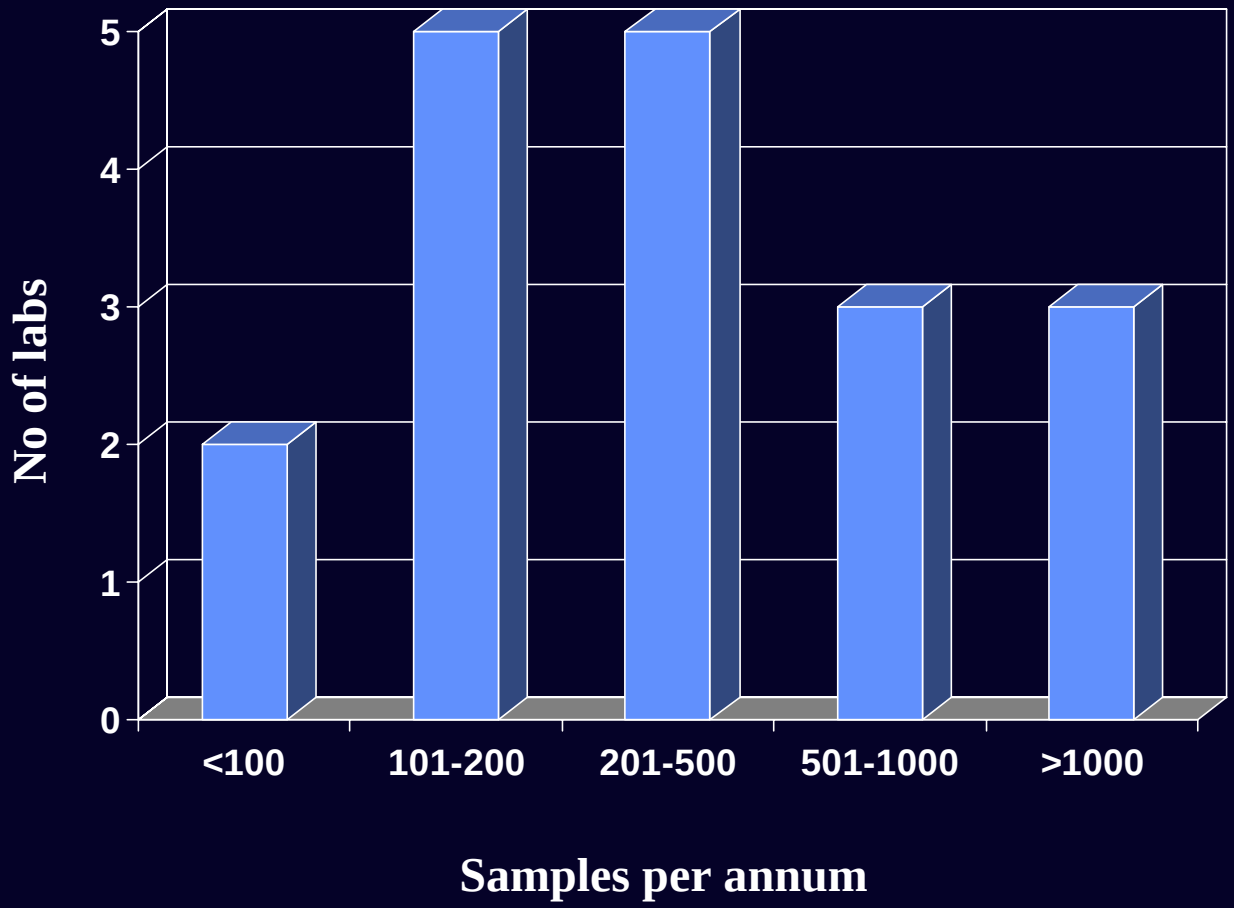
BCR-ABL survey results

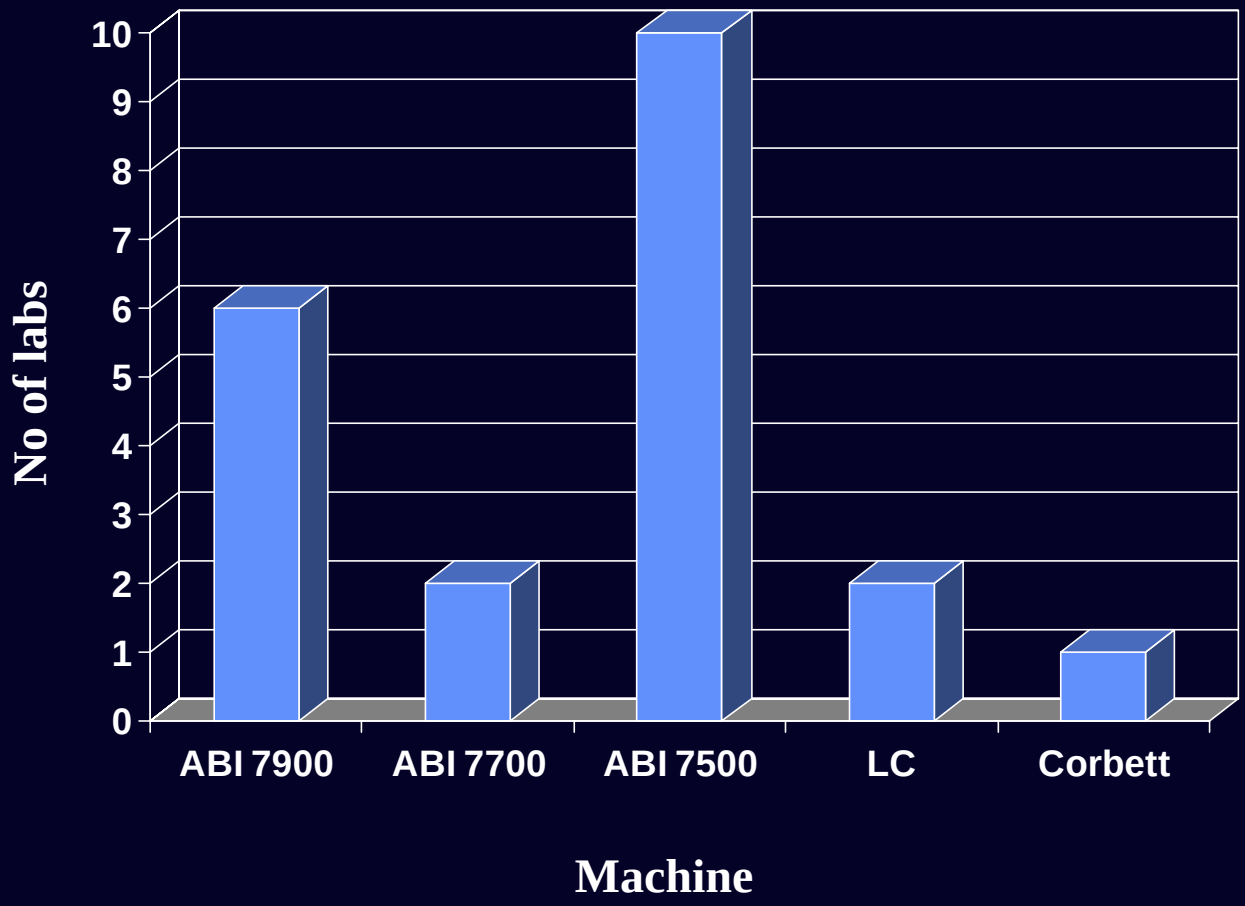
RQ-PCR testing labs

- **19 labs performing RQ-PCR for CML (18 surveys returned)**
  - **Exeter, Barts, Marsden, Leeds, Newcastle, Birmingham, Sheffield, Southampton, Liverpool, Manchester, Hammersmith, Plymouth, (King's)**
  - **Aberdeen, Edinburgh, Glasgow**
  - **Cardiff**
  - **Belfast**
  - **Dublin**
- **4 additional labs in England planning to set up, one in Scotland**

