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COPAS™ Technical Note TN01
C.elegans Survival Study

Effect of SYTOX® staining in combination with manual sorting or COPAS technology sorting on the mean survival of wild-type *C.elegans*.

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Objective

To confirm that COPAS sorting or exposure to the SYTOX® stain (Application Note *C.elegans* B02) has no significant effect on the viability of wild-type *C.elegans*. Specifically, we compared mean survival of nematodes that were transfered manually to separate adults from eggs and progeny with nematodes that were repeatedly flowed through a COPAS *BIOSORT*® (Figure 1; COPAS Protocol). These experiments were designed to determine what effect, if any, repeated passage through the COPAS flow cell and exposure to SYTOX® has on their lifespan. Two successive survival experiments were completed using nearly identical methods. A third experiment examined the effect on survival of continuous exposure to SYTOX® stain.

Figure 1. The COPAS *BIOSORT*® was used to sort *C.elegans* into 96 well microtiter plates.

Materials

Wild type N2 *C.elegans*, synchronized by bleaching and divided into two groups on agar plates.
M9 Buffer
Dissecting Microscope, (Leica)

Method

Large numbers of gravid adult N2 were treated with NaOH-Hypochloride (**SAMPLE PREPARATION PROTOCOL SPB05**) on the first day (day 0) to get a synchronous population of eggs at the start of the experiment. Approximately 10,000 eggs were distributed to 9 large Petri plates of NGM agar seeded with *E.coli* OP50. Larvae were grown at 16°C on day 1 to delay the start of reproduction, transferred on day 2, and maintained at 20°C on 100mm NGM plates throughout the rest of the experiment, through day 24.

When the experiment ended on day 24, at least 98% of the remaining worms in each set were dead. On day 4, the following procedure was used to separate young adult worms from their progeny: all animals on the plate were collected in M9 buffer, adult worms were separated from larvae and eggs by settling successively 4 times through M9 buffer in 15 mL tubes, suspended larvae and eggs were removed by pipette and discarded. Separation and removal of larvae by this procedure of allowing heavier adults to settle was repeated daily until no more progeny were produced. Some adults are lost by this procedure and some eggs and larvae do get carried over with the adults. These are removed manually from the adult population.

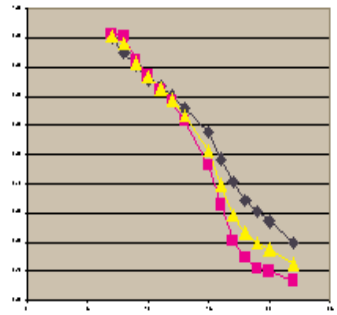

Figure 2. Control populations
Replicate 1 = blue,
Replicate 2 = purple
1 and 2 combined = yellow
Vertical axis = survival rate in %
Horizontal axis = days (1-24).

The worms were divided into 5 sets with each set further divided into 2 replicates. The sets were treated as follows:

1. **Control.** No exposure to COPAS or SYTOX® stain. Adult worms were collected daily and separated from larvae by settling, then returned to new, seeded NGM plates. Started with about 1000 worms per replicate. (see Figure 2)

2. **SYTOX® stained only.** No exposure to COPAS. The start point was with approximately 1000 worms per replicate. Adult worms were collected daily and separated from larvae by settling. On days 5,6,7,8,10,11,12,13 and 14, worms were exposed for 15 minutes to SYTOX® green stain (S-7020) at 5000:1 dilution in M9 buffer, followed by a wash in M9. (see Figure 3)

3. **COPAS only.** No exposure to SYTOX® stain. The start point was with about 1200 worms per replicate. Adult worms were collected and separated from larvae as above. On the same days listed for set 2 above, adults were placed in the COPAS sample cup in about 10 mL M9 buffer (stirring speed: 85 rpm), passed through the flow cell and collected directly into a sterile 250 mL beaker containing a small volume of M9 buffer placed under the outlet of the flow cell. All worms that exited the flow cell were collected without sorting (sorter valve off). The collected worms were settled in the collection beaker and the supernatant drawn off, then the worms were returned to new NGM plates. (see Figure 4)

4. **COPAS plus SYTOX® stain**. Started with about 1200 worms per replicate. On the same days listed above for set 3, adult worms were separated from larvae and stained with SYTOX® green as described. After 1x wash in M9, the worms were run through the COPAS as described above for set 3, then returned to NGM plates. (see Figure 5)

