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*BRIEF COMMUNICATION*

# Filters for Use with an Iodine–Quartz Lamp to Excite Immunofluorescence

A. H. TOMLINSON
*Public Health Laboratory, Radcliffe Infirmary, Oxford*

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In recent years the iodine–quartz lamp has been advocated as a cheap and convenient alternative to the mercury arc for exciting the fluorescence of fluorescein-labelled antibody. The iodine-quartz lamp is an incandescent filament source which has a very small output of ultraviolet light and relies for its efficiency on the blue-violet radiation, i.e. wavelengths between 400 and 500 m$\mu$.

Antibody conjugated with fluorescein isothiocyanate has a maximum absorption at a wavelength of 495 m$\mu$ and a peak emission of fluorescent light at 520–525 m$\mu$ (Fothergill, 1962; Brighton, 1966). An ideal primary filter would, therefore, have a high transmission at wavelengths shorter than 500 m$\mu$ and a very sharp cut-off above this wavelength. This exacting requirement is difficult to achieve because of the nature of light absorption by coloured materials. The secondary filter must pass light of wavelength 520 m$\mu$ and longer with a sharp cut-off at shorter wavelengths. This can be achieved by several glass and gelatine filters—as shown by data in the manufacturers' catalogues.

*Apparatus*

In order to compare mercury-arc and iodine–quartz illumination the two light sources were so arranged that, by rotating a specially mounted mirror, light from either source could be directed into the substage condenser.

The mercury source was a MED/250 watt burner in a Vickers housing, used with a Chance-Pilkington OX7 filter for blue light, or an OX7 plus a Chance-Pilkington OX1 for ultraviolet light.

The iodine–quartz lamp, 100 W, Atlas A1/209, was in a locally built lamp house fitted with a concave mirror behind the lamp, a collecting lens and a Chance-Pilkington ON 22 heat filter in front.

A Watson Bactil microscope was used with darkfield condenser and inclined monocular tube.

*Filters*

A critical test for the primary filter is to examine a microscopical preparation which has not been stained with fluorescein-conjugated antibody. Such a slide should not show any colouration which could be confused with fluorescein staining. It is important to note that this is a test of the primary filter only and no alteration of the secondary filter can be used to eliminate 'fluorescein-coloured' light transmitted by the primary filter.

Several glass primary filters used with the iodine–quartz lamp were unsatisfactory either because they did not excite fluorescence with sufficient brilliance, or because they gave confusing green colours with unstained preparations and poor contrast between antigen and background in stained sections.

The most satisfactory primary filter found was a combination of two Wratten gelatine filters, Nos. 32 and 38A, sandwiched between 2-inch square cover glasses. No. 32 is a magenta filter which has a transmission in the blue of 66 per cent at 460 m$\mu$, 21 per cent at 490 m$\mu$, falling to 0·13 per cent at 520 m$\mu$ (Kodak, 1961). In addition, it transmits orange-red light and the No. 38A gelatine was used to attenuate this; the combination transmits about 0·4 per cent in the range 600–640 m$\mu$. Extra attenuation of the orange can be achieved when necessary by adding either a CC 50C, or a 78A gelatine filter.

Satisfactory secondary filtration was given by a Wratten No. 12 which transmits only 1·5 per cent at 500 m$\mu$ rising to 55–88 per cent over the range of 520–550 m$\mu$.

## RESULTS

This filter combination has been used for several months to examine the following fluorescence stained antigens: *S. sonnei* in faecal smears; varicella/zoster virus in cultured human fibroblasts; mumps virus and Reo-3 virus in cultured monkey kidney cells; herpes simplex virus in cultured human amnion cells, and in frozen sections of mouse brain, human brain and guinea-pig skin.

Unstained preparations showed no green colouration at all. Specifically stained antigen was adequately bright and unstained parts of the preparation appeared as a darkground image in colours ranging from brown to orange, which gave excellent contrast with the green fluorescence. In the preparation of guinea-pig skin the keratinized layer and the connective tissue were unduly bright orange, but the addition of a CC 50C gelatine (very pale blue) to the primary filter reduced this to an acceptable level.

Comparison, effected by turning the mirror from one light source to the other, showed that the iodine–quartz lamp always gave as bright fluorescence as did the mercury arc. With many specimens, especially those weakly stained, the iodine–quartz illumination gave brighter fluorescence and with all preparations the colour contrast was better than with the mercury arc.

It has not been possible to make a direct comparison between iodine–quartz illumination and an HBO 200 arc.

This filter combination has not been extensively tested on commercial iodine–quartz equipment, but in a limited trial it performed well in the Gillett and Sibert 'Conference' microscope.

The suggested primary filter is not ideal because the combined transmission of the Nos. 32 and 38A is below 40 per cent at the wavelengths required for excitation. However, because it does not give any confusing green colouration and does produce good colour contrast, it is probably the best filter at present available for exciting immunofluorescence with an iodine–quartz lamp.

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