96), with a highly significant correlation (r = 0.95, p=0.0002). A Bland and Altman (22) plot of these data is
shown in Figure 1. The CVs of INR results in different centres ranged from 6% to 18.4% (mean 10.9%) for the 21 samples.

**CUC-S results**
A total of 11 samples were analysed by between 29 and 175 centres using CUC-S with ministrip during 2000 – 2002. Seven of these samples were also distributed to hospital laboratories in the main UK NEQAS programme and for these seven there was a highly significant correlation ($r = 0.96$, $p=0.003$) when compared to median INRs obtained in a large number of hospital laboratories using a variety of conventional thromboplastins. All 11 samples were also analysed by between 61 and 94 users of CUC

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**Figure 2:** INR results of the same sample analysed in 175 centres using CUC-S monitors (non-shaded columns). The number of centres who obtained each INR result is shown. The overall median INR was 2.6 with a CV of 9.0%. This was the lowest CV observed during the study period. The shaded columns show results obtained by CUC users when they analysed the same sample.

**Figure 3:** INR results of the same sample analysed in 130 centres using CUC-S monitors (non-shaded columns). The number of centres who obtained each INR result is shown. The overall median INR was 2.0 with a CV of 26.7%. This was the highest CV observed during the study period. The shaded columns show results obtained by CUC users when they analysed the same sample.
monitors. The mean INRs for the two types of monitor were practically identical (CUC – 2.95 and CUC 2.97, no significant difference, r = 0.98). The CV of CUC-S results in different centres ranged from 9.0 to 26.7% (mean 14.4%). The INR results obtained by individual CUC-S users for the samples with lowest and highest CVs are shown in Figures 2 and 3, which also show results obtained by CUC users with the same samples.

**TAS results obtained by UK NEQAS participants**

The TAS instrument was used with PT-one test strips which are calibrated for analysis of citrated plasma. A comparison with results obtained by other centres using conventional thromboplastins could be used to assess the accuracy.

A total of 49 samples were analysed by between seven and 20 centres during the period from 1996 to 2002. The mean TAS INR for the 49 plasmas was 3.14, which was significantly greater (paired t-test, p <0.01) from the mean of 2.99 obtained in >500 centres using conventional thromboplastins, a mean difference of 5.2%. A Bland and Altman plot of these data is shown in Figure 4, where each point shows the difference between TAS and conventional median INRs for each individual sample. The CV of TAS results in different centres ranged from 9.0 to 26.7% (mean 13.7%). The six-year period reviewed was divided into four periods, and the mean INRs of samples analysed in each period was calculated to assess whether the relationship between results with TAS and conventional methods altered over time. For the third time period (March 1999 – March 2000) results obtained by TAS users were significantly greater (paired t-test p<0.01) by 13.7%, with no significant differences for the other three periods. This analysis is shown in Table 1.

**Performance analysis**

Ninety-seven centres employing the CUC or CUC-S returned INR results for all eight samples in the four surveys during 2001. Fifty-one of these (54%) obtained INR results which were always within 15% of the median of all other users of the same monitor (within consensus). Thirty-six centres (38%) had a single result outwith consensus in the four surveys. The average proportion of centres whose results were outwith consensus in each survey was 9.8% for CUC and 11.0% for CUC-S. This compares with an average of 11.9% of hospital laboratories who were outwith consensus in the main UK NEQAS scheme during the

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**Table 1: INR results obtained by UK NEQAS participants using TAS monitors and the median of all conventional laboratory methods when analysing the same samples.** The 49 samples were divided into four groups based on the time period of analysis (group 1 being the first samples to have been analysed through to group 4 being the most recent).

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>N</th>
<th>Mean INR TAS</th>
<th>Mean INR All lab methods</th>
<th>P</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1996–1997</td>
<td>12</td>
<td>2.86</td>
<td>2.83</td>
<td>ns</td>
<td>1.1%</td>
</tr>
<tr>
<td>2</td>
<td>1997–1999</td>
<td>12</td>
<td>2.88</td>
<td>2.77</td>
<td>ns</td>
<td>6.7%</td>
</tr>
<tr>
<td>3</td>
<td>1999–2000</td>
<td>12</td>
<td>3.82</td>
<td>3.36</td>
<td>&lt;0.01</td>
<td>13.7%</td>
</tr>
<tr>
<td>4</td>
<td>2000–2002</td>
<td>13</td>
<td>3.04</td>
<td>3.00</td>
<td>ns</td>
<td>13.3%</td>
</tr>
<tr>
<td>All 4</td>
<td>1996–2002</td>
<td>49</td>
<td>3.15</td>
<td>2.99</td>
<td>&lt;0.01</td>
<td>5.4%</td>
</tr>
</tbody>
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same period. Only six centres had INRs outwith consensus in
two of the four surveys with two centres having this in three of
the four.

Discussion
The need for independent external assessment of INRs deter-
dined by conventional laboratory techniques is widely acknowl-
edged. There is increasing use worldwide of NPT or POC moni-
tors for monitoring coumarin anticoagulants, and it is not known
whether the same type of proficiency testing is possible or
necessary. We report here our long-term experience of an EQA
programme for health care professionals using POC monitors for
INR determination. During the period of study three types of
POC monitor were used by more than 10 centres participating in
the programme (CUC, CUC-S, TAS)

The CUC and CUC-S monitors were calibrated by the manu-
facturer for testing non-anticoagulated whole blood. The
samples employed for the EQA programme were lyophilised ci-
trated plasmas which were reconstituted and recalciﬁed by par-
ticipants prior to analysis. There was no signiﬁcant difference
between INRs of whole blood and recalciﬁed citrated plasma, al-
though results obtained on citrated plasma should not be used to
assess accuracy of INRs as determined on native whole blood
samples from patients. Other studies have reported that INRs ob-
tained with native capillary blood analysed using the CUC are in
acceptable agreement with conventional laboratory systems in
adults (11), and children (23).

