Supplementary Table 3. PCR techniques of the included studies

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| No | Article | Type of cells; Method of cell extraction; Method of RNA extraction; Amount of RNA used; cDNA synthesis technique; | Amplification technique; Quality control gene; Positive control; Negative control | Remarks |
| 1 | Biernaux C 1995[4] | **Type of cells:**   * CB: NR * PB: likely WBC because article stated: “In the ‘children’s WBCs…”   **Method of cell extraction:** NR  **Method of RNA extraction:** article referred to Chirgwin JM 1979[32]  **Amount of RNA used**:  4-10 µg of total RNA.In certain cases, the amount of total RNA involved in the RT reaction was increased to be consistent with the total RNA extracted from 108 cells (equivalent to approximately 100 mL of blood).  **cDNA synthesis technique:**  Using an optimized protocol described by Perkin Elmer/Cetus GeneAmp RNA PCR Kit (Emmeryville, CA). 40 pmol of 3' specific primer (c-ab1 a3) were used. | **Amplification technique:**  Nested PCR of M-BCR   * **Cycling condition:** 94°C/2 min 🡪 35 cycles (94°C/1 min, 62°C/1 min & 72°C/1 min) 🡪 72°C/5 min (same for 1st & 2nd run) * **Cycler:** NR * **1st run:** the total amount of cDNA in a final volume of 100 µL * **2nd run:** 10% of the 1st run product was involved into the 2nd run PCR run * **Detection of amplified DNA:** 10 µL of the reaction products were electrophoresed in TBE buffer, at 100 V for 1 hr at 4°C on 2% agarose gel staining with ethidium bromide. * **No. of replicate/subject:** NR * **Estimation of the minimal level of detection:** using the positive control diluted in negative control & submitted to nested PCR. The nested PCR products corresponding to these different dilutions of RNA *BCR-ABL1* were hybridized with radioactive complementary *BCR-ABL1* RNA. With their respective counted radioactivity, a chart was made that showed an exponential shape that is linear in semilogarithmic form for low concentrations from 1-50 copies. In parallel, samples from healthy individuals were submitted to the same cycles of amplification. The amount of amplified DNA obtained in these different samples was estimated by counting the radioactivity in each b& after Southern blotting & hybridization with a specific radioactively labeled *BCR-ABL1* probe. By reference to the amount of radioactivity obtained in different concentrations of internal control & the chart, the number of *BCR-ABL1* RNA copies in the samples could be estimated. In that way, the relative amount of the *BCR-ABL1* mRNA present in total RNA from WBCs could be estimated.   **Quality control gene:** *ABL1*. cDNA synthesis & PCR were performed in parallel with primers detecting the *ABL1* RNA.  **Positive control**: a reference *BCR-ABL1* RNA synthesized in vitro - 395-bp *BCR-ABL1* PCR product characterized by the presence of exon 3 of the *BCR* gene obtained from a CML patient in CP was cloned in the *Eco*RV site of the plasmid bluescript. The synthesis of the *BCR-ABL1* 395-bp fragment corresponding to the *BCR-ABL1* junction was transcribed from this plasmid using T7 polymerase (GIBCO BRL, Gr& Isl&, NY).  **Negative control:** yeast RNA | **Method validation:**   * Direct sequencing: 1 of the 18 b3a2 positive subjects was sequenced. * Repeated in 2 independent labs starting from RNA extraction with a complete set of new reaction products *(****Comments:*** *no. & method of selecting positive & negative sample send to each lab NR. Article just stated: “Some of the negative & positive samples have been reanalyzed using an identical procedure in 2 independent lab. 11 samples of RNA of normal adults were manipulated at random using exactly the same procedure.”)*   **Precaution:**1 |
