68 PROSTATE CANCER SCREENING USING PROSTATE SPECIFIC ANTIGEN EXTRACTED FROM DRIED BLOOD ON FILTER PAPER

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Prostate specific antigen (PSA) has recently been proposed, with the advent of increasingly sensitive assays, as a suitable marker to screen populations for occult prostate cancer.

Objective To simplify prostate cancer screening by validating the measure of PSA from blood dried on filter paper and subsequently sent through the mail.

Methods Serum obtained by venepuncture from individuals requesting PSA testing and paired dried blood spots sent separately through the mail were assayed for PSA using either a commercially available, highly sensitive automated assay (Immulite, third generation PSA) or a research assay based on time resolved fluorescence.

Results PSA purified as the free 30 kD protein (from seminal plasma) or as the 100,000 kD complex bound to and, α_1- antichymotrypsin was added to female whole blood to determine its distribution between intracellular and extracellular compartments. Concentrations in whole blood were the same as those found in the plasma corrected for hematocrit indicating that PSA remained outside the RBC in the extracellular compartment. We spotted spiked blood onto Schleicher and Schuell #903 filter paper, extracted the dried blood from five 3 MM2 punched-out disks into 6% BSA containing 0.05% Tween-20 and constructed calibration curves that showed a working assay range between 0.4 and 50 pg/L. Precision experiments conducted on spotted paper gave CV's <10%. Comparison of PSA levels determined between paired serum and mailed dried blood spots showed a consistent relationship.

Conclusions This study confirms that sensitive, precise and reliable PSA assays can be performed on dried blood spots on filter paper and may have value in screening programs where patient specimens are sent to the laboratory through the mail.

69 RESULTS OF A QC PROGRAM FOR ESTROGEN AND PROGESTERONE RECEPTOR (ER, PR) ASSAYS IN CANADA

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The ER and PR content of breast tumour cytosol extracts is used to select appropriate therapy for the breast cancer patient. These tests are performed in 14 laboratories in Canada. While each laboratory runs an in-house or kit control, external QC is not universal.

Objectives a. To establish the feasibility of providing an external QC program for all Canadian ER/PR testing sites. b. To assess the performance of these assays.

Methods Excess cytosol from routine ER/PR assays was lyophilized and distributed by mail. Assay details were collected by questionnaire. 16 QC samples, drawn from 4 different pools, were mailed to each laboratory in 1994. All results from each sample were expressed as a proportion of the all-laboratory mean for that sample. Within each laboratory, a proportional constant was chosen to bring the within-laboratory all-result mean to 1.0. The SD of the resultant pooled data from all labs was calculated and outliers beyond 3 SD's eliminated.

Results Lyophilized cytosols, distributed by regular mail, provide an inexpensive yet effective means of external QC for ER/PR assays. For ER assays, both immunochemical and DCC techniques were capable of providing satisfactory results, while for PR assays, the immunochemical technique was superior.

70 SYNTHESIS OF RNA PROBES FROM POLYMERASE CHAIN REACTION- GENERATED DNA TEMPLATES. APPLICATION TO THE DETECTION OF THE PHILADELPHIA TRANSLOCATION

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The most widely used approach for preparation of RNA probes involves cloning of the sequence of interest in a suitable vector containing T7 and/or SP6 promoters, digestion of the vector and transcription.

Objectives a. To synthesize RNA probes directly from PCR-generated DNA templates, thus avoiding cloning. b. To develop time-resolved fluorescence hybridization assays for the detection of the Philadelphia (Ph) translocation.

Methods mRNA was isolated from K562 cells (a Ph positive cell line) and, after reverse transcription, the diagnostically useful BCR-ABL sequences were amplified by PCR. The downstream primer was designed to contain the T7 promoter sequence at its 5' end. Therefore the T7 promoter is incorporated in the amplified product. Subsequently, the PCR mixture was transcribed in the presence of digoxigenin-UTP (dig-UTP). The RNA probes were used, directly, for hybridization. Hybridization was performed in streptavidin-coated wells. Biotinylated target DNA was immobilized and then hybridized to the RNA probe. The hybrids were detected with alkaline phosphatase-labeled antidigoxigenin antibody and fluorosalicylphosphate as substrate.

Results Hybridization was complete in 20 min at 42 °C. mRNA corresponding to a single Ph-positive cell in the presence of 500,000 normal cells can be detected with a signal/background ratio of 2. CVs were about 7%. Peripheral blood samples from four Ph-positive chronic myelogenous leukemia patients were tested and the results agreed with cytogenetics.

Conclusions The proposed method is an efficient alternative for synthesis of nonisotopically labelled RNA probes for highly sensitive hybridization assays.

71 AN AUTOMATED, CONTAINED, NONRADIOISOTOPIC PCR-BASED SYSTEM FOR DIRECT HIV-1 DETECTION IN BLOOD SAMPLES

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Clinical Diagnostic Systems, Inc., a Johnson and Johnson Company, has developed a rapid, automated PCR pouch system that employs the use of environmental separation of the amplification and detection reactions for carryover control. The disposable pouch contains an integrated colorimetric detection area for the multiplexed capture of coamplified PCR products.

Objectives Establish the sensitivity and specificity of the automated PCR pouch system using an assay developed for the detection of HIV-1 DNA sequences in clinical specimens.

Methods Blinded, randomized PBMC pellets from 66 HIV-1 seropositive and 26 HIV-1 seronegative individuals were lysed for 30 minutes in a proteinase containing buffer. The equivalent of 62.5µl of whole blood was amplified in the pouch system for 40 cycles using primer sets specific for HIV-1 *ltr* and *env* regions. An internal false negative control sequence was present in each reaction to monitor for the presence of inhibitors.

Results 66 seropositive specimens tested PCR positive and 24 seronegative specimens tested PCR negative. 2 additional seronegative samples indicated the presence of amplification inhibitors based upon internal false negative control results.

Conclusions This feasibility test indicates that the automated PCR pouch system is a rapid, sensitive and specific method for detecting HIV-1 DNA in clinical specimens.