

## **EABiotech Ltd.**

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### **Conventional Autoradiography detection of Tritiated(<sup>3</sup>H) and Carbon-14(<sup>14</sup>C) sugars on paper is compared to the Enhanced Autoradiography procedure.**

Mixtures containing D-[<sup>14</sup>C]glucose, D-[<sup>3</sup>H]mannose, L-[<sup>3</sup>H]arabinose and L-[<sup>3</sup>H]fucose were separated by descending paper chromatography on Whatman 3MM paper in ethyl acetate / pyridine / water (8:2:1) for 24 hours. Samples applied were (i) 1nCi of each monosaccharide, (ii) 10nCi of each monosaccharide, (iii) 100nCi of each monosaccharide. This procedure was carried out on three separate sheets of paper.

1. Chromatographic paper was placed in a cassette with X-ray film, placed in a -700 C freezer and exposed for 7 days, then developed. Figure 1( a ).
2. Chromatographic paper, before exposure to the film, was treated by a process called Fluorography. This is a process which improves the detection of Radio Isotopes on X-ray film, by saturation of the paper with a fluor(PPO). When the radioactivity on the surface of the paper interacts with the fluor, light is produced. Signal enhancement results because it is easier to detect light than to detect the beta particle directly. All other conditions were as described in (1) above. Figure 1( b ).
3. Chromatographic paper, before exposure to the film, was treated by the process called Enhanced Autoradiography. A wax scintillator was melted at low temperature (550C) into the chromatographic paper. The chromatographic paper where the wax was applied became translucent. Almost all of the radioactive decays , throughout the paper, were converted to light, and more easily be detected. All other conditions were as described in 1. above. Figure 1( c ).

The superior detection efficiency of Enhanced Autoradiography, over the other methods is obvious, by visual inspection.

Further enhancement could be achieved, by placing sample (3) and the X-ray film between two sheets of aluminium foil prior to loading them into the cassette.

The advantages of Enhanced Autoradiography are:

1. The separated components, D-[<sup>14</sup>C]glucose, D-[<sup>3</sup>H]mannose, L-[<sup>3</sup>H]arabinose and L-[<sup>3</sup>H]fucose shown on figure 1(c) (iii) may be cut out from the chromatographic paper, placed in a vial, and counted in a Liquid Scintillation Counter. In this way the amount of each component can be quantified.
2. There is no requirement for a liquid scintillation cocktail.

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3. After quantification as described in 1. above, the sample can be washed with an appropriate solvent, such as toluene or a toluene substitute, to remove the EA-Wax. The sample is left intact for further investigation.
4. Enhanced Autoradiography is so efficient that results that would normally take between 8 to 10 weeks to obtain can now be achieved in 3 days.
5. Due to the long exposure times required in the past, researchers increased the amount of radioactivity to try and speedup the process. The amount of activity now required can be reduced considerably, with great financial and material savings.

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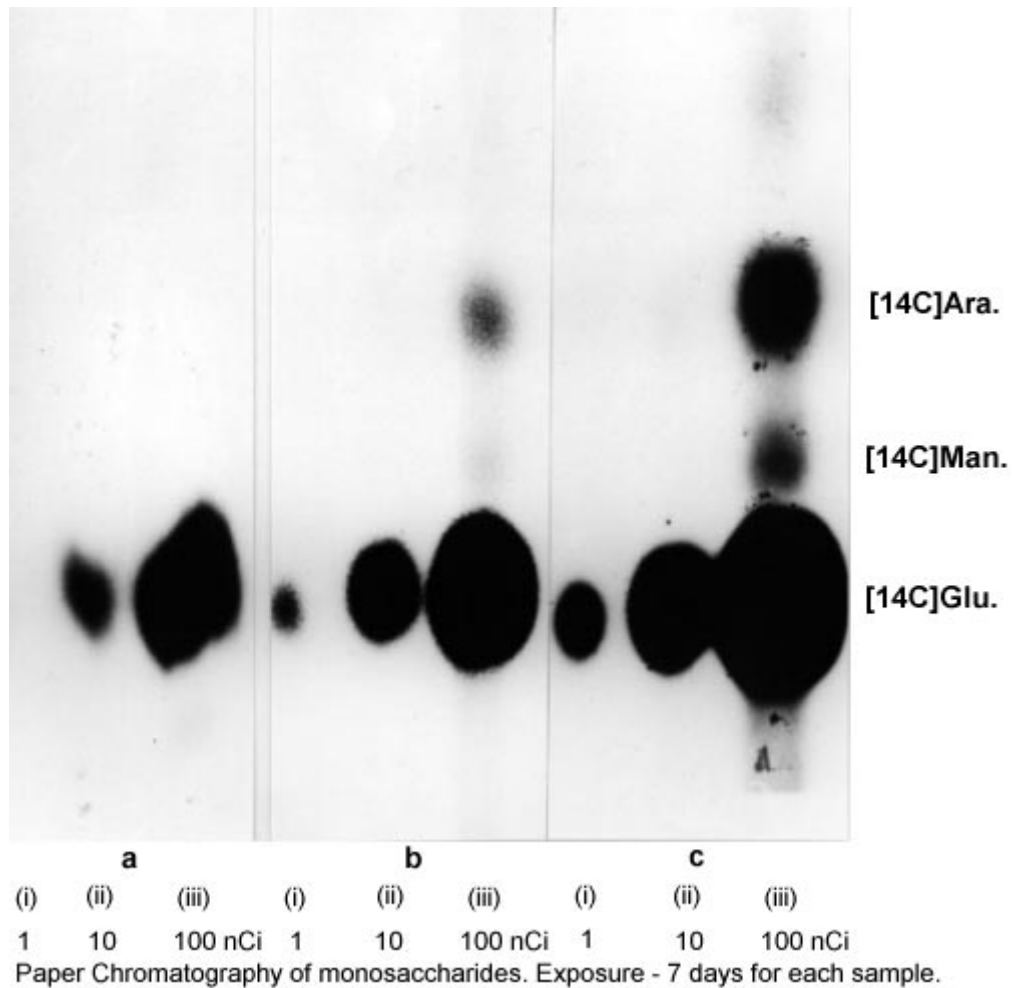


Figure 1: **The detection of radiolabelled sugars on paper chromatography. Comparison of the 3 methods used.** a) Autoradiography, b) Conventional Fluorography c) Enhanced Autoradiography.