| 2 | Bose S 1998[11] | **Type of cells:** WBC  **Method of cell extraction:** RBC lysis, & the isolated WBCs were washed twice in PBS & lysed in a GTC solution. Multiple aliquots of GTC lysates corresponding each to 107 WBCs were stored at -80°C until processed for RNA extraction.  **Method of RNA extraction:** article referred to Chomczynski P 1987[33] & Cross NC 1994[34]  **Amount of RNA used:** article referred to Chomczynski P 1987[33] & Cross NC 1994[34]  **cDNA synthesis technique:** article referred to Cross NC 1994[34] | **Amplification technique:**  Nested PCR of M-BCR & m-BCR (performed separately)   * 40 μL cDNA synthesis from each 107 cell aliquot was diluted in distilled H2O to 80 μL, & 1 μL was tested for quality control. The remaining 79 μL cDNA from each of 10 x 107 cell aliquot was then divided into 4 x 100 μL PCR tests. * **Cycling condition:** article referred to Cross NC 1996[35] * **Cycler:** article referred to Cross NC 1996[35] * **1st run:** article referred to Cross NC 1996[35] * **2nd run:** 1 μL of the 1st run product was involved into the 2nd run PCR run. article referred to Cross NC 1996[35] * **Detection of amplified DNA:** 5 μL of the 2nd run PCR products were electrophoresed through ethidium-bromide stained agarose gels, visualized, & photographed under UV light. * **No. of replicate/subject:** 40 * **Positive criteria:** ≥ 1 replicate * **Estimation of the minimal level of detection:** diluted cDNA preparations from cell lines previously standardized in a single test diagnostic protocols for reproducible detection of 1 leukemia cell in 105 to 106 nonhematopoietic cells (murine fibroblasts)   **Quality control gene:** *ABL1* (using one-step PCR amplification)  **Positive control**: BV173 for M-BCR, SD1 for m-BCR (each batch of PCR tests had 1 positive control)  **Negative control:** Murine cell line (NIH-3T3) (each batch of PCR tests had 4 negative controls derived from the RNA extraction, cDNA synthesis, & 1st run & 2nd run PCR blanks) | **Method validation:**   * Direct sequencing: likely in all the positive case as article stated: “For confirmation of the BCR-ABL nature of these products, representative fragments of each individual size were gel-purified & sequenced by conventional methods.”   **Precaution:**2 |
| 3 | Uckun FM 1998[12] | **Type of cells:** likely WBC as article stated: “Briefly, total cellular RNA was extracted…”  **Method of cell extraction:** NR  **Method of RNA extraction:** using the RNeasy total RNA isolation kit (Qiagen, Santa Clarita, CA). Article referred to Uckun FM 1993[36], Uckun FM 1994[37], & Gaynon PS 1997[38]. Negative controls were included.  **Amount of RNA used:** 20% of the total RNA sample  **cDNA synthesis technique:** with Moloney murine leukemia virus (MMLV) reverse transcriptase (GIBCOBRL, Gathersburg, MD) in the presence of dNTPs (= reaction mixture 1). | **Amplification technique:**  Nested PCR of m-BCR *(****comment:*** *NR, but it was m-BCR according to the sequence. Refer Supplementary Table 5 & Supplementary Figure 1 (b).)*   * **Cycling condition:** article referred to Uckun FM 1993[36] & Uckun FM 1994[37] * **Cycler:** article referred to Uckun FM 1993[36] & Uckun FM 1994[37] * **1st run & 2nd run:** article stated: “cDNA products were denatured, diluted in PCR buffer containing oligonucleotide primers & Amplitaq DNA polymerase (Perkin Elmer Cetus Corp, Norwalk, CT; = reaction mixture 2)”. Article referred to Uckun FM 1993[36] & Uckun FM 1994[37] * **Detection of amplified DNA:** PCR products were separated by electrophoresis in 1.2% agarose, transferred to nylon membranes, & hybridized with the oligonucleotide probes specific for internal sequences of *E2A-PBX1* or *BCR-ABL*. Article referred to Gaynon PS 1997[38]. * **No. of replicate/subject:** NR * **Estimation of the minimal level of detection:** NR   **Quality control gene:** *ABL1*  **Positive control**: RNA isolated from a patient (unique patient number [UPN] 100), with t(9;22)+ ALL  **Negative control:** Negative controls included PCR products from RNA-free reaction mixture 1 plus reaction mixture 2 (= negative control 1) & reaction mixture 1 containing RNA from RS4;11 cell line plus DNA polymerase-free reaction mixture 2 (= negative control 2).  *(****Comments:*** *all PCR assays were performed centrally in Children’s Cancer Group ALL Biology Reference Laboratory)* | **Method validation:** NR  **Precaution:**3 |