Sample throughput



Methodology: RQ-PCR machine



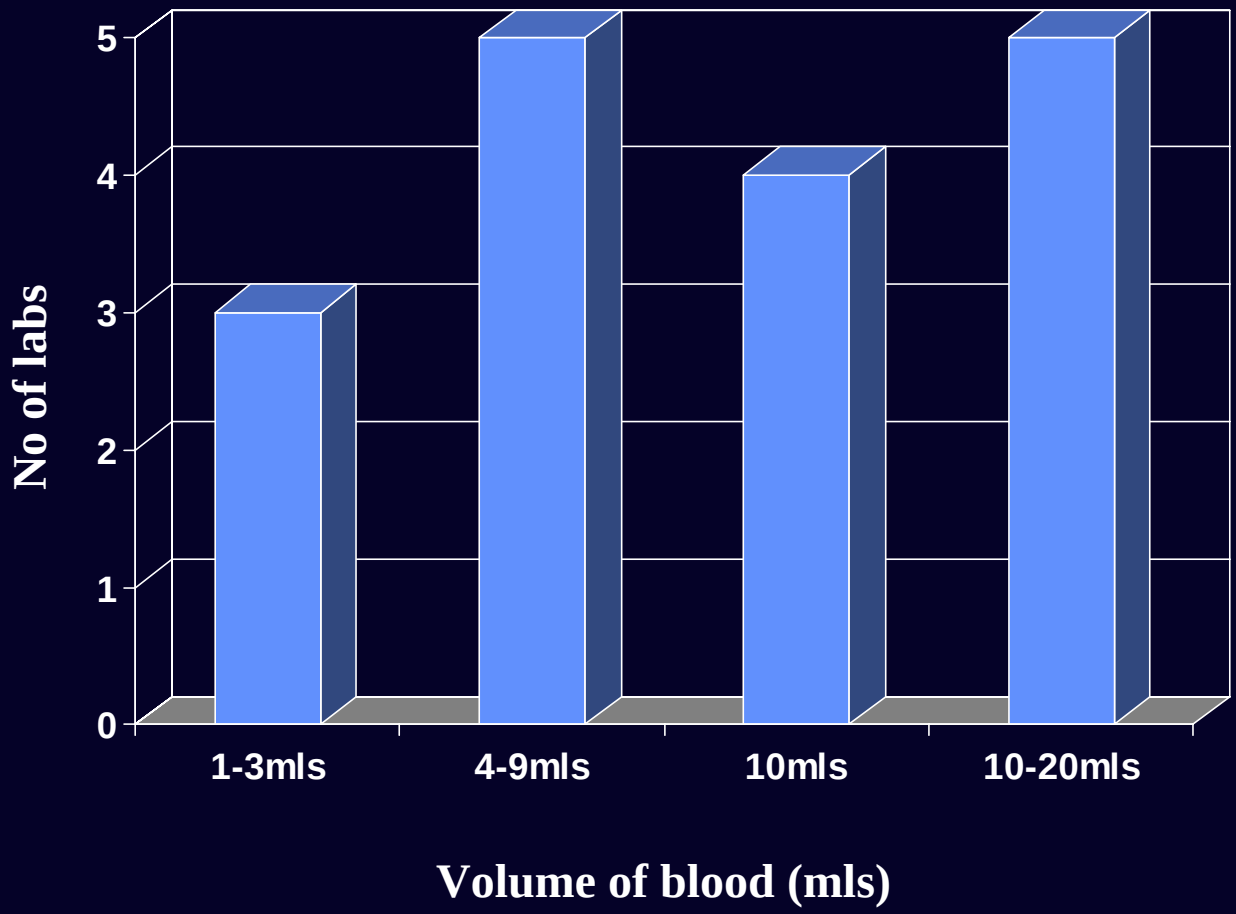
Methodology: primers and sample

- **Control gene: ABL (n=17), BCR (n=1)**
- **Control primers: EAC (n=12), HH (n=3), Mannheim (n=2), Adelaide (n=1)**
- **BCR-ABL primers: EAC (n=13), HH (n=2), Mannheim (n=2), Adelaide (n=1)**