Scoring death:

Beginning on day 8 (expt. 1) or day 7 (expt. 2), worms were scored daily, with a few days missed, to count the number that had died since the previous day. Before worms were washed from the previous day's NGM plates, each plate was scanned under the dissecting microscope to locate any worms that were not moving. Inert worms were prodded with a pick at least 3 times over about 10 seconds; any that failed to move were counted as dead and were picked off the plate. To calculate survival over the course of each experiment, only those worms that died and were removed from the plates or that were still alive at the end of the experiment were considered. During each experiment, an undetermined number of worms were lost during handling or by burrowing into the agar. These were not included in the calculation.

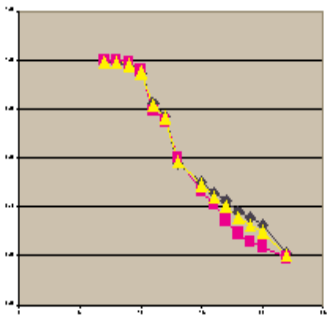

Figure 3. SYTOX® stained populations
Replicate 1 = blue,
Replicate 2 = purple
1 and 2 combined = yellow
Vertical axis = survival rate in %
Horizontal axis = days (1-24)

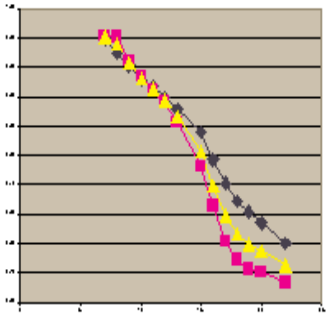

Figure 4. COPAS populations
Replicate 1 = blue,
Replicate 2 = purple
1 and 2 combined = yellow
Vertical axis = survival rate in %
Horizontal axis = days (1-24)

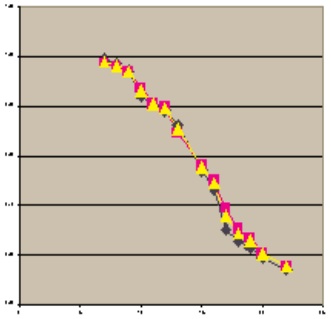

Figure 5. COPAS and SYTOX® populations
Replicate 1 = blue,
Replicate 2 = purple
1 and 2 combined = (yellow)
Vertical axis = survival rate in %
Horizontal axis = days (1-24)

Results

The SYTOX® test indicates that the chemical treatment does not affect the survival rate in either manual or COPAS sorting. Likewise, the COPAS methods do not show a significant effect versus the manual method on survival rate (see Figure 6).

Discussion

There are divergences of opinion in the literature concerning "typical" values for wild type mean survival. Kenyon (*C.elegans* II) reports survival for wild type N2 nematode strains ranging from 12-18 days. Experiences at Union Biometrica lie within this range. The Melov report in Science shows a mean survival of 21 +/- 6 days. Our results fall outside that range. A significant contribution may come from differences in wild type stocks between laboratories. Another contribution may come from the scoring technique. The slope of the survival results at the 50% point is the same as the wild type curves shown by Melov. The results suggest that neither exposure to COPAS sorting, nor exposure to the SYTOX® stain (see APPLICATION NOTE B02: *C.elegans* Sorting: Live vs. Dead) have significant effect on the survival of *C.elegans.*

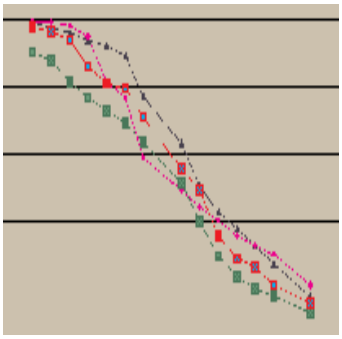

Figure 6. Comparison of the four different methods.
Manual = blue
SYTOX® alone = purple
COPAS alone = green
COPAS and SYTOX® = red
Vertical axis = survival rate in %
Horizontal axis = days (1-24)

References

Melov et all: Science. 2000 Sep 1; 289(5484):1567-9

Kenyon: *C.elegans* II. 1997. Chapter 28, pp. 791-813, Cold Spring Harbor Lab Press