| 4 | Ravetto PF 2003[13] | **Type of cells:** NR  **Method of cell extraction:** NR  **Method of RNA extraction:** using RNeasy Standard & Blood Mini Kits (Qiagen Ltd, Crawley, UK). The RNA was eluted in 40 µl of RNase free water; 1 µL of this was diluted 1/50 & used to measure the Optical Density 260/280 in order to obtain the yield, concentration, & purity of the RNA. *(****Comments:*** *average amount of RNA that authors obtained per ~0.5 mL sample was 6.9 µg.)*  **Amount of RNA used**:  1 µg aliquot of total RNA  **cDNA synthesis technique:** using Ready-To-Go RT-PCR beads (Amersham Pharmacia Biotech, Amersham. UK). Reactions were carried out in 0.2 mL tubes, each containing a lyophilised bead comprising the reagents required for performing a single tube RT-PCR reaction. To each tube 1 µg total RNA, 1.5 µL oligo(dT)12–18 primer (0.5 mg/ml), & 6 µL of 1st run PCR primers (5 mM) were added, & the volume brought to 60 µL with RNase free water. Reaction mixtures were vortexed to fully dissolve the beads, centrifuged, & incubated at 42°C for 1 hr to carry out cDNA synthesis. | **Amplification technique:**  Nested PCR of M-BCR & m-BCR (performed as multiplex)   * **Cycling condition:**   1st run - 94°C/3 min 🡪 32 cycles (94°C/30 sec, 58°C/30 sec & 72°C/30 sec) 🡪 72°C/3 min  2nd run - 94°C/3 min 🡪 38 cycles (94°C/15 sec, 55°C/15 sec & 72°C/15 sec) 🡪 72°C/3 min   * **Cycler:** PCR Express thermocycler (Hybaid, Middlesex, UK) * **1st run:** performed in the same tubes of cDNA synthesis * **2nd run:** 2 µL of 1st run product was involved into the 2nd run PCR run: 2 µL 10 x PCR buffer (200 mM Tris HCl, 500 mM KCl, pH 9.0), 2 µL primer mix (5 mM each), 3 µL dNTPs (2 mM each), 1.5 µL MgCl2 (25 mM), 1 µL W1 (1%), 0.1 µL Taq DNA polymerase (5 U/ml) (Gibco, Life Technologies Ltd, UK) & 8.4 µL H2O. * **Detection of amplified DNA:** 5 uL of each PCR product was electrophoresed in a 2% agarose gel, stained with ethidium bromide, & photographed under ultraviolet light (Biorad Gel Doc 2000, BioRad UK, Hemel Hempstead). The remaining PCR product was dot blotted onto positively charged nylon membranes (Hybond-N+, Pharmacia, Amersham, UK) using a vacuum manifold. The PCR products were denatured in situ with 0.5 M NaOH, 1.5 M NaCl followed by 1.5 M NaCl, 0.5 MTris HCl (pH 7.4), & the membranes baked at 80°C for two hr to fix the DNA. The fixed PCR products were then hybridised with 10 pmol of a translocation junction specific oligonucleotide probes labelled with 10 mCi g-32P-adenosine triphosphate (ATP) by T4 polynucleotide kinase (Promega, Southampton, UK).31–33 Hybridisation was carried out at 58°C overnight, & the membranes washed briefly at room temperature in 2´SSC/0.1% SDS followed by a second wash with tetramethyl ammonium chloride (TMAC) solution (3.0 M TMAC, 50 mM Tris-HCl pH 8.0, 2 mM EDTA pH 8.0, 0.1% SDS) at 58°C for 15 minutes. The membranes were sealed between plastic film, & autoradiography, to detect probe hybridisation, was carried out in real time on an InstantImager (Canberra Packard, Berkshire, UK). * **No. of replicate/subject:** 5 in 2nd run PCRs * **Positive criteria:** NR * **Estimation of the minimal level of detection:** RNA (1 µg) samples from the leukaemic reference cell lines carrying *BCR-ABL1* were serially diluted (10-1–10-6) with RNA from negative control cell line SV18, & subjected to nested PCR, then probed.   **Quality control gene:** β actin  **Positive control**: BV17330 expressing *BCR-ABL* obtained from the German Collection of Microorganisms & Cell Cultures, Braunschweig, Germany (http://[www.dsmz.de/](http://www.dsmz.de/)) & cultured in RPMI-1640 containing 10% fetal calf serum.  **Negative control:** Epstein-Barr virus transformed normal lymphoblastoid cell line (SV18), prepared from a normal donor, & not expressing the above fusion genes & cultured in RPMI-1640 containing 10% fetal calf serum. | **Method validation:**   * Direct sequencing   **Precaution:**   * Separate areas were used to set up PCRs, to carry out amplifications, & to analyse the PCR products. * Dedicated pipettes with aerosol resistant tips were used to prepare PCR mixtures. * In order to detect commonly expressed BCR-ABL transcripts, 2 pairs of 1st & 2nd run primers were used in multiplex reactions. |
