UK BTS/NIBSC STANDING ADVISORY COMMITTEE ON TRANSFUSION TRANSMITTED INFECTIONS (SACTTI)

CONFIDENTIAL

Minutes of the Special SACTTI Meeting on NAT held at North London Centre, (Training Suite, DBR) Monday 21 February 2000 at 11.00 am

Present:

SACTTI:	Dr C Bharucha (CB), Dr PE Hewitt (PEH), Dr JA Barbara (JAB), Dr B Dow (BD), Dr P Minor (PMi), Dr P Mortimer (PMo), Dr B McClelland (BMcC), Dr A Robinson (AR), Professor RS Tedder (RST)
NBA:	Professor JP Allain (JPA), Mr R Bedford (RB), Ms B Cant (BC), Dr V James (VJ), Dr E Love (EL), Dr S Moore (SM), Dr C Sims (CS), Dr K Soldan (KS)
NIBSC:	Dr J Saldanha (JS), Dr M Ferguson (MF)
WBS:	Dr H Hambley (HH), Dr R Hutton (RH)
MCA:	Dr M Kavanagh (MK)
NIBTS:	Mr J Savage (JS)
SNBTS:	Dr C Prowse (CP), Dr L Jarvis (LJ), Dr J Petrik (JP), Dr T Jordan (TJ)
BTSB/Ireland:	Dr J O'Riordan (JO'R)

Apologies:	Professor IM Franklin (SACTTI) Dr T Snape (SACTTI)

This Special SACTTI NAT meeting was set up to take stock of the current positions of the UK transfusion services with respect to nucleic acid testing, and to address the logistics of the next phase. These notes are a summary of points raised mainly at discussion during the meeting and should be used in conjunction with the copies of presentation hand-outs (attached).

National Updates

Dr Love described the current position of NAT screening within the NBS with national screening being performed at BPL using the Qiagen Biorobot extraction system together with the Roche Cobas Amplicor test. The public implementation of phase 1 was 1 September 1999 after various delays. Three zonal pooling laboratories have been set up together with 1 NAT testing site (BPL). As at end of 1999, 21,609 mini-pools containing 2,074,464 donations had been tested with only 1 serology negative/NAT positive donation identified (this donor later seroconverted). Phase 2 will involve the transfer of the current testing technology to the 3 zonal NAT labs - Brentwood (established, currently validating); Leeds (established, equipment installing); and Birmingham (to be established). Contingency arrangements between SNBTS and NBS have been fully tested and complex procedures for stock recall have been processed and tested successfully.

Dr Love explained how the NBS has a Project Steering Group established in October 1996. The NAT Steering Group is accountable to the NBS Executive on the strategy, approach, implementation and performance of NAT

screening. This Group receives guidance from the Scientific Group that provides expert advice and is aware of regulatory issues. SACTTI and NAT Steering Group must have close liaison during further developments in NAT.

Dr Prowse gave a brief overview of the SNBTS position. The SNBTS Medical and Scientific Committee (MSC) determines the policy of SNBTS with regard to NAT developments. Dr Prowse was a member of the MSC as are 3 SACTTI members (IMF, BMcC and BD) and therefore SACTTI should be well informed of SNBTS NAT developments. Up to now around 1,000,000 donations will have been tested through the Edinburgh laboratory. No serology negative NAT positive donation has been identified through mini-pooling. It should however be noted that maxi-pool testing immediately prior to mini-pool implementation did discover a serology negative/NAT positive donation. Nevertheless, the mini-pool PCR system had identified PCR positive donations - all of which had been confirmed as serological positives. The NIBSC working standard was used in every batch of tests and the 95% cut-off (determined by log dilutions) was 29 Geq/ml or 7.25IU/ml (ie approx 650 IU/ml at donation level). During the development of the assay a reagent batch release system had been introduced to satisfy Quality auditors. At present the system does suffer from some technical failures and IT transfer of data could be improved. SNBTS are working to a possible labile component release and a date of 1 September 2000 was mentioned with guarded optimism. As at February 1999, 3,037 pools had been tested from 285,661 donations with 52 initial reactives and 32 repeat reactives (all serologically

confirmed). Cellular release will depend greatly on the use of Lightcycler technology to reduce the turnaround time.