We studied the effect of using different calcium concen-
trations to recalciﬁed the test plasma before determining INR with
the CUC and minstrip. The INR results (n=13) obtained with the
CUC when test plasmas were recalciﬁed with 12.5 mM calcium
chloride were not signiﬁcantly different from the results ob-
tained by hospital laboratories who analysed the same plasma
samples in the UK NEQAS programme. This compares with
16.3 mM calcium used to recalciﬁed plasma for INR deter-
minations using CUC and TAS by Poller et al. (24). Calcium con-
centration has previously been shown to have an important in-
fluence on results of PT/INR determinations (25).

Eleven samples were analysed by users of CUC and also
CUC-S, and results were practically identical for the two types of
monitor (mean INRs 2.95 and 2.97, r = 0.98). The CV of INR re-
results in different centres using the CUC or the CUC-S monitors
was on average 10.9% and 14.4%, respectively. We have pre-
viously reported that for a sample with an INR of 2.5 the CVs ob-
tained by hospital laboratories who were using the same reagent
and instrument type were in the range 4.3% to 12.0% depending
on the test system (26). The spread of results in different POC
centres suggests that EQA is necessary in this setting. During
the period of assessment a number of problems were identiﬁed as a
consequence of outwith consensus results obtained in individual
centres as part of the programme, which could have led to inac-
curately reported INR results in patients. These included centres
who had failed to follow manufacturer’s instructions in respect to
test strip storage, and use of test strips for which the sealed foil
pouch had been punctured, providing further evidence that EQA
is necessary for these devices

Some POC centres obtained INR results which were
markedly different from the median value. Even for the test
sample with the best agreement between centres (lowest CV) the
INR results in individual centres varied from 1.9 to 4.5. This type
of variation occurred for all the samples distributed and for each
of the types of POC monitors employed. There are a number of
possible explanations for these marked discrepancies including
inaccuracy of the measurement system related to variation be-
tween different individual POC monitors, the calibration of the
test strip lot by the manufacturer, deterioration in the individual
test strips employed, or by inappropriately handled QC samples.

We performed a number of additional tests to investigate
whether the variations in INR in different centres might relate to
inappropriately handled QC samples. For CUC and CUC-S users
the diluents provided included calcium chloride, and we demon-
strated that INR results were dependant on the calcium concen-
tration employed as discussed above. Thus, if users did not
transfer all of the diluents provided, there would be a different
calcium concentration in the test material which in turn would
contribute to the variation in results obtained. We studied the ef-
effect of delay after addition of ﬁnal diluent and observed INR dif-
fences of approximately 4% for a delay of up to 7.5 min (the
time taken to perform three tests on the CUC device). The plas-
ma samples used for conventional laboratory INR deter-
minations were stable for at least two hours (data not shown)
after reconstitution with distilled water. Taken together our data
suggest that some variation in INRs in different centres could be
caused by inappropriately handled EQA samples. Use of differ-
cent lot numbers of test strips by participants may have con-
tributed to the variation observed, and small but statistically sig-
niﬁcantly different INRs have been reported for different lots of
CUC test strips (27). Clinically significant consistent monitor-
to-monitor variation has not been reported to our knowledge and
was not detected in our programme.

For each INR sample analysed, an average of 10–11% of
CUC and CUC-S results were more than 15% from the median of
INRs in all other centres using the same monitor. This is similar
to the proportion of hospital laboratories using conventional me-
ths with results outside these limits in the same time period.
We reviewed the results in individual centres over a series of four
surveys and more than half (54%) of centres had every result
within consensus with a further 38% having only a single INR re-
sult more than 15% different from the median. Only two centres
had this problem in three of the four surveys during this period,
and there was no centre with discrepancies of more than 15% in
all four of the surveys, suggesting that those centres who had dis-
crepant results were able to correct the problem and were subse-
quently able to obtain INRs which were similar to those in other
centres.

Up to 20 centres participating in the EQA programme used
the TAS or Rapidpoint Coag system during the period of study.
All centres used the test strip calibrated for use with citrated plas-
ma (PT-one). Over the six-year period of study the relationship
between INRs with TAS and conventional thromboplastins was
not constant. When the time period was divided into four, there
was one period during which TAS results were greater by 13.7%
on average. For the other three periods results were similar.
These relationships were unaffected by analysing the data after
exclusion of outliers. It has been suggested that an INR deviation of ±10% is clinically relevant (28). This discrepancy could be caused by an inaccuracy in INR determinations with the TAS or with the techniques employed by hospital laboratories, or both. This type of changing relationship confirms the need for continued vigilance of the relationship between results with different test systems through EQA to highlight where calibration effects may occur.

The data obtained by participants in the external quality assessment programme reported here confirm that proficiency testing is both possible and necessary for health care professionals determining INRs with the POC monitors employed by participants in our programmes. It remains to be established whether patients under self-management of oral anticoagulation with NPT monitors would benefit from participation in such a programme, although some data are available on this (29). Based on our results we recommend that health-care-professional users of the POC devices reported here participate regularly in independent external proficiency testing exercises.

Acknowledgements

The assistance of UK NEQAS participants is gratefully acknowledged.

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