| 5 | Hsu H 2004[14] | **Type of cells:** MNC  **Method of cell extraction:** Ficoll-separated PB & BM MNC  **Method of RNA extraction:** total RNA using commercially available reagents (RNAzol B; TEL-TEST, Inc, Friendswood, Tex).  **Amount of RNA used**: 2 µL aliquots of total RNA  **cDNA synthesis technique:** using 0.5 µL of oligo(dT) 15 primer & 1.5 µL of Moloney-murine leukemia virus RT RNase H (Promega, San Luis Obispo, Calif) in a total volume of 21.5 µL in accordance with manufacturer recommendations. | **Amplification technique:**  Nested PCR of M-BCR *(****comment:*** *NR, but it was M-BCR according to the sequence. Refer Supplementary Table 4 and Supplementary Figure 1 (a).)*   * **Cycling condition:** 95°C/5 min 🡪 20 cycles (95°C/30 sec, 62°C/30 sec & 72°C/60 sec) 🡪 72°C/7 min (same for 1st & 2nd run) * **Cycler:** Perkin-Elmer 2400 thermocycler (Applied Biosystems, Foster City, Calif) * **1st & 2nd run:** article just stated: “Each PCR reaction employed 300 ng of genomic DNA, 20 pmol of each primer, final volume of 50 µL of reaction mixture.” * **Detection of amplified DNA:** 10 µL aliquots of the nested PCR products were then analyzed in 1.5% agarose gels containing ethidium bromide & photographed under UV light. * **No. of replicate/subject:** NR * **Estimation of the minimal level of detection:** varied the ratio of K562 cells to Jurkat cells from 1 to 1:106 (total number of cells 108 in each sample) by means of serial dilution.   **Quality control gene:** glyceraldehyde 3-phosphate dehydrogenase  **Positive control**: K562 cells  **Negative control:** Jurkat, U937, HL-60 cells, & water | **Method validation:** NR  **Precaution:** NR |
| 6 | le Coutre P 2010[15] | **Type of cells:** NR  **Method of cell extraction:** separated by dextran sedimentation & lysed  **Method of RNA extraction:** article just stated: “RNA was isolated using a standard procedure”  **Amount of RNA used**: NR  **cDNA synthesis technique:** using random hexamer primers for total RNA (1 µg ⁄20 µL), & cDNA was stored at -20oC. For further analysis, 1.5 µg of RNA of samples with adequate *ABL* transcripts, determined by Taqman Analysis, were considered. | **Amplification technique:**  Nested PCR of M-BCR   * **Cycling condition:** Article just stated: “Detection of BCR-ABL transcripts was performed according to the st&ard procedures of our institution that are applied in diagnostic routine” * **Cycler:** Trio-Thermocycler, Biometra * **1st & 2nd run:** article just stated: “Detection of BCR-ABL transcripts was performed according to the standard procedures of our institution that are applied in diagnostic routine” * **Detection of amplified DNA:** NR * **No. of replicate/subject:** 2 * **Positive criteria:** NR * **Estimation of the minimal level of detection:** tested by dilution of K562 cells   **Quality control gene:** *ABL1*.Samples with adequate *ABL* transcripts were considered to be positive for c-ABL only after a 10-2 sample dilution showed *ABL* positivity comparable to a RNA (1.5 µg) sample isolated from HL60 cells.  **Positive control**: NR  **Negative control:** NR | **Method validation:**   * Positive samples were re-tested in an alternative lab using the identical PCR setting (Dr. Hans Joos, Invitek GmbH, 13125 Berlin-Buch, Germany, http://www.invitek.de).   **Precaution:** control & study samples were taken at different days & locations in our centers & processed separately to avoid cross-contamination between the two groups. |
| 7 | Song J 2011[16] | **Type of cells:** WBC  **Method of cell extraction:** NR  **Method of RNA extraction:** total RNA using QIAamp RNA Blood Mini Kit (Qiagen, Inc., Valencia, CA). ~8-16x106 WBC were used for total RNA extraction. **A**fter RNA extraction, DNase I (8U) treatment (Ambion, Inc., Austin, TX) was performed to remove residual DNA from the samples.  **Amount of RNA used**: 500 ng of total RNA  **cDNA synthesis technique:** using random primers in a 20 µL reaction using the RETROscript Reverse Transcription System (Ambion, Inc.). | **Amplification technique:**  Nested PCR of M-BCR & m-BCR (likely performed separately. Article stated: “Reaction for each genetic rearrangement were performed in triplicate.”)   * **Cycling condition:** 95°C/30 sec 🡪 35 cycles (94°C/30 sec, 65°C/1 min & 72°C/1 min) 🡪 72°C/10 min (same for 1st & 2nd run) * **Cycler:** PTC-100 Thermal cycler (MJ Research, Inc., Waltham, MA). * **1st run:** 3µL of each cDNA sample per reaction. Each 50µL PCR reaction contained 1X PCR buffer, 125 nmol of MgCl2, 40 nmol of dNTP mix, 200 pmol of each primer, & 1U of TaqDNA polymerase. * **2nd run:** 1 µL product of PCR 1st run was used as template in a 50 µL reaction performed under the same conditions as PCR 1st run. * **Detection of amplified DNA:** PCR products were visualized on 1% or 2% agarose gel for genomic DNA & cDNA templates, respectively. Bands were cut with a scalpel, & the DNA was purified using a QIAquick Gel Extraction Kit (Qiagen, Inc.) according to the manufacturer’s instructions. * **No of replicate:** 3. * **Positive criteria:** ≥1 replicate * **Estimation of the minimal level of detection:** Cell lines were used to determine the sensitivity of the nested PCR assay. To determine the sensitivity of nested PCR & the level of BCR-ABL p210 expression in healthy individuals, the BCR-ABL p210 (b3-a2)–positive cell line K562 was diluted with cultured fibroblast cells. Undiluted K562 tested positive for BCR-ABL p210 in both primary PCR & nested PCR, whereas the fibroblast cell line was negative for the BCRABL p210 transcripts in both primary & nested PCR.   **Quality control gene:** *ABL*  **Positive control:** *BCR-ABL*–positive cell line K-562 (CCL-243) from American Type Culture Collection (Manassas, VA).  **Negative control:** Nuclease-free water | **Method validation:**   * Direct sequencing *(****Comments****: article did not state which fusion gene was send for sequencing.)*   **Precaution:** Perform nested PCR in another lab in a different building to minimize the risk of contamination.  **Comments:** authors described qPCR methodology to study prevalence of M-BCR & m-BCR. However, the outcomes using qPCR was not reported (see Remarks in Table 2). Hence, the qPCR methodology was not extracted for the review. |
| 8 | Zuna J 2011[17] | **Type of cells:** NR  **Method of cell extraction:** NR  **Method of RNA extraction:** NR  **Amount of RNA used**: NR  **cDNA synthesis technique:** NR | **Amplification technique:** NR  **Quality control gene:** NR  **Positive control:** NR  **Negative control:** NR  *(****Comments:*** *this paper is Correspondence to Editor. No information on the PCR technique can be extracted except for information on precautionary measures taken in their study.)* | **Method validation:** NR  **Precaution:**4 |
| 9 | Boquett JA 2013[18] | **Type of cells:** WBCs  **Method of cell extraction:** NR  **Method of RNA extraction:** total cellular RNA was extracted from PB leukocytes using the Trizol reagent (Invitrogen, USA) according to manufacturer instructions. The material was then re­suspended in 18 µL sterile diethylpyrocarbonate-treated water.  **Amount of RNA used**: 6 µl of total RNA  **cDNA synthesis technique:** using the outer antisense primer & the cDNA GeneAmp RNA PCR synthesis kit (Perkin Elmer, USA). | **Amplification technique:**  Nested PCR of M-BCR   * **Cycling condition:** 93°C/2 min 🡪 35 cycles (93°C/40 sec, 60°C/30 sec & 72°C/40 sec) 🡪 72°C for 10 min (same for 1st & 2nd run) * Cycler: ThermalCycler Gradient, Eppendorf, USA * **1st & 2nd run:** article just stated: “20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 200 mL dNTP, & 1.25 U Taq polymerase” * **Detection of amplified DNA:** a fragment of 328 or 253 bp was generated in the presence or absence of exon b3, respectively. The amplified product was analyzed by electrophoresis on 10% polyacrylamide gel for 1 hr, & visualized by silver staining. * **No of replicate:** 3 * **Positive criteria:** NR * **Estimation of the minimal level of detection:** NR   **Quality control gene:** NR  **Positive control:** NR  **Negative control:** NR | **Method validation:** NR  **Precaution:** NR |
| 10 | Ismail SI 2014[19] | **Type of cells:** NR  **Method of cell extraction:** NR  **Method of RNA extraction:** RBC lysis & RNA isolation reagent TRIzol (Invitrogen Life Technologies, Carlsbad, CA, USA). RNA was extracted within a few hr of blood collection.  **Amount of RNA used**: 1 µg of total RNA  **cDNA synthesis technique:** using an RT system kit according to the manufac­turer's instructions (Promega, Madison, WI, USA). This kit utilized random hexamers & the MuLV RT enzyme. | **Amplification technique:**  Nested PCR of M-BCR & m-BCR (performed separately)   * Nested PCR was conducted to detect the presence of the three predominant bcr‑abl fusion transcripts (b2a2, b3a2 & e1a2). In this study, uniplex PCR was used instead of multiplex PCR. * **Cycling condition:** article referred to Cross NC 1994[34] & Nogva HK 1998[39] with minor modification * **Cycler:** NR * **Detection of amplified DNA:** nested PCR products (5 μL) were electrophoresed in 2% ethidium bromide stained agarose gels, visualized & images were captured (UVP, Cambridge, UK) under UV light. * **No of replicate:** NR * **Estimation of the minimal level of detection:** using 2 methods. (1) cells from the K562 *BCR‑ABL*‑positive cell line were serially diluted in MCF‑7 cells, which are *BCR‑ABL* negative. Dilutions were prepared in 10‑fold steps, from undiluted to a dilution of 10‑7. (2) *BCR‑ABL*‑positive RNA obtained from K562 cells was diluted in negative RNA from MCF‑7 cells. The dilutions were also prepared in 10‑fold steps, from undiluted to 10‑7.   **Quality control gene:** glyceraldehyde 3‑phosphate dehydrogenase  **Positive control:** a known positive CML sample for the *BCR-ABL1* transcript  **Negative control:** NTC was included in each PCR run | **Method validation:** NR  **Precaution:** NR  **Comments:** quality of the RNA was analyzed by electrophoresis gels & the concentration was determined spectrophotometrically (Bio‑Rad, Hercules, CA, USA). RNA was stored at ‑70˚C until use. |
| 11 | Kosik P 2017[22] | **Type of cells:** MNC  **Method of cell extraction:** MNC were isolated from 80–100 mL of CB, within 24 hr after birth by the standard gradient centrifugation using LymphoSepTM (MP Biomedicals, USA). No. of cells was assessed using autohematology analyzer (Mindray, BC-3000plus, China). Isolated CB MNC pellets were then shocked frozen in liquid nitrogen. Each cell pellet, containing ~107 MNC & provided in at least triplicates, was cryopreserved by a controlled rate freezer & stored in liquid nitrogen.  **Method of RNA extraction:** using RNAzol (Research Molecular Center, Ohio, USA) using standard protocol recommended by the manufacturer.  **Amount of RNA used**: 1 µg of total RNA  **cDNA synthesis technique:** following the manufacturer’s protocol (Thermo Scientific, St. Leon- Rot, Germany) | **Amplification technique:**  qPCR:   * **Reporter:** Taqman * **Cycling condition:** article referred to Gabert J 2003[40] * **Cycler:** * Skorvaga M 2014[20] reportd: RotorGene 2000 or BioRad CFX96 in * Kosik 2017[22] reported: BioRad CFX96 * **Amplification:** the qPCR contained 2 μL cDNA (100 ng RNA equivalent), 300 nM each primer, 200 nM probe (5′-fluorophore was FAM, 3′-quencher was BHQ1; synthesized by Merck (Darmstadt, Germany)), & * Skorvaga M 2014[20] reported: TaqMan universal PCR master mix from Applied Biosystems * Kosik ) 2017[22] reported: HOT FIREPol Probe qPCR mix from Solis BioDyne (Tartu, Estonia) * **Calibrator:** * Skorvaga M 2014[20] reported: prepared own plasmid standards, by religating ~10 pg of commercial pDNA, amplifying in *E.coli* & linearizing with a restriction endonuclease, from 1 to 100 copies per reaction * Kosik P 2017[22] reported: the plasmid standards with individual fusion genes subcloned into PCR II TOPO vector were from Ipsogen (Qiagen, Marseille, France) * **No of replicate:** triplicate * **Positive criteria:** ≥1 of 3   **Quality control gene:** *ABL1*  **Positive control:** NR  **Negative control:** NTC. Skorvaga M 2014[20] reported: “This finding along with fact that NTC ran in triplicates were found negative in all qPCR experiments suggest that the positivity of CB samples as detected in our lab was probably not caused by contamination during PCR. However, other sources of contamination, e.g. during isolation of MNC from UCB, RNA isolation, & cDNA synthesis cannot be excluded, although we followed strict precautionary measures in attempt to avoid contamination (Text S1).” | **Method validation:**5  **Precaution:** Skorvaga M 2014[20] reported: performed a qPCR experiment in a 96-well format (on BioRad CFX96 instrument) with only NTC, water & plasmid standards, & the result was that all NTC’s as well as water samples were clearly negative.  **Comments:**   * CB cells were irradiated with 60Co γ-rays on ice Theratron Elite 100 (Mds Nordion, Ottawa, Ontario, Canada) source. * Concentration & purity of isolated RNA was measured by Nanodrop N-1000 instrument (Thermo Scientific, Delaware, USA). |