Discussion

The latest Eurohep results suggest that the SNBTS system can detect 3IU/ml. SNBTS uses Nuclisens automated extraction with 2ml starting pool to obtain approx 30ul nucleic acid - 10ul required for RT and first round PCR, plan to use lightcycler for second round PCR. No difference was noted in sensitivities with different genotypes (although main comparison is genotypes 1 and 3). RST asked about the 20 initial reactives that failed to repeat - were they genuine negatives? It was pointed out that although theoretically a low level positive could account for the occasional unrepeatable initial reactive sample, such a sample would have a genome equivalent below the required sensitivity.

Dr Prowse then presented other NAT options eg HIV NAT, B19 etc. The Bac-T-Alert system can allow the extension of platelet concentrate shelflife - this can be used as an absolute release criterion (30hours old) or PCR result can be used to extend the shelflife.

Phase 2 validations have already been introduced in some German states, soon in USA, 1 centre in France, and in Austria, Netherlands and Canada. With regard to Phase 2 introduction in SNBTS, the delivery of samples is now the crucial step in the process. If the samples arrive by the middle of the

night of collection, pooling can be performed so that PCR extractions can commence early in the morning and negative results could be reported by 1pm the following day (using lightcycler for second round PCR).

Discussion

CP stated that it was hoped that SNBTS would have completed a pilot study by Easter 2000. However the logistics of sample delivery, staff conditions of service and timing of donation sessions had still to be debated. JO'R asked about failure rate and technical failures. Repeat testing from remains of nucleic acid extraction can be performed on some occasions. Re-extraction from the original mini-pool is sometimes required. If technical failure persists a further mini-pool preparation may be required. The BVDV control is added at pooling stage (copy number - 33/ml ie 66 copies/2ml). Around 25-30 mini-pools are processed per day - taking 3 runs on the nuclisens extractors (2). Technical failures and Internal Control failures had a rate of approximately 4%. Run failures (sensitivity) generally did not occur as the run control (1/day) was always detected. Extraction failures were recognised by no BVDV band in gel.

Mr R Bedford presented the plans to undertake phase 2 in NBS. He reminded the Group that although most components could eventually be released after NAT, granulocytes may need to be excluded. The inclusion of apheresis platelets in mini-pools or single PCR testing platelets to avoid extended quarantining because of technical failures was discussed. He

expressed concerns about the robustness of the current assay system. The reduction in size of the mini-pool from 96 to 48 may help to allay fears of losing most platelets held within one mini-pool. NBS plan to co-locate pooling and PCR testing. Mini-pooling needs to be performed by 6am in order that results are available by 1pm (day 1). Bac-T-Alert has already been assessed for extending the shelflife of platelets over long holiday periods. Run failures were at one time as high as 25-30% - currently around 5-10%. The problem of not getting a negative result to clear material has to be overcome otherwise there will be severe shortages and media attention will consequently be aroused. NBS does not envisage single donation NAT testing because of the nature of their service, ie too many new NAT laboratories would be required.

Discussion

It was announced that most USA centres and all Canadian centres were already NAT testing labile components - with no extension of shelf-life. It was pointed out that platelets will generally be older at issue in Phase 2 than currently. CB stated that there would be a joint SACTTI/SACBC meeting in the near future to address this issue of bacterial testing and efficacy. RB stated that NBS would not be able to meet a September 2000 deadline for introduction of Phase 2. Back-up contingency plans included the purchase of sufficient equipment to provide adequate back-up in case of failure together with ensuring each of the 3 NBS sites could theoretically test the entire NBS if necessary. JAB suggested that in the extreme circumstance of total assay

failure, single test TMA or even HCV Ag testing (see later) could be considered. The size of the mini-pool was continually being reduced in USA.

Dr Cate Sims explained that although the Cobas assay took only 12 samples, 3 of these samples were always controls - Pos Control that must be positive; Negative Control that must be negative and IC that must be reactive; and Low Positive Monitor Control (3.55 IU/ml) that was reactive in around 50-75% of runs.

Prof Tedder explained the concern he had when the IC gave a lower OD than normal. This suggested a loss of sensitivity and therefore required repeat testing of the appropriate mini-pool.

