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Letter to the Editor

Cross-channel contamination of prostaglandins during separation by thin-layer chromatography

Sir,

We wish to report a problem arising when samples containing substantially different amounts of prostaglandins (PGs) are run on adjacent channels on thin-layer chromatography (TLC) plates.

Whatman LK-5D TLC plates with 19 channels are often used to separate prostaglandins prior to radioimmunoassay [1, 2]. In our laboratory, urine after the addition of acetone, adjustment of pH to a value between 4.0 and 4.5 and extraction with chloroform, is spotted onto TLC plates. The chloroform--acetone extract is taken to dryness and reconstituted in methanol (100 μl) and applied to the TLC plate in 50-μl aliquots. Plates are developed in an organic phase of ethyl acetate—isooctane—acetic acid—water (80:50:16:90). Duplicates of each sample are assayed, and separated from each other on the plate by other samples, or by water blanks and a quality control of pooled urine. Blank values are in the range 10--20 pg/ml for thromboxane B_2 and PGE_2 and 10--50 pg/ml for 6-keto-$PGF_{1\alpha}$.

When measuring levels of 6-keto-$PGF_{1\alpha}$ of several ng/ml in rabbit urine we sometimes observed an increase in blank values from 50 to 600 pg/ml, and a corresponding increase in quality control urines from 374 pg to 800 pg. This only occurred when urines containing relatively large amounts of 6-keto-$PGF_{1\alpha}$ (of the order of 16 ng/ml) were run in channels adjacent to the blank or quality control. As this suggested that cross-channel contamination might be occurring, the following investigation was carried out.

Blanks and quality controls were either set up alongside channels that were unoccupied (A) or spotted with samples containing ng/ml quantities of PGE_2 and 6-keto-$PGF_{1\alpha}$ (B). Care was taken to apply the sample to the middle of the preadsorbent area no lower than 5 mm from the bottom of the plate to avoid flooding adjacent channels or contaminating the developing solvent. Our results show that there was definite contamination between adjacent channels resulting in high values for both blanks and quality control samples (Fig. 1). When the experiment was repeated using water blanks spiked with 10 ng of 6-keto-$PGF_{1\alpha}$ or PGE_2, the existence of cross-channel contamination was confirmed, indicating that the phenomenon was not limited only to those samples
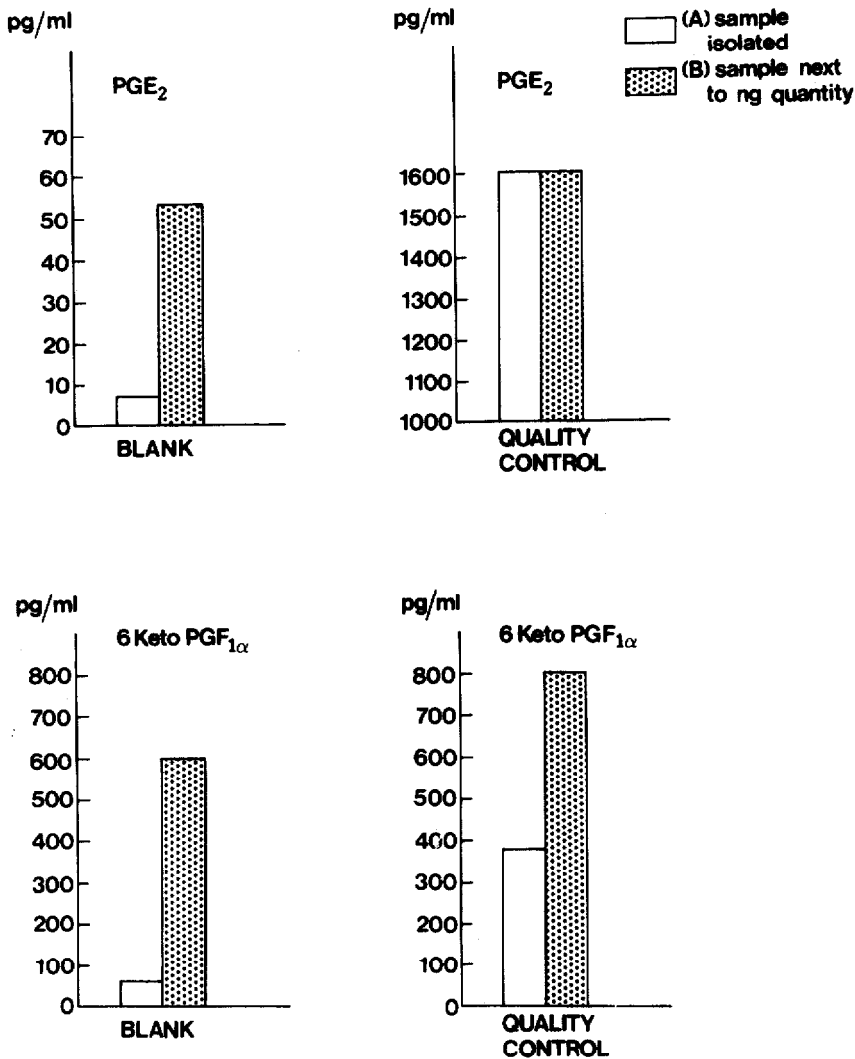
Fig. 1. Blank and quality control values for PGE_2 and 6-keto-$PGF_{1\alpha}$ when run in isolated channels or alongside channels containing ng/ml quantities of PGE_2 and 6-keto-$PGF_{1\alpha}$.

containing urinary extracts which may contain impurities affecting leaching from one channel to the next.

The problem was overcome by running blanks, quality controls and samples in duplicate in adjacent channels, leaving an unspotted channel between each pair of samples. The samples will then only cross contaminate their duplicates and this will be corrected by the estimation of recovery of the ^{3}H-labelled material added initially. This technique successfully eliminates the cross-channel contamination which previously gave rise to errors when samples in adjacent channels varied markedly in PG concentration. The only disadvantage of this procedure is the additional costs arising from the use of 11 rather than

the full 19 channels on the TLC plates. The phenomenon described may arise with substances other than prostaglandins for which these plates are used.

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