1Biernaux C 1995[4]

* Adhered to the guidelines recommended & adopted by the Molecular BiologyBMT Study Group.
* Samples were manipulated in a specific laminar flow oven, RNA extraction & RT-PCR were performed in a different room of the building, & all locations in which manipulations were performed were exposed to UV light between each experiment for a period of at least 20 hr.
* To rule out a possible error of the RT caused by the formation of artifactual heteroduplexes between the *BCR* & the *ABL* mRNAs that could serve as a template for the RT, the RNA preparations were heated at 90°C for 5 minutes before retrotranscription.
* The same RNA samples were also retro-transcribed with a thermostable RT working at 72°C after heating the RNA sample at 90°C (*rTrh* DNA polymerase; Perkin Elmer Cetus, Norwalk, CT). It should be noted that the different samples were manipulated at random in all steps of the analysis; nevertheless, positive & negative *BCR-ABL1* samples were found side by side in a same experiment, thus ruling out a possible intersample contamination.

2Bose S 1998[11]

* 6 stages (cell preparation, RNA extraction, cDNA synthesis, 1st run PCR, 2nd run PCR, & electrophoresis of PCR products) were each performed in 6 separate lab rooms in dedicated laminar air-flow cabinets.
* Mixes of reagents for cDNA synthesis & PCR amplification were prepared in bulk & stored as single-use aliquots with dedicated pipettes & plugged tips, in a PCR-free lab, by personnel not involved with handling cells &/or actual PCR procedures. Aliquots from all mixes were extensively tested on mock 45-tube PCR assays containing NTC before & at various intervals during the period of testing of actual cellular material & were always found to be contamination-free.
* Stocks from all the cell lines were analyzed by conventional cytogenetics & fluorescence in situ hybridization (FISH) with BCR & ABL probes14 to confirm the absence of a t(9;22).

3Uckun FM 1998[12]

* Article referred to Kwok S 1993[41] to prevent cross-contamination of samples.
* Prepared samples in a dedicated room separated from the room in PCR reactions were performed. UV germicidal lamps were used in biosafety hoods.
* Separate sets of supplies & pipetting devices for sample preparation & for setting up reactions. Used pipettes with aerosal-resistant tips. Wear gloves & change them frequently.
* Deionized water, buffer solutions, disposable pipette tips, & microcentrifuge tubes were autoclaved.
* All reagents, including oligonucleotides, were prepared, divided into aliquots, & stored in an area that is free of the PCR-amplified products.
* Mixed reagents before dividing them into aliquots. All PCR reagents were combined into a ‘‘premixture,’’ which was then pipetted into reaction tubes containing RNA/cDNA. Non-sample components, such as premixed dNTPs, primers, buffer, & enzyme, were added to the reaction tubes before sample cDNA (Molecular Bio-Products, San Diego, CA).