Dr Saldanha showed the results of 6 laboratories (RST, BPL (Qiagen/Cobas), SNBTS mini-pool(Nuclisens/In-house), SNBTS MRU (Roche), NIBSC and JPA(TMA)) with the 1999 Proficiency Panel. He had continually supplied working standard (97/844) to the the present NAT laboratories and was about to prepare a new batch of working reagent (99/738). A multiplex reagent (99/632) was also available that could be used for individual HIV, HBV, B19, HCV assays or any multiplex combination. He is planning to issue a new Proficiency Panel in the next few weeks. Dr Saldanha also offered to issue Quality Reports on the use of the working standards to the participating UK BTS laboratories. He also extended an invitation for a User's Forum to meet at NIBSC probably during June 2000.

Discussion

RST described the reasons for failures - extraction; analyte performance; amplification performance. Although SNBTS and NBS systems are different we still need to formulate terms that are common. The internal control differs between the systems. Use of the lightcyler should result in a similar readout to the Cobas. The definition of a "non-negative" result is important as this causes the main problems. CP offered to provide more up-to-date data on the SNBTS experience with technical problems (Appendix 1). RST questioned whether a positive control should be included in each run of 10 extractions on the Nuclisens extractor - however each extraction on the Nuclisens machine is unique and there have been no external QC (NIBSC run control) failures to indicate that this was necessary. The question of a software or mechanical failure that caused a percentage (eg 15%) increase in eluate of nucleic acid could theoretically result in loss in sensitivity. Similar problems had been encountered with ELISAs and newer "black box technologies" (eg PRISM). Both NBA KEG and SNBTS MTEG had looked at these systems to ensure they performed to the standards laid down and the Medicines Inspectorate had been satisfied with compliance. RST offered to contact CP (SNBTS) to work out terminology commonalities between the two present PCR mini-pool laboratories.

TMA Trial

Prof Allain presented his data on testing 50,000 first time donors with single donation TMA. EDTA tube was used for this exercise. Both HIV and HCV screening were done as a multiplex assay and individual tests were performed for confirmation. Ninety-one samples and 9 controls in each run. The system took 5 hours to get first result - 182 samples tested in 5.5 hours. With the 1999 NIBSC Profic Panel it appeared that around 20IU/ml of either genotype was detectable (Limited data). The assay suffered several problems including invalid samples, initial positives that failed to repeat and occasional invalid runs. The air conditioning in the laboratory accounted for most of the non-repeatable results. From 387 TMA runs, 8.8% (34) invalid runs were obtained - 4 (12%) due to technical failure, 26 (76%) due to calibrator failure and 4 (12%) ? haemolysis. Of 32,773 donations tested 478 (1.46%) had invalid results, 133 IRs and 24 RRs (22 conf HCV and 2 conf HIV). All were serologically confirmed positive. However one of the HCV samples was shown to be a mini-pool NAT negative (UK22770). He concluded that TMA's robustness required improvement to avoid invalid runs.

Discussion

The latter sample is currently undergoing further investigation in RST's laboratory and results are awaited.

Regulatory Aspects

Dr Saldanha gave a review of the Regulatory Aspects of NAT including CPMP/BWP/390/97 that was effective from 1 July 1999(HCV RNA run control 100iu/ml). The European Pharmacopeia 2000 supplement will include this. PEI introduced red cell release with sensitivity of 5000 IU/ml for a single blood donation. Austria started Sep 1999. In USA B19 NAT testing has gone through clinical trials. Such testing should reduce the contamination of pools with B19 to less than 10,000 Geq/ml in a manufacturing pool.

Risk Estimates

Dr Soldan presented data on estimates of risk of HCV, HIV and HBV in the NBS. Total risk (window period + false negative) is around 1in 0.6 million; 2.6 million and 0.6 million respectively.

HCV Antigen Assays

Dr Dow described the HCV Ag test manufactured by Ortho. This serological test detects HCV Ag in the same time frame as currently available serological assays. Through the use of seroconversion panels the HCV Ag assay has been shown to be only one day later than HCV PCR in detecting seroconversion. HCV Ag activity however will eventually decline on the appearance of c22 antibodies (analogous to HIV p24 Ag).

Dr Barbara presented HCV Ag data from France on haemodialysis patients. Results show that seroconversion occurs 2 days after HCV PCR is positive.

The specificity of the assay in France was shown to be 99.84% whereas N. London on screening 3000 donors 99.54% was obtained. A neutralisation assay was available and this generally showed >85% reduction with true positives. Due to time limitations further data on HCV Ag from SNBTS was provided as a handout.