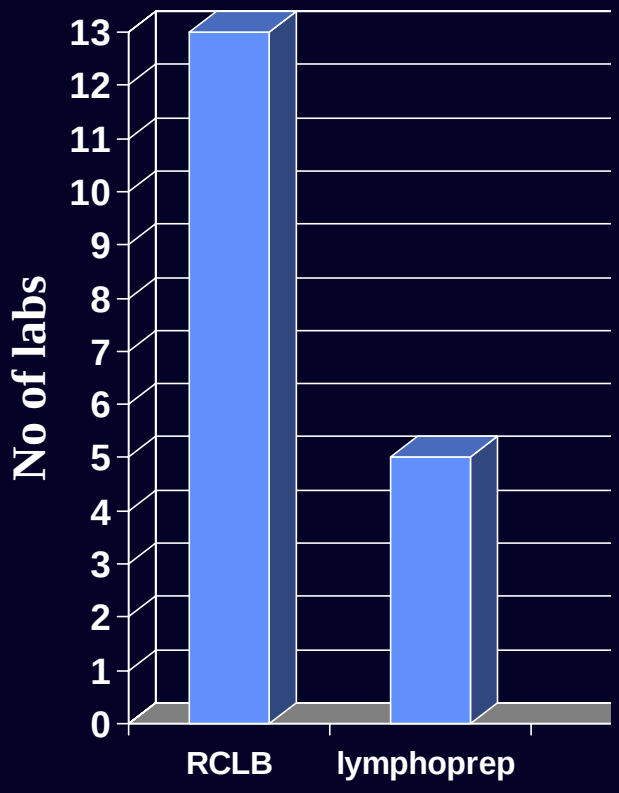
- **Determine transcript type prior to treatment?**
  - **Yes (n=14; if sample provided), no (n=3)**
  - **One lab mentioned quantitative level as patient-specific baseline**
- **Approximately 2% CML patients have BCR or ABL breakpoints that cannot be amplified with standard RQ-PCR primer sets.**

- **Anticoagulant?**
  - **EDTA (n=14), no preference (n=2), usually EDTA but hep/medium if cyto needed as well (n=2)**
  - **4 labs mentioned PCR problems with heparin**
  - **4 labs said simpler to use EDTA (other samples in EDTA/standard tubes used by phlebotomists)**

Methodology: sample volume



Removal of red cells and RNA extraction



Methodology

- **Number of extractions:**
  - **One RNA extraction; one cDNA synthesis (n=16)**
- **Amount of RNA:**
  - **not measured (n=9); 300ng (n=1); 1-2ug (n=6); 8-10ug (n=2)**
- **Reverse transcription:**
  - **Random hexamers (n=18)**
  - **MMLV (n=9), Superscript II (n=5), Superscript III (n=3), Thermoscript (n=1)**
- **Replicates BCR-ABL and control gene:**
  - **3 (n=12), 2 (n=5), 1 (n=1)**
- **Standard curve**
  - **Ipsogen plasmids (n=11), other plasmids (n=7), cell lines (n=1)**

Scoring results: negative

- **Most labs only called negative when all replicates negative**
- **If discordant results some labs repeat and/or perform nested PCR.**
- **Two labs said they routinely performed nested PCR on negative samples**

- **7 labs said they reported results as 'negative' or undetectable' with an indication of sensitivity from the number of control gene transcripts**
- **10 labs said they just reported results as 'negative' or undetectable'**

- **Acceptable value of ABL?**
  - **10,000 (n=4)**
  - **5,000 (n=2)**
  - **1,000 (n=3) or C_t>30 (n=1)**

  - **Should be aiming for >30,000 ABL transcripts in the same volume of cDNA that is analysed for BCR-ABL, but difficult to unambiguously define cut off for acceptability because depends on clinical context and previous results**

Scoring results: positive

- **Nearly all labs expressed results as %BCR-ABL/control gene**
- **Some also gave log reduction from patient baseline, previous sample or median untreated local patients**
- **Some also gave absolute values for BCR-ABL and control gene**

QC samples and run parameters

- **Nature, frequency and acceptance criteria highly variable**
  - **Cell line dilution (n=8)**
  - **Previous sample (n=8)**
  - **Plasmids (n=3)**
  - **Comparison of C_ts for standard curve (n=1)**

  - **Blanks, NTC, NAC all negative**
  - **C_ts of replicates <0.5 (n=3); <1 (n=5); <1.5 (n=4); <2 (n=1); SD < 3% (n=1)**
  - **Some labs pointed out that results are more variable at low levels and so need different criteria depending of number of transcripts detected.**

- **R^2>0.98 (7/11 who answered)**
- **Slope criteria highly variable between -3.0 to -4.0**
- **Threshold highly variable between 0.05 and 0.5**

Mutation analysis

- **9 labs set up as service**
- **1 research**
- **4 planning to set up**

- **9 service labs**
  - **Sequence whole region (n=5)**
  - **dHPLC whole region (n=2)**
  - **Pyrosequencing/ASO/sequencing T315, then sequencing whole region if negative (n=2)**

  - **One group routinely quantifies mutation level by pyrosequencing**