4Zuna J 2011[17]

* Routine strict precautions to prevent carryover contamination.
* Procedures were performed in the new lab where leukemic samples had never before been processed.
* Negative controls were included at all steps & positive controls were diluted.
* The positive control cDNA was added in a separate room when all other PCR vessels were tightly closed.

5Kosik P 2017[22]

Skorvaga M 2014[20] reported:

* In the same lab, some of the positive samples were further verified by nested PCR. *(****Comments:*** *further detail/result NR)*
* In the same lab,retest 15 positive samples (RotorGene 2000) using BioRad CFX96: started with isolation of total RNA by RNAzol method from never opened tubes with MNC pellets & all the steps were identical with those used in the first screening, except that the analysis was performed on BioRad CFX96 instrument. *(****Comments:*** *method of selecting 15 samples NR)*
* In different lab i.e. certified lab at NCI, Bratislava: were processed in an identical manner with 3 minor exceptions, namely (i) total RNA was isolated with Trizol (Invitrogen), (ii) the 39-quencher was TAMRA & (iii) RT qPCR instrument was RotorGene 3000. *(****Comments:*** *(i) method of selecting samples NR. Although Kosik P 2017 reported “About 1/4 of 20 positive samples were randomly selected for validation by a reference lab”, but this sentence is confusing. Hence disregarded. (ii) Data on no. of positive or negative sample for each fusion gene, TEL-AML1, MLL-AF4 & m-BCR send was contradicting in the text. In the section of Methodology, article stated: “For verification of CRI results, 20 selected samples in the form of 107 MNC frozen pellets…”, then in the section of Results, article stated: “All 7 selected CB samples tested positive for TEL-AML1 in CRI lab were found negative by a certified NCI lab. The negativity of 13 selected samples for this translocation was confirmed by NCI. Out of 18 BCR-ABL p190 positive samples, only 5 were confirmed at NCI. 2 CB samples were tested negative for this translocation in both lab. Single MLL-AF4 positive sample was not validated, in contrary out of 18 negative samples two were detected as positive at NCI.” Assuming 20 samples were send for each fusion gene, the no. stated for MLL-AF4 was only 18. (iii) From the statement, 13 TEL-AML1, 2 m-BCR & 18 MLL-AF4 negative sample, total 33, was send. But this is contradicting with the statement “In total, out of 32 samples tested negative in CRI lab, 29 were validated by NCI, resulting into ~90% validation rate of negative samples.”)*

Kosik P 2017[22] reported:

* Replicated qPCR verification run: *(****Comments:*** *unclear this was done in the same lab or NCI as in Skorvaga M 2014[20])* this validation was performed with the same screening method starting from a new frozen MNC aliquot for each proband. Samples from 90 PFG+ probands were repeatedly analyzed altogether in 229 qPCR runs. *(****Comments****: method of selecting 90 PFG+ probands NR. The no. 229 is contracting with other statement in the article: “positive samples were randomly selected for repeated screening 4 times while negative samples were repeated twice.” If according to this statement, it should be 90 x 4 = 360)*
* Direct sequencing: 22 samples, from which one was tested positive in 1 run, 17 in 2 runs, & 4 in 3 runs *(****Comments:*** *method of selecting 22 samples & how many sample for each fusion gene, TEL-AML1, MLL-AF4 & m-BCR NR)*

ALL, acute lymphoblastic leukaemia; BM, bone marrow; CB, umbilical cord blood; CP, chronic phase; EDTA, ethylenediaminetetraacetic acid; GTC, guanidinium thiocyanate; hr, hour(s); lab; laboratory; MNC, mononuclear cells; NR, not reported; NCI, National Cancer Institute; NTC, non‑template negative control; PB, peripheral blood; PBS, phosphate-buffered saline; PGF, pre-leukaemic fusion gene; RBC, red blood cell; RT-PCR, reverse transcriptase polymerase chain reaction; TBE, Tris/borate/EDTA, WBC, white blood cell