Discussion

RST stated that only 8 of 18 PCR positive ?seroconverters were detected by HCV Ag test. This would be discussed more fully at a future SACTTI meeting.

NAT as a Red Book Issue

Dr James reminded the group that the 'Red Book' provides guidelines for all the Medical Directors of the 4 UK BTS. She asked that SACTTI provide common guidelines for NAT testing that can be implemented in both NBS and SNBTS. These guidelines would be incorporated in the Red Guide and also in the Council of Europe Guidelines.

Dr Bharucha referred to the special SACTTI NAT meeting that had taken place in 1997. Despite the initial apprehension the successful UK-wide implementation of NAT was a tremendous achievement. The next phase should be introduced with a greater degree of commonality between the Blood Services and this should be developed under the continued auspices of SACTTI.

Any other Business

JPA asked if HCV Ag dilution studies should be performed. (answer No). KS estimated that in 8 years of HCV antibody testing there would probably have been 15 window period donors and 60 test failures.

CB closed the meeting asking for copies of overheads from speakers so that SACTTI members could debate the subject more fully at their March meeting.

BCDow/C Bharucha
26 February 2000

Key to sample designation

C1 HCV control (1:10 dilution 400 genome equivalents/ml)
C2 HCV control (1:100 dilution 400 genome equivalents/ml)
C3 HCV control (1:1000 dilution 400 genome equivalents/ml)
C4 HCV negative control

The C1 is our go/no-go control. It is always positive. Our C2 is generally positive (95%) and the C3 is positive on about 37% of occasions. The C4 is negative for HCV. All samples should be positive for BVDV however you would expect that the BVDV internal control is weaker when there is a strong HCV positive or the C1 control.

Samples with 8 digit numbers are pools of 95 samples. These are generally negative for HCV (but should be positive for BVDV).

1X BVDV = probes at 2um and 4um as recommended
2X BVDV = probes at twice the concentration recommended (4um and 8um)

NEW BVDV = new probes
OLD BVDV = old probes

	23.10.98 –22.3.99	23.3.99-13.9.99	14.9.99 – 30.12.99 New Nuclisens software introduced
No. pools tested	1587	2064	1542
Initial reactives (I/R)	25/1587 (1.57%)	34/2064 (1.65%)	15/1542 (0.97%)
Repeat reactives/ PCR positive confirmed	13/1587 (0.82%)	21/2064 (1.02%)	8/1542 (0.52%)
Non-repeat reactives	12/1587 (0.76%)	13/2064 (0.63%)	7/1542 (0.45%)
Extractor failures	50/1587 (3.15%)	85/2064 (4.12%)	22/1542 (1.42%)
Post-extraction BVDV (BVDV negative on repeat test	30/1587 (1.89%)	21/2064 (1.02%)	38/1542 (2.46%)
Pre-extraction BVDV (2 BVDV negative tests)	17/1587 (1.07%)	34/2064 (1.65%)	7/1542 (0.45%)
Total T/Fs	97/1587 (6.11%)	140/2064 (6.78%)	67/1542 (4.34%)

Technical Failure Rate in Routine SNBTS HCV NAT Testing

PCRFAIL.XLS	23 Oct 1998 to 22 March 1998	23 March 1998 to 13 September 1999	14 September 1999 to 30 December 1999	TOTAL
Pools Tested	**1587**	**2064**	**1542**	**5193**
initial reactive	25	34	15	**74**
%	1.58	1.65	0.97	1.42
repeat reactive (PCR confirmed)	13	21	8	**42**
%	0.82	1.02	0.52	0.81
unconfirmed initial reactive	12	13	7	**32**
%	0.76	0.63	0.45	0.62
Test failures	**97**	**140**	**67**	**304**
%	6.11	6.78	4.35	5.85
extractor failed	50	85	22	**157**
%	3.15	4.12	1.43	3.02
BVDV neg on rpt. test (post extract)	30	21	38	**89**
%	1.89	1.02	2.46	1.71
Both BVDV neg (pre extract)	17	34	7	**58**
%	1.07	1.65	0.45	1.12
CHECK T/F	**97**	**140**	**67**	**304**
Comment	Validation Phase	Routine with original Nuclisens Extractor softeware	New Software on Nuclisens Extractor	